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Yoran Le Strat

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Par

Yoran LE STRAT

**Etude de la communauté fongique associée à l'algue rouge
Palmaria palmata pour la production d'enzymes de dégradation
pariétale à visée biotechnologique**

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France

Rapporteurs avant soutenance :

Claire Hellio Professeure des universités, Université de Bretagne Occidentale
Anne Favel Maître de conférences des universités, Université Aix Marseille

Composition du Jury :

Président :	Bernard Offmann	Professeur des universités, Nantes Université
Examineurs :	Joël Fleurence	Professeur des universités, Nantes Université
	Justine Dumay	Professeure des universités, Nantes Université
	Thibaut Michel	Ingénieur de recherche, Greensea
Dir. de thèse :	Justine Dumay	Professeure des universités, Nantes Université

Invité(s)

Nicolas Ruiz Maître de conférences des universités, Nantes Université
Paul Délérès Maître de conférences des universités, Nantes Université

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Liste des abréviations

ACE : Enzyme de conversion de l'angiotensine

Act : Actine

AgNPs : Nanoparticules d'argent

AGOS : Oligosaccharides d'agar

AMTI : Aquaculture multitrophique intégrée

AOS : Oligosaccharides d'alginate

APC : Allophycocyanine

Arabino (milieu) : Milieu de culture minimum à l'arabinoxylane

ASE : Extraction par solvant accéléré

ASW : Eau de mer stérile artificielle

BCA (dosage) : Dosage à l'acide bichoninique

β -tub : β -tubuline

CMC (milieu) : Milieu de culture minimum à la carboxyméthyl cellulose

COS : Oligosaccharides de carraghénanes

CYA : "Czapeck yeast extract agar"

DCA : "Dextrose casein agar"

DHA : Acide docosahexaénoïque

DNS (dosage) : Dosage à l'acide dinitrosalicylique

EAE : Extraction assistée par enzymes

EF : Facteur d'élongation

EPA : Acide eicosapentaénoïque

Epi/endo : Non déterminée (épiphyte ou endophyte)

FOS : Oligosaccharides de fucoïdanes

HPP : Haute pression hydrostatique

IFREMER : Institut français de recherche pour l'exploitation de la mer

ISOMer : Institut des substances et organismes de la mer

ITS : "Internal transcribed spacers"

MAAs : Acides aminés analogues de la mycosporine

MAE : Extraction assistée par micro-ondes

MMS (collection) : Collection de souches fongiques marines

(MSP)-PCR : "(Mini/micro-satellite primed) - Polymerase chain reaction"

MUSCLE : "Multiple sequence comparison by log-expectation"

NCBI : "National center for biotechnology information"

PC : Phycocyanine

PCB : Phycocyanobiline

PDA : "Potato dextrose agar"

PE : Phycoérythrine

PEB : Phycoérythroiline

PEC : Phycoérythrocyanine

PUB : Phycourobiline

PXB : Phycobilivoline

ROS : Espèces réactives de l'oxygène

R-PE : R-phycoérythrine

RuBisCo : Ribulose-1,5-bisphosphate carboxylase / oxygénase

SAMBA : "Standardized and automated metabarcoding analyses"

SIDA : Syndrome de l'immunodéficience acquise

SFE : Extraction par fluide supercritique

SmF : "Submerged fermentation"

SSF : "Solid-state fermentation"

SWE : Extraction en eau subcritique

SWMD : "Seaweed metabolite database"

UAE : Extraction assistée par ultrasons

UPGMA : "Unweighted pair group method with arithmetic mean"

INTRODUCTION GENERALE

Extraction de R-Phycoérythrine d'algues rouges

L'intérêt des algues rouges en biotechnologies existe depuis plusieurs décennies et s'accroît notamment en Europe et en France, concernant par exemple la production de composés naturels d'intérêt et de métabolites à haute valeur ajoutée comme la R-phycoérythrine (R-PE), pigment photosynthétique surnuméraire spécifique des algues rouges.

Dans ce contexte, ce travail de thèse s'inscrit dans la continuité de plusieurs études menées au sein du laboratoire ISOMer, sur des problématiques en lien avec l'extraction et la purification de fractions protéiques ou de R-PE à partir de diverses algues rouges (Thèse de Claire Denis, Thèse de Mathilde Munier, Thèse de Trang N Guyen, Thèse de Cécile Le Guillard). Lors de ces travaux, l'extraction assistée par enzymes focalisée sur les protéines et pigments a été étudiée et optimisée par méthodologie des plans d'expériences sur les espèces *Palmaria palmata*, *Grateloupia turuturu*, *Mastocarpus stellatus* et *Gracilaria gracilis*. Ainsi, si les xylanases se sont avérées très efficaces sur *Palmaria palmata* (Dumay et al. 2013), il a été établi que l'utilisation des cellulases était plus adaptée que celle des xylanases pour la dégradation de *Gracilaria gracilis* (Nguyen et al. 2020). Si ces résultats montrent la pertinence de l'utilisation d'enzymes pour dégrader la paroi algale chez ces espèces, la déstructuration des matrices aglales par hydrolyse enzymatique semble plus compliquée à mettre œuvre chez d'autres espèces, comme *Grateloupia turuturu* par exemple (Denis et al. 2009). En effet, chez certaines espèces, il peut être intéressant de coupler les procédés enzymatiques à des procédés physiques, tels que les ultrasons (Le Guillard et al. 2016). Dans toutes ces études, il ressort qu'une mise au point spécifique et rigoureuse doit être mise en place concernant le procédé d'extraction assisté par enzyme. Parmi les paramètres les plus influents, le choix de l'enzyme utilisé est crucial. Si l'enzyme (ou les enzymes) est sélectionnée essentiellement en fonction de l'espèce étudiée, d'autres facteurs ont montré leur effet significatif sur la qualité des extraits obtenus (cycle de vie, environnement...). Lors de ces études, il a clairement été établi que les enzymes utilisées dans les procédés d'extraction étaient principalement de nature fongique.

Deux des espèces d'algues rouges les plus étudiées au sein du laboratoire ISOMer pour l'extraction de R-PE sont les espèces *Grateloupia turuturu* et *Palmaria palmata*, toute deux abondantes sur le littoral Atlantique français. Des rendements de R-PE atteignant jusqu'à 4,39 mg/g de matière sèche pour *Grateloupia turuturu* (Munier et al. 2013) et jusqu'à 12,36 mg/g de matière sèche pour *Palmaria palmata* (Dumay et al. 2013) ont notamment été obtenus. La R-PE une fois extraite peut ensuite être purifiée pour augmenter la valeur des extraits obtenus. Des travaux menés au laboratoire ont permis de mettre en place des protocoles en une étape utilisant la chromatographie échangeuse d'anions. Ainsi, pour *Grateloupia turuturu*, ce protocole a permis de purifier les extraits jusqu'à l'obtention d'un

indice de pureté de 2,89 (Munier *et al.* 2015). Sur *Gracilaria gracilis*, ce même protocole a permis d'avoir des extraits enrichis en R-PE avec un indice de pureté de 0,17 (Nguyen *et al.* 2020). Ces indices de pureté sont obtenus à l'aide du calcul du ratio A_{565}/A_{280} . En effet, la R-PE est caractérisée par un pic d'absorbance à 565 nm, qui est confronté à la quantité en protéines totales estimées selon une mesure d'absorbance à 280 nm. Des analyses approfondies sur les extraits produits indiquèrent que la R-PE pouvait représenter jusqu'à 0,05 % du poids sec total de l'algue chez *Palmaria palmata* (Denis *et al.* 2010) et que chez *Grateloupia turuturu*, elle restait stable jusqu'à 8h à la lumière ou en étant solubilisée dans des solutions de pH allant de 4 à 10 et pour une température allant jusqu'à 40°C (Munier *et al.* 2014).

D'après les données évoquées précédemment, il semble que l'algue rouge *Palmaria palmata* se caractérise par une production importante de R-PE par rapport aux autres algues, ou du moins soit caractérisée par des rendements d'extraction plus importants, compte tenu de l'efficacité du traitement enzymatique sur les parois de cette algue (Dumay *et al.* 2013). D'autres études mentionnent le fait que la R-PE peut représenter jusqu'à 0,03 mg/g de poids frais, et jusqu'à 16,9 % de la fraction protéique soluble (Joubert and Fleurence 2008), ou encore qu'elle peut représenter jusqu'à 12 % du poids sec de cette algue *Palmaria palmata* (Galland-Irmouli *et al.* 1999; Fleurence 2016; Fleurence *et al.* 2018).

Il semble donc que *P. palmata* est particulièrement prometteuse pour l'extraction assistée par enzymes de la R-PE. De plus, les procédés d'extraction mis en place et optimisés pour l'obtention de cette R-PE pourraient également être appliqués pour l'extraction de composés hydrosolubles, libérés lors de la destruction pariétale. En effet, il semble important de mentionner le fait que *P. palmata* est caractérisée par une quantité de protéines particulièrement importante par rapport aux autres macroalgues, pouvant représenter jusqu'à 35 % du poids sec de l'algue (Galland-Irmouli *et al.* 1999; Mouritsen *et al.* 2013; Dumay *et al.* 2014; Harnedy *et al.* 2014; Lee *et al.* 2017).

En parallèle, l'étude et la valorisation des champignons marins font partie d'une autre thématique principale portée par le laboratoire ISOMer. Ces travaux portèrent initialement sur l'isolement de champignons marins issus de coquillages, de sédiments et d'eau de mer, principalement représentés par les genres *Penicillium*, *Aspergillus*, *Trichoderma* et *Cladosporium* (Sallenave-Namont and Pouchus 2000). Dans le même temps, des études de bioaccumulation de toxines fongiques au sein des coquillages ont été menées, faisant part du risque d'empoisonnement par la consommation de coquillages contaminés par certains champignons marins comme *Trichoderma koningii* (Sallenave and Pouchus 1999; Sallenave-Namont and Pouchus 2000).

Ces études réalisées dans les années 2000 marquèrent le début de la création de la collection de champignons du laboratoire ISOMer, continuant à s'étoffer au fil des années, et à partir de laquelle des études de valorisation des métabolites d'intérêt ont été réalisées. Ainsi, l'isolement de ligérine a été effectué pour la première fois à partir d'une souche marine de *Penicillium*. Il s'agit d'un analogue de la fumagilline, un sesquiterpénoïde chloré aux activités cytotoxiques (Vansteelandt *et al.* 2013). Une production de griséofulvine, caractérisé par des propriétés antifongiques, a également été détectée à partir d'une souche marine de *Penicillium waksmanii* (Petit *et al.* 2004). Plusieurs études ont permis la détection et la caractérisation de nouveaux peptaibols, classe de peptides aux activités antibactériennes, antivirales, antifongiques et antiparasites, à partir de champignons marins appartenant notamment au complexe d'espèces *Trichoderma harzianum* (Ruiz *et al.* 2007; Carroux *et al.* 2013; van Bohemen *et al.* 2021). Un intérêt particulier a également été donné pour la détection de mycotoxines produites par des souches fongiques marines en zones conchylicoles et présentant un risque d'intoxication pour le consommateur. Par exemple, une souche d'*Aspergillus fumigatus* a montré une production de gliotoxine, aux effets cytotoxiques et immunosuppresseurs, pouvant être accumulée au sein de coquillages tels que la moule bleue (Grovel *et al.* 2003; Kerzaon *et al.* 2008).

Il semble tout naturel de se tourner aujourd'hui vers l'exploration de l'étude des activités enzymatiques de champignons marins au sein du laboratoire ISOMer. Ce projet de thèse s'inscrit donc à la croisée des deux grandes thématiques présentées ici à savoir **1) l'optimisation de l'extraction de la R-PE à partir de macroalgues rouges** et **2) la valorisation biotechnologique des champignons marins en utilisant comme algue modèle *Palmaria palmata***.

Utilisation d'enzymes xylanolytiques et cellulolytiques pour la dégradation de *P. palmata*

L'optimisation de l'extraction de R-PE à partir de *Palmaria palmata* porte sur plusieurs paramètres, qui ont précédemment été étudiés (Dumay *et al.* 2013). L'un des paramètres principaux est le choix des enzymes utilisées pour la dégradation de la matrice macroalgale ciblée. Certaines enzymes ont des activités multiples, certaines glucanases pouvant cibler des glucans et des xyloglucans par exemple, ces capacités de dégradation permettant de classer les enzymes dans les différentes familles des Glycosyl Hydrolases (Maclachlan and Brady 1994; Segato *et al.* 2017; Zhao *et al.* 2019). Cela étant, pour la dégradation d'un type de polysaccharide donné, comme la cellulose par exemple, l'enzyme correspondant à ce polysaccharide est utilisée pour sa dégradation (utilisation de cellulase pour la dégradation de cellulose). Ce principe de sélection des types d'enzymes à utiliser en fonction du substrat à dégrader explique l'utilisation de xylanases et de cellulases dans les procédés d'extraction de la R-PE par dégradation enzymatique à partir de *P. palmata*, le xylane et la cellulose étant

respectivement les principal et second polysaccharides composant la paroi cellulaire de cette algue (Morgan *et al.* 1980; Lahaye *et al.* 1993; Dumay *et al.* 2014; Naseri *et al.* 2020). L'utilisation conjointe de ces deux enzymes sur la dégradation de *P. palmata* a d'ailleurs fait l'objet de plusieurs études, aboutissant à des résultats contradictoires (Fleurence 1999, 2003; Joubert and Fleurence 2008). D'après ces auteurs, il semble que le caractère synergique de l'utilisation d'un mélange de xylanase et de cellulase pour la dégradation de l'algue dépende de la nature biologique de l'origine des enzymes. En effet, il semble que les xylanases et cellulases respectivement issues des champignons *Aspergillus aculeatus* et *Trichoderma resei* permettent une meilleure extraction de protéines que celles des champignons *Disporotrichum* sp. et *Trichoderma viride* (Fleurence 2003; Joubert and Fleurence 2008).

Il est cependant important de souligner le fait que tous les organismes synthétisant un certain type de polysaccharides ne vont pas synthétiser exactement la même molécule. Concernant le xylane, il a été montré que non seulement la structure variait d'une espèce à l'autre, mais que des variations pouvaient avoir lieu en comparant différentes parties de la même plante (Rennie and Scheller 2014; Biely *et al.* 2016). Il a d'ailleurs été montré que *P. palmata* présentait 4 types de xylanes différents (Jiao *et al.* 2012). Des différences ont également été notées entre les compositions des molécules de cellulose issues de macroalgues et celles de plantes terrestres (Bansemer *et al.* 2016).

Ainsi, un second degré de spécificité des enzymes pour leur substrat vient s'ajouter. Non seulement une enzyme caractérisée en tant que xylanase sera certainement plus efficace dans la dégradation du xylane que dans celle de la cellulose, mais cette xylanase pourra également cibler et dégrader de manière plus efficace un type de xylane en particulier. Il a effectivement été montré que le principe d'extraction assisté par enzymes est efficace dans l'obtention de composés tels que des protéines ou des pigments à partir de macroalgues, mais que l'extraction serait plus efficace en utilisant des enzymes caractérisées par une spécificité au substrat algal considéré, plutôt qu'en utilisant des enzymes non spécifiques (Charoensiddhi *et al.* 2017).

Afin d'améliorer l'extraction assistée par enzymes de R-PE à partir de l'algue rouge *P. palmata*, il semble particulièrement intéressant de rechercher des enzymes ciblant spécifiquement les polysaccharides de cette algue. Sur la base d'un principe de biomimétisme, il a été considéré que de telles enzymes pourraient être synthétisées par les divers microorganismes se développant au contact de l'algue considérée. Les microorganismes issus de *P. palmata*, par la nécessité d'adaptation à leur hôte, sont donc les candidats idéaux pour la production d'enzymes ciblant spécifiquement les polysaccharides de cette algue. Le manque de données sur les communautés fongiques marines et algicoles par rapport aux communautés bactériennes, ainsi que les avantages que présentent les champignons par rapport aux bactéries dans la production d'enzymes, ont motivé le choix d'une

analyse de la communauté fongique de l'algue rouge *Palmaria palmata* pour leur production d'enzymes polysaccharidases.

Organisation des travaux de thèse

Avant la description des travaux réalisés, un travail bibliographique (**CHAPITRE I**) a été réalisé afin d'établir le contexte scientifique général dans lequel s'inscrit cette thèse. Cette première partie aborde des thématiques et des sujets facilitant l'appréhension des travaux présentés par la suite. Dans un premier axe, les macroalgues et l'algue rouge *Palmaria palmata* en particulier ont été présentés à divers niveaux. Un second axe abordera la thématique générale des champignons marins, en particulier issus de macroalgues. Concernant les applications biotechnologiques de ces derniers, l'accent sera mis en particulier sur les potentiels enzymatiques des champignons marins dans le cadre de dégradation de polysaccharides, de protéines et de lipides de macroalgues. Ce dernier point fait l'objet d'un travail de review qui a été publié dans Journal of Applied Phycology.

Les trois chapitres suivants sont dédiés à la présentation des travaux réalisés dans le cadre de cette thèse.

Dans un premier temps (**CHAPITRE II**), l'étude de la communauté fongique associée à *Palmaria palmata* a été effectuée. L'étude a été menée sur une durée d'un an, et a permis d'étudier la structure de la diversité fongique associée à *P. palmata* dans son ensemble, mais également d'estimer les potentiels impacts de différents facteurs environnementaux et d'isolement sur cette diversité. Ainsi, la saisonnalité, le protocole d'isolement (distinguant les champignons épiphytes des champignons endophytes), la nature du milieu de culture utilisé pour l'isolement, et la température d'incubation forment les quatre paramètres considérés.

Dans un second temps (**CHAPITRE III**), un protocole de criblage des activités xylanase et cellulase a été mis au point et réalisé sur l'ensemble des souches fongiques cultivables obtenues dans la partie précédente. Ce protocole a pour objet principal de déterminer qualitativement la part de souches positives à ces deux activités enzymatiques au sein de la communauté fongique cultivable. L'effet potentiel des paramètres environnementaux et d'isolement mentionnés précédemment sur la production enzymatique par les souches fongiques a également pu être étudié.

Dans un troisième temps (**CHAPITRE IV**), certains isolats, répondant positivement aux tests qualitatifs portant sur les activités xylanase et cellulase, ont été sélectionnés pour des analyses quantitatives. Les activités enzymatiques cellulase et xylanase ont été mesurées sur six souches fongiques isolées à partir de *P. palmata*. Les extraits enzymatiques bruts issus de ces souches fongiques, et à partir desquels l'activité a été mesurée, ont ensuite été utilisés pour vérifier leur efficacité dans la dégradation de

cette algue rouge. Des résultats préliminaires dans l'extraction de R-PE à partir de *P. palmata* en utilisant ces extraits bruts enzymatiques fongiques ont été obtenus.

CHAPITRE I : ETAT DE L'ART

Palmaria palmata, une algue rouge particulière

MACROALGUES - Définition, taxonomie générale, historique des usages

Classification

Les algues sont des organismes à photosynthèse oxygénique, dont l'appareil végétatif est un thalle et dont les organes reproducteurs sont appelés cystes. Les végétaux marins sont en grande majorité représentés par les algues (Hurd 2014) qui, en termes de diversité, représentent le tiers des divisions du règne des plantes et constituent le groupe d'organismes vivants le plus diversifié sur Terre (Ioanou and Roussis 2009; Baweja et al. 2016). Les macroalgues sont classiquement divisées en trois lignées en fonction de leur pigmentation, les algues rouges (Rhodophyceae), vertes (Chlorophyceae) et brunes (Phaeophyceae) (Hurd 2014; Hamid et al. 2015). Parmi ces trois groupes, les Rhodophyceae sont caractérisées par la plus grande diversité avec 45 % des espèces totales de macroalgues, suivies par les Phaeophyceae puis les Chlorophyceae (Reddy et al. 2010; Patwary et al. 2021). Cette importante diversité des algues rouges a notamment été montrée dans une étude récente portant sur la distribution des macroalgues dans les eaux françaises d'outre-mer (Figure I. 1) (Stiger-Pouvreau and Zubia 2020).

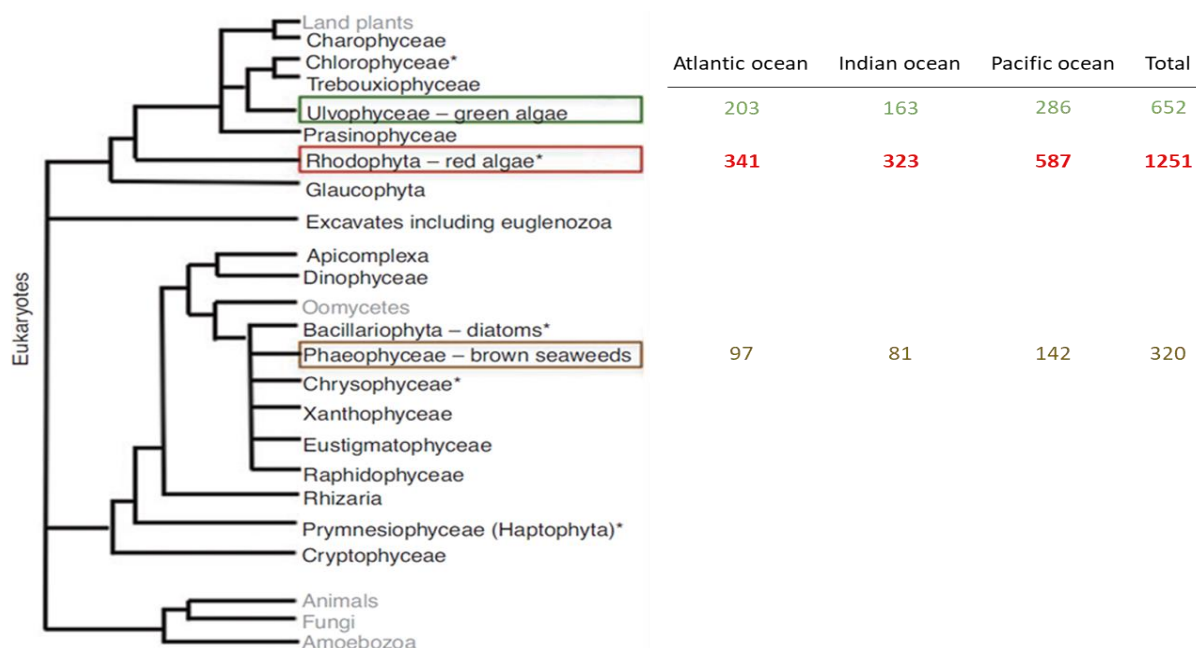


Figure I. 1 : Représentation de l'emplacement phylogénétique des trois grandes lignées d'algues (vertes, rouges et brunes) au sein des Eucaryotes. Le nombre d'espèces présentes dans les eaux françaises d'outre-mer a été renseigné pour chacune de ces trois lignées. D'après Stiger-Pouvreau and Zubia (2020).

Actuellement, environ 1600 espèces de macroalgues sont connues en Europe, classées dans un peu plus de 450 genres (Mineur et al. 2015). La base de données AlgaeBase (www.algaebase.org/) permet de visualiser la diversité et la taxonomie algale, ainsi que la biogéographie. Cette base de données

permet d'appréhender la diversité macroalgale à diverses échelles taxonomiques. La **Table I. 1** représente la quantité d'espèces différentes référencées pour chacune des grandes lignées d'algues (phylum), ainsi qu'un détail de la diversité des classes d'algues rouges qui seront présentées plus en détail par la suite.

Table I. 1 : Nombres d'espèces pour les trois lignées principales de macroalgues (algues rouges, brunes et vertes) et pour les sept classes d'algues rouges selon la base de données AlgaeBase.

Phylum	Classe	Effectif	Total
Rhodophyta (algues rouges)	Bangiophyceae	186	
	Compsopogonophyceae	73	
	Cyanidiophyceae	6	
	Florideophyceae	7088	7493
	Porphyridiophyceae	10	
	Rhodellophyceae	7	
	Stylonematophyceae	50	
Chlorophyta (algues vertes)			7033
Ochrophyta (algues brunes)			4474

Une estimation précise de la diversité macroalgale totale est difficile à réaliser car la découverte de nouvelles espèces est constante, ce qui tend à penser que de nombreuses espèces ou même de nombreux genres de macroalgues sont encore à découvrir. Cependant, la plupart des nouvelles macroalgues référencées en considérant une zone géographique en particulier comme l'Europe sont en fait des algues déjà observées ailleurs mais dont la présence dans cette zone géographique est nouvelle (Mineur *et al.* 2015). De plus, l'identification des algues au niveau morphologique peut parfois être difficile dans la mesure où des variations intraspécifiques dues aux conditions environnementales et à leur plasticité peuvent paraître plus importantes que des variations morphologiques interspécifiques (Norton *et al.* 1996). L'assignation spécifique des algues par des analyses moléculaires permet de lever ce verrou, notamment sur des algues d'intérêt économique (Lim *et al.* 2017).

Rôles écologiques des algues

Différentes tendances de répartition des espèces ont lieu sur le littoral, en fonction notamment de la localisation géographique, impliquant une importante diversité d'écosystèmes (Hurd 2014; Baweja *et al.* 2016). En ne considérant que les environnements côtiers, il est possible de différencier les estuaires, les lagons, les mangroves, les retenues d'eau, les marais salants, les côtes rocheuses, les plages de sable, les récifs coralliens, qui tous se caractérisent par des communautés macroalgales propres (Baweja *et al.* 2016). Les macroalgues ont divers rôles écologiques d'importance sur l'estran et dans

l'océan en général, constituant une source de nourriture et d'habitat pour d'autres espèces végétales, animales et microbiennes (Norton *et al.* 1996; Jueterbock *et al.* 2013; Casado-Amezúa *et al.* 2019).

Les macroalgues sont confrontées à plusieurs stress et développent des adaptations

Les zones littorales sont parfois décrites en tant qu'habitats aux conditions les plus rudes sur Terre à cause des rapides fluctuations environnementales qui y ont lieu (Hurd 2014). Les principaux facteurs environnementaux pouvant affecter les macroalgues, aussi connus en tant que facteurs abiotiques, peuvent être séparés entre les paramètres physiques et les paramètres chimiques (Hurd 2014; Baweja *et al.* 2016). Parmi les paramètres physiques, la température de l'eau, la quantité et la qualité de la lumière, la nature du substrat d'accroche de l'algue, ainsi que les contraintes dues aux vents et aux mouvements d'eau (courants, vagues) peuvent impacter le développement des macroalgues (Hurd 2014; Baweja *et al.* 2016; Lalegerie *et al.* 2020b; Ji and Gao 2021). Les principaux facteurs abiotiques chimiques pouvant affecter ces macroalgues sont la salinité (liée aux phénomènes de dessiccation), la disponibilité en nutriments en suspension, le pH de l'eau de mer, ainsi que la pollution (Hurd 2014; Baweja *et al.* 2016; Lalegerie *et al.* 2020b; Ji and Gao 2021). En plus des facteurs abiotiques, les facteurs biotiques correspondent aux interactions entre les macroalgues et leur microbiote (champignons, bactéries...) mais aussi certaines autres algues épiphytes et certains animaux tels que les brouteurs herbivores (Hurd 2014; Baweja *et al.* 2016; Lalegerie *et al.* 2020b). Ces interactions biologiques peuvent aussi bien avoir des effets bénéfiques pour l'algue que des effets néfastes (Hurd 2014). Pour contrer le stress biotique, des stratégies d'évitement peuvent être développées par les macroalgues. En effet, il est possible pour certaines algues d'échapper à leurs prédateurs potentiels, soit en se développant dans des endroits inaccessibles aux prédateurs (tels que des petites crevasses), soit en limitant par exemple leur période de croissance aux moments de faible prédation (la nuit) (Baweja *et al.* 2016). D'autres mécanismes existent comme l'adaptation de certaines algues aux herbivores brouteurs en développant des thalles calcaires ou le développement d'une tolérance à la prédation (Baweja *et al.* 2016). Cependant, la stratégie la plus connue et documentée est la production de composés bioactifs (Baweja *et al.* 2016; Lalegerie *et al.* 2020b). Les Rhodophyceae produiraient une diversité particulièrement importante de composés bioactifs avec plus de 1 600 composés identifiés (Hurd 2014; Abdul Malik *et al.* 2020). Cette diversité serait moindre pour les Phaeophyceae et encore plus réduite pour les Chlorophyceae, caractérisés par environ 1100 et 300 composés bioactifs identifiés respectivement (Hurd 2014). D'après la base de données Seaweed Metabolite Database (SWMD), 645, 220 et 32 métabolites auraient été obtenus à partir des algues rouges, brunes et vertes respectivement (Al Sharie *et al.* 2020).

Ces composés de réponse aux stress et liés à diverses adaptations aux conditions environnementales peuvent être issus du métabolisme primaire en appartenant à des classes de molécules telles que les

polysaccharides, les lipides, les protéines ou les pigments comme les phycobiliprotéines (dont la phycoérythrine) (Lalegerie *et al.* 2020b). Le métabolisme secondaire est aussi une source importante de molécules bioactives, les principales classes étant certainement les composés phénoliques (dont les phlorotannins) et les terpènes (Hurd 2014; Baweja *et al.* 2016; Lalegerie *et al.* 2020b). Les composés antioxydants ont fait l'objet de recherches particulièrement approfondies car le stress oxydant est souvent une conséquence des autres stress, et ces composants peuvent faire l'objet d'une valorisation dans la santé humaine. Les macroalgues synthétisent donc un panel de molécules aux propriétés antioxydantes telles que l'ascorbate (vitamine C), la vitamine A, la vitamine E, le glutathion, les caroténoïdes et les phycobiliprotéines, les polyphénols, etc. (Bischof and Rautenberger 2012; Contreras-Porcia *et al.* 2017). En plus de ces molécules actives, les macroalgues disposent d'un arsenal enzymatique de détoxification des ROS. Parmi ces enzymes il est possible de citer la superoxyde dismutase, l'ascorbate peroxydase, la glutathion réductase, la monodehydroascorbate réductase, la dehydroascorbate réductase ou la catalase (Bischof and Rautenberger 2012; Contreras-Porcia *et al.* 2017). La présence de halopéroxydases a aussi été observée chez certaines algues brunes (Bischof and Rautenberger 2012).

Ces conditions de vie difficile font partie de la vie des macroalgues depuis leur apparition sur Terre, leur métabolisme actuel est le résultat d'une adaptation progressive et d'une interaction constante avec leur environnement. Cependant, depuis plusieurs dizaines d'années, les activités humaines s'imposent comme une nouvelle et importante source de stress pour l'ensemble des écosystèmes du globe dont les littoraux (Norton *et al.* 1996). Ce stress anthropogénique consiste en un ensemble d'activités humaines dans les zones côtières comme l'introduction de macroalgues d'origine étrangères (Mineur *et al.* 2015). Cette introduction d'espèces étrangères en Europe est en augmentation exponentielle depuis les deux derniers siècles. Cependant, le suivi de la diversité n'étant que relativement récent, l'importation d'espèces potentiellement invasives date potentiellement de plus longtemps. Certaines algues européennes dites natives ne le seraient pas forcément mais cela reste difficile à évaluer (Mineur *et al.* 2015).

En plus de ces modifications de l'habitat à des échelles plus ou moins locales, les macroalgues doivent faire face à une multitude de stress secondaires résultant du changement climatique, perturbation globale à l'échelle de la planète liée aux activités humaines (Lalegerie *et al.* 2020b). En effet, une baisse de la présence des macroalgues est observée depuis les années 1990 principalement à cause d'une augmentation des températures des océans. Les macroalgues sont caractérisées par des températures optimales de croissance, de photosynthèse et de reproduction, qui se retrouvent ainsi fortement perturbées (Mineur *et al.* 2015; Casado-Amezúa *et al.* 2019). Par exemple, la température de surface de l'eau de mer en Bretagne connaît une augmentation de 0,35°C par décennie et ce réchauffement

est reconnu comme fortement responsable de la perturbation de la diversité des algues côtières (Gallon *et al.* 2014). En effet, il a été montré que les régions où la température augmentait le plus fortement correspondent aux régions dont la diversité en macroalgues est la plus perturbée (Gallon *et al.* 2014). Ces perturbations consistent en une augmentation de l'occurrence de certaines algues et une diminution ou disparition de certaines autres. Cette observation locale d'apparition/disparition d'espèces d'algues met en lumière un phénomène de migration de ces espèces suivant les évolutions de températures côtières (Jueterbock *et al.* 2013; Gallon *et al.* 2014). Cette migration vers le nord est estimée à une vitesse de 19 km/an dans l'Océan Atlantique Nord (Jueterbock *et al.* 2013). En effet, ce dernier semble marqué par un réchauffement plus rapide que tous les autres océans et il est estimé que les isothermes de température de surface remonteront d'environ 600 km vers le nord d'ici la fin du 21^{ème} siècle. L'isotherme d'été de 15°C s'est déjà décalée de 330 km vers le nord entre 1985 et 2000 (Jueterbock *et al.* 2013). Le comportement de trois espèces d'algues brunes (*Fucus serratus*, *F. vesiculosus* et *Ascophyllum nodosum*) a été modélisé face au dérèglement climatique et ces algues pourraient avoir totalement disparu des côtes françaises en 2100 (Jueterbock *et al.* 2013). Il peut être attendu qu'une telle disparition d'algues structurantes pour l'écosystème littoral dans sa globalité pourrait avoir des répercussions sur l'ensemble de cet écosystème. Certaines inconnues pourraient cependant faire varier ces prédictions. Par exemple, une hausse des températures pourraient accroître l'abondance d'herbivores brouteurs augmentant encore la pression effectuée sur les communautés d'algues. Par ailleurs, la plasticité et la capacité d'acclimatation est un autre paramètre difficile à prévoir et pouvant potentiellement permettre aux algues de résister partiellement aux augmentations de température. La migration de ces espèces vers le nord dépend également de leur capacité à tolérer une variation de la photopériode, et à supporter une entrée en compétition avec les macroalgues déjà présentes (Jueterbock *et al.* 2013).

Ces perturbations représentent d'importants paramètres à considérer, qui plus est dans une optique d'accroissement de l'utilisation des algues.

Utilisations des macroalgues

Alimentation humaine

Historiquement, les macroalgues sont principalement utilisées en l'alimentation humaine, en particulier dans les pays d'Asie comme le Japon, la Chine ou l'Indonésie (Ito and Hori 1989; Mabeau and Fleurence 1993; Sanjeewa *et al.* 2018; Hentati *et al.* 2020). La production en macroalgues de ces deux pays représente respectivement 48,7 et 36,6 % de la production mondiale (Hentati *et al.* 2020). L'Europe connaît depuis plusieurs décennies un important développement et une popularisation de l'usage des macroalgues en tant qu'aliment (Baweja *et al.* 2016; Sanjeewa *et al.* 2018; Afonso *et al.* 2019; Lopes *et al.* 2020), avec un marché caractérisé par une croissance exponentielle actuellement

autour de 7,5 % (Sahu *et al.* 2020). Bien que la diversité en macroalgues change d'une localisation à une autre, comme abordé précédemment, les macroalgues les plus consommées sont les algues brunes et plus particulièrement *Laminaria japonica*, *Pyropia yezeensis* et *Undaria pinnatifida*, suivies par les algues rouges et les algues vertes (Ito and Hori 1989; Nakhate and van der Meer 2021). Ces macroalgues ont une grande valeur nutritionnelle, avec un faible niveau calorique et une grande richesse en vitamines et en sels minéraux (Afonso *et al.* 2019; Leandro *et al.* 2019; Lourenço-Lopes *et al.* 2020; Nakhate and van der Meer 2021). Tous les minéraux essentiels tels que le sodium, le magnésium, le potassium, le phosphore ou le zinc se retrouvent chez les algues (Ito and Hori 1989) et leur production en calcium, fer et iode serait supérieure à celle de la plupart des plantes terrestres comestibles (Baweja *et al.* 2016). Les macroalgues produisent une large gamme de vitamines, aussi bien liposolubles qu'hydrosolubles (Ito and Hori 1989; Hamid *et al.* 2015). L'apport en protéines est relativement faible en ce qui concerne les algues brunes (3 à 29 % du poids sec total) mais est plus intéressant chez les algues vertes (9 à 30 % du poids sec total) et rouges (7 à 47 % du poids sec total) (Fleurence 1999; Baweja *et al.* 2016; Dumay and Morançais 2016; Afonso *et al.* 2019; Leandro *et al.* 2019; Lourenço-Lopes *et al.* 2020) et notamment chez les espèces *Porphyra tenera* et *Palmaria palmata* (Table I. 2) (Fleurence 1999).

Table I. 2 : Taux protéiques de plusieurs macroalgues comestibles utilisées en industrie. Issu de Fleurence (1999).

Espèce ou genre d'algue	<i>Palmaria palmata</i>	<i>Porphyra tenera</i>	<i>Ulva lactuca</i>	<i>Ulva pertusa</i>	<i>Laminaria digitata</i>	<i>Fucus sp.</i>	<i>Ascophyllum nodosum</i>
Quantité de protéine (% du poids sec)	8-35	33-47	10-21	20-26	8-15	3-11	3-15

Parmi les lipides totaux, qui ne représentent que de 1 à 2 % du poids sec des algues, les acides gras polyinsaturés tels que les omégas 3 ou omégas 6 sont particulièrement représentés (Ito and Hori 1989; Hamid *et al.* 2015) et à des proportions supérieures à celles rencontrées chez la plupart des plantes terrestres (Baweja *et al.* 2016). En plus d'être consommées fraîches et entières, les macroalgues sont largement utilisées en tant qu'additifs de certains aliments pour leur production en polysaccharides complexes présentant des propriétés gélifiantes et épaississantes (Mabeau and Fleurence 1993; Afonso *et al.* 2019; Leandro *et al.* 2019; Gomez *et al.* 2020; Lourenço-Lopes *et al.* 2020; Nakhate and van der Meer 2021). On retrouve ces hydrocolloïdes dans des préparations telles que les sirops, les crèmes glacées, les jus de fruits, les desserts, etc. (Nakhate and van der Meer 2021). Certains oligosaccharides dérivés de ces polysaccharides macroalgues ont également des applications dans le domaine alimentaire, notamment en améliorant le développement de bactéries du genre *Bifidobacterium* (Zhu *et al.* 2021).

Depuis plusieurs dizaines d'années, l'usage des macroalgues, traditionnellement limité à l'alimentation humaine, s'est grandement développé et diversifié notamment dans l'alimentation animale, l'agriculture, la production de biocarburants, la bioremédiation, ou dans les industries pharmaceutiques et cosmétiques *via* la production de composés bioactifs (**Figure I. 2**) (Baweja *et al.* 2016; Parkavi *et al.* 2020; Sahu *et al.* 2020; Nakhate and van der Meer 2021). Cela étant, elles sont encore sous-exploitées par rapport à leur réel potentiel (Baweja *et al.* 2016).

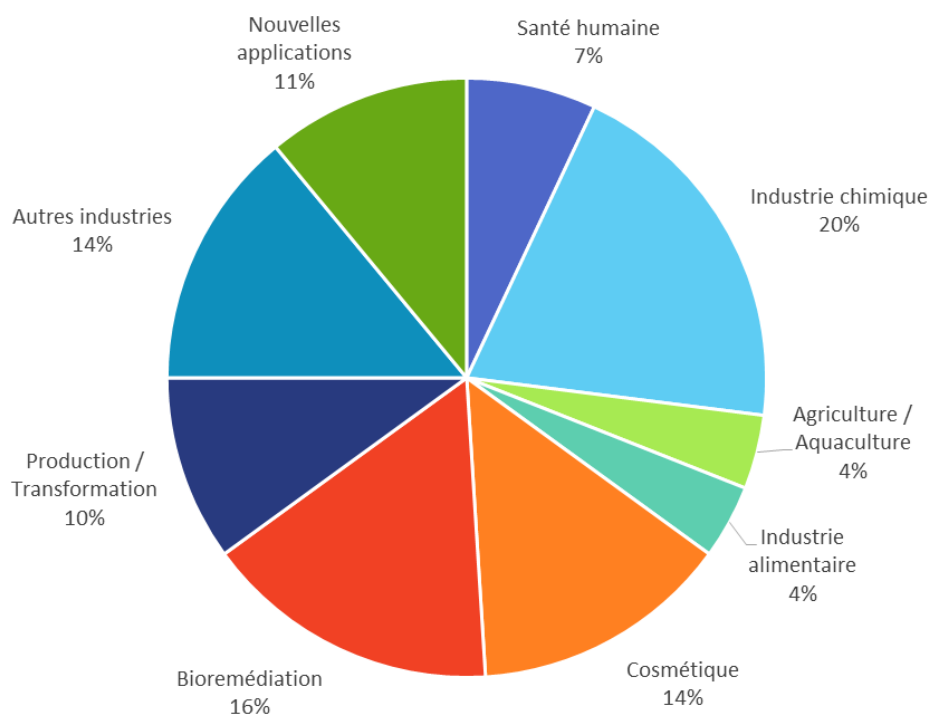


Figure I. 2 : Part des différents secteurs d'applications concernant l'utilisation des macroalgues en Europe. D'après Nakhate and van der Meer (2021).

Alimentation animale et agriculture

La qualité nutritionnelle des macroalgues évoquée précédemment rend leur utilisation dans l'alimentation animale particulièrement adaptée (Leandro *et al.* 2019; Yong *et al.* 2022). Par exemple, l'implémentation de macroalgues dans l'alimentation de ruminants s'accompagne d'une baisse de leur production de méthane (Yong *et al.* 2022). Les extraits algaux caractérisés notamment par certaines propriétés antibiotiques naturelles améliorent la santé des élevages qui en consomment, et peuvent améliorer la qualité du lait (Nakhate and van der Meer 2021). Certains composés macroalgaux tels que les oligosaccharides de carraghénanes sont utilisés en tant qu'additifs dans l'alimentation en aquaculture (**Table I. 3**) (Zhu *et al.* 2021). L'utilisation de macroalgues telle que l'algue brune *Ascophyllum nodosum* dans la fertilisation de cultures montre également plusieurs avantages (Baweja *et al.* 2016). Ainsi, la biomasse algale a une capacité naturelle de rétention de l'azote et du phosphore, ce qui réduit leur perte et augmente leur disponibilité (Zafar *et al.* 2022). Certains extraits issus de macroalgues (telles qu'*Ascophyllum nodosum*) peuvent permettre également d'augmenter le

rendement de culture (Yong *et al.* 2022; Zafar *et al.* 2022), d'améliorer les défenses naturelles des plantes contre les maladies et les insectes (Zhu *et al.* 2021; Zafar *et al.* 2022), de stimuler la germination (Baweja *et al.* 2016; Leandro *et al.* 2019; Zafar *et al.* 2022) et le développement des systèmes aériens et racinaires (Baweja *et al.* 2016; Leandro *et al.* 2019; Zhu *et al.* 2021; Zafar *et al.* 2022). Ces extraits ont aussi montré des implications dans l'augmentation de la tolérance à certains stress abiotiques pour les cultures tels que la sécheresse, le gel ou les hautes températures (Baweja *et al.* 2016; Leandro *et al.* 2019; Zhu *et al.* 2021; Zafar *et al.* 2022). Des phytohormones telles que l'acide abscissique, les auxines, les gibbérellines ou les polyamines sont également présentes dans ces extraits, ainsi que des micronutriments qui peuvent agir en tant que régulateurs de croissance et d'agents de murissement (Baweja *et al.* 2016; Zafar *et al.* 2022). Ces extraits algaux présentent également des avantages de biodégradabilité et de non-toxicité par rapports aux fertilisants conventionnels (Zafar *et al.* 2022).

Biocarburants et bioplastiques

La biomasse macroalgale présente une vitesse de croissance plus importante que la biomasse végétale terrestre, et la culture de macroalgues ne nécessite pas de substrat terrestre, ce qui rend leur usage économique et sans compétition territoriale avec la culture des plantes terrestres (Baweja *et al.* 2016; Laurens *et al.* 2020; Parkavi *et al.* 2020; Sahu *et al.* 2020; Kostas *et al.* 2021). Ces avantages sont non seulement intéressants pour l'utilisation des macroalgues dans l'agriculture et l'alimentation animale, mais également pour leur utilisation dans la production de biocarburants (Parkavi *et al.* 2020; Sahu *et al.* 2020; Nakhate and van der Meer 2021; Yong *et al.* 2022) ou de bioplastiques (Parkavi *et al.* 2020; Sahu *et al.* 2020; Yong *et al.* 2022). En effet, la fixation du CO₂ par les macroalgues est particulièrement efficace et la présence extrêmement rare de lignine dans leurs parois cellulaires permet une dégradation plus aisée par rapport aux plantes terrestres. Les macroalgues ne nécessitent par ailleurs pas forcément d'apports en nutriments (Laurens *et al.* 2020; Sahu *et al.* 2020; Kostas *et al.* 2021). En plus de la production de biocarburants, les macroalgues auraient également un intérêt dans la production d'énergie solaire, et plus spécifiquement dans la composition de cellules photovoltaïques (Sahu *et al.* 2020). Actuellement, la pollution plastique est particulièrement préoccupante car omniprésente sur l'ensemble du globe et présente des risques sanitaires liés à une toxicité affectant notamment les animaux, dont les humains (Yong *et al.* 2022). Une production de bioplastiques permettrait de s'affranchir de l'industrie pétrochimique et de la toxicité due à la composition chimique des plastiques conventionnels (Yong *et al.* 2022). En effet, les polysaccharides algaux tels que les alginates, carraghénanes ou agars peuvent être utilisés pour la formation de matériaux précurseurs dans la formation de bioplastiques (**Table I. 3**) (Lim *et al.* 2021; Yong *et al.* 2022). Leurs performances (durabilité, résistance physique, résistance à la chaleur) sont améliorées lorsqu'ils sont combinés avec d'autres matériaux biodégradables, formant ainsi un plastique composite (Yong *et al.* 2022). Ainsi, des

algues telles que *Kappaphycus alvarezii* et *Gelidium sesquipedale* ont été utilisées pour la production de films plastiques par des procédés de production écologiques sans extraction chimique (Lim *et al.* 2021). Bien qu'il s'agisse d'une technique particulièrement prometteuse, d'autres travaux sont nécessaires pour aboutir à un bioplastique viable et utilisable dans le futur (Lim *et al.* 2021; Yong *et al.* 2022).

Bioremédiation

L'importante capacité d'adsorption de minéraux tels que l'azote et le phosphore évoquée précédemment fait des macroalgues de bons candidats pour la bioremédiation et plus précisément le traitement des eaux usées (Arumugam *et al.* 2018; Wang *et al.* 2020). Ces eaux usées sont des co-produits de nombreuses industries (textiles, pétrochimiques, aquaculture, agriculture...) et contiennent des composés particulièrement polluants (Arumugam *et al.* 2018). Des méthodes physiques, chimiques et biologiques existent pour le traitement de ces eaux usées. Les traitements biologiques sont les plus avantageux en termes de coût, de simplicité et d'écologie (Wang *et al.* 2020). Ainsi, les macroalgues ont montré des capacités d'adsorption de certaines molécules polluantes comme les composés phénoliques grâce à une adsorption à leur surface *via* des liaisons hydrogènes entre ces phénols et les groupes hydroxyles des polysaccharides tels que les alginates (**Table I. 3**) (Arumugam *et al.* 2018; Wang *et al.* 2020). Certaines macroalgues ont également montré des capacités d'adsorption pour les colorants synthétiques ou les métaux lourds (Arumugam *et al.* 2018). Les algues brunes semblent particulièrement efficaces dans la neutralisation du toluène et du benzène (Wang *et al.* 2020). Cette biomasse produite pour le traitement des déchets pourrait ensuite être valorisée pour la production de biocarburants par exemple (Wang *et al.* 2020).

Table I. 3 : Synthèse non-exhaustive des applications biotechnologiques et activités biologiques de composés issus de macroalgues.

Application	Nature de molécule	Effet	Type ou espèce d'algue	Références
Alimentation animale (additifs)	Oligosaccharides de carraghénanes		Algues rouges	(Zhu <i>et al.</i> 2021)
Agriculture	Extraits algaux et hormones (acide abscissique, auxines...)	Amélioration du rendement, stimulation des défenses naturelles, résistance aux stress	<i>Ascophyllum nodosum</i> (algue brune)	(Baweja <i>et al.</i> 2016; Leandro <i>et al.</i> 2019; Zhu <i>et al.</i> 2021; Yong <i>et al.</i> 2022; Zafar <i>et al.</i> 2022)
Bioplastiques	Alginates, carraghénanes, agars		Algues rouges (<i>Kappaphycus alvarezii</i> , <i>Gelidium sesquipedale</i>) et brunes	(Lim <i>et al.</i> 2021; Yong <i>et al.</i> 2022)
Bioremédiation	Alginates	Adsorption de polluants	Algues brunes	(Arumugam <i>et al.</i> 2018; Wang <i>et al.</i> 2020)
Production de composés bioactifs	Carraghénane, agar, porphyrane (polysaccharides)	Antioxydant, antibactérien, immunomodulateur, antiviral, anticoagulant, antithrombotique, anticancéreux, anti-hyperlipidémique, anti-inflammatoire	Algues rouges	(Hentati <i>et al.</i> 2020; Lourenço-Lopes <i>et al.</i> 2020)
	Alginate, fucane, laminarine (polysaccharides)	Antioxydant, antibactérien, immunomodulateur, antiviral, anticoagulant, antithrombotique, anticancéreux, anti-inflammatoire, photoprotecteur, neuroprotecteur	Algues brunes	(Hentati <i>et al.</i> 2020; Lourenço-Lopes <i>et al.</i> 2020)
	Ulvane, rhamnane, arabinogalactane (polysaccharides)	Antioxydant, antiviral, anticancéreux, anticoagulant, antithrombotique, hypocholestérolémique	Algues vertes	(Hentati <i>et al.</i> 2020; Lourenço-Lopes <i>et al.</i> 2020)
	Chlorophylles (pigment)	Antioxydant, photoprotecteur	Algues rouges, brunes, vertes	(Lourenço-Lopes <i>et al.</i> 2020)
	Caroténoïdes (pigment)	Antioxydant, photoprotecteur, anti-âge	Algues rouges, brunes, vertes	(Lourenço-Lopes <i>et al.</i> 2020)
	Phycobiliprotéines (pigment)	Antioxydant, anti-inflammatoire, hépatoprotecteur, antidiabétique, antihypertenseur, immunomodulateur, anti-âge, neuroprotecteur	Algues rouges	(Lourenço-Lopes <i>et al.</i> 2020)

Table I. 3 : Suite.

Application	Nature de molécule	Effet	Type ou espèce d'algue	Références
Production de composés bioactifs	Autres métabolites primaires (protéines, lipides), et secondaires (hormones, terpènes, composés phénoliques...)	Antioxydant, antimicrobien, antiviral, antifongique, anticoagulant, anti-inflammatoire, antidiabétique, antitumoral, photoprotecteur, neuroprotecteur, anti-âge	Algues rouges, brunes, vertes	(Leandro <i>et al.</i> 2019; Lourenço-Lopes <i>et al.</i> 2020)

Synthèse de composés bioactifs d'intérêt

La production de médicaments et de principes actifs par les macroalgues est également très étudiée. L'intérêt que ces macroalgues constituent dans les biotechnologies en lien avec la santé s'est développé dans les dernières décennies (Mineur *et al.* 2015; Shannon and Abu-Ghannam 2019; Torres *et al.* 2019). Les propriétés gélifiantes et épaississantes de polysaccharides macroalgaux tels que les alginates, les agars ou les carraghénanes présentent non seulement des applications dans le secteur alimentaire mais également dans diverses formulations cosmétiques ou pharmaceutiques, où ils sont utilisés par exemple en tant qu'agents véhicules ou stabilisateurs (Leandro *et al.* 2019; Shannon and Abu-Ghannam 2019; Torres *et al.* 2019; Gomez *et al.* 2020; Hentati *et al.* 2020; Lourenço-Lopes *et al.* 2020). De plus, ces polysaccharides (et en particulier les polysaccharides sulfatés) peuvent aussi présenter des activités biologiques d'intérêt cosmétiques et pharmaceutiques, telles que des propriétés photo-protectrices, antioxydantes, antibactériennes, antivirales, antitumorales, immunomodulatrices, anticoagulantes, antithrombotiques ou anti-inflammatoires (**Table I. 3**) (Leandro *et al.* 2019; Shannon and Abu-Ghannam 2019; Gomez *et al.* 2020; Hentati *et al.* 2020; Lourenço-Lopes *et al.* 2020; Zhu *et al.* 2021; Yong *et al.* 2022). Certains polysaccharides algaux présenteraient également des propriétés neuroprotectrices, en particulier contre la maladie d'Alzheimer (Bauer *et al.* 2021). Cependant, ces polysaccharides sont peu solubles ce qui limite leur biodisponibilité et donc leur usage appliqué. Néanmoins, leur dégradation permet l'obtention d'oligosaccharides, conservant les activités des polysaccharides dont ils sont issus tout en étant plus solubles et donc beaucoup plus viables (Zhu *et al.* 2021). Les études sur les propriétés des oligosaccharides marins se développent continuellement depuis 2005 (Zhu *et al.* 2021). Parmi ces oligosaccharides, les oligosaccharides d'alginate (AOS), d'agar (AGOS), de carraghénanes (COS) ou de fucoïdanes (FOS) présenteraient des activités comparables à celles énoncées précédemment pour les polysaccharides (Zhu *et al.* 2021). Il a été montré que les AOS pourraient aussi améliorer la production de collagène au niveau de la peau humaine, stimuler la croissance des cellules endothéliales ou des kératinocytes, et dégrader les biofilms bactériens. De plus, les AGOS auraient des propriétés

prébiotiques intéressantes et leurs capacités hydratantes pourraient avoir des applications en cosmétique, tandis que les FOS pourraient avoir des applications dans les maladies vasculaires (Zhu *et al.* 2021).

D'autres composés bioactifs ont montré un intérêt dans le traitement de maladies comme le cancer (Nakhate and van der Meer 2021; Yong *et al.* 2022), le SIDA (Yong *et al.* 2022), les maladies vasculaires (Yong *et al.* 2022), les maladies neurodégénératives (Nakhate and van der Meer 2021) ou le diabète (Yong *et al.* 2022). Par ailleurs, des composés vermifuges tels que l'acide α -kainique, des composés halogénés aux propriétés anti-carcinogéniques ou des molécules aux propriétés anti-ulcères tels que la porphosine et la vercoyosine ont été extraits d'algues rouges (Ito and Hori 1989). Des activités antihypertensives et hypocholestérolémiques ont été associées à des composés algaux tels que la laminine et la β -homobétaïne respectivement (Ito and Hori 1989). Tout comme les plantes, les macroalgues ont la capacité de synthétiser des phytostérols, le plus connu étant probablement le fucostérol caractérisé par des activités antidiabétiques, anti-ostéoporotiques, et antioxydantes (Hamid *et al.* 2015).

Les pigments naturels issus de macroalgues tels que les chlorophylles, les caroténoïdes et les phycobiliprotéines présentent également des activités antioxydantes, antidiabétiques, antihypertensives, immunomodulatrices, anti-inflammatoires, anti-âge, photoprotectrices ou hépatoprotectrices (**Table I. 3**) (Lourenço-Lopes *et al.* 2020) mais ont également par nature des propriétés colorantes, ce qui les rend particulièrement attractifs dans le domaine de la cosmétologie (Torres *et al.* 2019; Parkavi *et al.* 2020). En dehors de ces pigments, l'intérêt des molécules marines et particulièrement celles issues des macroalgues est depuis quelques années en croissance dans le domaine de la cosmétique et des soins thérapeutiques, on parle de phycocosmétique ou de phycothérapie (Baweja *et al.* 2016; Nakhate and van der Meer 2021). Parmi les nombreuses biomolécules à intérêt cosmétique issues des macroalgues, les acides aminés analogues de la mycosporine (MAAs) présentent d'intéressantes capacités de protection contre les rayons UV et sont donc utilisées dans des écrans solaires (Nakhate and van der Meer 2021). Cet intérêt croissant des composés actifs issus de macroalgues n'est pas uniquement dû à leur efficacité, mais également à une augmentation d'un intérêt général des consommateurs pour les produits naturels au profit des composés de synthèse (Leandro *et al.* 2019; Lourenço-Lopes *et al.* 2020). En effet, il a par exemple été montré que les pigments de synthèse pouvaient présenter des effets néfastes sur la santé humaine et sur l'environnement, tandis que des pigments naturels tels que les phycobiliprotéines montrent au contraire des bénéfices pour la santé humaine et sont respectueuses de l'environnement (Lourenço-Lopes *et al.* 2020). Plus généralement, les composés naturels présentent tout un panel d'avantages par rapport aux composés synthétiques, tels qu'une meilleure biodisponibilité, une faible toxicité sur

la santé et pour l'environnement, ainsi qu'une production moins coûteuse (Fiset *et al.* 2008; Goswami and Naik 2014; Jacobsen *et al.* 2019; Jesumani *et al.* 2019; Lomartire *et al.* 2021).

RHODOPHYTES - Taxons les plus connus et utilisés, usages principaux, métabolites d'intérêt

Caractéristiques des algues rouges

L'importante diversité spécifique des algues rouges par rapport aux algues brunes et vertes s'accompagne d'une forte diversité morphologique, là encore plus importante que chez les Phaeophyceae et les Chlorophyceae (Saunders and Hommersand 2004).

Au niveau morphologique, anatomique et structural, les algues rouges se distinguent par certaines caractéristiques. La présence de réserves glucidiques sous forme de granules d'amidon floridéen constitués uniquement d'amylopectine et situés dans le cytoplasme caractérise également les algues rouges (Yoon *et al.* 2017). Ces dernières se caractérisent aussi par la présence de pigments hydrosolubles de nature protéique : les phycobiliprotéines. Phycoérythrines, phycocyanines et allophycocyanines sont organisées en structures appelées phycobilisomes et localisées au niveau de la surface externe des thylakoïdes (Freshwater *et al.* 1994; Yoon *et al.* 2017).

Classification et taxonomie

Traditionnellement et jusqu'au début des années 1990, la taxonomie des algues rouges s'élaborait selon leur pigmentation, et selon les caractéristiques anatomiques, structurales ou reproductives énoncées précédemment. Cependant, cela résulta en l'élaboration d'une taxonomie instable, avec des groupes polyphylétiques tels que les Bangiophycideae (Ragan *et al.* 1994), et une organisation taxonomique générale pouvant varier d'une publication à une autre. Par exemple, lorsque certains auteurs évoquaient trois ordres de Bangiophycideae et dix-sept ordres de Florideophycideae (Freshwater *et al.* 1994), d'autres citaient quatre ordres de Bangiophycideae et quatorze ordres de Florideophycideae (Ragan *et al.* 1994).

Ainsi, les deux études de Freshwater *et al.* (1994) et de Ragan *et al.* (1994) montrent le commencement d'une taxonomie des algues rouges par analyses moléculaires, et plus précisément en se basant sur les gènes *rbsS* et *rbsL* de la RuBisCo (Ribulose-1,5-bisphosphate carboxylase / oxygénase), et sur celui de la sous unité ribosomale, respectivement. Depuis, de nombreuses nouvelles études ont été réalisées, mêlant ou non aux analyses moléculaires des analyses morphologiques, se basant sur un ou plusieurs marqueurs moléculaires, pour effectuer des mises à jour de la taxonomie des algues rouges (Saunders and Hommersand 2004; Lim *et al.* 2017; Yoon *et al.* 2017). Actuellement, une organisation du phylum des Rhodophyta en deux sous-phylums et sept classes est largement acceptée par la communauté

scientifique (**Table I. 4**) (Yoon *et al.* 2017). Il apparait que la classe des Florideophyceae est la plus diversifiée en termes d'espèces, avec plus de 6750 espèces, ce qui représente 95 % de toutes les algues rouges. La quasi-totalité des représentants de cette classe se retrouve dans le milieu marin (Yoon *et al.* 2017).

Table I. 4 : Classification taxonomique actuelle des algues rouges jusqu'au niveau de l'ordre (Yoon et al. 2017). Les * permettent de retracer l'identité taxonomique de l'algue rouge *Palmaria palmata* (Famille : *Palmariaceae*).

Phylum	Sous-phylum	Classe	Sous-classe	Ordre
Rhodophyta*	Cyanidiophytina	Cyanidiophyceae		Cyanidiales
	Rhodophytina*	Bangiophyceae		Bangiales
		Compsopogonophyceae		Compsopogonales Erythropeltidales Rhodochaetales
		Florideophyceae*	Hildenbrandiophycidae	Hildenbrandiales
			Nemaliophycidae*	Acrochaetiales Balbianiales Balliales Batrachospermales Colaconematales Entwisleiales Nemaliales Palmariales* Rhodachlyales Thoreaales
			Corallinophycidae	Corallinales Hapalidiales Rhodogorgonales Sporolithales
			Ahnfeltiophycidae	Ahnfeltiales Pihelliales
			Rhodymeniophycidae	Acrosymphytales Bonnemaisoniales Ceramiales Gelidiales Gigartinales Gracilariales Halymeniales Nemastomatales Peyssonneliales Plocamiales Rhodymeniales Sebdeniales
		Porphyridiophyceae		Porphyridiales
		Rhodellophyceae		Dixoniellales Glaucosphaerales Rhodellales
		Stylonematophyceae		Rufusiales Stylonematales

Ecologie des algues rouges

Les algues rouges sont représentées aussi bien par des espèces autotrophes (indépendantes) qu'hétérotrophes (épiphytes parasites) (Baweja *et al.* 2016). Elles se développent principalement dans les eaux tempérées et tropicales (Baweja *et al.* 2016) mais peuvent se retrouver dans des régions froides (Freshwater *et al.* 1994). Environ 10 % du total des espèces de Rhodophyceae présentes sur Terre se retrouvent dans l'Océan Atlantique Nord (Tittley 2002). Parmi 707 espèces de macroalgues recensées sur les côtes Atlantiques françaises, 403 appartenaient aux Rhodophyceae (contre 183 pour les Phaeophyceae et 121 pour les Chlorophyceae) (Burel *et al.* 2019). Localement, les algues rouges sont principalement retrouvées dans la zone intertidale (Lalegerie *et al.* 2020b) mais peuvent se retrouver au niveau des limites hautes de la zone de balancement des marées, ainsi qu'à plusieurs centaines de mètres de profondeur dans les eaux claires tropicales (Yoon *et al.* 2017). Cette capacité à vivre en eaux profondes est notamment liée à leur large spectre d'absorption lumineuse grâce à la synthèse de divers pigments dont les phycobiliprotéines, en plus de la chlorophylle a (Baweja *et al.* 2016).

Phycobiliprotéines et R-Phycoérythrine

Ces pigments, et en particulier la phycoérythrine, présentent de nombreux intérêts biotechnologiques dans la valorisation de ces algues rouges. Il existe quatre types de phycobiliprotéines, seuls pigments hydrosolubles de macroalgues et présents uniquement chez les algues rouges. Il s'agit de la phycoérythrine (PE), la phycocyanine (PC), l'allophycocyanine (APC) et la phycoerythrocyanine (PEC) (Martínez-Oyanedel *et al.* 2004; Dumay *et al.* 2014; Hamid *et al.* 2015; Baweja *et al.* 2016; Miyabe *et al.* 2017).

Structurellement, les phycobiliprotéines sont composées de deux sous-unités protéiques différentes α et β . Ces sous-unités α et β peuvent s'associer pour former un monomère ($\alpha\beta$) et trois de ces monomères peuvent à leur tour s'associer en disque pour former un trimère ($\alpha\beta$)₃. L'association de deux trimères l'un sur l'autre forme un hexamère ($\alpha\beta$)₆ et correspond à la structure globale d'une phycobiliprotéine (**Figure I. 3**) (Dumay *et al.* 2014; Miyabe *et al.* 2017).

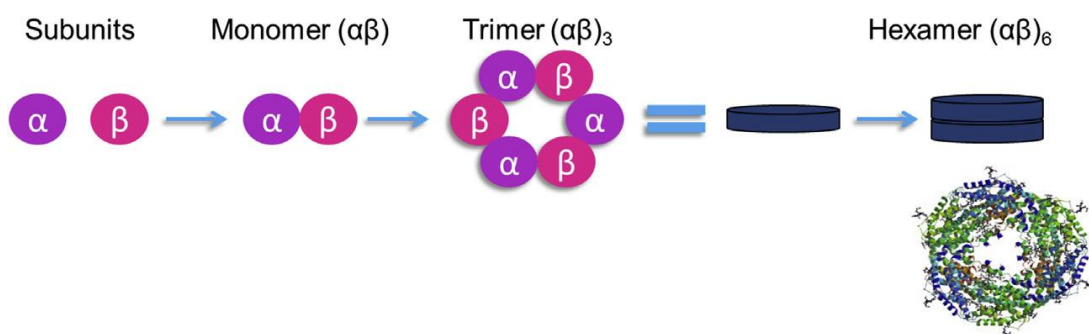


Figure I. 3 : Modalités d'assemblage des sous unités protéiques des phycobiliprotéines. Issu de Dumay *et al.* (2014).

Concernant la phycoérythrine, une troisième sous-unité protéique γ peut être associée à cet hexamère et stabilise l'ensemble de la structure en se fixant au centre ce qui forme un polymère $(\alpha\beta)_6\gamma$ (Fleurence 2003; Niu *et al.* 2006; Liu *et al.* 2009; Dumay *et al.* 2014).

Les couleurs des phycobiliprotéines sont induites par la présence de chromophores, qui vont varier selon les espèces et familles. Il existe quatre chromophores différents, la phycobilivoline (PXB), la phycocyanobiline (PCB), la phycoérythrobiline (PEB) et la phycourobiline (PUB), responsables respectivement d'une coloration violette, bleue, rouge et bleue / turquoise (Martínez-Oyanedel *et al.* 2004; Dumay *et al.* 2014). A chaque monomère (α , β et γ) de chaque phycobiliprotéine se fixe au moins un chromophore *via* une liaison thioéther et chaque type de phycobiliprotéine est associé à un nombre et type précis de chromophores (Martínez-Oyanedel *et al.* 2004; Niu *et al.* 2006; Dumay *et al.* 2014).

Il existe plusieurs types de phycoérythrines, dont la composition en chromophores diffère selon le type d'organisme considéré. La R-phycoérythrine (R-PE) est la principale phycobiliprotéine retrouvée chez les algues rouges, et est ainsi responsable de leur coloration (Dumay *et al.* 2013, 2014). Ce pigment est caractérisé par une longueur d'onde d'absorption maximum (λ_{max}) qui peut varier selon la littérature considérée mais est de l'ordre de 490 - 580 nm (Niu *et al.* 2006; Liu *et al.* 2009; Dumay *et al.* 2013; Miyabe *et al.* 2017; Sfriso *et al.* 2018). Ces variations peuvent être expliquées par le fait que la R-PE présente une structure douée d'une certaine plasticité qui peut donc être sensiblement modifiée selon les conditions environnementales comme le pH ou la nature du solvant (Jiang *et al.* 1999; Martínez-Oyanedel *et al.* 2004; Liu *et al.* 2009; Bharmoria *et al.* 2020). La R-PE est caractérisée par un poids moléculaire générique de 240 kDa (Dumay *et al.* 2014; Sfriso *et al.* 2018).

Les caractéristiques et la stabilité structurelle de la R-PE en font une molécule à haute valeur ajoutée en biotechnologies. En effet, en dehors de son effet colorant rose pouvant trouver des applications dans diverses industries (alimentation, peinture, cosmétiques), sa fluorescence lui confère de nombreux potentiels dans des technologies tels que la cytométrie en flux, l'immunofluorescence ou l'immunophénotypage (Fleurence 1999; Dumay *et al.* 2013, 2014; Sfriso *et al.* 2018; Bharmoria *et al.* 2020). Les activités des pigments algaux dont les phycobiliprotéines ont déjà été abordées, mais la R-PE spécifiquement a montré une importante diversité de bioactivités telles que des activités antitumorales, anti-âges, anti-inflammatoires, antioxydantes, antidiabétiques, antiparasites, immunosuppressives et antihypertensives (Fleurence 2003; Dumay *et al.* 2013, 2014; Miyabe *et al.* 2017; Leandro *et al.* 2019; Lourenço-Lopes *et al.* 2020; Rosales-Mendoza *et al.* 2020). La R-PE présente également un certain potentiel dans l'élaboration de cellules photovoltaïques (Bharmoria *et al.* 2020), ainsi que dans les nanotechnologies et plus précisément en association avec des nanoparticules

d'argent Ag⁰, dans la composition de matériaux composites à haute conductivité électrique (Bekasova *et al.* 2017).

Intérêt biotechnologique de molécules issues d'algues rouges

Cet important potentiel biotechnologique des phycobiliprotéines n'est pas restreint à la phycoérythrine (Ioanou and Roussis 2009; Bedoux *et al.* 2014a; Bhowmick *et al.* 2020; Rosales-Mendoza *et al.* 2020; Kostas *et al.* 2021). En effet, les phycocyanines peuvent montrer des activités anticancéreuses, anti-inflammatoires, antioxydantes, immunomodulatrices et neuroprotectrices, tandis que les allophycocyanines ont des activités antioxydantes, anti-inflammatoires, antitumorales, antivirales et hépatoprotectrices (Kostas *et al.* 2021).

En ce qui concerne les caroténoïdes, ils peuvent présenter en plus de leur pouvoir colorant des activités antioxydantes, anti-inflammatoires, antitumorales et peuvent être utilisés pour limiter les troubles ophtalmologiques et les maladies chroniques. Ils sont donc non seulement utilisés dans l'alimentation humaine mais aussi en tant qu'additifs dans l'alimentation animale, dans la cosmétologie et la pharmacologie (Kostas *et al.* 2021; Rana and Gupta 2021).

En plus des pigments, les algues rouges présentent une grande diversité de molécules bioactives d'intérêt, dont les grandes familles ont déjà été évoquées précédemment (Bouhlal *et al.* 2013; Hardouin *et al.* 2014; Pal *et al.* 2014; Rico *et al.* 2017; Sanjeewa *et al.* 2018). Cette diversité en composés d'intérêt est plus importante que chez les algues brunes et vertes (Fleurence *et al.* 2018; Abdul Malik *et al.* 2020; Ismail *et al.* 2020) et s'accompagne d'une meilleure valeur commerciale générale (Reddy *et al.* 2010). Les activités biologiques de ces métabolites secondaires sont souvent liées, pour l'algue, à des mécanismes de défenses contre les stress biotiques, coûteux en énergie mais apportant d'importants bénéfices dans la lutte contre les attaques microbiennes notamment (Yoon *et al.* 2017).

Les polysaccharides sulfatés d'algues rouges, tels que les agars ou les carraghénanes, constituent les composés principaux pour lesquels ces algues sont produites, car ils présentent d'intéressantes propriétés gélifiantes, épaississantes et stabilisantes en tant qu'hydrocolloïdes dans l'industrie alimentaire mais également dans la formulation de diverses préparations cosmétiques ou pharmacologiques (Ioanou and Roussis 2009; Sanjeewa *et al.* 2018; Lopes *et al.* 2020; Kostas *et al.* 2021; Rana and Gupta 2021). En plus de ces propriétés physiques, ces polysaccharides d'algues rouges présentent de nombreuses bioactivités d'intérêt pharmacologique (Rico *et al.* 2017; Ismail *et al.* 2020; Rosales-Mendoza *et al.* 2020; Elmajnaoui 2021; Kostas *et al.* 2021; Rana and Gupta 2021). Ces polysaccharides sulfatés peuvent notamment être utilisés dans le traitement de l'hypertension (Kostas *et al.* 2021). Les agars et carraghénanes d'algues rouges telles que *Grateloupia indica* ou

Porphyra yezoensis auraient des activités anticoagulantes comparables à celles de l'héparine qui peut provoquer des effets indésirables (Ismail *et al.* 2020; Elmajnaoui 2021; Rana and Gupta 2021). D'une manière générale, la bioactivité de ces polysaccharides d'algues rouges est liée à leur degré de sulfatation (Kostas *et al.* 2021). Comme abordé précédemment, certaines de ces activités caractérisent également les oligosaccharides issus de ces polysaccharides (Rana and Gupta 2021; Zhu *et al.* 2021).

Les algues rouges sont particulièrement intéressantes pour leur importante quantité de protéines par rapport aux algues brunes ou vertes (Fleurence *et al.* 2018; Kostas *et al.* 2021; Rana and Gupta 2021). Par exemple, des espèces telles que *Porphyra yezoensis* ou *Palmaria palmata* contiennent une quantité de protéines particulièrement importante, pouvant représenter 47 et 35 % de leur poids sec total respectivement (Fleurence *et al.* 2018). En plus d'une valeur nutritive liée à leur composition en acides aminés essentiels (Kostas *et al.* 2021), divers peptides ou protéines extraits d'algues rouges sont caractérisés par des activités antioxydantes (Ismail *et al.* 2020; Kostas *et al.* 2021), antihypertensives (Fitzgerald *et al.* 2014; Ismail *et al.* 2020), antivirales (Rosales-Mendoza *et al.* 2020), analgésiques (Ismail *et al.* 2020) et anticariogéniques (Yu *et al.* 2019). Certains acides aminés et certaines protéines d'algues rouges peuvent aussi avoir des activités diminuant le taux de cholestérol, inhibant la précipitation de calcium, améliorant les fonctions hépatiques et aidant à traiter des problèmes neurophysiologiques tels que l'épilepsie, les maladies d'Alzheimer et de Parkinson (Kostas *et al.* 2021).

Les polyphénols et autres composés phénoliques pouvant être halogénés (comme les bromophénols) sont également caractérisés par de nombreuses bioactivités d'intérêt pour la médecine et la pharmacologie. Parmi celles-ci, il est possible de citer des activités antioxydantes (Yuan *et al.* 2005b; Ismail *et al.* 2020; Elmajnaoui 2021; Kostas *et al.* 2021; Rana and Gupta 2021), anti-inflammatoires (Elmajnaoui 2021), anti-cancer (Yuan *et al.* 2005b; Ismail *et al.* 2020; Kostas *et al.* 2021), antithrombotiques (Elmajnaoui 2021), analgésiques (Ismail *et al.* 2020), antidiabétiques (Ismail *et al.* 2020; Kostas *et al.* 2021), antivirales (Rana and Gupta 2021) ou photoprotectrices (Bedoux *et al.* 2014b). Ces polyphénols d'algues rouges auraient également des activités antimicrobiennes, antibactériennes (notamment contre des bactéries ayant développé des résistances à certains antibiotiques conventionnels) et antifongiques, qui pourraient varier en fonction de la saison, de la phase de croissance de l'algue ou de l'efficacité des méthodes d'extraction (Bhowmick *et al.* 2020; Ismail *et al.* 2020; Elmajnaoui 2021).

La faible proportion en lipides totaux au sein des macroalgues d'une manière générale a déjà été abordée. Cette faible proportion se retrouve donc également chez les algues rouges pour lesquelles cette fraction représente 1 à 3 % du poids sec total. Cependant une grande partie de cette fraction lipidique est représentée par des acides gras polyinsaturés essentiels (Mabeau and Fleurence 1993;

(Kostas *et al.* 2021; Rana and Gupta 2021). Parmi ces acides gras essentiels, l'acide eicosapentaénoïque (EPA) et l'acide docosahexaénoïque (DHA), ont plusieurs fonctions dans l'immunomodulation, dans la régulation de facteurs de transcription, et dans le traitement certaines maladies oculaires, neuro-dégénératives ou auto-immunes (Kostas *et al.* 2021). Par ailleurs, les fractions lipidiques issues des algues rouges peuvent également montrer diverses propriétés biologiques antioxydantes (Rico *et al.* 2017; Ismail *et al.* 2020; Lopes *et al.* 2020; Elmajnaoui 2021), antimicrobiennes (Stabili *et al.* 2012; Rico *et al.* 2017; Bhowmick *et al.* 2020; Ismail *et al.* 2020; Elmajnaoui 2021), antivirales (Rico *et al.* 2017; Ismail *et al.* 2020), antidiabétiques (Ismail *et al.* 2020), et anti-inflammatoires (Ismail *et al.* 2020; Lopes *et al.* 2020; Elmajnaoui 2021). Les acides gras polyinsaturés issus d'algues rouges permettraient aussi de lutter contre les problèmes cardiovasculaires (Ismail *et al.* 2020; Elmajnaoui 2021; Kostas *et al.* 2021) et de prévenir certains cancers (Rico *et al.* 2017; Lopes *et al.* 2020; Elmajnaoui 2021; Kostas *et al.* 2021). Ces capacités antitumorales se retrouvent notamment chez les phospholipides d'algues rouges. Ces derniers auraient également des bienfaits sur le système nerveux central (Elmajnaoui 2021). Les stérols composent une famille de lipides ayant également des activités antioxydantes, antivirales, antifongiques et antibactériennes et sont présents chez les algues rouges (Kostas *et al.* 2021).

Les terpènes sont des molécules non polaires constituées d'unités isoprènes et représentent l'une des plus grandes familles de métabolites secondaires. Plusieurs algues rouges, notamment des genres *Laurencia*, *Plocamium* ou *Chondrococcus*, ont la capacité de synthétiser des terpènes, pouvant être halogénés (Kostas *et al.* 2021; Rana and Gupta 2021). Ces molécules bioactives ont montré des activités antimicrobiennes (Ismail *et al.* 2020; Elmajnaoui 2021; Kostas *et al.* 2021; Rana and Gupta 2021), antivirales (Rana and Gupta 2021) anti-inflammatoires (Elmajnaoui 2021; Rana and Gupta 2021), antioxydantes (Elmajnaoui 2021), antithrombotiques (Elmajnaoui 2021), analgésiques (Ismail *et al.* 2020), cytotoxiques (Kostas *et al.* 2021), antituberculeuses (Kostas *et al.* 2021), antihelminthiques (Rana and Gupta 2021) et anti-tumorales (Ismail *et al.* 2020; Kostas *et al.* 2021).

Les acides aminés analogues de la mycosporine (MAAs) constituent une famille de petits métabolites secondaires hydrosolubles produits par les algues rouges (*Chondrus crispus*, *Palmaria palmata*, *Gelidium* spp., *Pyropia* spp., *Grateloupia lanceola*) et présentant notamment des propriétés antioxydantes (Ismail *et al.* 2020; Kostas *et al.* 2021), osmoprotectrices (Kostas *et al.* 2021) et photoprotectrices (Ismail *et al.* 2020; Kostas *et al.* 2021; Rana and Gupta 2021). Ils sont donc notamment utilisés en cosmétologie pour des soins et la protection de la peau (Kostas *et al.* 2021).

Le potentiel nutritif de ces macroalgues marines, dont les algues rouges, est notamment dû à leur richesse en sels minéraux tels que le calcium, le magnésium, le phosphore, le potassium, le sodium,

ainsi que le fer et l'iode qui sont particulièrement présents (Mabeau and Fleurence 1993; Dumay *et al.* 2014; Lopes *et al.* 2020; Naseri *et al.* 2020; Kostas *et al.* 2021; Rana and Gupta 2021). Le zinc présent dans certaines algues rouges peut aussi aider à combattre certaines maladies cardiovasculaires, les maladies dégénératives et le cancer (Elmajnaoui 2021). Ces algues sont également intéressantes en tant que source de vitamines (Mabeau and Fleurence 1993; Lopes *et al.* 2020; Naseri *et al.* 2020; Rana and Gupta 2021). En effet, la plupart des algues rouges sont caractérisées par une grande quantité de vitamines A, B1, B2 et B12 et les vitamines C, E et K1 peuvent aussi être particulièrement synthétisées en fonction de l'espèce d'algue considérée (Mabeau and Fleurence 1993; Kostas *et al.* 2021).

Certaines études portant sur les activités biologiques des algues rouges ne déterminent pas l'activité d'un composé précis mais portent sur celle de l'extrait obtenu à partir de l'algue considérée. Il peut s'agir le plus souvent d'extraits aqueux, éthanoliques ou méthanoliques. Ainsi, par exemple, des extraits méthanoliques de plusieurs algues rouges dont *Gracilaria gracilis* ont montré des activités antioxydantes et antibactériennes (Hmani *et al.* 2021), un extrait méthanolique de *Kappaphycus* sp. a montré des activités antioxydantes (Araújo *et al.* 2020), un extrait éthanolique de *Grateloupia elliptica* a montré des activités anti-obésité (Elmajnaoui 2021), un extrait aqueux de *Sarcodia ceylanica* a montré des activités antioxydantes et antidiabétiques (Elmajnaoui 2021), un extrait d'algue rouge de *Solieria filiformis* riche en polyphénols a montré une activité antivirale (Ismail *et al.* 2020). Divers extraits d'algues rouges présentent également des activités antihelminthiques et anti-inflammatoires (Rana and Gupta 2021), antifongiques contre certains types de champignons pathogènes humains, des effets bénéfiques pour la santé de la peau, avec des activités adoucissantes, éclaircissantes, de restauration de l'élasticité, ainsi que des effets bénéfiques pour la prévention de maladies neurodégénératives comme la maladie d'Alzheimer (Ismail *et al.* 2020).

Palmaria palmata - Caractéristiques, écologie, principaux composés d'intérêt

Écologie, morphologie, cycle de vie de *P. palmata*

Palmaria palmata (Linné) F. Weber & D. Mohr (1805) est une algue rouge sur laquelle ces travaux de thèse se sont concentrés d'après les objectifs explicités dans l'**INTRODUCTION GÉNÉRALE**. Cette algue appartient à la famille des Palmariaceae et sa taxonomie est présentée au niveau du **Table I. 4**. Il s'agit d'une algue très commune au niveau des côtes rocheuses de l'hémisphère Nord (Grote 2019). Cette algue peut se retrouver de manière particulièrement abondante au niveau des côtes de l'Océan Atlantique Nord (Hill 2008; Li *et al.* 2015; Baweja *et al.* 2016), de l'Arctique jusqu'au Portugal concernant les côtes est, et jusqu'au Connecticut (USA) concernant les côtes ouest (Pereira 2015; Grote 2019). Des analyses moléculaires et phylogénétiques montrent par ailleurs une divergence génétique de populations de cette algue entre les côtes est et ouest de l'Océan Atlantique, qui

remonterait à l'époque du Pléistocène (0,126 - 0,781 millions d'années) (Li *et al.* 2015). Localement, *P. palmata* peut se développer sur un substrat rocheux (développement épilithique) ou sur d'autres macroalgues (développement épiphytique) telles que *Laminaria hyperborea*, *Laminaria digitata* ou *Fucus* sp. (Hill 2008; Garbary *et al.* 2012; Mouritsen *et al.* 2013; Grote 2019). Elle se développe généralement en bas de la zone intertidale (Grote 2019) mais peut parfois se retrouver en haut de l'estran et jusqu'à 20 mètres de profondeur (Hill 2008; Grote 2019).

P. palmata possède un thalle plat se présentant sous la forme de frondes palmées, en fourches aux extrémités arrondies, et mesurant généralement entre 20 et 50 cm de long et avec des lames entre 3 et 8 cm de large (Figure I. 4) (Hill 2008; Mouritsen *et al.* 2013; Baweja *et al.* 2016; Grote 2019).



Figure I. 4 : Photographies de thalles de l'algue rouge *Palmaria palmata*. © Yoran Le Strat.

La couleur de l'algue se situe dans les tons brun-rouge sombre et pourpres, et sa texture rappelle celle du cuir (Hill 2008; Mouritsen *et al.* 2013; Baweja *et al.* 2016). Un stipe court relie le thalle à un petit crampon discoïde qui permet la fixation de l'algue à son substrat (Hill 2008; Mouritsen *et al.* 2013; Grote 2019). Cette algue est pérenne avec une nouvelle croissance chaque année, à condition que ce crampon fragile reste fixé à son substrat car elle n'a pas la capacité de se refixer (Hill 2008; Grote 2019). Il est ainsi recommandé, pour une récolte durable et responsable, d'éviter d'arracher l'algue mais de privilégier une découpe au niveau du stipe. Le cycle de vie de *P. palmata* est connu depuis 1980 et est présenté Figure I. 5 (van derMeer and Todd 1980).

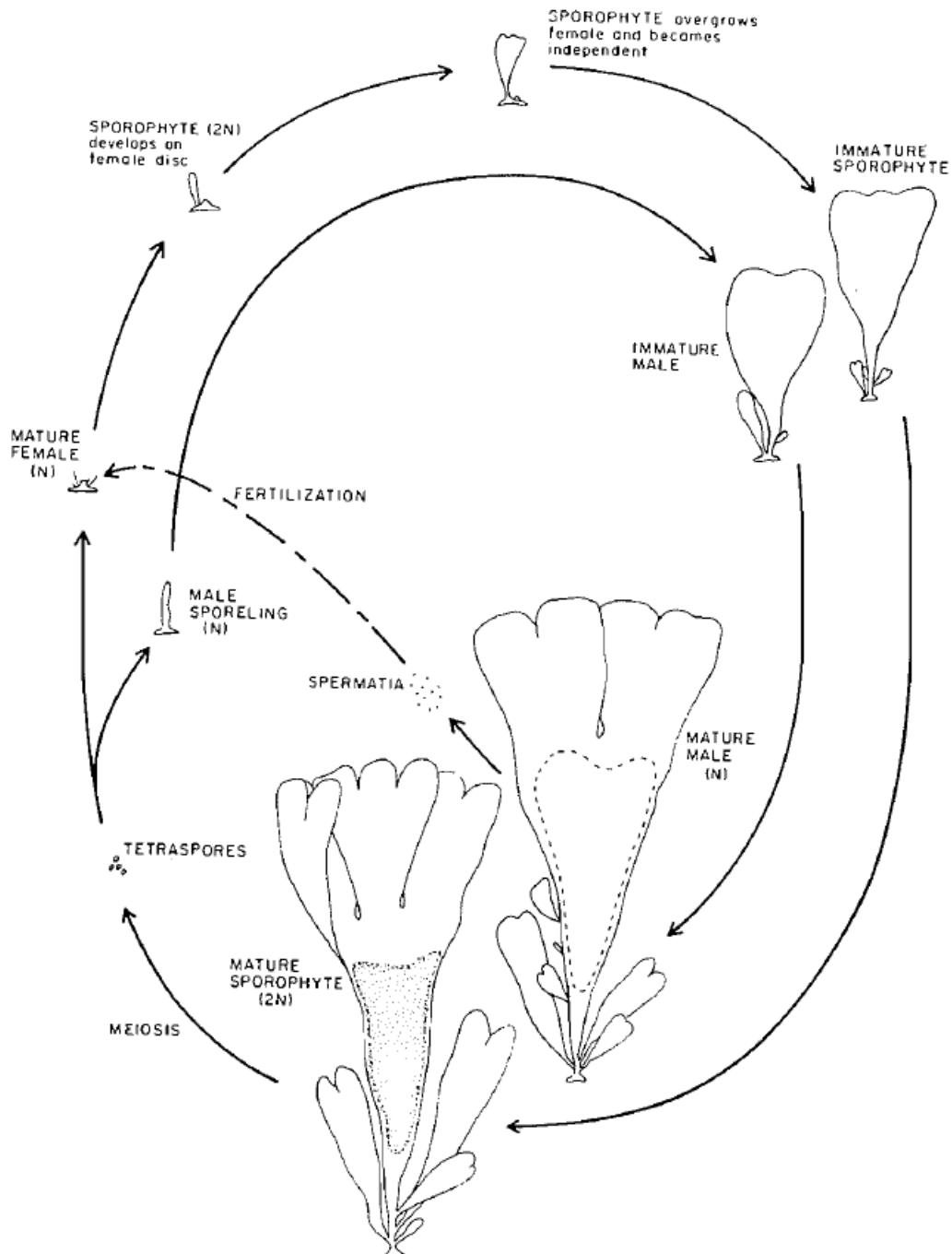


Figure 1. 5 : Cycle de vie et de reproduction de l'algue rouge *Palmaria palmata*. Issu de *van derMeer and Todd (1980)*.

Il s'agit d'un cycle haplodiplontique fortement hétéromorphe, caractérisé par un gamétophyte femelle encroûtant très réduit, de l'ordre du millimètre, et par un gamétophyte mâle macroscopique semblable au tétrasporophyte (*van derMeer and Todd 1980; Hill 2008; Baweja et al. 2016; Grote 2019*). Après fécondation du gamète femelle par le gamète mâle, le tétrasporophyte diploïde se développe directement à partir du gamétophyte femelle et va finalement le recouvrir (*van derMeer and Todd 1980; Hill 2008; Grote 2019*). Après une période de maturation d'un an, des spores tétrasporangiales vont se développer au niveau du tétrasporophyte ce qui va permettre momentanément leur

distinction avec les gamétophytes mâles (Grote 2019). Les spores relarguées par les tétrasporophytes matures vont se fixer à un substrat et donner lieu à une nouvelle génération de gamétophytes femelles et mâles (van derMeer and Todd 1980; Hill 2008; Grote 2019).

Culture et usages de *P. palmata*

Les espèces du genre *Palmaria* sont aussi connues sous le nom vernaculaire de "dulse" (Baweja et al. 2016). Ce terme "dulse" en français, se change en "dillisk" en gaélique et en "tellesk" en breton, ce qui laisse penser à une origine commune celtique et ancienne de cette appellation (Mouritsen et al. 2013). En effet, *P. palmata* est l'une des rares macroalgues dont il a été montré que la consommation en Europe existe depuis plusieurs centaines voire milliers d'années. Ainsi, son utilisation dans l'alimentation remonterait à 5 millénaires en Irlande, et au moins autant en Bretagne. Sa consommation était assez diversifiée, dans des soupes, des ragouts ou avec du pain. Des traces de l'utilisation et de la commercialisation de cette algue remontant aux périodes vikings sont aussi présentes dans les pays scandinaves tels que la Norvège ou l'Islande (Mouritsen et al. 2013).

L'algue rouge *P. palmata* est réputée comme étant l'une des plus intéressantes au niveau gustatif pour l'alimentation humaine (Mouritsen et al. 2013) et présente également une valeur nutritionnelle très intéressante et une richesse particulière en protéines, lipides, vitamines et minéraux notamment (Grote 2019). Des études continuent de se développer pour explorer et améliorer les qualités gustatives de l'algue. Par exemple, Stévant et al. (2020) ont récemment montré les avantages procurés par un processus de séchage partiel (20 % d'humidité résiduelle) par rapport à un séchage complet (6 % d'humidité résiduelle) dans le développement des saveurs de l'algue. Etant particulièrement bien adaptée aux conditions environnementales caractérisant l'Océan Atlantique, de petites productions de cette algue se développent dans quelques pays européens tels que l'Irlande, la France (Bretagne), l'Espagne et plus récemment le Danemark (Mouritsen et al. 2013; Grote 2019) mais il n'existe pas encore de culture à échelle industrielle et à visée commerciale pour cette algue en Europe (Grote 2019).

Cependant, comme mentionné précédemment, le marché des macroalgues est en pleine et constante expansion à l'échelle mondiale, notamment concernant *P. palmata* qui est l'une des plus connues et des plus commercialisées avec notamment les genres *Porphyra*, *Himanthalia*, *Undaria* et *Ulva* (Grote 2019; Lalegerie et al. 2020a). Cette augmentation de la demande peut localement perturber les stocks naturels dans le cadre du ramassage, et créer une pression sur les communautés d'algues. Une optimisation des protocoles de cultures, en pleine mer ou sur terre en bacs, est donc nécessaire pour satisfaire cette demande croissante (Grote 2019). Cette culture des macroalgues se fait traditionnellement manuellement en attachant des fragments de thalle à un substrat (Le Gall 2004;

Grote 2019). Plus récemment, des techniques propres à chaque algue selon leur cycle de reproduction se basent sur la fixation des spores au substrat, ce qui augmente l'efficacité et le rendement de la culture, notamment chez *P. palmata* (Le Gall 2004).

La culture des algues peut être connectée avec d'autres types d'aquaculture comme celles de bivalves ou de poissons qui vont produire les nutriments nécessaires au développement des algues. Ces dernières, quant à elles, vont agir en tant qu'agents de filtration. On parle d'aquaculture multitrophique intégrée (AMTI) (Grote 2019). L'algue rouge *P. palmata* constitue un bon candidat dans le cadre de ces AMTI mais des travaux de recherches sont encore en cours et nécessaires pour l'optimisation de ce procédé qui l'intégrerait (Grote 2019; Sahu et al. 2020).

La filtration et le traitement des eaux usées issues d'aquaculture reposent notamment sur une capacité de fixation et d'accumulation de certains composés par cette algue, qui peut donc être utilisée dans le traitement d'autres types d'eaux usées (Sahu et al. 2020), en agissant notamment en tant que bioaccumulateur d'azote et de phosphore pouvant se retrouver en forte concentration dans les eaux estuariennes (Tremblay-Gratton et al. 2018). *P. palmata* a montré également une capacité à fixer les métaux lourds par adsorption sur les polysaccharides (Prasher et al. 2004; Hill 2008; Li et al. 2011).

P. palmata peut également être utilisée dans l'industrie des biocarburants due à sa forte production de biomasse (Grote 2019; Gallagher et al. 2021), dans l'alimentation animale, notamment pour les vaches et les chevaux (Grote 2019) mais aussi pour les animaux marins comme le saumon Atlantique (Wan et al. 2016), ou dans la production de biofilms consommables biodégradables et non polluants qui isolent l'aliment (Albertos et al. 2019).

Métabolites d'intérêt principaux et bioactivités

L'importante production de protéines par *P. palmata* par rapport aux autres macroalgues a précédemment été évoquée. En effet, la quantité de protéines produites par *P. palmata* représente généralement autour de 20 % du poids sec de l'algue mais peut atteindre jusqu'à 35 %, et dépend de sa localisation géographique et de la saison (Galland-Irmouli et al. 1999; Mouritsen et al. 2013; Dumay et al. 2014; Harnedy et al. 2014; Lee et al. 2017). Cette variation saisonnière de la quantité en protéines synthétisée par *P. palmata* se note par une production particulièrement importante en hiver et au printemps, puis plus faible en été et en automne (Galland-Irmouli et al. 1999; García-Bueno et al. 2014). Cette variation serait notamment corrélée à celle de la quantité en nutriments disponibles dans l'environnement (Joubert and Fleurence 2008). D'une manière générale, les valeurs protéiques quantitatives de *P. palmata* sont comparables à celles du soja ou du poulet et sont plus élevées que pour la plupart des autres macroalgues à l'exception du nori (*Porphyra* spp.) (Galland-Irmouli et al. 1999; Mouritsen et al. 2013; Sahu et al. 2020). Concernant la richesse qualitative de cette fraction

protéique et la composition en acides aminés, *P. palmata* se caractérise par une quantité d'acides aminés essentiels (46 % de la fraction totale d'acides aminés) comparable à celle retrouvés dans les œufs (Galland-Irmouli *et al.* 1999; Dumay *et al.* 2014). Les acides aminés les plus présents sont l'alanine, l'acide aspartique, l'acide glutamique et la glycine (Mouritsen *et al.* 2013). La fraction protéique est également affectée de manière qualitative par des changements saisonniers, avec notamment un manque de thréonine et de lysine en été (Galland-Irmouli *et al.* 1999; Dumay *et al.* 2014). En plus d'une valeur nutritionnelle intéressante, cette fraction protéique montre des bioactivités d'intérêt médical et pharmacologique, notamment antioxydantes (Harnedy *et al.* 2014, 2017; Beaulieu *et al.* 2016). Ces extraits protéiques et peptidiques auraient également des effets bénéfiques pour traiter des maladies neurodégénératives ainsi que le diabète (Harnedy *et al.* 2014; McLaughlin *et al.* 2020). De plus, des extraits protéiques et peptidiques issus de *P. palmata* ont montré des activités inhibitrices de l'enzyme de conversion de l'angiotensine (ACE) impliquée dans le système de régulation de la pression artérielle et auraient donc des propriétés anti-hypertensives (Harnedy *et al.* 2014; Miyabe *et al.* 2017; Seca and Pinto 2018). Cette activité anti-hypertensive de *P. palmata* peut également être due à des peptides impliqués dans l'inhibition de la rénine, premier constituant du système rénine-angiotensine-aldostérone (Fitzgerald *et al.* 2012). Ces extraits pourraient avoir des applications dans l'alimentation à effets bénéfiques sur la santé ("Functional food") (Beaulieu *et al.* 2016). Par exemple, l'implémentation d'un extrait peptidique de *P. palmata* aux propriétés anti-hypertensives (inhibition de la rénine) dans l'élaboration du pain a montré non seulement une absence d'altération des caractéristiques sensorielles et de la texture de ce dernier, mais également une conservation de l'activité anti-hypertensive à l'issue du procédé de fabrication (Fitzgerald *et al.* 2014). Ces diverses bioactivités liées aux fractions protéiques de *P. palmata* dépendent, tout comme la composition protéique de l'algue, de la saisonnalité et des conditions de culture (Beaulieu *et al.* 2016).

En plus de son importante production de protéines, *P. palmata* synthétise d'autres composés d'intérêts tels que des acides gras polyinsaturés, et plus spécifiquement l'EPA. Cet acide gras a des capacités de protection vis-à-vis de maladies cardiovasculaires et neurodégénératives, et est caractérisé par des activités antioxydantes, antitumorales, antimicrobiennes, antivirales et anti-inflammatoires, avec des applications dans des domaines tels que l'alimentation, la cosmétologie et la pharmacologie (Lopes *et al.* 2019). Quantitativement, les acides gras sont faiblement représentés chez *P. palmata* comme la plupart des autres macroalgues (jusqu'à 2 % du poids sec total), mais cette algue présente qualitativement une grande proportion d'EPA par rapport aux autres macroalgues, ce qui induit un ratio oméga 3 / oméga 6 particulièrement intéressant (Mouritsen *et al.* 2013; Grote 2019; Lopes *et al.* 2019). La saisonnalité ainsi que le mode de conservation de ces acides gras peut faire varier la proportion en EPA (Mouritsen *et al.* 2013).

En ce qui concerne les pigments, les phycobiliprotéines semblent particulièrement bioactives, avec des activités antitumorales, anti-inflammatoires et antioxydantes montrées chez *P. palmata* (Lee *et al.* 2017; Nishida *et al.* 2020). Plus particulièrement, par sa composition en acides aminés, la R-PE de *P. palmata* serait partiellement responsable de l'activité anti-hypertensive due à l'inhibition de l'ACE évoquée précédemment (Miyabe *et al.* 2017). La synthèse de pigments est affectée par la saisonnalité, avec une décroissance de la quantité de pigments en été, qui coïncide avec une augmentation de l'intensité lumineuse (Lalegerie *et al.* 2020a).

P. palmata est caractérisée par une intéressante production de vitamines, comme les vitamines C et E (Ferraces-Casais *et al.* 2012), et de minéraux essentiels, en particulier le fer et l'iode (Mouritsen *et al.* 2013; Grote 2019). Des tests et mesures de bioactivité ont aussi été menés sur des extraits de *P. palmata*, et ont notamment montré des potentiels antioxydants et antimicrobiens liés à la synthèse de composés phénoliques (Yuan *et al.* 2005b, 2005a; Cox *et al.* 2010; Wang *et al.* 2010), des effets bénéfiques dans le traitement de cancer ou de maladies cardiovasculaires et neurodégénératives (Grote 2019), ou bien une utilité en tant que substrat pour la production d'acide succinique par la bactérie *Escherichia coli* (Olajuyin *et al.* 2016). Cette macroalgue rouge montre aussi des propriétés photoprotectrices et antioxydantes grâce à la synthèse de MAAs, utilisés dans des écrans solaires en cosmétique, permettant de réduire les impacts néfastes de la lumière sur la peau, et favorisant le renouvellement de cette dernière (Lalegerie *et al.* 2020a; Nishida *et al.* 2020). Des xylo-oligomères issus de la dégradation du xylane, polysaccharide principal de *P. palmata*, montrent aussi des effets prébiotiques sur les bactéries intestinales (Yamamoto *et al.* 2019; Nishida *et al.* 2020).

Procédés d'extraction (focus sur l'EAE et sur la R-PE)

Palmaria palmata est caractérisée par une paroi cellulaire constituée majoritairement de xylane, et de cellulose en plus faible quantité (Morgan *et al.* 1980; Lahaye *et al.* 1993; Dumay *et al.* 2014; Naseri *et al.* 2020). Le xylane consiste en une chaîne principale de résidus de D-xylose reliés par des liaisons β -1,3 et β -1,4, sur laquelle peuvent se lier d'autres chaînes de xylane ou de cellulose, tandis que la cellulose est faite de résidus de D-glucose reliés en chaîne par des liaisons β -1,4 (Morgan *et al.* 1980; Lahaye *et al.* 1993). Ces polysaccharides pariétaux et intracellulaires compartimentent la cellule, limitent l'accès aux composés d'intérêt qu'elle contient, et développent des interactions ioniques avec les protéines (Joubert and Fleurence 2008; Denis *et al.* 2009; Wang *et al.* 2010; Harnedy and Fitzgerald 2013b; Dumay *et al.* 2014; Sfriso *et al.* 2018). Or, parmi ces composés présents chez *P. palmata*, il a été montré que les protéines solubles et plus particulièrement la R-PE montrent des applications variées et à fort potentiel biotechnologique. Afin d'améliorer l'accès à de tels composés, il est nécessaire de développer des méthodes d'extraction efficace, permettant de détruire ces

polysaccharides et de limiter leur viscosité, tout en conservant l'intégrité des molécules ciblées (Wang *et al.* 2002; Niu *et al.* 2006; Denis *et al.* 2009; Wang *et al.* 2010; Harnedy and Fitzgerald 2013b).

Parmi les techniques d'extraction actuelles pouvant être utilisées au niveau des matrices macroalgales, il est possible de citer l'extraction par solvant accéléré (ASE) (Santos *et al.* 2019), l'extraction par fluide supercritique (SFE) (Heffernan *et al.* 2016; Cikoš *et al.* 2018; Santos *et al.* 2019), l'extraction assistée par ultrasons (UAE) (Harnedy and Fitzgerald 2013b; Le Guillard *et al.* 2015; Garcia-Vaquero *et al.* 2017; Cikoš *et al.* 2018; Santos *et al.* 2019), l'extraction en eau subcritique (SWE) (Cikoš *et al.* 2018), ou l'extraction assistée par micro-ondes (MAE) (Garcia-Vaquero *et al.* 2017; Cikoš *et al.* 2018; Santos *et al.* 2019). L'extraction de la R-PE à partir de matrices algales telles que *P. palmata* consiste conventionnellement en l'application d'un choc osmotique ou en la réalisation d'un broyage à l'azote liquide (Fleurence 2003; Denis *et al.* 2009; Harnedy and Fitzgerald 2013b), suivie par une solubilisation dans un tampon phosphate (Denis *et al.* 2009; Dumay *et al.* 2015; Nguyen *et al.* 2018). Cependant, cette technique prend beaucoup de temps, n'est pas optimale et ne peut pas être appliquée et utilisée à l'échelle industrielle à cause notamment de son coût élevé (Fleurence 2003; Dumay *et al.* 2014; Nguyen *et al.* 2018).

Il semble donc que l'une des techniques les plus efficaces pour l'extraction de protéines et plus particulièrement la R-PE à partir de macroalgues rouges telles que *P. palmata* est l'extraction assistée par enzymes (EAE) (Fleurencel *et al.* 1995; Dumay *et al.* 2013; Harnedy and Fitzgerald 2013b). Cette technique, consistant en une digestion enzymatique ciblée des polysaccharides d'une matrice donnée, est la plus utilisée en ce qui concerne l'extraction de la R-PE (Dumay *et al.* 2014; Sfriso *et al.* 2018). En effet, plusieurs études ont été réalisées sur l'utilisation de cette EAE sur l'algue rouge *P. palmata* (Fleurence 1999, 2003; Joubert and Fleurence 2008; Dumay *et al.* 2013, 2014; Naseri *et al.* 2020), mais également sur les algues rouges *Gracilaria gracilis* (Nguyen *et al.* 2020), *Grateloupia turuturu* (Denis *et al.* 2009) ou *Gelidium pusillum* (Mittal and Raghavarao 2018).

Les conditions de la réaction ainsi que la nature des enzymes sélectionnée doivent être sélectionnées de manière à optimiser l'extraction (Harnedy and Fitzgerald 2013b). Ainsi, certains paramètres tels que le temps de la réaction, le ratio enzyme / substrat et la température d'incubation sont particulièrement importants (Nguyen *et al.* 2020). Un protocole d'extraction de la R-PE à partir de *P. palmata* a ainsi été optimisé par méthodologie des plans d'expériences en utilisant une xylanase à un ratio enzyme / substrat de 320, pour une réaction de 17,8 minutes, et à une température de 2°C. Cette optimisation a permis d'obtenir une concentration en R-PE de 12,36 g/kg de matière sèche (Dumay *et al.* 2013). La spécificité de substrat des enzymes utilisées a aussi son importance. En effet, la paroi cellulaire des macroalgues est constituée de divers polysaccharides dont la nature change en fonction de l'espèce

considérée. Les enzymes utilisées doivent donc être adaptée à la matrice macroalgale (Fleurence 2003; Nguyen *et al.* 2020). Il a été montré que le rendement d'extraction de la R-PE à partir de l'algue rouge *Gracilaria gracilis* était particulièrement important lorsqu'une cellulase était utilisée (Nguyen *et al.* 2020), tandis que l'utilisation d'enzymes xylanolytiques semble particulièrement adaptée pour l'extraction de la R-PE à partir de *P. palmata* (Dumay *et al.* 2013). Cela indique que l'efficacité de l'enzyme choisie est spécifiquement liée à la composition structurale de la paroi cellulaire de chaque algue, celles de *Gracilaria gracilis* et *Palmaria palmata* étant majoritairement constituées de cellulose et de xylane, respectivement (Fleurence 2003; Dumay *et al.* 2013; Nguyen *et al.* 2020). Concernant *P. palmata*, il a été précédemment mentionné que bien que le xylane constitue la majeure partie de sa paroi cellulaire, celle-ci est également partiellement constituée de cellulose. Ainsi, il a été montré que l'utilisation simultanée de xylanase et de cellulase résulte en un meilleur rendement d'extraction en protéines et en R-PE que lorsqu'elles sont utilisées séparément (Fleurence 2003; Joubert and Fleurence 2008; Mittal and Raghavarao 2018). L'effet synergique de ces enzymes peut être expliqué par le fait que les fibres de cellulose sont recouvertes par des couches de xylane. Ainsi, la dégradation de la cellulose par l'utilisation de cellulases seule est limitée par le xylane, et les xylanases ne dégradent pas la cellulose mais augmentent l'exposition de la cellulose aux cellulases (Mittal and Raghavarao 2018). Cela étant, l'action synergique de ces deux enzymes semble dépendre de leur origine biologique (Fleurence 1999, 2003).

En dehors de son efficacité, l'EAE présente de nombreux avantages en permettant un procédé d'extraction respectueux de l'environnement, sans solvants polluants, caractérisé par des extraits de bonne qualité (Hardouin *et al.* 2014; Charoensiddhi *et al.* 2017). De plus, les enzymes sont des catalyseurs qui sont donc réutilisables après le procédé d'extraction, elles sont également non toxiques et biodégradables (Hardouin *et al.* 2014). Cette technique d'extraction peut aussi être utilisée en association avec d'autres techniques d'extraction impliquant les micro-ondes ou les ultrasons pour augmenter encore le rendement (Harnedy and Fitzgerald 2013b; Dumay *et al.* 2014; Hardouin *et al.* 2014; Le Guillard *et al.* 2015). Plus récemment, un procédé d'EAE a été appliqué en association avec une technologie de haute pression hydrostatique (HPP) sur l'extraction de divers composés tels que les protéines, les polyphénols et les polysaccharides solubles à partir de *P. palmata* (Suwal *et al.* 2019).

Cependant, quelques verrous subsistent pour l'utilisation de cette technique à échelle industrielle. En effet, les enzymes peuvent se montrer moins stables que certains catalyseurs chimiques et le ratio enzyme / substrat est souvent trop important pour une viabilité économique à une échelle plus importante que celle du laboratoire (Harnedy and Fitzgerald 2013b; Hardouin *et al.* 2014). Un autre paramètre pourrait consister à utiliser des enzymes particulièrement spécifiques et adaptées aux polysaccharides constituant la paroi cellulaire de *P. palmata* (Harnedy and Fitzgerald 2013b).

Un intérêt actuel croissant pour les champignons marins

CHAMPIGNONS MARINS - Origine et définition, habitats, principaux taxons et potentiel biotechnologique

Origine et phylogénie des champignons

Les champignons (Mycètes, Fungi) ont longtemps été assimilés à des plantes mais constituent en réalité un règne à part, plus proche de celui des animaux (Baldauf 1999). Ce regroupement des champignons et des animaux en un clade et partageant un ancêtre commun qu'ils ne partagent pas avec les plantes est notamment visible au niveau moléculaire. Ainsi, les champignons forment avec les animaux (ainsi qu'avec d'autres organismes eucaryotes) le groupe des Opisthokontes (Figure I. 6) (Baldauf 1999; Andrews and Harris 2007; Raghukumar 2017).

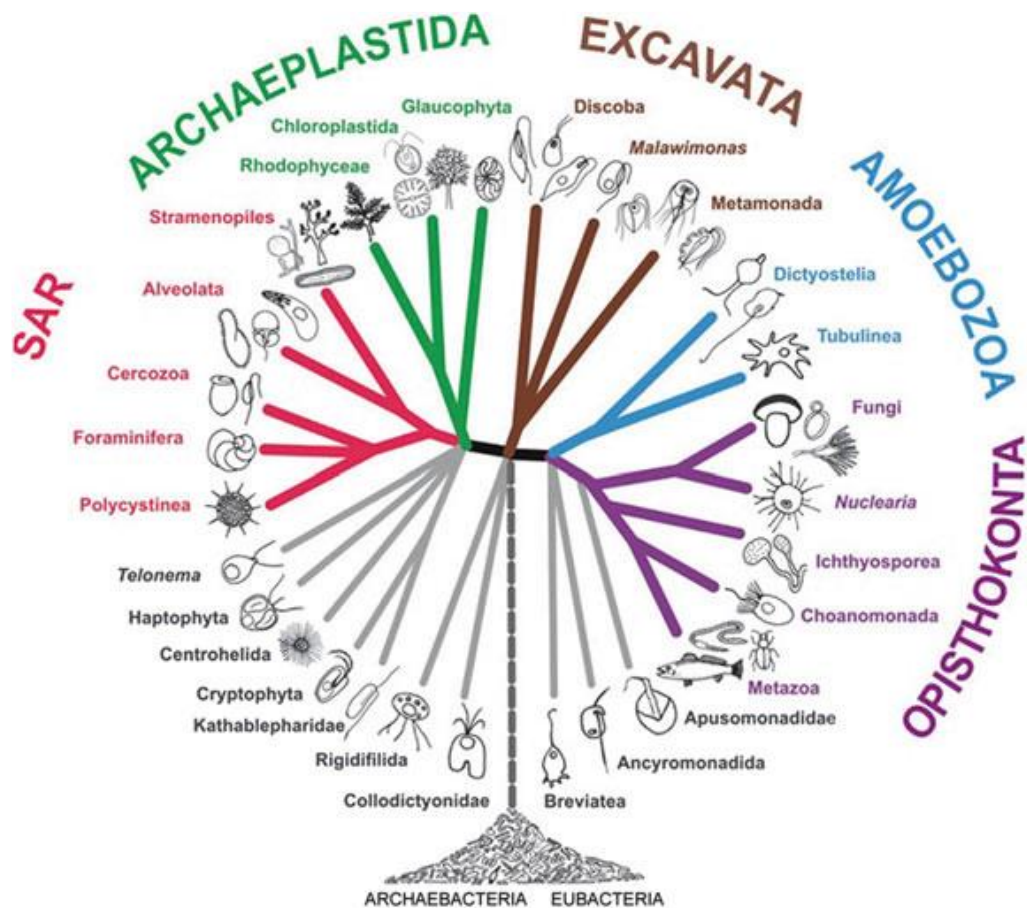


Figure I. 6 : Arbre phylogénétique général des eucaryotes. Le clade des "Fungi" correspond aux règne des Mycètes. Issus de Raghukumar (2017).

La divergence des champignons avec les animaux n'est pas clairement établie mais daterait d'environ 1 milliard d'années au moins. La dernière divergence majeure concerne les phylums des Ascomycota (ascomycètes) et des Basidiomycota (basidiomycètes), qui aurait eu lieu il y a environ 0,5 milliards d'années (Andrews and Harris 2007). Au total, neuf lignées principales de champignons peuvent être définies : Opisthosporidia, Chytridiomycota, Neocallimastigomycota, Blastocladiomycota, Zoopagomycota, Mucoromycota, Glomeromycota, Basidiomycota et Ascomycota (Figure I. 7). Cette taxonomie des champignons a connu de multiples changements et évolutions depuis leur définition par Linné qui les avait associés au règne des végétaux (Naranjo-Ortiz and Gabaldón 2019). En plus d'être issus de la divergence la plus récente, les Ascomycota (ascomycètes) et les Basidiomycota (basidiomycètes) sont les deux phylums les plus répandus et les plus diversifiés (Raghukumar 2017).

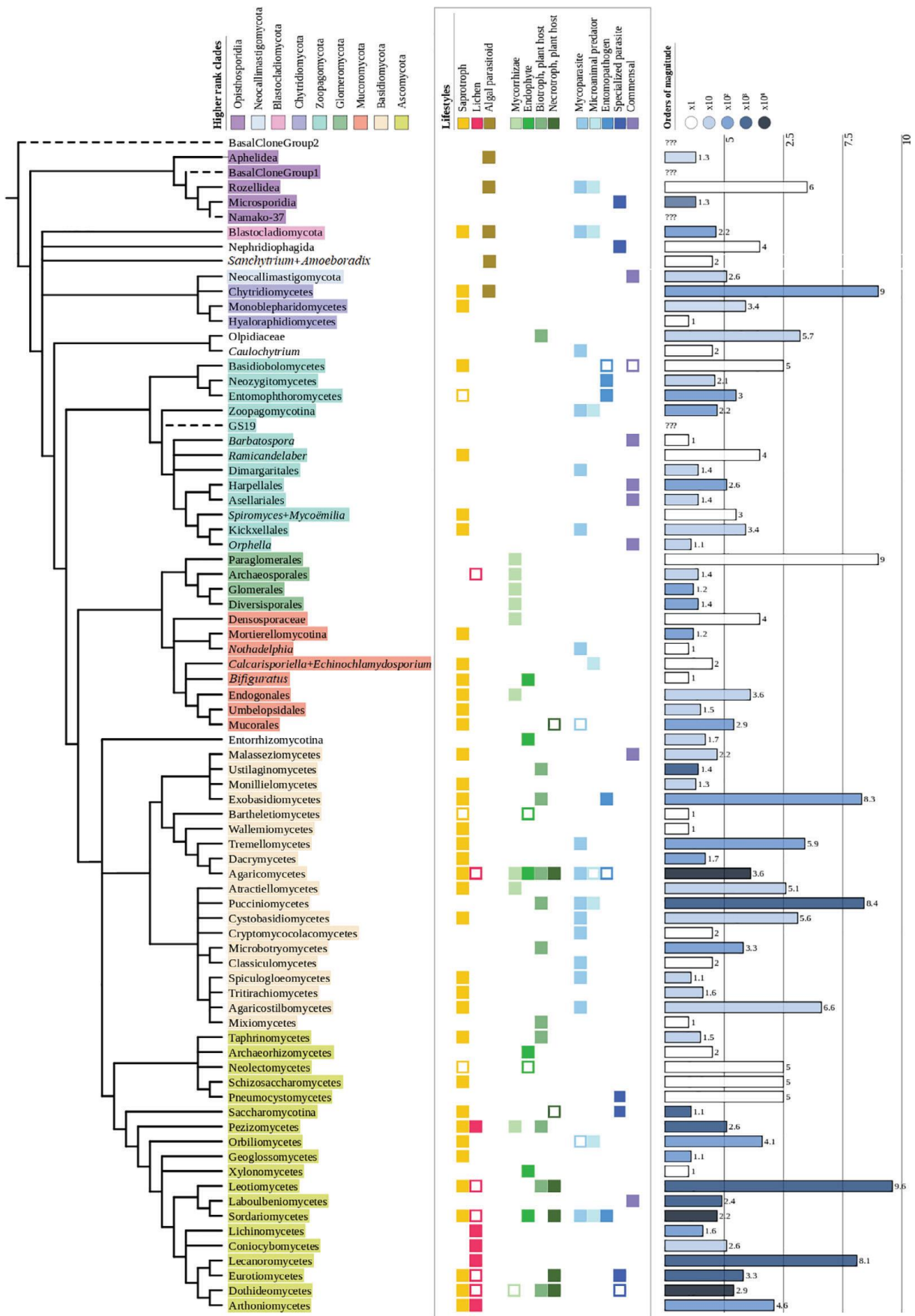


Figure 1. 7 : Arbre phylogénétique du règne des champignons. La première colonne représente les différentes classes de champignons, clustérisées selon les principaux phylums en fonction de la couleur. La seconde colonne représente les différents styles de vie présents pour chaque clade (les carrés vides représentent des styles de vies anecdotiques ou hypothétiques). La troisième colonne représente le nombre d'espèces décrites dans chaque groupe (les ordres de grandeurs peuvent beaucoup varier d'un clade à l'autre, d'où la présence de couleurs différentes). Issu de [Naranjo-Ortiz and Gabaldón \(2019\)](#).

Caractéristiques générales des champignons

Les champignons sont des organismes eucaryotes non vascularisés (Baldauf 1999; McConnaughey 2014). Ils sont hétérotrophes vis-à-vis du carbone, osmotrophes et chimio-organotrophes en excréant des exoenzymes dans le milieu extérieur permettant la digestion de macromolécules (polysaccharides, protéines, lipides) issues des substrats sur lesquels ils se développent en molécules plus petites qui peuvent être absorbées par le champignon (Baldauf 1999; Kavanagh 2005; Andrews and Harris 2007; McConnaughey 2014). Les champignons sont également non-diazotrophiques et ne peuvent pas non plus fixer l'azote gazeux. Ils ont donc besoin d'azote sous forme de sels (comme le sel d'ammonium) ou de matière organique azotée comme les acides aminés (Kavanagh 2005). Ils peuvent avoir des modes de vie en aérobie, en anaérobie ou en anaérobie facultative (Kavanagh 2005; Andrews and Harris 2007).

Les champignons peuvent se développer sous forme unicellulaires et correspondent ainsi aux levures et / ou sous la forme d'organismes pluricellulaires connus sous l'appellation de champignons filamenteux. Certains champignons peuvent être dimorphiques et se présenter sous forme unicellulaire ou filamenteuse selon les conditions de croissance (Kavanagh 2005). Ces deux formes sont répandues dans la plupart des lignées de champignons dont notamment les Ascomycota et Basidiomycota (Nieuwenhuis and James 2016; Raghukumar 2017). En ce qui concerne les champignons filamenteux, ils sont comme leur nom l'indique composés de filaments à croissance apicale nommés hyphes. L'ensemble de ces hyphes compose la masse du champignon, appelée mycélium (Kavanagh 2005; McConnaughey 2014). Chez les champignons les plus évolués, les hyphes sont cloisonnés (ou septés) par des parois transversales (les septa) séparant des structures appelées "articles", qui ressemblent à des cellules et peuvent contenir un ou plusieurs noyaux. C'est le cas des ascomycètes et basidiomycètes. A l'inverse, chez les champignons les moins évolués, les filaments sont non-cloisonnés (coenocytiques) et plurinucléés (Figure I. 8).

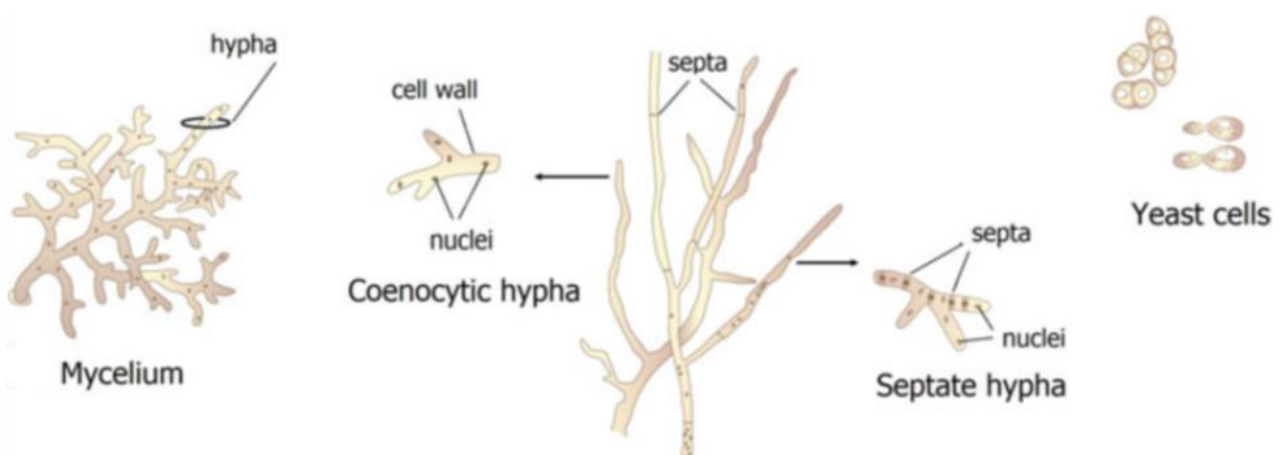


Figure I. 8 : Représentation schématique de diverses structures de croissance fongique. Issu de Pournou (2020).

Cycles de vie et reproduction chez les champignons (*focus* sur ascomycètes et basidiomycètes)

Les champignons sont également caractérisés par une grande diversité de cycles de vie et de nombreuses modalités différentes de reproduction sexuée et asexuée. La quasi-totalité des champignons peuvent se reproduire de manière sexuée mais ce type de reproduction est souvent bien moins fréquent que la reproduction asexuée ou multiplication végétative (Andrews and Harris 2007; Nieuwenhuis and James 2016). La plupart des cycles de reproduction des champignons consiste en une phase haploïde et une phase diploïde. La reproduction sexuée commence généralement par une reconnaissance et fusion des hyphes haploïdes de deux individus, nommée anastomose. Les cytoplasmes des deux individus fusionnent mais les deux noyaux restent séparés dans la cellule ainsi formée. L'hyphe produit par cette union va former des sporanges, cellules où les spores vont se former après la fusion des deux noyaux. Les spores sont relarguées afin de former de nouvelles hyphes et le cycle se répète. La reproduction asexuée ou multiplication végétative peut se réaliser de deux manières, soit par fragmentation du mycélium, soit par la production de "spores" asexuées appelées conidies (McConaughy 2014).

Les deux phylums majeurs des champignons sont les Ascomycota et les Basidiomycota, formant le groupe des Dikarya. Ils sont caractérisés par un mode de reproduction sexuée impliquant une fusion des hyphes des deux individus sans fusion des noyaux, d'où leur nom (Naranjo-Ortiz and Gabaldón 2019). Comparativement aux basidiomycètes, les hyphes dikaryotiques issues de l'anastomose des ascomycètes sont généralement plus réduits et ont une durée de vie plus courte (Naranjo-Ortiz and Gabaldón 2019). La **Figure I. 9** représente un cycle de vie type des ascomycètes, en se basant sur le genre *Aspergillus* (Ellena et al. 2020).

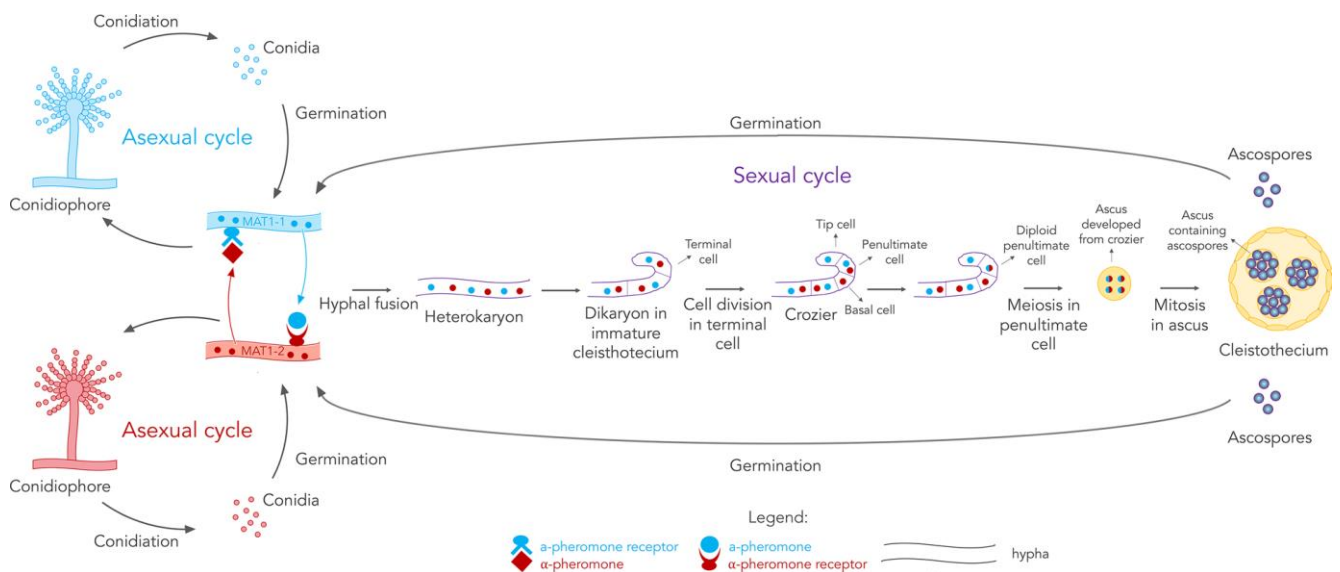


Figure I. 9 : Représentation schématique des phases de reproduction sexuée et asexuée et de leur interconnexion chez le genre *Aspergillus*, Ascomycota. Issu de Ellena et al. (2020).

Les hyphes dicaryotiques résultent en la formation de structures en forme de sacs, nommées asques, d'où l'appellation d'ascomycètes, qui contiennent généralement 8 ascospores issues de la reproduction sexuée (McConnaughey 2014; Nieuwenhuis and James 2016; Naranjo-Ortiz and Gabaldón 2019). La fusion des noyaux et la méiose n'a lieu qu'au moment de la production des spores, la phase diploïde est donc très réduite. (Nieuwenhuis and James 2016). La reproduction asexuée est aussi largement répandue chez les ascomycètes, que ce soit par fragmentation du mycélium ou par la production de conidies, se développant sur des structures spécialisées, les conidiophores (McConnaughey 2014; Nieuwenhuis and James 2016; Raghukumar 2017; Naranjo-Ortiz and Gabaldón 2019). Ces conidiophores sont caractérisés par une importante variabilité d'une espèce à l'autre et peuvent servir dans l'assignation taxonomique morphologique de souches fongiques (**Figure I. 10**).

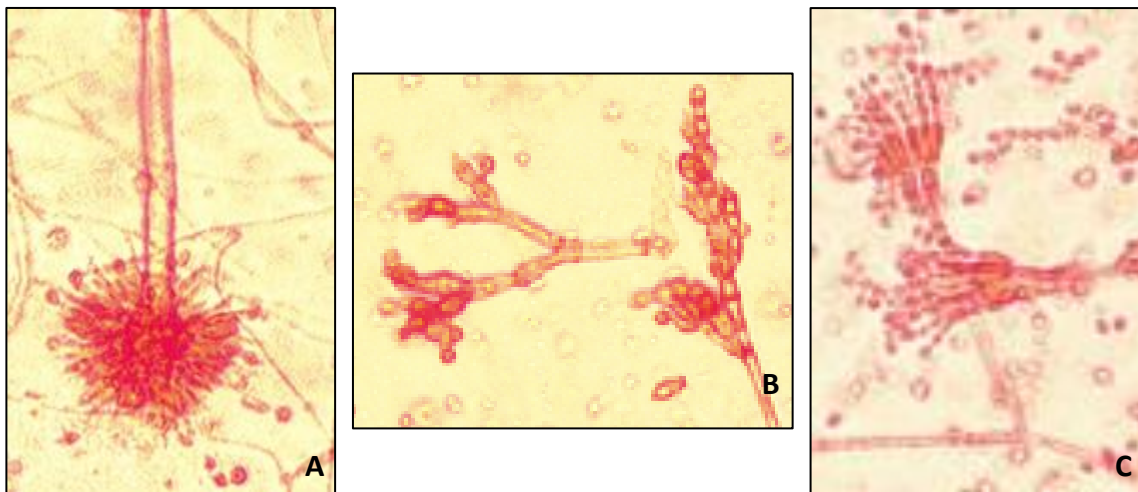


Figure I. 10 : Conidiophores d'ascomycètes permettant une assignation taxonomique au niveau du genre. *Aspergillus* (A), *Cladosporium* (B) et *Penicillium* (C). © Yoran Le Strat.

Les basidiomycètes forment le second phylum le plus riche en termes d'espèces (environ 32 000 espèces décrites) et présentent une grande variété de modes de vie et de cycles cellulaires (Naranjo-Ortiz and Gabaldón 2019). La principale caractéristique de ce phylum est la production de cellules reproductrices en forme de massue plus ou moins allongées, les basides, d'où ils tirent leur nom. Quatre basidiospores sont généralement externalisées de ces basides (McConnaughey 2014; Raghukumar 2017; Naranjo-Ortiz and Gabaldón 2019). La **Figure I. 11** suivante représente le cycle de vie type des basidiomycètes en se basant sur le genre *Coprinus* (Raghukumar 2017).

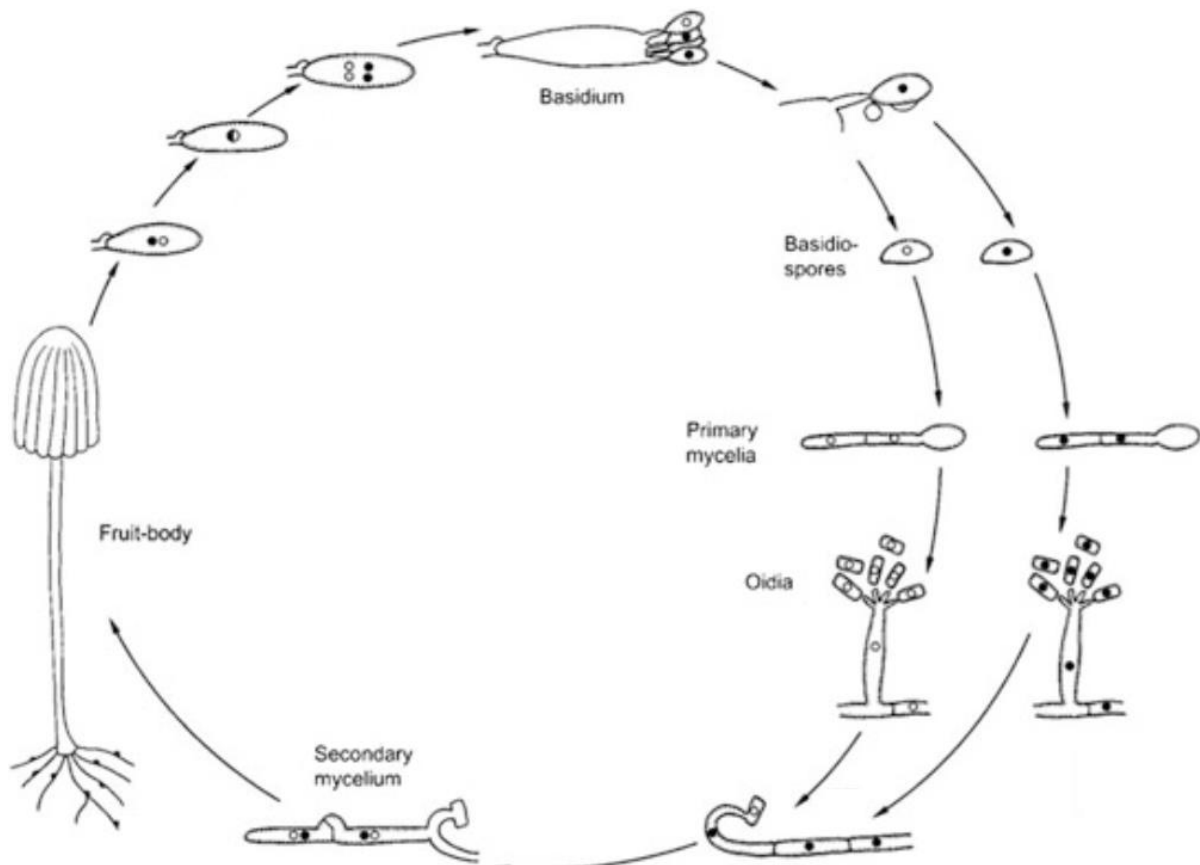


Figure I. 11 : Représentation schématique du cycle de vie chez le genre *Coprinus*, Basidiomycota. Issu de *Raghukumar (2017)*.

Les champignons sont théoriquement capables de se développer à partir du moment où de l'eau et une source de carbone sont disponibles (*Gladfelter et al. 2019*). Certains champignons sont même caractérisés en tant qu'oligotrophes et se développent avec un apport en nutriments très limité, ce qui leur permet notamment de coloniser des environnements particulièrement pauvres (*Kavanagh 2005*). Ils ont donc pu coloniser la majorité si ce n'est tous les environnements terrestres et aquatiques, notamment marins (*Andrews and Harris 2007*). Tandis que les champignons terrestres sont bien décrits, les champignons marins restent peu étudiés par rapport aux autres microorganismes marins. Cela est principalement dû au fait que les champignons au sens large sont fréquemment considérés en tant que strictement terrestres (*Rédou et al. 2016*).

Les champignons marins

Les champignons marins ont été définis pour la première fois en 1961, sur la base de leur capacité à se développer dans des conditions semblables à celles caractérisant l'eau de mer, et donc notamment en présence de salinité (*Johnson and Sparrow 1961*). Après une vingtaine d'années, cette définition a été discutée et remise en question par le fait que certains champignons terrestres étaient capables de survivre, se développer et se reproduire dans de telles conditions marines (*Kohlmeyer and Kohlmeyer 1979*). Ces auteurs ont donc fait la distinction entre champignons marins obligatoires, ne pouvant

sporuler (et donc se développer) que dans des conditions marines ou estuariennes, et champignons marins facultatifs, étant des champignons supposés terrestres mais caractérisés par une adaptation aux conditions de vie marine dans lesquelles il leur était possible de sporuler et de proliférer (Kohlmeyer and Kohlmeyer 1979). On peut distinguer les champignons marins obligatoires, considérés en tant que vrais champignons marins des champignons marins facultatifs (capables de se développer dans des conditions terrestres) mais cela tend à sous-estimer la biodiversité fongique marine réelle et globale. De plus, certains champignons marins facultatifs isolés à partir d'environnement marins ont été caractérisés par des adaptations claires à leur environnement, notamment avec des interactions avec le reste de la communauté microbienne marine, mais ne pouvaient toujours pas être considérés pleinement en tant que champignons marins (Rédou *et al.* 2016; Pang and Jones 2017). Face à ces confusions, Pang and Jones (2017) ont établi une nouvelle définition, utilisée actuellement par la plupart de la communauté scientifique et dans le cadre de cette thèse, décrivant trois moyens de définir en tant que marine une souche fongique : 1) le champignon est capable de croître et / ou de sporuler dans un environnement marin (sur substrat), 2) le champignon développe des relations symbiotiques avec d'autres organismes marins ou 3) le champignon montre une adaptation ou une évolution au niveau génétique en réponse à l'environnement marin, ou est métaboliquement actif dans cet environnement.

Encore aujourd'hui le terme de "champignon dérivé de la mer" (marine-derived fungus) est souvent utilisé lorsqu'il est difficile de déterminer si le champignon est strictement marin ou non (Bugni and Ireland 2004; Ameen *et al.* 2021). Depuis la première colonisation de l'environnement terrestre par les champignons, de nombreuses transitions entre les habitats marins et terrestres ont eu lieu, ce qui a mené à diverses adaptations. Ainsi, l'origine des champignons marins est toujours en discussion et pourrait varier d'un taxon fongique à un autre (Gonçalves *et al.* 2022).

Sous-représentation des études sur les champignons marins par rapport aux champignons terrestres et aux bactéries marines

Malgré leur rôle central reconnu dans la santé et dans les maladies de la biosphère terrestre, la fonction et le potentiel des champignons marins est bien moins renseigné (Gladfelter *et al.* 2019). Ainsi, alors que la surface des océans correspond aux deux tiers de la planète, les microorganismes qui y résident sont moins étudiés, connus et exploités que les microorganismes terrestres (Wainwright *et al.* 2017). La quantité totale d'espèces fongiques différentes serait de l'ordre de 2,2 à 3,8 millions. Avec environ 120 000 espèces décrites jusqu'à présent, cela représente 3 à 8 % de la totalité des espèces estimées (Hawksworth and Lücking 2017; Jones *et al.* 2019). En 2008, sur environ 100 000 champignons reconnus, seulement 500 correspondaient à des champignons marins, ce qui ne représentait que 0,5 % du total des champignons (Hawksworth and Lücking 2017). Aujourd'hui,

environ 1500 espèces de champignons marins ont été décrites (Ameen *et al.* 2021) ce qui représenteraient moins de 1 % des champignons totaux décrits (Gonçalves *et al.* 2022). Il a par ailleurs été rapporté que la biomasse totale des champignons marins serait supérieure à celle des bactéries, en particulier dans les environnements riches en carbone organique. Il est estimé qu'environ 1 % des champignons marins totaux ont été identifiés (Gladfelter *et al.* 2019) alors qu'en moyenne un millilitre d'eau de mer contiendrait plus d'un millier de cellules fongiques (Rédou *et al.* 2016). Les champignons marins sont faiblement étudiés par rapport aux autres microorganismes marins et la distribution globale et la diversité des champignons marins sont relativement peu connues (Jones *et al.* 2019).

Principaux taxons chez les champignons marins

Les Ascomycota représentent le phylum majoritaire dans l'environnement marin en termes de représentativité, suivis par les Basidiomycota (Blackwell 2011; Manohar and Raghukumar 2013; Rédou *et al.* 2016; Agusman and Danging 2017; Amend *et al.* 2019; Sarma 2019; Abdul Malik *et al.* 2020; Ameen *et al.* 2021; Gonçalves *et al.* 2022). Les Ascomycota marins sont principalement représentés au niveau du sous-phylum par les Pezizomycotina, les Saccharomycotina et les Taphrinomycotina et au niveau de la classe par les Dothideomycetes, les Eurotiomycetes, les Sordariomycetes, les Leotiomycetes, et les Saccharomycetes (Rédou *et al.* 2016; Gonçalves *et al.* 2022). Les Basidiomycota marins sont principalement représentés, quant à eux, au niveau du sous-phylum par les Agaricomycotina, les Pucciniomycotina et les Ustilaginomycotina et au niveau de la classe par les Agaricomycetes, les Agaricostilbomycetes, les Atractiellomycetes, les Cystobasidiomycetes, les Exobasidiomycetes, les Microbotryomycetes, les Tremellomycetes, les Ustilaginomycetes et les Wallemiomycetes (Rédou *et al.* 2016; Gonçalves *et al.* 2022). Parmi les Ascomycota marins, plusieurs ordres semblent particulièrement représentés tels que les Microascales, les Pleosporales, les Eurotiales et les Saccharomycetales (Jones *et al.* 2019). Les genres les plus représentés sont *Cladosporium*, *Fusarium*, *Aspergillus*, *Penicillium*, *Gliocladium*, *Microsphaeriopsis*, *Paecilomyces*, *Phoma*, *Phomopsis*, *Trichoderma* et *Ulocladium* (Bugni and Ireland 2004). Avec l'augmentation des études et des données concernant la diversité des champignons marins, une base de données dédiée à ces derniers a été créée récemment (www.marinefungi.org) et permet d'accéder notamment à des détails de classification et à des descriptions de champignons marins (Jones *et al.* 2019).

Diversité d'habitats pour les champignons marins

Il est traditionnellement établi que les formes ancestrales de champignons ont apparues dans l'océan. A l'échelle de la planète, les champignons ont été trouvés dans tous les types d'environnements marins dans lesquels ils ont été recherchés (Gladfelter *et al.* 2019). Les champignons sont donc communément répandus dans le milieu marin au sens large, dont la colonne d'eau, la glace marine, les fonds

océaniques dont les sources hydrothermales, les sédiments, le bois flotté, les algues, les mangroves, les coraux, les animaux invertébrés, les mammifères marins ou les détritits (Le Calvez *et al.* 2009; Damare *et al.* 2012; Tisthammer *et al.* 2016; Agrawal *et al.* 2018; Amend *et al.* 2019; Gladfelter *et al.* 2019; Ameen *et al.* 2021; Zeghal *et al.* 2021; Zeng *et al.* 2021; Gonçalves *et al.* 2022). La **Figure I. 12** permet d'illustrer cette grande diversité de substrats et d'environnements caractérisant les champignons marins.

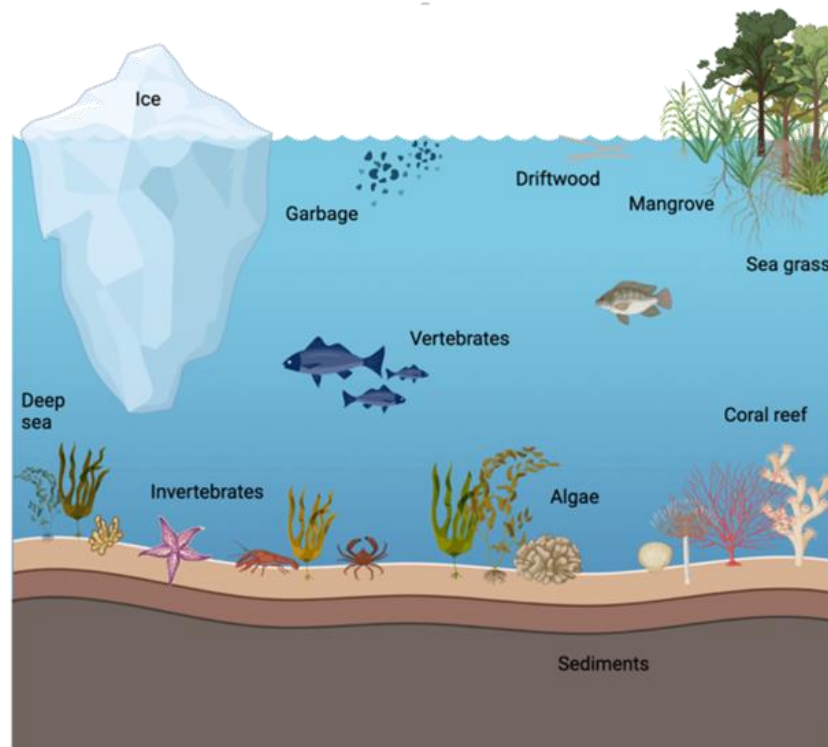


Figure I. 12 : Représentation de la diversité des habitats des champignons marins. Issu de Gonçalves *et al.* (2022).

Bien qu'ils soient ubiquistes, certains champignons marins ne se retrouvent qu'au niveau d'une localisation géographique précise, ou en association avec un substrat en particulier (Gonçalves *et al.* 2022).

Selon les études, les souches fongiques peuvent être séparées selon différents critères liés à leur habitat. Par exemple, selon Hugues (1974), les communautés fongiques se diversifient selon le climat, la température et les zones géographiques (arctique, antarctique, tempérée, subtropicale et tropicale). Cette différenciation géographique de la diversité fongique a été observée concernant les champignons marins planctoniques, avec une diversité fongique plus forte dans certaines localisations comme la mer Baltique et la mer Méditerranée (Hassett *et al.* 2020). La structuration de ces communautés fongiques dépend des conditions environnementales de chaque zone géographique, avec par exemple une augmentation de la part des Chytridiomycetes dans la diversité fongique globale

lorsque la salinité varie significativement par rapport à la moyenne océanique (Hassett *et al.* 2020). L'effet de la localisation géographique sur la diversité fongique a été vérifié plus récemment mais la localisation verticale, selon la profondeur de l'eau, aurait un impact supérieur sur cette distribution fongique (Tisthammer *et al.* 2016). Ainsi, dans plusieurs études, la distinction entre champignons marins côtiers et champignons marins des profondeurs a été établie selon les caractéristiques propres à chaque habitat (température, intensité lumineuse, pression, diversité biologique associée...) (Manohar and Raghukumar 2013; Sarma 2019). Ainsi, il a été montré que les sédiments marins profonds sont caractérisés par une diversité fongique plus faible que les environnements côtiers, mais présentent de nombreuses souches appartenant à des espèces connues du milieu terrestre (Manohar and Raghukumar 2013; Rédou *et al.* 2016). Cependant, d'autres habitats des profondeurs océaniques comme les sources hydrothermales sont quant à eux caractérisés par une forte diversité fongique (Rédou *et al.* 2016). Depuis les années 1970, les sources hydrothermales sont étudiées notamment pour les communautés bactériennes associées. Il faudra attendre la fin des années 2000 pour que les chercheurs s'intéressent aux communautés fongiques. Cela reflète l'idée générale et erronée que les champignons sont des organismes uniquement terrestres (Le Calvez *et al.* 2009). Certains environnements côtiers tels que les mangroves ou les récifs coralliens ont également été décrits comme source d'importante diversité fongique (Manohar and Raghukumar 2013; Sarma 2019).

Il est intéressant de noter que tous les habitats potentiels pour les champignons marins ne sont pas étudiés de manière égale. Par exemple, les environnements côtiers, la colonne d'eau et les sources hydrothermales seraient moins étudiés que les environnements profonds concernant la diversité fongique à l'échelle moléculaire (Sarma 2019). De plus, les champignons marins isolés à partir des éponges et des algues sont particulièrement bien documentés en comparaison avec les autres habitats fongiques marins (Rédou *et al.* 2016). Ainsi, au-delà d'une sous-estimation de la diversité marine fongique globale dans l'absolu, les zones les moins étudiées pourraient apparaître à tort comme ayant une diversité fongique plus faible que d'autres zones plus étudiées.

Utilisations biotechnologiques des molécules de champignons marins (métabolites bioactifs)

Le haut potentiel des champignons dans le domaine de la santé et de la pharmacologie est de plus en plus étudié. Les champignons marins ne sont pas en reste. Ils produisent par exemple une plus grande diversité de terpènes bioactifs que les bactéries (Gozari *et al.* 2021). De plus, ces terpènes peuvent présenter des halogénations qui améliorent leur activité, notamment antimicrobiennes, antivirales, antiprolifératives, anti-algues, anti-inflammatoires, antihypertensives, antiépileptiques, antihyperlipidémique ou inhibitrices d'adhésion cellulaire (Gozari *et al.* 2021).

En effet, l'une des applications majeures des champignons marins réside dans leur production de composés bioactifs et de métabolites secondaires (ou spécialisés) d'intérêt qui peuvent ne pas être retrouvés chez leurs homologues terrestres (Bugni and Ireland 2004; Raghukumar 2008). Jusqu'aux années 1980, les champignons marins étaient largement négligés car estimés peu abondants, et pour lesquels l'appartenance au milieu marin était sujette à discussion. Jusqu'aux années 1992, seulement 15 molécules d'intérêts issues de champignons marins étaient connues, alors qu'ils y en a aujourd'hui des milliers (Agrawal *et al.* 2018; Ibrar *et al.* 2020). En effet, l'utilisation de champignons isolés à partir d'environnements marins et la découverte de nouveaux produits naturels est en constante croissance depuis les années 1990 (Rédou *et al.* 2016; Sarasan *et al.* 2017; Nicoletti and Andolfi 2018; Varrella *et al.* 2021). Toutefois, ce réservoir constitue encore une source sous-estimée de métabolites secondaires d'intérêt (Shin 2020). Les champignons sont, d'un point de vue phylogénique, plus diversifiés que les bactéries et se caractérisent par un génome plus grand composé notamment de clusters de gènes impliqués dans la production de composés d'intérêt. Bien que le nombre de produits naturels issus de champignons marins soit en constante augmentation, il est encore bien inférieur à celui décrit chez des bactéries marines (Giddings and Newman 2022).

Parmi les composés bioactifs d'intérêt synthétisés par les champignons marins et principalement issus du métabolisme secondaire, il est possible de citer par exemple les terpènes, les polykétides, les alcaloïdes, les exopolysaccharides, les dérivés du shikimate, les mycosporines, les quinones, les stéroïdes mais aussi des lipides et des peptides non ribosomiaux (Thatoi *et al.* 2013; Hasan *et al.* 2015; Agrawal *et al.* 2018; Bramhachari *et al.* 2019; Shin 2020; Giddings and Newman 2022). Ces molécules issues des champignons marins ont été particulièrement étudiées durant les dernières années et présentent un large spectre d'activités biologiques, détaillées dans la **Table I. 5**.

Table I. 5 : Représentation des diverses activités biologiques recensées à partir du métabolome des champignons marins.

Activités biologiques de composés fongiques	Références bibliographiques
Antiviraux	Jones <i>et al.</i> (2008, 2019); Damare <i>et al.</i> (2012); Thatoi <i>et al.</i> (2013); Hasan <i>et al.</i> (2015); Bramhachari <i>et al.</i> (2019); Hyde <i>et al.</i> (2019); Shin (2020); Ameen <i>et al.</i> (2021); Varrella <i>et al.</i> (2021); Giddings and Newman (2022)
Antifongiques	Damare <i>et al.</i> (2012); Hasan <i>et al.</i> (2015); Bramhachari <i>et al.</i> (2019); Hyde <i>et al.</i> (2019); Jones <i>et al.</i> (2019); Ameen <i>et al.</i> (2021); Varrella <i>et al.</i> (2021); Giddings and Newman (2022)
Antibactériens	Jones <i>et al.</i> (2008, 2019); Damare <i>et al.</i> (2012); Dobretsov <i>et al.</i> (2013); Thatoi <i>et al.</i> (2013); Hasan <i>et al.</i> (2015); Agrawal <i>et al.</i> (2018); Bramhachari <i>et al.</i> (2019); Gladfelter <i>et al.</i> (2019); Hyde <i>et al.</i> (2019); Ibrar <i>et al.</i> (2020); Shin (2020); Ameen <i>et al.</i> (2021); Varrella <i>et al.</i> (2021); Giddings and Newman (2022)
Anti-algues	Bramhachari <i>et al.</i> (2019)
Insecticides	Bramhachari <i>et al.</i> (2019)
Anticancéreux	Damare <i>et al.</i> (2012); Thatoi <i>et al.</i> (2013); Hasan <i>et al.</i> (2015); Agrawal <i>et al.</i> (2018); Bramhachari <i>et al.</i> (2019); Gladfelter <i>et al.</i> (2019); Hyde <i>et al.</i> (2019); Jones <i>et al.</i> (2019); Shin (2020); Ameen <i>et al.</i> (2021); Varrella <i>et al.</i> (2021); Giddings and Newman (2022)
Anti-inflammatoires	Damare <i>et al.</i> (2012); Thatoi <i>et al.</i> (2013); Hasan <i>et al.</i> (2015); Ibrar <i>et al.</i> (2020); Ameen <i>et al.</i> (2021); Varrella <i>et al.</i> (2021); Giddings and Newman (2022)
Antiallergiques	Ibrar <i>et al.</i> (2020); Giddings and Newman (2022)
Cytotoxiques	Jones <i>et al.</i> (2008); Damare <i>et al.</i> (2012); Bramhachari <i>et al.</i> (2019); Ibrar <i>et al.</i> (2020); Ameen <i>et al.</i> (2021); Varrella <i>et al.</i> (2021)
Antioxydants	Thatoi <i>et al.</i> (2013); Agrawal <i>et al.</i> (2018); Bramhachari <i>et al.</i> (2019); Ameen <i>et al.</i> (2021)
Photoprotecteurs	Agrawal <i>et al.</i> (2018)
Antidiabétiques	Thatoi <i>et al.</i> (2013); Hyde <i>et al.</i> (2019); Ameen <i>et al.</i> (2021)
Immunomodulateurs	Thatoi <i>et al.</i> (2013); Hyde <i>et al.</i> (2019)
Neuroprotecteurs	Damare <i>et al.</i> (2012)
Antituberculeux	Ibrar <i>et al.</i> (2020)

Parmi ces activités appliquées au domaine de la santé, certains métabolites montrent aussi des intérêts en cosmétologie avec par exemple des effets contre le vieillissement de la peau, la capacité d'effet blanchissant sur la peau *via* notamment une inhibition de l'activité tyrosinase ou la lutter contre des infections de la peau comme l'acné (Agrawal *et al.* 2018; Hyde *et al.* 2019). Certains composés antimicrobiens peuvent également être utilisés pour lutter contre le fouling, en étant potentiellement utilisés en synergie avec d'autres composés fongiques anti-biofilms bactériens (Jones *et al.* 2008; Dobretsov *et al.* 2013; Hyde *et al.* 2019). D'autres métabolites issus de champignons marins sont

également utilisés dans l'agriculture, dans l'amélioration de la productivité des plantes, de leur croissance et de leur résistance à différents stress ou en tant que bio-fertilisateurs (Hyde *et al.* 2019). Certaines molécules bioactives permettent aussi de lutter contre divers pathogènes, insectes ou nématodes (Thatoi *et al.* 2013; Hyde *et al.* 2019). Certaines autres molécules produites par les champignons marins peuvent être utilisées en tant que conservateurs ou en tant que tensioactifs naturels dont les applications se sont récemment étendues à divers secteurs industriels telles que l'alimentation, la pharmacologie et l'industrie pétrolière (Damare *et al.* 2012; Hyde *et al.* 2019). Certains champignons marins peuvent également synthétiser des pigments d'intérêt tels que de l'hydroxyanthraquinone ou bien de l'azaphilone (Hyde *et al.* 2019; Venil *et al.* 2020).

Les principales activités biologiques issues des champignons marins, les principales familles moléculaires auxquelles elles sont associées ainsi que leurs potentielles applications industrielles sont présentées **Figure I. 13**.



Figure I. 13 : Principales classes de métabolites produits par les champignons marins, ainsi que leurs principales activités biologiques et leurs principales applications. Issu de Gonçalves *et al.* (2022).

Bien que de nombreuses activités biologiques aient été décrites chez les champignons marins, très peu de ces composés ont été commercialisés (Ameen *et al.* 2021). Dans le domaine de la pharmacologie, seule la plinabuline isolée à partir d'une souche marine d'*Aspergillus* et aux propriétés anticancéreuses est actuellement en essais cliniques (Gonçalves *et al.* 2022).

Les enzymes de champignons marins : des molécules à très hauts potentiels

En dehors de la production de molécules bioactives, les champignons, et en particulier les champignons marins sont particulièrement intéressants pour leur production d'enzymes à intérêt industriel (Thatoi *et al.* 2013). Les environnements marins diffèrent des environnements terrestres en ce qui concerne les facteurs physicochimiques tels que la salinité, la pression, la lumière (quantitativement et qualitativement) ainsi que la température (Zhang and Kim 2010; Bonugli-Santos *et al.* 2015). Une adaptation à ces facteurs est nécessaire pour les organismes marins, ce qui inclue notamment la synthèse d'enzymes adaptées aux hautes salinités mais également aux températures extrêmes (Shah *et al.* 2019; Varrella *et al.* 2021). Les enzymes marines sont également caractérisées par des affinités de substrats différentes que pour les enzymes terrestres (Trincon 2011; Arnosti *et*

al. 2014). En fonction du substrat considéré, les enzymes marines pourraient donc permettre une meilleure dégradation que leurs homologues terrestres. Plusieurs oxydoréductases marines peuvent également être caractérisées par une meilleure résistance aux solvants organiques en plus d'une tolérance aux salinités importantes (Trinconne 2011). L'intérêt industriel pour de telles enzymes marines, comme les polysaccharidases, les protéases et les lipases n'a cessé de croître depuis plusieurs années (Zhang and Kim 2010).

Certains bioprocédés comme la production d'éthanol ou de biocarburants à partir de biomasse algale nécessitent une étape de désalinisation lorsque des enzymes terrestres sont utilisées. L'utilisation d'enzymes halophiles marines, et notamment de champignons marins, permettrait de passer outre cette étape de désalinisation (Gamauf *et al.* 2007; Trinconne 2011, 2017; Thatoi *et al.* 2013; Hyde *et al.* 2019). En effet, les champignons marins sont les principaux microorganismes permettant la dégradation de structures lignocellulosiques et de composés aromatiques dans le milieu marin (Ameen *et al.* 2021).

Le traitement de divers effluents dans le cadre de la bioremédiation est également plus efficace en utilisant des enzymes marines par rapport aux enzymes terrestres (Raghukumar *et al.* 2004; Damare *et al.* 2012; Trinconne 2017; Hyde *et al.* 2019). Il a été montré que les champignons pouvaient être utilisés pour dégrader les hydrocarbures ou les eaux usées issus d'industries textiles, cosmétiques, du papier ou de l'alimentation mais aussi les plastiques comme le polyuréthane par exemple (Damare *et al.* 2012; Thatoi *et al.* 2013; Venkateswara Sarma 2018; Gladfelter *et al.* 2019; Hyde *et al.* 2019; Singh *et al.* 2020; Zeghal *et al.* 2021). Ainsi, toutes les surfaces du milieu marin sont colonisées par des microorganismes, dont les plastiques, qui semblent montrer une communauté microbienne associée différente de celles associées à d'autres substrats naturels (Zeghal *et al.* 2021). Les principales enzymes impliquées dans ce procédé de dégradation des plastiques et des hydrocarbures correspondraient aux enzymes lignocellulolytiques (Damare *et al.* 2012; Venkateswara Sarma 2018; Zeghal *et al.* 2021). Les champignons peuvent également transformer les métaux lourds en métalloïdes et en composés organométalliques, ce qui modifie leur mobilité et leur toxicité (Gadd 2007; Singh *et al.* 2020). En plus d'un potentiel en bioremédiation, les champignons peuvent être utilisés en tant que bioindicateurs d'une pollution aux métaux lourds, une corrélation existant entre la quantité de ces métaux présents au niveau du substrat de croissance et au sein du champignon (Gadd 2007). Ces capacités à métaboliser les métaux peuvent également avoir des applications dans le domaine de la santé. Par exemple, les champignons marins sont prometteurs dans la synthèse de nanoparticules d'argent (AgNPs), présentant des activités antimicrobiennes, antivirales, antidermatophytiques, anti-inflammatoires, antitumorales, hépatoprotectrices, cytotoxiques, antihypertensives et immunomodulatrices (Khan *et al.* 2018).

Ainsi, l'intérêt d'étudier les enzymes marines s'étend à d'autres domaines tels que la santé, l'alimentation ou la production de produits chimiques tels que les détergents (Damare *et al.* 2012; Trincone 2017; Shah *et al.* 2019). Elles peuvent être utilisées dans l'extraction de composés d'intérêt présents chez divers organismes marins (Trincone 2017). En plus de se démarquer par d'importantes activités enzymatiques, les champignons présentent l'avantage d'excréter leurs enzymes dans le milieu extérieur, contrairement aux bactéries (Schatz 1984; Grant and Rhodes 1992). Cette excrétion induit une disponibilité directe des enzymes dans le milieu de culture sans nécessiter d'étape d'extraction. Bien que la production enzymatique par les champignons marins présente plusieurs avantages par rapport aux bactéries marines, les enzymes marines bactériennes sont encore bien plus documentées que celles des champignons marins (de Vries *et al.* 2020).

Une part importante du potentiel des enzymes fongiques marines concerne leurs applications en lien avec la dégradation des polysaccharides issus de macroalgues. Ainsi, la plupart des types d'applications des enzymes fongiques marines évoquées peut cibler spécifiquement des substrats macroalgaux (Nicoletti and Andolfi 2018; de Vries *et al.* 2020). Ce potentiel biotechnologique des enzymes fongiques marines sur les macroalgues sera développé plus dans le détail dans la suite de ce manuscrit. Il est attendu que les champignons les plus à même de produire des enzymes capables de cibler les polysaccharides macroalgaux sont les champignons constituant le mycobiote de ces macroalgues.

CHAMPIGNONS ISSUS D'ALGUES (ALGICOLES) - Ecologie, modes de vie, principaux taxons et production d'enzymes de dégradation des polysaccharides, protéines et lipides algaux

Rôles écologiques des champignons marins au sein des algues

Les macroalgues présentent comme toutes les plantes une communauté fongique propre avec qui elles interagissent pour leur croissance, leur défense, leur développement et leur apport en nutriments (Rédou *et al.* 2016). Cette association entre les macroalgues et leur communauté microbienne peut être considérée comme un super-organisme constituant un holobionte (Egan *et al.* 2013; Rédou *et al.* 2016). Plusieurs relations mutualistes peuvent exister entre des champignons et leur hôte macroalgal, impliquant des bénéfices pour les deux organismes (Bugni and Ireland 2004; Rédou *et al.* 2016; Raghukumar 2017; Nicoletti and Andolfi 2018; Menaa *et al.* 2020). Cela peut même aller jusqu'à une interdépendance de l'hôte et du champignon, avec par exemple l'algue brune *Ascophyllum nodosum* et le champignon *Mycosphaerella ascophylli* qui ne peuvent pas être trouvés séparément dans la nature (Bugni and Ireland 2004). Certains autres champignons mutualistes permettent à leur hôte de mieux résister à la dessiccation (Raghukumar 2017). Les champignons algicoles sont également souvent caractérisés en tant que saprophytes lorsqu'ils se développent et sont isolés à partir de

biomasse algale morte (Suryanarayanan 2012; Raghukumar 2017; Nicoletti and Andolfi 2018; Menaa *et al.* 2020). Cependant, la diversité des champignons algicoles ainsi que leurs interactions avec leurs hôtes macroalgues sont encore clairement sous-étudiés, notamment par rapport aux bactéries, pour permettre d'établir des tendances claires (Suryanarayanan 2012; Garzoli *et al.* 2018; Menaa *et al.* 2020). Le rôle précis de ces champignons marins dans la dégradation de biomasse n'est pas encore non plus clairement établi (Gomaa *et al.* 2015). De plus amples études sur les communautés fongiques sont nécessaires, notamment en ce qui concerne les algues rouges et vertes qui sont moins étudiées que les algues brunes en ce qui concerne leur mycobiome associé (Suryanarayanan 2012; Raghukumar 2017; Garzoli *et al.* 2018).

Techniques d'étude du microbiote associé aux macroalgues

Lors des dernières décennies, l'intérêt général autour des communautés microbiennes associées aux algues s'est développé (Friedrich 2012). La grande majorité des études sur cette diversité microbienne issue des macroalgues (algicole) porte encore aujourd'hui sur la communauté bactérienne (Egan *et al.* 2013; Rédou *et al.* 2016). Les communautés bactériennes associées avec la surface des macroalgues ont divers rôles de protection pour ces macroalgues, avec des propriétés antifouling et de prévention contre une colonisation par des microorganismes pathogènes (Egan *et al.* 2013). Etant donné leur rôle protecteur sur la surface des algues, les bactéries épiphytes sont bien plus étudiées que les bactéries endophytes (Egan *et al.* 2013). En effet, depuis 2016, au moins 32 études ont été publiées en rapport avec la diversité bactérienne épiphyte de macroalgues (Florez *et al.* 2017).

Divers facteurs influencent la diversité des communautés fongiques associées aux macroalgues, tels que la salinité, la localisation géographique du prélèvement, ainsi que la nature biologique du substrat algal ainsi que la synthèse potentielle de composés antifongiques (Suryanarayanan 2012; Wainwright *et al.* 2019). Concernant la diversité des communautés fongiques associées à des algues brunes, rouges et vertes, certains auteurs décrivent une absence de tendance claire permettant de d'établir la présence d'une diversité fongique plus importante chez un groupe d'algue par rapport aux autres (Suryanarayanan *et al.* 2010; Suryanarayanan 2012; Flewelling *et al.* 2013). Cette observation est contradictoire avec les conclusions issues d'une étude récente selon laquelle les algues brunes seraient caractérisées par une diversité fongique plus importante que les algues rouges et vertes (Abdul Malik *et al.* 2020). Par ailleurs, il semble particulièrement difficile de définir un cœur de diversité fongique constant et précis pour une espèce de macroalgue donnée compte tenu de la grande variabilité caractérisant les résultats de diversité obtenus lors de divers prélèvements (Tourneroché *et al.* 2020).

Plusieurs champignons semblent généralement associés aux macroalgues, appartenant aux genres *Acremonium*, *Alternaria*, *Arthrinium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Geomyces*, *Penicillium* et

Phoma (Abdul Malik *et al.* 2020). La tendance selon laquelle les champignons marins sont principalement représentés par les ascomycètes se vérifie ainsi chez les macroalgues (Rédou *et al.* 2016). Des précautions doivent cependant être prises concernant ces données générales. En effet, la plupart des études portant sur les communautés fongiques de macroalgues se concentrent sur les communautés endophytes avec l'élimination des épiphytes (Abdul Malik *et al.* 2020). Par ailleurs, la stratégie de culture de champignons privilégie principalement l'apparition et le développement de souches ubiquistes et à croissance rapide. Pour obtenir une idée plus exhaustive de la communauté fongique cultivable totale, en prenant aussi en compte les souches à développement lent, il est conseillé de procéder à des isolements par dilution de l'échantillon (Rédou *et al.* 2016). De plus, la majorité des études portant sur la diversité de ces communautés fongiques associées aux macroalgues se limite aux portions cultivables, non obligatoirement représentatives de la diversité totale réelle (Rédou *et al.* 2016; Agusman and Danging 2017). Chez les champignons marins issus d'algues, cette portion cultivable représenterait moins de 2 % de la communauté fongique totale associée à l'algue (Garzoli *et al.* 2018). En effet, une proportion très importante de souches fongiques nécessite un ensemble de conditions particulières pour se développer, qui ne sont pas forcément transposables en laboratoire (Rédou *et al.* 2016). Ainsi, les spores de certaines espèces fongiques ne germent qu'en cas de faible apport nutritionnel. Pour isoler ces espèces il est conseillé de réaliser des milieux de cultures avec différentes concentrations en substrat, dont certaines très faibles. Un autre biais occasionné par la méthode d'isolement sur milieu de culture est l'absence des conditions environnementales et notamment les potentielles communications entre certaines espèces fongiques et les cellules d'autres organismes marins, qui peuvent être nécessaires pour la germination des spores (Rédou *et al.* 2016). Une approche complémentaire moléculaire permet donc de compléter la diversité d'une communauté fongique et de vérifier la pertinence écologique de la diversité obtenue par culture, notamment en utilisant les séquences Internal Transcribed Spacer (ITS) en tant que marqueurs moléculaires, qui sont probablement les plus répandues et les plus utilisées pour les études de diversité fongique (Suryanarayanan 2012; Rédou *et al.* 2016; Garzoli *et al.* 2018). Cependant, dans le cadre d'études dont le but est l'usage concret de champignons marins pour leurs applications ou leur production de composés d'intérêt, l'utilisation de d'approches culturelles pour isoler le plus possible de souches fongiques candidates est indispensable (Flewelling *et al.* 2013; Hong *et al.* 2015; Albakosh *et al.* 2016; Lee *et al.* 2019).

Comme mentionné précédemment, les champignons marins ont de nombreuses applications biotechnologiques en tant que producteurs de métabolites d'intérêt et de composés bioactifs. Une majorité de souches fongiques isolées à partir de macroalgues semblent caractérisées par des activités antimicrobiennes prometteuses (Zhang *et al.* 2009; Suryanarayanan *et al.* 2010; Flewelling *et al.* 2013;

Hong *et al.* 2015; Sarasan *et al.* 2017; Kamat *et al.* 2020). La synthèse d'autres composés aux propriétés antioxydantes (Suryanarayanan *et al.* 2010; Suryanarayanan 2012; Hong *et al.* 2015; Sarasan *et al.* 2017; Kamat *et al.* 2020), antitumorales (Sarasan *et al.* 2017; Kamat *et al.* 2020), anti-algues ou anti-insectes (Suryanarayanan *et al.* 2010; Suryanarayanan 2012) ont été décrites chez des souches fongiques algicoles. Par ailleurs, ces bioactivités fongiques pourraient être sous-estimées notamment par l'assignation abusive de certaines activités aux algues sans réelle confirmation qu'il s'agisse de bioactivités directement liées à l'algue ou au microbiote (Gnavi *et al.* 2017).

Les champignons algicoles ont également été caractérisés en tant que producteurs prometteurs d'enzymes hydrolytiques telles que les polysaccharidases (Ogaki *et al.* 2019). Des activités cellulases intéressantes ont été décrites (Hong *et al.* 2015; Lee *et al.* 2019), ainsi que des activités β -glucosidases et endoglucanases par exemple (Lee *et al.* 2019). De nombreuses autres activités enzymatiques retrouvées chez les champignons marins ont été discutées dans plusieurs revues bibliographiques (Damare *et al.* 2012; Velmurugan and Lee 2012; Bonugli-Santos *et al.* 2015; Balabanova *et al.* 2018; Mzui and Ch 2018; de Vries *et al.* 2020). Concernant l'extraction de R-PE à partir d'algues rouges, toutes les enzymes utilisées selon l'ensemble des études portant sur ce sujet sont des enzymes commerciales. Le marché global des enzymes a été évalué à environ 10 milliards de dollars US en 2019, et est caractérisé par une augmentation annuelle entre 5 et 7 % (Filiatrault-Chastel *et al.* 2021; Singh *et al.* 2021). Les cellulases, représentant 20 % du marché des enzymes industrielles (Singh *et al.* 2021), font partie depuis plusieurs décennies des enzymes les plus commercialisées avec les xylanases, les protéases, les amylases et les pectinases (Bajaj and Mahajan 2019a; Tushar and Dutta 2020; Bhardwaj *et al.* 2021; Ejaz *et al.* 2021). Plusieurs cocktails enzymatiques ont été testés et comparés pour l'extraction de protéines chez *P. palmata*. En utilisant des combinaisons d'enzymes commerciales, il est possible d'extraire jusqu'à 90 % des protéines de l'algue, la combinaison la plus efficace étant celle regroupant les enzymes Celluclast® et Alcalase®, toutes deux à 0,2 % (pds./pds.) (Naseri *et al.* 2020). La majorité des enzymes commerciales utilisées en industrie provient des champignons (Østergaard and Olsen 2011; Hardouin *et al.* 2014; Filiatrault-Chastel *et al.* 2021). L'efficacité enzymatique de ces champignons semble supérieure à celle des bactéries (Schatz 1984; Grant and Rhodes 1992). Par exemple, sur l'ensemble des enzymes utilisées avec succès sur *P. palmata* pour l'extraction de composés antioxydants, la majorité était d'origine fongique (Wang *et al.* 2010). De plus, les champignons présentent l'avantage d'excréter leurs enzymes dans le milieu extérieur, ce qui permet de s'affranchir d'une étape d'extraction dans la production d'enzymes par rapport aux enzymes bactériennes.

Cependant, il semble qu'aucune étude bibliographique n'ait jusqu'à présent été réalisée sur les capacités des champignons marins à produire des enzymes ciblant spécifiquement les polysaccharides

de macroalgues. Ainsi, la section suivante présente un travail de revue bibliographique réalisé dans le cadre de cette thèse de doctorat, portant sur les capacités des champignons marins à synthétiser des enzymes dégradant les polysaccharides, protéines et lipides issus de macroalgues.



Marine fungal abilities to enzymatically degrade algal polysaccharides, proteins and lipids: a review

Yoran Le Strat¹ · Nicolas Ruiz¹ · Joël Fleurence¹ · Yves-François Pouchus¹ · Paul Délérís¹ · Justine Dumay¹ 

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Abstract

Over the last decades, metabolites with biotechnological application produced by marine resources and notably macroalgae have seen increasing interest. Among these metabolites, many require the use of efficient extraction processes to reach a sufficiently high yield for industrial development. One of the more promising extraction processes currently used is the extraction assisted by enzymes, which can be coupled with other extraction techniques. However, most of the commercial enzymes used for the extraction of molecules of interest from marine material are enzymes obtained from terrestrial microorganisms and with limited substrate specificity. The efficiency of the extraction could then be increased by using more specific enzymes according to the targeted matrix. Marine fungi are particularly promising for the production of degradation enzymes and their interest recently increased, but they are still less studied than marine bacteria. A state of the art on the degradation enzymes from marine fungi is proposed, and more specifically on polysaccharide, protein and lipid degradation enzymes.

Keywords Seaweeds · Marine fungi · Enzymatic activities · Polysaccharidases · Proteases · Lipases

Introduction

Marine fungi were first defined in 1961, based on their ability to grow in seawater conditions (Johnson and Sparrow 1961). After two decades, this definition was modified due to the ability of some fungal strains to grow under conditions comparable with those found in marine environments (Kohlmeyer and Kohlmeyer 1979). These authors then defined obligate and facultative marine fungi: the former needing marine or estuarine conditions to sporulate and the latter being fungal strains isolated from terrestrial habitats able to grow and sporulate in marine conditions. Even though this definition has been widely used in the last decades, it has some ambiguities considering those fungal strains isolated from the marine environment but able to grow under terrestrial conditions which are considered as facultative fungi. “True” marine fungi have been then linked only with obligate marine fungi, discriminating the facultative ones, leading to an underestimation of the whole marine fungal biodiversity. Moreover, some facultative marine fungi

isolated from the marine environment are characterized by clear adaptations in the marine environment and have developed interactions with marine microbial communities, but still could not be considered as real marine fungi (Pang *et al.* 2016; Rédou *et al.* 2016). The currently used definition has been given by Pang *et al.* (2016) and describes three ways to consider a fungal strain as a marine fungus: “(1) it is able to grow and/or sporulate (on substrata) in marine environments; (2) it forms symbiotic relationships with other marine organisms; or (3) it is shown to adapt and evolve at the genetic level or be metabolically active in marine environments”.

Fungal strains have been found in every marine environment investigated. Fungi seem to be widespread in the marine environment, including the water column, sediments, driftwood, algae, invertebrates and mammals (Tisthammer *et al.* 2016; Amend *et al.* 2019). Marine fungi isolated from sponges and algae are particularly well documented compared with those isolated from other marine habitats. Indeed, sponges correspond to the most studied substrate, and algae correspond to the second most studied one for marine fun-

✉ Justine Dumay
justine.dumay@univ-nantes.fr

¹ Nantes Université, Institut Des Substances Et Organismes de La Mer, ISOMER, UR 2160, F-44000 Nantes, France

Chapitre I : Etat de l'art

gal isolation (Rédou *et al.* 2016). Studies can have different criteria to separate fungal strains according to habitat diversity. For example, Hugues (1974) separated marine fungal communities according to the climate, tempera and biogeographical zones (arctic, antarctic, temperate, subtropical, tropical). Geographic location seems indeed to have an influence on the marine fungal distribution, but the use of environmental factors like water depth would be more pertinent (Tisthammer *et al.* 2016). In most studies, the distinction can then be established between coastal and deep-sea marine fungi because of the different characteristics of such habitats (temperature, light, pressure, biological diversity...) (Manohar and Raghukumar 2013; Satyanarayana *et al.* 2019). Then, it appears that deep marine sediments have lower diversity than coastal ones, but with many cultivable fungal strains belonging to species also found in terrestrial environments (Manohar and Raghukumar 2013; Rédou *et al.* 2016). However, other deep-sea habitats such as hydrothermal vents seem to be characterized by a high fungal diversity (Rédou *et al.* 2016). Coastal environments such as mangroves or coral reefs also show a high fungal diversity (Manohar and Raghukumar 2013; Satyanarayana *et al.* 2019). All putative marine fungal habitats are not studied equally: coastal, water column and hydrothermal vents environments would be less studied than deep-sea regions (Satyanarayana *et al.* 2019). Beyond an absolute underestimation of global marine fungal diversity, the less studied marine regions would then putatively appear wrongly as having poor fungal diversity than more studied regions.

The phyla Ascomycota and Basidiomycota are the most represented considering the fungal strains isolated from marine environments (Manohar and Raghukumar 2013; Rédou *et al.* 2016; Amend *et al.* 2019) and a predominance of Ascomycota has been highlighted in several studies (Satyanarayana *et al.* 2019). Among these Ascomycota, several orders are particularly represented in marine fungi such as Microascales, Pleosporales, Eurotiales and Saccharomycetales (Jones *et al.* 2019). At the genus level, *Aspergillus*, *Cladosporium*, *Fusarium*, *Gliocladium*, *Microsphaeriopsis*, *Paecilomyces*, *Penicillium*, *Phoma*, *Phomopsis*, *Trichoderma* and *Ulocladium* seem to be particularly represented (Bugni and Ireland 2004). In response to the increasing studies on marine fungal diversity, a database on marine fungi (www.marinefungi.org) has been created recently and provides classification details and descriptions of marine fungi (Jones *et al.* 2019).

Concerning algal fungal diversity, the great majority belong to Ascomycota (Menezes *et al.* 2010; Suryanarayanan 2012; Gnavi *et al.* 2017; Olafsson 2017; Garzoli *et al.* 2018; Nicoletti and Andolfi 2018; Kamat *et al.* 2020). Among these Ascomycota, species belonging to several genera such as *Aspergillus*, *Cladosporium* and *Penicillium* are dominant and exhibit a broad algal host

specificity (Suryanarayanan 2012; Raghukumar 2017; Abdul Malik *et al.* 2020). They cope particularly easily with environmental factors (such as saline conditions), exhibit easy cultivable potential and high growth rates compared with other fungal species (Bugni and Ireland 2004; Suryanarayanan 2012). Some other species are restricted to only several different hosts (Raghukumar 2017), and several fungal species are so adapted to their macroalgal host metabolites that they cannot grow in their absence. This is the case of *Acremonium fuci* of which spore germination is possible only in the presence of tissue of the macroalga *Fucus serratus* (Suryanarayanan 2012; Nicoletti and Andolfi 2018).

Algal fungi can develop various kinds of interactions with their algal host. Fungi are often linked with infections and diseases in the marine environment and are then characterized as parasites (Bugni and Ireland 2004; Suryanarayanan 2012; Raghukumar 2017; Nicoletti and Andolfi 2018; Mena *et al.* 2020). These parasitic fungi can either be characterized as obligate or facultative (opportunistic), and can be restricted to several hosts, suggesting coevolution between hosts and pathogens (Raghukumar 2017). Some mutualistic relationships can exist between fungi and the macroalgal host, with benefits to both organisms (Bugni and Ireland 2004; Raghukumar 2017; Nicoletti and Andolfi 2018; Mena *et al.* 2020). For example, the brown alga *Ascophyllum nodosum* and the fungus *Mycosphaerella ascophylli* cannot be found separated in nature (Bugni and Ireland 2004). Some other mutualistic fungi allow to their host to cope with desiccation (Raghukumar 2017). Marine fungi also often develop on dead macroalgal biomass and are described as saprobes (Suryanarayanan 2012; Raghukumar 2017; Nicoletti and Andolfi 2018; Mena *et al.* 2020).

However, the diversity of algal fungi and their interactions with the algal hosts are probably still not enough studied, notably compared with bacteria, to establish clear tendencies (Suryanarayanan 2012; Garzoli *et al.* 2018; Mena *et al.* 2020). The role of these marine fungi in the degradation of macroalgal biomass is also not well established (Gomaa *et al.* 2015). Further fungal community inventory need to be performed, notably considering red and green seaweeds on which fungal diversities are less documented than brown seaweeds (Suryanarayanan 2012; Raghukumar 2017; Garzoli *et al.* 2018). Moreover, even if the use of molecular-based protocols is increasing, most studies on algal fungal diversity has been performed on the cultivable community which is known to represent less than 2% of the total fungal diversity (Garzoli *et al.* 2018). It would therefore be important to complement culture-based studies with molecular-based studies to exhibit a more exhaustive fungal diversity. Moreover, these studies on fungal diversity should also be performed on varied seaweed structures and organs which could harbour differences in fungal diversity, and in varied seasons which can influence the diversity of fungal communities (Suryanarayanan 2012).

Marine fungi, particularly algal ones, can be consid-

ered as sources of new compounds of biotechnological interest which cannot be found in their terrestrial counterpart (Bugni and Ireland 2004; Raghukumar 2008). They are notably known as producers of antiviral, antifungal, anti-bacterial or anticancer natural products (Jones *et al.* 2019). More specifically, several fungal strains isolated from macroalgae exhibit promising bioactivities such as antioxidant (Suryanarayanan *et al.* 2010; Suryanarayanan 2012; Hong *et al.* 2015; Sarasan *et al.* 2017; Kamat *et al.* 2020), anti-microbial (Zhang *et al.* 2009; Suryanarayanan *et al.* 2010; Flewelling *et al.* 2013; Hong *et al.* 2015; Sarasan *et al.* 2017; Kamat *et al.* 2020), antitumor (Sarasan *et al.* 2017; Kamat *et al.* 2020), anti-algal or anti-insect (Suryanarayanan *et al.* 2010; Suryanarayanan 2012) activities. Marine environments differ from terrestrial ones by several physicochemical factors, such as salinity, pressure, light condition or temperature (Zhang and Kim 2010; Bonugli-Santos *et al.* 2015). Marine organisms need to cope with these factors notably with the synthesis of enzymes adapted to high concentrations of salinity but also to high temperatures (Shah *et al.* 2019). Marine enzymes also have substrate affinities which differ from the ones of terrestrial enzymes (Trincon 2011; Arnosti *et al.* 2014). According to the targeted substrate, marine enzymes could then exhibit higher performances than terrestrial ones. Several marine oxidoreductases also exhibit higher resistance to organic solvents, besides the tolerance of high concentrations of salinity (Trincon 2011). These marine enzymes, such as polysaccharidases, proteases and lipases, have gained interest over several years in their corresponding industrial applications (Zhang and Kim 2010). Several bioprocesses such as ethanol production from marine algal biomass, need a desalination step when terrestrial enzymes are used. The use of halophilic enzymes derived from the marine environment would then allow this desalination step to be ignored (Trincon 2011). Treatments of various effluents are also more effective by using marine enzymes compared with terrestrial ones (Raghukumar *et al.* 2004). Marine enzymes are also increasingly used in several other domains such as medicine, food supplements, beverages and production of chemicals (Shah *et al.* 2019). Fungal enzymes are currently particularly well represented in biotechnological applications. A recent report by de Vries *et al.* (2020) shows that more than 50% of commercial enzymes with biotechnological interest have been obtained from fungi. Besides their high enzymatic activities (Schatz 1984; Grant and Rhodes 1992), fungi possess the advantage, compared with bacteria, to excrete enzymes in the external medium. This enzymatic external excretion allows the recovery of enzymatic crude extract without supplementary extraction protocol.

The use of fungi isolated from marine environments in biotechnologies and the finding of new natural products has been increasing since the 1990s (Rédou *et al.* 2016; Sarasan *et al.* 2017; Nicoletti and Andolfi 2018). Among these natural products, enzymes of degradation (of polysaccharides, proteins, lipids, nucleic acids...) from marine fungi are of particular interest compared with their terrestrial counterparts due to their higher specific activity (Balanova *et al.* 2018a) or because they are active under particular conditions such as high salinity, high pressure, acid and alkaline pH (Pointing *et al.* 1998; Huang *et al.* 2004; Wang *et al.* 2007; Atalla *et al.* 2013; Mzui and Ch 2018; Ogaki *et al.* 2019). Even if marine fungi seem to be characterized by several advantages compared with marine bacteria according to enzyme production, marine bacterial enzymes are largely better documented than marine fungal ones (de Vries *et al.* 2020). Polysaccharidases have numerous interests in several industrial fields. More specifically, the use of this kind of enzyme on algal biomasses is particularly interesting in food industries, biofuel production, pre- and probiotic production, cosmetics and pharmaceuticals, paper and textile industries, and for bioplastic production (Nicoletti and Andolfi 2018; de Vries *et al.* 2020). Marine polysaccharides, particularly sulfated polysaccharides from marine algae, can exhibit interesting activities that seem to be increased when the polysaccharide is partially degraded, highlighting another interest about polysaccharidases (Synytsya *et al.* 2015; de Vries *et al.* 2020). Proteinases and lipases are also degrading enzymes with industrial application even if polysaccharide degrading enzymes are more diverse because of the great diversity of natural polysaccharides.

The majority of enzymes of interest from marine fungi correspond to polysaccharide degrading enzymes, such as cellulases, xylanases or amylases. A first part will then briefly describe the structures and functions of the principal macroalgal polysaccharides targeted by these fungal enzymes. Nevertheless, several studies have also studied marine fungal proteases and lipases (Damare *et al.*, 2012). This review aims to summarize data and literature available on the algal polysaccharide, protein and lipid degrading enzymes secreted by marine fungi. Several of these references have been published from the 1980s even if the majority of them have been obtained during the last decade. Characteristics such as optimal temperature and pH, specific activity or enzymatic molecular weight are particularly focused on.

Principal polysaccharides found in marine macroalgae

Seaweed polysaccharides can have three main different roles for the alga: some polysaccharides have a role of structuring of the cell and are mainly found in the cell wall, some other

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polysaccharides have a role of food stock for the alga and some other are found in the cell matrix with several roles among which the prevention of desiccation (Usov 2011; Synytsya *et al.* 2015). These latter polysaccharides have other protective effect for the alga and are in part responsible of the algal flexibility (Percival 1979). Almost all of them are sulphated polysaccharides and are deeply studied for their application in human health (Kuznetsova *et al.* 2020). Moreover, many of these sulphated polysaccharides are widely used in other domain such as food, cosmetic or pharmaceutical industries thanks to their gelling and thickening properties (Renn 1997; Jiao *et al.* 2011; Dobrinčić *et al.* 2020; Kuznetsova *et al.* 2020).

Chlorophyceae

Chlorophyceae have a physiology and polysaccharide composition more similar to terrestrial plants than the Rhodophyceae and Phaeophyceae. This similarity is for example highlighted by the presence of starch as food reserve in the Chlorophyceae but not in the Phaeophyceae or Rhodophyceae (with the exception of Floridean starch) even when it shows several differences with terrestrial starch (Percival 1979). Principal structural polysaccharides of the Chlorophyceae are xylan, mannan and cellulose (Percival 1979; Hong *et al.* 2014). Sulphated polysaccharides from green seaweed have heterogeneous structures particularly complex and numerous compared with other seaweed (Jiao *et al.* 2011). Their functions are diverse and can enhance the flexibility as well as the resistance of the alga. They also have a role of defence against pathogens and allow water retention (Percival 1979). The best known of the sulphated polysaccharides of green seaweeds is certainly ulvan, for which precise structure can change according to the considered algal species, the environment or the season (Jiao *et al.* 2011; Synytsya *et al.* 2015; Kuznetsova *et al.* 2020). Other sulphated polysaccharides from green seaweeds, such as sulphated rhamnan, galactan, arabinogalactan or mannan, have been studied (Wang *et al.* 2014).

Rhodophyceae

The main polysaccharide with a food reserve function in the Rhodophyceae is floridean starch. It differs from terrestrial or green seaweed starch by the absence of amylose, consisting of small granules kind of amylopectin (Percival 1979; Usov 2011).

Xylan is particularly represented in red seaweeds, with several forms that are not observed in green or brown seaweeds (Synytsya *et al.* 2015). Moreover, this polysaccharide has a role of food storage in red seaweeds in addition to the structural role also observed in green and

brown seaweeds (Percival 1979). Another structural polysaccharide, also found in green and brown seaweeds, is cellulose although it generally only represents about 10% of the cell wall (Usov 2011). The other main structural polysaccharide found in Rhodophyceae is mannan, which can be found in the cell matrix as is xylan (Usov 2011).

One main group of water-soluble sulphated polysaccharides in the Rhodophyceae, the sulphated galactans, is separated into two subgroups: agarans and carrageenans (Ahmadi *et al.* 2015). The latter exists mainly in three different forms namely ι -, κ - and λ -carrageenans according to their sulphation level (Usov 2011; Kuznetsova *et al.* 2020). Both agarans and carrageenans are composed of galactose residues, but the former is more precisely composed of L-galactose and the latter is composed of D-galactose (Jiao *et al.* 2011; Usov 2011; Synytsya *et al.* 2015). Hybrid polysaccharides with agaran and carrageenan parts have been observed in some red algae (Jiao *et al.* 2011; Usov 2011). As for the Chlorophyceae, there is a great diversity of sulphated polysaccharides in Rhodophyceae, such as sulphated xylogalactan or xylomannan for example (Jiao *et al.* 2011).

Phaeophyceae

Laminarin is the principal storage product for brown seaweeds and is one of the most important carbon sources in marine environments (Percival 1979; Sanjeeva *et al.* 2017). Contrarily to Rhodophyceae and Chlorophyceae, Phaeophyceae do not synthesize starch as a storage product but form laminarin. Laminarin structure consists in a chain of 1,3-linked β -D-glucose residues with several branched β -D-glucose residues (Ahmadi *et al.* 2015; Sanjeeva *et al.* 2017; Dobrinčić *et al.* 2020).

Alginate is the most abundant polysaccharide found in brown seaweeds and is located in the cell wall (Ahmadi *et al.* 2015; Synytsya *et al.* 2015). It is structurally composed of β -D-mannuronic acid and α -L-guluronic acid with varying proportions resulting in a diversity of alginates (Synytsya *et al.* 2015; Dobrinčić *et al.* 2020; Kuznetsova *et al.* 2020). Cellulose has also been described in brown seaweeds with a structural function.

Fucans are the main sulphated polysaccharides situated in the algal matrix in the Phaeophyceae. They have a great structural diversity, and algal species can even be composed of different kinds of fucans (Jiao *et al.* 2011; Ahmadi *et al.* 2015). Fucose is the main component of fucans, but the structural complexity and diversity of this polysaccharide are due to the additional presence of monosaccharides such as glucose, galactose, xylose, mannose or uronic acids (Jiao *et al.* 2011; Sanjeeva *et al.* 2017; Kuznetsova *et al.* 2020). "Simple fucoidans" are only composed of a chain of sulphated fucose residues, but many other forms of fucoidans have been described with great heteropolysaccharidal and structural diversity (Synytsya *et al.* 2015; Sanjeeva *et al.* 2017; Dobrinčić *et al.* 2020). Generally, the characterization and the

precise description of seaweed polysaccharides is difficult to establish because of a great amount of derivatives and diversity even in the same species, and because of the effect of several factors such as the season or the algal age on their structure.

Enzymes of polysaccharide degradation from marine fungi

As in several other scientific domains, comparison of enzymatic activity values from a publication to another can be difficult because of a diversity in methodologies. Indeed, even if the dinitrosalicylic acid (DNS) method is the most widespread method of measuring polysaccharidase activity, some other methods can be used. Moreover, the substrate used for the activity can vary. Another source of diversity is fungal culturing methods, which differ widely in the literature because of fungal growth conditions. Those are defined by many variables (substrate, ionic solution, temperature, pH, solid/liquid medium...) and are responsible for different enzyme production. For example, according to the aim of the study, the substrate on which fungi are grown before enzymatic extraction can be a commercial preparation of polysaccharide targeted by an enzyme of interest, an alga (from which fungi can be isolated) pre-processed and incorporated into the medium, or food industry by-products of economic interest. On the other hand, in some studies the crude enzymatic activity is measured, while in others the enzymatic extract will be purified, using once again very diverse methods (Raghukumar *et al.* 2004; Li *et al.* 2007; D'Souza-Ticlo *et al.* 2009; Qianqian *et al.* 2011; Singh *et al.* 2011; Wu *et al.* 2011; Rong *et al.* 2015; Jmel *et al.* 2020). Here, we will describe all marine fungal enzymatic activities referenced in the literature degrading polysaccharides with few comparisons due to the reasons mentioned above. As enzymatic mechanisms are complex and generally monospecifically studied, it can be assumed that the same enzyme can exhibit various activities, and consequently a fungal strain can also have various enzymatic activities. For example, cellulose belonging to the glucan polysaccharide family, a cellulase could also be described as glucanase.

In this review, we describe activities as published in the literature.

A representative part of the whole data detailed further is synthesized in Table 1, focusing on data obtained from 2000 organized in four groups: firstly glucanase, secondly galactanases, thirdly other polysaccharide hydrolases and fourthly polysaccharide-degrading enzymes with a non-hydrolytic mechanism. When data were available supplementary information has been given, being enzymatic

activity, specific activity, enzymatic kinetics (V_m and K_m), molecular weight, and optimal temperature and pH. The ranges of enzymatic activities (and specific activities) were elaborated by selecting the highest activities of every study. There can be an important gap between the highest activity values reported, but this can be explained by the fact that they come from diverse studies, with many variables which could have an impact on the enzymatic activity.

Then, in order to highlight the diversity of some of these variables, the marine fungal species, their principal natural origins and the substrates used for their enzymatic production are summarized in Table 2. These data, such as Table 1, focus on the best polysaccharide-degrading marine fungal strains of every study and on the literature published from 2000.

The diversity of activities, of optimal pH and temperature, along with the marine fungal species that may be particularly promising for polysaccharide degradation, as well as other several tendencies, are further discussed in detail.

Hydrolases

Glucan degradation

The majority of studies dealing with marine fungal enzymes degrading polysaccharides concern glucans. Among these studies, many describe enzymes with glucanase or glucosidase activities but without specific association with a particular polysaccharide—most enzymes involved in polysaccharide degradation are not specific and can degrade several types of polysaccharides with different efficiencies. Thus, a glucanase could both degrade glucans and xyloglucans, and this ability is a characteristic participating in its classification in the Glycosyl Hydrolases families (Maclachlan and Brady 1994; Segato *et al.* 2017; Zhao *et al.* 2019). Natural algal glucans targeted by marine fungal enzymes of degradation are laminarin, cellulose and starch. Their structures and the general mode of action of glucanases are presented in Fig. 1. The use of commercial preparations of cellulose for fungal growth to measure glucan degradation capacities is particularly widespread compared with other natural glucans such as laminarin, or amylose (Fig. 1b).

Glucanases can be classified according to two principal operating modes allowing the degradation of glucan into glucosides, small chains of glucose residues. Endoglucanases represent enzymes allowing degradation of polymers of glucan targeting osidic linkages into the chain, while exoglucanases specifically hydrolyze the ends of the polysaccharide chain (Fig. 1a). In order to completely degrade glucosides into glucose molecules, organisms degrading glucans need to synthesize glucosidases. As for glucanases, glucosidases can target either glucoside α - or β -linkages and thus defined as α -glucosidases and β -glucosidases (Fig. 1a).

Table 1 Summary of marine fungal enzymes degrading polysaccharide described from 2000 and their principal characteristics. Only data concerning highest enzymatic activities obtained from the studies or bringing information on enzymes characteristics (Vm, Km, molecular weight, optimal temperature and pH) are presented (1 U = 1 μmol min⁻¹)

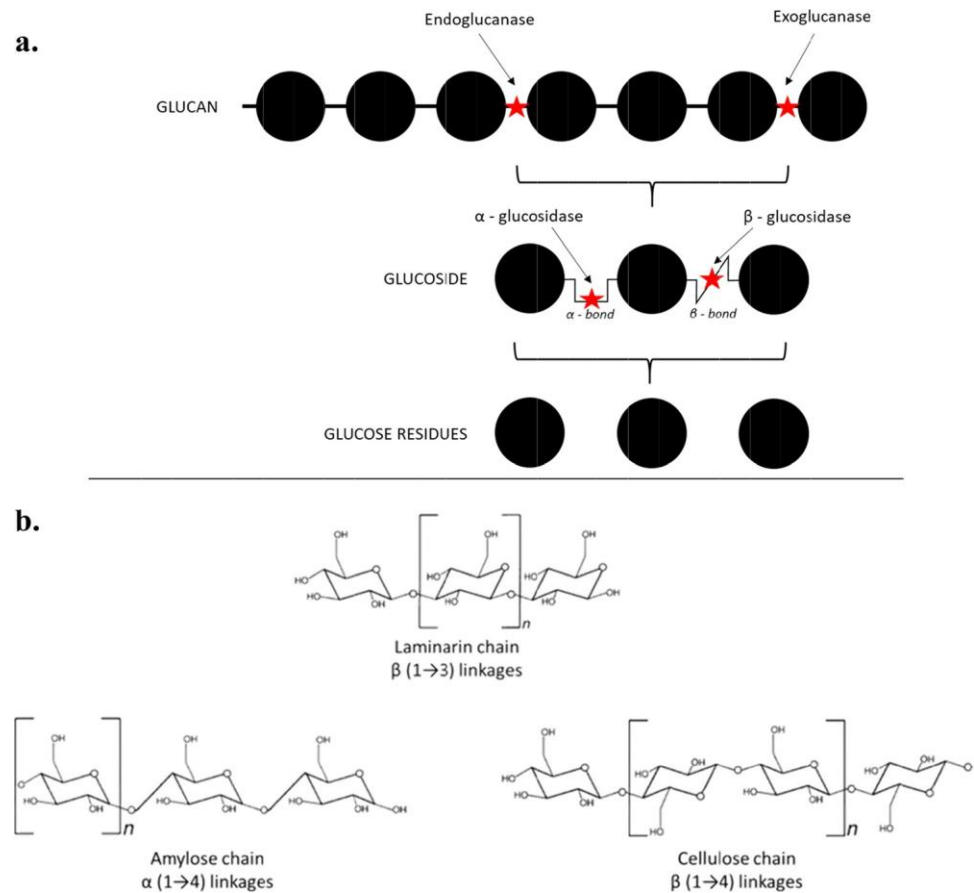
Polysaccharide-degrading specificity	Highest enzymatic activity (U mL ⁻¹)	Highest specific activity (U mg ⁻¹)	Vm (U mg ⁻¹)	Km (mM)	Molecular weight (kDa)	Optimal temperature (°C)	Optimal pH	References
Glucosidase	0.22–80	110	381.1 (U mg ⁻¹)	0.140	107	35–70	4–7	Elyas et al. 2010; Ravindran et al. 2010; Hong et al. 2015; Lee et al. 2015, 2019; Karray et al. 2016; Méndez-Lífer et al. 2018; Patyshakuliyeva et al. 2020
Cellulase	0.15–21.4	0.011–30.1	0.57 (U L ⁻¹)	0.070	56–67	40–60	4–7.5	Ravindran et al. 2010; Xue et al. 2012a; Torres and dela Cruz 2013; Li et al. 2014; Trivedi 2015, p. 201; Hong et al. 2015; Rong et al. 2015; Lee et al. 2015, 2019; Karray et al. 2016; Ghazal Mustafa et al. 2016; Patyshakuliyeva et al. 2020; Jmel et al. 2020
Laminarinase	0.22	0.010–13.5		0.002–0.005	54–56	40–60	4.4–7.5	Burtseva et al. 2003, 2006; Balabanova et al. 2018b; Patyshakuliyeva et al. 2020
Amylase		297.3–120×10 ³	0.25 (mg min ⁻¹ mL ⁻¹)	0.059	98	30–60	4.5	Li et al. 2007; Burtseva et al. 2010; Gonçalves et al. 2013
Galactosidase		42×10 ³						Burtseva et al. 2010
Agarase	0.182–7.69	20			39–44	40–50	7–8	Kawaroe et al. 2015; Gomaa et al. 2015, 2017; Furbino et al. 2018; Fawzy 2020
Carrageenase	0.015–17.49							Furbino et al. 2018; Patyshakuliyeva et al. 2020
Xylosidase	0.01	0.0024						Thirunavukkarasu et al. 2015; Patyshakuliyeva et al. 2020
Xylanase	2.924–629.6	2.2–10×10 ³	2688 (U mg ⁻¹)		18–38	25–55	3.5–9	Raghukumar et al. 2004; Hou et al. 2006; Torres and dela Cruz 2013; Del-Cid et al. 2014; Thirunavukkarasu et al. 2015; dos Santos et al. 2016; Korkmaz et al. 2017; Wu et al. 2018; Patyshakuliyeva et al. 2020
Fucosidase	0.00185							Shvetsova et al. 2014
Fucoiidanase	0.006–4	0.25–0.3×10 ³	2.02–6.55 (mg min ⁻¹ mL ⁻¹)	0.036–0.139	64–180	40–60	6–7	Burtseva et al. 2010; Qianqian et al. 2011; Wu et al. 2011; Gomaa et al. 2015, 2019; Hifney et al. 2019; Patyshakuliyeva et al. 2020
Mannosidase		5.5×10 ³						Burtseva et al. 2010
Alginate lyase	24–860	67.24–85		0.227	95	35	6.5	Burtseva et al. 2010; Singh et al. 2011; Gomaa et al. 2015, 2019

Table 2 Summary of marine fungal species exhibiting polysaccharide-degrading abilities described from 2000, along with their environmental origin and the substrates used for their growth and enzymatic production. Only the best polysaccharide-degrading strains of each study are reported

Polysaccharide-degrading specificity	Fungal origins	Fungal identifications	Substrates for fungal growth	References
Cellulolytic	Mangrove debris (wood, leaves), macroalgae (<i>Agarum</i> sp., <i>Fucus</i> sp., <i>Sargassum</i> sp., <i>Ulva</i> sp.), marine sediments, seawater	<i>Arthrinium saccharicola</i> , <i>Aspergillus</i> sp., <i>A. niger</i> , <i>Aureobasidium pullulans</i> , <i>Candida</i> sp., <i>Chaetomium</i> sp., <i>Cladosporium sphaerospermum</i> , <i>Fusarium equiseti</i> , <i>Penicillium chrysogenum</i> , <i>P. madriti</i> , <i>Phomopsis</i> sp., <i>Scopulariopsis brevicaulis</i> , <i>Trichoderma paraviridescens</i>	Macroalgae (<i>Ulva lactuca</i> , <i>U. fasciata</i> , <i>Agarum clathratum</i>), purified substrates (cellulose, carboxymethyl cellulose), raw materials (cotton seeds, rice bran, wheat bran)	Elyas et al. 2010; Ravindran et al. 2010; Xue et al. 2012a; Torres and dela Cruz 2013, p. 2; Li et al. 2014; Trivedi 2015; Hong et al. 2015; Rong et al. 2015; Lee et al. 2015, 2019; Balabanova et al. 2018b; Patyshakuliyeva et al. 2020
Laminarinolytic	Macroalgae (<i>Fucus</i> sp.), marine sediments	<i>Beauveria felina</i> , <i>Chaetomium indicum</i> , <i>Penicillium brevicompactum</i>	Macroalgae (<i>Laminaria digitata</i>), laminarin, rice bran	Burtseva et al. 2003; Balabanova et al. 2018b; Patyshakuliyeva et al. 2020
Amylolytic	Marine sediments, ocean depths	<i>Aureobasidium pullulans</i> , <i>Penicillium solitum</i> , <i>Scopulariopsis brevicaulis</i>	Starch, rice bran	Li et al. 2007; Burtseva et al. 2010; Gonçalves et al. 2013; Balabanova et al. 2018b
Agarolytic	Macroalgae (<i>Palisada perforata</i> , <i>Georgiella confluens</i>), marine sediments, seawater	<i>Beauveria felina</i> , <i>Cladosporium</i> sp., <i>Curvularia lunata</i> , <i>Dendryphiella arenaria</i> , <i>Hypocreaceae</i> sp.	Macroalgae (<i>Gelidium latifolium</i> , <i>Palisada perforata</i>), agar, rice bran	Kawaroe et al. 2015; Goma et al. 2015, p. 20, 2017; Balabanova et al. 2018b; Furbino et al. 2018; Fawzy 2020
Carrageenanolytic	Macroalgae (<i>Iridaea cordata</i> , <i>Fucus</i> sp.), marine sediments	<i>Beauveria felina</i> , <i>Clonostachys rosea</i> , <i>Penicillium</i> sp.	Macroalgae (<i>Laminaria digitata</i>), carrageenan, rice bran	Balabanova et al. 2018b; Furbino et al. 2018; Patyshakuliyeva et al. 2020
Xylanolytic	Mangrove leaves and sediments, macroalgae (<i>Fucus</i> sp., <i>Sargassum wightii</i>), marine sponge (<i>Drummacidon reticulatum</i>), marine sediments	<i>Aspergillus tubingensis</i> , <i>A. ustus</i> , <i>Beauveria felina</i> , <i>Cladosporium</i> sp., <i>Penicillium chrysogenum</i> , <i>Phoma</i> sp., <i>Trichoderma harzianum</i> , <i>T. paraviridescens</i> , <i>T. pleuroticola</i>	Macroalgae (<i>Ulva lactuca</i>), purified xylan (from beechwood, birchwood, oat, rice), rice bran	Raghukumar et al. 2004; Hou et al. 2006; Torres and dela Cruz 2013, p. 201; Del-Cid et al. 2014; Thirunavukkarasu et al. 2015; dos Santos et al. 2016; Korkmaz et al. 2017; Balabanova et al. 2018b; Wu et al. 2018; Patyshakuliyeva et al. 2020
Fucoxidolytic	Macroalgae (<i>Fucus</i> sp., <i>Padina pavonica</i> , <i>Palisada perforata</i>), marine sediments	<i>Beauveria felina</i> , <i>Cladosporium salinae</i> , <i>Dendryphiella arenaria</i> , <i>Fusarium</i> sp., <i>Trichoderma paraviridescens</i>	Macroalgae (<i>Cystoseira trinodis</i> , <i>Laminaria digitata</i> , <i>Sargassum</i> sp.), fucoidan (from <i>Sargassum latifolium</i> , <i>Fucus vesiculosus</i>), rice bran	Qianqian et al. 2011; Wu et al. 2011; Goma et al. 2015, p. 20, 2019; Balabanova et al. 2018b; Hifney et al. 2019; Patyshakuliyeva et al. 2020
Mannanolytic	Marine sediments	<i>Beauveria felina</i>	Rice bran	Balabanova et al. 2018b
Alginolytic	Marine macroalgae (<i>Cystoseira myrica</i> , <i>Dicoryta dichotoma</i> , <i>Palisada perforata</i>), marine sediments	<i>Acrophilophora</i> sp., <i>Aspergillus oryzae</i> , <i>Beauveria felina</i> , <i>Dendryphiella arenaria</i> , <i>Setosphaeria rostrata</i>	Macroalgae (<i>Sargassum</i> sp.), sodium alginate, rice bran	Singh et al. 2011; Goma et al. 2015, 2019; Balabanova et al. 2018b

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Fig. 1 a. General mechanisms of glucan degradation with glucanases with a first step of degradation in glucosides followed by a second step of degradation of glucosides in glucose residues. Disks represent glucose, red stars represent link breaking by the corresponding enzyme. **b.** Structural composition of natural glucans targeted by marine fungal enzymes (adapted from Allen *et al.* (2001); Kadokawa (2012); Olsson and Westm (2013))



Studies dealing with the characterization of glucan degrading enzymes from marine fungi (endoglucanases, exoglucanases and glucosidases) describe strains isolated from diverse habitats such as water column, marine sediments, mangrove, sponges, algae... However, concerning algae, studies principally describe fungal communities of brown algae (Burtseva *et al.* 2003, 2006; Hong *et al.* 2015; Lee *et al.* 2015, 2019; Patyshakuliyeva *et al.* 2020). That can be explained by two phenomena. First, enzymes obtained from fungi isolated from brown algae are more studied than enzymes obtained from fungi isolated from other algal families. Furthermore, the glucanase family includes enzymes targeting the most common polysaccharides composing brown algae such as cellulose or laminarin.

All studies characterizing glucanase activities perform assays on these kinds of polysaccharides as substrates. These glucanase activities and properties will be discussed further according to their substrate, being cellulose, laminarin and starch.

However, concerning glucosidases, studies are mostly performed using p-nitrophenyl- β -D-glucopyranoside (pNPG) as substrate, which is not specific with any natural polysaccharide but has colorimetric properties allowing the

enzymatic assays. Moreover, marine fungal glucosidases are less studied than marine fungal glucanases.

Nevertheless, an important diversity of marine fungal strains exhibit β -glucosidase activities (Pointing *et al.* 1999; Hong *et al.* 2015; Lee *et al.* 2015; Patyshakuliyeva *et al.* 2020). Among marine fungal strains in which β -glucosidase activity has been studied, the genera *Arthrinium*, *Penicillium*, *Hypoxylon*, *Trichoderma*, *Aspergillus* and *Fusarium* seem particularly interesting because their β -glucosidase activity has been exhibited in several studies (Pointing *et al.* 1999; Burtseva *et al.* 2010; Hong *et al.* 2015; Lee *et al.* 2015, 2019; Patyshakuliyeva *et al.* 2020).

The optimal temperatures of most β -glucosidases obtained from marine fungi range from 50 to 60 °C (MacDonald and Speedie 1982; MacDonald *et al.* 1985; Ravindran *et al.* 2010; Karray *et al.* 2016). However, some β -glucosidase activities from marine fungi are optimal at 70 °C (Méndez-Líter *et al.* 2018) or at 35 °C (Elyas *et al.* 2010). Optimal pH ranges from 4 to 7 (Elyas *et al.* 2010; Ravindran *et al.* 2010; Karray *et al.* 2016; Méndez-Líter *et al.* 2018). Concerning salinity, a strain of *Aspergillus niger* isolated from seawater was characterized by an optimal NaCl concentration of 6% for its β -glucosidase activity (Xue *et al.* 2012b).

Cellulases

Cellulose is the most abundant natural polysaccharide and is widely present in terrestrial as well as marine plants. This polysaccharide is made of D-glucose linked in a chain by β -1,4 bonds (Fig. 1b). The interests in the characterization of new cellulose-degrading enzymes are multiple. Three main kinds of cellulase are found in fungi with different and complementary functions (Acharya *et al.* 2008). Endoglucanases hydrolyze the internal chain of the cellulose releasing multiple smaller chains, exoglucanases target the reducing and non-reducing ends of the cellulose chain resulting in cellobiose production and β -glucosidases release glucose residues from cellobiose. All of these enzymatic activities have been studied in marine fungi (Pointing *et al.* 1999; Ravindran *et al.* 2010; Hong *et al.* 2015; Lee *et al.* 2015, 2019; Patyshakuliyeva *et al.* 2020). However, according to the study and considering the methodology applied, the detailed enzymatic activity may not be more specific about and limited to “cellulase” activity, corresponding in fact to the cellulolytic complex or parts of it. Using cellulase for enzymatic hydrolysis could be of great interest in complex biological matrices due to the wide spectrum of efficient substrates that can be degraded.

Species belonging to fungal genera *Trichoderma* and *Aspergillus* are of particular interest considering their cellulase production, and other fungal genera such as *Penicillium* and *Humicola* are also known as cellulase producers (Kuhad *et al.* 2011; Gupta *et al.* 2015; Imran *et al.* 2016; Kunamneni 2016; Kavanagh 2017; Balabanova *et al.* 2018a; Shah *et al.* 2019; Singh *et al.* 2021). However, several other marine fungi produce only a low level of cellulase, or no cellulase at all, due to their specificity for a non-cellulose substrate (Balabanova *et al.* 2018a). When cultured on non-marine substrates, marine fungi have cellulolytic activities comparable with terrestrial fungal strains (Balabanova *et al.* 2018a).

Cellulase enzymes represent the third place in industry share (15%) after amylase (25%) and protease (18%) (Singh *et al.* 2021), and the industrial interest of these enzymes has greatly increased during the two past decades (Bhardwaj *et al.* 2021).

Cellulases have application in the food industry and particularly in the production of plant juices and oil (they can be used in collaboration with xylanase and pectinase for extraction and clarification) as well as in baking (increasing bread quality) (Kuhad *et al.* 2011; Imran *et al.* 2016; Kunamneni 2016; Kavanagh 2017; Shah *et al.* 2019; Siqueira *et al.* 2020; Srivastava *et al.* 2020; Bhardwaj *et al.* 2021; Ejaz *et al.* 2021; Singh *et al.* 2021). Cellulases have various other applications in industries such as laundry detergents (Vala *et al.* 2019; Barzkar and Sohail 2020), textiles (Trivedi *et al.* 2016; Vala *et al.* 2019; Barzkar and Sohail 2020; Singh *et al.* 2021),

leather (Trivedi *et al.* 2016) and pharmaceuticals (Singh *et al.* 2021). They are also used in the pulp and paper industry, particularly in the bleaching process as an alternative to traditional chlorine chemicals which exhibit disadvantages in terms of health and environment (Raghukumar 2008; Trivedi *et al.* 2016; Vala *et al.* 2019; Singh *et al.* 2021).

These cellulases also have applications directly linked with macroalgal uses. Indeed, these enzymes can be used in the degradation of seaweed wastes to produce plant bio-fertilizers in agriculture (Barzkar and Sohail 2020; Singh *et al.* 2021). They have also gained interest in the production of third-generation biofuels, allowing saccharification of macroalgal biomass (Vala *et al.* 2019; Barzkar and Sohail 2020). Marine fungi are particularly promising in biofuel production compared with marine bacteria or commercial enzymes (Trivedi *et al.* 2016; Vala *et al.* 2019). For example, species of *Aspergillus* have been particularly studied for the production of bioethanol from green seaweeds (Ghazal Mustafa *et al.* 2016; Karray *et al.* 2016; Girisha *et al.* 2017), but other genera are also studied for this bioethanol production, like *Trichoderma* or *Cladosporium* (Trivedi 2015; Girisha *et al.* 2017).

Advantages of marine microbial enzymes compared with terrestrial ones have already been mentioned. Marine microbial cellulase presents a particularly interesting stability at high or low temperatures, high pressure and salinity, and acid or alkaline pH (Barzkar and Sohail 2020).

Most of the studies of cellulolytic activities of marine fungal strains concern carboxymethyl cellulase (CMCase) (Meyers and Reynolds 1959; Torres and dela Cruz 2013; Li *et al.* 2014; Rong *et al.* 2015; Trivedi 2015; Karray *et al.* 2016). Carboxymethyl cellulose (CMC) is a modified cellulose produced with alkali cellulose and monochloroacetic acid (Hollabaugh *et al.* 1945) and is notably more soluble than classic kinds of cellulose and is easier to use as a carbon substrate. As CMC is not found in nature and fungal cellulases allow the degradation of CMC, CMCase activity is usually measured for the screening of cellulase activity. Indeed, some studies mention a “cellulase activity” when using CMC as enzyme substrate (Meyers and Reynolds 1959; Torres and dela Cruz 2013; Li *et al.* 2014; Trivedi 2015). Cellulolytic activities obtained using CMC as substrate will then be considered as cellulase in the present paragraph. The use of such pure cellulose substrates generally allows better cellulase production than by using other raw substrates (e.g., rice straw, wheat straw or sugar-cane bagasse), but pure substrates can also be characterized by a low solubility and structural complexity, and they are more expensive (Bhardwaj *et al.* 2021). Some studies then aim to determine the preferred raw substrate to obtain the highest enzymatic activity. For example, the use of cotton seeds in culture allowed the highest exoglucanase, endoglucanase and β -glucosidase activities to be obtained by solid fermentation of a strain of *Chaetomium* sp. isolated from mangrove wood

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(Ravindran *et al.* 2010).

As cellulases are glucanases allowing the degradation of cellulose, these cellulases can exhibit more specific endo-, exoglucanase or glucosidase activities. However, several cellulases can exhibit both endo- and exoglucanase activities (Gupta *et al.* 2012). Of the marine fungal cellulolytic enzymes, endoglucanases appear to be particularly well documented and represented compared with exoglucanases or glucosidases (Luo *et al.* 2005).

Marine fungal cellulases can be obtained from strains isolated from various habitats such as algae (Hong *et al.* 2015; Lee *et al.* 2015; Trivedi 2015; Patyshakuliyeva *et al.* 2020), sponges (Baker *et al.* 2009), mangrove habitats (Pointing *et al.* 1999; Ravindran *et al.* 2010; Torres and dela Cruz 2013), sediments (Balabanova *et al.* 2018b) and seawater (Li *et al.* 2014; Rong *et al.* 2015).

Optimal temperature and pH for cellulase activity can depend on the nature of considered cellulase (endoglucanase, exoglucanase or β -glucosidase) and the marine fungal species (Pointing *et al.* 1999). Generally, cellulase activities seem to be highest with a temperature ranging from 50 to 60 °C (MacDonald and Speedie 1982; MacDonald *et al.* 1985; Pointing *et al.* 1999; Ravindran *et al.* 2010; Santos *et al.* 2020) with several exceptions of optimal temperatures at 40 °C, notably for CMCase activities (Rong *et al.* 2015; Trivedi 2015). Optimal pH values for marine fungal cellulase generally are about 5–6, with several optimal pH values at 4 or even 9 (Meyers and Reynolds 1959; Pointing *et al.* 1999; Ravindran *et al.* 2010; Peng *et al.* 2011; Rong *et al.* 2015; Trivedi 2015; Santos *et al.* 2020). Rong *et al.* (2015) have characterized the molecular size of a marine fungal CMCase obtained from the *Aureobasidium pullulans* 98 strain, isolated from seawater in China, with a result of 67 kDa. A marine fungal strain of *Aspergillus terreus* has been described as producing a smaller cellulolytic enzyme (endoglucanase), at 56 kDa (Jmel *et al.* 2020).

The evolution of cellulase activity according to the concentration of NaCl was observed for a strain of *Ulocladium chartarum* isolated from salt marshes in Egypt. The cellulase activity slightly increases from 10 to 30 g L⁻¹ NaCl in the culture medium (0.215 and 0.259 U mL⁻¹ respectively) then decreases with 40 g L⁻¹ NaCl (0.124 U mL⁻¹) (Sallam *et al.* 1988). Some other fungi have an increasing cellulolytic capacity with a salinity increasing (*Savoryella lignicola*, *Stagonospora* sp., *Trematosphaeria striatispora*), while the opposite is observed for others (*Helicascus kanaloanus*, *Lignincola laevis*) (Pointing *et al.* 1998).

As mentioned, strains of *Aspergillus* seem to be particularly well represented in studies dealing with marine fungal cellulases (Ravindran *et al.* 2010; Xue *et al.* 2012a; Karray *et al.* 2016; Girisha *et al.* 2017; Santos *et al.* 2020). Moreover, *Aspergillus* strains exhibited higher cellulase

specific activity (18.6 U mg⁻¹) than species of *Penicillium* or *Rhizopus* (Ghazal Mustafa *et al.* 2016). Recently, an endoglucanase obtained from an *Aspergillus terreus* strain isolated from the green alga *Ulva* sp. was potentially interesting by its high specific activity (30.1 U mg⁻¹) and its high thermostability (Jmel *et al.* 2020). The use of this enzyme, compared with other commercial endoglucanases, is characterized by a higher degradation capacity of the alga *Ulva* sp. because this endoglucanase targets specifically the complex structure of the alga (Jmel *et al.* 2020). Even if *Aspergillus* sp. strains exhibit CMCase activities, it does not mean that these activities are always particularly high. As an example, fungi isolated from mangrove leaves in the Philippines showed that the highest CMCase activity was reached by a *Phomopsis* sp. strain (21.4 U mL⁻¹), while that of an *Aspergillus* sp. strain was 7.8 U mL⁻¹. *Colletotrichum* sp., *Penicillium* sp. and *Paecilomyces* sp. strain activities were also higher than that of *Aspergillus* sp. (Torres and dela Cruz 2013). *Aspergillus* is a genus widespread in the terrestrial environment, where cellulose is particularly present. It is difficult to know if terrestrial fungal strains have inherited and developed their cellulose-degrading capacity from the marine fungal strains having colonized the land, or if this ability has appeared in terrestrial fungal strains which would then colonize marine habitats. Interestingly, cellulase activities exhibited by *Penicillium* strains were almost as high as *Aspergillus* ones, *Penicillium* also being ubiquitous and widespread in terrestrial environments (Ghazal Mustafa *et al.* 2016). In a screening study of marine fungal strains associated with the brown alga *Agarum clathratum*, it has been shown that among the 233 isolated strains, belonging to 89 species, the highest cellulase activity was observed from *Penicillium madriti* with values higher than 0.5 U mL⁻¹ (Lee *et al.* 2019). Fungi belonging to *Penicillium* genus are generally characterized by a good halotolerance, which is highlighted by Lee *et al.* (2015) where a strain of *Penicillium chrysogenum* was characterized by an increase of cellulase activity of 126% at 0.5 mM NaCl compared with no salt medium. However, the most active species was *Trichoderma hamatum* exhibiting a cellulase activity of 0.57 U mL⁻¹ (Lee *et al.* 2015). In a recent study, a strain of *Trichoderma para-viridescens* exhibited a cellulase activity of 0.15 U mL⁻¹, being 2 to 10 times higher than those of other strains belonging to *Cladosporium*, *Epicoccum*, *Clonostachys*, *Penicillium* and *Rhizopus* genera (Patyshakuliyeva *et al.* 2020). Some other marine fungal species exhibit interesting cellulolytic activities, including *Hypoxylon oceanicum* (Pointing *et al.* 1999) and *Arthrinium* sp. (Hong *et al.* 2015). In addition to filamentous fungi, yeasts are also reported for cellulolytic activity such as strain of *Aureobasidium* (Torres and dela Cruz 2013; Rong *et al.* 2015; Karray *et al.* 2016). Moreover, some strains of *Candida* isolated from seawater in China exhibited higher CMCase activity (1.311 U mL⁻¹) than the filamentous *Ascomycota* sp. and *Cladosporium* sp. (respectively 1.133 U mL⁻¹ and 0.843

U mL⁻¹) (Li *et al.* 2014).

Several factors have been found to be responsible of variation of cellulolytic activities, in addition with the nature of the substrate previously discussed (Table 2). The optimal incubation period has an impact on cellulase activity of *Scopulariopsis brevicaulis*, which was more than two times higher after 7 days than 4 days of incubation, unlike *Beauveria felina* which exhibited low and no cellulase activity after 4 and 7 days, respectively (Balabanova *et al.* 2018b). Two main kinds of industrial fungal fermentations exist, being based on liquid media (submerged fermentation) or solid media (solid-state fermentation). Fungal enzyme production seems higher with fermentation performed on solid media (Vinięgra-González *et al.* 2003; Hölker *et al.* 2004; Dutta *et al.* 2008; Ravindran *et al.* 2010; Rodríguez-Jasso *et al.* 2013). The activity of cellulolytic enzymes was also influenced by the amount of cellulose available and by the presence or absence of other enzymes such as the β -glucosidase which induced endoglucanase synthesis. Indeed, it has been observed that specific inhibition of β -glucosidase in the *Trichoderma reesei* QM9414 resulted in an inhibition of its endoglucanase activity (Kubicek 1987). Besides the enzymatic activity, growth rate also greatly influences the biotechnological potential of marine fungal strains, which can be limited considering strains with slow growth (Baker *et al.* 2009). Moreover, in order to enhance the activity of fungal enzymes, mixed cultures are being currently developed (Singh *et al.* 2021). Indeed, fungi not only excrete cellulase for the degradation of lignocellulosic compounds but a large set of enzymes with multiple polysaccharide targets. Different kinds of cellulase are usually used synergistically because of the lower efficiency obtained with a unique cellulase (Imran *et al.* 2016). Mixed cultures reduce the chance of contamination of the culture medium and can enhance enzyme production and efficiency (Bhardwaj *et al.* 2021; Singh *et al.* 2021).

Laminarinases

Some 1,3- β -glucanases can specifically target laminarin which is a polysaccharide composed of a linear chain of glucose linked in majority with β -1,3 bonds (Fig. 1b). The main sources of laminarinases are bacteria, fungi and algae (Huang *et al.* 2021). The principal biotechnological function of laminarinase is the production of laminarin-derived oligosaccharides which are more soluble in water than native polysaccharides and can exhibit several bioactivities of interest such as antioxidant, prebiotic, immunoregulatory, anti-inflammatory, radioprotective and antitumor activities (Trincone 2018; Usoltseva *et al.* 2020; Jagtap and Manohar 2021, p. 2021; Huang *et al.* 2021). These laminarinases also have applications in food industries

(in brewing or wine making) (Usoltseva *et al.* 2020), in agriculture (as plant biocontrol agents) (Trincone 2018; Usoltseva *et al.* 2020) or in cosmetics (Huang *et al.* 2021). They are also used in the conversion of algal biomass into fermentable sugars for the production of bioethanol, but only a few studies have been performed on the subject since 2018 (Perez *et al.* 2018; Trincone 2018; Usoltseva *et al.* 2020; Huang *et al.* 2021; Rocher *et al.* 2021). Indeed, practical use of these laminarinases is just beginning (Usoltseva *et al.* 2020).

A diversity of fungal strains degrade laminarin, including several particularly promising ones such as strains of *Dendryphiella salina*, whose capacity of degrading laminarin has been described in several studies with enzymatic activities up to 0.032 U mL⁻¹ (Schatz 1984; Grant and Rhodes 1992). *Cercospora salina* has been reported to exhibit a specific activity of 34 U mg⁻¹ (Chesters and Bull 1963). A strain of *Penicillium brevicompactum* showed an activity of 0.22 U mL⁻¹ (Patyshakuliyeva *et al.* 2020). While optimal pH for the laminarinase activity of *D. salina* was 4.6 (Grant and Rhodes 1992), this enzymatic activity from *Chaetomium indicum* and *Trichoderma aureviride* was stable for pH in the range 4.5–7.5 (Burtseva *et al.* 2006). This suggests that marine fungal laminarinases tend to be preferentially active in acidic pH values. The optimal temperature for the laminarinase activity of *Chaetomium indicum* and *T. aureviride* has been recorded at 45 and 40 °C, respectively (Burtseva *et al.* 2006). An exoglucanase activity observed in a marine strain of *C. indicum* has been associated with an enzyme of 54 kDa (Burtseva *et al.* 2003). This exoglucanase shows a higher substrate affinity for laminarin than for other tested substrates (i.e. agar, laminarin oligosaccharides, glucose, etc.).

The incubation period for fungal growth before the measurement of the enzymatic activity seems to impact laminarinase activity. For example, *Beauveria felina* laminarinase specific activity was two times higher at 7 days of incubation (0.0100 U mg⁻¹) than at 4 days (0.5 × 10⁻³ U mg⁻¹) (Balabanova *et al.* 2018b). However, enzymatic specific activity increasing with the fungal incubation time is not observed for all enzymes. The opposite tendency characterized the fucoidanase and fucosidase activities. On the other hand, substrate nature does not seem to always influence the enzymatic activity of fungal strains. Enzymatic activities, including laminarinases, obtained from strains cultivated on *Laminaria* or *Ulva* matrices did not show significant differences (Patyshakuliyeva *et al.* 2020). This study demonstrated that most fungal strains grow preferentially on an algal matrix-based medium than a glucose-based medium. Growing on an algal medium could be of interest when specific algal degrading enzymes are targeted further.

Amylases

Starch has two principal components, namely amylose (a linear polysaccharide made of α -1,4 linked glucose units (Fig. 1b) and amylopectin (a polysaccharide made of an α -1,4 linked glucose backbone with α -1,6 linked glucose branching). Starch is not particularly represented in marine algae. Some green algae have been studied for starch and belong to the genus *Ulva*. Furthermore, one class of red seaweed (Bangioophyceae) is characterized by starch granules (Love *et al.* 1963; Percival 1979; Yu *et al.* 2002). Even if some data concerning amylases obtained from marine fungi are presented below, none of these fungi are indicated as isolated from an alga. Strains of interest have been isolated from sponges (Mohapatra *et al.* 1998), marine sediments (Gonçalves *et al.* 2013; Balabanova *et al.* 2018b), deep-sea (Li *et al.* 2007) or are taken from marine microorganism collections (Burtseva *et al.* 2010; Wang *et al.* 2016). However, amylases obtained from marine environments could have interests in the treatment of terrestrial starch which is the main source of starch (Prabhu *et al.* 2019), because of their particular characteristics.

The starch saccharification process performed by amylases is used in several industries such as textile (notably during the desizing process) (Homaei *et al.* 2016a; Trincone 2018; Vashist *et al.* 2019; Ahmed *et al.* 2020), leather (Trincone 2018), food (notably in bakery) (Homaei *et al.* 2016a; Suriya *et al.* 2016; Barone *et al.* 2019; Vashist *et al.* 2019; Ahmed *et al.* 2020), feed (by enhancing the protein concentration) (Vashist *et al.* 2019; Ahmed *et al.* 2020), detergent (Suriya *et al.* 2016; Trincone 2018; Ahmed *et al.* 2020), fermented beverages and distilleries (Homaei *et al.* 2016a; Vashist *et al.* 2019; Ahmed *et al.* 2020), paper industry (Homaei *et al.* 2016a; Suriya *et al.* 2016; Vashist *et al.* 2019), pharmaceuticals (Trincone 2018, p. 20; Barone *et al.* 2019; Ahmed *et al.* 2020) and chemicals (Trincone 2018; Barone *et al.* 2019; Ahmed *et al.* 2020). Amylases are also used in waste treatment (Trincone 2018; Vashist *et al.* 2019), in biofuel production from starch biomass (Homaei *et al.* 2016a; Suriya *et al.* 2016; Barone *et al.* 2019) and in the processing of glucose and fructose syrups (Homaei *et al.* 2016a; Suriya *et al.* 2016). The use of amylase in industries using traditional chemicals can replace these, which has health and environmental benefits (Barone *et al.* 2019).

Fungal sources of amylases mainly belong to the genera *Aspergillus*, *Penicillium* or *Rhizopus* (Kango *et al.* 2019).

The optimal temperature for the activity of amylases obtained from marine fungi seems to be similar to those for other glucanase enzymes (60 °C) (Mohapatra *et al.* 1998; Li *et al.* 2007). Indeed, even if all glucan degrading enzymes can degrade different specific polysaccharides, it can be expected that they all have similarities in their global mechanism, including the temperature of activity. However,

an amylase obtained from a strain of *Penicillium solitum* isolated from marine sediments of Antarctica exhibited its highest activity according to the enzymatic zone of clearance on solid culture medium at a temperature of 30 °C. However, without a higher tested temperature, it is difficult to define any optimal temperature (Gonçalves *et al.* 2013). Nevertheless, the cold active ability of this *P. solitum* amylase could have interests in cold processes in the pharmaceutical, cosmetic and chemical industries (Gonçalves *et al.* 2013).

It seems that optimal pH for the activity of amylases is acidic, with pH values ranging from 4.5 to 5 (Mohapatra *et al.* 1998; Li *et al.* 2007). This tendency of acid functioning enzymes obtained from marine fungi seems globally shared between all kinds of enzymes.

Of the 15 strains of marine fungi obtained from a microorganism collection (Marine microorganisms of the Pacific Institute of Bioorganic Chemistry) showing an amylase activity, the two most active strains belonged to the species *Penicillium chrysogenum* (Burtseva *et al.* 2010). Moreover, the majority of strains exhibiting amylase activity belonged to *Penicillium*. The amylase ability of this genus is also highlighted by Gonçalves *et al.* (2013) who showed a promising amylase activity from a strain of *Penicillium solitum*. Interestingly, the *Penicillium* genus is also widespread in terrestrial plants, in which starch is commonly present (Ellis *et al.* 1998; Belyuchenko 2016).

Amylase activity was obtained from two strains of *Scopulariopsis brevicaulis* and *Beauveria felina* isolated from marine sediments. The highest specific activity (0.5×10^{-3} U mg^{-1}) was observed after 7 days of incubation in *S. brevicaulis*, with a value more than twice higher than the one obtained after 4 days of incubation (0.2×10^{-3} U mg^{-1}) (Balabanova *et al.* 2018b). Other marine fungal strains of *Aureobasidium pullulans*, *Mucor* sp. and *Pestalotiopsis* sp. have shown promising amylase activities (Mohapatra *et al.* 1998; Li *et al.* 2007; Wang *et al.* 2016).

An amylase specific activity of 120×10^3 U mg^{-1} has been obtained from a strain of *Penicillium chrysogenum* (Burtseva *et al.* 2010). The addition of laminarin in the fungal culture medium seems to increase amylase activities (as well as most other enzyme activities) in a strain of *Penicillium canescens* (Burtseva *et al.* 2010).

Few studies dealing with marine amylases are available compared with terrestrial ones and marine amylases are poorly characterized. However, it has been shown that an amylase obtained from the marine yeast *Aureobasidium pullulans* was characterized by a molecular weight of 98 kDa and composed of two subunits of 65 and 33 kDa (Li *et al.* 2007). It seems that marine amylases are more tolerant to salinity than terrestrial ones (Mohapatra *et al.* 1998). In this study, the amylase reached its optimal activity with 2% NaCl, and a salinity increase to 9% halves this activity.

The high salinity would be part of extreme conditions for which marine amylases and more generally marine enzymes may present an important interest notably in starch saccharification.

Several marine fungal strains belonging to *Calcarisoprium*, *Tritirachium*, *Bartalinia*, *Penicillium*, *Scopulariopsis* and *Pestalotiopsis* grew on starch similar to their growth on glucose, showing the efficiency of amylases produced. A strain of *Pestalotiopsis* sp. exhibited the best affinity with starch (Wang *et al.* 2016).

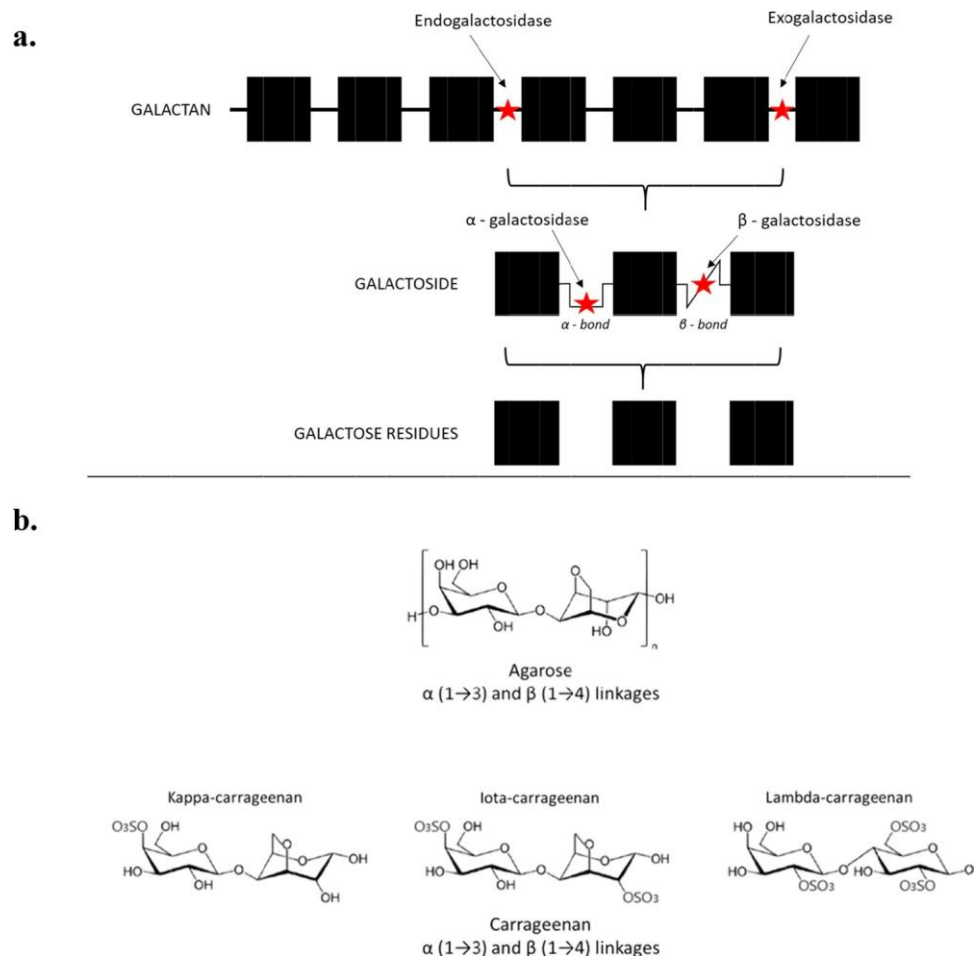
The production of amylases by marine filamentous fungi seems to be less important than by those obtained by marine bacteria or marine yeasts. Indeed, considering 161 marine strains isolated from Antarctica (sediments, plants, algae, water, animal faeces, ice, etc.) tested at 20 °C (151 bacteria, 31 yeasts and 10 filamentous fungi), any filamentous fungi exhibited amylase activity contrarily to bacteria and yeasts, among which 51% and 3%, respectively, exhibited amylase activity (Loperena *et al.* 2012).

Galactan degradation

Galactans are polymers of galactose, as glucans correspond to glucose polymers. However, galactans are less common than glucans in nature; therefore, they are less studied and data dealing with galactan degrading enzymes in the literature are fewer than those for glucan degrading ones. Nevertheless, several algal galactan molecules are targeted by marine fungal enzymes, namely agarose and carrageenan. Their structures and the general mode of action of galactanases are presented in Fig. 2.

As for cellulose and more widely glucan degradation, galactan degradation occurs by the action of three types of enzymes: endogalactanases, exogalactanases and galactosidases. The first two target respectively the side chain and the end of the galactan molecule (Fig. 2a). The name of galactanase is defined according to the nature of the targeted galactan, i.e. agarases and carrageenases which will respectively cleave agar and carrageenan. The third, the galactosidases, are responsible for the degradation of galactosides into galactose units (Fig. 2a) (Yoshimi *et al.* 2017).

Fig. 2 a. General mechanisms of galactan degradation with galactanases with a first step of degradation in galactosides followed by a second step of degradation of galactosides in galactose residues. Squares represent galactose, red stars represent link breaking by the corresponding enzyme. **b.** Structural composition of natural galactans targeted by marine fungal enzymes (adapted from Henares *et al.* (2010); Ven and Rani (2012))



Galactosidases

The α -galactosidase, β -galactosidase and α -AcN-galactosaminidase specific activities found in *Beauveria felina* ranged from 1.2×10^{-3} to 2.4×10^{-3} U mg⁻¹. Interestingly, the α -galactosidase activity was twice as high after 4-day incubation than after 7-day incubation, showing that some enzymatic activities seem to decrease after a short period of incubation (Balabanova *et al.* 2018b). *Scopulariopsis brevicaulis* also exhibited β -galactosidase and α -AcN-galactosaminidase specific activities, respectively 2.5×10^{-3} and 1.7×10^{-3} U mg⁻¹ (Balabanova *et al.* 2018b).

In a study screening some enzymatic activities from marine fungal strains belonging to the three genera *Aspergillus*, *Geomyces* and *Penicillium*, only *Penicillium* strains showed β -galactosidase activity (although not all *Penicillium* strains exhibited enzymatic activity). A strain of *Penicillium implicatum* exhibited a noticeable β -galactosidase specific activity (42×10^3 U mg⁻¹), at least twice higher than the β -galactosidase activity of the other tested strains of *Penicillium* (Burtseva *et al.* 2010).

Agarases

Agar is composed of two structures, namely agarose (a polysaccharide constituted by a repetition of a disaccharide made of D-galactose and 3,6-anhydro-L-galactopyranose (Fig. 2b) and agaropectin (a mixture of smaller sulfated molecules), and is widely used in many industries (agronomy, cosmetics, pharmacology, microbiology...) because of its gelling and thickening properties (Armisen *et al.* 1991; Armisen and Gaiatas 2009). As for carrageenan, agar is produced by red seaweeds (Armisen *et al.* 1991; Armisen and Gaiatas 2009). Industrial processes entailing the use of agarases can be performed by native or recombinant enzymes. Agar-oligosaccharides and neoagar-oligosaccharides are then obtained after agarose hydrolysis by native and recombinant agarases respectively (Jahromi and Barzkar 2018; Trincone 2018). These oligosaccharides can exhibit several bioactivities such as prebiotic, anti-obesity, anti-diabetic, immunomodulatory, skin whitening, skin moisturizing, anti-inflammatory, antioxidant, antibacterial and antitumor activities (Jahromi and Barzkar 2018; Vashist *et al.* 2019; Park *et al.* 2020; Jagtap and Manohar 2021). These bioactivities have particular interests in industries such as pharmaceutical, cosmetic, medical or food (Muffler *et al.* 2015; Beygmoradi and Homaei 2017; Jahromi and Barzkar 2018; Birolli *et al.* 2019; Vashist *et al.* 2019; Park *et al.* 2020). These agarases are also used in molecular biology for the recovering of DNA fragments in agarose gels (Muffler *et al.* 2015; Beygmoradi and Homaei 2017; Jahromi and Barzkar 2018; Trincone 2018) and are promising in the production of biofuel (Martin *et al.* 2014; Park *et al.* 2020).

Agarases have direct applications on seaweed, being used for the preparation of algal protoplasts for genetic engineering (Jahromi and Barzkar 2018; Trincone 2018), for the control of red algal blooms (Beygmoradi and Homaei 2017), for the prevention of biofouling (Beygmoradi and Homaei 2017) or for the recovery of algal compounds of interest such as unsaturated fatty acids, pigments or vitamins by the hydrolysis of the algal cell wall (Jahromi and Barzkar 2018). Marine agarases generally exhibit thermostability as well as halostability which can be worthwhile in several industries (Jahromi and Barzkar 2018). There is a great gap between knowledge in marine bacterial enzymes and marine fungal enzymes, with few data on the latter, which highlights great opportunities in these research fields (Gomaa *et al.* 2017; Birolli *et al.* 2019).

As for carrageenan, galactan units of agar polymers are linked by β and α alternated bonds (Fig. 2b), which explain the differentiation between α -agarases and β -agarases (Flament *et al.* 2007; Gomaa *et al.* 2017). Marine fungal strains of the genera *Beauveria*, *Scopulariopsis* and *Cladosporium* have been described as degrading agar (Gomaa *et al.* 2015; Balabanova *et al.* 2018b; Furbino *et al.* 2018). Moreover, the majority of agarolytic marine fungal strains isolated from algae are associated with red seaweeds, although some also have been isolated from brown seaweeds (Gomaa *et al.* 2015, 2017; Furbino *et al.* 2018; Fawzy 2020).

The optimal temperature of agarases obtained from marine fungi ranges from 40 to 50 °C (Kawaroe *et al.* 2015; Gomaa *et al.* 2017; Fawzy 2020). The optimal pH, contrarily to glucanases, seem to be more alkaline for agarases, ranging from 7 to 8 (Kawaroe *et al.* 2015; Fawzy 2020).

According to Balabanova *et al.* (2018b), isolates *Beauveria felina* and *Scopulariopsis brevicaulis* exhibited agarase specific activities of 0.4×10^{-3} U mg⁻¹ and 0.04×10^{-3} U mg⁻¹, respectively. These activity values seem particularly low compared with other enzymes screened in this study for these fungal strains. Among nine marine fungi isolated from macroalgae, a strain of *Cladosporium* sp. isolated from the red seaweed *Georgiella confluens* exhibited the highest agarase activity (6.55 U mL⁻¹) (Furbino *et al.* 2018). The first described fungal agarase came from a strain of Hypocreaceae, isolated from seawater and using the red seaweed *Gelidium latifolium* as a substrate for the fungal incubation (Kawaroe *et al.* 2015). Two bands of proteins were obtained by SDS-PAGE, at 39 and 44 kDa. To the best of our knowledge, it is the first reference describing the molecular weight of a marine fungal agarase, but it seems that this agarase molecular weight is close to those found for marine bacteria (Kawaroe *et al.* 2015).

Gomaa *et al.* (2015) obtained agarase activities from marine fungi isolated from several seaweeds, principally red seaweeds. The fungal isolates were incubated using *Palisada perforata* as carbon substrate, and interestingly, the highest agarase activities obtained corresponded to marine fungi isolated from

P. perforata. The highest agarase specific activity was reached by a *Curvularia lunata* strain (isolated from *P. perforata*) at 20 U mg⁻¹. Other marine strains of *Aspergillus terreus*, *Setosphaeria rostrate* and *Dendryphiella arenaria* also showed promising agarase activities. According to Gomaa *et al.* (2015), the studied algicolous fungi exhibited higher agarase activities than other non-algicolous fungi, highlighting the adaptation of these closely related fungi to their algal substrate. Moreover, the agarase and amylase activities of marine fungi associated with *P. perforata* seem to have a synergetic influence, allowing a more efficient degradation of the alga.

Carrageenases

Carrageenan is a polymer of galactose residues alternatively linked with α -1,3 and β -1,4 bonds (Fig. 2b). Three main types of carrageenans are found according to their structural organization and sulfation degree and correspond to kappa-, iota- and lambda-carrageenan (De Ruiter and Rudolph 1997) (Fig. 2b). Carrageenans are of particular interests in some industries (agronomic, cosmetics, pharmaceuticals...) due to their numerous properties like gelling and thickening (Necas and Bartosikova 2013; Campbell and Hotchkiss 2017; Furbino *et al.* 2018). These carrageenans are only produced by red seaweeds (De Ruiter and Rudolph 1997; Rocha de Souza *et al.* 2007), but some marine fungi isolated from brown seaweeds show carrageenase activity (Furbino *et al.* 2018; Patyshakuliyeva *et al.* 2020). This observation highlights the fact that marine fungal species can be characterized by enzymatic strategies allowing them to develop on different substrates.

One of the main industrial use of carrageenase is the synthesis of oligosaccharides from carrageenan polysaccharides (Ghanbarzadeh *et al.* 2018; Zhu *et al.* 2018a). Indeed, carrageenan exhibits interesting bioactivities, but its therapeutic use is limited by the low tissue-penetrating ability and bioavailability due to high molecular weight and viscosity. Oligosaccharides have similar bioactivities and exhibit better bioavailability and solubility (Ghanbarzadeh *et al.* 2018). These oligosaccharides have antitumor, antiviral, anticoagulant, immunoregulatory and antioxidant activities (Chauhan and Saxena 2016; Beygmoradi and Homaei 2017; Ghanbarzadeh *et al.* 2018; Trincone 2018; Zhu *et al.* 2018a; Vashist *et al.* 2019; Jagtap and Manohar 2021). Carrageenases are used in several industries such as the food, detergent, textile, pharmaceuticals and chemicals industries (Chauhan and Saxena 2016; Beygmoradi and Homaei 2017; Ghanbarzadeh *et al.* 2018; Zhu *et al.* 2018a; Birolli *et al.* 2019) and can also be used as plant defence stimulators in agriculture (Ghanbarzadeh *et al.* 2018), and seem promising for bioethanol

production (Chauhan and Saxena 2016; Ghanbarzadeh *et al.* 2018; Zhu *et al.* 2018a).

Carrageenases also have application in marine biotechnology and environment. Indeed, they are used in seafood processing (Beygmoradi and Homaei 2017), for the isolation and preparation of algal protoplasts (Chauhan and Saxena 2016; Ghanbarzadeh *et al.* 2018; Zhu *et al.* 2018a), in the treatment of seaweed wastes (used as industrial raw material and as depolluting agents) (Chauhan and Saxena 2016; Ghanbarzadeh *et al.* 2018; Zhu *et al.* 2018a), in the prevention of red algal blooms (Chauhan and Saxena 2016) and in the recovery of compounds of interest such as proteins from macroalgae (Chauhan and Saxena 2016).

Some marine fungal genera are regularly studied for their carrageenase activities, such as *Beauveria*, *Penicillium* or *Cladosporium* (Balabanova *et al.* 2018b; Furbino *et al.* 2018; Patyshakuliyeva *et al.* 2020). Carrageenase activity has been measured in both *Beauveria felina* and *Scopulariopsis brevicaulis* (Balabanova *et al.* 2018b). The tendency of *B. felina* to reach its maximum carrageenase enzymatic activity level early (4 days of incubation) has been observed, as for the α -galactosidase activity. The maximum carrageenase activity of *S. brevicaulis* is reached after 7 days of incubation. However, with values of 0.02×10^{-3} and 0.13×10^{-3} U mg⁻¹ obtained after an incubation of 7 days respectively for *B. felina* and *S. brevicaulis*, carrageenase specific activity is particularly low compared with other screened enzymatic activities (Balabanova *et al.* 2018b).

Of the four algae from which fungi have been isolated and screened for their carrageenase activity, three belonged to the Rhodophyceae (*Georgiella confluens*, *Iridaea cordata*, *Palmaria decipiens*) and one to the Phaeophyceae (*Ascoseira mirabilis*) (Furbino *et al.* 2018). This observation suggested that carrageenase activity is mostly found in Rhodophyceae-associated fungi, which are characterized by presence of carrageenan unlike Phaeophyceae and Chlorophyceae. Considering the marine fungal strains exhibiting carrageenase activity, the genus *Penicillium* was the most represented. Furthermore, a *Penicillium* sp. strain isolated from the red alga *I. cordata* exhibited the highest carrageenase activity (17.5 U mL⁻¹), at least twice higher than the other strains (Furbino *et al.* 2018). In another study, among fungi isolated from the brown alga *Fucus* sp., the strain characterized by the highest carrageenase activity (0.015 U mL⁻¹) was a strain of *Clonostachys rosea* (Patyshakuliyeva *et al.* 2020). The other carrageenase activities of the study ranged from 0.0045 to 0.0115 U mL⁻¹. It is important to highlight the fact that there are many variables (i.e. fungal species, fungal isolation, conservation, incubation, enzymatic extraction protocol, activity measurement...) which could explain these different carrageenase activities between both studies. Nevertheless, it may be interesting to perform studies to determine if marine fungi isolated from red seaweeds are

more interesting with respect to carrageenase activity than marine fungi isolated from brown seaweeds.

Other hydrolases

Even if many polysaccharides targeted by marine fungal polysaccharidases belong either to glucans or galactans, several other polysaccharides can be also hydrolyzed, such as xylan, fucan and mannan. Their structures and the global hydrolyze mechanism which allows the degradation of these polysaccharides are presented Fig. 3.

Xylan degrading enzymes

Xylan is a polymer of xylose residues linked by β -1,4 osi- dic bonds, putatively branched with α -arabinofuranose or α -glucuronic acids residues (Fig. 3b). Xylan is particularly represented in red seaweeds and is widely distributed in terrestrial plants, in both dicotyledons (such as beechwood) and

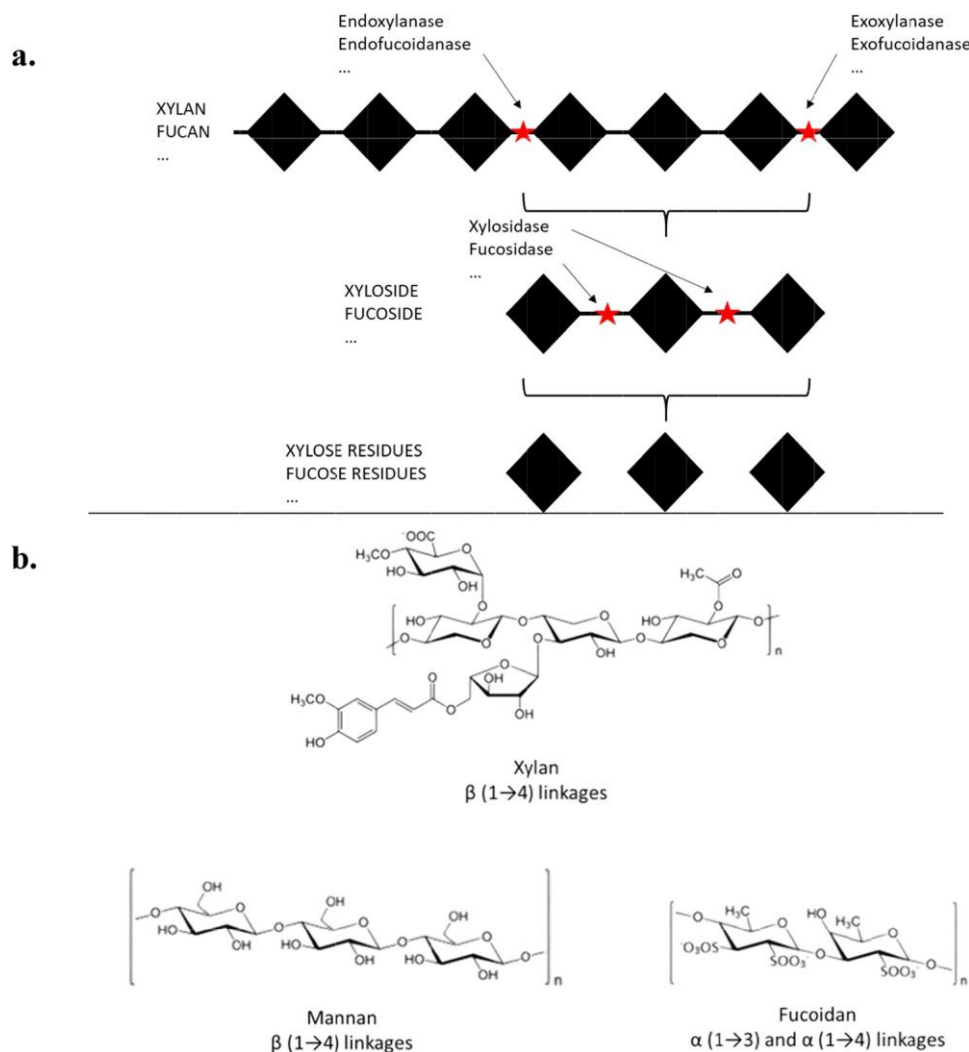
monocotyledons (such as corn) (Synytsya *et al.* 2015; Jensenet *al.* 2018; Hsieh and Harris 2019).

Xylan degrading enzymes can be separated between xylanases and xylosidases. Xylanases (endo- and exoxylanases) target the xylan chains to produce xylosides, oligosaccharides of xylan, while xylosidases degrade these xylosides to produce xylose (Fig. 3a).

Xylan is a principal component of the polysaccharide cell wall of some plants and algae. The main sources of xylanases for industrial uses are fungi and bacteria (Burlacu *et al.* 2016). Among fungi, several genera are particularly promising for xylanase production, such as *Aspergillus*, *Trichoderma*, *Penicillium* and *Fusarium* (Motta *et al.* 2013; Burlacu *et al.* 2016; Bajaj and Mahajan 2019; Singh *et al.* 2019). Fungal xylanase activities are generally higher than bacterial ones (Burlacu *et al.* 2016).

Xylanases, including marine ones, can be used in numerous industries and applications, such as food (notably for extraction and clarification of fruit juices, wine, brewing, bakery, dairy, marine food, extraction of coffee, plant oils

Fig. 3 a. General mechanism of other polysaccharide degradation with hydrolases with a first step of polysaccharide degradation in “osides” followed by a second step of degradation of “osides” in “oses”. Diamonds represent sugar residues (xylose, fucose and mannose), and red stars represent link breaking by the corresponding enzyme. **b.** Structural composition of natural other polysaccharides targeted by marine fungal enzymes (adapted from Rogowski *et al.* (2014); Anbuhezian *et al.* (2015); Bonechi *et al.* (2017))



and starch) (Polizeli *et al.* 2005; Burlacu *et al.* 2016; Beygmoradi and Homaei 2017; Trincone 2018; Alokika and Singh 2019; Singh *et al.* 2019; Vashist *et al.* 2019; Qeshmi *et al.* 2020), pulp and paper biobleaching (Raghukumar *et al.* 2004; Polizeli *et al.* 2005; Burlacu *et al.* 2016; Beygmoradi and Homaei 2017; Alokika and Singh 2019; Singh *et al.* 2019; Vashist *et al.* 2019; Qeshmi *et al.* 2020), textile (Polizeli *et al.* 2005), animal feed (including fish feed) (Polizeli *et al.* 2005; Burlacu *et al.* 2016; Alokika and Singh 2019; Singh *et al.* 2019; Vashist *et al.* 2019; Qeshmi *et al.* 2020), biofuel production (notably in the saccharification of seaweed biomass) (Burlacu *et al.* 2016; Trincone 2018; Alokika and Singh 2019; Singh *et al.* 2019; Qeshmi *et al.* 2020), detergent (Burlacu *et al.* 2016), waste treatment (Burlacu *et al.* 2016; Trincone 2018), plant biocontrol (Burlacu *et al.* 2016), chemicals (Polizeli *et al.* 2005) and pharmaceuticals (Polizeli *et al.* 2005; Alokika and Singh 2019; Birolli *et al.* 2019; Singh *et al.* 2019; Qeshmi *et al.* 2020).

Marine xylanases are mostly obtained from bacteria and fungi, being particularly interesting in terms of high temperature and pH tolerance (Qeshmi *et al.* 2020). These marine xylanases can be used for the production of algal protoplasts (Qeshmi *et al.* 2020) and for the extraction of soluble metabolites of interest, such as the R-Phycocyanin extracted from red algae for example (Dumay *et al.* 2014).

These xylan degrading enzymes from marine sourced microorganisms are poorly studied compared with terrestrial ones. Moreover, marine fungal xylan degrading enzymes seem more efficient than marine bacterial ones (Haltrich *et al.* 1996; Bergquist *et al.* 2002; Polizeli *et al.* 2005; Thirunavukkarasu *et al.* 2015). These observations can explain the interest concerning xylan degrading enzymes from fungi isolated from marine environments for their use in industries.

Xylanases

The optimal temperature for xylanase activity in marine fungi ranges from 45 to 55 °C (Raghukumar *et al.* 2004; Torres and dela Cruz 2013; Del-Cid *et al.* 2014; dos Santos *et al.* 2016; Korkmaz *et al.* 2017; Wu *et al.* 2018). However, a cold-adaptive xylanase obtained from a marine strain of *Penicillium chrysogenum* has shown an optimal temperature of 25 °C (Hou *et al.* 2006), highlighting the high diversity considering the specificities even in the same enzyme family. In general, polysaccharide degrading enzymes from marine fungi seem to have optimal pH values slightly acidic as is also the case concerning xylanases with optimal pH values varying between 5 and 6 (Hou *et al.* 2006; Del-Cid *et al.* 2014; Thirunavukkarasu *et al.* 2015; dos Santos *et al.* 2016; Korkmaz *et al.* 2017; Wu *et al.* 2018). A xylanase obtained from a marine strain of *Aspergillus* sp. showed optimal activity at pH 3.5 (Raghukumar *et al.* 2004). Nevertheless, several xylanases from marine fungi can also be characterized by

optimal pH values more neutral or even slightly alkaline (Torres and dela Cruz 2013).

Xylanases can be obtained from marine fungi isolated from different habitats like marine sponges (Del-Cid *et al.* 2014; dos Santos *et al.* 2016; Korkmaz *et al.* 2017), marine sediments (Hou *et al.* 2006; Korkmaz *et al.* 2017; Wu *et al.* 2018), mangroves (Raghukumar *et al.* 1994, 2004; Torres and dela Cruz 2013; Wu *et al.* 2018) and seaweeds (Thirunavukkarasu *et al.* 2015; Patyshakuliyeva *et al.* 2020). Among marine fungal xylanases, most of them have been obtained from filamentous fungi, but some marine yeast strains seem to present interesting xylanase activities (Duarte *et al.* 2013; Torres and dela Cruz 2013).

Among the marine fungal strains characterized by a promising xylanase activity, the genera *Trichoderma* and *Aspergillus* seem to be particularly efficient for xylan degradation (Raghukumar *et al.* 2004; Torres and dela Cruz 2013; Thirunavukkarasu *et al.* 2015; dos Santos *et al.* 2016; Korkmaz *et al.* 2017; Patyshakuliyeva *et al.* 2020). Indeed, terrestrial strains of these two genera are currently mainly used for the production of commercial xylanases (Torres and dela Cruz 2013; Korkmaz *et al.* 2017). The important xylan degrading capacity of a marine *Trichoderma harzianum* should be due to its high xylanase and xylosidase activities. The presence of 2% of NaCl in the culture medium increased the xylanase activity of this strain compared with no salt control culture, showing the adaptability of *T. harzianum* to its marine environment (Thirunavukkarasu *et al.* 2015). Marine strains of *Trichoderma* are not only promising for the production of xylanases and xylosidases but also glucanases and β -glucosidases (Patyshakuliyeva *et al.* 2020). Indeed, xylanase-producing marine fungi seem to generally synthesize also cellulases (Luo *et al.* 2005). These two kinds of enzymes have complementary activities in the degradation of natural polysaccharides. According to Patyshakuliyeva *et al.* (2020), marine fungi do not need to exhibit enzymatic activities perfectly complementary with the polysaccharides of the substrate to allow its degradation. Indeed, the presence of enzymes targeting polymers which are widespread in algal cell walls (like xylanases or cellulases) would be sufficient to allow the fungal growth on diverse algal biomasses (Patyshakuliyeva *et al.* 2020). In the study of Thirunavukkarasu *et al.* (2015), the second most important xylanase producer was an *Aspergillus terreus* strain isolated from a seaweed (*Cymodocea serrulata*), with specific activity values of 0.7 U mg⁻¹. In addition to the fact that *Aspergillus* is a genus particularly interesting for xylanase production, the study of dos Santos *et al.* (2016) highlighted also the importance of fungal culture condition to optimize the enzymatic activity (with an increase of xylanase activity from 49.4 to 629.6 U mL⁻¹ thanks to culture optimization). Other marine fungal strains of the genera *Penicillium* or *Cladosporium*, for example, are described in several studies exhibiting xylanase

activities of interest (Hou *et al.* 2006; Torres and dela Cruz 2013; Del-Cid *et al.* 2014; Patyshakuliyeva *et al.* 2020).

Several xylanases obtained from marine fungi of genera such as *Phoma*, *Penicillium*, *Cladosporium*, *Trichoderma* or *Paecilomyces* showed better activity in the presence of NaCl concentrations close to those of seawater than without any salinity (Raghukumar *et al.* 2004; Torres and dela Cruz 2013; Korkmaz *et al.* 2017; Wu *et al.* 2018). A strain of *Trichoderma pleuroticola* (08CK001) was characterized by sixfold xylanase production in a culture medium supplemented with 24.4% artificial seawater (Korkmaz *et al.* 2017). An important factor of fungal culture conditions impacting xylanase activity is the substrate used. Pure xylan is the most evident substrate to enhance the xylanase activity, but for large-scale enzymatic production, this substrate is not economically viable. Other kinds of substrates of interest in the enhancement of enzymatic activities include agricultural by-products or co-products (Table 2). Considering a marine *Cladosporium* sp. strain screened by Del-Cid *et al.* (2014), the highest xylanase activities were obtained using beechwood and birchwood xylylans (specific activities of 200 and 160 U mg⁻¹, respectively), but the most promising alternative substrate was the wheat bran.

Marine fungal strains characterized by xylanase activity can produce several xylanases. It is the case for an *Aspergillus niger* strain isolated from mangrove (Raghukumar *et al.* 2004), which appears to have two different xylanases (13 and 18 kDa). The enzyme molecular weight differs according to the fungus. A marine fungal xylanase obtained from a strain of *Penicillium chrysogenum* was characterized by Hou *et al.* (2006) with a molecular weight of 38 kDa. This molecular weight is about two times higher than both xylanases obtained from *Aspergillus niger* (18 and 13 kDa) characterized by Raghukumar *et al.* (2004). A xylanase produced by a mangrove fungal strain of *Phoma* sp. has an intermediate theoretical molecular weight of 24.4 kDa (Wu *et al.* 2018).

Xylosidases

There are fewer studies on xylosidases from marine fungi than on xylanases. This can be explained in part by the fact that some studies characterize the general xylanolytic activity, degrading the xylan to xylose, whereas others make the distinction between the activities of the different actors of the xylan degrading complex (principally endoxylanases, exoxylanases and xylosidases).

Due to this lack of studies available in the literature, no data have been found on optimal temperature and pH of marine fungal xylosidase, as well as data on enzyme molecular weight. However, concerning salinity tolerance, xylosidase specific activity increased respectively by 3.2, 2.1 and 1.8 with 1.5, 2 and 2.5% NaCl compared with media without supplemented NaCl in *Trichoderma harzianum*

strain MTCC10344 isolated from *Sargassum wightii* (Thirunavukkarasu *et al.* 2015). Another study on *T. harzianum*, *Curvularia tuberculata*, *Gonatophragmium mori* and *Aspergillus terreus* marine strains, showed that the xylosidase specific activity was maintained from 0 to 1.2 M NaCl, without any significant increase (Sengupta *et al.* 2017).

Thirunavukkarasu *et al.* (2015) found that the marine fungal strain showing the most important xylosidase specific activity (2.4×10^{-3} U mg⁻¹) was *Trichoderma harzianum*. This genus seems to be particularly interesting with respect to xylosidase activity (Thirunavukkarasu *et al.* 2015; Sengupta *et al.* 2017; Patyshakuliyeva *et al.* 2020). In the study of Patyshakuliyeva *et al.* (2020), the *Trichoderma paraviri-descens* strain CBS 143,790 FP-027-C5 isolated from *Fucus* sp. was characterized by the greatest xylosidase activity (0.01 U mL⁻¹) compared with the other screened marine fungal strains. Some of these strains seem to exhibit a little preference for the use of *Ulva lactuca* as a substrate compared with *Laminaria digitata*.

Fucoïdanases

Fucoïdanes are sulfated polysaccharides principally constituted of fucose alternatively linked by α (1 → 3) and α (1 → 4) bonds (Fig. 3b) and they are mainly found in brown macroalgae (Morya *et al.* 2012; Zayed and Ulber 2019).

Endo- and exo-fucoïdanases degrade fucoïdan and respectively target the backbone and the ends of the polymeric chain, resulting in oligosaccharides themselves degraded in monosaccharides (fucose) by fucosidases (Fig. 3a).

The presence of fucoïdanase in marine fungi was discussed in 2009 (Holtkamp *et al.* 2009), but here we provide information from several more recent studies. However, despite the biotechnological potential of fucoïdanes, studies on the properties of fucoïdanases are scarce because of the limited description of microbial producers of this enzyme (Gurpilhares *et al.* 2016; Balabanova *et al.* 2018a).

Besides the role of fucoïdanases in the description of fucoïdan structures (Kusaykin *et al.* 2015), recent studies exhibited bioactivities of fucoïdan oligosaccharides obtained with fucoïdanases and fucosidases such as anticancer (Trincon 2018) and immunoregulatory activities (Jagtap and Manohar 2021). Moreover, fucosylated oligosaccharides can be involved in physiological processes such as immuneresponse, signal transduction, early embryogenesis, growth regulation, apoptosis, adhesion of pathogens, extravation of leukocytes and maturation and interaction of gametes. Fungal fucosidases are mainly synthesized by *Aspergillus*, *Fusarium* and *Penicillium* genera (Guzmán-Rodríguez *et al.* 2019). Considering all the enzymes described in the present review, fucoïdan-degrading enzymes are certainly the ones for which properties and industrial applications are the least studied.

Habitats of fucoidanase producing marine fungi seem to be limited to red and brown macroalgae (Gomaa *et al.* 2015, 2019; Hifney *et al.* 2019; Patyshakuliyeva *et al.* 2020), and marine sediments (Qianqian *et al.* 2011; Wu *et al.* 2011; Balabanova *et al.* 2018b).

Optimal temperatures of marine fungal fucoidanase range from 40 to 60 °C (Qianqian *et al.* 2011; Wu *et al.* 2011; Hifney *et al.* 2019). The optimal pH values are slightly acidic to neutral, ranging from 6 to 7, which is a little less acidic than other marine fungal enzymes previously discussed (Qianqian *et al.* 2011; Wu *et al.* 2011; Hifney *et al.* 2019). Concerning optimal salinity, a culture optimization protocol for *Dendryphiella arenaria* enzymatic production showed that increasing NaCl concentrations have a negative impact on fucoidanase production. However, fungal growth could be inhibited without supplemented NaCl. Thus, a salinity of 1.5% NaCl seems to allow the best fucoidanase activity (Gomaa *et al.* 2019).

Dendryphiella seems to be a particularly interesting marine fungal genus for fucoidanase activity (Wu *et al.* 2011; Gomaa *et al.* 2019; Hifney *et al.* 2019). However, marine strains belonging to other widespread fungal genera such as *Penicillium*, *Trichoderma*, *Aspergillus* or *Cladosporium* have also been described for their fucoidanase activities (Burtseva *et al.* 2010; Gomaa *et al.* 2015; Patyshakuliyeva *et al.* 2020).

Among the six marine fungal strains isolated from the brown alga *Fucus* sp., fucoidanase activity was detected in *Trichoderma paraviridescens* strain CBS 143,790 FP-027-C5 (6×10^{-3} U mL⁻¹) and *Rhizopus oryzae* strain CBS 143,788 FP-027-B4 (4.5×10^{-3} U mL⁻¹) (Patyshakuliyeva *et al.* 2020). Gomaa *et al.* (2015) observed the highest fucoidanase specific activity (18 U mg⁻¹) in a *Cladosporium salinae* strain isolated from *Padina pavonica*. Interestingly, all of the isolated strains were grown on a *Sargassum*-based medium, but marine fungal strains isolated from *Sargassum* sp. did not show the highest enzymatic activities (Gomaa *et al.* 2015). Indeed, the three most active strains were isolated from the red seaweeds *Padina pavonica* and *Palisada perforata*. Thus, a specific association with the alga does not seem to necessarily allow a better degradation compared with fungi isolated from other habitats, at least concerning fucoidan degradation (Gomaa *et al.* 2015).

The molecular weight of marine fungal fucoidanase seems to be variable. Two fucoidanases have been characterized with molecular weights of 64 kDa and 180 kDa, obtained respectively from marine strains of *Fusarium* sp. (Qianqian *et al.* 2011) and *Dendryphiella arenaria* (Wu *et al.* 2011).

According to Gomaa *et al.* (2019), the main factors allowing a better fucoidanase activity were the supplementation with fucoidan and urea in the culture media. Even if the increase of salt seemed to exert a negative impact on

fucoidanase activity, it was also necessary for the growth of the studied fungal strain *Dendryphiella arenaria* isolated from *Palisada perforata*.

Fucosidases

As previously mentioned, fucosidases allow the release of monosaccharides from the oligosaccharides produced by fucoidanases degrading fucoidans. These enzymes are still poorly explored considering marine fungi. As for some other enzymatic activities poorly explored in marine fungi such as xylosidases or mannanases, no data are available for fucosidase on optimal temperature, pH values and molecular weights.

Shvetsova *et al.* (2014) have reported the growth of a *Fusarium proliferatum* strain on a solution of algal fucoidans. The marine origin of this strain is not clear, but the fucosidase activity was measured and reached 1.85×10^{-3} U mL⁻¹. In another study, the marine fungal isolates *Beauveria felina* and *Scopulariopsis brevicaulis* exhibited fucosidase activities (Balabanova *et al.* 2018b).

Mannanases

Mannan is a linear polysaccharide composed of mannose residues linked by β -1,4 linkages (Fig. 3b). This polysaccharide is produced by terrestrial plants but also by marine algae and bacteria (Frei and Preston 1964; Huizing and Rietema 1975; Recalde *et al.* 2012; Kokoulin *et al.* 2020). The backbone of this polysaccharide is randomly hydrolyzed by mannanases with the production of β -1,4-manno-oligomers. The hydrolysis of both these oligomers and mannan molecule, with the release of mannose residues, is realized by mannosidase which targets the non-reducing ends of these molecules (Fig. 3a) (Malgas *et al.* 2015).

No data on marine mannanases has been found and only a few studies describing enzymatic activities of marine fungi have measured mannosidase activity (Burtseva *et al.* 2010; Balabanova *et al.* 2018b). Indeed, mannanase from the marine environment was first mentioned in 2006 from the abalone *Haliotis discushannai* (Ootsuka *et al.* 2006; Trincon 2018). However, the use of mannan-degrading enzymes is well described, and they have applications in numerous industries such as pulp and paper in the biobleaching process (Dhawan and Kaur 2007; van Zyl *et al.* 2010; Chauhan *et al.* 2012), detergent (Dhawan and Kaur 2007; van Zyl *et al.* 2010; Chauhan *et al.* 2012), food (in the hydrolysis of coffee extracts, oil extraction from coconut, maceration and clarification of fruit juices) (Dhawan and Kaur 2007; van Zyl *et al.* 2010; Chauhan *et al.* 2012; Kango *et al.* 2019), animal feed (notably as fish feed additive) (Dhawan and Kaur 2007; van Zyl *et al.* 2010; Chauhan *et al.* 2012; Kango *et al.* 2019),

textile (Dhawan and Kaur 2007) and pharmaceuticals (manno-oligosaccharides and mannoses residues synthesized by mannanases are of interest as prebiotics and in urinary tract infection, or in several abdominal disorders) (van Zyl *et al.* 2010; Chauhan *et al.* 2012). They also have supplementary applications in oil, gas or biofuel industry (van Zyl *et al.* 2010; Chauhan *et al.* 2012), and as antifouling by removing bacterial biofilms (Chauhan *et al.* 2012).

Among the fungal producers of mannanases, the best ones belong to the genera *Aspergillus*, *Agaricus*, *Trichoderma* and *Sclerotium* (Dhawan and Kaur 2007; Kango *et al.* 2019).

According to Balabanova *et al.* (2018b), the mannosidase activity of *Beauveria felina* is higher after 7 days of incubation than after 4 days of incubation. The mannosidase specific activity after 7 days of incubations was comparable between the two marine fungal species tested (*B. felina* and *Scopulariopsis brevicaulis*) with values respectively at 1.5×10^{-3} and 1.4×10^{-3} U mg⁻¹. Mannosidase activity has also been detected in two marine fungal strains of *Penicillium chrysogenum* and *P. canescens* grown on a fucoidan-based medium (Burtseva *et al.* 2010).

Lyases

The great majority of marine fungal polysaccharidases described in the literature belong to the hydrolase family. However, one important polysaccharide, alginate, is degraded by other kinds of enzymes characterized by a lyase catalytic reaction.

Alginate lyases

Alginases, also known as alginate lyases, allow the degradation of alginate. These enzymes target the β -1,4 linkages between the β -D mannuronic acid and α -L-guluronic acid units constituting alginate (Fig. 4). Alginate lyases are produced by several marine organisms including brown seaweeds, marine molluscs, bacteria, viruses, cyanobacteria and fungi (Zhu *et al.* 2018b; Dharani *et al.* 2020; Gao *et al.* 2021). They can be used as antibiotics due to their ability to degrade the polymeric substances found in some bacterial biofilms (Dharani *et al.* 2020; Cao *et al.* 2021; Gao *et al.* 2021). Alginate lyases have also application in biofuel production by the saccharification of brown seaweed biomass (Beygmoradi and Homaei 2017; Trincone 2018; Zhu *et al.* 2018b; Dharani *et al.* 2020) and in production of oligosaccharides with many application notably in plant biocontrol (promoting plant growth, conferring plant resistance, inhibition of pathogen growth, being environmental-friendly fertilizer and biopesticide) (Zhu *et al.* 2018b; Dharani *et al.* 2020; Zhang *et al.* 2020; Cao *et al.* 2021) and in pharmaceuticals (immunomodulatory, anti-inflammatory, antimicrobial, antioxidant, anticoagulant, prebiotic, antitumor,

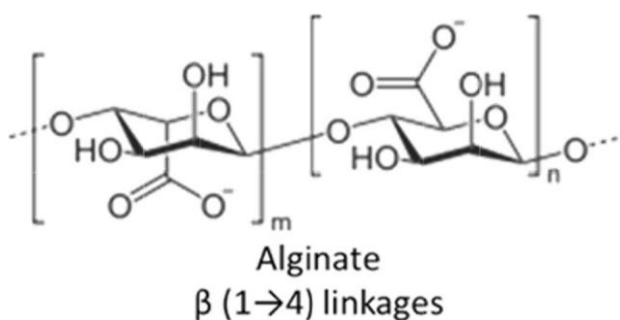


Fig. 4 Structure of alginate (from Claudio-Rizo *et al.* (2018))

anti-hypertensive, antidiabetic and neuroprotective activities) (Beygmoradi and Homaei 2017; Trincone 2018; Dharani *et al.* 2020; Zhang *et al.* 2020; Cao *et al.* 2021; Gao *et al.* 2021; Jagtap and Manohar 2021). They can also be used for the production of food additives (Beygmoradi and Homaei 2017; Dharani *et al.* 2020; Cao *et al.* 2021).

A marine fungal strain of *Paradendryphiella salina* (isolated from *Fucus serratus*) only differs from terrestrial strains by the presence of alginate lyase concerning the degradation of brown algae (Pilgaard *et al.* 2019). This highlights marine fungal adaptation at the species level, with an enzymatic activity linked to the specific habitat of the marine strain.

The optimal temperature for marine fungal alginase activity seems to be lower than for other marine fungal polysaccharide degrading enzymes. The highest alginase specific activity of a marine strain of *Aspergillus oryzae* (67.2 U mg⁻¹) was obtained at 35 °C (Singh *et al.* 2011). The tendency of slightly acidic preference generally observed for polysaccharide degrading enzymes of marine fungi also characterizes alginases. The optimal pH ranges from 6 to 6.5 according to the species and the studies (Wainwright and Sherbrock-Cox 1981; Singh *et al.* 2011).

Two fungal genera, *Dendryphiella* and *Aspergillus*, have been reported to exhibit alginase activity (Wainwright and Sherbrock-Cox 1981; Schaumann and Weide 1990; Burtseva *et al.* 2010; Singh *et al.* 2011; Gomaa *et al.* 2015, 2019). Gomaa *et al.* (2015) obtained the highest alginase specific activity of 85 U mg⁻¹ with strains of *Acrophilophora* sp. and *Setosphaeria rostrata*. Interestingly, these two active strains were isolated from brown and red seaweed, respectively. Thus, even if alginate is particularly present in brown seaweeds, the difference of alginate degrading capacity between these two strains isolated from red and brown seaweeds remains unclear (Gomaa *et al.* 2015). Nevertheless, it is quite difficult to compare enzymatic activities of marine fungal strains which are obtained from different habitats. Indeed, many studies are focused on the activity of one marine fungal species or genus.

In general, marine fungi are able to grow with NaCl, but at high concentrations, enzymes are inhibited. The impact of salinity on alginate degradation depends on the fungal species. The alginate degrading capacity of *Dendryphiella arenaria* is inhibited by 1% NaCl, while a salinity ranging from 1 to 3% NaCl stimulates *Dendryphiella salina* alginate degrading capacity (Wainwright and Sherbrock-Cox 1981). These results for *D. arenaria* should be discussed with another study showing that a 4% NaCl concentration was optimal to obtain the highest alginase activity (24 U mL⁻¹) (Gomaa *et al.* 2019). These results suggest intraspecific variability or could be due to variations between protocols and assays.

One alginate lyase of *Aspergillus oryzae* has been characterized and is constituted of two subunits of 40 and 50 kDa (Singh *et al.* 2011).

Lyases in marine fungal species are poorly documented, except for some studies on alginate lyases. One strain of *Thalassochytrium gracilariopsis* isolated from *Gracilariopsis* sp. presented an α -glucan lyase (Nielsen *et al.* 2000). However, considering promising enzymes such as ulvan lyases (currently studied for biofuel production from green seaweed), no data are available from marine fungi, and almost all studies describe marine bacterial activities.

Enzymes of protein degradation from marine fungi

Proteases can generally be differentiated between exopeptidases (peptidases) and endopeptidases (proteinases) (Kango *et al.* 2019). On the basis of the functional group of their active site, proteases can be subgrouped into cysteine, aspartic, serine and metallo-proteases (Kango *et al.* 2019).

Fungal proteases have several benefits against bacterial ones and major fungal protease producers belong to *Aspergillus*, *Cephalosporium*, *Fusarium*, *Rhizopus* and *Penicillium* genera (Naveed *et al.* 2021).

Proteases, including marine ones, are used in food industry (cheese manufacturing, baking, brewing and dairy industries, fruit juice and coffee processing and meat tenderization) (Muffler *et al.* 2015; Srilakshmi and Madhavi 2015; Homaei *et al.* 2016b; Duarte *et al.* 2018; Mzui and Ch 2018; Vashist *et al.* 2019; Naveed *et al.* 2021), in detergent industry (Zhang and Kim 2010, 2012; Muffler *et al.* 2015; Srilakshmi and Madhavi 2015; Homaei *et al.* 2016b; Beygmoradi and Homaei 2017; Mzui and Ch 2018; Vashist *et al.* 2019; Barzkar and Sohail 2020; Naveed *et al.* 2021), in leather industry (Zhang and Kim 2010, 2012; Muffler *et al.* 2015; Srilakshmi and Madhavi 2015; Homaei *et al.* 2016b; Beygmoradi and Homaei 2017; Mzui and Ch 2018; Barzkar and Sohail 2020; Naveed *et al.* 2021), in pharmaceuticals (treatments of several disorders such as ulcers, cancer, cystic fibrosis, cardiac and

digestive disorders, and inflammation and exhibiting antioxidant, anticancer and antibacterial bioactivities) (Zhang and Kim 2010, 2012; Muffler *et al.* 2015; Srilakshmi and Madhavi 2015; Homaei *et al.* 2016b; Beygmoradi and Homaei 2017; Duarte *et al.* 2018; Mzui and Ch 2018; Barone *et al.* 2019; Vashist *et al.* 2019; Barzkar and Sohail 2020; Naveed *et al.* 2021), in cosmetic industry (as skin peeling and softening) (Vashist *et al.* 2019; Naveed *et al.* 2021), in textile industry (silk degumming) (Srilakshmi and Madhavi 2015; Homaei *et al.* 2016b; Duarte *et al.* 2018; Mzui and Ch 2018; Vashist *et al.* 2019; Naveed *et al.* 2021), in chemical industry (Barone *et al.* 2019; Naveed *et al.* 2021) and in waste management (Muffler *et al.* 2015; Vashist *et al.* 2019; Naveed *et al.* 2021). Marine proteases are also used in contact lens cleansing, in marine waste treatment and as antifouling and antibiofilm agents (Barzkar and Sohail 2020).

However, data on the characterization or application of marine fungal proteases are still limited; the first study describing a protease from an endophytic marine fungus was published in 2014 (Budiarto *et al.* 2014).

Marine fungal proteases are of particular interest for their ability to be active in extreme conditions such as salinity, some proteases being active with values up to 3.5 M NaCl (Lario *et al.* 2015), and low temperatures, several proteases exhibiting an optimal temperature of 25 °C (Pazgier *et al.* 2003; Turkiewicz *et al.* 2003; Zhu *et al.* 2009; Mzui and Ch 2018). They can also be active in acidic (Li *et al.* 2010; Lario *et al.* 2015) or alkaline (Zhu *et al.* 2009; Jasmin *et al.* 2010; Mzui and Ch 2018) conditions, some proteases exhibiting optimal activities with pH values ranging from 3.5 to 11.

Proteolytic enzymes from marine fungi are less documented than polysaccharide degrading enzymes (Mzui and Ch 2018). As for the previous subsections, enzymatic activities are not easily comparable between studies, because of the diversity of fungal species, habitats and used methodology to obtain activities. However, contrarily to the numerous substrates putatively used for the study of polysaccharide degrading enzymes, the majority of marine fungal protease assays are carried out using casein (or azocasein). The substrate is of course adjusted according to the targeted protease activity. Thus, gelatin or keratin are used in the case of gelatinase or keratinase studies, respectively (Pisano *et al.* 1964; El-Gendy 2010). Nevertheless, most of the studies cited in this review only measure the marine fungal protease activity without substrate specificity.

Marine fungi exhibiting protease activities have been isolated from various habitats such as algae (Chaud *et al.* 2016), sediments (Chellappan *et al.* 2006), corals (El-Gendy 2010) or column water (Li *et al.* 2010). Among the studies characterizing marine fungal proteases, several have focused on marine yeasts belonging to genera like *Rhodotorula*, *Aureobasidium*, *Leucosporidium* or *Metschnikowia* (Pazgier *et al.* 2003; Turkiewicz *et al.* 2003; Chi *et al.* 2007;

Li *et al.* 2010; Duarte *et al.* 2013; Lario *et al.* 2015; Chaud *et al.* 2016). Considering marine filamentous fungi, common genera have been studied such as *Aspergillus* (Damare *et al.* 2006; Kamat *et al.* 2008) or *Penicillium* (Zhu *et al.* 2009; El-Gendy 2010; Park *et al.* 2016; Wang *et al.* 2016). However, species such as *Xylaria psidii* and *Engyodontium album* have been described by several studies for their protease activity (Chellappan *et al.* 2006, 2011; Jasmin *et al.* 2010; Budiarto *et al.* 2014; Indarmawan *et al.* 2016).

Marine fungal proteases are generally characterized by an optimal temperature between 45 and 60 °C (Chellappan *et al.* 2006, 2011; Damare *et al.* 2006; Chi *et al.* 2007; El-Gendy 2010; Lario *et al.* 2015). However, other marine fungal proteases have been studied because of their lower optimal temperature. Then, several marine fungal species have shown their highest protease activity at 40 °C (Li *et al.* 2010), 35 °C (Zhu *et al.* 2009), 25 °C (Pazgier *et al.* 2003; Turkiewicz *et al.* 2003) or even 10 °C (Chaud *et al.* 2016). Cold environments such as polar zones as the Antarctic are of particular interest in the targeting of cold-adapted marine fungal proteases.

Optimal pH for marine fungal proteases seemed to be very variable from acidic to alkaline values according to the species, while an acidic tendency characterized polysaccharide degrading enzymes. Proteases (subtilase or keratinase) optimal activities have been observed at neutral pH (Pazgier *et al.* 2003; Turkiewicz *et al.* 2003; El-Gendy 2010; Budiarto *et al.* 2014; Samuel *et al.* 2018), while other optimal activities have been observed between pH values 9 and 11 (Chellappan *et al.* 2006, 2011; Damare *et al.* 2006; Chi *et al.* 2007; Zhu *et al.* 2009; El-Gendy 2010). Interestingly, marine fungal (yeast) species of *Rhodotorula* or *Metschnikowia* contain proteases with acidic optimal pH values of 4–5 and 3.5, respectively (Li *et al.* 2010; Lario *et al.* 2015; Chaud *et al.* 2016). To the best of our knowledge, no marine filamentous fungal protease with acid affinity has been reported in the literature.

Most of the described marine fungal proteases have a molecular weight ranging from 30 to 40 kDa (Pazgier *et al.* 2003; Turkiewicz *et al.* 2003; Chellappan *et al.* 2006, 2011; Damare *et al.* 2006; Zhu *et al.* 2009; El-Gendy 2010; Jasmin *et al.* 2010; Lario *et al.* 2015). El-Gendy (2010) purified a smaller keratinase of 19 kDa from a marine *Penicillium* sp. isolate, while a 71 kDa extracellular protease has been described from an isolate of *Xylaria psidii* (Budiarto *et al.* 2014). Interestingly, an isolate of this species has been described to express three proteases of 56.6, 89.1 and 162.2 kDa (Indarmawan *et al.* 2016).

The effect of the carbon source for fungal growth seems to have an impact on protease activity. However, protease activities depend on marine fungal species screened. Maltose is the preferential carbon source for protease production by *Colletotrichum capsici* and *Curvularia lunata* (Samuel

et al. 2018), while glucose and corn starch allow the highest protease activity of *Metschnikowia reukaufii* (72.5 U mL⁻¹) and *Aureobasidium pullulans* (7.2 U mL⁻¹), respectively (Chi *et al.* 2007; Li *et al.* 2010). It does not seem to be one specific interesting carbon source for enzymatic production. However, each fungal isolate has different affinities when agroindustrial residues such as rice, wheat or barley straw are used as substrate (El-Gendy 2010).

The effect of NaCl on marine fungal protease activity depends on the species considered. Some protease activities decrease at once with the adding of NaCl (Chellappan *et al.* 2011; Chaud *et al.* 2016), but others keep activity at low NaCl concentrations from 0.1 to 1 M (Damare *et al.* 2006) or even with a high amount of NaCl such as 3.5 M maintaining 40% of the initial activity (Lario *et al.* 2015). However, it is important to know that seawater does not only contain NaCl but many other salts and minerals. Marine fungal growth with supplemented artificial seawater is greater than with NaCl enriched medium (Chaud *et al.* 2016).

The protease activity is different considering the marine fungal species but the substrate used for enzymatic assays can also have a great influence. As mentioned above, casein is the main substrate used for protease activity measurement. Indeed, it has been shown that compared with haemoglobin, gelatin or bovine serum albumin (BSA), proteolytic activity was higher using casein as a substrate (Chellappan *et al.* 2011). On the other hand, more than half of the marine filamentous fungi screened by Loperena *et al.* (2012) were characterized by a gelatin degrading capacity but no casein degrading capacity. A large spectrum of substrate potentially targeted by the proteases increase their application and their interest (Chellappan *et al.* 2011).

Enzymes of lipid degradation from marine fungi

Different types of lipolytic enzymes can be found in microorganisms, such as lipases, esterases or phospholipases, targeting respectively long and medium triglycerides, short triglycerides and phosphoglycerides (Albayati *et al.* 2020). These hydrolyses result in the production of shorter molecules such as free fatty acids and glycerol notably. They can be either in activated or inactivated forms (Patnala *et al.* 2016; Geoffroy and Achur 2018).

Microbial lipases have numerous advantages, being easily modified genetically, and exhibit a great diversity of characteristics and specificity (Filho *et al.* 2019). Among these microbial lipases, fungal ones are particularly promising for industrial use because of their ease of production, specificities and stabilities (Navvabi *et al.* 2018; Filho *et al.* 2019; Chandra *et al.* 2020; Melani *et al.* 2020). The most cited fungal genera for lipase production are *Aspergillus*,

Trichoderma, *Rhizopus*, *Penicillium*, *Mucor*, *Geotrichum* and *Fusarium* (Navvabi *et al.* 2018; Melani *et al.* 2020). Lipases from marine environments are particularly promising even if they are less studied than terrestrial ones (Patnala *et al.* 2016; Mzui and Ch 2018; Navvabi *et al.* 2018; Filho *et al.* 2019).

Lipases, including marine ones, are mainly used in food and nutrition (particularly in fat and oil industry, for the production of a cocoa butter equivalent, in the bakery industry, in dairy industry, in egg processing, in fruit juices, for fish preservation or as biosensors) (Zhang and Kim 2010, 2012; Patnala *et al.* 2016; Beygmoradi and Homaei 2017; Navvabi *et al.* 2018; Filho *et al.* 2019; Vashist *et al.* 2019; Chandra *et al.* 2020; Melani *et al.* 2020). They are also used in the wastewater treatment (Basheer *et al.* 2011; Patnala *et al.* 2016; Filho *et al.* 2019; Vashist *et al.* 2019; Chandra *et al.* 2020; Melani *et al.* 2020), animal feed (Navvabi *et al.* 2018), detergent (Zhang and Kim 2010, 2012; Beygmoradi and Homaei 2017; Navvabi *et al.* 2018; Chandra *et al.* 2020), paper production (Zhang and Kim 2010, 2012; Navvabi *et al.* 2018; Vashist *et al.* 2019; Chandra *et al.* 2020), textile (Navvabi *et al.* 2018; Vashist *et al.* 2019; Chandra *et al.* 2020), leather (Vashist *et al.* 2019; Chandra *et al.* 2020), pharmaceuticals (treatment of cardiovascular diseases, obesity, anxiety, inflammation and pain) (Beygmoradi and Homaei 2017; Navvabi *et al.* 2018; Barone *et al.* 2019; Vashist *et al.* 2019; Chandra *et al.* 2020; Melani *et al.* 2020), cosmetics (Zhang and Kim 2010, 2012; Vashist *et al.* 2019; Chandra *et al.* 2020), agriculture (Beygmoradi and Homaei 2017; Chandra *et al.* 2020) and biofuel production (Navvabi *et al.* 2018; Filho *et al.* 2019; Chandra *et al.* 2020; Melani *et al.* 2020).

Substrates used for the lipolytic assays are principally either olive oil or paranitrophenol derivatives (paranitrophenol palmitate or p-nitrophenyl laurate). The different enzymatic substrates are then chosen according to their availability and using easiness and do not necessarily correspond to the lipidic substrate specifically targeted by the studied enzyme. However, activity values from a study to another are still uneasily comparable.

Marine fungi are generally more resistant to physico-chemical constraints than their terrestrial counterparts (Patnala *et al.* 2016; Navvabi *et al.* 2018). Among these resistances, marine fungal lipases can be characterized by a salinity tolerance (Huang *et al.* 2004; Geoffry and Achur 2018) and can be cold-adapted (Wang *et al.* 2007; Mzui and Ch 2018; Wentzel *et al.* 2019). Lipases, producing glycerol from lipids, are of particular interest for cold resistance and cell survival because of the cryoprotectant effect of this compound (Duarte *et al.* 2013). A study of extracellular enzymes produced by microorganisms in Antarctica has shown that 40% of filamentous fungi were producing lipases when incubated at 4 °C, while only 13% of yeasts produced this enzyme (Loperena *et al.* 2012).

This observation needs to be compared with another study on marine yeasts isolated in Antarctica in which 46% of yeasts isolates were able to produce lipases with incubation at 15 °C (Duarte *et al.* 2013).

Most of the marine fungal species described as lipolytic have been isolated from seawater near India (Potumarthi *et al.* 2008; Basheer *et al.* 2011; Lanka *et al.* 2016; Geoffry and Achur 2018) or Antarctica (Loperena *et al.* 2012; Duarte *et al.* 2013; Wentzel *et al.* 2019). However, some lipolytic marine fungi have also been isolated from sediments, seaweed or fish (Wang *et al.* 2007; Farha and Hatha 2019). According to Duarte *et al.* (2013), a majority of lipase producing marine yeasts have been isolated from lichens and marine sediments (compared with other animal or algal sources). Almost half of the studies on marine fungal lipases have described yeast lipases from species like *Aureobasidium pullulans*, *Rhodotorula mucilaginosa* or *Candida* sp. (Wang *et al.* 2007; Liu *et al.* 2008; Potumarthi *et al.* 2008; Duarte *et al.* 2013; Li *et al.* 2014; Wentzel *et al.* 2019). Marine filamentous fungi with lipase activity belong mainly to common genera such as *Penicillium*, *Cladosporium*, *Aspergillus* or *Fusarium* (Basheer *et al.* 2011; Li *et al.* 2014; Lanka *et al.* 2016; Wang *et al.* 2016; Geoffry and Achur 2018).

The optimal temperatures for marine fungal lipases are lower than those observed for proteases and most of the polysaccharidases. Indeed the highest optimal temperature, observed in *Geotrichum marinum*, *Aspergillus awamori* and several yeast species, only reached 40 °C (Huang *et al.* 2004; Wang *et al.* 2007; Basheer *et al.* 2011). The variation of optimal temperature is particularly low compared with proteases or polysaccharidases, with a lower optimal temperature for lipase activity at 35 °C (Wang *et al.* 2007; Liu *et al.* 2008). The few studies characterizing the optimal temperature for marine fungal lipases could explain the weak variation observed.

Optimal pH values of marine fungal lipases seem also to be constant from a study to another with optimal pH values neutral and varying between 6 (for *Rhodotorula mucilaginosa*) and 8.5 (for *Aureobasidium pullulans* and *Fusarium solani*) (Wang *et al.* 2007; Liu *et al.* 2008; Geoffry and Achur 2018). Optimal pH values ranging from 7 to 8 have been observed for lipases extracted from *Aspergillus awamori*, *Geotrichum marinum* and several yeast species (Huang *et al.* 2004; Wang *et al.* 2007; Basheer *et al.* 2011). Three marine fungal lipases have been purified and their molecular weight characterized. Lipases from *Aureobasidium pullulans* and *Geotrichum marinum* have similar molecular weights of 63.5 and 62 kDa respectively (Huang *et al.* 2004; Liu *et al.* 2008), and a lipase from *Aspergillus awamori* has a molecular weight of 90 kDa (Basheer *et al.* 2011).

Even if lipases are not the most described enzymes from marine fungi, it seems that this enzymatic activity is particularly high in several cases. Indeed, an isolate of *Penicillium* sp. has been tested on several enzymatic activities including gelatinase, amylase, phytase, pectinase and lipase. The lipolytic activity seemed to be the most important compared with other enzymes like amylase or cellulase, based on the enzymatic zone of clearance on a solid culture medium (Farha and Hatha 2019). This kind of promising marine fungal lipolytic activity has also been detected in four marine fungi isolated in China (*Cladosporium* sp., *Rhodospiridium* sp., *Candida* sp. and unidentified *Ascomycota*) which have exhibited lipolytic activity higher than cellulase activity. In this study, the highest lipase specific activity was obtained for *Candida* sp. strain PKU Y8 with 21.94 U mL⁻¹ (Li *et al.* 2014). A strain of *Leucosporidium scottii* was characterized by the highest lipase activity of 0.23 U mL⁻¹ (Duarte *et al.* 2013). Among nine marine yeast producing lipases, *Candida intermedia* and *Pichia guilliermondii* showed the highest specific activities of 0.042 and 0.0436 U mg⁻¹, respectively (Wang *et al.* 2007).

As lipases are induced by lipidic substrates (Basheer *et al.* 2011), several studies have reported the influence of different oils as the main carbon source in the fungal culture medium. Peanut oil (Liu *et al.* 2008) or rice bran oil (Basheer *et al.* 2011) have shown particularly interesting results with a lipase production higher than using other lipidic substrates. In the first report of lipase produced by marine-derived yeasts, olive oil resulted in the highest lipase specific activity in five of the nine screened strains, with values up to 0.2 U mg⁻¹, compared with other lipidic substrates such as lard, peanut oil and soybean oil (Wang *et al.* 2007). Supplementation with 1% of molasses also seemed to induce the lipase activity of a *Rhodotorula mucilaginosa* isolate (Potumarthi *et al.* 2008).

Conclusion

Marine microorganisms are of obvious biotechnological interest and have applications in many diverse industries. Among them, bacteria are still clearly more documented than fungi, but there is increased interest in fungi. Indeed, new marine fungal metabolites are regularly and currently discovered, highlighting the richness of these organisms.

This review provided an overview of polysaccharide, lipid and protein degrading enzymes obtained from marine fungi. Because of the great diversity of marine polysaccharides, and especially marine algae, and because of their numerous applications, marine fungal polysaccharidases begin to be well documented compared with proteases and lipases. Fungi from different marine habitats have shown a wide variety of polysaccharidases, some being specifically

necessary for fungal growth and development, while others apparently inherited from terrestrial ancestors.

Few data on polysaccharidases, proteases and lipases of marine fungi are available compared with marine bacterial ones, but this scope of research is growing. Unfortunately, enzymatic activity values, as well as kinetic parameters, are not easily comparable between studies, principally because of differences in fungal growth protocols or because of the use of various units of activity. Standardization of protocols and assays would permit better data comparisons, but several issues exist. For example, methods are continuously evolving to allow better and sharper results, fungal strains can have different substrate affinity for a same enzyme and it can lead to a loss of information from previous studies with non-standardized results.

Fungal genera such as *Aspergillus*, *Penicillium*, *Cladosporium* or *Trichoderma* seemed to be particularly well represented considering the highest polysaccharidase activities. This could be partially explained by their ease of isolation and growth in lab conditions. It should then be interesting to go further in the characterization of other marine polysaccharidases of *Aspergillus*, *Penicillium*, *Cladosporium* or *Trichoderma* species because there should be enough data in the literature to discuss the results. On the other hand, it is important to not only focus on easy growing species because this ability is not obligatory linked with high and interesting polysaccharidase activities.

Marine fungal polysaccharidases exhibit optimal temperatures of about 40–60 °C and pHs ranging from 4 to 7, with some exceptions. They are generally characterized by molecular weights ranging from 40 to 60 kDa, with some heaviest enzymes about 100 kDa. Marine fungal polysaccharidases are also particularly studied for their resistance against saline conditions. In most cases, low concentrations of NaCl ranging from 1 to 3% resulted in high enzymatic activities, whereas high NaCl concentrations have inhibitory effects. When cultured on non salted media, the growth and development of marine fungi are generally lower than by using saline conditions, leading to a loss of enzymatic activity. This can be explained by the marine adaptation of the studied fungal strains.

This review focused on marine fungal polysaccharidases targeting macroalgal polymers, but marine fungi, notably the ones isolated from macroalgae, can exhibit other polysaccharidase activities. Several data and reports exist on hydrolases such as pustulanases, dextranases, pectinases or chitinases. Other enzymes such as oxidoreductases, involved in the degradation of lignocellulosic substrates and particularly the ones found in mangroves, are also well documented, being laccases, manganese-peroxidases and lignin-peroxidases.

Most of the marine fungal species studied for polysaccharidase activities have been isolated from marine sediments or

marine algae. However, even if fungal strains have a marine origin, they can be able to produce enzymes active in the presence of terrestrial substrate. Indeed, most of the marine fungal species have also terrestrial counterparts from which they could have inherited abilities to degrade terrestrial polysaccharides. This observation explains the fact that in several studies cited in this review, marine fungal growths carried out on terrestrial agricultural by-products such as rice or wheat bran for example. Indeed, their ability to degrade terrestrial polysaccharides, added to their particularly interesting resistance to extreme physicochemical conditions compared with their terrestrial counterparts, explains the great interest of marine fungi for agricultural by-product valorization.

Data on marine fungal proteases and lipases are still very scarce. Predominant marine filamentous fungal genera cited above for polysaccharidase activities such as *Penicillium* or *Aspergillus* are also represented in studies on proteases and lipases. Optimal temperatures for marine fungal proteases seem more diverse than for polysaccharidases even if there is still a tendency around 40–60 °C. Lipase optimal temperature seems lower, with the highest optimal activity around 40 °C. Contrarily to polysaccharidases, marine fungal proteases seem to be characterized by neutral to alkaline optimal pH, while marine fungal lipase optimal pHs seem to be generally neutral. Molecular weights of protease are generally about 30–40 kDa, a little lower than polysaccharidases. On the contrary, the smallest marine fungal lipase has been characterized with a molecular weight of 62 kDa. All conclusions, observations and tendencies concerning marine fungal lipases and proteases need to be qualified considering the small amount of available data.

Many fields of application linked to enzyme use still need to be optimized, highlighting a great interest in the development of research on specific and highly active enzymes. Moreover, studies on marine fungal enzymes targeting specific polysaccharides such as mannan are still scarce, as are studies on proteases or lipases. Many unexplored marine habitats could then be sources of fungal species characterized by a production of new enzymes of great interest.

These studies could enhance the applications which already exist in numerous industries such as human food, animal feed, agriculture, cosmetics, pharmaceuticals, chemicals, detergent, textile, leather, pulp and paper, biofuels or in waste management. They could also potentially allow the development of new biotechnologies, notably in applied phycology. Indeed, marine fungal enzymes, and particularly algal ones, would be of particular interest in the specific degradation of macroalgal polysaccharides. This degradation process occurs in several applications such as the extraction of bioactive molecules from macroalgae, the treatment of seaweed wastes notably for the production of fertilizers in agriculture, the production of biofuels of third

generation, the preparation of protoplasts for algal genetic engineering, the prevention of algal bloom or biofouling. This implication of marine fungal enzymes in algal biotechnologies is particularly both recent and promising.

It has been shown in this review that marine fungi are a great source of enzymes responsible for the degradation of polysaccharides, proteins and lipids. These enzymes are produced by a wide variety of fungi and are promising because of their high activities. Assuming that marine fungi associated with a specific alga would exhibit a particularly promising ability to degrade this alga, studying the fungal community and its enzymatic potential on algae of biotechnological interest would enhance the efficiency of these technologies. Moreover, several processes such as the use of synergistic enzymes or marine fungal co-cultures can enhance the enzymatic degradation potential. Still, two economic challenges remain, being the low reusability potential of enzymes, and their production prices, along with a continuous need of discovery of new physicochemically stable enzymes.

Declarations

Conflict of interest The authors declare no competing interests.

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CHAPITRE II : DIVERSITÉ FONGIQUE
ASSOCIÉE À *P. PALMATA*

Préface

Le point de départ de ces travaux de thèse a été l'élaboration d'un protocole de récolte et de traitement des thalles de *Palmaria palmata* afin d'isoler les champignons issus de l'algue.

La **Figure II. 1** récapitule les grandes étapes de ce protocole détaillé dans ce chapitre et élaboré en croisant plusieurs protocoles d'isolement fongiques marins précédemment publiés, tels que ceux issus des travaux de [Bovio et al. \(2018\)](#), [Zuccaro et al. \(2003\)](#) et [Zhang et al. \(2009\)](#).

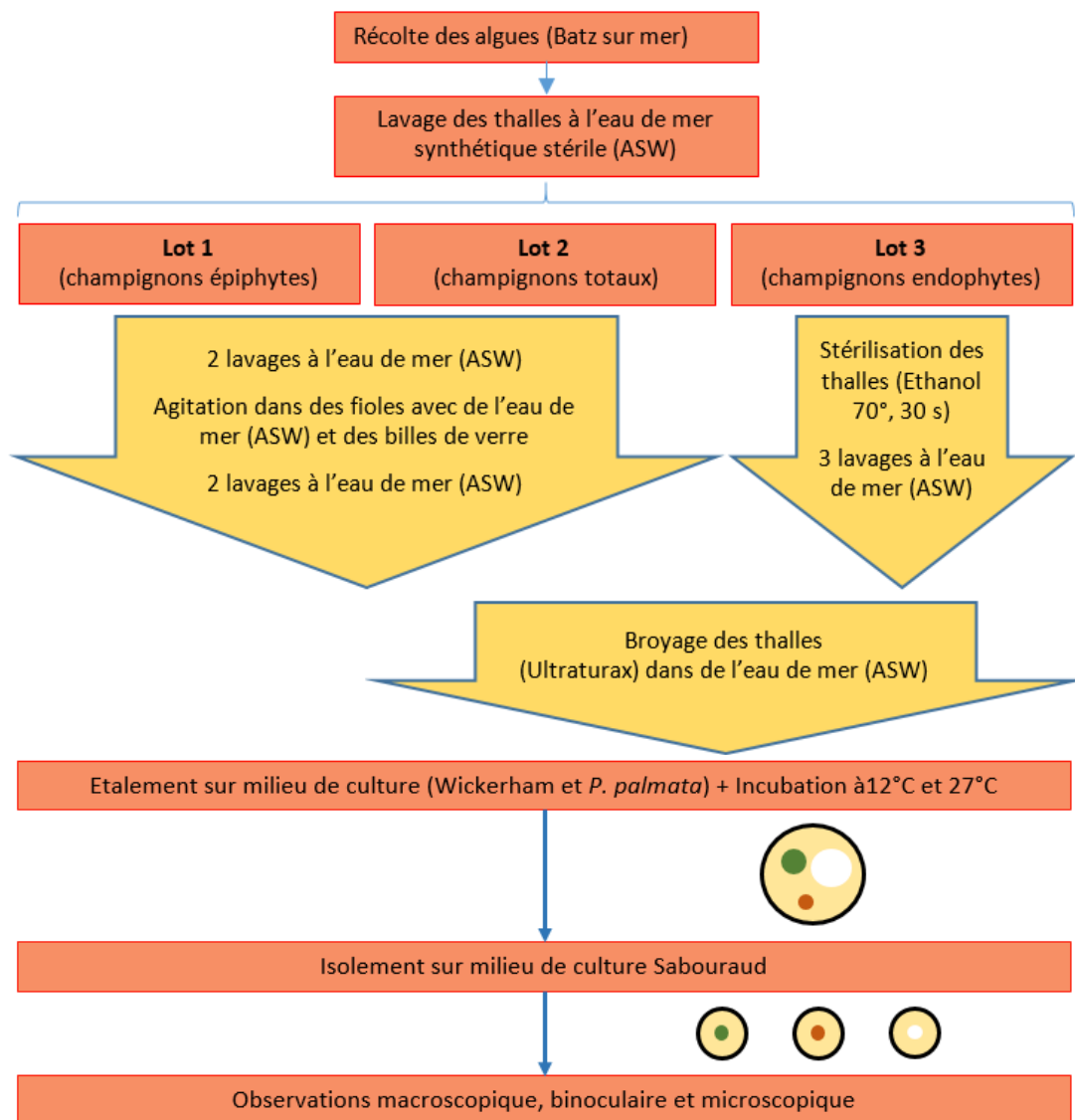


Figure II. 1 : Représentation schématique des principales étapes du protocole de récolte et d'isolement des souches fongiques à partir de l'algue rouge *Palmaria palmata*.

Ce protocole final n'a cependant pas été établi directement, plusieurs améliorations et ajustements ont dû être effectués au début des travaux d'isolement. Par exemple, les premiers isollements n'ont été réalisés que sur des milieux non-sélectifs, comparables au milieu Wickerham. Il s'agit des milieux "Potato Dextrose Agar" (PDA : glucose 20 g/L, extrait de pomme de terre 4 g/L, agar 15 g/L, ZnSO₄ 0,01 g/L, CuSO₄ 0.005 g/L, chloramphénicol 0,05 g/L) et "Czapeck Yeast extract Agar" (CYA : K₂HPO₄ 1 g/L, extrait de levure 5 g/L, saccharose 30 g/L, agar 15 g/L, chloramphénicol 0,05 g/L, NaNO₃ 0,03 g/L, KCl 0,5×10⁻³ g/L, MgSO₄ 7H₂O 0,5×10⁻³ g/L, FeSO₄ 7H₂O 10×10⁻⁶ g/L, ZnSO₄ 7H₂O 10×10⁻⁶ g/L, CuSO₄ 5H₂O 5×10⁻⁶ g/L). Par ailleurs, la constitution d'un cocktail d'antibiotiques intégré aux milieux de cultures a dû être adaptée pour contrer efficacement les contaminations bactériennes récurrentes lors des premiers essais, et perturbant les isollements fongiques.

Ainsi, les cinq premières récoltes d'algues (du 09/11/2018 au 16/05/2019) furent associées à un protocole d'isolement antérieur à celui présenté **Figure II. 1**. Cinquante-sept souches fongiques furent néanmoins isolées et intégrées à la mycothèque marine du laboratoire ISOMer sous les références MMS1621 à 1677 (**Table II. 1**). Elles n'ont toutefois pas été intégrées aux travaux présentés dans la suite de ce chapitre.

Table II. 1 : Souches isolées des récoltes d'algues selon le protocole initial.

Récoltes	Dates	Nombres d'isolats	Références MMS
01	09/11/2018	15	1621 – 1635
02	26/11/2018	15	1636 – 1650
03	10/12/2018	10	1651 – 1660
04	22/03/2019	12	1661 – 1672
05	16/05/2019	5	1673 – 1677
		Total 57	

A ces 57 souches fongiques viennent donc s'ajouter 321 souches supplémentaires, obtenues selon le protocole final détaillé **Figure II. 1** et listées dans le **Table II. 2**.

Table II. 2 : Souches isolées des récoltes d'algues selon le protocole standardisé.

Récoltes	Dates	Nombres d'isolats	Références MMS
06	19/06/2019	29	1678 – 1706
07	18/07/2019	30	1707 – 1736
08	16/09/2019	19	1737 – 1755
09	13/11/2019	16	1756 – 1771
10	09/01/2020	46	1772 – 1817
11	11/02/2020	39	1818 – 1856
12	25/05/2020	63	1857 – 1919
13	22/06/2020	79	1920 – 1998
		Total 321	

Ces 321 souches furent isolées à partir de 8 missions de récoltes d'algues différentes, deux pour chaque saison sur une période d'une année, de l'été 2019 au printemps 2020. Cependant, la pandémie de Covid-19 a perturbé l'organisation de ces missions de récoltes, et la deuxième récolte du printemps 2020 n'a pu être réalisée que le 22 juin, ce qui ne correspond pas rigoureusement à une récolte de printemps mais qui sera considérée comme telle.

Au total, ce sont donc 386 champignons qui ont été isolées à partir de *P. palmata* durant ces travaux de thèse. Chaque souche a été observée macroscopiquement et microscopiquement, donnant lieu selon les observations à un premier niveau d'assignation taxonomique au niveau du genre, en fonction des structures de reproduction asexuées (conidiophores) détectées. Beaucoup de souches n'ont pas pu être assignées de cette manière car n'étant constituées que d'un mycélium stérile. Les photographies macroscopiques et microscopiques des 386 souches, ainsi que leurs assignations, sont présentées en **Annexe 1**.

Les 321 souches isolées à partir des 8 dernières récoltes ont donc été obtenues selon le protocole standardisé (**Figure II. 1, Table II. 2**). Pour ces souches, l'assignation taxonomique a été confirmée et affinée par un protocole de séquençage de marqueurs moléculaires tels que les ITS (Internal Transcribed Spacers), plus robuste que les assignations morphologiques. Toutefois, 13 souches ont dû être exclues de l'étude à cause de problèmes de séquençage et d'assignation contradictoires. Ainsi, dans ce chapitre est présentée la diversité fongique caractérisée par les 308 souches qui constituent la communauté cultivable identifiée de *P. palmata*. La structuration de cette communauté fongique en fonction des paramètres environnementaux (saisons), écologique (localisation dans l'algue en épiphytes ou endophytes) et cultureux (température d'incubation et nature du milieu de culture utilisés pour l'isolement) est également étudiée.

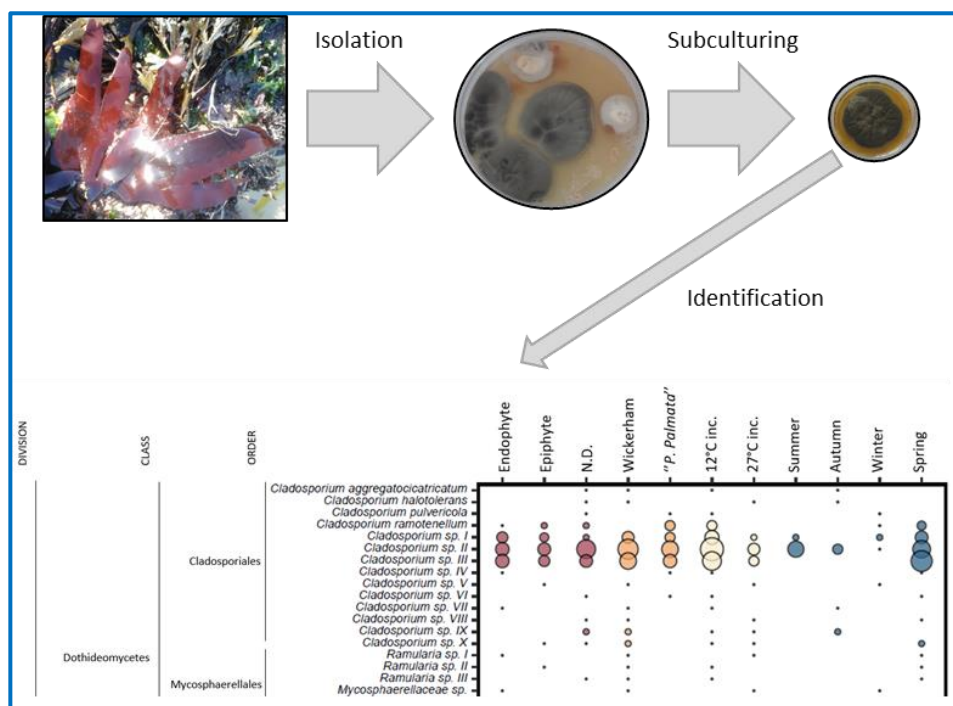
The cultivable mycobiota of the red seaweed *Palmaria palmata*: occurrence, diversity and temporal variation

Yoran Le Strat¹, Nicolas Ruiz¹, Paul Déleris¹, Thibaut Robiou du Pont¹, Samuel Bertrand¹, Stella Debaets², Gaëtan Burgaud², Justine Dumay¹

¹ Nantes Université, Institut des Substances et Organismes de la Mer (ISOMER), UR 2160, F-44000, Nantes, France ; yoran.le-strat@univ-nantes.fr; nicolas.ruiz@univ-nantes.fr; paul.deleris@univ-nantes.fr; thibaut.robious@univ-nantes.fr; samuel.bertrand@univ-nantes.fr; justine.dumay@univ-nantes.fr

² Univ Brest, INRAE, Laboratoire Universitaire de Biodiversité et Écologie Microbienne, F-29280 Plouzané, France; stella.debaets@univ-brest.fr; gaetan.burgaud@univ-brest.fr

Graphical abstract



Abstract

The present study aimed at achieving the first description of the cultivable fungal community associated with the red alga *Palmaria palmata* and its dynamic over a period of one year. The experimental plan for culturing was designed to capture the broadest possible representation of *in situ* fungal populations, considering the composition of culture media, the incubation temperature and the distinction between epiphytes and endophytes. Thalli of *P. palmata* were sampled from Valentin beach

in Batz-sur-Mer (France) twice for each season between 2019 and 2020. Fungal isolation was performed using 2 contrasted culture media, one aspecific enriched Wickerham medium and one specifically designed “*P. palmata*” medium to mimic *in situ* conditions, along with two different incubation temperatures (12°C and 27°C) and a distinction between epiphytes and endophytes. This exhaustive experimental plan generated 308 fungal isolates. Macroscopic and microscopic observations were processed on every fungal isolate for preliminary morphological observations and further dereplicated and identified using molecular approaches, leading to the identification of 147 taxa. The cultivable fungal diversity of *P. palmata* is dominated by Ascomycota (80 %) with few representatives affiliated to the phylum Basidiomycota (20 %). The three classes Dothideomycetes, Eurotiomycetes and Sordariomycetes dominate the fungal diversity, representing 34.4, 28.2 and 21.8 % of the whole cultivable diversity in terms of isolate’s number. The genera *Cladosporium*, *Penicillium* and *Aspergillus* appear dominant both in terms of isolate’s number (28.2, 20.8 and 7.1 %) and of species richness (9.5, 21.1 and 6.8 %). Described fungal diversity was significantly structured according to seasons. Culture conditions such as incubation temperature and culture medium also influenced this cultivable fungal diversity. This study is the first global study of composition of cultivable fungal community associated with *P. palmata*.

1. Introduction

Palmaria palmata, also called dulse, sea lettuce flakes, creatnach or söl (English/Irish/Scottish/Icelandic), is a red seaweed growing on the northern coasts of the Atlantic, from Portugal to Arctic, and Pacific Oceans (Pereira 2015). The earliest record of this macroalga species for food consumption is ~1,500 years ago (Indergaard 1991), with traditional uses reported in Iceland, Ireland and Brittany back to the fifth century as food but also medicine (Mouritsen *et al.* 2013). The amount of proteins in macroalgae depends on many factors, such as species, location, time of harvest, along with extraction protocols (O’ Brien *et al.* 2022). Protein content of red seaweeds such as *P. palmata* can reach up to 47 % of dry weight (dw), which appears of high interest in comparison to other seaweeds such as green seaweeds with protein contents reaching 26 % dw and brown seaweeds reaching 15 % dw, but also in comparison to traditional protein sources including milk (~3 % dw), chicken (~27 % dw) or soybeans (~35 % dw) (Fleurence 1999; Galland-Irmouli *et al.* 1999; Mouritsen *et al.* 2013; Fleurence *et al.* 2018; Tanna and Mishra 2018; Naseri *et al.* 2020; Pliego-Cortés *et al.* 2020). The amount of fatty acids is low (1.8 – 2 % of dry weight) but is one-half composed of unsaturated fatty acids (Mouritsen *et al.* 2013). Several studies have shown specific compounds of *P. palmata* to bear health-related properties such as polyphenols, chlorophyll a and phycobiliproteins (Yuan *et al.* 2005b), but also bioactive peptides with bioactivities including antioxidant activities (Harnedy *et al.* 2017) or even anti-hypertensive activities (Fitzgerald *et al.* 2014).

Over the past years, there was an increasing interest about the microbial communities associated to macroalgae and more precisely on the key roles of these microbiota in the algal growth that have led to consider the algae and its microbiome as a unique entity defined as the holobiont (Egan *et al.* 2013). The great majority of studies have targeted bacterial communities (Florez *et al.* 2017; Roth-Schulze *et al.* 2018; Selvarajan *et al.* 2019; Tourneroché *et al.* 2020; Pei *et al.* 2021), overlooking the mycobiota associated with seaweeds, which remain largely unknown. While the estimated number of fungal species in the marine environment reach 10,000 (Jones 2011), only 1,112 species were described in 2017 (Gnavi *et al.* 2017) in numerous habitats from coastal waters to the deep biosphere (Burgaud *et al.* 2022). Marine fungi are clearly less known than their terrestrial counterparts even though they appear abundant and important with non-trivial ecological roles in marine ecosystems, and particularly from coastal zones including macroalgal habitats (Gnavi *et al.* 2017; Goecke and Imhoff 2017; Ogaki *et al.* 2019; Stien 2020). While still limited, the interest on marine fungi associated with macroalgae has increased during the last decade (Ogaki *et al.* 2019) either in terms of diversity but also on putative ecological roles. Most studies have described the fungal communities associated to brown seaweeds using culture-based approaches, even though this approach is only partial, and has allowed to generate less than 2 % of the total marine fungal diversity to date (Hong *et al.* 2015; Krishna Priya and Padmakumar 2017; Garzoli *et al.* 2018; Lee *et al.* 2019; Wainwright *et al.* 2019; Tourneroché *et al.* 2020). The great majority of macroalgae-derived fungi belonged to the phylum Ascomycota (Menezes *et al.* 2010; Gnavi *et al.* 2017; Goecke and Imhoff 2017; Garzoli *et al.* 2018; Kamat *et al.* 2020), which appears consistent since the marine fungal diversity is largely dominated by ascomycetes (Jones 2011). More specifically, some genera such as *Penicillium*, *Aspergillus* or *Cladosporium* seem particularly represented probably because of their halotolerance, osmotolerance, cultivation efficiency but also high adaptive potential (Suryanarayanan *et al.* 2010; Gnavi *et al.* 2017; Krishna Priya and Padmakumar 2017; Garzoli *et al.* 2018; Lee *et al.* 2019; Kamat *et al.* 2020; Stien 2020). The ecological roles of endophytic fungi associated with macroalgae were defined as parasitic or saprobic (Suryanarayanan *et al.* 2010) but this definitely needs further investigation using more in-depth approaches such as meta-omics to delve deeper into the functions of the algal mycobioime.

Several factors seem to influence the fungal diversity associated with macroalgae such as the salinity, geographical sampling zone and algal structure or antifungal compound synthesis (Suryanarayanan 2012; Wainwright *et al.* 2019). Even if brown algae might be characterized by a higher cultivable fungal diversity compared with red and green seaweeds, several other studies comparing these three macroalgal lineages concluded on the absence of clear trend allowing to define one macroalgal family as the greatest reservoir of fungal biodiversity (Suryanarayanan *et al.* 2010; Suryanarayanan 2012; Flewelling *et al.* 2013; Abdul Malik *et al.* 2020). Moreover, it seems particularly difficult to define a core

of fungal diversity even for a single macroalgal species because of a great variability in terms of diversity between the different sampling sites (Tourneroché *et al.* 2020). This definitely calls for more investigations to highlight clear representation of the cultivable mycobiota of seaweeds, uncharted seaweeds being a focused priority.

To the best of our knowledge, no global study has yet been carried out on the composition of the cultivable fungal community associated with the red macroalga *P. palmata*. To date, studies focused on microbial community associated with *P. palmata* only targeted bacteria or only sporadically mentioned the presence or isolation of few fungal isolates (Liot *et al.* 1993; Moore *et al.* 2002; Flewelling *et al.* 2013). In this frame, aim of this study is to achieve the first description of the cultivable fungal community structure of the red alga *P. palmata* and its seasonal dynamic over a period of one year. Fungal isolation was performed using 2 contrasted culture media, one aspecific enriched Wickerham medium and one specifically designed “*P. palmata*” medium to mimic *in situ* conditions, along with two different incubation temperatures (12°C and 27°C) and a distinction between epiphytes and endophytes.

2. Material and methods

2.1. Seaweed sampling

Palmaria palmata sampling was performed in the west coast of France at Valentin beach in Batz-sur-Mer (47.276222, -2.495004) between 2019 and 2020 over one year. A total of eight periods of sampling were carried out, with approximately two samplings per season at the following dates (dd/mm/yyyy): 19/06/2019, 18/07/2019, 16/09/2019, 13/11/2019, 09/01/2020, 11/02/2020, 25/05/2020 and 22/06/2020. During algal sampling, the temperature was measured and seawater samplings were performed to wash the seaweeds in the laboratory.

2.2. Fungal isolation

Three protocols were performed in order to specifically isolate (i) epiphytic, (ii) endophytic, and (iii) both epiphytic and endophytic isolates without distinction from algal thalli of *P. palmata*, using the best sterile and aseptic conditions possible to avoid contamination. These protocols were inspired by several other protocols of marine fungal isolation (Zuccaro *et al.* 2003; Zhang *et al.* 2009; Bovio *et al.* 2018). Culture controls were realised with the absence of algal contact on culture medium. Regarding the isolation of epiphytic fungal isolates, algal thalli were first washed twice with sterile artificial seawater (ASW), then washed with ASW using glass beads and with agitation to gently remove residual debris or aspecific microorganisms. Thalli were then once again washed twice with ASW and spread on solid culture media in 15 cm-diameter Petri dishes. The isolation of specifically endophytic fungal

isolates started with a chemical disinfection in a bath of ethanol 70 % of algal thalli during 30 seconds to eliminate all epiphytic microorganisms. Thalli were then washed three times with ASW and then ground using a T25 Ultra-Turrax instrument (IKA® Werke GmbH & Co. KG, Staufen, Germany) in ASW. The crushed material were then spread on solid culture media in 15 cm-diameter Petri dishes. The protocol allowing the isolation of both epiphytic and endophytic fungal strains (cited in the present study as "epi/endo") started as for the specifically epiphytic isolation protocol, with ASW washes, the use of glass beads and agitation, and new ASW washes. Then, thalli were ground using Ultra-Turrax in ASW. The crushed material were then spread on solid culture media in 15 cm-diameter Petri dishes.

Two different media, the aspecific enriched Wickerham medium (Wickerham 1946) and the specifically designed "*Palmaria palmata*" medium to mimic *in situ* conditions, were used for the incubation protocols described above. Wickerham medium composition was glucose 10 g/L (Rectapur, VWR-Avantor, Rosny-sous-Bois, France), yeast extract 3 g/L, malt extract 3 g/L, agar 17 g/L (Condalab Madrid, Spain), peptone 5 g/L, (Biokar Diagnostics, Beauvais, France), sea salts 30 g/L (Reef Crystals, Aquarium Systems, Strasbourg, France), chloramphenicol 0.5 g/L (Merck Chimie, Fontenay sous Bois, France) and penicillin G sodium salt 0.2 g/L (Sigma-Aldrich, Saint-Quentin-Fallavier, France). Specific "*Palmaria palmata*" sterile medium consisted on *P. palmata* lyophilized powder 15.6 g/L, agar 17 g/L, sea salts 30 g/L, chloramphenicol 0.5 g/L and penicillin G 0.2 g/L. Two incubation temperature were also applied at 12°C and 27°C. Each incubation protocol was made in triplicate.

Fungal colonies (identified as yeasts or filamentous fungi based on macro- and microscopic appearance) that grew on the inoculated plates were isolated under sterile conditions on DCA medium (dextrose 40 g/L, enzymatic digest of casein 10 g/L, agar 15 g/L, Condalab) at the same incubation temperature used for enrichment. Each isolate was then subcultured and stored at 4°C and added to the laboratory fungal collection (from MMS1621 to MMS1998). The subcultured isolate was incubated at 12°C or 27°C and was observed as mentioned later after growth.

2.3. Fungal identification

Fungal identification was first performed on the basis of macroscopic and microscopic morphological features leading to an identification at the genus level when conidiophores were visible. Complementary identification were made using a molecular approach. The DNA of all the fungal isolates was extracted using the FastDNA Spin Kit (MP Biomedicals, Illkirch, France) following manufacturer's recommendations and the extracted DNA concentration was measured with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Illkirch, France) and standardized at 7.5 ng/μL. DNA extracts were stored at -20°C. A MSP-PCR (Mini/Micro-Satellite Primed PCR) protocol was used as a dereplication approach to cluster similar fungal isolates, as described by [Rédou et al. \(2015\)](#).

Briefly, the M13 microsatellite primer (5'-GAGGGTGGCGTTCT-3') was chosen to perform the PCR leading to the amplification of different numbers and sizes of DNA fragments according to the degree of replication of the M13 sequence in each fungal isolate. The amplification of the mini/micro-satellite sequences allowed the assignation of a specific fingerprint for every fungal isolate. The PCR reaction was realized in a 25 µL mix, containing 1X of PCR buffer (Promega, Charbonnières-les-Bains, France), 2 mM of MgCl₂ (Promega), 0.8 µM of the M13 primer (Sigma-Aldrich), 0.4 mM of dNTP (Promega), 0.025 U/µL of Taq Polymerase (Promega) and 15 ng of fungal genomic DNA. Amplifications consisted in an initial denaturation step at 95°C for 5 min, followed by 40 cycles of 45 s at 93°C, 60 s at 50°C and 60 s at 72°C, and a final elongation step at 72°C for 6 min. PCR products were revealed by electrophoresis with a negative control PCR product (DNA replaced by sterile distilled water) and a molecular size marker (1 kb). The visualisation of DNA bands constituting M13 profiles were carried out under ultraviolet light using a Quantum ST4 (Vilber, Marne-la-Vallée, France). M13 genetic fingerprints were analysed using Bionumerics software version 6.6 (Applied Maths NV, Sint-Martens-Latem, Belgium). The Dice coefficient was used to group all the fingerprints according to their similarities and the UPGMA method were used for the clustering. An arbitrary cut-off at 90 % of sequence similarity were used to design the different clusters. In addition of each unique isolate, one sequence per cluster was selected for DNA sequencing.

The internal transcribed spacers (ITS) was chosen to sequence the previously selected isolates. Primers used for the amplification were ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3'), in forward and reverse position respectively. The PCR reaction was performed on 10 ng of DNA for each selected isolate with 25 µL mix containing 1X of PCR buffer, 2 mM of MgCl₂, 0.2 µM of both ITS4 and ITS5 primers (Thermo Fisher Scientifics), 0.2 mM of dNTP, 0.025 U/µL of Taq Polymerase (Promega). The amplification consisted in an initial denaturation step at 95°C for 5 min, followed by 29 cycles of 60 s at 95°C, 60 s at 55°C and 60 s at 72 °C, and a final elongation step at 72°C for 10 min. PCR products were send to Eurofins Scientific (Ebersberg, Germany) for sequencing using ITS4 and ITS5 primers. For each fungal strain, both forward and reverse sequences were aligned by *de novo* process using Geneious software (2021.2.2 version, www.geneious.com) to design a consensus sequence. The consensus sequences were pulled in the Blast function of the NCBI database (default algorithm: max target sequences: 100; expect threshold: 0.05; word size: 28; max matches in a query range: 0; match/mismatch scores: 1,-2; gap costs: linear; filter: low complexity region; mask: mask for lookup table only) and assignations were then performed for each fungal isolate, when possible. Based on ITS markers, the assignation of several fungal isolates was limited to the genus level and supplementary analyses were performed using other gene markers in order to obtain a better accuracy (**Table II. 3**).

Table II. 3: Supplementary molecular markers used for fungal identification.

Markers	Primer's names	Sequences	<i>Cladosporium</i>	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Verticillium</i>
Act	ACT-512F	5'-ATGTGCAAGGCCGGTTTCGC-3'	X	X		
	ACT-783R	5'-TACGAGTCCTTCTGGCCCAT-3'				
EF1a	EF1-f	5'-ATGGGTAAGGAGACAAGACTCA-3'	X			X
	EF1-r	5'-TGGAGATACCAGCCTCGAAC-3'				
β-tub	Bt2a	5'-GGTAACCAAATCGGTGCTGCTTTC-3'			X	
	Bt2b	5'-ACCCTCAGTGTAGTGACCTTGGC-3'				
18S	nu-ssu-1196	5'-TCTGGACCTGGTGAG-3'				X
	nu-ssu-0814	5'-TTAGCATGGAATAATRRATAGGA-3'				

The PCR reaction was performed on 40 ng, 10 ng and 15 ng of genomic DNA for actin, EF1a and β-tubulin and 18S sequencing, respectively. The PCR reaction for actin sequencing was performed in 25 μL mix, containing 1X of PCR buffer, 2 mM of MgCl₂, 0.5 μM of both ACT-512F and ACT-783R primers (Thermo Fisher Scientifics), 0.2 mM of dNTP, 0.05 U/μL of Taq Polymerase and 40 ng of genomic DNA. The amplification consisted in an initial denaturation step at 95°C for 8 min, followed by 29 cycles of 15 s at 95°C, 20 s at 58°C and 60 s at 72°C, and a final elongation step at 72°C for 5 min. The PCR reaction for EF1a sequencing was performed in 30 μL mix, containing 1X of PCR buffer, 1.5 mM of MgCl₂, 0.3 μM of EF1-f and EF1-r primers (Thermo Fisher Scientifics), 0.2 mM of dNTP, 0.025 U/μL of Taq Polymerase and 10 ng of genomic DNA. The amplification consisted in an initial denaturation step at 95°C for 5 min, followed by 29 cycles of 60 s at 95°C, 90 s at 62°C and 60 s at 72°C, and a final elongation step at 72°C for 5 min. The PCR reaction for β-tubulin sequencing was performed in 25 μL mix, containing 1X of PCR buffer, 1.5 mM of MgCl₂, 0.2 μM of both Bt2a and Bt2b primers (Thermo Fisher Scientifics), 0.2mM of dNTP, 0.025 U/μL of Taq Polymerase and 10 ng of genomic DNA. The amplification consisted in an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 60 s at 94°C, 60 s at 61°C and 60 s at 72°C, and a final elongation step at 72°C for 5 min. The PCR reaction for 18S sequencing was performed in 25 μL mix, containing 1X of PCR buffer, 1.5 mM of MgCl₂, 0.2 μM of both nu-ssu-1196 and nu-ssu-0814 primers (Thermo Fisher Scientifics), 0.2 mM of dNTP, 0.025 U/μL of Taq Polymerase and 15 ng of genomic DNA. The amplification consisted in an initial denaturation step at 95°C for 5 min, followed by 29 cycles of 60 s at 95°C, 60 s at 55°C and 90 s at 72°C, and a final elongation step at 72°C for 7 min. PCR products were send to Eurofins Scientific (Ebersberg, Germany) for sequencing using corresponding primers and both forward and reverse sequences were aligned by *de novo* process using Geneious software to design a consensus sequence. For each molecular marker, consensus sequences were aligned using MUSCLE software (<https://www.ebi.ac.uk/Tools/msa/muscle/>) and rigorous identical sequences were clustered in taxa. All fungal assignments were performed on the basis of the best hit displaying query coverage and identity higher than 99 % or on the highest

values when the best match was lower. Fungal classification was proposed according to Mycobank (<http://www.mycobank.org>) and the Index Fungorum (<http://www.indexfungorum.org>) databases.

2.4. Statistical analysis

The significance of the impact of the four studied variables (ecological habitat, incubation medium, incubation temperature, and seasons) on the fungal diversity was evaluated by using the Pearson's Chi-squared test. This evaluation was performed at three taxonomical levels being the division, the class and the genus (or the most accurate taxonomical assignment). Specific χ^2 tests for independence of variables were processed taking into consideration the number of isolates and the number of taxa at different taxonomic levels (phylum, class and genus).

3. Results

3.1. Global fungal diversity associated with *P. palmata* (phylum, class and genus level)

A total of 308 fungal isolates belonging to 147 different taxa were obtained including 276 isolates (90 %) of Ascomycota (118 taxa, representing 80 % of the total diversity) and 32 isolates (10 %) of Basidiomycota (29 taxa, representing 20 % of the total diversity) (**Annexe 2**). The assignation of fungal isolates was sometimes established at genus or higher taxonomic levels (family, order, class, subdivision). Global diversity of fungal isolates at the class level is summarized in **Figure II. 2**.

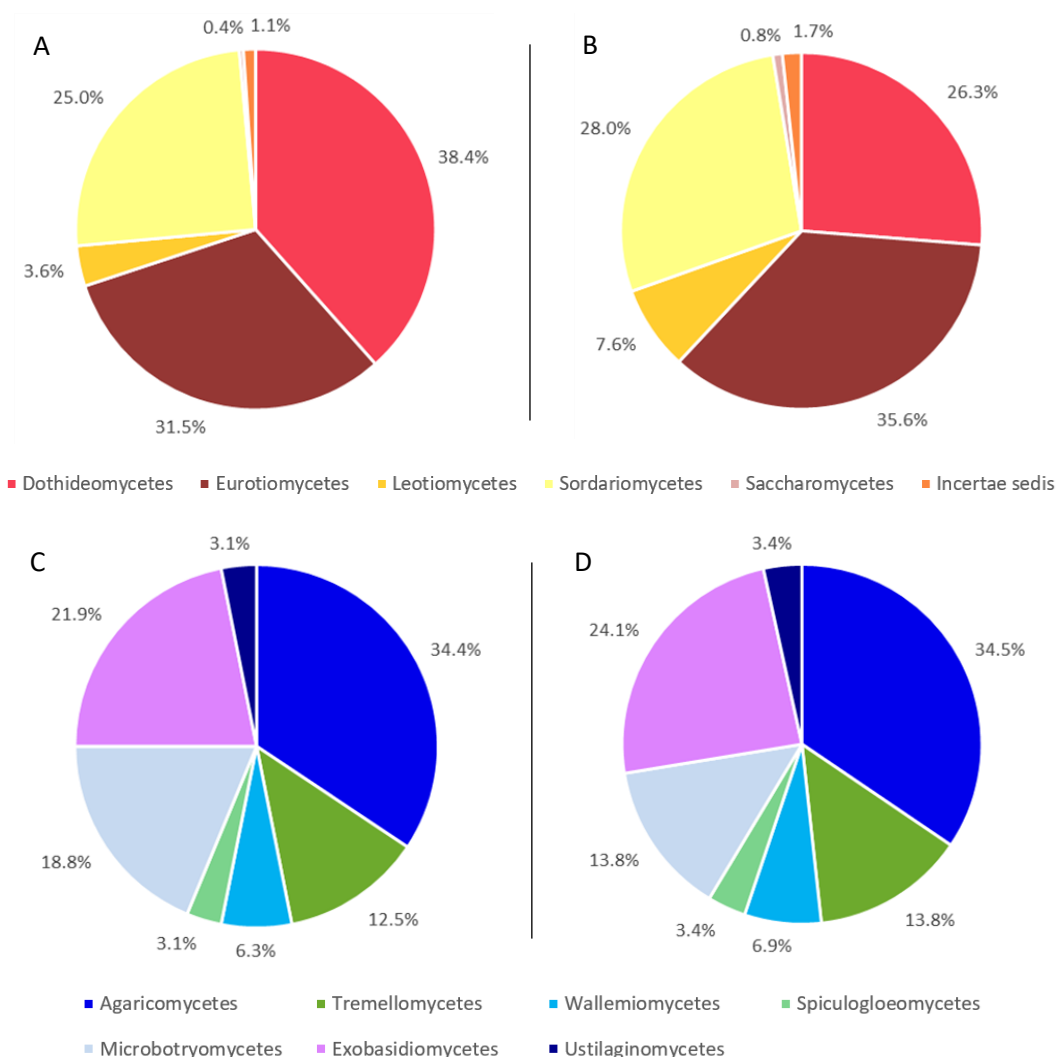


Figure II. 2: Fungal class diversity from *Palmaria palmata*. Quantitative distribution of the 276 Ascomycota strains (90 % of total strains) (A). Qualitative distribution of the 118 Ascomycota taxa (80 % of total taxa) (B). Quantitative distribution of the 32 Basidiomycota strains (10 % of total strains) (C). Qualitative distribution of the 29 Basidiomycota taxa (20 % of total taxa) (D).

Differences in terms of abundance and species richness could be noticed between fungal classes. Interestingly, Dothideomycetes isolates represented 38.4 % of total isolates from Ascomycota (**Figure II. 2A**) but this percentage dropped to 26.3 % considering the number of taxa (**Figure II. 2B**). Comparatively, Eurotiomycetes were characterized, within Ascomycota, by an abundance of 31.5 % and a specific richness reaching 35.6 %. Sordariomycetes were the third major class of Ascomycota, representing 25 % in terms of isolate's number and 28 % considering the number of taxa. The four major fungal classes belonging to Basidiomycota were Agaricomycetes, Exobasidiomycetes, Microbotryomycetes and Tremellomycetes, representing 34.4 %, 21.9 %, 18.8 % and 12.5 % of Basidiomycota isolates, and 34.5 %, 24.1 %, 13.8 % and 13.8 % of Basidiomycota taxa, respectively (**Figure II. 2C, D**). Interestingly, global trends between abundance and species richness at the class

taxonomic level, either for Ascomycota or Basidiomycota phyla, appear comparable, clearly indicate a relatively high diversity.

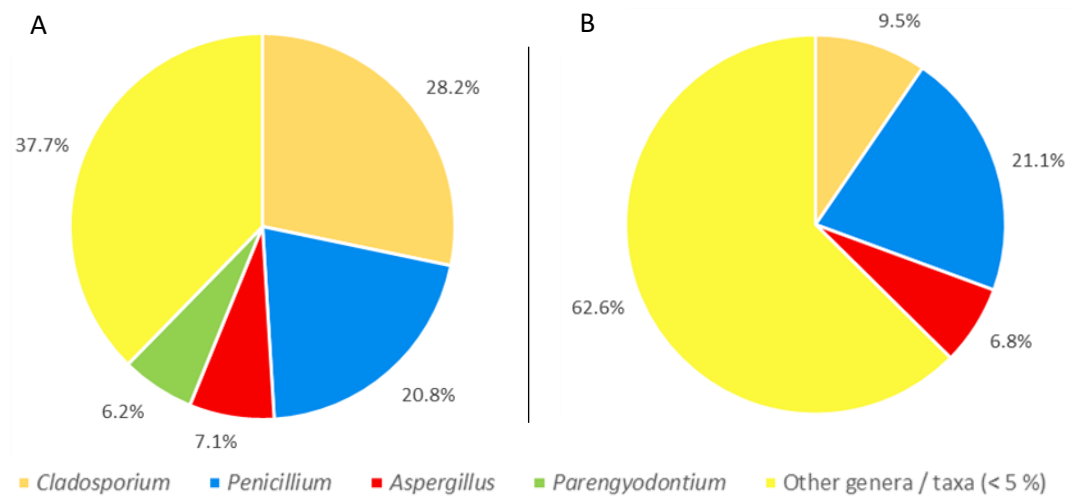


Figure II. 3: Main fungal genera from *Palmaria palmata*. Quantitative distribution of the main genera (represented by more than 5% of total isolates) (A). Qualitative distribution of the main genera (represented by more than 5% of total taxa) (B).

At the genus level, the cultivable fungal diversity appears characterized by four predominating genera affiliated to the phylum Ascomycota, *i.e.* *Cladosporium*, *Penicillium*, *Aspergillus* and *Parengyodontium* with 28.2%, 20.8%, 7.1%, and 6.2% of total fungal isolates, respectively (**Figure II. 3**). Several taxa of these major genera were over-represented by a great amount of isolates as for example for *Cladosporium* sp. I, II and III (**Annexe 2**). Other genera were represented by less than 5% of the global amount of fungal strains such as *Acremonium* (3.2%), *Calcarisporium* (2.3%), *Lecanicillium* (1.6%), *Stachybotrys* (1.6%) and *Sporobolomyces* (1.3%) (data not shown), but all together covered 37.7% of the diversity, clearly highlighting the non-trivial occurrence of low-abundant taxa associated to *P. palmata*. The high representation of several genera in terms of isolate numbers is not necessarily linked with their representation in terms of taxon numbers (**Figure II. 3**). Then, *Cladosporium* genus represented 28.2% of total isolates but 9.5% of total taxa. Similarly, *Parengyodontium* genus represented 6.2% of total isolates by only one taxon (*Parengyodontium album*). *Penicillium* genus was similarly represented considering isolate or taxon numbers, as well as *Aspergillus*.

3.2. General impact of isolation and environmental parameters on the global fungal community

Isolation and culture of fungi from *P. palmata* were performed through four variables: location in alga (epiphyte, endophyte and "epi/endo"), temperature (12°C and 27°C), culture medium (Wickerham or

"*P. palmata*" medium) and sampling period (summer 2019, autumn 2019, winter 2019 and spring 2020). The impacts of these factors on the number of isolates are indicated in **Figure II. 4**, considering the total fungal community without taxonomic distinction.

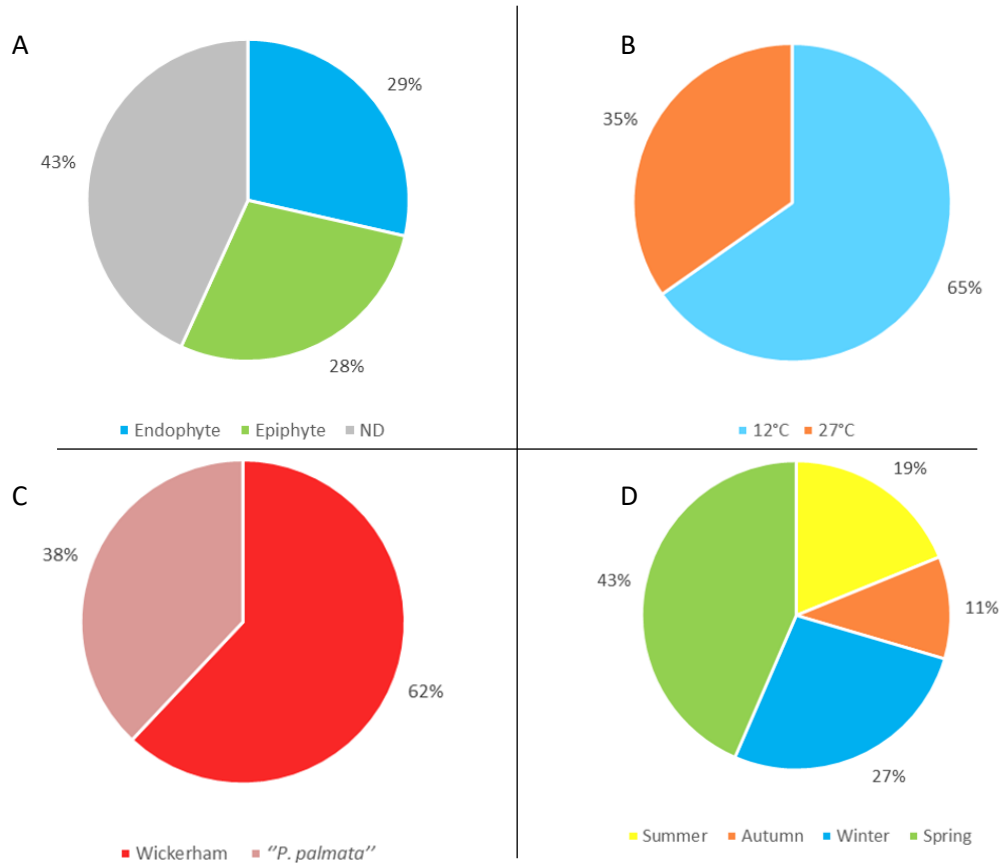


Figure II. 4: Distribution of the global fungal community of *Palmaria palmata* according to the algal location (A), incubation temperature (B), culture medium (C) and sampling period (D).

Both epiphytic and endophytic cultivable fungal communities associated with *P. palmata* were characterized by similar proportion of isolates over one year, with 87 (28 %) epiphytes and 88 (29 %) endophytes isolates. Here, 133 isolated (43 %) have been obtained with the non-selective isolation protocol and could thus not be attributed neither to epiphytic nor endophytic communities. The best incubation conditions to allow the obtention of numerous isolates seemed to correspond to the use of Wickerham medium and a temperature of 12°C.

The impact of seasonality on sampling seems to have less contrasted impact on the amount of isolates. Spring was the season with the highest amount of isolates, whereas autumn was the season characterized by the lowest one, with 43.6 % and 10.7 % of total isolates, respectively. Summer and winter appear as intermediates with 18.8 % and 26.9 % of total isolates, respectively. Without any intention to establish early conclusion, the dynamic of fungal abundance appears to peak during spring

and then to decrease from summer to autumn before starting increasing from winter to peak again during spring. Whether this trend is periodic and resilient needs further investigation.

3.3. Impacts of isolation and environmental parameters on the cultivable fungal diversity

The relative repartition of each fungal taxon according to the four studied parameters (ecological habitat, culture medium, incubation temperature and season) is represented as a bubble plot in **Figure II. 5.**

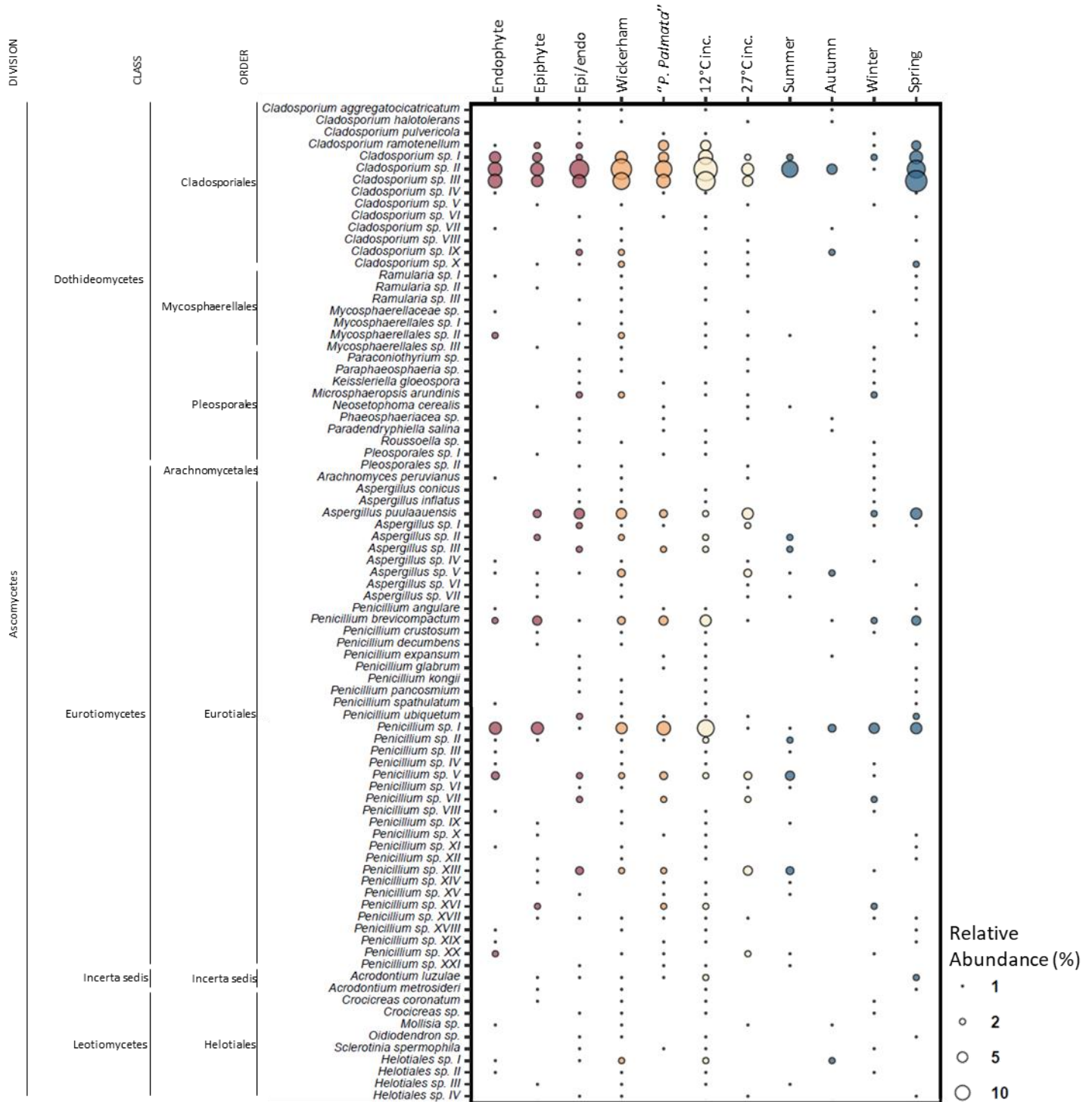


Figure II. 5: List of the cultivable fungi isolated from *Palmaria palmata*. The Bubble plot represents the classification of each taxa isolated (Division, Order, Class) and their relative abundance according to the different parameters assessed: ecological habitat (red), culture medium (orange), incubation temperature (yellow) and season (blue).

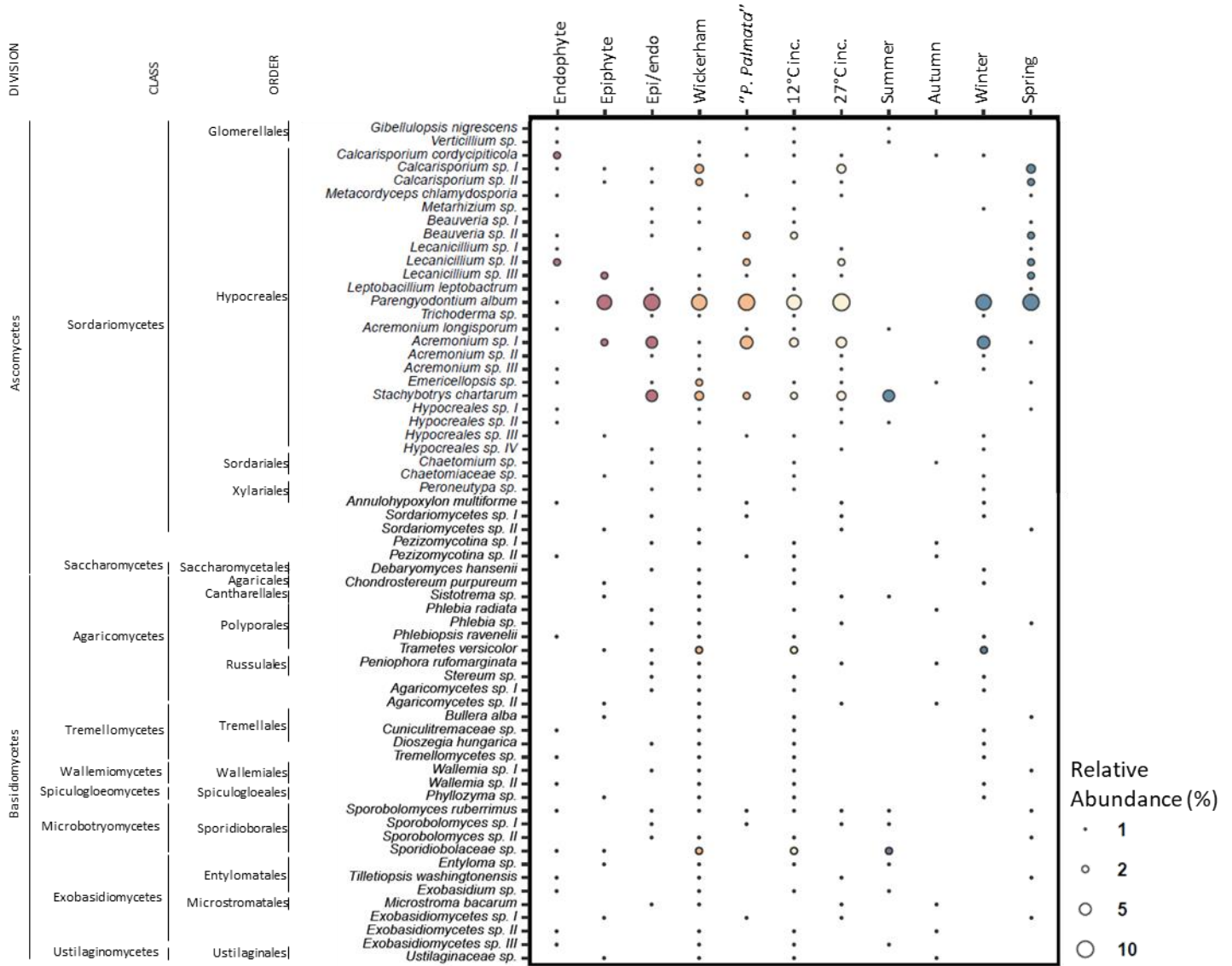


Figure II. 5: Cont.

3.3.1. Influence of variables on fungal diversity

3.3.1.1. Temperature

Temperature has a real impact on culturing efficiency. An incubation temperature of 12°C led to the isolation of 105 taxa, represented by 255 isolates, while an incubation temperature of 27°C generated 63 taxa, represented by 211 isolates (**Figure II. 4**). Interestingly, the overlap between these 2 conditions is low with only 21 taxa, meaning ~14 % of the diversity, but represented by 158 isolates. These overlapping taxa are mainly Ascomycota and mostly represented by ubiquitous genera such as *Cladosporium*, *Penicillium*, *Aspergillus*, *Acremonium* / *Emericellopsis*, or *Calcarisporium*. The conducted χ^2 tests for independence of variables did not show dependence between temperatures and the established diversity at the phylum level (neither in terms of isolate's number nor in terms of number of taxa) but showed dependence at the class level only when considering the isolate's number (p-value < 0.05) and a high dependence when focusing on the three main classes Dothideomycetes, Eurotiomycetes and Sordariomycetes (p-value < 0.001). At the genus level with a specific focus on the three main genera, *i.e.* *Cladosporium*, *Penicillium* and *Aspergillus*, the χ^2 tests for independence of variables did show a high dependence when considering the isolate's number (p-value < 0.001) but not when considering the number of taxa.

Taxa with the most isolates obtained through an incubation at 12°C corresponded to *Cladosporium* sp. II, *Cladosporium* sp. III, *Penicillium* sp. I and *Cladosporium* sp. I (**Figure II. 5**), with 28, 18, 14 and 10 strains isolated, respectively. Six isolates of *Penicillium brevicompactum* were obtained at 12°C. All the five isolates of *Cladosporium ramotenellum* and the two isolates of taxa *Aspergillus* sp. III, *Acrodontium luzulae*, *Trametes versicolor*, *Beauveria* sp. II, Helotiales sp. I, *Aspergillus* sp. II, *Penicillium* sp. XVI, *Penicillium* sp. II and Sporidiobolaceae sp. were obtained with a 12°C incubation. Many of the above-mentioned taxa belonged to Eurotiomycetes and Dothideomycetes, corresponding to the two major cultivable fungal classes associated with *P. palmata* (**Figure II. 2**). *Trametes versicolor* and Sporidiobolaceae sp. taxa, with a putative preference to a 12°C incubation temperature, belonged to basidiomycetes.

Only 116 strains were obtained through an incubation at 27°C. Among the taxa characterized by a more important number of isolates obtained with a 27°C than a 12°C temperature incubation, 11 and 6 strains belonged to *Parengyodontium album* and *Aspergillus puulaauensis*, respectively (**Figure II. 5**). All the four strains of *Penicillium* sp. XIII were isolated at a 27°C incubation temperature. Six other taxa were characterized by a total of two or three isolates, all isolated at 27°C. They corresponded to *Aspergillus* sp. V, *Calcarisporium* sp. I, *Aspergillus* sp. I, *Penicillium* sp. VII, *Lecanicillium* sp. II, *Penicillium* sp. XX. Even if *Cladosporium* sp. II and sp. III taxa were characterized by 7 and 5 strains isolated at 27°C, respectively, they also counted 28 and 18 strains isolated at 12°C, respectively. All taxa with a putative

27°C incubation temperature preference belonged to Eurotiomycetes or Sordariomycetes classes (Ascomycota), but no Dothideomycetes was detected.

3.3.1.2. Culture medium

Culture medium composition has an important impact on culturing rate. The use of Wickerham media led to the isolation of 115 taxa, represented by 267 isolates, while the use of the *P. palmata* medium generated 52 taxa, represented by 199 isolates (**Figure II. 4**). Interestingly, the overlap between these 2 media is low with only 20 taxa, meaning ~14 % of the diversity, but represented by 158 isolates. These overlapping taxa are mainly ascomycetes and represented by ubiquitous genera such as *Cladosporium*, *Aspergillus*, *Penicillium*, *Acremonium*, *Parengyodontium* and *Stachybotrys*. The conducted chi² tests for independence of variables showed dependence between the variable “culture medium” and the established diversity at the phylum level, either in terms of isolate’s number (p-value < 0.001) and number of taxa (0.006). At the class level, the conducted chi² tests only showed dependence in terms of isolate’s number (p-value < 0.05). Chi² tests with a focus on the three main classes and the three main genera did not show any dependence.

Within the 35 isolates of *Cladosporium* sp. II and the 23 of *Cladosporium* sp. III, 21 and 14 were isolated from the Wickerham culture medium (**Figure II. 5**). Moreover, among the 12 isolates of *Cladosporium* sp. I and the 8 isolates of *Aspergillus puulaauensis*, 7 and 5 were isolated from Wickerham culture medium, respectively. The twelve taxa of *Aspergillus* sp. V, *Calcarisporium* sp. I, *Cladosporium* sp. IX, *Microsphaeropsis arundinis*, *Calcarisporium* sp. II, *Cladosporium* sp. X, *Emericellopsis* sp., *Mycosphaerellales* sp. II, *Trametes versicolor*, Helotiales sp. I, *Aspergillus* sp. II, Sporidiobolaceae sp. were characterized by a total or two or three isolates exclusively obtained on Wickerham medium. The three main classes Eurotiomycetes, Sordariomycetes and Dothideomycetes characterizing fungal community of *P. palmata* were represented among the above-mentioned taxa.

Among the 15 isolates of *Penicillium* sp. I and the 7 isolates of *Acremonium* sp. I, 9 and 6 strains were isolated from “*P. palmata*” culture medium, respectively. All the 5 isolates of *Cladosporium ramotenellum* were isolated from “*P. palmata*” culture medium. *Penicillium* sp. VII, *Lecanicillium* sp. II, *Aspergillus* sp. III, *Beauveria* sp. II and *Penicillium* sp. XVI were exclusively isolated with ‘*P. palmata*’ medium and belong to Ascomycota and either to Eurotiomycetes, Sordariomycetes or Dothideomycetes.

3.3.1.3. Location in the algae (endophyte / epiphyte)

Location in the algae seems to have a limited importance on culturing rate. The endophytic-based approach led to the isolation of 54 taxa, represented by 88 isolates, while the epiphytic-based approach generated 49 taxa represented by 87 isolates (**Figure II. 4**). The non-targeted approach

(epi/endo) led to the isolation of 77 taxa represented by 133 isolates, clearly highlighting an improved isolation rate. However, the endo-/epiphytic approaches generated 40 and 32 taxa that were not revealed by the non-targeted approach, respectively. This seems to underline the importance of the diversification of culture conditions to access a higher scale of fungal representatives. However, this can also be weighed a bit when considering χ^2 tests of independence since no dependence was visualized either at the phylum, class or genus levels, and also when considering the number of isolates or of taxa, clearly strengthening the idea that sharp analyses distinguishing endophyte and epiphyte did not impact culturing efficiency.

Among the 147 total taxa, 54 were characterized by at least one endophytic strain and belonged to both Basidiomycota and Ascomycota. Endophyte cultivable fungi were mainly represented by *Cladosporium* and *Penicillium* genera (**Figure II. 5**). Among the total isolates of *Cladosporium* sp. II (35) and sp. III (23), nine strains were characterized as endophytes in both cases. Seven and six endophytic strains of *Penicillium* sp. I and *Cladosporium* sp. I were obtained, respectively.

Considering epiphytic communities associated with *P. palmata*, 49 of the 147 total taxa were characterized by at least one epiphytic strain. Basidiomycetes and ascomycetes were both represented. Among the 35 isolates of *Cladosporium* sp. II and the 19 isolates of *Parengyodontium album*, eight strains were isolated as epiphytic strains in both cases and numerous epiphytes also belonged to *Aspergillus* and *Penicillium* genera (**Annexe 2**).

Finally, the isolation protocol without distinction between epiphytes and endophytes (epi/endo) allowed the isolation of the majority of the strains. Indeed, of the 308 total isolates, 133 could not be characterized neither as epiphyte nor as endophyte, and among the 147 total taxa, 77 counted at least one isolate without associated location. *Cladosporium* sp. II, *Parengyodontium album* and *Cladosporium* sp. III counted 18, 10 and 8 isolates without associated location, respectively. All the five *Stachybotrys chartarum* isolates could not be defined as endophytic or epiphytic.

3.3.1.4. Seasonality

Seasonality has a dramatic impact on culturing rate. A split between summer/autumn and winter/spring is clearly visible based on both number of taxa and isolate's number (**Figure II. 4**). Sampling during winter and spring led to the isolation of a close number of taxa, 59 and 56 respectively. However, sampling during spring allowed the isolation of 134 isolates, way higher compared to the 83 isolates obtained during the winter isolation campaign. χ^2 tests for independence of variables confirmed these trends with p-values close to the threshold of significance at the phylum level (p-value = 0.059) but clearly lower at the class with and without a specific focus on the three main classes (p-values = 0.0008 and 0.004, respectively) and genus level targeting the 3 main genera (p-value < 0.005).

Sampling during summer and autumn led to the isolation of 32 and 24 taxa, represented by 58 and 33 isolates, respectively. Interestingly, ~90 % of the fungal taxa were obtained at a unique season (representing 129 taxa), clearly highlighting the seasonal dynamics of this cultivable fungal community structure.

The season with the lowest number of isolates was autumn, with only 33 isolates among the 308 total strains. The taxa *Cladosporium* sp. IX and Helotiales sp. I were both characterized by a total of two strains isolated in autumn.

The isolates of *Stachybotrys chartarum*, *Penicillium* sp. II, V, XIII, *Aspergillus* sp. II, III, Sporidiobolaceae sp. were principally obtained in summer compared with the three other seasons (**Figure II. 5**). Regarding *Penicillium* sp. V and XIII, only one strain was not isolated during summer in both cases. All the five isolates of *Stachybotrys chartarum* corresponded to summer isolates. No Dothideomycetes taxon was observed within summer isolates.

Among the 7 isolates of *Acremonium* sp. I, 6 were obtained in winter. *Penicillium* sp. VII, XVI, *Microsphaeropsis arundinis*, and *Trametes versicolor* were characterized by a total of two strains isolated in winter. These strains belong to the three major classes of *P. palmata* fungal communities, corresponding to Eurotiomycetes, Sordariomycetes and Dothideomycetes, with the exception of the Basidiomycota taxon *Trametes versicolor*.

The totality of the 23 strains of *Cladosporium* sp. III was isolated in spring (**Figure II. 5**). Among the 12 isolates of *Cladosporium* sp. I, the 8 isolates of *Aspergillus puulaauensis* and the 5 isolates of *Cladosporium ramotenellum*, 8, 6 and 4 strains were obtained in spring, respectively. All the three isolates of *Calcarisporium* sp. I were characterized as spring isolates. *Lecanicillium* sp. II, III, *Beauveria* sp. II, *Penicillium ubiquestum*, *Acrodontium luzulae*, and *Calcarisporium* sp. II were characterized by a total of two strains isolated in spring. Eurotiomycetes, Sordariomycetes, and Dothideomycetes classes were represented among the taxa. No Basidiomycota taxon was detected in spring isolates.

3.3.2. Description of the behaviour of the main taxa according to the season and isolation parameters

Within the 147 total taxa, only 14 were characterized by more than two isolates and corresponded to *Acremonium* sp. I, *Aspergillus puulaauensis*, *Aspergillus* sp. V, *Calcarisporium* sp. I, *Cladosporium ramotenellum*, *Cladosporium* sp. I, II, III, *Parengyodontium album*, *Penicillium brevicompactum*, *Penicillium* sp. I, V, XIII and *Stachybotrys chartarum*.

Acremonium sp. I was represented by 7 isolates, on which six were obtained on "*P. palmata*" medium and six were obtained in winter. The isolates of *Acremonium* sp. II and III were also obtained in winter,

but were isolated on Wickerham medium. Both taxa *Aspergillus puulaauensis* and *Aspergillus* sp. V seemed to be characterized by a majority of strains isolated on Wickerham medium and incubated at 27°C. Six of the eight strains of *Aspergillus puulaauensis* were isolated in spring. A majority of *Aspergillus* strains could not be associated neither with epiphytic nor endophytic habitat, but for the ones that can be, a majority was epiphytic. Among the 22 strains of *Aspergillus*, 16 were isolated on Wickerham medium. The three strains of *Calcarisporium* sp. I were also isolated on Wickerham medium, incubated at 27°C and obtained in spring. The four main taxa of *Cladosporium* were *Cladosporium ramotenellum*, *Cladosporium* sp. I, II and III and were represented by 5, 12, 35 and 23 isolates, respectively. All of these taxa were characterized with a majority of isolates obtained with a 12°C incubation temperature. Regarding the culture medium, *Cladosporium* sp. II and III were represented by a majority of strains isolated from Wickerham medium, whereas all the five isolates of *Cladosporium ramotenellum* were isolated from "*P. palmata*" medium. All these four taxa were mainly isolated in spring. *Parengyodontium album* strains were more associated with epiphytic habitat than endophytic one but it cannot be determined for 10 strains. However, the totality of *Parengyodontium album* isolates was obtained either in winter or in spring. The four principal taxa of *Penicillium* were *Penicillium brevicompactum*, *Penicillium* sp. I, V and XIII and were respectively composed of 7, 15, 5 and 4 isolates. Four on the seven strains of *Penicillium brevicompactum* were epiphytes, whereas 3 among the 5 strains of *Penicillium* sp. V were endophytic. A great majority of strains of both *Penicillium brevicompactum* and *Penicillium* sp. I were isolated with a 12°C incubation temperature but all the 4 isolates of *Penicillium* sp. XIII were obtained with a 27°C incubation temperature. Both *Penicillium brevicompactum* and *Penicillium* sp. I taxa mainly obtained with a 12°C incubation temperature were characterized with a majority of strains obtained in winter and spring. *Penicillium* sp. V and XIII strains were mainly isolated in summer. Within the 33 remaining strains belonging to the *Penicillium* genus, 26 were obtained with a 12°C incubation temperature and 14 were isolated in spring. Considering the five strains of *Stachybotrys chartarum*, they all were isolated in summer but without any other tendency.

Considering the genus level, some genera were characterized with a high amount of underrepresented taxa, counting only one or two isolates. Thus, except genera previously mentioned, *Acrodontium*, *Beauveria*, *Lecanicillium*, *Ramularia* and *Sporobolomyces* were characterized by more than two isolates.

All strains of *Acrodontium* and *Beauveria* were isolated in spring with a 12°C incubation temperature. *Lecanicillium* genus was characterized by 5 strains all isolated in spring, and 4 of them at the 27°C incubation temperature. The three strains of *Ramularia* were isolated on Wickerham medium and from spring isolates. Among the 4 strains of *Sporobolomyces*, which was the most represented genus of Basidiomycota, two were obtained in spring and two in summer.

4. Discussion

4.1. General structuration of *P. palmata* fungal community

Among the 147 fungal taxa, corresponding to 308 isolates, 118 belonged to Ascomycota and 29 to Basidiomycota. The predominance of the phylum Ascomycota has been widely assessed by previous studies focusing on different marine habitats. Algae-inhabiting fungi are following a similar trend and are also mostly affiliated to the ascomycetes (Jones 2011; Agusman and Danging 2017; Abdul Malik *et al.* 2020; Ahmed *et al.* 2021). In the present study, the main fungal were Dothideomycetes, Eurotiomycetes and Sordariomycetes whereas Leotiomycetes and Saccharomycetes represented a minority of isolates. These three main Ascomycota classes were already often recorded as major classes or at least as abundant in several marine environments such as sediments, seawater and corals (Xu *et al.* 2014, 2018; Zhang *et al.* 2015; Li *et al.* 2016, 2019; Tisthammer *et al.* 2016; Luo *et al.* 2020; Yang *et al.* 2020; Baltar *et al.* 2021; Marchese *et al.* 2021). They are also known as main classes considering fungal isolates from marine plants and macroalgae (Panzer *et al.* 2015; Vallet *et al.* 2018; Jones *et al.* 2019; Kamat *et al.* 2020). These three classes were notably described as clearly dominant by considering the fungal community of the green alga *Flabellia petiolata* (Gnavi *et al.* 2017) and the brown alga *Padina pavonica* (Garzoli *et al.* 2018).

At the genus level, a great diversity of fungi has been observed in the marine environment. Among the genera commonly found, *Acremonium*, *Alternaria*, *Arthrinium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Geomyces*, *Gliocladium*, *Microsphaeriopsis*, *Paecilomyces*, *Penicillium*, *Phoma*, *Phomopsis*, *Trichoderma* and *Ulocladium* can be cited (Sallenave-Namont and Pouchus 2000; Bugni and Ireland 2004; Matallah-Boutiba *et al.* 2012; Abdul Malik *et al.* 2020). In the present study, the three genera *Cladosporium*, *Penicillium* and *Aspergillus* were the most represented in terms of isolate numbers and taxonomic diversity. The predominance and ubiquity of these three genera has already been mentioned in the literature considering marine environment such as deep sediments, corals or seagrasses (Panno *et al.* 2013; Li *et al.* 2016; Subrmaniyan *et al.* 2018; Xu *et al.* 2018; Jones *et al.* 2019; Yang *et al.* 2020). *Aspergillus*, *Cladosporium* and *Penicillium* were also dominant in macroalgae, where they were characterized by a broad host specificity (Suryanarayanan 2012; Abdel-Gawad *et al.* 2014; Raghukumar 2017; Abdul Malik *et al.* 2020; Kamat *et al.* 2020). For example, *Aspergillus* and *Cladosporium* genera dominated among 96 fungal algicolous isolates and *Cladosporium* and *Penicillium* were dominant considering seven Antarctic macroalgae (Furbino *et al.* 2018; Sahoo *et al.* 2021). However, caution must be taken with these observations. Indeed, these studies performed on algicolous fungal communities were mostly focused on endophytic fungi with the elimination of epiphytic ones (Abdul Malik *et al.* 2020). Regarding basidiomycetes, a comparative description with

the literature is uneasy because this phylum is represented by only one or two isolates in the present study. Nevertheless, the observed relative dominance of Agaricomycetes class among Basidiomycota has already been observed in marine environment and more precisely in Arctic sediments (Zhang *et al.* 2015).

The low taxonomic resolution of some isolates was due to lack of comparable sequences in database or because distinction between various assignments was not possible, which explain the presence of several distinct clades for some genera. Nevertheless, the aim of the present study was to give an overview of the *P. palmata* fungal community.

To the best of our knowledge, *Acremonium longisporum*, *Acrodontium luzulae*, *Acrodontium metrosideri*, *Annulohyphoxylon multiforme*, *Arachnomyces peruvianus*, *Aspergillus inflatus*, *Bullera alba*, *Calcarisporium cordycipiticola*, *Cladosporium pulvericola*, *Crocicreas coronatum*, *Dioszegia hungarica*, *Exobasidium* sp., *Keissleriella gloeospora*, *Leptobacillium leptobactrum*, *Metacordyceps chlamydosporia*, *Microstroma bacarum*, *Neosetophoma cerealis*, *Penicillium angulare*, *Penicillium pancosmium*, *Penicillium spathulatum*, *Peniophora rufomarginata*, *Phlebia radiata*, *Phlebiopsis ravenelii*, *Phyllozoma* sp. and *Sclerotinia spermophila* seem to be reported for the first time from marine environment. The 63 remaining taxa have already been described from marine environment (**Annexe 2**).

4.2. Global impacts of environmental and isolation parameters on the amount of isolated fungi

Conditions of incubation have clear influence on the number of growing isolates, as previously highlighted using χ^2 tests of independence. Indeed, the number of fungal strains isolated on Wickerham and with an incubation temperature of 12°C was almost twice higher than the number of fungal strains isolated on "*P. palmata*" medium and with an incubation temperature of 27°C, respectively. Wickerham is a rich culture medium which can easily be used for the isolation and growth of marine fungi (Kjer *et al.* 2010; Yue *et al.* 2015; Fan *et al.* 2019). In the other hand, the presence of lyophilisate of *P. palmata* as unique carbon source in the "*P. palmata*" medium, is a limiting factor for marine aspecific isolates or with low abilities to grow in such a poor and specific medium. It was then expected to observe more fungal developments on the non-selective Wickerham medium than on the selective "*P. palmata*" one. Regarding the parameter temperature, an incubation at 12°C seems to fit more with the general sea temperature at Batz-sur-Mer compared with an incubation at 27°C. This could explain the preference of a growth at 12°C for a majority of the isolates. Moreover, it has already been shown that temperature for marine fungal optimal development was generally comparable to

the temperature of environmental site (Overy *et al.* 2019; Pasqualetti *et al.* 2020), strengthening the idea of the importance of mimicking at best *in situ* conditions.

Three isolation protocols have been performed with two selective protocols (either for the strict isolation of epiphytes or endophytes) and one non-selective protocol (epi/endo). Independently of taxonomic assignation, more fungal isolates were obtained by performing the non-selective protocol than both selective isolation protocols, which resulted in comparable numbers of isolates. The non-discriminating protocol almost led to the same amount of isolated strains than the sum of those obtained with epiphytic and endophytic protocols. However, the spreading of some strains on the culture medium can inhibit the detection and the isolation of slower strains. Indeed, by taking into account the total fungal community of *P. palmata*, culture media of the non-selective protocol were frequently quickly recovered by one or two fungal fast-growers, before any possible development of putative other slow-growing isolates. Further studies could be realized by applying a dilution-to-extinction methodology to reduce the amount of these fast-growing isolates and then facilitate slow-growers isolation (Rédou *et al.* 2016). Application of this methodology was not possible in our work already dealing with a complete isolation protocol. Moreover, it is important to stay cautious with the fact that there is a great biological variability from a biological sampling to another, notably concerning fungal cultivation and isolation, resulting in a low reproducibility of the isolation procedure (Franco *et al.* 2000; Parsa *et al.* 2018, 201; Tourneroché *et al.* 2020).

Seasonality had a great and significant impact on the fungal isolation rate from *P. palmata*. Nevertheless, the low repeatability previously mentioned must also be taken into account when comparing the number of isolates obtained according to the season. Spring seemed to be the best season to isolate a great number of fungal strains associated with *P. palmata* (134 isolates), followed by winter, summer, and autumn characterized by 83, 58 and 33 isolates, respectively. Particular caution is required in the case of spring isolate over-representation, because for each season two samplings were performed at different dates. Due to the Covid-19 pandemic, the second spring sampling was performed on the 22nd of June 2020. This sampling would then rigorously correspond to a summer sampling but have been considered as a spring sampling. Moreover, the high isolation potential on spring might be an artefact due to the preference of only several easily cultivable isolates, such as *Cladosporium* sp., which were then over-represented compared with other cultivable isolates.

4.3. Effects of environmental and isolation parameters on the structuration of the fungal community diversity

Besides having an influence on the global fungal isolation, the different parameters of the present study could be related with the amount of isolates obtained at various taxonomic levels. Potential

effects of environmental and isolation parameters will be discussed on the three main classes of Ascomycota corresponding to Dothideomycetes, Eurotiomycetes and Sordariomycetes. The other Ascomycota classes and the ones of Basidiomycota seemed not to be represented by sufficient numbers of strains to be rigorously compared. Environmental and isolation parameters effects will also be discussed at the genus level and principally for the three main genera *Aspergillus*, *Cladosporium* and *Penicillium*. Because only one genus of Basidiomycota (*Sporobolomyces*) was represented by more than two isolates, the taxonomic structuration of this division was uneasy to define according to the studied environmental and isolation parameters.

The effect of incubation temperature on the development of marine fungi is studied from several decades (Curran 1980). Considering the three main fungal classes (Dothideomycetes, Sordariomycetes and Eurotiomycetes), their diversity in terms of isolate number was significantly related with the incubation temperature (p-value < 0.05), as well as the genus level by considering the three most represented genera *Cladosporium*, *Penicillium* and *Aspergillus* (p-value < 0.05). In the present study, taxa belonging to Sordariomycetes seemed to be preferentially isolated at 27°C while taxa belonging to Dothideomycetes and Eurotiomycetes seemed to be mainly isolated at 12°C. Temperature seems to be a major parameter influencing the diversity of fungal communities (Li et al. 2016, 2019; Cheung et al. 2018). Interestingly, Dothideomycetes should be particularly adapted to low temperatures, compared with other classes such as Eurotiomycetes and Sordariomycetes (Li et al. 2016, 2019). Fungi belonging to Dothideomycetes, Eurotiomycetes and Sordariomycetes isolated from the green alga *Flabellia petiolata* were mostly isolated at 25°C (compared with 15°C). This preferential incubation temperature may be partially explain by the fact that this study on cultivable fungal community associated with *Flabellia petiolata* was performed in Mediterranean Sea (Gnavi et al. 2017), which may be warmer than Atlantic Ocean and more precisely Atlantic French coasts. Moreover, the low temperature preference of Dothideomycetes observed in the present study may be due to the high amount of *Cladosporium* isolates (representing 87 % of the Dothideomycetes), preferentially isolated at 12°C. Indeed, from a study on fungi isolated from deep-sea sediments, *Cladosporium* isolates appeared particularly psychrophilic compared to other species (Wei et al. 2018).

It is well established that the nature of the culture medium has an influence on the isolation of marine fungi and impact the observed cultivable diversity (Overy et al. 2019). Algal-based culture medium has already been used for the growth of marine fungi (Gnavi et al. 2017; Garzoli et al. 2018; Fawzy et al. 2020; Landeta-Salgado et al. 2021). Nevertheless, to the best of our knowledge, the present study is the first one using the macroalga *P. palmata* as carbon source in a culture medium for isolation of fungi. Comparative studies on marine fungal diversity obtained by using a non-selective medium in one hand, and a selective algal-based medium in the other hand are particularly limited (Gnavi et al.

2017; Garzoli *et al.* 2018). The three main Ascomycota classes Dothideomycetes, Sordariomycetes and Eurotiomycetes revealed in the present study seemed to be characterized by a slight preference for the non-selective Wickerham culture medium, which corresponds to the general observation on the global fungal community. Nevertheless, within these three fungal classes, several fungal taxa were recorded to be either mainly isolated from Wickerham or "*P. palmata*" media albeit no significant impact of the nature of the culture medium on the fungal diversity was detected (a significant influence was only detected at the phylum level with a p-value of 0.0058). In the previously mentioned study on fungal community associated with *Flabellia petiolata*, one non-selective and one algal-based selective media were used for the fungal isolation. Contrary to the present study, there was comparable number of strains isolated from both selective and non-selective media, including fungi belonging to Dothideomycetes, Eurotiomycetes and Sordariomycetes (Gnavi *et al.* 2017). Divergences may be explained by the nature of the macroalgal host, by different environmental conditions and by slight differences in the isolation protocol (algal thalli sonication, homogenate filtration, incubation temperature, etc.). Results of the study of Garzoli *et al.* (2018) on the fungal cultivable diversity isolated from *Padina pavonica* are more in accordance with the present study, where a majority of the taxa were isolated from a non-selective medium and a minority from an algal-based one. Regarding basidiomycetes, in the present study, no taxa seemed to be preferentially isolated on the selective "*P. palmata*" medium. Indeed, a significant impact of the nature of culture medium on the diversity in terms of isolate number at the taxonomic level of division was established (p-value < 0.05). It can then be hypothesized that basidiomycetes and particularly Agaricomycetes would not be well adapted to *P. palmata*.

Algicolous fungi can either be characterized as epiphytes when they colonize the macroalgal surface, or as endophytes when they develop in the inner macroalgal tissues. The endophytic fungal communities are more studied than epiphytic ones (Abdel-Gawad *et al.* 2014; Abdul Malik *et al.* 2020). Three protocols were conducted in the present study in order to selectively isolate either epiphytes, endophytes, or fungal strains without selectivity. A majority of isolates were obtained by performing the non-selective isolation protocol (133 isolates on a total of 308). It was then not possible to establish clear repartitions and trends on the preferred ecological habitat of observed taxa. For example, *Parengyodontium album* was characterized by 8 epiphytic and 1 endophytic strains, but also by 10 isolates belonging to the non-determined location. A previous study described *Engyodontium* (syn. *Parengyodontium*) as a major macroalgal endophyte, but all isolates were obtained from a green alga and no strain was isolated from red algae (Sarasan *et al.* 2020). Besides, only referring on selective isolation procedures, epiphytes and endophytes are generally similarly distributed among taxonomic groups. It was notably the case regarding the main classes Dothideomycetes, Eurotiomycetes and

Sordariomycetes. According to the present study, *Cladosporium*, *Penicillium* and *Aspergillus* could be considered as major endophytic genera compared with other fungal genera but this over-representation was due to an absolute domination, also extended to epiphytes strains. It has already been observed that fungal strains of *Penicillium* and *Aspergillus* isolated from macroalgae were over-represented in both epiphytic and endophytic communities (Abdel-Gawad *et al.* 2014; Fan *et al.* 2019). In the present study, the parameter of ecological habitat (epiphyte, endophyte, epi/endo) was not significantly related with the algicolous fungal diversity, neither at the division level, nor at the class level, nor at the genus level.

The season is often related with the temperature which has been previously mentioned as impacting the marine fungal diversity (Wang *et al.* 2021). Moreover, the season influences the macroalgal protein and carbohydrate composition, notably in *P. palmata* (Galland-Irmouli *et al.* 1999; Dumay *et al.* 2014; Laurens *et al.* 2020; Sasaki and Yoshikuni 2022). As mentioned before, the substrate composition also influences the development of fungi and the observed diversity in terms of isolate number, which is then expected to change according to variations in macroalgal host composition. In the present study, the impact of season on the fungal diversity was significant at both class and genus level (p -value < 0.05) and was near the significance limit at the division level (p -value = 0.05898). At the class level, both Dothideomycetes and Sordariomycetes had not particular affinity with summer, except for *Stachybotrys chartarum* for which the 5 strains were isolated in summer. Regarding Eurotiomycetes, several taxa of *Aspergillus* and *Penicillium* genera were mostly isolated during summer. Only 33 isolates were obtained during autumn among the 308 total fungal isolates. Winter was not particularly related with any isolation preference at the class level, except considering Agaricomycetes, the main class of Basidiomycota, for which 9 strains among the 15 total isolates were obtained. Considering ascomycetes, several taxa such as *Microsphaeropsis arundinis*, *Acremonium* sp. I, or belonging to *Penicillium* genus may be related by a preferred isolation in winter but no particular trends were observed at the class level. *Cladosporium* and most generally Dothideomycetes were recorded as mainly isolated from seawater in winter (Wang *et al.* 2021). The over-representation of spring isolates compared with other seasons was principally due to *Cladosporium* strains, which represented 41 % of the whole isolates obtained during spring. Because this genus represented 82 % of the Dothideomycetes in terms of number of isolates, this latter had a clear preference for spring. Nevertheless, this spring preference was also observed for both Eurotiomycetes and Sordariomycetes. In contrast to the present study, Abdel-Gawad *et al.* (2014) established that autumn and winter would result in a higher algicolous fungal diversity than spring and summer. The present observations and conclusions performed comparing fungal isolates diversity with seasons must be cautiously considered even if a significant impact of the seasonality was established on the fungal diversity in terms of isolate

number at both class and genus levels. Total isolate numbers obtained from each season were not similar, which constitute an interesting result but make uneasy a rigorous comparison of diversity between all the four seasons.

4.4. Environmental and isolation parameters impacts on main fungal taxa

On the total of 147 fungal taxa isolated from *P. palmata*, only 14 were characterized by more than two isolates on which descriptive trends according to the different environmental and isolation parameters were possible. These 14 taxa belonged to 7 genera, corresponding to *Acremonium*, *Aspergillus*, *Calcarisporium*, *Cladosporium*, *Parengyodontium*, *Penicillium* and *Stachybotrys*.

The *Acremonium* genus seemed particularly related with a winter isolation and the principal taxa (*Acremonium* sp. I) was mainly isolated from "*P. palmata*" medium. This observed selective adaptation of *Acremonium* for a macroalgal host has already been documented, with *Acremonium fuci* conidia germinating only in presence of *Fucus serratus* tissues (Zuccaro *et al.* 2003; Teixeira *et al.* 2019).

The isolation of *Aspergillus* isolates seemed not to be particularly related neither to the incubation temperature, nor to the season. Nevertheless, *Aspergillus* has previously been described as main genus isolated in summer in seawater compared with spring and winter (Wang *et al.* 2021). This over-representation of *Aspergillus* isolates during summer, compared with other taxa, has also been observed in macroalgae, but this genus was similarly isolated during the three remaining seasons (Abdel-Gawad *et al.* 2014). Regarding the effect of the nature of the culture medium, *Aspergillus* isolates seemed to be preferentially isolated from non-selective Wickerham medium and characterized as more epiphytic than endophytic, even if the observed differences were not significant. This potential epiphytic strategy, along with a low affinity with the "*P. palmata*" selective medium may be related to a global strategy of superficial widely non-selective colonization of this genus. Interestingly, in Sarasan *et al.* (2020), *Aspergillus* isolates had not any host preference among the three macroalgal phyla, and were obtained from 7 to the 11 studied macroalgae. This ability to colonize a wide host variety has also been observed in mangrove, where *Aspergillus* has been described as the most speciose fungal genus (Devadatha *et al.* 2021). Furthermore, this genus has already been characterized in marine environments as ubiquitous with various ecological strategies such as saprophytic, endophytic or pathogenic (Ramírez-Camejo *et al.* 2012; Burge *et al.* 2013; Wong Chin *et al.* 2021).

The four main *Cladosporium* taxa were preferentially isolated at 12°C from spring isolates. It has already been reported that marine-derived strains of *Cladosporium* were particularly adapted to low temperatures (Gonçalves *et al.* 2013; Del-Cid *et al.* 2014; Duarte *et al.* 2018). Even if more *Cladosporium* strains were isolated from the non-selective Wickerham medium compared with the selective "*P. palmata*" medium, the difference was not significant. Moreover, no particular link could

be established considering the impact of the isolation protocol on the structuration of this fungal genus within *P. palmata*.

Even if *Parengyodontium album* was one of the most represented taxa in the present study, no clear impact of the different parameters of this study was observed. However, all 19 *Parengyodontium album* isolates were obtained during winter and spring.

Several *Penicillium* taxa were characterized by opposite trends in response to environmental and isolation factors in the present study, but global trends can be mentioned. All taxa represented at least by two isolates were isolated from "*P. palmata*" medium, suggesting here a host adaptation. Even if several taxa, such as *Penicillium* sp. XIII, were mainly isolated at 27°C, 75 % of the total strains were isolated at 12°C. It has already been reported that marine-derived *Penicillium* strains were particularly adapted to low temperatures (Gonçalves *et al.* 2013; Del-Cid *et al.* 2014; Duarte *et al.* 2018). The principal marine fungal genus found in association with mussels and marine sediments was *Penicillium* and isolates were mostly obtained with a 12°C incubation temperature when compared with 27°C (Matallah-Boutiba *et al.* 2012). In the present study, most of the *Penicillium* taxa were isolated during winter and spring, but several taxa (*Penicillium* sp. V and XIII) seemed preferentially observed in summer isolates. Spring and winter preference of marine *Penicillium* strains has already been documented (De Moura Sarquis and De Oliveira 1996). Moreover, it has been previously observed in marine sediments that winter resulted in higher *Penicillium* diversity compared with summer (Park *et al.* 2019). Whether representatives of the *Penicillium* genus are physiologically adapted to low temperatures remains an open question and needs further investigation.

Traditionally, the majority of studies on the diversity of marine fungal communities are mainly based on cultivable fungal diversity (Goecke and Imhoff 2017). Nevertheless, recent molecular-based studies on marine fungal diversity associated with macroalgae revealed that total diversity would be composed equally by ascomycetes and basidiomycetes (Tourneroché *et al.* 2020). The diversity of cultivable fungi would then not necessarily be representative of total diversity, even if it could be the case according to the substrate (Zuccaro *et al.* 2008). Comparison between cultivable and non-cultivable fungal communities may allow to give supplementary information and discussions about the ecological pertinence of cultivable diversity compared with total diversity. Confrontation of the present cultivable results with an investigation on the non-cultivable fungal diversity associated with *P. palmata* by metabarcoding approach would then allow to determine the ecological pertinence of this cultivable diversity.

Fungal strains isolated from macroalgae have been described as producers of bioactive compounds with numerous biological activities such as antimicrobial, antioxidant, antitumoral, antialgal or

insecticide activities (Zhang *et al.* 2009; Suryanarayanan *et al.* 2010; Suryanarayanan 2012; Flewelling *et al.* 2013; Hong *et al.* 2015; Sarasan *et al.* 2017; Kamat *et al.* 2020). Algicolous fungi have also been characterized as promising producers of hydrolyzing enzymes such as cellulolytic, β -glucosidase and endoglucanase activities (Hong *et al.* 2015; Lee *et al.* 2019; Ogaki *et al.* 2019). Many other enzymatic activities linked with marine fungi have been discussed in several bibliographic reviews (Damare *et al.* 2012, 201; Velmurugan and Lee 2012; Bonugli-Santos *et al.* 2015; Balabanova *et al.* 2018; Mzui and Ch 2018; de Vries *et al.* 2020; Le Strat *et al.* 2022). It would then be of particular interest to determine the biotechnological potentials of the fungal strains isolated from *P. palmata* and more precisely by focusing on their xylanolytic and cellulolytic activities considering that cell wall of *P. palmata* is mainly composed of xylan and cellulose (Morgan *et al.* 1980; Lahaye *et al.* 1993; Dumay *et al.* 2014; Naseri *et al.* 2020).

5. Conclusion

In conclusion, the present study is the first wide description of the cultivable mycobiota of the red alga *Palmaria palmata*. This work has resulted in a large collection of 308 fungal isolates belonging to 147 taxa. Ascomycetes were considerably more isolated than basidiomycetes and were mainly represented by the three classes Dothideomycetes, Eurotiomycetes and Sordariomycetes. An incubation at 12°C based on the non-selective Wickerham culture medium was particularly efficient for isolating a significant number of fungi, in comparison with an incubation at 27°C on a selective "*P. palmata*" medium. Interestingly, Sordariomycetes seemed to be preferentially isolated at 27°C, while Dothideomycetes and Eurotiomycetes at 12°C. According to the various isolation protocols performed in this study, most of the isolates could not be characterized neither as epiphytes nor as endophytes. The influence of season on fungal diversity in terms of isolate number differed according to the considered taxa but was significant at both class and genus levels. The genera *Cladosporium*, *Penicillium* and *Aspergillus* highlighted in the present study can see their roles strengthened as ubiquitous in the marine environment, since *P. palmata*, as many other marine habitats and marine algae, appears as hosting such taxa in large majority. The present study was performed on one year. It would be interesting to verify the observed seasonal trends by performing another comparable work on another year. Finally, fungi isolated from *Palmaria palmata* could represent promising producers of hydrolyzing enzymes such as xylanolytic and cellulolytic activities.

Postface

Nous venons de décrire la diversité d'une vaste communauté fongique associée à *P. palmata*. Certaines conclusions ont pu être établies quant à la structuration de cette communauté cultivable dans son ensemble et son évolution en fonction de différents paramètres environnementaux et culturels. Cependant, la fonge cultivable ne représente qu'une infime partie de la diversité totale. En effet, chez les champignons marins issus d'algues, cette partie cultivable ne représenterait que jusqu'à 1 ou 2 % de la communauté fongique totale (Agusman and Danging 2017; Garzoli et al. 2018).

Cette problématique a été prise en compte dès le début de ces travaux et a motivé l'élaboration d'un protocole basé sur une approche de metabarcoding afin d'évaluer le profil de diversité de la communauté fongique totale associée à *P. palmata*. Ce profil de diversité totale avait vocation à être confronté à la diversité cultivable précédemment décrite, et à déterminer la pertinence écologique de cette dernière.

Ainsi, lors des missions de récoltes réalisées pour l'isolement fongique à partir de thalles de *P. palmata*, certains thalles ont été conservés pour l'analyse de la fonge totale. Pour chaque récolte, un carré d'environ 1 cm² a été découpé dans 10 thalles algaux différents, puis a été rincé à l'eau distillée. Les 10 fragments d'algues ainsi traités ont ensuite été stockés à -20°C afin de servir ultérieurement de matrice pour l'extraction d'ADN total. Parallèlement, lors de chaque récolte d'algues, de l'eau de mer correspondant au site de récolte a été prélevée puis filtrée au laboratoire à travers un filtre Whatman 47 µm afin de retenir les éléments macroscopiques. Le filtrat récupéré a ensuite été filtré de nouveau à travers un filtre 0,4 µm afin de retenir l'ADN total en suspension dans l'eau et comprenant l'ADN fongique. Ces derniers filtres ont été conservés à -20°C et leur but était de servir de témoin caractérisant le bruit de fond d'ADN fongique de l'environnement marin étudié pour chaque récolte d'algue.

Afin de limiter le nombre d'échantillons à utiliser *via* le protocole de metabarcoding, une étude de communauté fongique totale par saison a été envisagée, contrairement à l'étude de la communauté fongique cultivable caractérisée par deux missions d'isolement par saison. Ainsi, seuls les échantillons d'algues préparés suite aux récoltes du 19/06/2019 (été), du 16/09/2019 (automne), du 09/01/2020 (hiver) et du 25/05/2020 (printemps) ont effectivement été utilisés pour l'analyse metabarcoding. La **Figure II. 6** résume le nombre d'échantillons sur lesquels le protocole a été effectué.

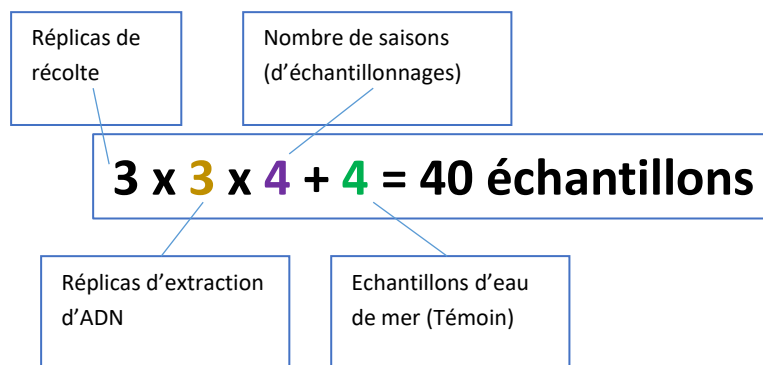


Figure II. 6: Récapitulatif du nombre réduit d'échantillons d'ADN à utiliser pour le metabarcoding sur la base d'un échantillonnage par saison pendant 1 an.

Après avoir finalisé les campagnes d'échantillonnage, 12 échantillons d'algue (3 échantillons par saison, préservés à -20°C) et 4 filtres d'ADN (un témoin par saison, préservé à -20°C) ont été envoyés au prestataire MrDNA pour (i) une étape d'extraction d'ADN total, et (ii) une seconde étape de metabarcoding (amplification de la région ITS2 et séquençage MiSeq). Cette dernière est basée sur une amplification de la région ITS2 (Internal Transcribed Spacers) en utilisant des amorces spécifiques aux champignons (5.8S-Fun (5'-AACTTTYRCAAYGGATCWCT-3') en amorce *forward* et ITS4-Fun (5'-AGCCTCCGCTTATTGATATGCTTAART-3') en amorce *reverse*) (Taylor and Cunliffe 2016). L'approche consiste à amplifier et séquencer les amplicons fongiques correspondant à la région ITS2 et à les assigner via la base de données UNITE (Nilsson et al. 2019). Ensuite, une analyse bioinformatique devait être réalisée via le workflow SAMBA de l'IFREMER afin de révéler (i) la diversité alpha, c'est-à-dire la diversité au sein des échantillons via les indices de diversité (Chao1, Shannon, InvSimpson) et d'équitabilité (Pielou), et (ii) la diversité beta, c'est-à-dire la comparaison entre les échantillons tout en intégrant les paramètres environnementaux afin de révéler leur impact potentiel sur la diversité. *In fine*, les diversités moléculaire et culturelle devaient être comparées afin d'obtenir plus d'informations sur la pertinence écologique des différents taxons mis en évidence par approche culturelle.

Des données brutes ont bien été générées par le prestataire MrDNA. Cependant, l'analyse bioinformatique a mis en évidence un certain nombre de biais et notamment (i) une qualité très faible des séquences ("reads") et (ii) une incohérence non expliquée concernant leur longueur et celles des séquences sens à 250pb et des séquences anti-sens à 300pb (Figure II. 7).

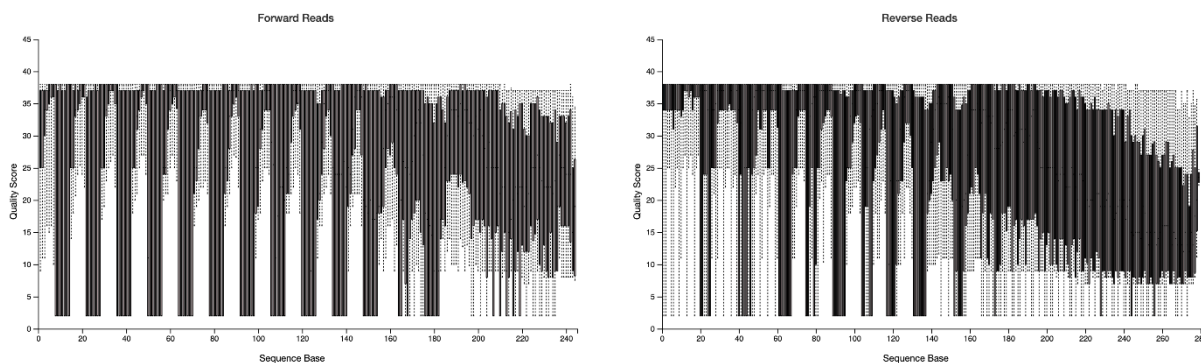


Figure II. 7: Analyse des séquences de données brutes. Représentation du score de qualité et de la taille des séquences pour les séquences sens (Forward Reads) et anti-sens (Reverse Reads).

A l'heure actuelle, ces données de diversité fongique totale issues de *P. palmata* sont donc difficilement interprétables et les raisons concrètes de ces problèmes ne sont pas encore connues et nécessitent des investigations complémentaires. Toutefois, un stock d'échantillons d'algues des récoltes concernées ayant été conservés à -20°C , il reste envisageable de reconduire ces travaux afin d'obtenir cette diversité fongique totale et la confronter à la diversité cultivable décrite dans ce chapitre.

Cette absence de données exploitables sur la diversité fongique totale n'a pas entravé la poursuite des travaux de cette thèse et notamment sur la valorisation des champignons isolés de *P. palmata* pour leur production d'enzymes xylanolytiques et cellulolytiques ciblant la dégradation pariétale de l'algue. Cette étude est présentée dans le chapitre suivant (**CHAPITRE III**).

CHAPITRE III : CRIBLAGE ENZYMATIQUE DE LA FONGE CULTIVABLE

Préface

Les enzymes produites par les microorganismes présentent un important intérêt dans diverses industries. L'un des principaux facteurs limitant à leur utilisation concrète est cependant le coût de production des enzymes qui est souvent élevé. La découverte et la sélection de souches de microorganismes (notamment fongiques) caractérisées par une production enzymatique, ainsi que l'application de techniques de culture permettant une importante production enzymatique constituent des pistes à emprunter pour réduire ces coûts de production (Chapla *et al.* 2010; Motta *et al.* 2013). Le potentiel de dégradation de polysaccharides de nouvelles souches fongiques pourrait être étudié et comparé au cas par cas avec les enzymes actuellement commercialisées (Filiatrault-Chastel *et al.* 2021). Il semble donc particulièrement important de mener des études de criblage d'activités enzymatiques sur de nouvelles souches fongiques à haut potentiel xylanolytiques et cellulolytiques (Bajaj and Mahajan 2019b).

Le chapitre précédent a décrit l'obtention d'un grand nombre de souches fongiques à partir de l'algue rouge *P. palmata* lors de collectes réalisées pendant une année. Cette algue rouge contient une importante quantité de R-PE, pigment photosynthétique rouge à fort potentiel biotechnologique et à haute valeur ajoutée (Dumay *et al.* 2013). L'optimisation de l'extraction de ce pigment par procédé enzymatique constitue l'un des axes de recherche du laboratoire ISOMer. Dans cette optique d'optimisation, l'utilisation de souches fongiques issues de *P. palmata* pourrait constituer une stratégie intéressante pour dégrader cette algue. En effet, pour pouvoir se développer sur ce substrat algal les champignons associés produisent vraisemblablement des enzymes permettant la dégradation de ce substrat.

Ainsi, les plus de 300 souches isolées à partir de *P. palmata* ont fait l'objet d'un vaste criblage qualitatif enzymatique, caractérisant plus spécifiquement la présence ou non d'activité xylanolytique et / ou cellulolytique. La **Figure III. 1** présente succinctement les différentes étapes de ce criblage à partir des souches fongiques isolées de *P. palmata*. Ces différentes étapes, les milieux de culture et le protocole dans son ensemble sont décrits plus en détail dans la suite de ce chapitre.

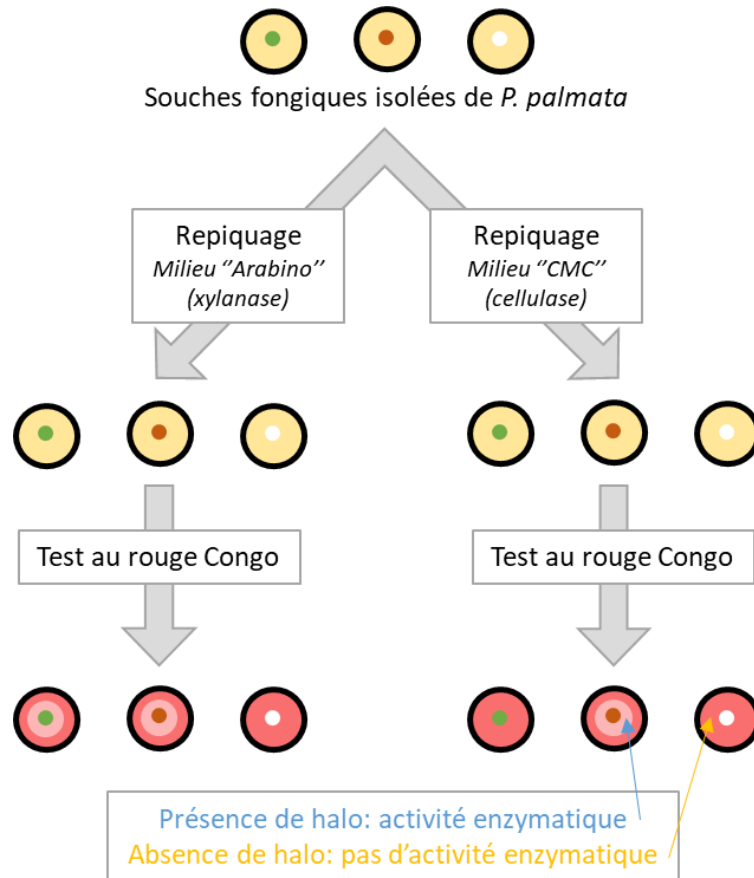


Figure III. 1 : Etapes principales du criblage enzymatique appliqué à l'ensemble des souches fongiques. Les milieux "Arabino" et "CMC" sont respectivement composés d'arabinoxylane et de carboxyméthylcellulose. L'utilisation de ces deux sources de carbone permet de cribler les activités xylanase et cellulase respectivement.

L'avancée en parallèle des travaux dans le cadre de la thèse a conduit à la réalisation d'ajustements sur la caractérisation des souches. En effet, les 308 souches criblées dans ce chapitre ne correspondent pas exactement aux 308 souches constituant la communauté fongique évaluée au niveau taxonomique dans le **CHAPITRE II (Figure III. 2)**.

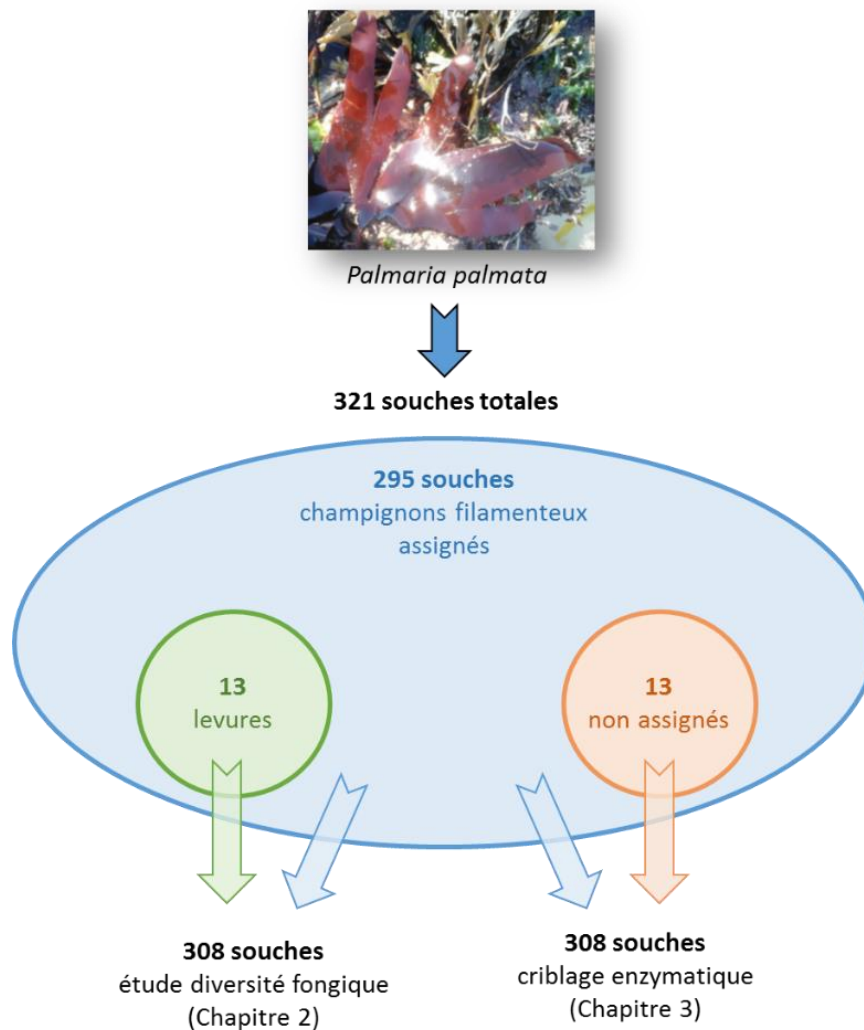


Figure III. 2 : Communautés fongiques étudiées associées à *Palmaria palmata*.

Le protocole d'assignation taxonomique détaillé dans le **CHAPITRE II** a porté sur un ensemble de 321 souches dont 308 ont été attribuées à divers niveaux taxonomiques et ont fait l'objet de l'étude. Parmi ces 308 souches, 295 correspondaient à des champignons filamenteux et 13 correspondaient à des levures. Les 13 souches non assignées et donc exclues de l'étude sur la diversité correspondaient quant à elles uniquement à des champignons filamenteux. Ces dernières, ont tout de même été intégrées dans la présente étude portant sur les impacts des paramètres environnementaux et d'isolement sur les activités enzymatiques des souches fongiques. En conséquence, les 308 souches étudiées dans le **CHAPITRE II** correspondent aux 321 souches totales sans les 13 souches non assignées et les 308 souches criblées dans ce chapitre correspondent aux 321 souches totales sans les 13 souches de levures. La correspondance totale du nombre de souches non assignées avec celui des levures est absolument fortuite.

Enzymatic screening of xylanolytic and cellulolytic activities among the cultivable fungal community isolated from *Palmaria palmata*

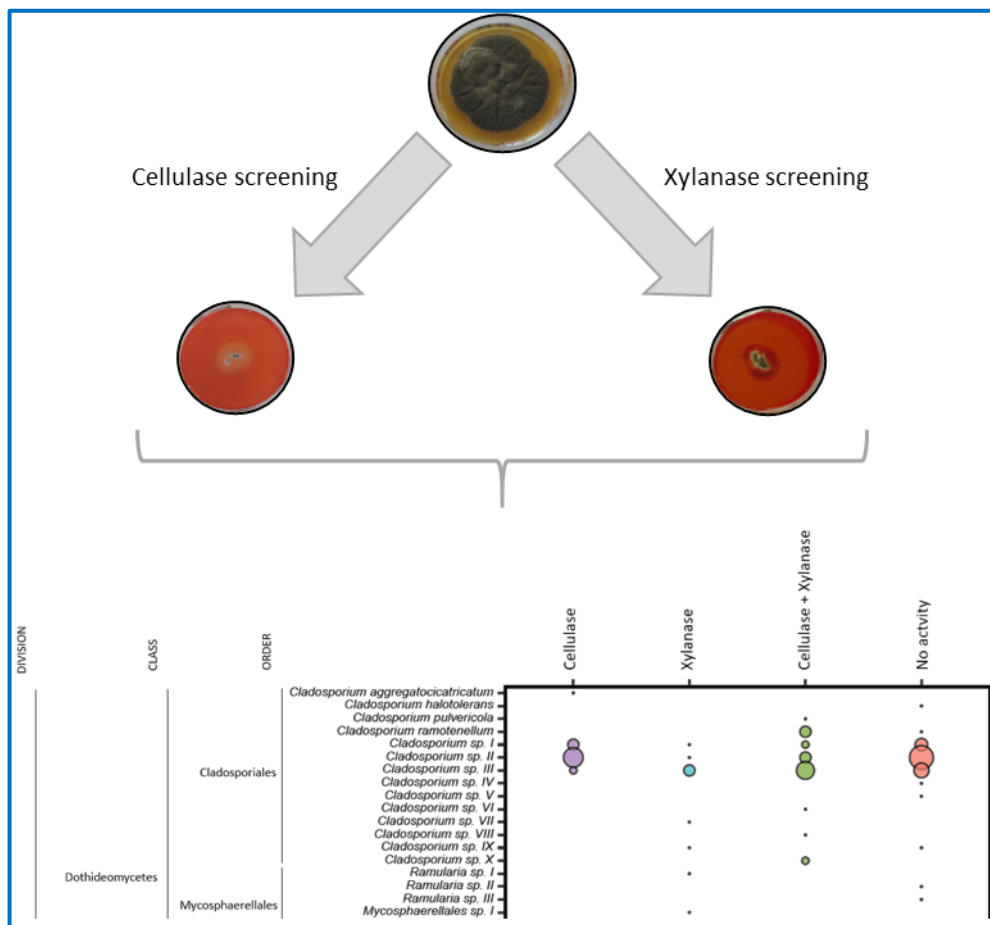
Yoran Le Strat¹, Paul Déleris¹, Nicolas Ruiz¹, Thibaut Robiou du Pont¹, Samuel Bertrand¹, Amélie Saumonneau², Gaëtan Burgaud³, Justine Dumay¹

¹ Nantes Université, Institut des Substances et Organismes de la Mer (ISOMER), UR 2160, F-44000, Nantes, France ; yoran.le-strat@univ-nantes.fr; paul.deleris@univ-nantes.fr; nicolas.ruiz@univ-nantes.fr; thibaut.robiou@univ-nantes.fr; samuel.bertrand@univ-nantes.fr; justine.dumay@univ-nantes.fr

² Nantes Université, Cellule de compétence D-ZYME, CAPACITÉS, F-44000, Nantes, France ; amelie.saumonneau@capacites.fr

³ Univ Brest, INRAE, Laboratoire Universitaire de Biodiversité et Écologie Microbienne, F-29280, Plouzané, France ; gaetan.burgaud@univ-brest.fr

Graphical abstract



Abstract

Fungal enzymes have potential biotechnological interests notably considering their strong ability to degrade various lignocellulosic biomasses. Here, we report the first large-scale screening of cellulolytic and xylanolytic activities of more than 300 fungal strains isolated from the red macroalga *Palmaria palmata*. Fungal polysaccharide degradations were assessed using a qualitative enzymatic assay based on a Congo red protocol. Enzymatic activities were investigated according to the fungal taxonomic assignation and various environmental and isolation parameters. Among the 308 total fungal isolates, 186 were enzymatically active. Among them, 113 exhibited both enzymatic activities and the remaining strains exhibited either strict cellulase or xylanase activity. Fungal genera *Penicillium*, *Aspergillus* and *Cladosporium* were over-represented and particularly characterized by recurrent xylanase and cellulase activities. Seasonality and isolation parameters seemed to impact the occurrence of enzymatically active fungal strains isolated from *P. palmata*.

1. Introduction

From several millennia, fungi have been used in food production such as Asian traditional fermented food, baking, brewing, cheese, and cultivated mushrooms (Bennett 1998; Østergaard and Olsen 2011; Money 2016). From the middle of the 20th century, other fungal applications were investigated, in domains such as antibiotic, enzyme or organic acid production (Bennett 1998; Horgan and Murphy 2017). Indeed, fungi produce a large variety of compounds of interest such as proteins, enzymes, lipids and polysaccharides (Horgan and Murphy 2017; Yadav 2019). The use of fungi in industry is mainly linked to their enzymatic activities (Money 2016). Besides the great efficiency and the potential of enzymes in numerous applications, they also present a great sustainability by replacing previous procedures, chemicals or technics hazardous for nature or presenting various toxicities (Dhiman *et al.* 2008; McKelvey and Murphy 2017; Shah *et al.* 2019; Yakubu *et al.* 2019; Tushar and Dutta 2020; Ejaz *et al.* 2021). The use of enzymes can present several advantages such as cost-effectiveness, better efficiency, non-toxicity, better product quality, eco-friendly processes, and reduced process time (Yadav 2019). Interestingly, the majority of total industrial and commercial enzymes are produced by fungi (Østergaard and Olsen 2011; McKelvey and Murphy 2017; Filiatrault-Chastel *et al.* 2021). Global enzyme market was evaluated at ~10 billion US \$ in 2019, with an annual increase estimated between 5 and 7 % (Filiatrault-Chastel *et al.* 2021; Singh *et al.* 2021).

Fungal enzymes of biotechnological interests are mainly linked with the degradation of lignocellulosic biomass, being composed of lignin, cellulose and hemicellulose (Andlar *et al.* 2018). Cellulose is the principal natural polysaccharide (Siqueira *et al.* 2020; Bhardwaj *et al.* 2021) and xylan is the second one in terms of renewable carbon on earth (Polizeli *et al.* 2005; Dhiman *et al.* 2008; Chapla *et al.* 2010;

Motta *et al.* 2013). Due to the complexity of lignocellulosic structures, a complex enzymatic arsenal must be developed to perform an efficient degradation (Andlar *et al.* 2018). Micro-organisms are of particular interest considering this polysaccharide degradation, because they can be easily cultured under controlled conditions, at low cost and using few space, with a short time of fermentation (Motta *et al.* 2013; Ejaz *et al.* 2021). Fungi have the advantage of an extracellular enzymatic production and a higher production than other microorganisms (Motta *et al.* 2013; Imran *et al.* 2016; Bajaj and Mahajan 2019b; Siqueira *et al.* 2020; Bhardwaj *et al.* 2021).

Enzymes of biotechnological interest are mainly represented by the class of hydrolases (Østergaard and Olsen 2011; Money 2016) and cellulases and xylanases are among the most used enzymes in many industrial sectors (McKelvey and Murphy 2017). Numerous studies have reported the biotechnological interests of fungal cellulases and xylanases in pulp and paper industry processes, in textile industry, in the production of biofuel, in food and beverage industries, in animal feed, in the extraction of bioactive compounds of pharmaceutical interest, in detergent industries, in agriculture and for waste management (Dhiman *et al.* 2008; Chapla *et al.* 2010; Kuhad *et al.* 2011; Singh *et al.* 2016, 2021; Tushar and Dutta 2020; Ejaz *et al.* 2021).

Fungi of *Trichoderma*, *Penicillium* and *Aspergillus* genera have particularly interesting biomass-degrading capacities and are of particular industrial interest (Money 2016; Filiatrault-Chastel *et al.* 2021; Meyer 2021). The genera *Aspergillus*, *Trichoderma*, *Penicillium* and *Humicola* are particularly known as cellulase producers (Kuhad *et al.* 2011; Imran *et al.* 2016; Kunamneni 2016; McKelvey and Murphy 2017; Shah *et al.* 2019; Singh *et al.* 2021). Likewise, the genera *Aspergillus*, *Trichoderma* and *Fusarium* have been described as xylanase producers (Motta *et al.* 2013; Bajaj and Mahajan 2019b).

The potential of marine fungi in biotechnologies, including enzyme-related researches, is well established and has increased during the last years but is still poorly explored and developed compared with terrestrial fungal biotechnology. Nevertheless, marine-derived fungal enzymes, besides forming a great source of diversity, have several advantages against terrestrial-derived ones. Indeed, marine fungal enzymes may have affinities notably with substrates specifically found in marine environments and be used in several processes involving algal biomass degradation without desalinization step (Trincone 2011; Arnosti *et al.* 2014; Le Strat *et al.* 2022). Moreover, halophilic enzymes are particularly stable in high saline conditions but also in high temperatures and they are characterized by a tolerance against organic solvents. These marine enzymes are used for several domains such as medicine, food supplement, beverage, wastewater treatment, remediation, biotransformation and production of chemicals (Shah *et al.* 2019).

Marine fungal enzymes would also be suitable for the extraction conditions of macroalgal products for which biotechnological interests is increasing during the last decades (van der Loos *et al.* 2019). Indeed, macroalgae are characterized by subtle and complex associations with microbial communities (van der Loos *et al.* 2019; Abdul Malik *et al.* 2020). Microorganisms and notably fungi of the macroalgal holobiont could then produce enzymes particularly specific and efficient for the macroalgal cell wall degradation. Nevertheless, one of the greatest limiting factor in the use of microbial enzymes is the high cost of enzyme production. The discovery and selection of microbial strains with a high enzymatic production as well as growth technics allowing a great release of enzymes would reduce this cost (Chapla *et al.* 2010; Motta *et al.* 2013). It is of particular interest to screen and describe more microorganisms which produce both xylanases and cellulases notably because of their synergistic actions (Bajaj and Mahajan 2019b). The use of both enzymes in the extraction of compounds of biotechnological interests from some organisms such as *P. palmata* is particularly appropriated because of a cell wall composed mostly of xylan and cellulose.

Among Rhodophyceae, *Palmaria palmata* is an edible alga found in the coastal waters of the North Atlantic and particularly popular in Ireland, Brittany (France), Maine (USA) and Nova Scotia (Canada) (Lee *et al.* 2017). *P. palmata* is characterized by a fast growth and a high protein content, reaching more than 30 % of its dry weight, giving to this macroalga a good potential as food ingredient (Harnedy and Fitzgerald 2013b; Lee *et al.* 2017). Major part of these proteins are phycobiliproteins, including R-phycoerythrin, which show notably anti-inflammatory and antioxidant activities (Lee *et al.* 2017; Nishida *et al.* 2020). Besides proteins, *P. palmata* synthesizes other compounds of interests such as polyunsaturated fatty acids and more specifically eicosapentaenoic acid (EPA) having numerous health benefits and applications in food, cosmetics and pharmaceuticals (Lopes *et al.* 2019). Extracts of *P. palmata* were used for diverse assays and measurements, exhibiting for example an antioxidant activity over a long period of time (Yuan *et al.* 2005a), photoprotective activities by the synthesis of mycosporin-like amino acids or prebiotic effects on enteric bacteria (Nishida *et al.* 2020). Among several conventional methods of protein extraction, enzymatic assisted extraction (EAE) would be particularly promising, and even more in alkaline conditions. However, conditions of extraction and nature of selected enzymes must be specifically optimized for cell disruption and protein recovery, for each macroalgal species (Harnedy and Fitzgerald 2013a).

In the best of our knowledge, no data is available on the fungal community of *P. palmata* until our recently study (**CHAPITRE II**). This fungal community could be a great source of new marine fungal strains exhibiting enzymatic activities of biotechnological potential, particularly adapted for the extraction of compounds of interest from their host *P. palmata*. Then, the aim of this present study

was to screen the total cultivable fungal community of *P. palmata* previously obtained in order to reveal either cellulolytic and xylanolytic activities.

2. Materials and methods

2.1. Fungal strains screened

A total of 321 marine fungal strains were isolated from *P. palmata* in Batz-sur-Mer (France) over a period of one year. Briefly, eight algal samplings were performed between June 2019 and June 2020 with two samplings per season. Three different isolation procedures were performed for each sampling, allowing the distinction between 1) epiphytic fungal community, 2) endophytic fungal community, 3) total fungal community. Incubation of fungi were performed using two distinct culture media, aspecific enriched Wickerham medium and specifically designed "*P. palmata*" medium, and two distinct incubation temperature, being 12°C and 27°C (**CHAPITRE II**). Every fungal isolates was then associated with precise sampling date, algal location, incubation medium, and incubation temperature.

Fungal strains appearing during incubation were then isolated on DCA medium (detailed composition in **CHAPITRE II**). After a growth at 27°C or 12°C (according to the previous temperature of incubation), the fungal isolates were stored at 4°C and subcultures could be performed for the identification or the enzymatic assays.

Isolations allowed an enrichment of the laboratory fungal collection (from MMS1621 to MMS1998) and these strains were used for the following described enzymatic screening.

2.2. Culture media used for qualitative enzymatic assays

Carboxymethyl cellulose based medium (CMC medium) and arabinoxylane based medium (Arabino medium) were used for the qualitative cellulolytic and xylanolytic assays, respectively. CMC medium was made of sodium carboxymethyl cellulose 5 g/L (Sigma-Aldrich, St. Quentin Fallavier, France), agar 20 g/L (Condalab, Madrid, Spain), K₂HPO₄ 1 g/L (Sigma-Aldrich, St. Quentin Fallavier, France), MgSO₄ 2.4 g/L (Prolabo, Borj Louizir, Tunisia), NH₄NO₃ 2.4 g/L (Merck, Darmstadt, Germany), reef crystal sea salts 36 g/L (Aquarium Systems, Sarrebourg, France). Arabino medium was made of wheat arabinoxylane 5 g/L (Megazyme, Wicklow, Ireland), agar 20 g/L, K₂HPO₄ 1 g/L, MgSO₄ 2.4 g/L, NH₄NO₃ 2.4 g/L, reef crystal sea salts, 36 g/L. These culture media were prepared in 60 mm diameter Petri dishes.

2.3. Screening of cellulolytic and xylanolytic filamentous fungi

The following protocol was adapted from the study of [Meddeb-Mouelhi et al. \(2014\)](#). Marine fungal strains were subcultured on both CMC and Arabino media, with carboxymethyl cellulose and arabinoxylane as sole carbon source. Incubation was performed at 12°C or 27°C according to the temperature of incubation associated to each isolated strain (**CHAPITRE II**), until fungal colonies reached 1 – 2 cm of diameter. A volume of 1 – 2 mL of Congo red dye solution 0.2 % (Kuhlmann, Paris, France) was added on the agar surface until complete flooding of the fungal colonies. The fixation of the Congo red occurred during 15 min and the solution was then discarded, followed by a first wash with distilled water. Then, four consecutive rinsing were performed using NaCl 1M solution (VWR Chemicals, Lutterworth, United Kingdom), with an agitation of 2 min at 125 rotations per min on rotating agitator (Edmund Bühler GmbH, Bodelshausen, Germany) for every rinsing step. Congo red is a dye fixing with polysaccharides such as carboxymethyl cellulose or arabinoxylane. A clearing halo formation surrounding the fungal colonies characterized the enzymatic activity of the tested strain, either xylanase or cellulase, according to the carbon source of the culture medium. Although visual observation can be subjective, potential moderate (+) or high (++) activities were qualitatively estimated based on the intensity of the clearing halo formation. Strains were non-active when no clearing halo formation appeared.

2.4. Statistical analysis

The significance of the effect of both fungal taxonomy and the four studied variables (ecological habitat, incubation medium, incubation temperature, and seasons) on the enzymatic activities of fungal strains was evaluated by using the Pearson's Chi-squared test. This evaluation was performed at three taxonomical levels being the division, the class and the genus (or the most accurate taxonomical assignation). Specific χ^2 tests for independence of variables were processed taking into consideration the number of isolates at different taxonomic levels (phylum, class and genus).

3. Results

3.1. Algicolous fungal abilities to degrade cellulose and xylan

The enzymatic activity was qualitatively estimated by the presence of a clear halo surrounding the fungal colonies, after the coloration with Congo red. Filamentous fungi, which represent the major part of total isolated fungi (308 on 321 total strains) were either characterized by no halo, highlighting an absence of enzymatic activity (xylanase and cellulase), or by halos with various intensities and widths, highlighting a presence of an enzymatic activity. Regarding yeasts strains, when halos were

observed, they were particularly thin and uneasily exploitable. Hence, the present work has been limited on the enzymatic screening of filamentous fungi.

Among all the filamentous isolates associated with *P. palmata*, 61 % of all the 308 strains were characterized by the apparition of a clear area around active colonies, after Congo red coloration, at least for one of both tested enzymatic activities, being cellulase and xylanase (**Figure III. 3**). Indeed, 37 % of the strains screened exhibited both cellulase and xylanase activities, while strict cellulase or xylanase active strains were similarly represented by 11 % and 13 % of total isolates, respectively.

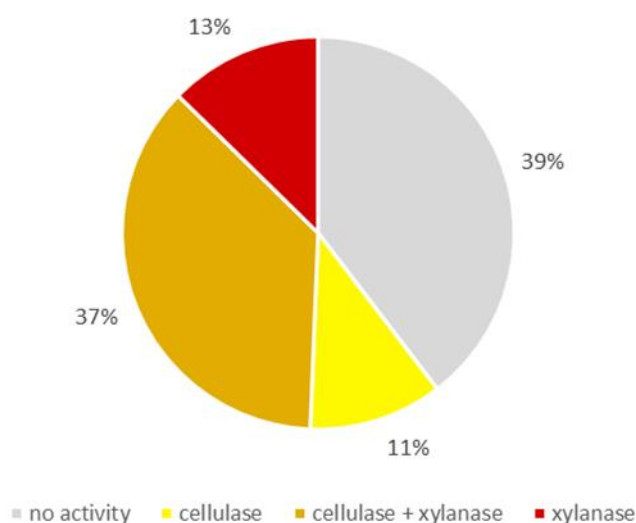


Figure III. 3: Repartition of the 308 filamentous fungal isolates associated with *Palmaria palmata* according to their enzymatic activities.

Both intensity and size of the clear area varied between strains and potentially allowed to determine strains of particular interest according to their enzymatic activities (**Figure III. 4**).

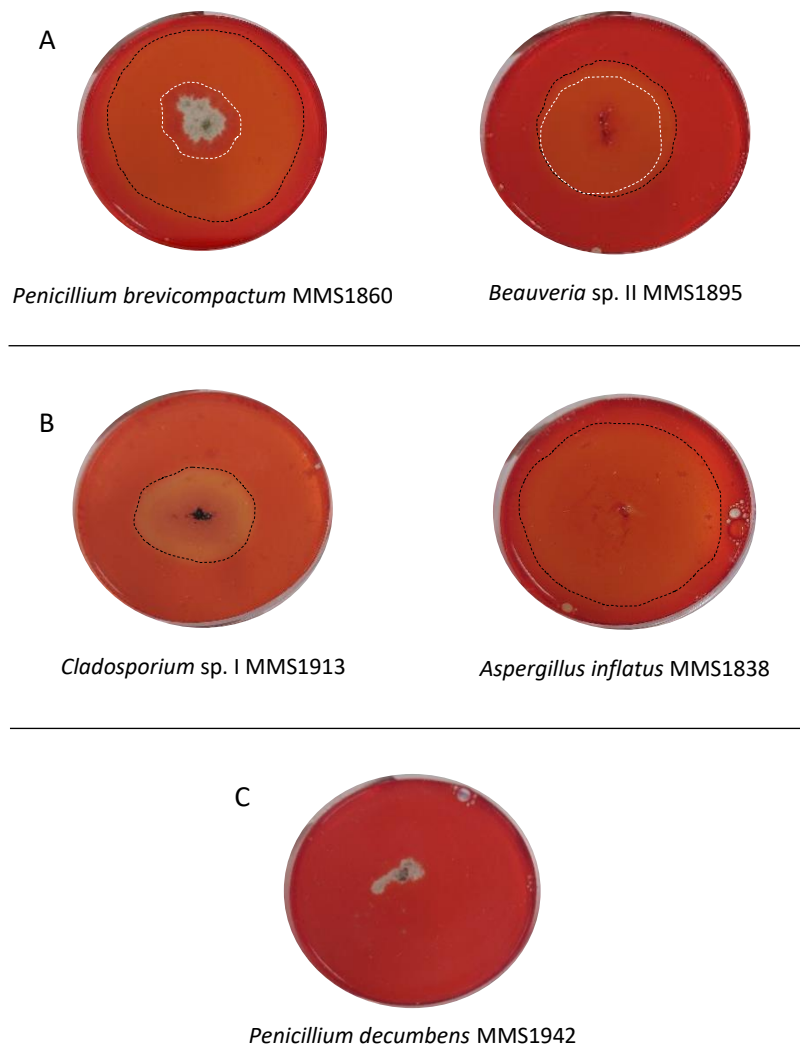


Figure III. 4: Qualitative enzymatic Congo red assays. Different sizes of xylanase halo formations (A). Different intensities of cellulase halo formations (B). Mycelial development of an isolate on CMC medium without halo (C). White dotted lines represent mycelial limits and black dotted lines represent halo limits.

There was a great diversity of enzymatic halos between all screened strains, notably according to the difference between area of halo and of fungal mycelium. This difference was represented in **Figure III. 4A**, MMS1860 strain being characterized by a xylanolytic halo particularly extended beyond mycelium limits compared with MMS1895. The intensity of enzymatic halos also differed from one fungal strain to another. For example, a particularly intense cellulolytic halo characterized the MMS1913 strain while MMS1838 cellulolytic halo was tenuous (**Figure III. 4B**).

Focusing on this halo intensity and on the basis of visual estimations, enzymatically active isolates were separated between two groups. Highly active strains were characterized by a clearer halo than moderate active strains. Activities of every strain were then qualitatively indicated on the **Annexe 3**. Information about the incubation temperature, the incubation culture medium, the location into the

alga and the sampling season characterizing each isolate were also given for each fungal strain isolated from *P. palmata*.

On the basis of these qualitative observations, 33 and 46 isolates resulted in a particularly clear cellulolytic and xylanolytic halos, respectively. Most of these strains exhibited a clear halo only either for cellulase or xylanase assay, but they were generally characterized by both enzymatic activities. Only five isolates among the 33 with cellulolytic activity and ten among the 46 with xylanolytic activity were characterized by a single enzymatic activity. Among the 308 fungal isolates screened, *Aspergillus* sp. III MMS1733, *Cladosporium* sp. II MMS1738, *Acremonium* sp. II MMS1777, *Penicillium* sp. V MMS1782, *Cladosporium ramotenellum* MMS1855 and MMS1959, *Penicillium brevicompactum* MMS1910, *Cladosporium* sp. VIII MMS1956 and *Cladosporium* sp. I MMS1980 were the nine most promising strains according to their enzymatic activity. They all were characterized by a particularly clear halo on both cellulase and xylanase assays (**Annexe 3**).

Considered enzymatically inactive strains either developed on CMC or Arabino media without any halo after enzymatic assays with Congo red, or did not develop at all on these media (**Figure III. 4C**).

3.2. Taxonomy-wise and environmental distributions of the enzymatic activities of the tested fungal strains

Ascomycota was the most represented phylum but several Basidiomycota were detected. Dothideomycetes, Sordariomycetes and Eurotiomycetes were the main classes described (**Figure III. 5**). The major genera found in association with *P. palmata* were *Cladosporium*, *Penicillium*, and *Aspergillus*. Other fungal classes were detected, being Agaricomycetes, Leotiomycetes, Saccharomycetes, Tremellomycetes, Agaricostilbomycetes, Microbotryomycetes, Exobasidiomycetes, Ustilaginomycetes and Wallemiomycetes. Most of the 147 taxa composing the cultivable fungal diversity of *P. palmata* were only represented by one or two isolates. The most represented taxon was *Cladosporium* sp. II, with 35 isolates. On the 147 total taxa, 136 corresponded to filamentous fungi. Then, the following part comparing the enzymatic activity (cellulase and xylanase) with taxonomic assignation of fungal strains was limited to the 295 isolates composing the 136 filamentous fungal taxa characterized and isolated from *P. palmata* (**Figure III. 5**).

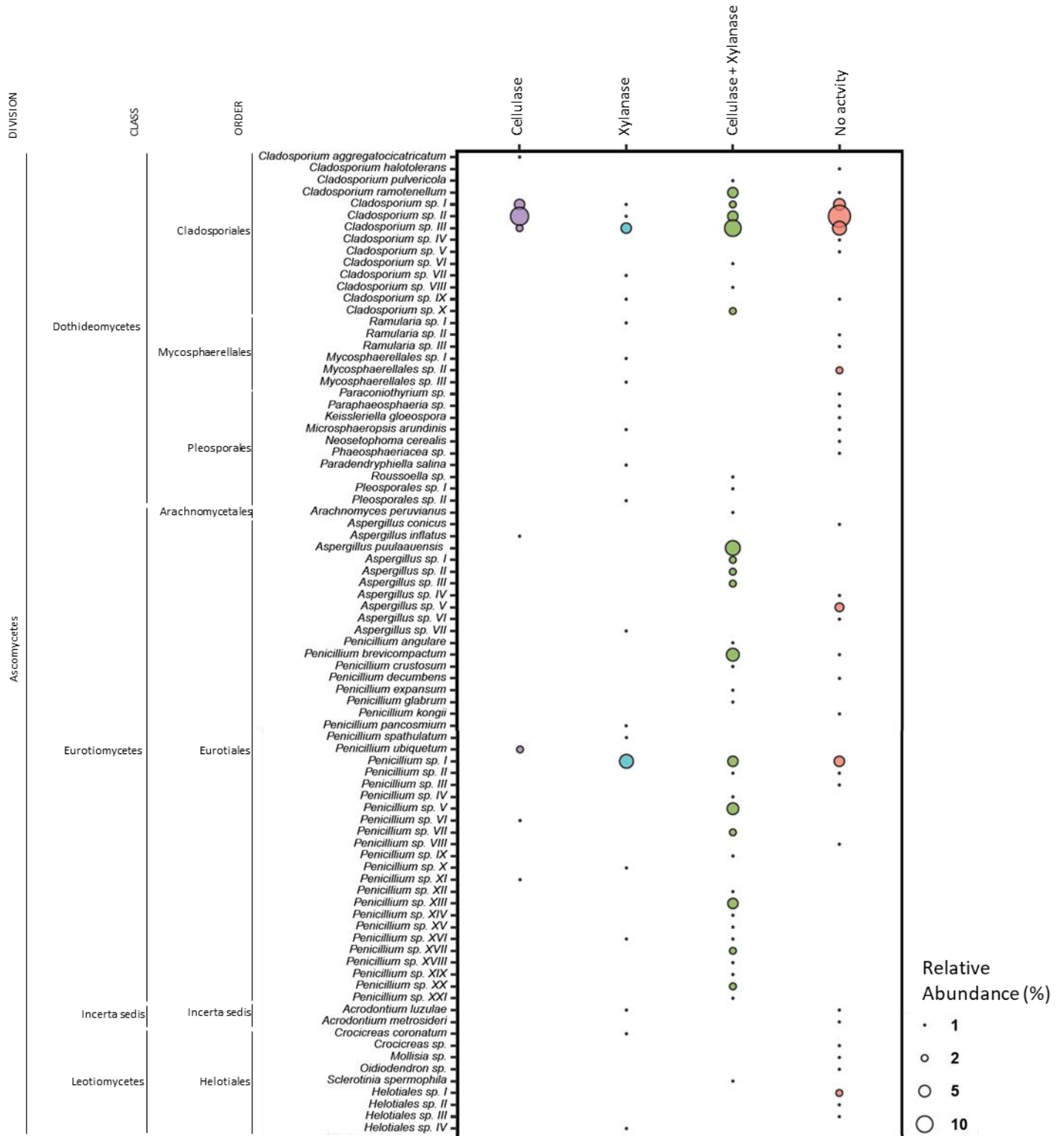


Figure III. 5: List of the cultivable fungi isolated from *Palmaria palmata*. The Bubble plot represents the classification of each taxa isolated (Division, Order, Class) and their relative abundance according to the enzymatic activity: cellulase (purple), xylanase (blue), cellulase + xylanase (green) and no activity (orange).

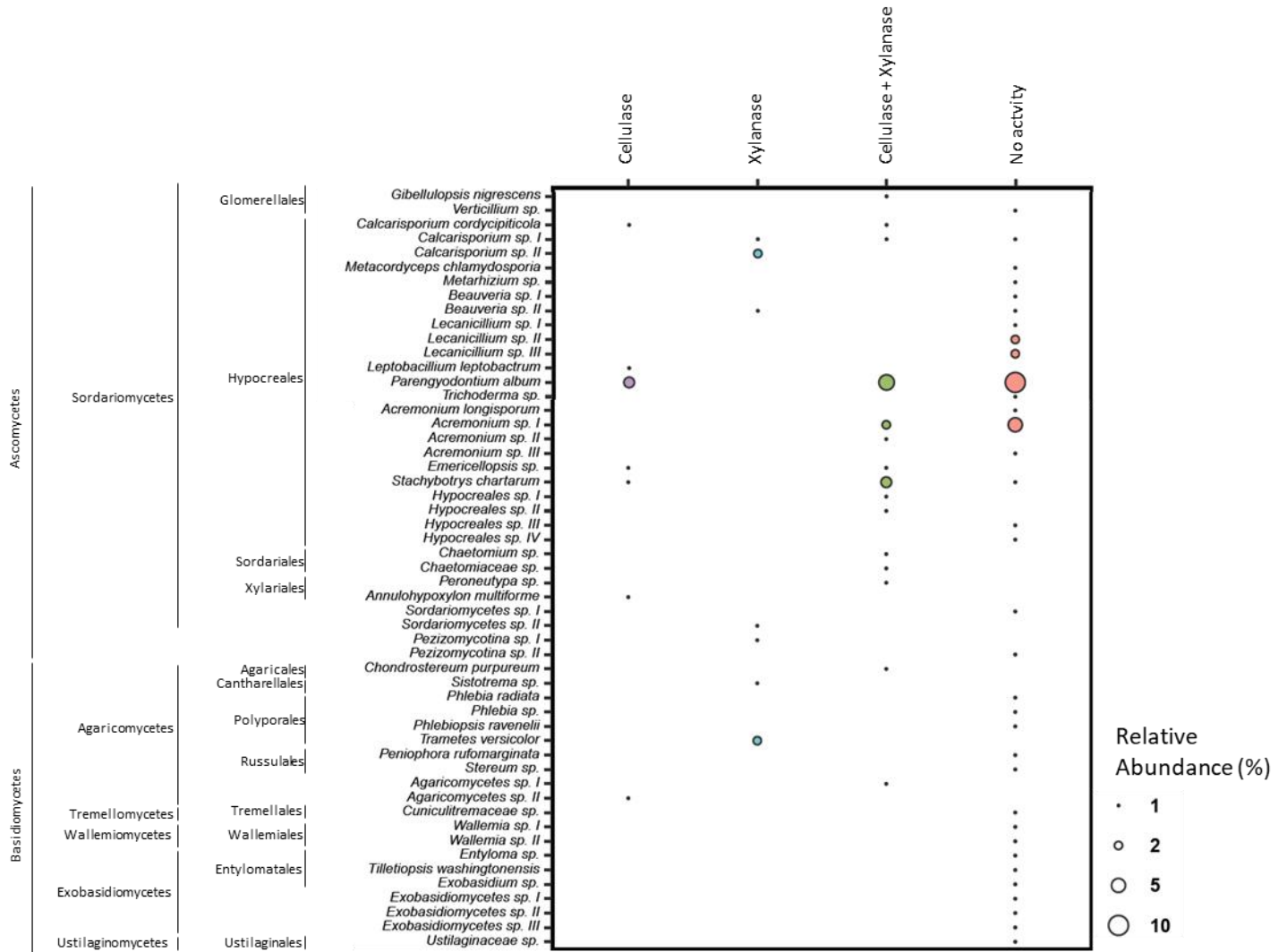


Figure III. 5: Cont.

3.2.1. Main enzymatically active taxa in terms of cellulases and xylanases among *P. palmata* associated fungi

Fourteen taxa among the 136 filamentous fungal taxa were characterized by more than two isolates (**Figure III. 5**). These taxa were *Acremonium* sp. I, *Aspergillus puulaauensis*, *Aspergillus* sp. V, *Calcarisporium* sp. I, *Cladosporium ramotenellum*, *Cladosporium* sp. I, II, III, *Parengyodontium album*, *Penicillium brevicompactum*, *Penicillium* sp. I, V, XIII and *Stachybotrys chartarum*. The taxonomy had a real impact on the enzymatic activities of fungal strains. Among the 295 fungal strains taxonomically described, 176 were characterized by at least one enzymatic activity and 119 were characterized by no activity. The conducted χ^2 tests for independence of variables showed dependence between the enzymatic activity and the fungal taxonomy at phylum level (p -value = 0.01). The significance of this dependence was higher regarding class (p -value = 1.8×10^{-6}) and genus level (p -value = 5.7×10^{-5}).

Only 15 taxa on the 136 filamentous taxa were characterized by at least one isolate exhibiting strict cellulase activity (and no xylanase activity) (**Figure III. 5**). The main taxon considering the strict cellulase activity was *Cladosporium* sp. II, with 12 of the 17 enzymatically active strains exhibiting only cellulase activity. *Cladosporium* sp. I, *Parengyodontium album* and *Cladosporium* sp. III were characterized by 4, 3 and 2 strictly cellulolytic strains on 7, 9 and 16 total enzymatically active strains respectively. The two isolates of *Penicillium ubiquetum* were characterized by strict cellulase activity. All these strictly cellulolytic taxa belonged to the three main fungal classes, Dothideomycetes, Sordariomycetes and Eurotiomycetes, except one Basidiomycota strain belonging to the taxon Agaricomycetes sp. II.

On the 136 filamentous fungal taxa associated with *P. palmata*, 27 taxa were characterized by at least one fungal strain exhibiting strict xylanase activity (and no cellulase activity) (**Figure III. 5**). On the 11 enzymatically active strains of *Penicillium* sp. I taxon, 7 were characterized by a strict xylanase activity. Both *Trametes versicolor* and *Calcarisporium* sp. II taxa counted a total of two strains exhibiting strictly xylanase activities. Moreover, on the 7 *Calcarisporium* isolates, only one did not exhibit any enzymatic activity. Considering *Cladosporium* sp. III, 4 of the 16 enzymatically active strains were characterized by a strict xylanase activity. The majority of strictly xylanolytic isolates belonged to Dothideomycetes or Eurotiomycetes. Other classes of Ascomycota were represented, being Sordariomycetes and Leotiomycetes, and two taxa exhibiting strictly xylanolytic isolates belonged to Agaricomycetes (Basidiomycota).

As mentioned before, most enzymatically active strains were characterized by both cellulase and xylanase activity. Indeed, 52 of the 136 filamentous fungal taxa were characterized by isolates with both enzymatic activities (**Figure III. 5**). This corresponded to 113 isolates exhibiting cellulolytic and xylanolytic activities, on a total of 186 enzymatically active strains. The taxa *Aspergillus puulaauensis*,

Penicillium brevicompactum, *Penicillium* sp. V, *Cladosporium ramotenellum* and *Penicillium* sp. XIII were characterized by 8, 6, 5, 4 and 4 enzymatically active strains, all exhibiting both xylanase and cellulase activities. *Cladosporium* sp. III, *Parengyodontium album* and *Stachybotrys chartarum* taxa were characterized by a majority of enzymatically active strains exhibiting both enzyme activities. *Acremonium* sp. I, *Aspergillus* sp. I, II, III, *Cladosporium* sp. X and *Penicillium* sp. VII, XVII, XX were taxa characterized by a total of two enzymatically active strains with both cellulase and xylanase activities. A great majority of isolates exhibiting both activities belonged to the classes of Dothideomycetes, Sordariomycetes and Eurotiomycetes. One isolate belonging to Leotiomycetes (Ascomycota) and two to Agaricomycetes (Basidiomycota) were also characterized by both enzyme activities.

It is important to notice that even if some taxa were characterized by numerous enzymatically active strains, this amount of active strains needed to be compared with inactive strains. Indeed, *Cladosporium* sp. II and III were characterized by a total of 17 and 16 active strains, respectively, and then seemed to exhibit a similar enzymatic potential. However, the latter taxa counted 7 additional strains without any enzymatic activity while the former was characterized by 18 isolates without any enzymatic activity, which represented the majority of this taxon strain amount. Similarly, among 10 total isolates of *Acremonium*, only 3 were enzymatically active, and among 19 total isolates of *Parengyodontium album*, only nine were active. All the three isolates of *Aspergillus* sp. V taxon and the two isolates of Helotiales sp. I, *Lecanicillium* sp. II, *Lecanicillium* sp. III and *Mycosphaerellales* sp. II did not exhibit any enzymatic activity. Fifty taxa represented by only one isolate were not characterized by any enzymatic activity. Interestingly, while the part of basidiomycetes was particularly low considering enzymatically active strains as presented previously, there were 15 taxa on the 55 without any enzymatic activity.

Further descriptions could be established regarding the three main genera isolated from *P. palmata*, *Aspergillus*, *Cladosporium* and *Penicillium*. The enzymatic profiles of the two *Aspergillus* taxa characterized by more than two isolates were strictly different. Indeed, all the 8 strains of *Aspergillus puulaauensis* exhibited both cellulase and xylanase activities while all the 3 isolates of *Aspergillus* sp. V were inactive. Six of the 11 remaining strains of the *Aspergillus* genus were characterized by both cellulase and xylanase activities. Interestingly, all isolates of each *Aspergillus* taxon were characterized by one precise enzymatic category, either strictly cellulolytic (*Aspergillus inflatus*), or strictly xylanolytic (*Aspergillus* sp. VII), or with both (*Aspergillus puulaauensis*, *Aspergillus* sp. I, II, III) or without activities (*Aspergillus conicus*, *Aspergillus* sp. IV, V, VI). *Cladosporium* taxa seemed to have various enzymatic profile. Considering the most represented taxa in term of number of isolates, *Cladosporium ramotenellum* and *Cladosporium* sp. III were characterized by a majority of strains with both cellulase and xylanase activities. Both *Cladosporium* sp. I and II were

characterized by a majority of strains without any enzymatic activity, but considering active strains they exhibited principally a strict cellulase activity. The other *Cladosporium* taxa were represented by a total of 12 isolates, among which 8 were characterized by one enzymatic activity and 5 by both cellulase and xylanase activities. Considering the four *Penicillium* taxa characterized by more than two isolates, *Penicillium brevicompactum*, *Penicillium* sp. V and XIII were represented by a total of 16 strains including 15 strains exhibiting both cellulolytic and xylanolytic. Almost half of *Penicillium* sp. I isolates were principally only xylanolytic, but 4 strains exhibited both enzymatic activities and 4 others were inactive. Among the 33 remaining isolates, 20 were characterized by both cellulase and xylanase activities. Five of these 33 isolates were inactive, while 4 were strictly cellulolytic and 4 others were strictly xylanolytic.

3.2.2. Impacts of environmental factors on the distribution of the marine fungal xylanolytic and cellulolytic activities

We previously described that cultivable fungal strains could present preferences according to factors such as incubation temperature or culture medium (**CHAPITRE II**). For example, it has been shown that more strains were isolated with a 12°C temperature incubation on a Wickerham medium than 27°C on the selective "*P. palmata*" medium. This could then lead to an under-estimation of the impact of such over-represented conditions on the strain enzymatic profiles. Then, considering each condition, the amount of strains belonging to a given enzymatic category was reported on the total amount of strains isolated with the considered factor. This allows to free of any bias due to strain cultivation (**Figure III. 6**).

The four studied variables (ecological habitat, incubation medium, incubation temperature, and seasons) seemed to have a limited impact on the presence of enzymatic activity. Regarding all the three ecological habitats, they were almost equally distributed among all enzymatic profiles. This observation was similar regarding the incubation temperature and the culture medium. Chi² tests of independence resulted in an absence of dependence between these three variables and the enzymatic activities of fungal strains. Nevertheless, seasons were unequally represented regarding the four enzymatic profiles. For example, almost 50 % of cellulolytic and xylanolytic strains were respectively obtained in summer and autumn, relatively to the total amount of strains in every seasons. The chi² tests of independence then resulted in a dependence between season and enzymatic activity of fungal strains (p-value < 0.001).

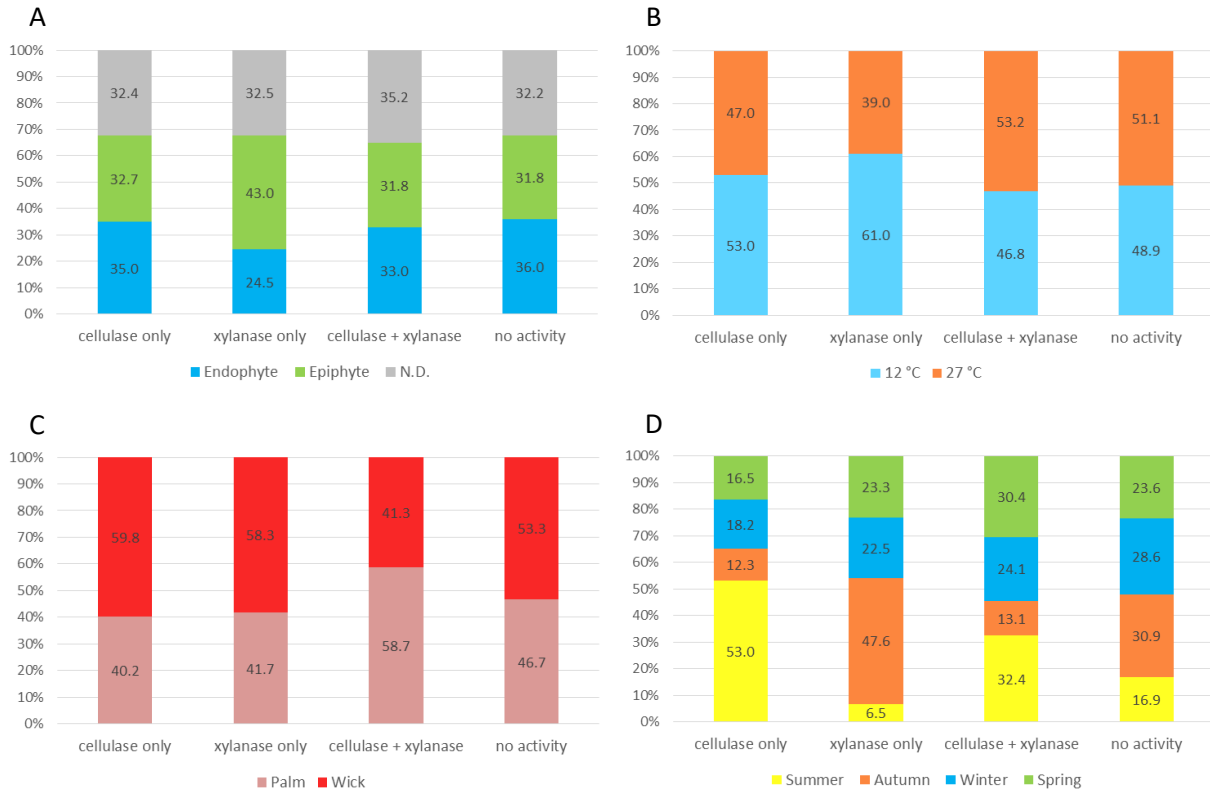


Figure III. 6: Distribution of the four enzymatic categories of the fungal strains isolated from *Palmaria palmata* according to environmental and isolation parameters. Fungal location in alga (A), incubation temperature (B), incubation culture medium (Palm = “*P. Palmata*” medium, Wick = Wickerham medium) (C) and season of sampling (D). Percentages reported the amount of strains belonging to enzymatic categories on the total amount of strains associated with the factor value.

The strict cellulase activity of fungal strains isolated from *P. palmata* were not related with the location in the alga (Figure III. 6A) nor with the incubation temperature (Figure III. 6B). However, strict cellulolytic strains seemed to be slightly more found as endophytes with a 12°C incubation temperature than as epiphytic and with a 27°C temperature, respectively. Considering the culture medium, almost 60 % of cellulolytic strains in relative percentage were related with Wickerham medium (Figure III. 6C). Interestingly, more than the half of strictly cellulolytic strains were from sampling performed in summer, relatively to the total amount of strains in every seasons (Figure III. 6D).

Fungal isolates exhibiting a strict xylanase activity seemed to be more epiphytic than endophytic, with relative percentage of 43 % and 24.5 %, respectively (Figure III. 6A). A temperature of 12°C and a Wickerham culture medium seemed to be more indicated for the isolation of strictly xylanolytic fungal strains compared with incubation temperature of 27°C and “*P. palmata*” culture medium (Figure III. 6B, C). Nevertheless, there was not significant dependence between these three variables and the enzymatic activities. While strict cellulolytic strains were preferentially isolated in summer, strict xylanolytic ones should be preferentially isolated in autumn (Figure III. 6D). Indeed, in relative abundance, autumn sampling represented almost 50 % of strict xylanolytic strains.

Isolates with both enzymatic activities did not seem to be particularly related to the location in alga (**Figure III. 6A**). An opposite non-significant tendency was observed on strains with both enzymatic activities considering the temperature and nature of the culture medium used for the strain isolation from *P. palmata*, in comparison to the 2 previous categories (xylanolytic and cellulolytic strains). Indeed, a relative abundance of 53.2 % and of 58.7 % of strains with both activities were related with a 27°C incubation and an isolation on “*P. palmata*” medium respectively (**Figure III. 6B, C**). However, these strains were preferentially isolated in summer (32.4 %) and spring (30.4 %) compared with winter and autumn.

Interestingly, strains without any enzymatic activity seemed to be particularly equally distributed among the conditions of the different studied factors compared with strains with enzymatic activities. Nonetheless, inactive strains should be more endophytic than epiphytic and they would preferentially be isolated at 27°C on a Wickerham culture medium. Almost 40 % of enzymatically inactive strains were relatively found in autumn.

4. Discussion

4.1. General abilities of *P. palmata* derived fungal community to degrade cellulose and xylan

Several studies on screening of cellulase or xylanase activities from algicolous communities were previously performed (Abiodun *et al.* 2015; Hong *et al.* 2015; Lee *et al.* 2015, 2019; Patyshakuliyeva *et al.* 2019). Nevertheless, because of the low amount of hemicellulose in brown algae, screening of xylanase activity of marine fungi isolated from brown macroalgae are less conclusive than cellulase screening (Hong *et al.* 2015). A screening of xylanase activity, along with cellulase one, on marine fungal communities associated with red macroalgae then presented a particular interest. Cellulases and xylanases have already been detected and measured in marine fungal strains isolated from red seaweeds (Lee *et al.* 2015; Thirunavukkarasu *et al.* 2015). More than 60 % of total fungal isolates exhibited at least one enzymatic (cellulase or xylanase) activity. Nevertheless, among these 186 enzymatically active fungal isolates, 61 % exhibited both cellulase and xylanase activities. These xylanolytic and cellulolytic capacities of fungi isolated from *P. palmata* could be explained by the polysaccharidal composition of this macroalga. *P. palmata* cell wall is principally composed of xylan, while cellulose is also present but in lower amount (Lahaye *et al.* 1993; Fleurence *et al.* 1995; Deniaud *et al.* 2003). To colonize and develop on this macroalga, fungi then need to present the ability to degrade polysaccharides composing its cell wall.

4.2. Relationship between fungal taxonomy and enzymatic profiles

Because of the cell wall of *P. palmata* composed of xylan and cellulose, isolates exhibiting both xylanase and cellulase activities would then be more promising in macroalgal degradation than isolates characterized by only one of these enzymatic activities. On the 186 enzymatically active fungal strains, a majority of 113 isolates exhibited both xylanase and cellulase activities, which corresponded to 52 taxa. Several taxa belonging to the Trichocomaceae, such as *Aspergillus puulaauensis*, *Penicillium brevicompactum* and *Penicillium* sp. V, were the most promising in terms of production of both xylanase and cellulase activities because almost all strains of these taxa were characterized by this double activity. This observation was in accordance with numerous studies qualifying species of both *Penicillium* and *Aspergillus* genera as great producers of cellulases and xylanases (Kuhad *et al.* 2011; Motta *et al.* 2013; Gupta *et al.* 2015; Imran *et al.* 2016; Kunamneni 2016; Bajaj and Mahajan 2019b; Shah *et al.* 2019; Singh *et al.* 2021). Some other taxa counted numerous strains with both activities, such as *Cladosporium* sp. III or *Parengyodontium album*, but they were also characterized by many inactive strains. The difference of intensity between halos was previously mentioned and particularly strains displaying clear ones have been selected on the basis of this difference. Interestingly, among the nine strains having the clearest enzymatic halos for both xylanase and cellulase activities, 5 belonged to *Cladosporium* genus, while *Penicillium* and *Aspergillus* genera previously cited as particularly promising were represented by 2 and 1 strains, respectively. This could be partially explained by the over-representation of the *Cladosporium* genus in *P. palmata* fungal community with 87 on the 308 total screened strains. As well as *Penicillium* and *Aspergillus* genera, *Cladosporium* species are widespread in marine environment (Sallenave-Namont and Pouchus 2000; Da Silva *et al.* 2008; Matallah-Boutiba *et al.* 2012; Imhoff 2016; Cunliffe *et al.* 2017; Bovio *et al.* 2018; Maamar *et al.* 2020; Le Strat *et al.* 2022) and are characterized by promising polysaccharidases including cellulase and xylanase (Del-Cid *et al.* 2014; Korkmaz *et al.* 2017; Patyshakuliyeva *et al.* 2019; Le Strat *et al.* 2022). These characteristics of *Cladosporium* genus could partially explain its domination against other fungal genera. This genus may also be particularly well adapted to colonization of *P. palmata*. Several taxa, such as *Cladosporium* sp. II or III, contained strains with an important diversity in terms of enzymatic activities, either with both cellulase and xylanase activities, with only one of these activities, or without activity.

Because xylan is the principal polysaccharide constituting the cell wall of *P. palmata*, strict xylanolytic fungal strains are expected to be more efficient for macroalgal degradation than strict cellulolytic ones. Referring only to the substrate affinity, it would be expected to observe a majority of strict xylanolytic strains compared with strict cellulolytic ones. However, strict xylanolytic and cellulolytic strains are found in similar amount in the fungal community associated with *P. palmata*. This equilibrium could

be partially explained by the fact that cellulose is most widespread than xylan in environment (Motta *et al.* 2013; Siqueira *et al.* 2020; Bhardwaj *et al.* 2021), notably regarding seaweeds where cellulose is widely synthesized by all main groups of macroalgae (Phaeophyceae, Rhodophyceae and Chlorophyceae) contrarily to xylan (Engel *et al.* 1965; Usov 2011; Le Strat *et al.* 2022). This could potentially lead to a global over-representation of cellulase enzymes in marine fungal species compared with other activities such as xylanase. In the present study, most of taxa composed of strictly xylanolytic or strictly cellulolytic strains were also composed of fungal strains either exhibiting a double activity and/or no activity. No clear tendency could be established on the propensity of one taxa or one species to be characterized either by a strict xylanolytic or strict cellulolytic activity. However, considering strains characterized by the clearest enzymatic halos, *Penicillium* strains were the most numerous in terms of xylanolytic activity, while *Cladosporium* strains were the most numerous in terms of cellulolytic activity.

Most of taxa characterizing the fungal community of *P. palmata* reported in this study were composed of only one isolate. That did not mean that these taxa cannot exhibit highly active xylanases or cellulases. Moreover, some taxa such as *Parengyodontium album* and *Acremonium* sp. I, were characterized by a majority of inactive strains.

4.3. Effect of environmental and isolation parameters on fungal enzymatic activity

Seasonal variation in the polysaccharidal composition of *P. palmata* have already been reported (Morgan *et al.* 1980; Joubert and Fleurence 2008). It has already been discussed that substrate has an impact on the enzymatic production of microorganisms. It could then be expected that the enzymatic production by algicolous fungal community, as well as its global structure, vary according to the season. These expectations, poorly documented on marine microbial communities, have already been observed on terrestrial ones (Findlay and Watling 1998; Eswaran and Khandeparker 2019; Ullah *et al.* 2021; Zhang *et al.* 2021). The present study presented a preferential isolation of fungal strains exhibiting both cellulase and xylanase in summer and spring, while strictly xylanolytic strains were mainly isolated in autumn and strictly cellulolytic ones were mainly isolated in summer. Further investigations on the precise polysaccharidal structure evolution according to the season in *P. palmata* are needed to test hypotheses that xylan and cellulose may be particularly available for fungal degradation in autumn and in summer, respectively.

Besides environmental factors impacting sampled macroalgal biomass and related fungal community, parameters of isolation and incubation protocols could have an influence on the distribution of xylanolytic or cellulolytic activities among the fungal isolates.

Protocol of isolation discriminating epiphytic and endophytic strains did not shown any impact on the isolation of fungal strains characterized by both cellulolytic and xylanolytic activities. Moreover, most isolates presented in this study were neither characterized as endophytes nor epiphytes but obtained with a non-discriminating protocol. To the best of our knowledge, very few reports on terrestrial fungi and no report on marine fungi have been performed about the comparative ecological structures and enzymatic activities between endophytic and epiphytic species associated with a defined substrate (Osono and Takeda 1999; Santamaría and Bayman 2005; Kharwar *et al.* 2010; Corrêa *et al.* 2014).

Parameters of temperature incubation and nature of culture medium seemed to have several various tendencies on strains characterized by one activity (either cellulase or xylanase) or strains with both activities. These tendencies were only based on observations and did not resulted from significant dependence between incubation parameters and enzymatic activities. Fungal isolates with strict xylanolytic activities seemed to be slightly preferentially isolated at 12°C compared with 27°C, with a factor 1.6. Nevertheless, incubation temperature did not any impact on the occurrence of fungal strains characterized either by strict cellulolytic activity, or both xylanolytic and cellulolytic activities, or no activity. Interestingly, fungal strains characterized by only one enzymatic activity, xylanase or cellulase, were 1.4 and 1.5 times more isolated from Wickerham medium than from "*P. palmata*" medium. Contrarily, fungal strains displaying both cellulase and xylanase activities were 1.4 times more isolated from "*P. palmata*" medium than from Wickerham medium. The "*P. palmata*" culture medium seemed to slightly promote the isolation of fungal strains displaying both cellulase and xylanase activities. This could be explained by the selective properties of this medium composed of natural macroalgal polysaccharides without any addition of other sugars such as glucose, which is present in the non-selective Wickerham medium. Besides a selection process, it has already been demonstrated that cellulose and xylan can have an enhancing effect on the cellulase and xylanase production, respectively (Motta *et al.* 2013; Bhardwaj *et al.* 2021). Even if data are particularly scarce regarding relationship between marine fungal enzyme production and their substrate of isolation, several studies have shown that the enzymes produced by microorganisms can be dependent on their growth substrate (Misic and Fabiano 2005; Ahmed and Bibi 2018; M'barek *et al.* 2019; Liu *et al.* 2021). Besides potential slight effects of incubation parameters on the selection of strains exhibiting both cellulase and xylanase activities (no significant dependence in the present study), it has been established that marine fungal strains with xylanolytic abilities also widely exhibited cellulase activities (Luo *et al.* 2005). It is important to notice that both parameters did not appear to be interdependent. Indeed, independently of the medium or the season, there were constantly almost twice more fungal strains isolated with a 12°C incubation compared with 27°C incubation.

These observations and results could be useful in the elaboration of isolation and incubation protocols applied for algicolous fungi, according to the targeted application. Then, an incubation at 27°C on “*P. palmata*” medium had a particular interest for isolating strains with both cellulase and xylanase activities which would allow a better *P. palmata* biomass degradation compared with strictly cellulolytic or xylanolytic strains. On the contrary, the isolation of strains with either strict cellulolytic or xylanolytic activities could be enhanced by an incubation at 12°C on Wickerham culture medium. This can be useful in some industries such as paper ones, where the application of xylanase without cellulase is required (Raghukumar *et al.* 2004; Raghukumar 2008). Nevertheless, it still has to be confirmed whether these results can be transposed to other macroalgal biomasses. Further similar works on other macroalgae could then be useful to determine the best procedure to obtain highly active fungal strains in terms of enzymes and according to various substrates.

Other algicolous fungal communities adapted to their polysaccharides matrixes should then be obtained and characterized by various specific enzymatic profiles. Moreover, the present study focused on filamentous fungi because the enzymatic assays performed were not relevant considering yeast isolates. An adapted screening protocol for polysaccharidase production from yeasts should be tested and applied to evaluate their enzymatic potentials. Besides, the enzymatic activities observed in fungal strains seemed to be related with seasons. Even if some studies described variations of the global polysaccharide fractions of *P. palmata* according to the season, no precise data specified the seasonal evolution of polysaccharide composition of this alga, which could putatively be linked to the enzymatic activities of the associated fungi.

5. Conclusion

In conclusion, a great diversity of taxa have been detected in the present study to synthesize cellulolytic and xylanolytic enzymes. To the best of our knowledge, the present study is the first large-scale screening of both cellulase and xylanase activities performed on marine fungal community associated with a red macroalga. Among the 308 filamentous fungal strains isolated from *P. palmata*, 186 were characterized by at least one enzymatic activity among xylanase and cellulase. Moreover, among these 186 enzymatically active strains, 113 isolates exhibited a double activity (cellulase and xylanase). Several fungal genera ubiquitous in the marine environment such as *Penicillium*, *Aspergillus* or *Cladosporium* were over-represented in the present study and particularly characterized by recurrent xylanase and cellulase activities. According to the present work, isolation and incubation protocols could have an influence on the enzymatic profiles of the fungal strains isolated. Then, fungal strains displaying both cellulase and xylanase activities were mainly isolated during spring and summer. This isolation of fungal strains with a double enzymatic activity seems also enhanced by the use of the

specific culture medium "*P. palmata*". Nevertheless, there remains a great lack of data considering the screening of enzymatic potential of algicolous fungal strains. Detection and characterization of marine fungal strains specifically associated with macroalgal biomass could result in the discovery of new enzymes particularly adapted to these substrates. These enzymes may then be of great interest in the extraction of biotechnologically valuable compounds from these macroalgae. In order to characterize more precisely the enzymatic potential of fungal strains isolated from *P. palmata*, quantitative enzymatic assays could be performed notably on the nine fungal strains appearing as the best xylanolytic and cellulolytic producers on the basis of enzymatic halos obtained through qualitative assays. These strains are *Aspergillus* sp. III MMS1733, *Cladosporium* sp. II MMS1738, *Acremonium* sp. II MMS1777, *Penicillium* sp. V MMS1782, *Cladosporium ramotenellum* MMS1855 and MMS1959, *Penicillium brevicompactum* MMS1910, *Cladosporium* sp. VIII MMS1956 and *Cladosporium* sp. I MMS1980. Besides enzymatic activities, it would be of practical interest to estimate the degradation potential of these strains on *P. palmata* biomass. Indeed, an important degradation potential could make of these strains promising candidates for the extraction of compounds of interest such as R-phycoerythrin.

Postface

La méthode de criblage d'activité enzymatique est basée sur une culture fongique sur milieu solide colorée par le rouge Congo et par l'observation visuelle d'un halo de décoloration. Selon l'activité enzymatique visée (xylanolytique ou cellulolytique), le milieu ne contenait soit que de l'arabinoxylane soit que de la carboxymethylcellulose (CMC) comme source de carbone, respectivement. Cette notion de "seule source de carbone" peut être biaisée par la présence d'agar, agent solidifiant du milieu, bien qu'elle soit mentionnée ainsi dans de nombreuses publications sur lesquelles nous nous sommes basés pour adapter le protocole de criblage (Del-Cid *et al.* 2014; Meddeb-Mouelhi *et al.* 2014; Shabeena Anjum *et al.* 2017; Wu *et al.* 2018; Chung *et al.* 2019). La plupart des réponses négatives aux tests de criblage enzymatique étaient dues à une absence totale de développement de la souche testée, et donc également à une absence de halo. Cependant, quelques exceptions ont concerné des souches qui se développaient sur les milieux minimum CMC et arabinoxylane mais ne présentaient pas de halo de décoloration. Cette absence de halo enzymatique caractérisait une absence d'activités xylanolytiques ou cellulolytiques et la seule autre source de carbone connue du milieu était donc l'agar.

Pour vérifier cette hypothèse, des tests de repiquage de plusieurs souches fongiques sur un milieu solide dont la seule source de carbone était l'agar ont été réalisés. Il en a résulté que la quasi-totalité des souches testées se sont développées, pour la plupart très faiblement et tardivement, sur ces milieux minimum d'agar. Deux hypothèses ont pu être émises. La première suppose que l'ensemble des souches se développant sur ce milieu minimum d'agar présentent une activité agarase. La seconde explique le développement de ces souches sur ce milieu par la présence de sources nutritives en très faibles quantités dans l'agar ou le sel d'eau de mer (crystal reef) utilisés, sous la forme de sucres simples tels que le glucose. La première hypothèse semble la moins probable car il en résulterait que la communauté fongique associée à *P. palmata*, dont la fraction polysaccharidale est composée essentiellement de xylane, soit caractérisée par plus de souches agarolytiques que de souches xylanolytiques. La seconde hypothèse semble plus probable au vu du caractère très réduit et tardif du développement de la plupart des souches fongiques sur milieu minimum d'agar. De plus, il serait possible d'expliquer le bon développement de quelques rares souches à partir des potentielles impuretés de ce milieu par le fait que certaines espèces fongiques produisent des spores dont la germination est particulièrement induite dans des conditions de faible ressources nutritives (Rédou *et al.* 2016). Des tests de dosages de sucres réducteurs menés sur la poudre d'agar ou le sel d'eau de mer utilisés pourraient être effectués afin de vérifier cette dernière hypothèse.

Ce type de questionnement ne se pose pas dans le cas de cultures liquides car il y est plus aisé de respecter rigoureusement le principe d'unique source de carbone. En général, la production d'enzymes

par les champignons se réalise soit par un tel protocole de fermentation en solution ("submerged fermentation", SmF) soit en utilisant un substrat solide ("solid state fermentation", SSF) comme ceux utilisés pour le criblage enzymatique. Le protocole de SmF est adapté aux échelles industrielles car le contrôle de la croissance et des paramètres physicochimiques est aisé, et car il permet d'obtenir une bonne reproductibilité ainsi qu'une bonne pureté enzymatique (Polizeli *et al.* 2005; Motta *et al.* 2013; Kunamneni 2016). Par ailleurs, le protocole par SSF présente également de nombreux avantages par rapport à la SmF, tels que la facilité d'adaptation de la souche fongique pour le substrat, une plus faible contamination bactérienne, des besoins plus faibles en eau, un équipement plus simple, une meilleure reproduction de l'environnement naturel, une meilleure viabilité économique liée notamment à un besoin plus faible en énergie, ainsi que des meilleurs rendements en enzymes (Beg *et al.* 2001; Polizeli *et al.* 2005; Dhiman *et al.* 2008; Chapla *et al.* 2010; Motta *et al.* 2013; Gupta *et al.* 2015; Imran *et al.* 2016; Money 2016; Shah *et al.* 2019; Bhardwaj *et al.* 2021; Ejaz *et al.* 2021; Singh *et al.* 2021). Ce dernier type de fermentation a donc été choisi pour la culture et la production enzymatique des souches fongiques caractérisées par une double activité xylanolytique et cellulolytique. Concernant la production de cellulase et de xylanase par les champignons, les substrats purs (cellulose ou xylane) ainsi que les substrats bruts (paille de riz, de blé, ...) peuvent être utilisés. L'utilisation de substrats purs induit souvent une meilleure production enzymatique mais est plus chère que l'utilisation de substrats bruts, qui peuvent induire la production d'enzymes mais peuvent aussi être caractérisés par la présence de composés inhibiteurs comme les composés phénoliques (Maheswari and Chandra 2000; Motta *et al.* 2013; Bhardwaj *et al.* 2021).

L'usage de ces substrats bruts permet également de se rapprocher de l'environnement naturel des champignons. Pour ces différentes raisons, le milieu de culture utilisé dans la suite de cette étude n'est constitué que de biomasse de *P. palmata* et d'eau distillée. Le protocole de fermentation mis en place pour la production enzymatique des souches fongiques sélectionnées suite au criblage enzymatique, a ensuite été considéré dans le chapitre suivant (**CHAPITRE IV**).

CHAPITRE IV : QUANTIFICATION DES
ACTIVITÉS ENZYMATIQUES -
DÉGRADATION DE *P. PALMATA*

Préface

A l'issue du travail de criblage enzymatique, 18 souches ont été sélectionnées pour être cultivées afin de caractériser de manière putative et quantitative les activités cellulase et xylanase. Ces souches, appartenant à la collection MMS correspondent à *Stachybotrys chartarum* MMS1679, *Aspergillus* sp. III MMS1733, *Cladosporium* sp. II MMS1738, *Acremonium* sp. II MMS1777, *Penicillium* sp. V MMS1782, *Aspergillus* sp. I MMS1785, *Peroneutypa* sp. MMS1817, *Pleosporales* sp. I MMS1822, *Arachnomyces peruvianus* MMS1853, *Cladosporium ramotenellum* MMS1855, *Penicillium* sp. XVIII MMS1906, *Hypocreales* sp. I MMS1908, *Penicillium brevicompactum* MMS1910, *Cladosporium* sp. VIII MMS1956, *Cladosporium ramotenellum* MMS1959, *Cladosporium* sp. I MMS1980, *Parengyodontium album* MMS1982 and *Penicillium* sp. XIX MMS1986. Les souches sélectionnées ont été cultivées selon le protocole décrit dans ce chapitre sur milieu DCA en fonction de l'observation microscopique sur la production des conidies et sur la capacité de croissance sur milieu solide, seules 6 souches ont ensuite été utilisées pour la caractérisation de l'activité enzymatique (**Figure IV. 1**).

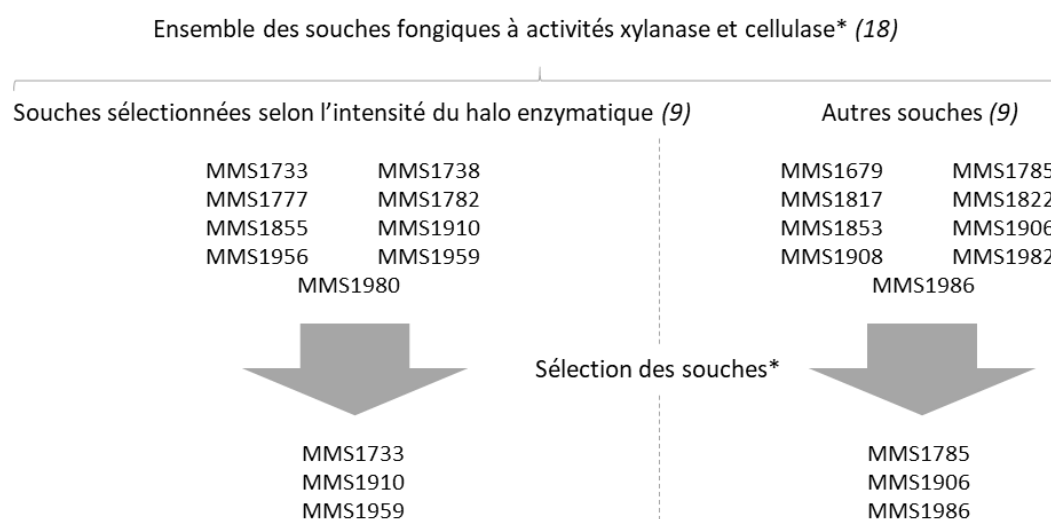


Figure IV. 1 : Sélection des souches fongiques utilisées dans ce chapitre. Les nombres entre parenthèses représentent le nombre de souches. * Sélection effectuée en fonction de la vitesse de croissance, du taux de recouvrement du milieu de culture, et du degré de sporulation.

En plus de la quantification des activités enzymatiques, les six souches fongiques sélectionnées ont fait l'objet de tests de digestion de la paroi de *P. palmata* afin d'en extraire les protéines solubles totales ainsi que la R-PE. Ces différentes étapes sont succinctement présentées dans la **Figure IV. 2** et détaillées dans la suite du manuscrit.

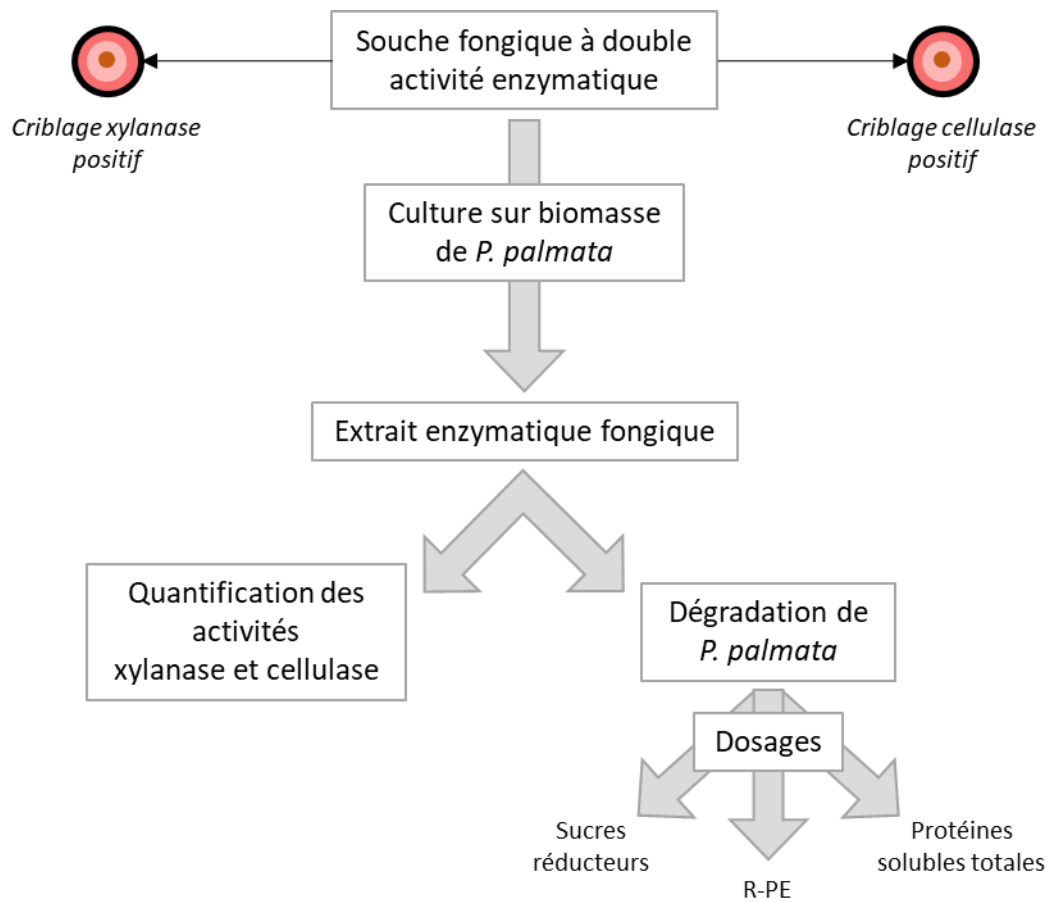


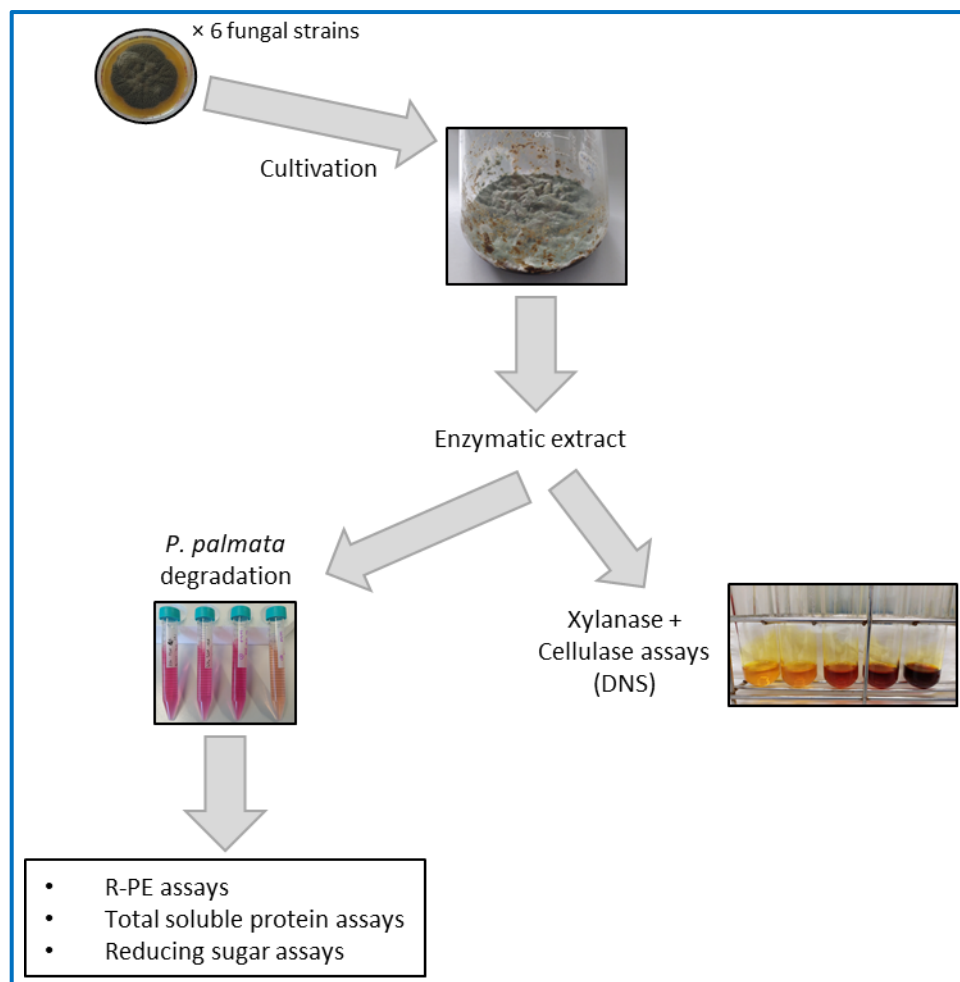
Figure IV. 2 : Etapes principales de la quantification des activités enzymatiques et des dosages effectués suite à la dégradation de *Palmaria palmata*. Ces étapes ont été appliquées sur l'ensemble des six souches fongiques sélectionnées précédemment.

Quantification of xylanolytic and cellulolytic activities of fungal strains isolated from *P. palmata* for macroalgal biomass degradation and R-phycoerythrin extraction

Yoran Le Strat¹, Margaux Mandin¹, Nicolas Ruiz¹, Thibaut Robiou du Pont¹, Emilie Ragueneau¹, Alexandre Barnett¹, Paul Déleris¹, Justine Dumay¹

¹ Nantes Université, Institut des Substances et Organismes de la Mer (ISOMER), UR 2160, F-44000, Nantes, France ; yoran.le-strat@univ-nantes.fr; margaux.mandin@etu-univ-nantes.fr; nicolas.ruiz@univ-nantes.fr; thibaut.robious@univ-nantes.fr; emilie.ragueneau@univ-nantes.fr; alexandre.barnett@univ-nantes.fr; paul.delersis@univ-nantes.fr; justine.dumay@univ-nantes.fr

Graphical abstract



Abstract

The aim of the present study was to quantify both xylanolytic and cellulolytic activities of enzymatic extracts obtained from six *Palmaria palmata* derived fungal strains. Degradation of *P. palmata* biomass by these enzymatic extracts was also investigated, focused on soluble protein and R-PE extraction. Enzymatic extracts were obtained by solid state fermentation based on culture media only made of *P. palmata* biomass. Then, extracts were enzymatically assayed by using DNS method and macroalgal degradation abilities were evaluated by measuring reducing sugar release. Soluble proteins and R-PE recovery yields were evaluated through BCA and spectrophotometric assay, respectively. Control experiment was performed using commercial xylanase regularly used for *P. palmata* cell wall degradation. Various enzymatic activities were obtained according to fungal isolates up to 978 U/mL for xylanase and 50 U/mL for cellulase. It was also observed that enzymatic extract obtained allowed high degrading abilities, with 4 of the 6 fungal strains assessed exhibiting at least equal results as the commercial enzymes for the reducing sugar release. In the same way, all the 6 strains allowed the same soluble protein extraction yield and 4 of them led to an improvement of R-PE extraction. Several strains seemed particularly promising for R-PE recovery such as *Penicillium* sp. MMS 1906, *Aspergillus* sp. MMS1733 and *Penicillium brevicompactum* MMS1910.

1. Introduction

Seaweeds are traditionally consumed in Asian country for a long time (Mabeau and Fleurence 1993; Sanjeewa *et al.* 2018). Their wide use in Europe is more recent (consumption of seaweeds as vegetables and condiments is authorized from 1990 in France) and is gradually increasing from several decades, notably because of an increasing demand of healthy food, with low ecological impact (Mabeau and Fleurence 1993; Sanjeewa *et al.* 2018; Lopes *et al.* 2020).

Algae may be the most diverse group of organisms alive, representing one third of the total divisions of the plant kingdom (Ioanou and Roussis 2009). Among macroalgae, red seaweeds (Rhodophyceae) are characterized by the highest specific diversity, representing 45 % of total seaweed species, followed by brown (Phaeophyceae) and green ones (Chlorophyceae) (Reddy *et al.* 2010; Patwary *et al.* 2021). Red seaweeds are also the main macroalgal group in terms of commercial values (Reddy *et al.* 2010) and in terms of secondary metabolite production (Abdul Malik *et al.* 2020). Hydrocolloids of red seaweeds, being agars and carrageenans, are not only used in food but also in pharmaceuticals (Ioanou and Roussis 2009; Sanjeewa *et al.* 2018). Indeed, in addition to the useful thickening and gelling properties in formulation, these macroalgal sulphated polysaccharides can be characterized by biological activities such as antiviral ones (Rico *et al.* 2017; Rosales-Mendoza *et al.* 2020). Besides hydrocolloids, red seaweeds are also widely known to synthesize a great diversity of bioactive

compounds with biotechnological interests and health-related properties, such as pigments, lipids or polyphenols as example (Bouhlal *et al.* 2013; Hardouin *et al.* 2014; Pal *et al.* 2014; Rico *et al.* 2017; Sanjeeva *et al.* 2018).

Red seaweeds would be more promising than green and brown macroalgae according to their protein contents (Fleurence 1999; Galland-Irmouli *et al.* 1999; Le Guillard *et al.* 2015; Afonso *et al.* 2019; Leandro *et al.* 2019; Lourenço-Lopes *et al.* 2020). The red seaweed *P. palmata* is one of the best macroalgal protein producer with a protein fraction representing up to 35 % of the dry weight (Mabeau and Fleurence 1993; Fleurence 1999; Dumay *et al.* 2014; Naseri *et al.* 2020). Quantitatively, these protein levels can be higher than the ones observed in soybean (Fleurence 1999; Dumay *et al.* 2014). From a qualitative point of view, essential amino acids represent 46 % of total amino acids from *P. palmata* and composition can be compared with the one of soybean or eggs (Fleurence 1999; Galland-Irmouli *et al.* 1999; Dumay *et al.* 2014). However, the access and the digestibility of these proteins are limited by polysaccharides such as xylan in *P. palmata*, which are antinutritional (Galland-Irmouli *et al.* 1999; Dumay *et al.* 2014). One of the protein of interest synthesized by Rhodophyceae, including *P. palmata*, is the phycoerythrin pigment, belonging to phycobiliproteins, and used notably as immunofluorescence dye (Fleurence 1999).

Phycobiliproteins are water soluble algal pigments which can represent 20 % of algal dry weight and which can be separated between phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC) and phycoerythrocyanin (PEC) (Jiang *et al.* 1999; Martínez-Oyanedel *et al.* 2004; Niu *et al.* 2006; Dumay *et al.* 2014; Miyabe *et al.* 2017). These phycobiliproteins are composed of two different subunits, α and β , and only PE possesses another γ subunit in its central cavity, which allow a higher structural stability (Fleurence 2003; Niu *et al.* 2006; Liu *et al.* 2009; Dumay *et al.* 2014). Phycobiliproteins are organized into phycobilisomes which allow the light energy transfer and are located on the external membrane of thylakoids (Niu *et al.* 2006; Dumay *et al.* 2014). These phycobilisomes are composed of a core, linked with thylakoids by polypeptides, and composed of APC. The other structure is the rod, composed of PC next to the core and of PE at the other extremity (Martínez-Oyanedel *et al.* 2004; Dumay *et al.* 2014; Miyabe *et al.* 2017). These structures are linked with polypeptides (Martínez-Oyanedel *et al.* 2004). The light harvesting is performed by PE, and the energy is transferred *via* PC and APC to chlorophyll in the reaction centre (Jiang *et al.* 1999; Bharmoria *et al.* 2020). R-PE is the major phycobiliprotein in red seaweeds and is responsible of their coloration (Dumay *et al.* 2013, 2014).

Phycobiliproteins are not harmful to human as being ingested or applied to external surface, they are widely used in food and cosmetics and as photosensitizer for tumour treatment (Niu *et al.* 2006). More specifically, R-PE is characterized by an important biotechnological interest. It can be used as a pink

colorant, particularly in food industry, and as fluorescent probe in several technologies such as flow cytometry, fluorescent immunoassays or immunophenotyping (Fleurence 1999; Dumay *et al.* 2013, 2014; Sfriso *et al.* 2018; Bharmoria *et al.* 2020). R-PE was also characterized by several bioactivities such as antitumoral, anti-ageing, anti-inflammatory, antioxidant, antidiabetic, antiparasitic, immunosuppressive and antihypertensive ones (Fleurence 2003; Dumay *et al.* 2013, 2014; Miyabe *et al.* 2017; Leandro *et al.* 2019; Lourenço-Lopes *et al.* 2020; Rosales-Mendoza *et al.* 2020). It also have potential in application in solar cells (Bharmoria *et al.* 2020). R-PE potential in nanotechnologies is also highlighted by a study exhibiting the great enhancement of its electrical conductivity with an association of AgO nanoparticles in the R-PE channel (Bekasova *et al.* 2017). The price of R-PE generally vary between 180 and 250 US \$/mg and it depend principally on the R-PE purity (Fleurence 2003).

However, the R-PE uses are still limited to small scales by several issues about its extraction, purification and conservation but various strategies are currently in development to cope with these problems. One of the biggest issues for the R-PE extraction, which also concern all soluble proteins and metabolites of interest from macroalgae, is the presence of cell wall polysaccharides having a retention effect and intracellular polysaccharides exhibiting ionic interactions with proteins (Joubert and Fleurence 2008; Denis *et al.* 2009; Wang *et al.* 2010; Harnedy and Fitzgerald 2013a; Sfriso *et al.* 2018). In *P. palmata*, these polysaccharides are mostly xylan and cellulose, in a lower amount (Morgan *et al.* 1980; Lahaye *et al.* 1993; Dumay *et al.* 2014; Naseri *et al.* 2020). Conventional process of R-PE extraction consisted in osmotic shock or liquid nitrogen grinding but these technics were time consuming, not very efficient and not applicable at industrial scale due to their cost (Fleurence 2003; Dumay *et al.* 2014; Nguyen *et al.* 2018). Currently, the most widespread technic for R-PE extraction is the enzyme-assisted extraction (EAE) (Dumay *et al.* 2014; Sfriso *et al.* 2018). EAE is a particularly promising process, widely used in macroalgal metabolite recovery, as an eco-friendly process, with high yield of products and a good quality (Hardouin *et al.* 2014; Charoensiddhi *et al.* 2017). EAE process allows the extraction of many inaccessible compounds due to polysaccharides cell wall, and can be used in association with other extraction approaches such as microwave or ultrasound (Harnedy and Fitzgerald 2013a; Dumay *et al.* 2014; Hardouin *et al.* 2014).

Several studies about enzymatic assisted extraction use on the R-PE recovery from *P. palmata* were performed (Fleurence 1999, 2003; Joubert and Fleurence 2008; Dumay *et al.* 2013, 2014; Naseri *et al.* 2020). R-PE enzyme assisted extraction has also been studied in other red seaweeds such as *Gracilaria gracilis* (Nguyen *et al.* 2020), *Grateloupia turuturu* (Denis *et al.* 2009) or *Gelidium pusillum* (Mittal and Raghavarao 2018). The cell walls of red seaweeds is composed of various polysaccharides which differ from a species to another and are responsible of the use of adapted enzymes in order to solubilize compounds of interest such as R-PE and other proteins (Fleurence 2003; Nguyen *et al.* 2020).

It has been previously shown that the use of both xylanase and cellulase would allow an optimal extraction compared with the use of only one enzyme (Fleurence 2003; Joubert and Fleurence 2008). This synergistic effect seems not to be characteristic of all cellulases and xylanases, it would depend on the biological origin of enzymes used for extraction (Fleurence 1999).

Moreover, even if commercial enzymes have been successfully studied for the R-PE extraction from *P. palmata* by degrading its cell wall, the importance of the enzymatic specificity for the substrate has previously been mentioned. Indeed, EAE seems to be particularly efficient using algal-specific enzymes (Charoensiddhi *et al.* 2017). Marine enzymes including cellulases would be particularly industrially promising compared with terrestrial ones by exhibiting stabilities toward several physicochemical parameters such as pressure, pH, temperature, salinity, metals and chemicals or solvents (Barzkar and Sohail 2020). Among marine enzyme producers, fungi would be particularly interesting, compared with bacteria, by excreting enzymes in the extracellular medium and exhibiting higher enzymatic activities (Motta *et al.* 2013; Imran *et al.* 2016; Bhardwaj *et al.* 2021), as well as having several abilities such as crystalline cellulose degradation (Barzkar and Sohail 2020).

The fungal diversity associated with *P. palmata* have recently been studied, including cultivable strains (**CHAPITRE II**). Both xylanase and cellulase activities were screened from this cultivable fungal community associated with *P. palmata* (**CHAPITRE III**). Six fungal strains from *P. palmata* were selected and quantitatively assayed for xylanase and cellulase activities. Enzymatic extracts obtained from selected fungi were also tested on the R-PE recovery from *P. palmata*.

2. Material and methods

2.1. Fungal strains

A large collection of marine fungal isolates has been previously obtained and characterized from the red alga *Palmaria palmata* (**CHAPITRE II**). These strains have been deposited in the laboratory Marine Mycological Strain collection (MMS). The algal thalli were sampled at Batz-sur-Mer (France) between June 2019 and June 2020. Briefly, fungal isolation was performed using one aspecific enriched Wickerham medium and one specifically designed "*P. palmata*" medium to mimic in situ conditions, along with two different incubation temperatures (12°C and 27°C). Distinction between epiphytes and endophytes was performed according to different treatments of the thalli. Fungal identification was performed using ITS (Internal Transcribed Spacers) as principal molecular marker and completed with supplementary sequencing data (18S ribosomal nuclear subunit, β -tubulin or actin). An enzymatic qualitative screening targeting cellulolytic and xylanolytic activities has been previously performed on all these strains (**CHAPITRE III**). Based on this screening, six strains were selected in order to putatively

and quantitatively characterize cellulase and xylanase activities. The **Table IV. 1** summarises all informative data regarding the present studied strains.

Table IV. 1: Strains studied for the enzymatic production. Additional strain information of location in alga (Epi/endo = non-determined location), incubation temperature, isolation culture medium (Wick = Wickerham medium, Palm = “*P. palmata*” medium) and season of sampling is given according to previous work (**CHAPITRE II**).

Fungal isolate	Molecular marker	Location in alga	Incubation temperature (°C)	Isolation culture medium	<i>P. palmata</i> sampling season
<i>Aspergillus</i> sp. MMS1733	ITS / actin	epi/endo	12	Palm	summer
<i>Aspergillus</i> sp. MMS1785	ITS	epi/endo	27	Palm	winter
<i>Penicillium</i> sp. MMS1906	ITS / β -tubulin	endophyte	12	Wick	spring
<i>P. brevicompactum</i> MMS1910	ITS / β -tubulin	endophyte	12	Wick	spring
<i>Cladosporium</i> <i>ramotenellum</i> MMS1959	ITS / actin / elongation factor (ef1)	epi/endo	12	Palm	spring
<i>Penicillium</i> sp. MMS1986	ITS / β -tubulin	endophyte	12	Palm	spring

2.2. Strain cultivation and preparation of conidia suspension

Fungal strains were grown in 50 mL conical flask on 15 – 20 mL of Dextrose Casein Agar medium (DCA, Condalab, Madrid, Spain) composed of 40 g/L dextrose, 10 g/L mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1), 15 g/L agar. After 14 days of incubation at 27°C, a conidial suspension was prepared with the addition of 5 mL of sterile distilled water and 0.1 mL of Tween 80 in the tube of fungal growth. Conidial suspensions were standardized at 10⁶ conidia/mL and were then aliquoted and stored at -20°C.

2.3. Enzyme production

A sterile culture medium based on lyophilised powder of *P. palmata* at 80 % humidity was prepared for the fungal enzyme production. Similar fungal culture media at 80 % humidity have already been used for enzymatic production, with various carbon sources (Gao *et al.* 2008; Rodríguez-Jasso *et al.* 2013). For fungal growth and enzymatic production, 1.5 mL of conidial suspension was added on all the surface of culture medium prepared with 20 g of lyophilised powder of *P. palmata* (1 g of wet alga corresponded to 0.15 g of dry alga). Such culture was performed in triplicate for each strain and incubated 7 days at 27°C. Biological negative control was realized with the incubation of culture medium without any conidia suspension. After incubation, 35 mL of sterile distilled water was added in the fungal cultures and homogenisation was performed using a blender (Virtis 23, New York, USA). The mash was then centrifuged 15 min at 9500 rpm (SL 8R, Thermo Scientific™, Waltham, USA) and

the supernatant was filtered through a glass microfiber filter at 1.2 μm retention porosity (Whatman™, Buckinghamshire, United Kingdom) using a vacuum pump (Millipore, Burlington, USA). Glycerol 87 % w/w (PlusOne, St George, USA) was added to reach a final concentration of 10 % in the filtrate which is then stored at -80°C . This filtrate corresponded to the enzymatic extract used for the following tests.

2.4 Protein quantification

Protein quantifications were performed using bicinchoninic acid (BCA) method with the Pierce™ BCA Protein Assay Kit (Thermo Scientific™) according to the manufacturer instructions. Measurements were performed on ELx800 UV Universal Microplate Reader (BioTek, Winooski, USA).

2.5. Enzymatic assays

Enzymatic assays were performed on enzymatic extracts to determine both cellulase and xylanase activities of every selected strain, using the dinitrosalicylic acid (DNS) method (Bailey *et al.* 1992). Briefly, this method allows the measurement of reducing sugar production by enzymatic extract from a substrate being sodium carboxymethyl cellulose (Sigma-Aldrich, St. Louis, USA) and wheat arabinoxylan (Megazyme, Wicklow, Ireland) for cellulase- and xylanase-assays, respectively. Both substrates, sodium carboxymethyl cellulose and arabinoxylan, were prepared at 1 % in 0.05 M pH 5.3 sodium citrate buffer (citric acid 21 g/L, Acros Organics, Geel, Belgium, and sodium citrate tribasic dihydrate 29.6 g/L, Sigma-Aldrich, St. Louis, USA). A solution of DNS 1 % was prepared (DNS, Sigma-Aldrich, St. Louis, USA, 1 %; Phenol, Fluka™, Honeywell, Charlotte, USA, 0.2 %; sodium sulphite, Sigma-Aldrich, 0.05 %; NaOH, Fluka™, 1 %). Commercial enzymatic solutions were used as control, being solutions of 1 % cellulase and 1 % xylanase from *Trichoderma longibrachiatum* (Sigma-Aldrich, St. Louis, USA). Reaction consisted in mixing 1.8 mL of substrate solution with 200 μL of enzymatic solution (fungal extract or control), heating this mix at 50°C during 5 min, adding 3 mL of 1 % DNS solution, heating the mix at 100°C during 15 min, and reading the OD at 540 nm (Shimadzu, Kyoto, Japan) after a cooling step of tubes in cold water. One unit of enzyme activity was defined as the amount of enzyme allowing the release of 1 μmol of product (xylose for xylanase and glucose for cellulase) per minute in assay condition.

2.6. R-PE extraction from *P. palmata*

In a previous study, commercial xylanase powder concentration were defined as optimal at 17.8 g/kg dw (35.6 mg in 200 mL of solution of reaction) (Dumay *et al.* 2013). This commercial extract was used at this concentration for positive control. To standardize the R-PE extraction conditions for the six selected fungal strains, a volume of enzymatic extract allowing a xylanase activity equivalent to 35.6

mg of commercial xylanase was used. These volumes were established using results of DNS assays realized on arabinoxylan as substrate and described in **Table IV. 2**.

Table IV. 2: Composition of reaction solution of R-PE extraction for each fungal strain. The last line corresponds to the positive control.

Fungal isolate	Volume of fungal extract (mL)	Volume of acetate buffer (mL)	Cut <i>P. palmata</i> thalli (g)
<i>Aspergillus</i> sp. MMS1733	44.5	155.5	15
<i>Aspergillus</i> sp. MMS1785	14	186	15
<i>Penicillium</i> sp. MMS1906	32.3	167.7	15
<i>P. brevicompactum</i> MMS1910	16.6	183.4	15
<i>Cladosporium ramotenellum</i> MMS1959	39.5	160.5	15
<i>Penicillium</i> sp. MMS1986	29.6	170.4	15
Positive control	35.6 mg *	200	15

*mass of commercial xylanase powder from *T. longibrachiatum*

The R-PE extraction from *P. palmata* was performed on thalli sampled the 14th July 2021 and stored at -20°C after washing. The reaction consisted in the degradation of 15 g of cut fresh thalli of *P. palmata* occurring in an acetate buffer 50 mM pH 5 (anhydrous acetic acid, Thermo Scientific; sodium acetate, Sigma-Aldrich) with the addition either of 35.6 mg of commercial xylanase or corresponding volume of enzymatic extract (**Table IV. 2**), in a total volume of 200 mL in 200 mL enzymatic reactors (Fisher Scientific, Wheaton, USA). The reaction was performed during 320 min at 24°C with Polystat 36 (Fisher Scientific), in darkness and with an agitation of 250 rpm (Biostir® Wheaton, Millville, USA) according to [Dumay et al. \(2013\)](#). Solution was then roughly filtered through a fine sieve and was centrifuged 20 min at 4°C and 9500 rpm (SL 8R, Thermo Scientific). The supernatant corresponding to R-PE extract was aliquoted and stored at -20°C.

2.7. Dosage of R-PE, proteins and reducing sugars

Algal lysates obtained after *P. palmata* enzymatic degradation were used to determine reducing sugar, R-PE and protein concentrations using the following protocols. Reducing sugar were assayed using DNS method as mentioned above but without the first step of enzymatic incubation. R-PE assays were performed by spectrophotometric method and the OD measurements were done using a Shimadzu UV1900 spectrophotometer (Shimadzu, Kyoto, Japan). The following formula was used to obtain the R-PE concentration in mg/mL: $[R-PE] = 0.1247 [(A_{564} - A_{730}) - 0.4583 (A_{618} - A_{730})]$, according to [Sampath-Wiley and Neefus \(2007\)](#). Data of R-PE and soluble protein concentrations initially expressed in mg/mL were converted in mg/g dw based on the previously mentioned equivalence 1 g wet alga = 0.15 g dry alga.

2.8. Statistical analysis

Statistical analyses were performed using Rstudio (RStudio Team, 2020) and R software (R Core Team, 2020). The significances of differences between values of enzymatic activities, specific activities, reducing sugar, R-PE and total soluble protein concentrations were established by using pairwise Student test from rstatix package (Kassambara 2020). This test was the only solution to test the equality of expected results and not the stochastic equality. Similarity between strains is highlighted on the graphs by similar letter for similar data ($p\text{-value} \leq 0.05$).

3. Results

3.1. Xylanase and cellulase activities quantifications

The six fungal strains selected were tested for cellulase and xylanase activities by DNS assays. Results of enzymatic and specific activities were represented in **Figure IV. 3** and **Figure IV. 4**, respectively.

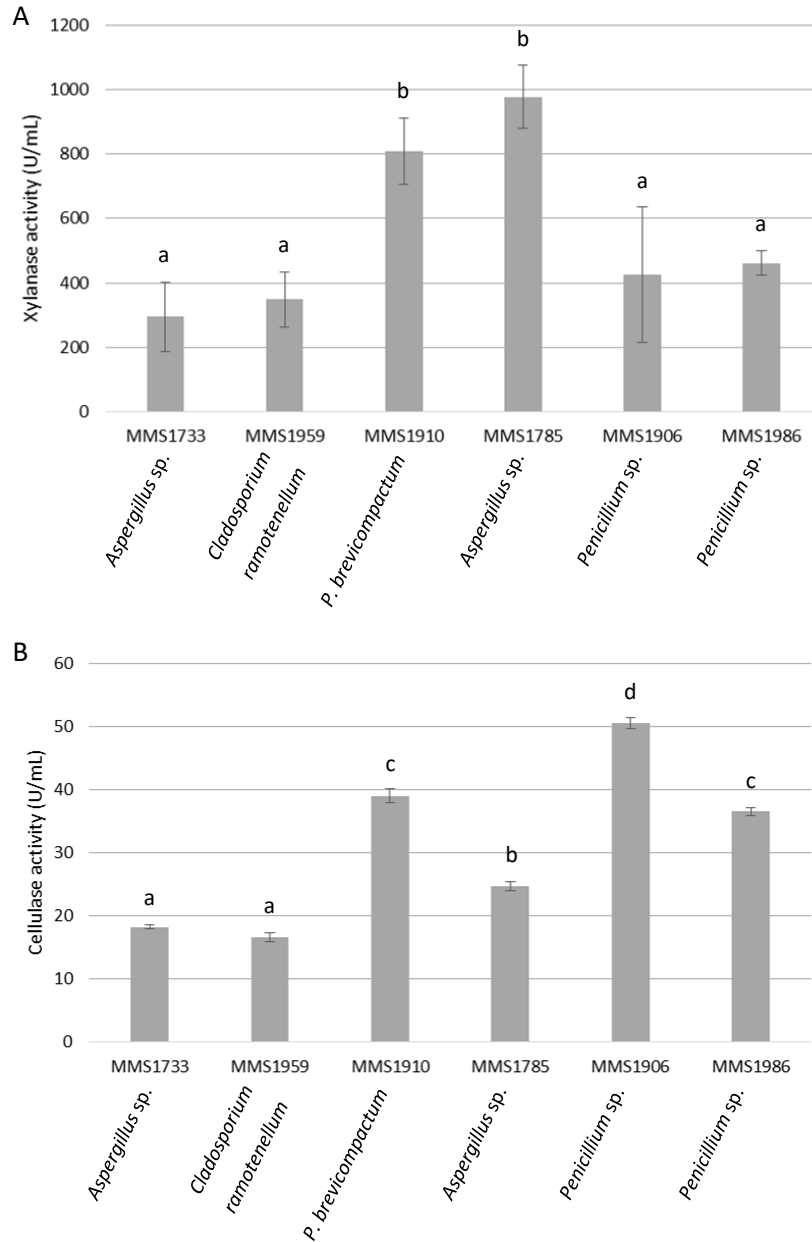


Figure IV. 3: Xylanase (A) and cellulase (B) activity in U/ml ($1U = 1 \mu\text{mol}/\text{min}$) of the six tested strains. Letters represent clustering of non-significantly different values according to post-hoc pair-wise Student test.

Regarding the xylanase activity assays (**Figure IV. 3A**), both *Aspergillus sp.* MMS1785 and *Penicillium brevicompactum* MMS1910 exhibited xylanase activities significantly different and at least 2.1 and 1.8 times higher than the four other isolates, respectively ($p\text{-value} < 0.01$). Standard deviations for xylanase activities were more important than considering cellulase ones. Then, no clear distinction could be done between MMS1733, MMS1986, MMS1906 and MMS1959 according to their xylanase activities which varied between 295.4 and 461.8 U/mL. The two highly active isolates MMS1910 and MMS1785 exhibited xylanolytic activities at 809.7 and 978 U/mL respectively.

Regarding cellulase assays, the three best cellulolytic isolates belonged to *Penicillium* genus with activities higher than 35 U/mL, and two intermediate strains belonged to *Aspergillus* genus with cellulase activities ranging from 24.7 to 36.5 U/mL **Figure IV. 3B**. The isolate characterized by the lowest cellulase activity was the one assigned as *Cladosporium ramotenellum* (16.6 U/mL). Clear significant distinctions could be done between these three highly cellulolytic strains (MMS1906, MMS1910 and MMS1986) and the three other strains (MMS1959, MMS1733 and MMS1785) (p -value < 0.001). The three *Penicillium* isolates with the highest cellulase activities, were also characterized as endophytic. Nonetheless, the location of the three other strains could not be determined neither as endophytic nor epiphytic. The best cellulolytic strain was MMS1906 with an activity at 50.5 U/mL.

The specific activities of all the six strains were measured for both cellulase and xylanase (**Figure IV. 4**). They ranged from 94.4 (MMS1733) to 253.7 (MMS1785) U/mg for xylanase and from 5.8 (MMS1733) to 15.1 (MMS1906) U/mg for cellulase.

Based on these results, no clear and significant distinction could be done between xylanase specific activities of MMS1733, MMS1959, MMS1910, MMS1906 and MMS1986 strains, with values ranging from 94.4 and 174.2 U/mg (**Figure IV. 4A**). Nevertheless, the MMS1785 strain stayed the best xylanolytic one with a specific activity reaching 253.7 U/mg, significantly higher than xylanase specific activities of other strains.

Regarding cellulase, the three strains MMS1733, MMS1959 and MMS1785 exhibited the lowest specific activities, ranging from 5.8 to 6.9 U/mg (**Figure IV. 4B**). The three other strains, MMS1910, MMS1986 and MMS1906 were characterized by cellulolytic specific activities significantly different between themselves, and to the three previously mentioned strains. They exhibited cellulase specific activity at 8.4, 10.7 and 15.1 U/mg, respectively. The strain MMS1906 was then characterized by both highest enzymatic and specific activities regarding cellulase.

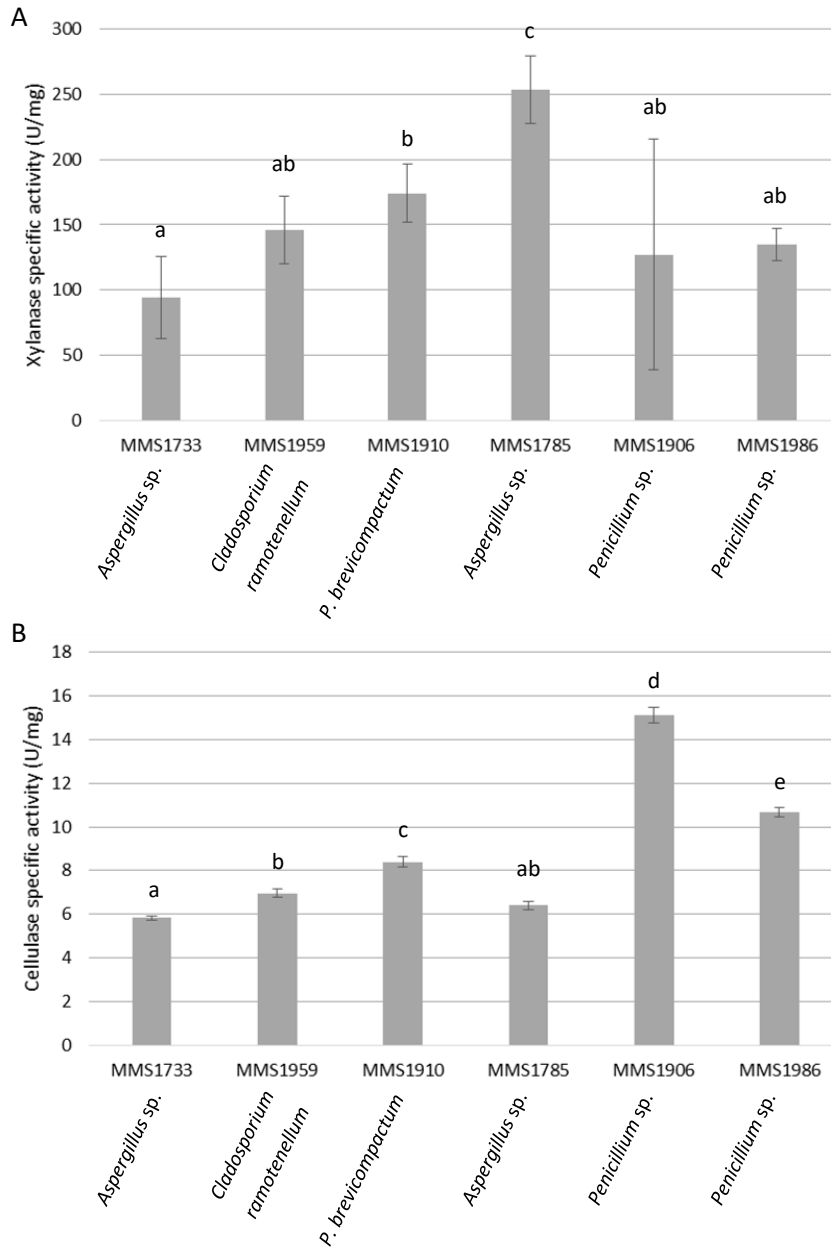


Figure IV. 4: Xylanase (A) and cellulase (B) specific activity in U/mg (1U = 1 μ mol/min) of the six tested strains. Letters represent clustering of non-significantly different values according to post-hoc pair-wise Student test.

Comparing both cellulase and xylanase (enzymatic and specific activities), it appeared that all fungal strains clearly exhibited highest xylanase than cellulase activity (**Figure IV. 3, 4**). The strain *Penicillium* sp. MMS1906 was characterized by a xylanolytic activity 8.4 times higher than cellulolytic one, being the lowest gap between cellulolytic and xylanolytic activity compared with other isolates. The greatest difference between cellulolytic and xylanolytic activities was detected with *Aspergillus* sp. MMS1785, with a xylanolytic activity 39.6 times higher than cellulolytic one.

3.2. Reducing sugars, R-PE and total proteins amounts from *P. palmata* lysates

Following the enzymatic digestion protocol of *P. palmata* with the enzymatic fungal extracts (or with commercial xylanase for positive control), we assessed the amount of reducing sugar, R-PE, and total protein released.

Reducing sugar extraction varied between 13.5 mM and 19.2 mM according to the six fungal isolates (**Figure IV. 5**). Enzymatic extracts from strains MMS1733, MMS1986 and MMS1906 permitted to reach similar concentrations of reducing sugar in algal filtrate at 19.2, 19 and 18.7 mM, respectively. They were significantly different to reducing sugar concentrations obtained with the other fungal strains (p -value < 0.05). MMS1785, MMS1910 and MMS1959 were characterised by a respective decrease regarding reducing sugar release which was not significantly different. The positive control resulted in reducing sugar extraction lower than the ones obtained by using MMS1733, MMS1986 and MMS1906 but not significantly different (17.3 mM). The negative control without enzyme was significantly different to all the other assays, resulting in reducing sugar extraction at least 20 times lower than by using fungal extracts.

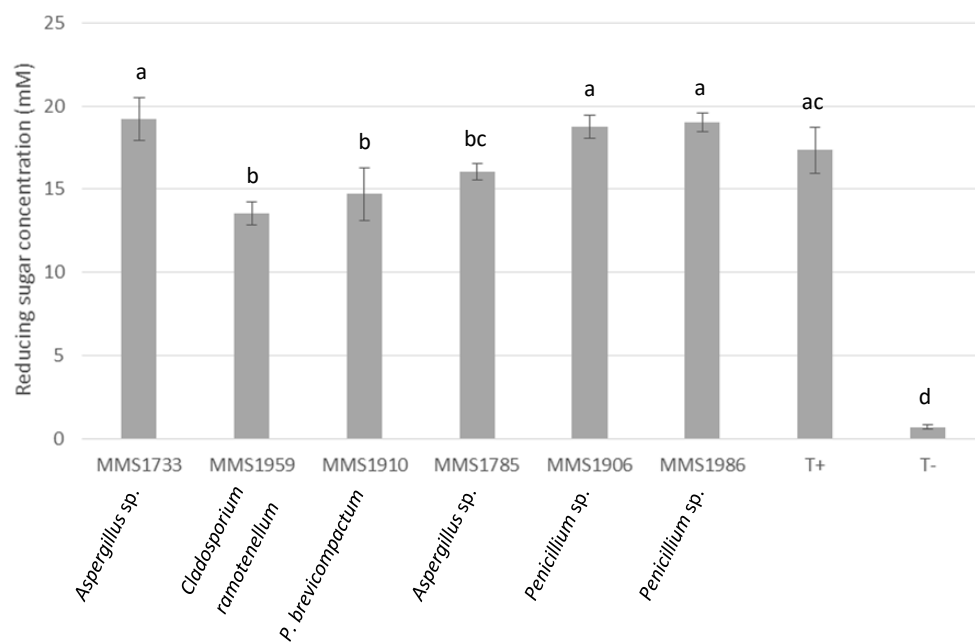


Figure IV. 5: Reducing sugar concentration (mM) in the algal lysates after enzymatic digestion. Negative control (T-) corresponded to an algal lysate obtained without enzyme added in the reactor, while positive control (T+) corresponded to an algal lysate obtained with addition of commercial xylanase in the reactor. Letters represent clustering of non-significantly different values according to post-hoc pair-wise Student test.

The **Figure IV. 6** represents R-PE and total soluble protein releases obtained with all the six fungal extracts. Determination of these releases were performed by using spectrophotometric method (Sampath-Wiley and Neefus 2007) and BCA assays respectively, on fungal extracts obtained after the algal thallus degradation. These releases were compared with positive control (commercial xylanase) and negative control (no enzyme).

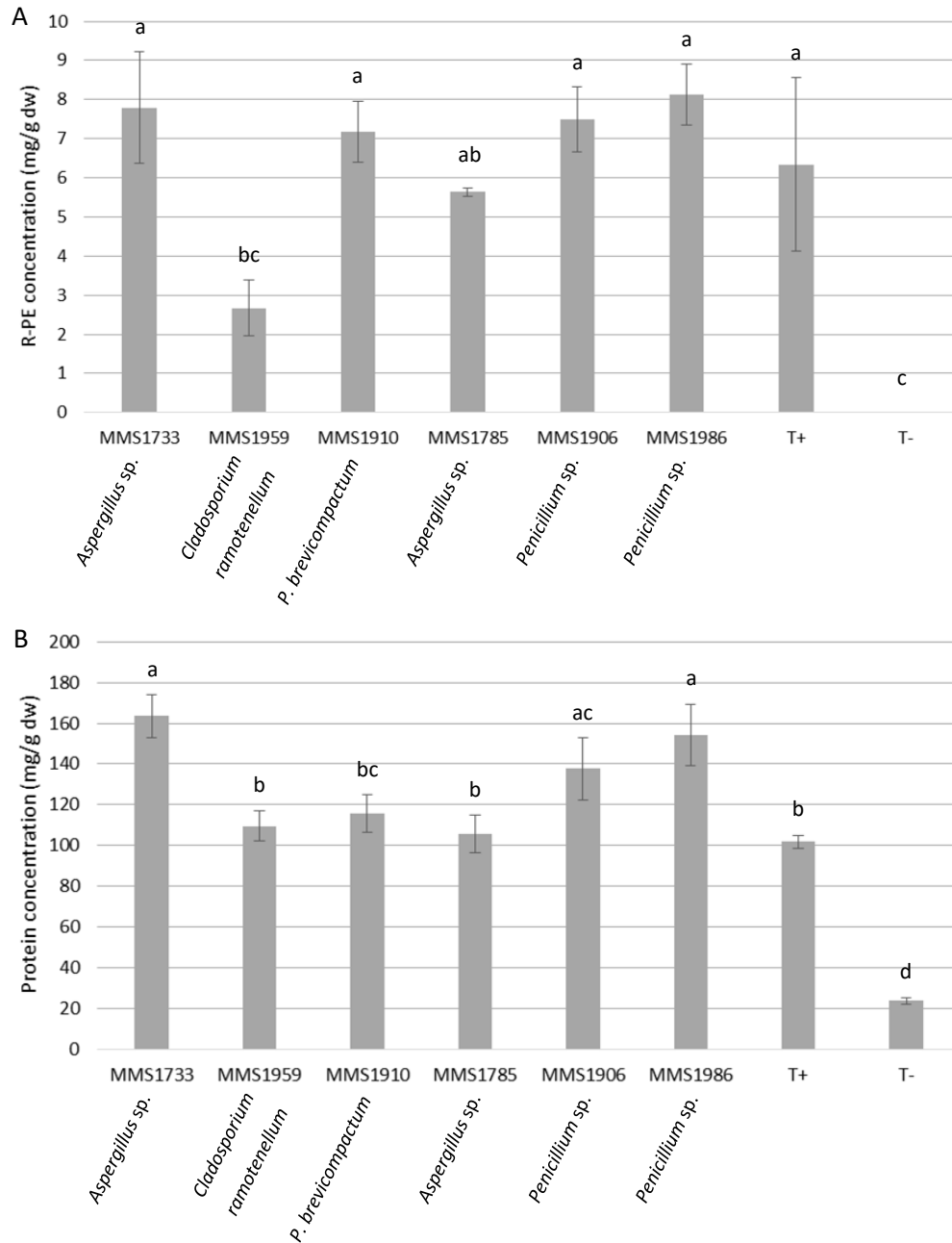


Figure IV. 6: R-PE (A) and soluble protein (B) dosage from algal lysates after enzymatic digestion (mg/g dw). R-PE concentration was determined according to Sampath-Wiley and Neefus (2007) method. Negative control (T-) corresponded to an algal lysate obtained with no enzyme added in the reactor, while positive control (T+) corresponded to an algal lysate obtained with addition of commercial xylanase in the reactor. Letters represent grouping of non-significantly different values according to post-hoc pair-wise Student test.

The algal lysates obtained using enzymatic extract of MMS1986, MMS1733, MMS1906 and MMS1910 seemed to be similarly characterized by the highest amounts of R-PE release, at 8.1, 7.8, 7.5 and 7.2 mg/g dw, respectively (**Figure IV. 6A**). Algal degradation using MMS1959 enzymatic extract led to the lowest R-PE concentration in enzymatic reactor (2.67 mg/g dw), significantly different to the R-PE concentrations of the four previously mentioned strains (p -value < 0.01). The enzymatic extract of MMS1785 allowed an intermediate R-PE release (5.63 mg/g dw). The positive control resulted in a R-PE release of 6.3 mg/g dw. This value was lower than the ones obtained by using fungal extracts of MMS1986, MMS1733, MMS1906 and MMS1910 but not significantly different. It must be highlighted that this positive control was characterized by an important standard deviation. No R-PE release was obtained with the negative control without any enzyme.

The highest total protein recovery was obtained using the enzymatic extracts of MMS1733 and MMS1986, with protein concentrations of 163.5 and 154.3 mg/g dw (**Figure IV. 6B**). The use of enzymatic extracts of strains MMS1785, MMS1959 and MMS1910 was characterized by low total protein contents in the lysate with concentrations of 105.5, 109.6 and 115.8 mg/g dw. Total protein contents obtained with these three strains were significantly different to the ones obtained with MMS1733 and MMS1986 strains (p -value < 0.01). An intermediate total protein concentration in algal lysate of 138 mg/g dw was observed using MMS1906 enzymatic extract. The positive control resulted in a total protein release of 101.7 mg/g dw. This value was significantly lower than the ones obtained by using fungal extracts of MMS1733, MMS1986 and MMS1906 (p -value < 0.01). The negative control without any enzyme resulted in a total protein release of 23.6 mg/g dw, significantly lower than the ones obtained by using fungal extracts (p -value < 0.001).

4. Discussion

4.1. *Aspergillus*, *Penicillium* and *Cladosporium* marine fungal genera as promising xylanase and cellulase producers

The six fungal strains selected for a description of their cellulolytic and xylanolytic potential, as well as for their ability to degrade the red seaweed *P. palmata* and to recover R-PE, belonged to the three fungal genera *Penicillium*, *Cladosporium* and *Aspergillus*. These fungal genera are widely distributed in environment, notably marine ones, as easily cultivable fungi. More specifically, *Aspergillus*, *Cladosporium* and *Penicillium* are dominant among all Ascomycota genera widely colonizing macroalgae, and they are characterized by a broad algal host specificity (Suryanarayanan 2012; Raghukumar 2017; Abdul Malik *et al.* 2020; Le Strat *et al.* 2022).

In a recent review, we have realized a state of the art on the polysaccharidase abilities of marine-derived fungi, focused on macroalgal polysaccharides (Le Strat *et al.* 2022). Among various aptitudes in the degradation of macroalgal polysaccharides, fungal species belonging to *Aspergillus*, *Cladosporium* and *Penicillium* genera were recorded to exhibit both cellulolytic and xylanolytic activities (Balabanova *et al.* 2018; Barzkar and Sohail 2020; Le Strat *et al.* 2022).

4.2. Xylanolytic and cellulolytic profiles of fungal strains related to their macroalgal degradation abilities and R-PE recovery

Besides enzymatic assays applied on every six fungal strains, enzymatic extracts were then used for *P. palmata* biomass degradation. Extraction yield of reducing sugars; R-PE and soluble proteins were then estimated. The R-PE concentration of macroalgal lysates were obtained by using Sampath-Wiley and Neefus (2007), optimised from the protocol set up by Beer and Eshel (1985). Conversions of enzymatic activities into specific activities were performed by using total protein contents in fungal enzymatic extracts. These values of protein contents varied slightly from 3.1 to 3.9 regarding MMS1733, MMS1906, MMS1986 and MMS1785 (data not shown). This could explain that trends of fungal strain enzymatic activities between themselves are globally conserved regarding specific activities. Nevertheless, MMS1959 and MMS1910 enzymatic extracts were characterized by total protein concentrations of 2.4 and 4.6 mg/mL, respectively (data not shown). This relative high protein concentration of MMS1910 enzymatic extract resulted in a decrease in enzymatic relative potential of this isolate compared with other fungal strains by considering enzymatic specific activity (U/mg) rather than enzymatic activity (U/mL).

The results obtained in the present study can be compared with previous ones obtained in our laboratory on *P. palmata* degradation with cellulolytic and xylanolytic enzymes. Regarding R-PE recovery, enzymatic assisted extraction without optimisation resulted in up to 4 mg/g dw or 0.35 mg/g ww R-PE concentrations (Fleurence 2003; Joubert and Fleurence 2008). After optimisation of the extraction protocol adapted for the use of commercial xylanase of *T. longibrachiatum*, the R-PE yield reached 12.36 mg/g dw (Dumay *et al.* 2013). In the present study, the highest R-PE yield reached 8.1 mg/g dw (MMS1986). Nevertheless, it is important to notice that the positive control used in the present study, expected to allow a 12.36 mg/g dw R-PE yield (Dumay *et al.* 2013), resulted in a R-PE recovery almost twice lower, at only 6.3 mg/g dw. Enzymatic extracts of MMS1733, MMS1910, MMS1906 and MMS1986 allowed a R-PE recovery higher than with commercial xylanase.

Regarding soluble protein recovery, previous enzymatic extraction without optimisation led to protein yields below 2.5 mg/g ww (Joubert and Fleurence 2008). After optimisation of the extraction protocol adapted for the use of commercial xylanase, the soluble protein yield reached 30.58 mg/g dw (Dumay

et al. 2013). In the present study, the positive control corresponding to this commercial *T. longibrachiatum* xylanase resulted in a protein recovery of 101.7 mg/g dw. This important gap between protein recovery values of the present study and the previous ones could be explained by dissimilarities in protocols used for protein assays. Indeed, protein assays of the present study were performed by using BCA methodology while the Bradford methodology was used in [Dumay *et al.* \(2013\)](#). Interestingly, all fungal enzymatic extracts resulted in obtaining higher soluble protein yields compared to positive control, up to 163.5 mg/g dw for MMS1733.

The use of fungal enzymatic extracts allowed better macroalgal degradation, R-PE or soluble protein recoveries compared to the commercial xylanase used as positive control. Thus, it seems that the xylanase activities from *P. palmata* associated fungal strains present a better efficiency and specificity compared with the *T. longibrachiatum*. The potential presence of several enzymes in extracts could result in an overestimation of their polysaccharidal degrading abilities compared with a sole xylanase used as control. The choice of a mono-activity enzyme positive control was nevertheless motivated by several reasons, including the facts that *P. palmata* cell wall was mainly composed of xylan, and that this commercial *T. longibrachiatum* xylanase has already been used, resulting in a great degradation potential of *P. palmata* biomass after optimisation of the process ([Dumay *et al.* 2013](#)). It could then be expected that the addition of other enzymes such as commercial cellulase would have result only in a limited increase in macroalgal degradation. As mentioned before, it has already been shown that the use of combined cellulase and xylanase resulted in a better R-PE extraction from *P. palmata* ([Fleurence 2003](#); [Joubert and Fleurence 2008](#)), but the precise R-PE extraction yield increase due to cellulase addition compared with sole xylanase has not been investigated. Besides, these results would depend on the biological origin of the combined xylanase and cellulase ([Fleurence 2003](#); [Joubert and Fleurence 2008](#)). The choice of a sole xylanase positive control was also justified by the fact that in comparable studies characterizing xylanase or cellulase activities from marine fungal enzymatic extracts, the positive control was either a sole commercial enzyme ([Jmel *et al.* 2020](#)), or was inexistent ([Raghukumar *et al.* 2004](#)). Moreover, a standardization protocol were performed in the present study to allow a comparison as rigorous as possible between fungal extracts and commercial positive control.

It would then be of high interest to further characterize the enzymatic composition of these fungal extract beyond the quantification of both xylanolytic and cellulolytic activities. Moreover, optimisation can be envisage on protocol of fungal culture, by investigating conditions resulting potentially in a higher production of enzymes, such as incubation time, incubation temperature, humidity percentage or volume of conidial suspension. Optimization could also be performed on the protocol of macroalgal degradation and R-PE recovery, by modulating reaction parameters such as fungal enzymatic extract concentration, time and temperature of reaction, or amount of macroalgal biomass.

It was previously mentioned that MMS1785 and MMS1906 exhibited respectively the highest xylanase (978 U/ml) and cellulase (50.5 U/ml) activities. These two strains were then expected to allow a good macroalgal biomass degradation and consequently a good R-PE release, particularly regarding MMS1785 because *P. palmata* cell wall is mainly composed of xylan (Morgan *et al.* 1980; Lahaye *et al.* 1993; Dumay *et al.* 2014; Naseri *et al.* 2020). Nevertheless, while enzymatic extract of MMS1906 resulted in both high reducing sugar concentration (18.7 mM) and high R-PE concentration (7.5 mg/g dw) in the macroalgal lysate compared with other strains as expected, this was not the case for MMS1785 enzymatic extract. The weak *P. palmata* degradation ability of this strain was also highlighted by the lowest total protein recovery (105 mg/g dw) obtained by using its enzymatic extract. In comparison, MMS1906 was associated with a relatively high total protein concentration in the lysate, reaching 138 mg/g dw. These unexpected results could be explained by variability in the enzymatic substrate specificity of MMS1785. Indeed, the nature of the substrate is known to greatly affect the efficiency of enzymes, notably marine fungal enzymes (Raghukumar *et al.* 2004; Mostafa *et al.* 2014; Patyshakuliyeva *et al.* 2019; Atalla *et al.* 2020). In the present study, wheat arabinoxylan was used as substrate for DNS assays because of its good water solubility and commercial availability, and its use resulted in the attribution of a high xylanase activity to MMS1785. Then, considering the obtained results, the xylanase activity of MMS1785 enzymatic extract should be lower by using *P. palmata* purified xylan than by using commercial wheat arabinoxylan. Interestingly, reducing sugar, R-PE and total protein concentrations seemed similar between MMS1785 and the xylanase positive control. It is also possible that the low R-PE and total protein concentrations observed in the macroalgal lysate generated by the MMS1785 enzymatic extract should be related to a potential proteolytic degradation. Indeed, enzymatic extracts were not purified and proteases could putatively partially degrade *P. palmata* proteins, including R-PE (Nikolaeva *et al.* 1999; Bienert *et al.* 2006; Parmar *et al.* 2011; Gomaa *et al.* 2015).

Interestingly, opposite results were obtained considering MMS1733. Similarly to MMS1959, MMS1733 exhibited particularly low cellulase and xylanase activities compared with other strains, at 295.4 and 18.2 U/mL, respectively. Nevertheless, the enzymatic extract of this strain allowed to obtain high reducing sugar, R-PE and protein concentrations. That could be explained by similar arguments explaining the results of MMS1785. Indeed, contrarily to MMS1785, MMS1733 should have excreted xylanolytic and cellulolytic enzymes with low affinity for substrates used in DNS assays (wheat arabinoxylan and carboxymethyl cellulose, respectively), but with a great affinity with *P. palmata* polysaccharides, resulting in an important release of reducing sugars. The high relative rate of protein recovery (including R-PE) could be explained by this highly specific enzymatic affinity, but also putatively by a low proteolytic activity. Nevertheless, the ratio (R-PE / total proteins) obtained with

MMS1733 did not seem particularly high compared with other isolates. Interestingly, both MMS1733 and MMS1785 are *Aspergillus* species. In the literature, several *Aspergillus* cellulase or xylanase were characterized as exhibiting high substrate specificities (Madhu *et al.* 2009; Oh *et al.* 2018; Chen *et al.* 2019; Sulyman *et al.* 2020). It could then be hypothesized that the present algicolous strains belonging to this genus would synthesize enzymatic arsenals with high substrate specificities.

The isolate MMS1986 was comparable with MMS1906 previously discussed. Reducing sugar, total protein and R-PE concentrations obtained by using both enzymatic extracts were similar and among the highest compared with the other fungal isolates. Indeed, the use of both strains for these three assays gave results not significantly different. Moreover, MMS1986 was the fungal isolate which allowed the highest R-PE recovery (8.1 mg/g dw). Both MMS1906 and MMS1986 were *Penicillium* isolates. Similarities between these both strains regarding their enzymatic strategies may be notably due to their taxonomic proximity. Several relative recent studies have demonstrated the relationship between genetic distance of fungal strains and their enzymatic profile, including *Penicillium* genus (Tedersoo *et al.* 2012; Benoit *et al.* 2015; Horta *et al.* 2018; Barrett *et al.* 2020). Nevertheless, the natural habitats would also greatly influence the genome evolution and notably the enzymatic profile (Benoit *et al.* 2015).

Compared with all the other fungal isolates, MMS1959 seemed to be the worse candidate for *P. palmata* degradation. As mentioned before, this isolate exhibited the lowest relative cellulase and xylanase activities and its use was characterized by the lowest reducing sugar and R-PE concentration. This strain might also excrete proteases potentially in part responsible of the low amount of recovered proteins. Among all the tested fungal strains, MMS1959 was the only one belonging to the *Cladosporium* genus. Interestingly, this genus was the major genus in terms of strain number isolated from *P. palmata* (CHAPITRE II). Further investigations on the other *Cladosporium* isolates obtained from this macroalga may either allow descriptions of other various enzymatic profiles, or result in the observation of a homogeneous enzymatic degradation strategy.

The strain *Penicillium brevicompactum* MMS1910 was the most promising considering its absolute enzymatic production. Indeed, it exhibited the second highest cellulase and xylanase activities. Nevertheless, the macroalgal degradation performed with this enzymatic extract did not seem of particular interest, resulting in a total soluble protein concentration quite low compared with the other fungi screened. However, it allowed a good R-PE recovery (7.2 mg/g dw) and the use of this fungal strain resulted in the best ratio (R-PE / total proteins), with R-PE corresponding to 6 % of total protein content.

Even if the use of purified commercial enzymes is currently principally used in industry, the use of the extracted secretome can then permit, besides an economically viable deletion of purification procedure, to target complex substrates with the presence of several enzymes, potentially acting in synergy (Beg *et al.* 2001; Imran *et al.* 2016; Filiatrault-Chastel *et al.* 2021). An optimization of the polysaccharide degradation and a reduced risk of contamination of the fungal culture medium by unwanted microorganisms can be performed by fungal co-cultivation (Bhardwaj *et al.* 2021; Filiatrault-Chastel *et al.* 2021; Singh *et al.* 2021). Because of the advantages given by the use of fungal co-cultivation on the enzymatic degradation of biological substrates (Mostafa *et al.* 2014), the industrial use of secretomes obtained from co-cultivation of the fungal strains investigated in the present study may be a promising way to investigate in the R-PE recovery from *P. palmata*.

Other analyses of interest would consist in measurement and screening of potential proteolytic activities present in each fungal enzymatic extract and then resulting notably in partial R-PE degradation, as discussed above.

As previously mentioned, it seems to have correlation between the enzymatic degrading abilities of *P. palmata* biomass degradation and the genetic assignation of fungal strains. Further investigations on the remaining enzymatically active fungal isolates previously described (**CHAPITRE II**) may be necessary to validate or not this hypothesis. This could also allow the investigation of the potential correlations between fungal isolation, incubation or environmental factors and cellulolytic and xylanolytic algicolous fungal abilities.

5. Conclusion

In conclusion, to the best of our knowledge, it is the first study on the use of enzymes of *P. palmata* associated fungi in the degradation of its own biomass for biomolecules recovery. The present study has resulted in the quantification of both cellulolytic and xylanolytic activities of six fungal isolates derived from this red macroalga. The fungal culture process using only *P. palmata* biomass as substrate succeeded in the production of enzymatically active fungal secretomes. The use of these fungal secretomes gave results of high interest in the cell wall degradation and the R-PE recovery. Several strains seemed more promising than the others such as *Penicillium* sp MMS1906, *Aspergillus* sp. MMS1733 and *Penicillium brevicompactum* MMS1910. Interestingly, the most promising isolates in terms of cell wall degradation and R-PE recovery did not necessarily correspond to the isolates characterized by the highest enzymatic activities. Further works and investigations are required notably in the optimisation of the uses of fungal secretomes in the R-PE recovery from *P. palmata*.

Postface

L'observation de halos enzymatiques intenses obtenus lors des travaux de criblage (**CHAPITRE III**) a motivé la sélection de souches pour leur quantification enzymatique. Parmi les souches dont les activités xylanolytiques et cellulolytiques ont été quantifiées, les isolats MMS1733, MMS1910 et MMS1959 se sont révélés potentiellement intéressants par rapport aux autres souches présentant des halos enzymatiques de dosage qualitatif plus faibles. Cependant, concernant les deux activités enzymatiques testées, les souches MMS1906 et MMS1785 ont permis d'obtenir respectivement les plus hautes valeurs d'activités cellulolytiques et xylanolytiques. Aucune de ces deux souches n'appartenait au groupe de souches caractérisées par des halos enzymatiques particulièrement intenses. Par ailleurs, la souche MMS1959 a été caractérisée par des activités enzymatiques parmi les plus faibles, et son utilisation semble la moins prometteuse en matière de dégradation de *P. palmata* et d'extraction de R-PE.

Dans la littérature, les résultats obtenus pour les activités enzymatiques microbiennes entre les tests quantitatifs au DNS et les tests qualitatifs au rouge Congo sont généralement corrélés, avec une correspondance entre la zone de clarté et les valeurs quantifiées d'activités. Cependant, plusieurs exceptions ont été relevées, dues à des fluctuations des paramètres expérimentaux, dont le temps d'incubation (Ahmad *et al.* 2013b, 2013a; Meddeb-Mouelhi *et al.* 2014; Sahu *et al.* 2018). En effet, étant donné la grande diversité des communautés fongiques, cela implique d'importantes variations dans les taux de croissance, induisant une variation du temps d'incubation entre les souches pour ce qui est du protocole de coloration au rouge Congo. Selon Abiodun *et al.* (2015), la possible absence de corrélation entre les zones de clartés obtenues avec la coloration au rouge Congo et les valeurs d'activités enzymatiques des champignons testés devrait limiter l'utilisation de ce genre de tests qualitatifs à un usage d'indicateur initial menant à des procédés de caractérisation enzymatiques plus précis.

Ainsi, l'obtention de souches fongiques caractérisées par un halo enzymatique ou une zone de clarté particulièrement intense n'assure pas que ces souches soient effectivement les plus actives. Parmi les 112 autres isolats présentant une double activité xylanolytique et cellulolytique et n'ayant pas été sélectionnés pour une quantification d'activité, certaines pourraient posséder des activités comparables ou même plus importantes que les six souches dont les activités ont été quantifiées. Bien que le protocole de criblage se limite vraisemblablement ainsi à une dimension qualitative, il a tout de même permis une présélection de 112 souches doublement actives sur les 308 souches testées au total.

Dans le cadre de l'étude, 9 souches fongiques issues du criblage ont été présélectionnées pour une quantification enzymatique en fonction de la forte intensité de leur halo de décoloration. A part la souche MMS1777, qui appartient au genre *Acremonium*, les 8 autres souches sélectionnées appartiennent aux genres majoritaires décrits chez *P. palmata* (**CHAPITRE II**), à savoir *Cladosporium*, *Aspergillus* et *Penicillium*. Neuf autres souches caractérisées par une double activité xylanolytique et cellulolytique mais par des halos plus faibles ont également été choisies parmi ces genres connus pour leurs potentiels enzymatiques (Del-Cid *et al.* 2014; Korkmaz *et al.* 2017; Patyshakuliyeva *et al.* 2019; Le Strat *et al.* 2022).

Par ailleurs, les espèces des genres *Trichoderma*, *Penicillium* et *Aspergillus* ont des capacités de dégradation de biomasses particulièrement intéressantes (Money 2016; Filiatrault-Chastel *et al.* 2021; Meyer 2021) en tant que producteurs de cellulase (Kuhad *et al.* 2011; Gupta *et al.* 2015; Imran *et al.* 2016; Kunamneni 2016; McKelvey and Murphy 2017; Shah *et al.* 2019; Singh *et al.* 2021) et xylanases (Motta *et al.* 2013; Bajaj and Mahajan 2019b).

Le genre *Trichoderma* est également connu pour avoir des capacités de dégradation de biomasses végétales importantes liées à la production de xylanases et cellulases, notamment utilisée pour la dégradation de la paroi de *P. palmata* (Dumay *et al.* 2013). Les *Trichoderma* semblent donc théoriquement bien adaptés à *P. palmata*. De manière assez surprenante, ce genre n'est que très peu présent au sein de la communauté fongique de *P. palmata* (une seule souche identifiée sur un total de 308) alors qu'il a été décrit dans divers environnements marins tels que les sédiments, les bivalves ou les éponges (Sallenave-Namont and Pouchus 2000; Poirier *et al.* 2007; Matallah-Boutiba *et al.* 2012; Ruiz *et al.* 2013; Mohamed-Benkada *et al.* 2016; Korkmaz *et al.* 2017). Toutefois, bien que des isolats aient été isolés à partir de macroalgues comme *Sargassum wightii* (Thirunavukkarasu *et al.* 2015), *Fucus* sp. (Patyshakuliyeva *et al.* 2019), *Agarum cribosum* (Lee *et al.* 2015) ou *Laurencia intermedia* (Notarte *et al.* 2018), ce genre ne semble pas particulièrement représenté dans les communautés fongiques associées aux macroalgues. Les raisons expliquant cette absence de *Trichoderma* chez *P. palmata*, pourtant largement présent dans le milieu marin et présentant d'importants potentiels xylanolytiques, seraient particulièrement intéressantes à explorer.

Les résultats issus de cette étude montrent néanmoins la pertinence de l'exploration de la fonge associée à une algue pour la recherche d'activités enzymatiques efficaces pour la déstructuration de sa paroi. Ainsi, les extraits obtenus à partir de culture de champignon en fermentation solide montrent des potentialités de dégradation pariétale supérieures à celle obtenue avec l'enzyme commerciale obtenue à partir de *Trichoderma longibrachiatum*. L'extraction de la R-Phycoérythrine en utilisant les extraits enzymatiques obtenus en laboratoire sont prometteurs et devraient être poursuivis d'une

part, par l'étude du changement d'échelle pour la fermentation et d'autre part, par l'optimisation des conditions d'hydrolyse et la purification des extraits algaux enrichis en protéines et pigments.

CONCLUSIONS GÉNÉRALES ET PERSPECTIVES

Le travail présenté dans le cadre de cette thèse consiste à étudier les communautés fongiques associées à l'algue rouge *Palmaria palmata* et à mettre en évidence des souches fongiques à fort potentiel biotechnologique pour la production d'enzymes spécifiques dégradant les parois de l'algue, notamment dans l'optique d'extraction de composés à haute valeur ajoutée comme la R-phycoérythrine (R-PE).

Pour répondre à ces objectifs, cette thèse s'est donc déclinée en trois parties principales. Tout d'abord, l'étude de la diversité des communautés fongiques cultivables de *P. palmata* a été étudiée, au cours de laquelle les impacts de divers paramètres environnementaux, écologiques et culturels ont été évalués. Ensuite, un criblage enzymatique qualitatif portant sur les activités xylanolytiques et cellulolytiques a été réalisé sur l'ensemble des souches fongiques isolées. Enfin, suite à ce criblage, certaines souches fongiques ont été sélectionnées pour une caractérisation quantitative de leurs activités enzymatiques, ainsi que pour des tests d'extraction de la R-PE à partir de thalle de *P. palmata*.

Considérant les communautés fongiques cultivables de *P. palmata*, une vaste campagne de prélèvements d'algues a été réalisée sur une année à raison de deux échantillonnages par saison. Les champignons ont été isolés en croisant différents paramètres écologiques (localisation épiphyte, endophyte ou indéterminée), environnementaux (saison de prélèvement) et culturels (température d'incubation de 12 ou 27°C, milieu de culture d'isolement non-sélectif Wickerham ou à base d'algue lyophilisée). Un total de 308 souches fongiques a pu être ainsi identifié, correspondant à 147 taxons différents, avec une prédominance des Ascomycota (276 souches et 118 taxons) sur les Basidiomycota. A ce jour, il s'agit de la plus importante collection de champignons obtenue à partir d'une algue rouge. Au sein des Ascomycota, les classes majoritairement observées correspondent aux Dothideomycetes, Sordariomycetes et Eurotiomycetes et à l'échelle du genre, *P. palmata* est caractérisée par une prédominance des genres *Cladosporium*, *Penicillium* et *Aspergillus*. Les impacts de la saisonnalité et des paramètres d'isolement sur la structuration de cette communauté fongique ont été mis en évidence à l'échelle du phylum, des classes et des genres prédominants. Globalement, un isolement sur milieu non-spécifique Wickerham et à une température d'incubation de 12°C permet d'obtenir un plus grand nombre de souches que l'utilisation du milieu spécifique à base d'algue "*P. palmata*" et une température de 27°C. De même, les algues récoltées au printemps semblent plus intéressantes en termes de quantité de champignons isolés contrairement à celles récoltées en automne. Il serait intéressant de tester la répétabilité de ces résultats sur une année supplémentaire, ce qui permettrait d'étudier la structuration de la communauté fongique cultivable de *P. palmata* sur une temporalité plus importante, et d'estimer de potentielles variations d'une année à l'autre. De plus, d'autres paramètres pouvant potentiellement impacter cette diversité seraient intéressants à explorer (cycle de vie de l'algue, zone géographique...). Par ailleurs, des études complémentaires par approche de

metabarcoding, amorcées mais à ce jour pas encore finalisées, devront être explorées pour confronter la diversité fongique cultivable à la diversité fongique totale associée à cette algue. La caractérisation de la fonge associée à *P. palmata* pourrait également être étendue à celles d'autres macroalgues présentant des intérêts biotechnologiques de par leur synthèse de composés bioactifs spécifiques. Enfin, dans le contexte actuel de recherche de nouvelles molécules bioactives, la collection de champignons issus de *P. palmata* pourrait représenter un réservoir non négligeable de métabolites d'intérêt à cribler, en lien avec les thématiques de recherche du laboratoire.

Le potentiel enzymatique des souches fongiques isolées en se focalisant sur leurs activités xylanolytiques et cellulolytiques potentielles a ensuite été étudié dans l'optique d'améliorer la dégradation enzymatique de *P. palmata*. Ces travaux représentent le premier criblage à grande échelle de la totalité d'une communauté fongique cultivable associée à une algue rouge. Sur la totalité des 308 souches fongiques criblées, 186 souches (61 %) sont caractérisées par au moins une activité enzymatique (cellulase et/ou xylanase) et parmi ces 186 souches, 113 sont caractérisées par une double activité xylanolytique et cellulolytique et semblent donc particulièrement adaptées à *P. palmata*. Cette double activité enzymatique s'est retrouvée chez plusieurs taxons mais semble caractériser en particulier ceux appartenant aux genres *Penicillium* et *Aspergillus*. Les relations entre taxonomie et profil enzymatique ont été étudiées, ainsi que l'impact de la saisonnalité et des paramètres d'isolement sur l'occurrence des activités enzymatiques. Bien que l'impact du milieu de culture sur l'activité enzymatique des souches fongique ne soit pas significativement établi, l'utilisation du milieu non-sélectif Wickerham pour l'isolement des souches semble permettre d'obtenir plus de souches ne présentant qu'une seule activité enzymatique par rapport au milieu "*P. palmata*". L'utilisation de ce dernier permet quant à lui d'isoler plus de souches présentant une double activité. Cela pourrait être dû au caractère sélectif de ce milieu "*P. palmata*", favorisant le développement de souches adaptées aux polysaccharides de l'algue. Il a également été montré que des isollements en été et en automne favorisent l'obtention de souches fongiques à activités cellulase seule et xylanase seule par rapport aux autres saisons, respectivement. Les souches fongiques à double activité enzymatiques ont quant à elles été principalement isolées en été et au printemps. L'intérêt de renouveler des travaux sur les communautés fongiques pourrait également permettre de consolider les tendances d'activités enzymatiques observées par criblage chez les champignons en fonction de la saison. En parallèle, il serait intéressant de confronter de potentielles tendances de production saisonnière d'enzymes par les champignons associée à *P. palmata*, avec une évolution saisonnière précise de la proportion des différents polysaccharides présents chez cette algue par rapport aux polysaccharides totaux. En dehors de la saison, d'autres paramètres semblent pouvoir influencer le potentiel enzymatique des souches isolées de *P. palmata* tels que la température et la nature du milieu d'incubation. Ces influences

pourraient être différentes pour des souches fongiques isolées d'autres algues d'intérêt biotechnologique.

Enfin, l'analyse quantitative des activités enzymatiques des champignons ayant répondu positivement au criblage enzymatique a été réalisée. Six souches appartenant aux genres *Aspergillus*, *Penicillium* et *Cladosporium* ont été sélectionnées afin de quantifier leurs activités xylanase et cellulase. Pour cela, un procédé de fermentation sur matrice solide à base d'algue a été optimisé pour les cultures fongiques et la production d'extraits enzymatiques. A partir de ces productions enzymatiques, une quantification des xylanases et cellulases a pu être réalisée ainsi que des tests préliminaires de dégradation des thalles de *P. palmata* afin d'extraire la R-PE. D'une manière générale, les souches fongiques testées ont montré des activités xylanolytiques au moins dix fois supérieures à leurs activités cellulolytiques. L'usage d'extraits enzymatiques fongiques a permis d'obtenir pour certaines souches de meilleurs rendements d'extraction en R-PE et en protéines solubles que ceux obtenus par le témoin positif (xylanase commerciale de *T. longibrachiatum*). Ainsi, les souches *Aspergillus* sp. MMS1733, *Penicillium* sp. MMS1906 et *Penicillium brevicompactum* MMS1910 semblent particulièrement intéressantes pour l'extraction de la R-PE. Cette dernière partie a ainsi permis de mettre en exergue l'intérêt de l'usage de secrétomes fongiques pour la dégradation pariétale de macroalgues et l'extraction de R-PE. De plus amples travaux de recherche sont nécessaires notamment pour évaluer le potentiel biotechnologique réel de ces souches, notamment par des procédés d'optimisation à divers niveaux tels que la culture fongique ou lors des réactions enzymatiques. Il serait notamment envisageable d'étudier les effets d'un mélange des secrétomes fongiques pouvant avoir des effets synergiques sur ce potentiel d'extraction assisté par enzymes, et d'étudier l'effet de la co-culture de plusieurs souches enzymatiquement actives sur l'intensité des activités des extraits enzymatiques. Par ailleurs, il serait également intéressant d'étendre les protocoles de quantification enzymatique et d'extraction de protéines solubles et de R-PE à d'autres souches fongiques isolées de *P. palmata*. En effet, il serait ainsi possible d'évaluer de potentiels liens entre la taxonomie et le profil enzymatique des souches. Enfin, l'obtention d'activités particulièrement efficaces suite à l'utilisation d'une souche prometteuse et à l'optimisation de sa production enzymatique pourrait motiver divers procédés de purifications enzymatiques pour réaliser une caractérisation fine d'enzymes à haut potentiel industriel.

En conclusion, ce travail a permis de décrire pour la première fois les communautés fongiques cultivables de l'algue rouge *P. palmata* et de mettre en évidence le fort potentiel biotechnologique des souches pour une production enzymatique ciblée sur la dégradation pariétale de matrices algales complexes. Il s'inscrit donc dans une progression de la recherche fondamentale mais possède aussi une dimension appliquée et économique en s'alignant dans les problématiques d'optimisation de

l'extraction de la R-PE à partir de *Palmaria palmata* portées par le laboratoire IsoMer. Enfin, ces travaux s'inscrivent dans un intérêt écologique global, avec pour objectif de favoriser l'usage de pigments naturels par rapport à ceux issus de la pétrochimie, et de promouvoir des procédés d'extraction respectueux de l'environnement tels que l'extraction assisté par enzymes. Leur caractère novateur implique de nombreuses autres investigations et pistes de recherche en perspective, aussi bien en lien avec la recherche fondamentale qu'avec la recherche appliquée et les biotechnologies.

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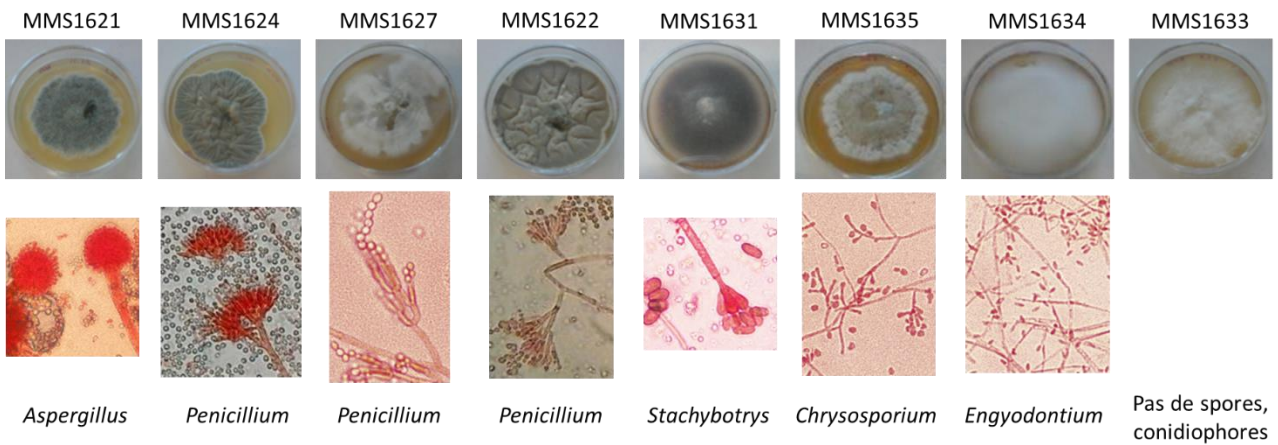
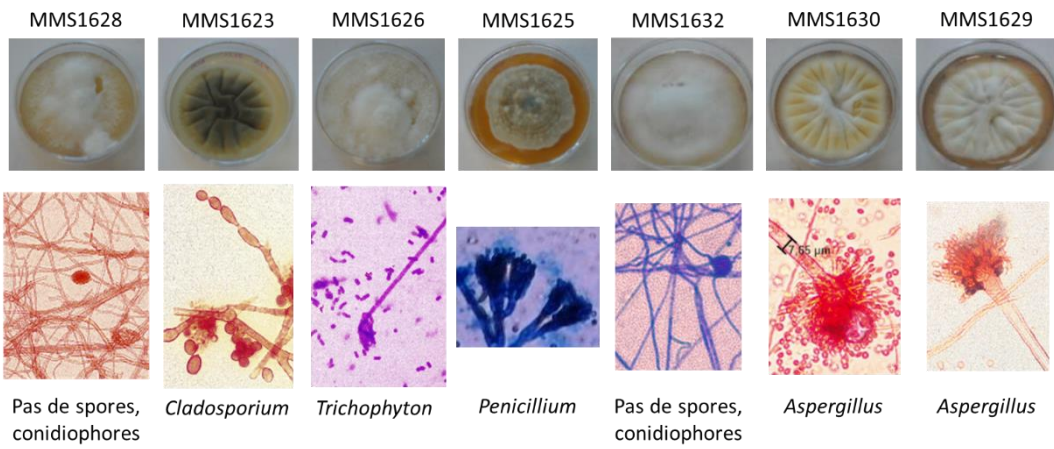
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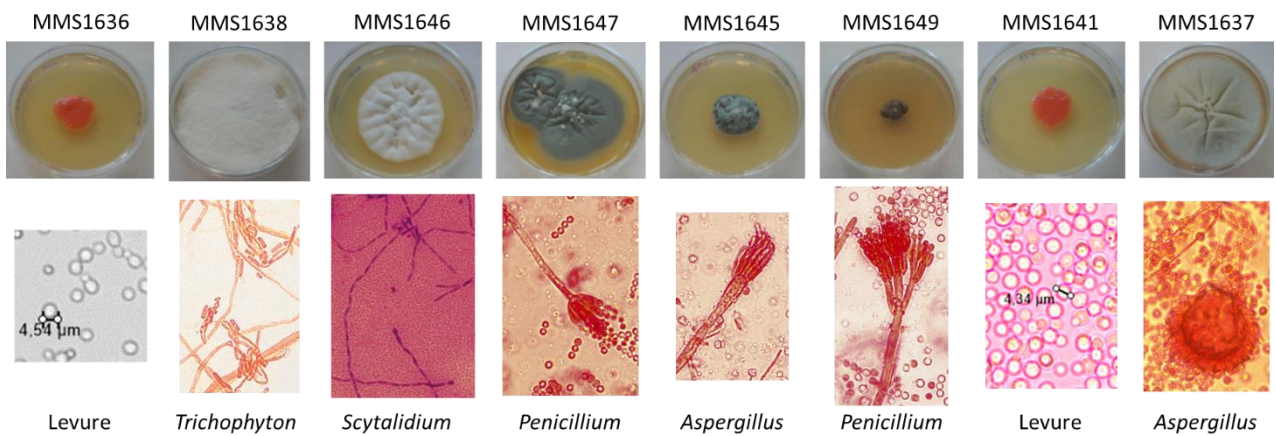
ANNEXES

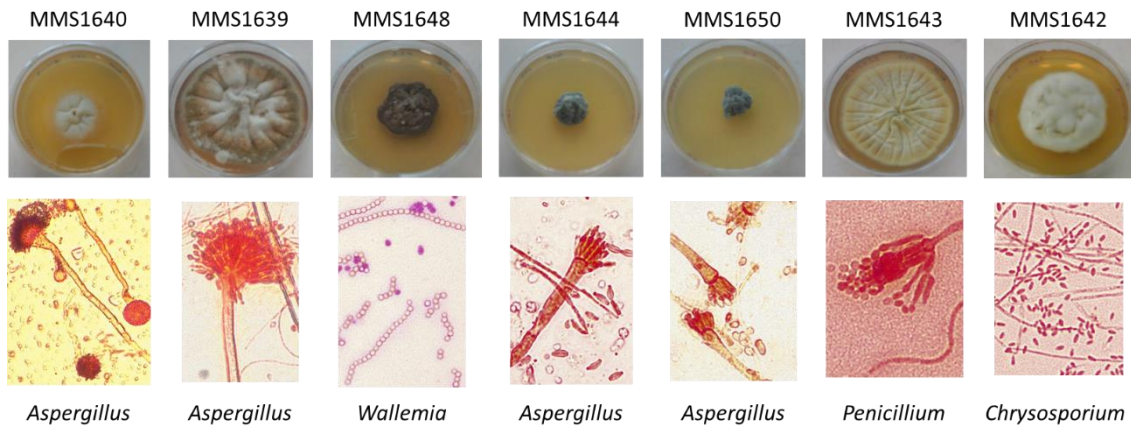
Annexe 1 : Photographies macroscopiques et microscopiques des 386 souches totales

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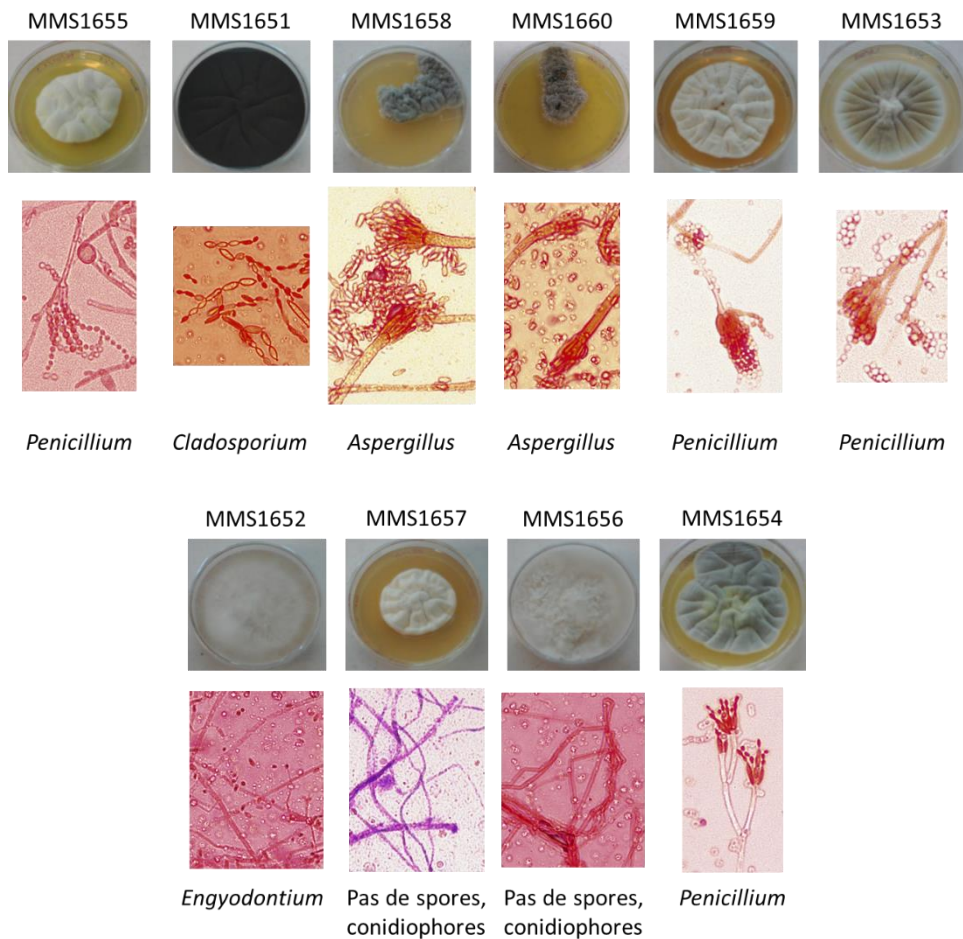


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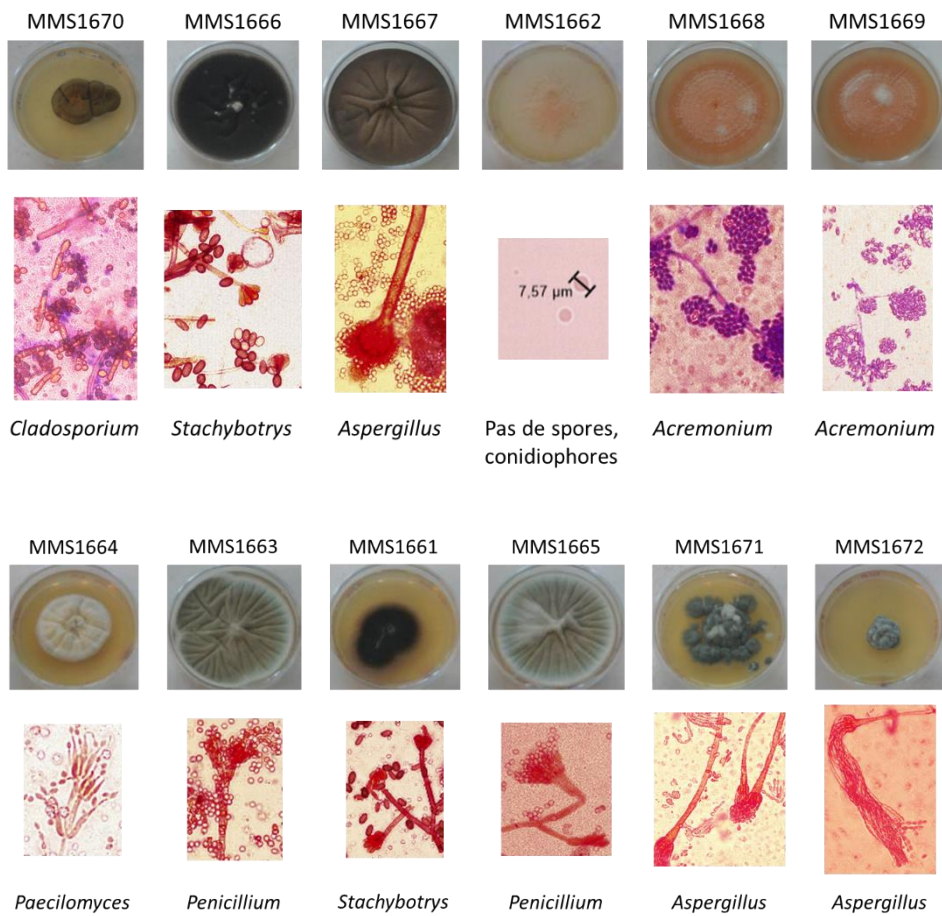




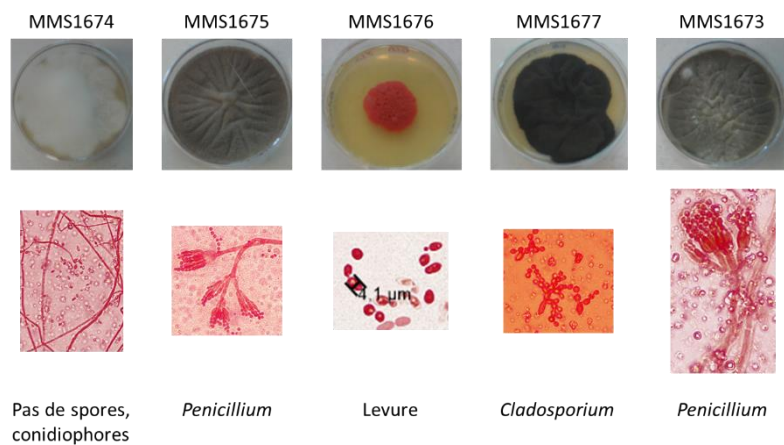
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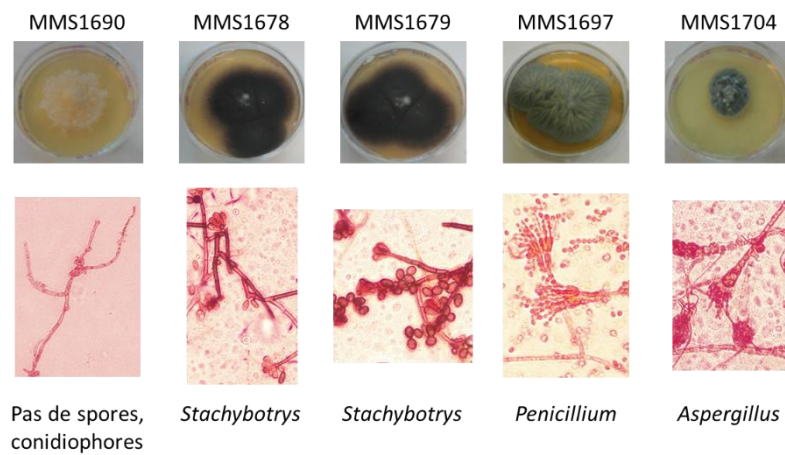
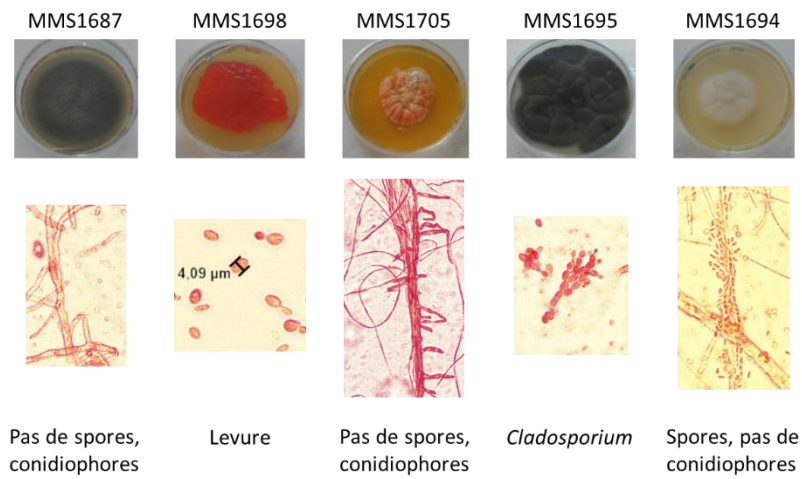
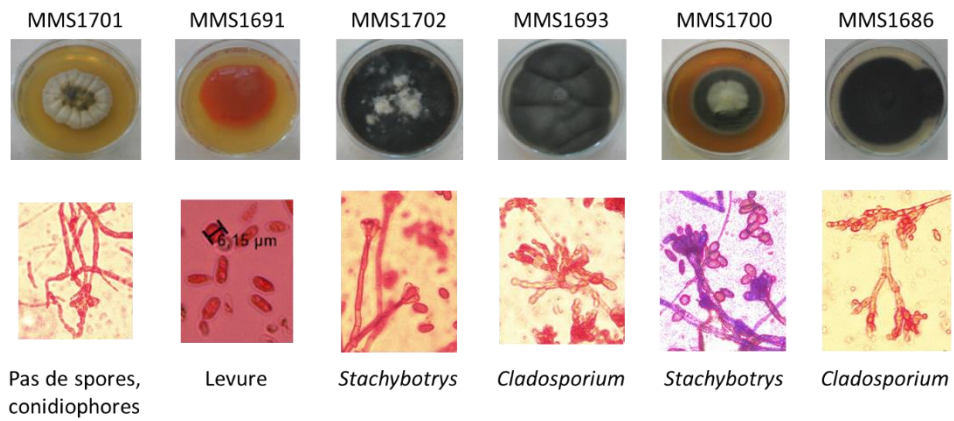
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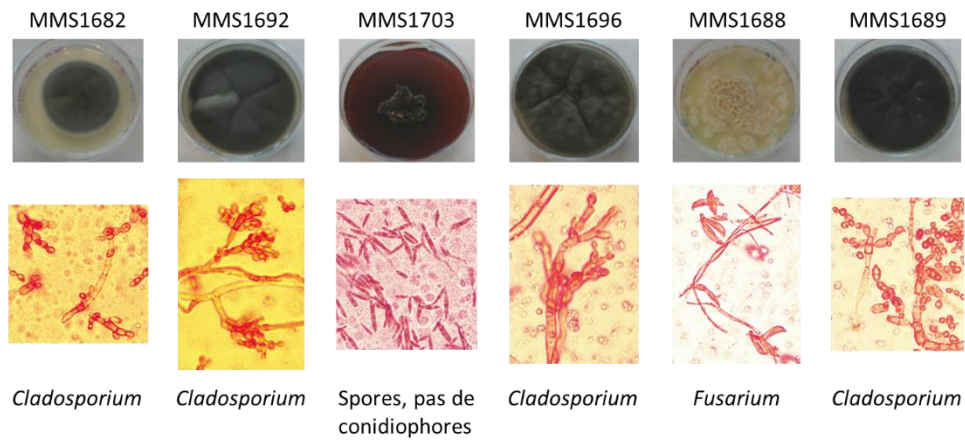
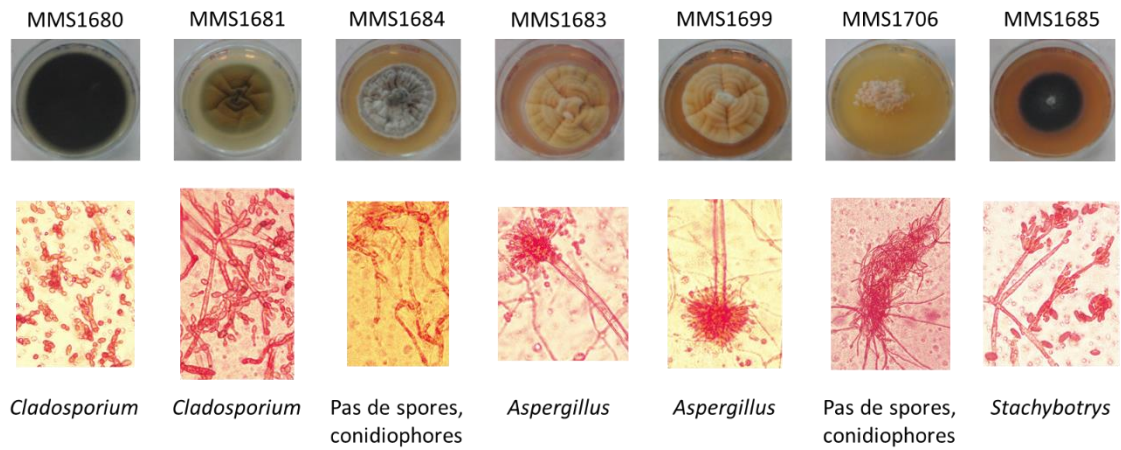


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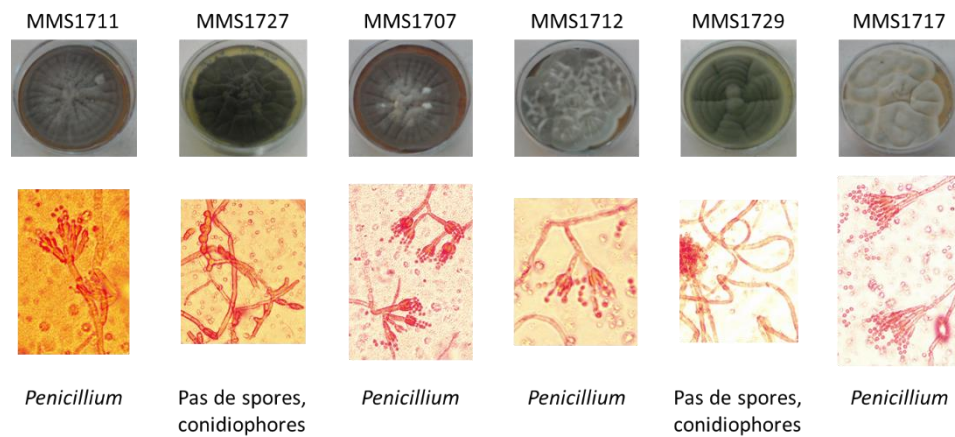









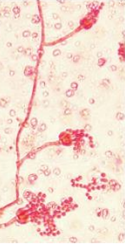
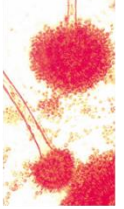
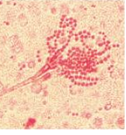
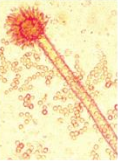
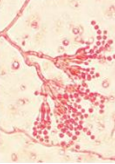






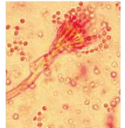
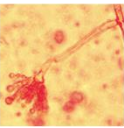
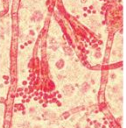
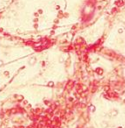
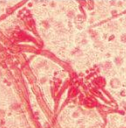
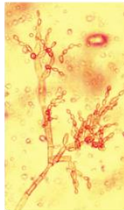
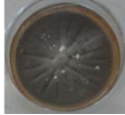
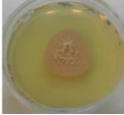

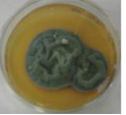
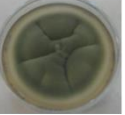
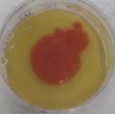
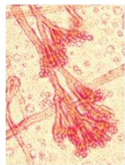

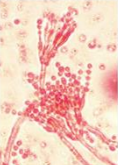
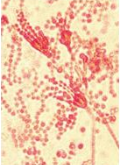
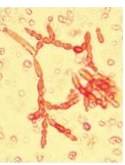

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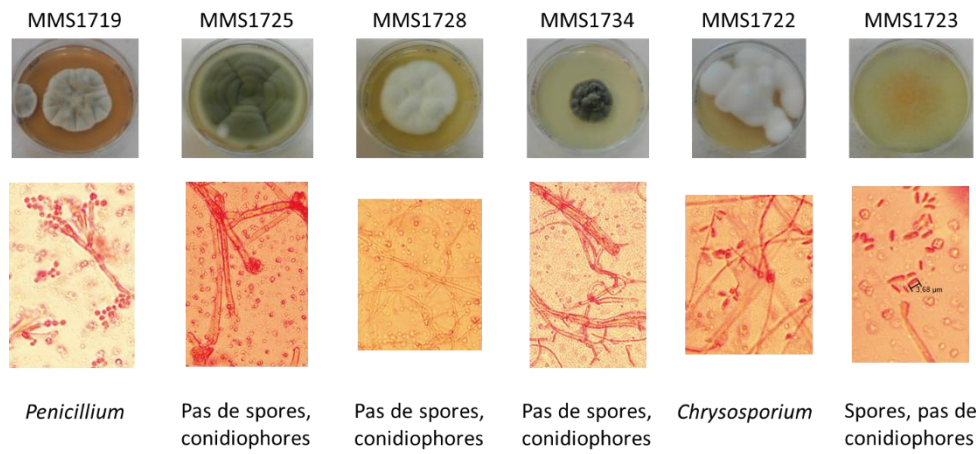




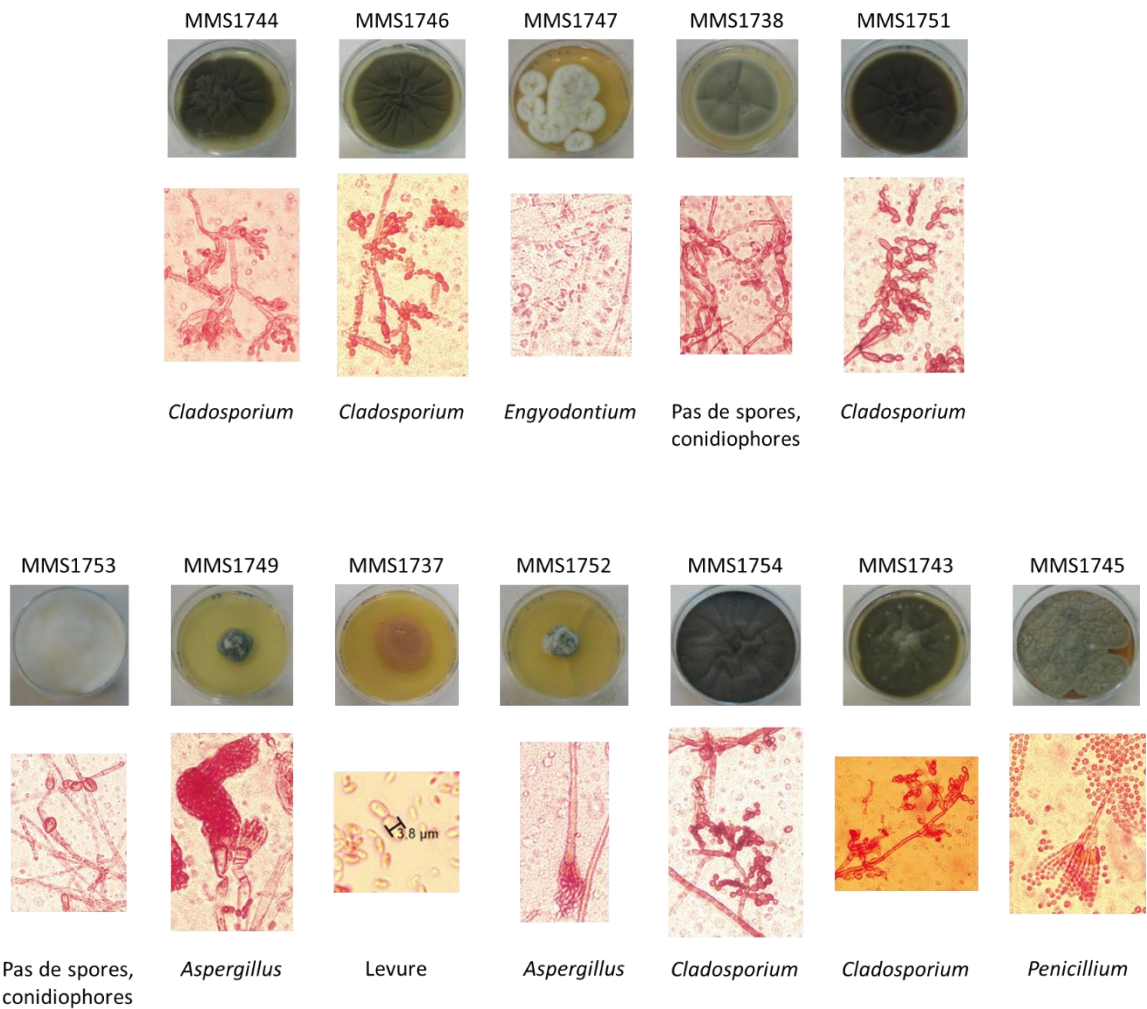
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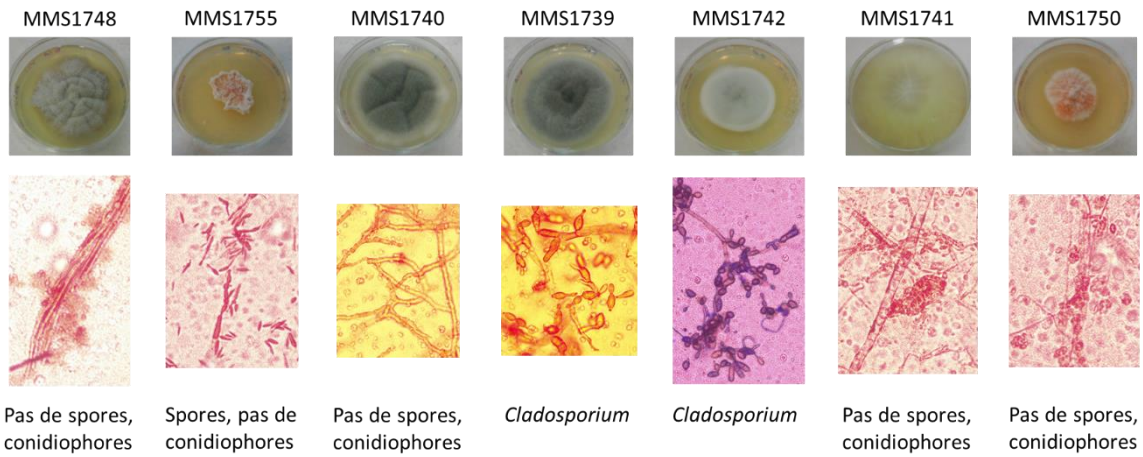


MMS1720	MMS1718	MMS1733	MMS1731	MMS1735	MMS1721
					
					
Pas de spores, conidiophores	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Penicillium</i>
MMS1715	MMS1726	MMS1709	MMS1714	MMS1710	MMS1713
					
					
<i>Penicillium</i>	<i>Aspergillus</i>	<i>Penicillium</i>	Pas de spores, conidiophores	<i>Penicillium</i>	<i>Cladosporium</i>
MMS1708	MMS1736	MMS1724	MMS1732	MMS1716	MMS1730
					
					
<i>Penicillium</i>	Spores, pas de conidiophores	<i>Penicillium</i>	<i>Penicillium</i>	<i>Penicillium</i>	Levure

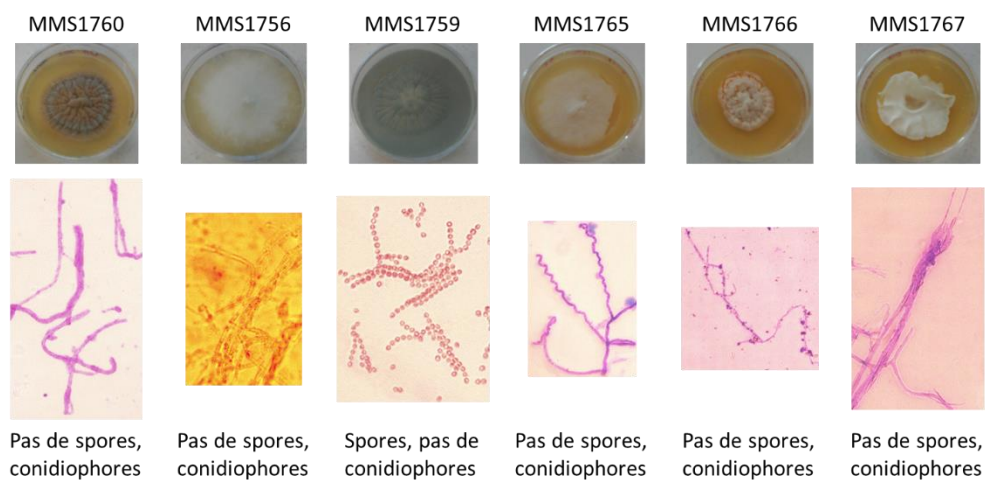
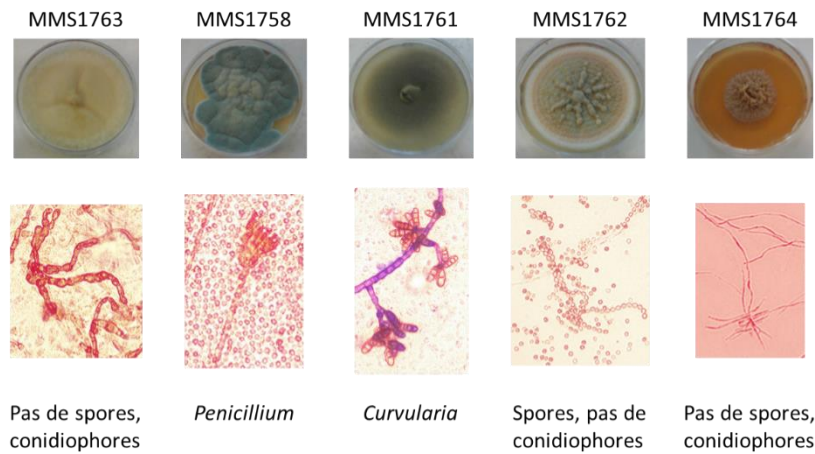


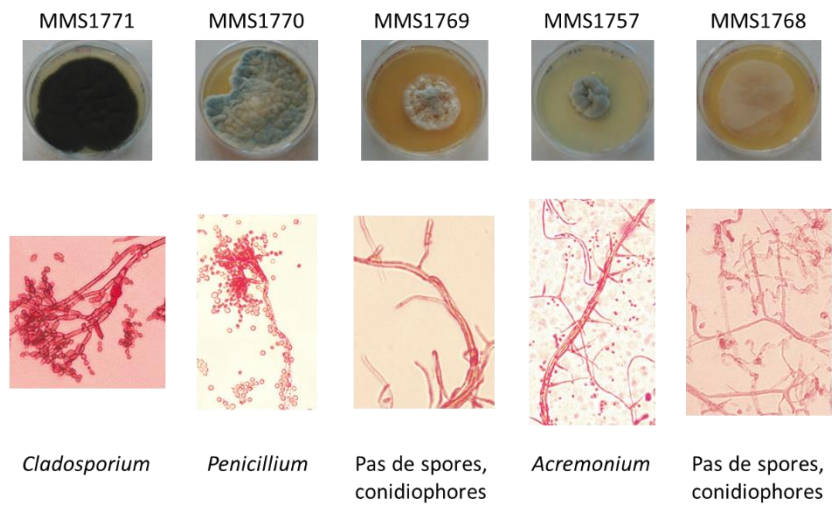
Récolte 8 (16/09/2019)



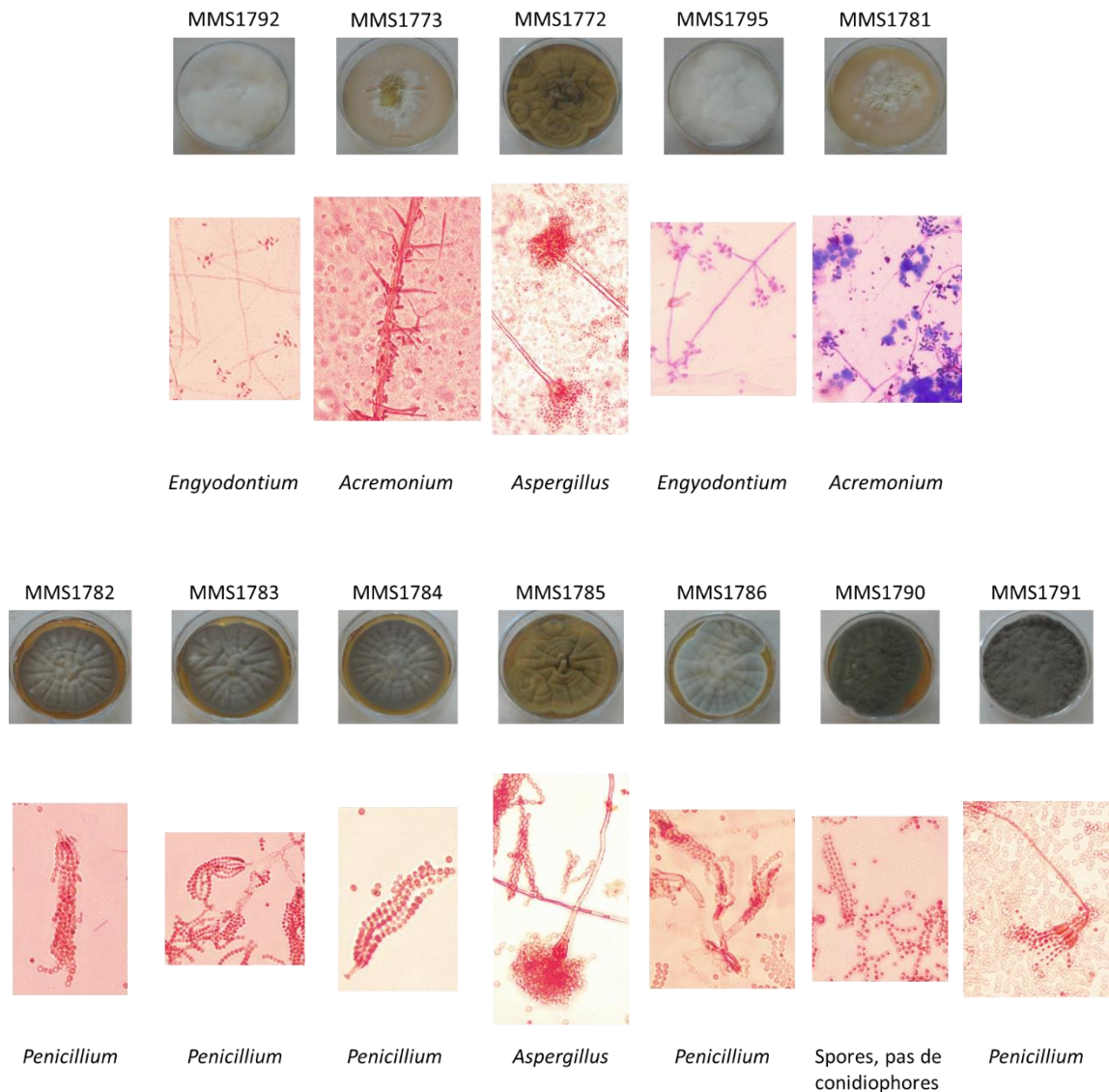


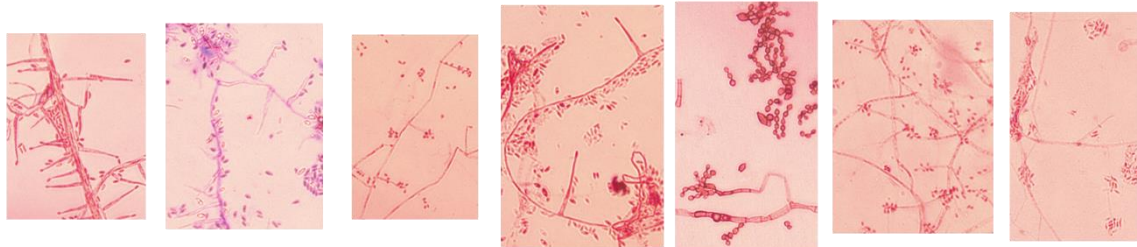
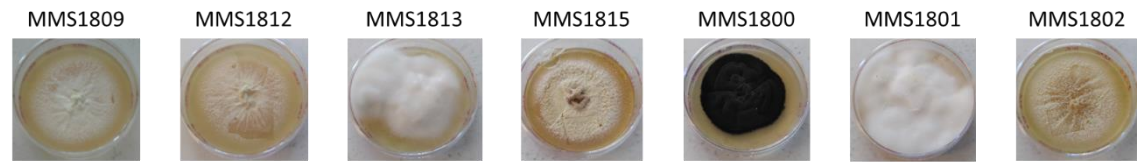
Récolte 9 (13/11/2019)



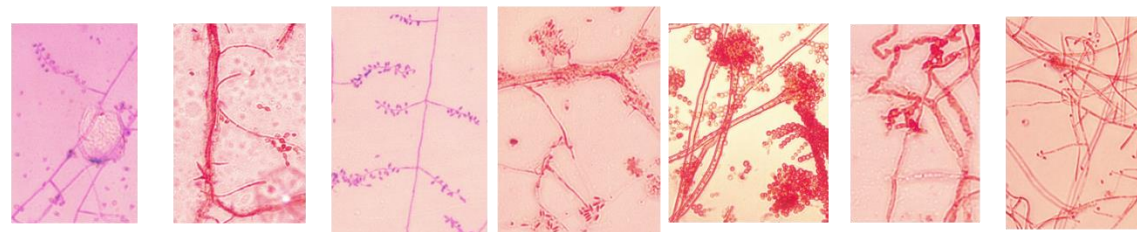
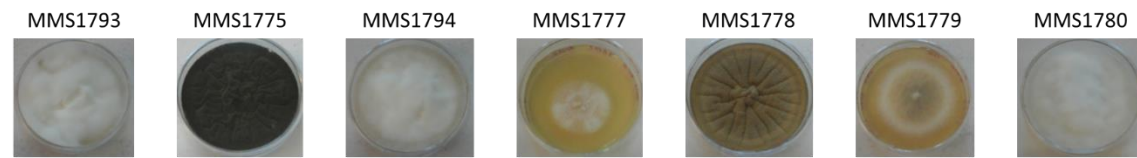


Récolte 10 (09/01/2020)

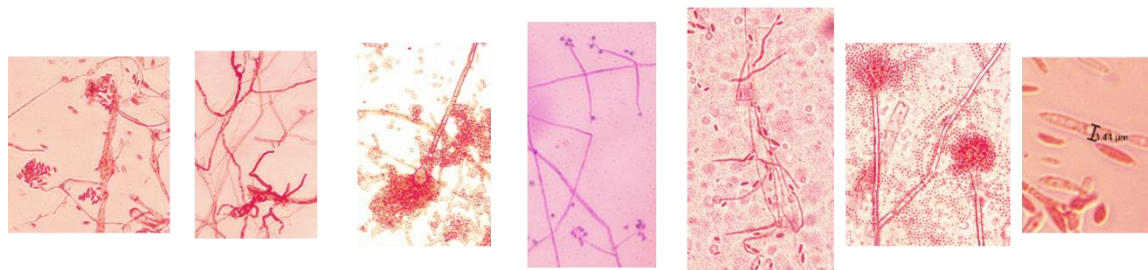




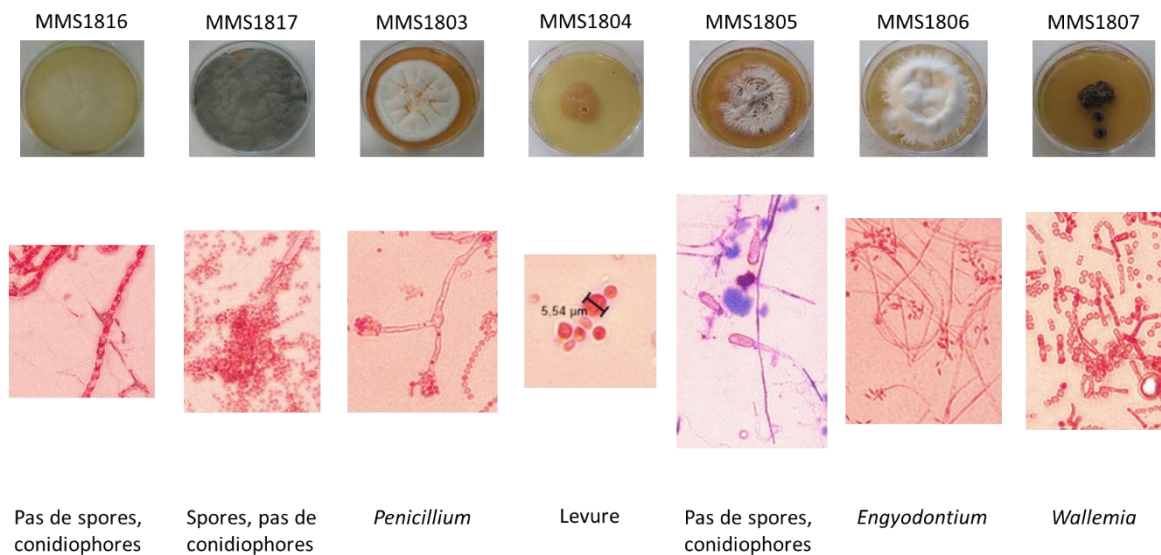
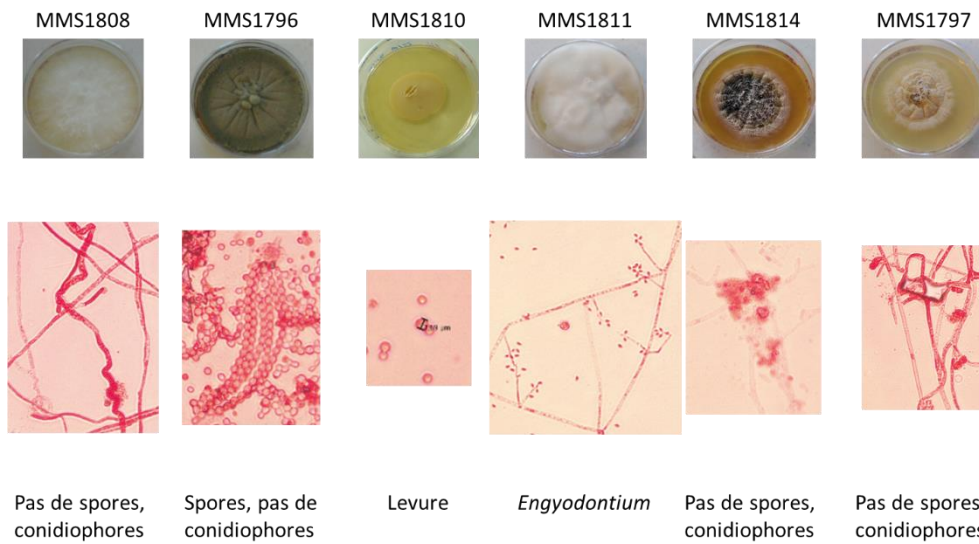
<i>Acremonium</i>	<i>Acremonium</i>	<i>Engyodontium</i>	<i>Acremonium</i>	<i>Cladosporium</i>	<i>Engyodontium</i>	<i>Acremonium</i>
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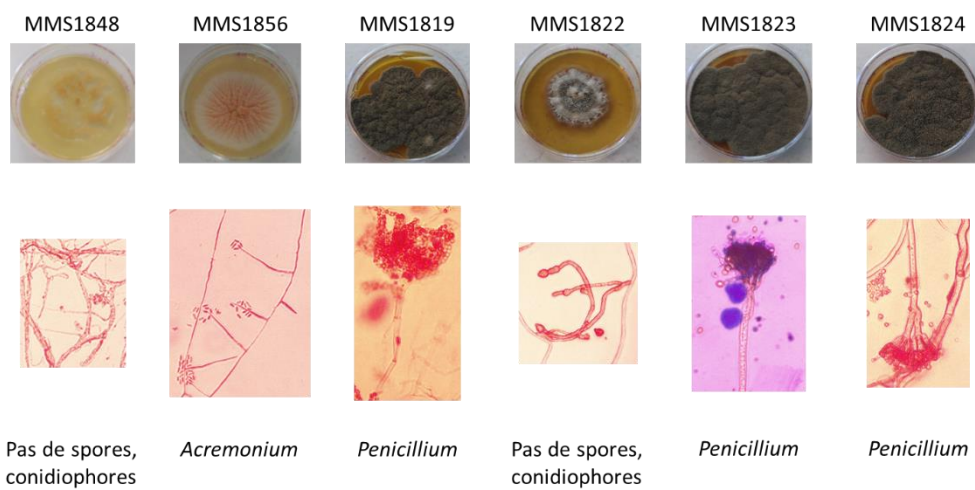
<i>Engyodontium</i>	<i>Cladosporium</i>	<i>Engyodontium</i>	<i>Acremonium</i>	<i>Aspergillus</i>	Pas de spores, conidiophores	<i>Engyodontium</i>
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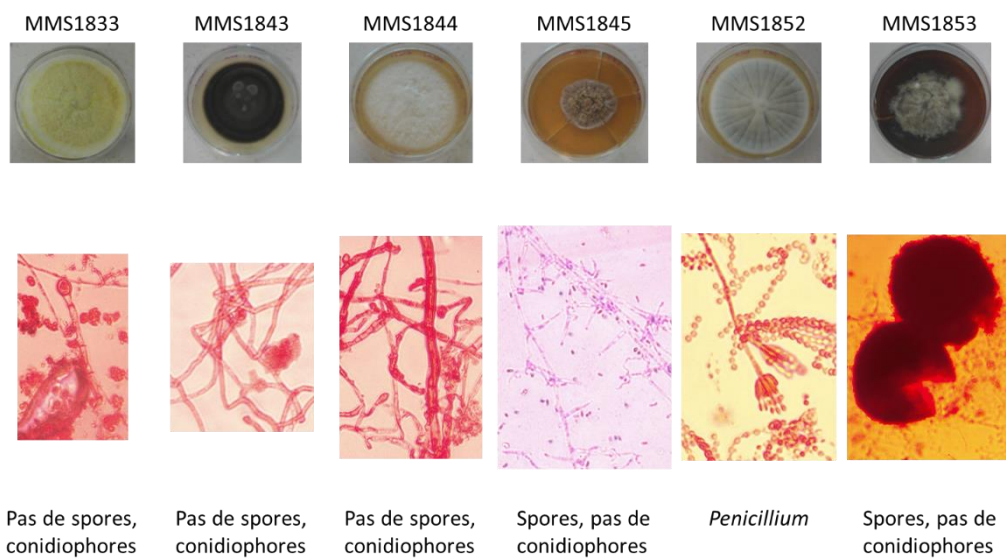
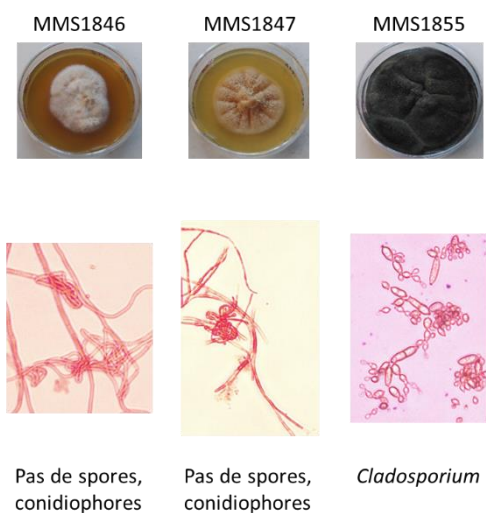
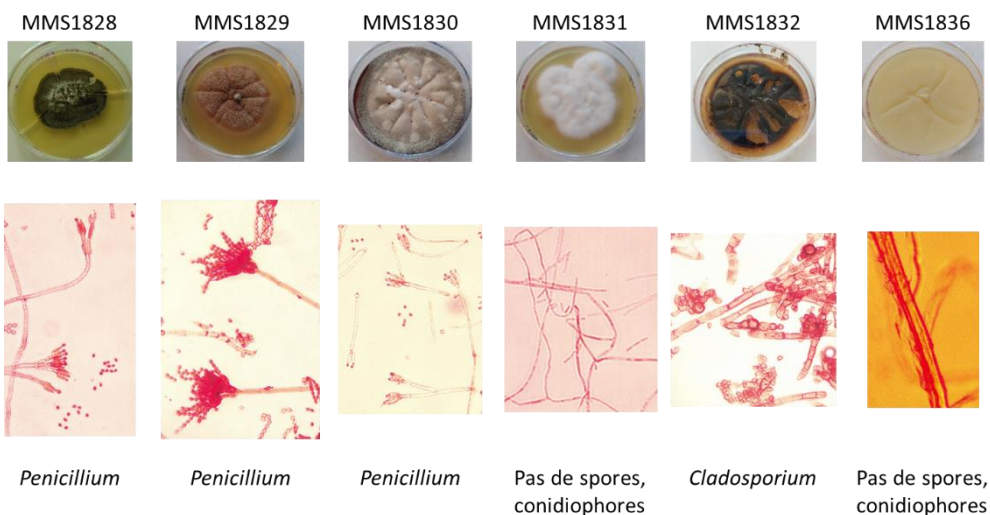


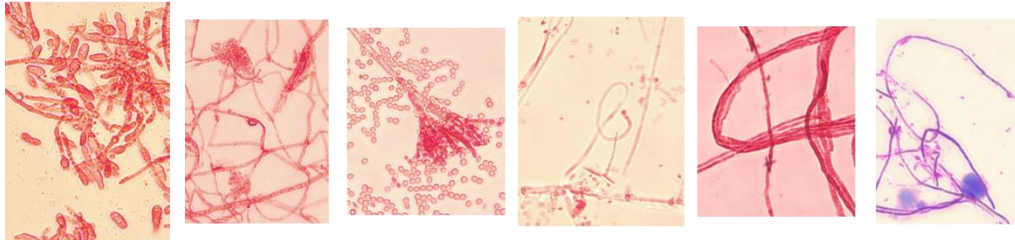
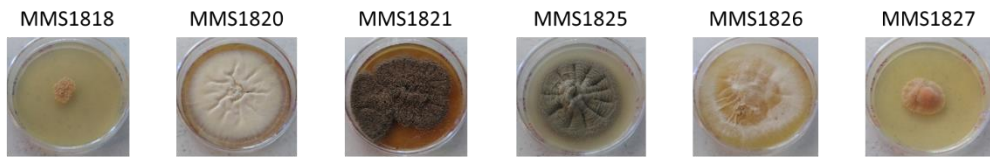
<i>Acremonium</i>	Pas de spores, conidiophores	<i>Aspergillus</i>	<i>Engyodontium</i>	<i>Acremonium</i>	<i>Aspergillus</i>	Levure
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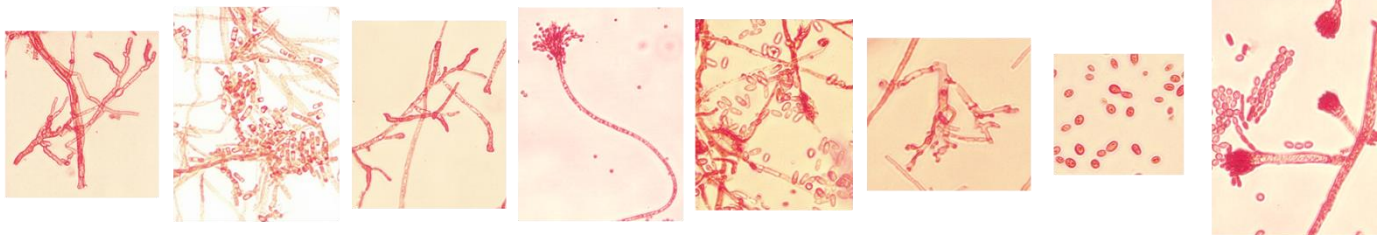
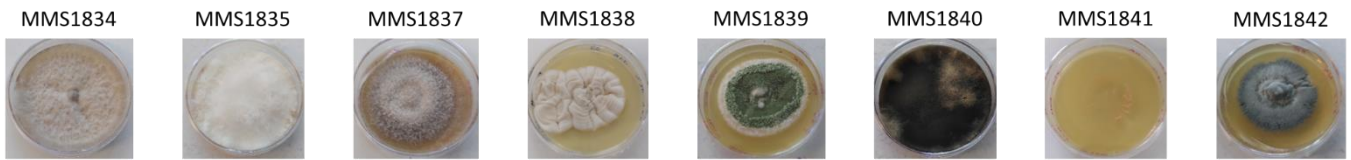
Récolte 11 (11/02/2020)



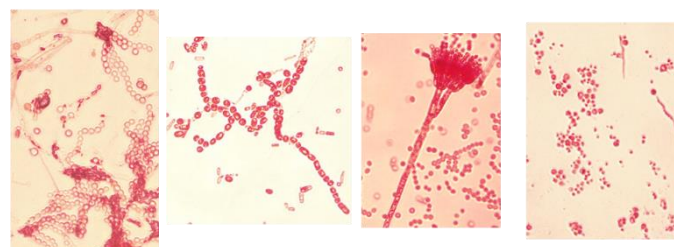
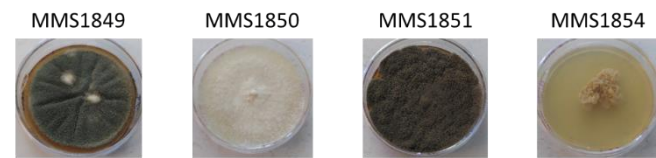




MMS1818: Spores, pas de conidiophores
 MMS1820: Pas de spores, conidiophores
 MMS1821: *Penicillium*
 MMS1825: Pas de spores, conidiophores
 MMS1826: Pas de spores, conidiophores
 MMS1827: Pas de spores, conidiophores

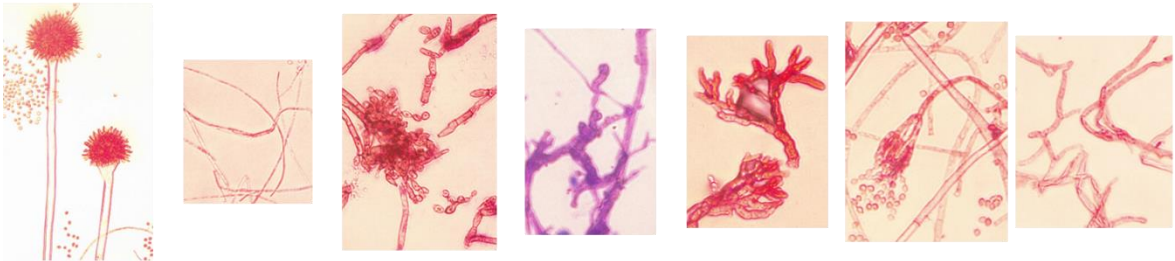


MMS1834: Pas de spores, conidiophores
 MMS1835: Spores, pas de conidiophores
 MMS1837: Pas de spores, conidiophores
 MMS1838: *Aspergillus*
 MMS1839: Spores, pas de conidiophores
 MMS1840: Pas de spores, conidiophores
 MMS1841: Levure
 MMS1842: *Aspergillus*

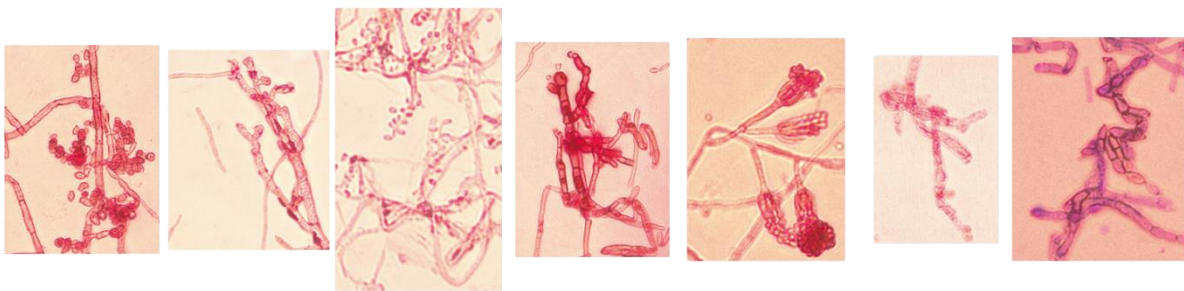
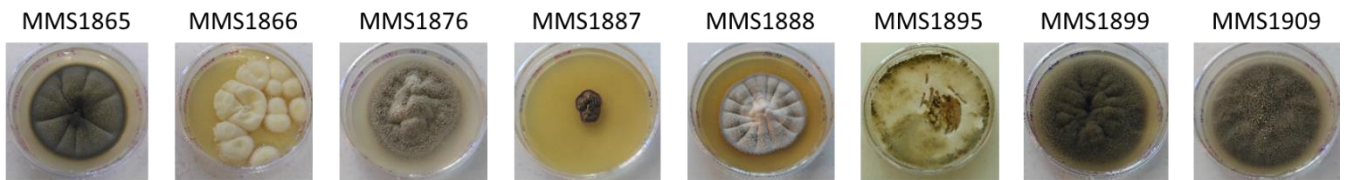


MMS1849: Spores, pas de conidiophores
 MMS1850: Spores, pas de conidiophores
 MMS1851: *Penicillium*
 MMS1854: Spores, pas de conidiophores

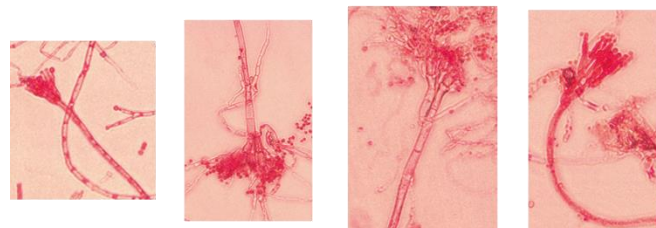
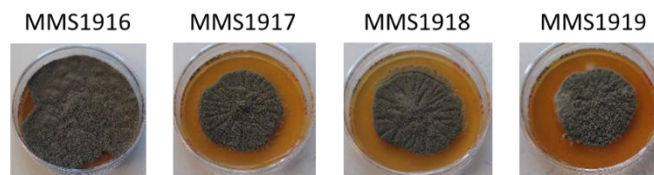
Récolte 12 (25/05/2020)



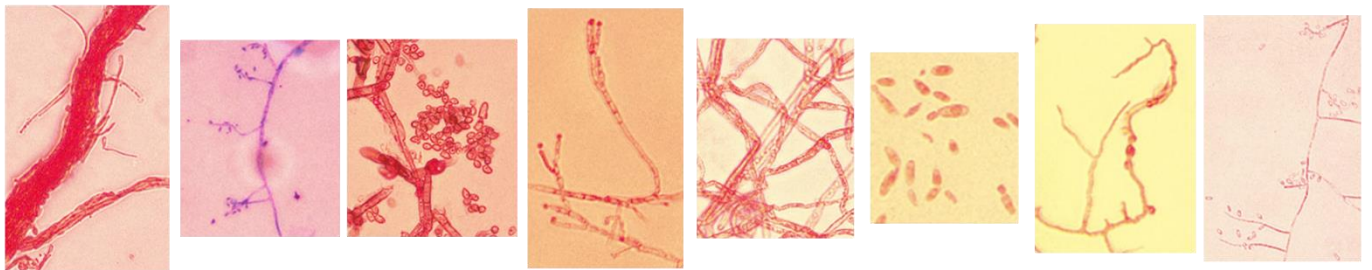
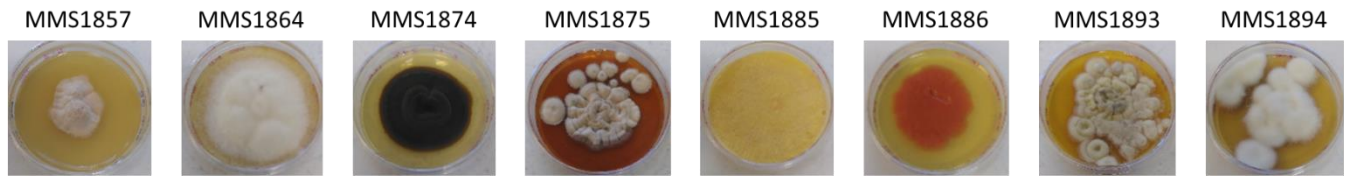
Aspergillus Pas de spores, conidiophores *Cladosporium* Pas de spores, conidiophores Pas de spores, conidiophores *Penicillium* Pas de spores, conidiophores



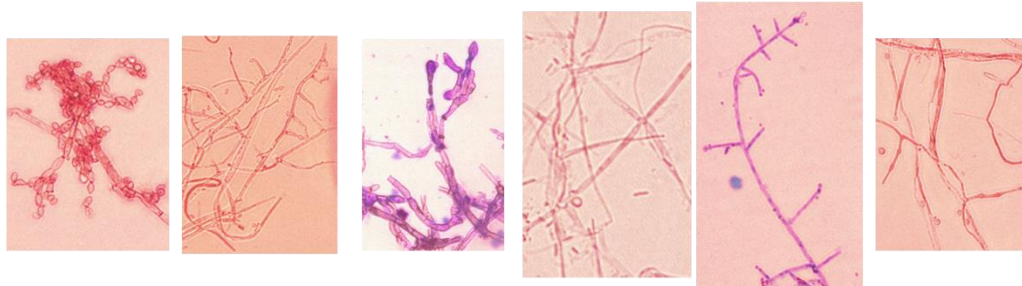
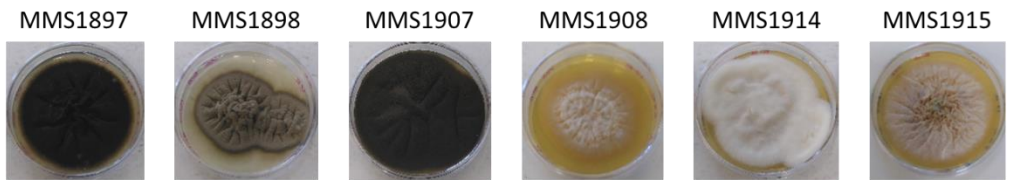
Cladosporium Pas de spores, conidiophores *Engyodontium* *Cladosporium* *Penicillium* Pas de spores, conidiophores *Cladosporium*



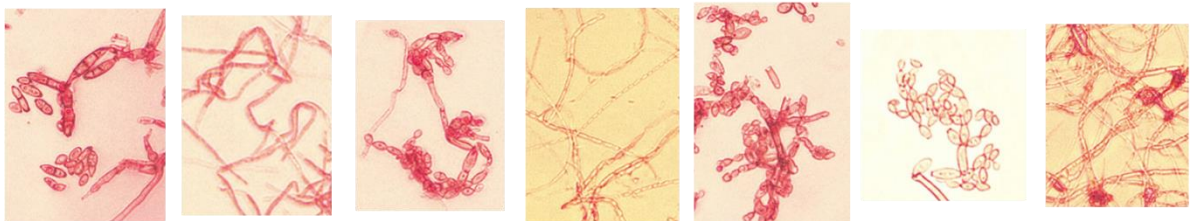
Penicillium *Penicillium* *Penicillium* *Penicillium*



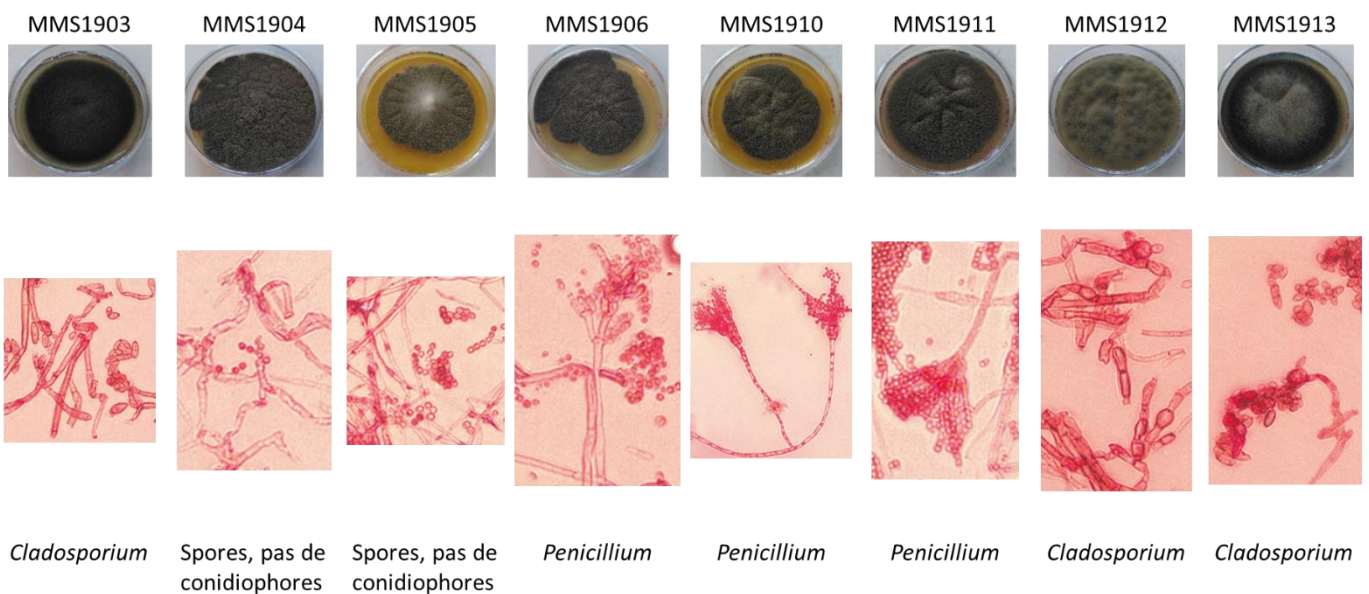
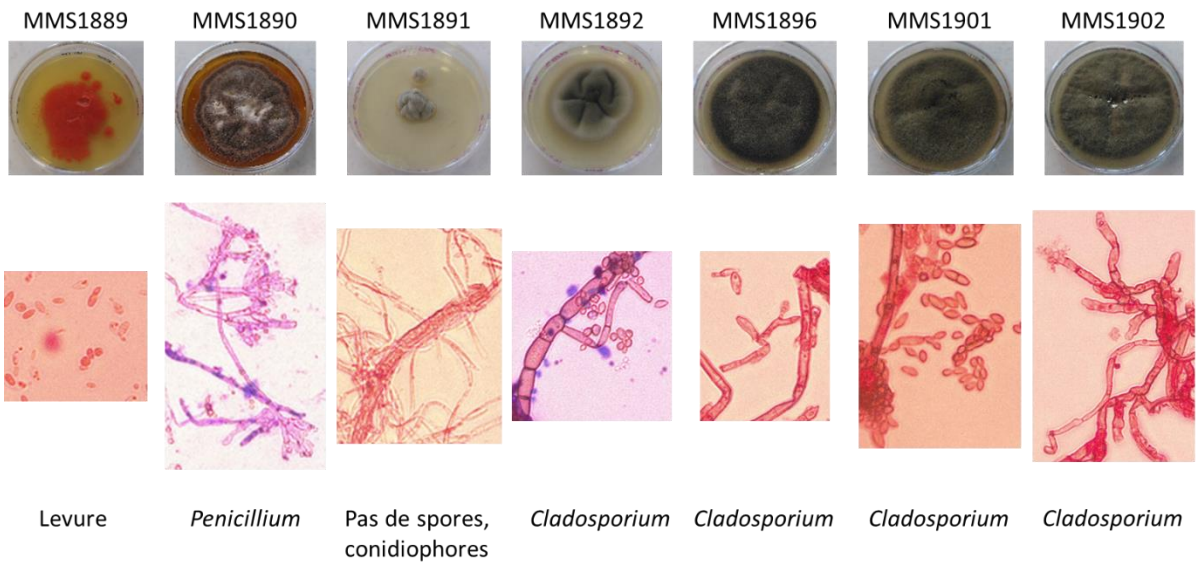
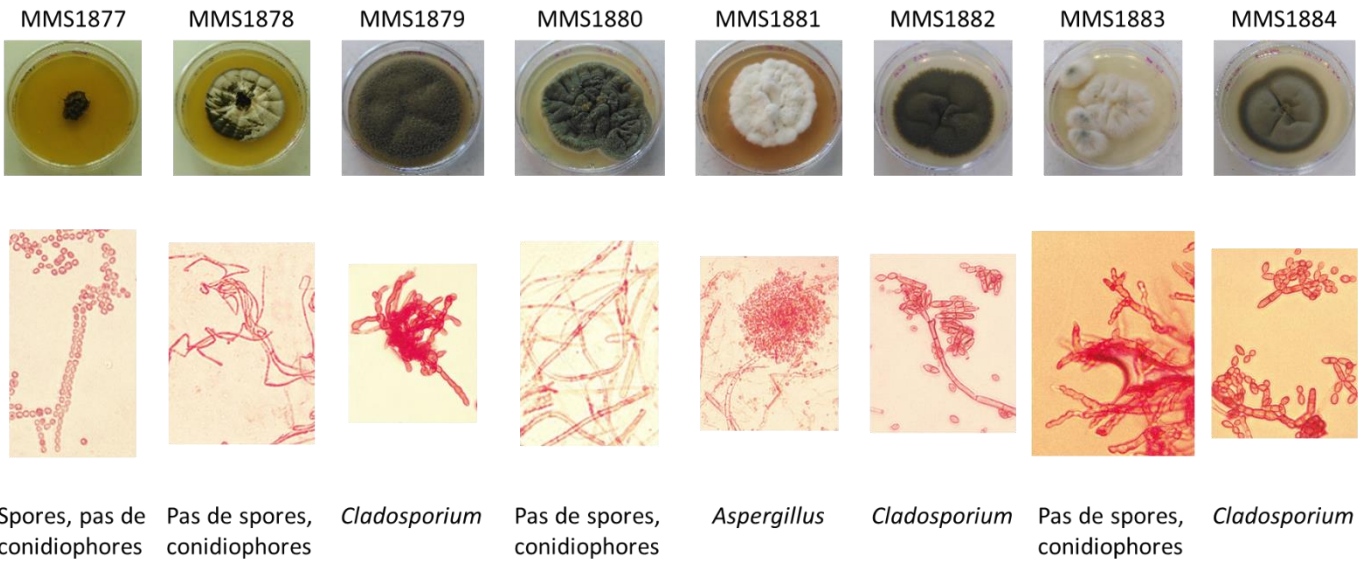
MMS1857: Pas de spores, conidiophores
 MMS1864: *Engyodontium*
 MMS1874: *Cladosporium*
 MMS1875: Pas de spores, conidiophores
 MMS1885: Pas de spores, conidiophores
 MMS1886: Levure
 MMS1893: Pas de spores, conidiophores
 MMS1894: *Engyodontium*



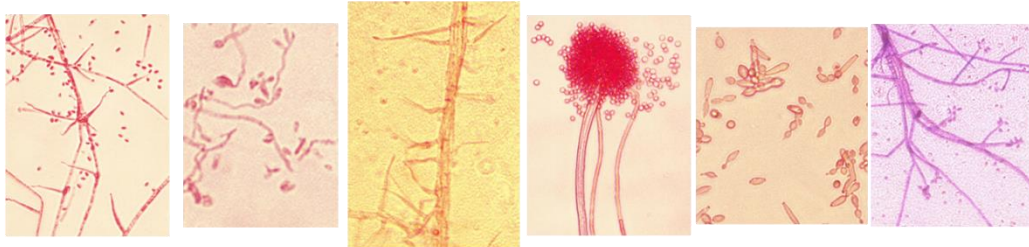
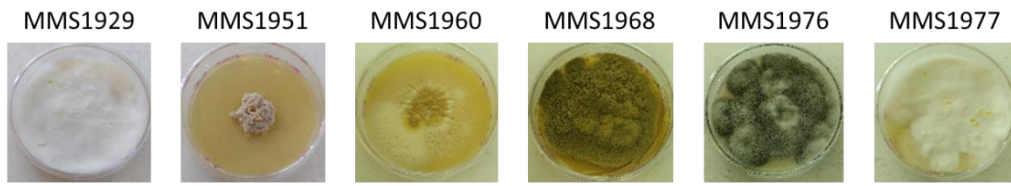
MMS1897: *Cladosporium*
 MMS1898: *Acremonium*
 MMS1907: *Cladosporium*
 MMS1908: *Acremonium*
 MMS1914: Pas de spores, conidiophores
 MMS1915: Pas de spores, conidiophores



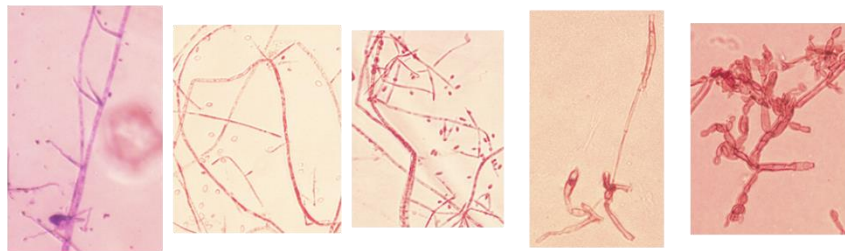
MMS1863: *Cladosporium*
 MMS1867: Pas de spores, conidiophores
 MMS1868: *Cladosporium*
 MMS1870: Pas de spores, conidiophores
 MMS1871: *Cladosporium*
 MMS1872: *Cladosporium*
 MMS1873: Pas de spores, conidiophores



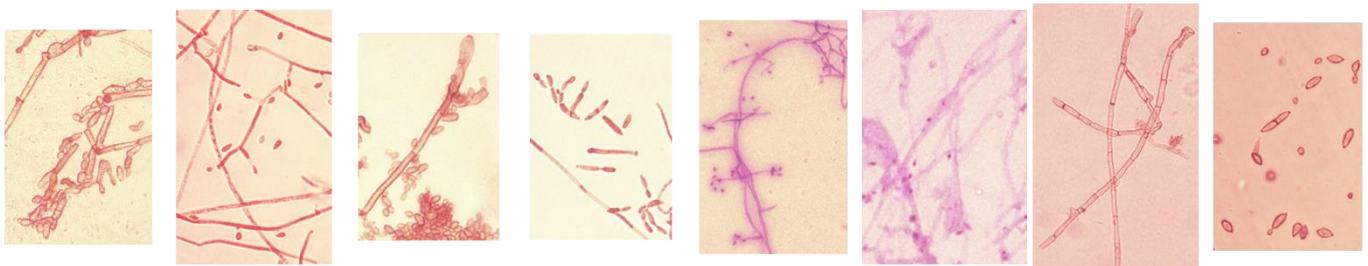
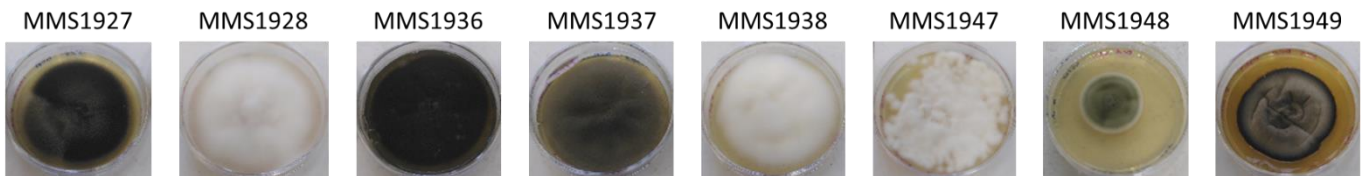
Récolte 13 (22/06/2020)



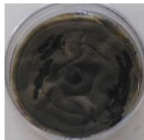
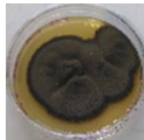



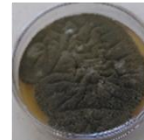

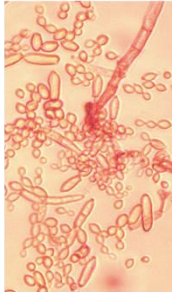
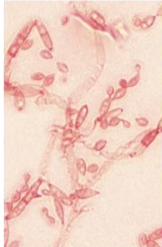
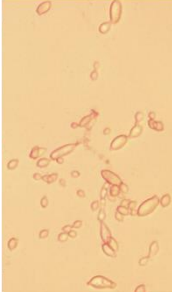
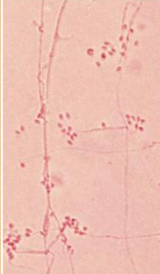
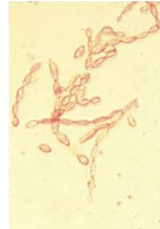

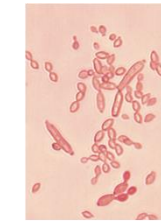
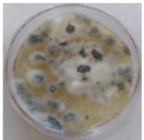


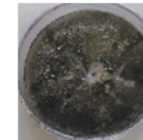
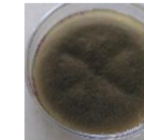
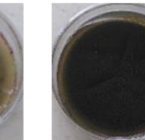
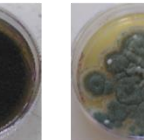
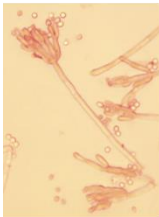
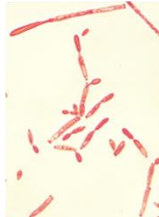
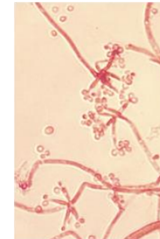
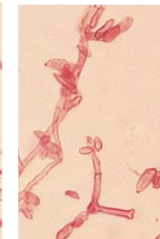
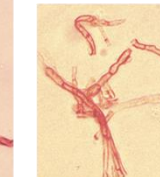
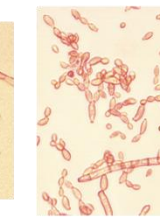
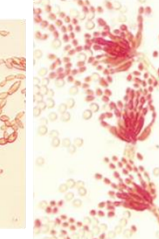

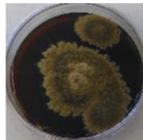
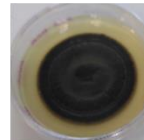
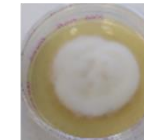

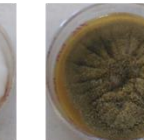

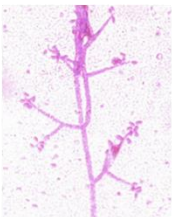
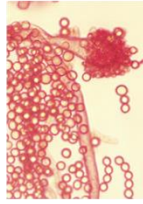
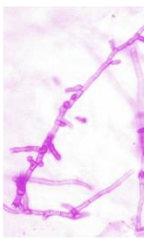
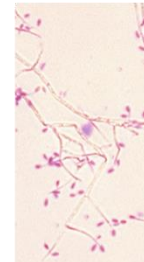
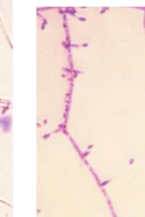

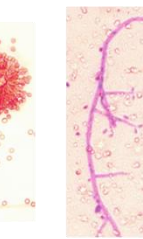
Engyodontium Spores, pas de conidiophores *Acremonium* *Aspergillus* *Cladosporium* *Engyodontium*

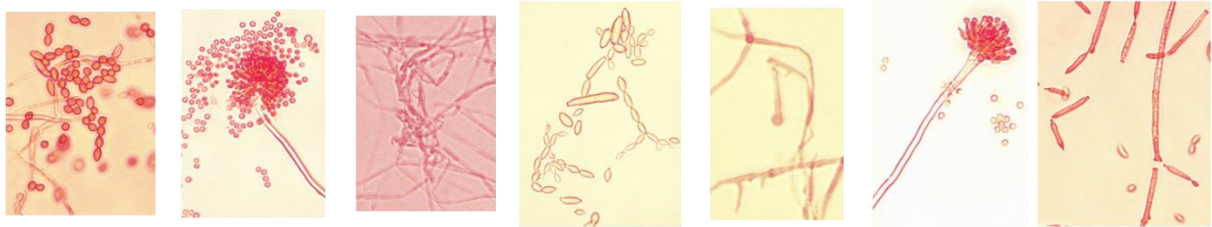
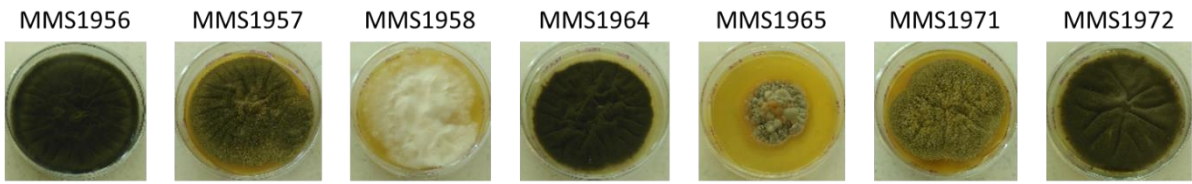


Engyodontium Spores, pas de *Engyodontium* *Cladosporium* *Cladosporium*
conidiophores conidiophores

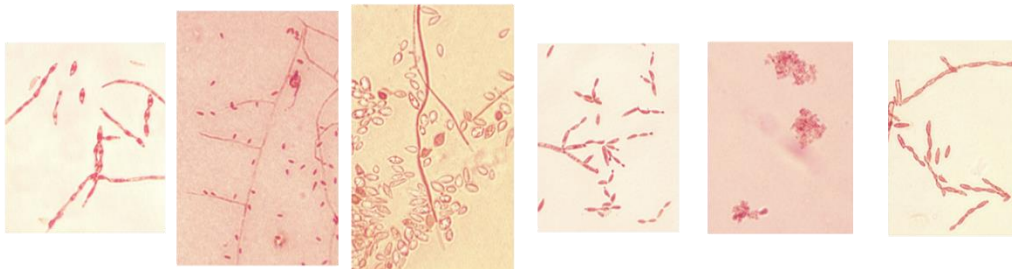
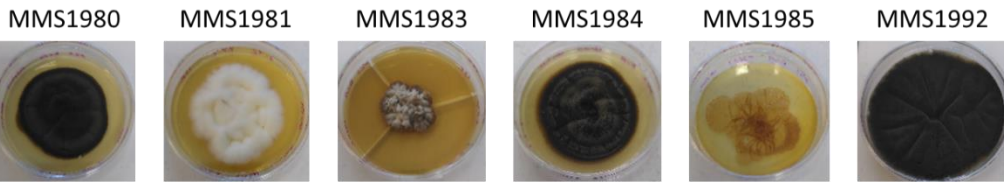


Cladosporium Spores, pas de *Cladosporium* *Cladosporium* *Engyodontium* Pas de spores, Pas de spores, *Cladosporium*
conidiophores conidiophores conidiophores conidiophores conidiophores

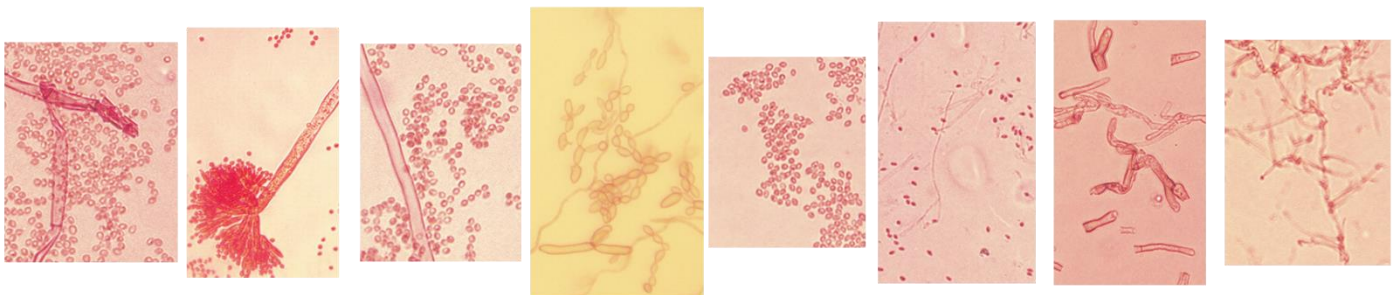
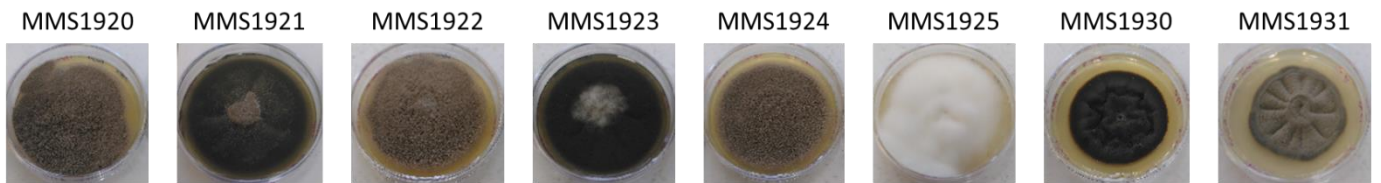
MMS1950	MMS1959	MMS1966	MMS1967	MMS1973	MMS1974	MMS1975
						
						
<i>Cladosporium</i>	<i>Cladosporium</i>	<i>Cladosporium</i>	<i>Engyodontium</i>	<i>Cladosporium</i>	<i>Penicillium</i>	<i>Cladosporium</i>
MMS1986	MMS1987	MMS1988	MMS1993	MMS1994	MMS1995	MMS1996
						
						
<i>Penicillium</i>	<i>Cladosporium</i>	<i>Engyodontium</i>	<i>Cladosporium</i>	Pas de spores, conidiophores	<i>Cladosporium</i>	<i>Penicillium</i>
MMS1926	MMS1932	MMS1933	MMS1934	MMS1935	MMS1945	MMS1946
						
						
<i>Engyodontium</i>	<i>Aspergillus</i>	Pas de spores, conidiophores	<i>Engyodontium</i>	Spores, pas de conidiophores	<i>Aspergillus</i>	<i>Engyodontium</i>



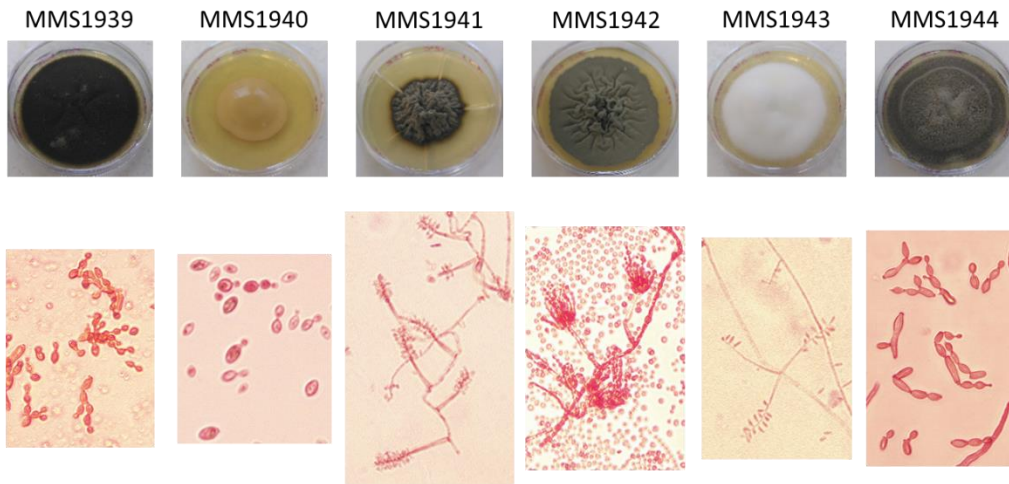
Cladosporium *Aspergillus* Pas de spores, conidiophores *Cladosporium* Pas de spores, conidiophores *Aspergillus* *Cladosporium*



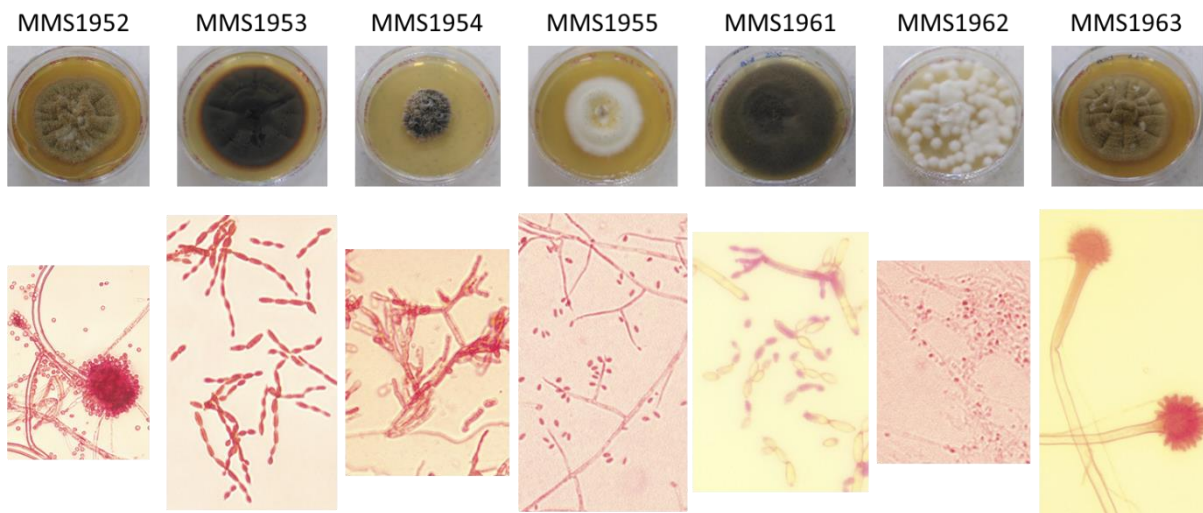
Spores, pas de conidiophores *Engyodontium* Spores, pas de conidiophores *Cladosporium* Pas de spores, conidiophores *Cladosporium*



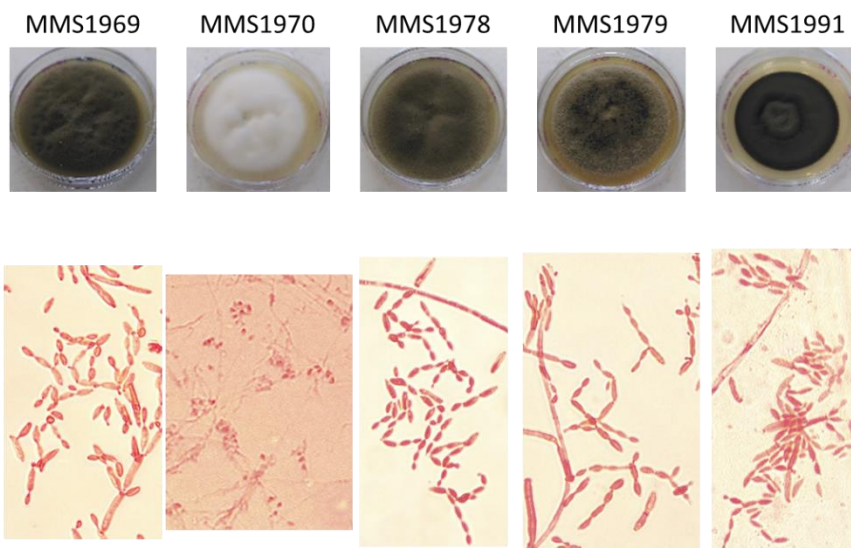
Spores, pas de conidiophores *Aspergillus* Spores, pas de conidiophores *Cladosporium* Spores, pas de conidiophores Spores, pas de conidiophores *Cladosporium* Pas de spores, conidiophores



Cladosporium *Levure* *Engyodontium* *Penicillium* *Engyodontium* *Cladosporium*



Aspergillus *Cladosporium* Pas de spores, conidiophores *Engyodontium* *Cladosporium* Pas de spores, conidiophores *Aspergillus*



Cladosporium *Engyodontium* *Cladosporium* *Cladosporium* *Cladosporium*

Annexes

Annexe 2 : Description of the cultivable fungal diversity associated to *P. palmata* according to the four variables: location in the alga (Endo: endophytes, Epi: epiphytes, Epi/Endo: non-determined location), incubation temperature, culture medium (Wick: Wickerham medium, Palm: "P. palmata" medium) and season of sampling (Sum : summer, Aut: autumn, Win: winter, Spr: spring).

Assignment	Total	Endo	Epi	Epi/Endo	12°C	27°C	Wick	Palm	Sum	Aut	Win	Spr	References
<i>Acremonium longisporum</i>	1	1	0	0	1	0	0	1	1	0	0	0	*
<i>Acremonium</i> sp. I	7	0	2	5	3	4	1	6	0	0	6	1	Zuccaro <i>et al.</i> (2004)
<i>Acremonium</i> sp. II	1	0	0	1	0	1	1	0	0	0	1	0	
<i>Acremonium</i> sp. III	1	1	0	0	0	1	1	0	0	0	1	0	
<i>Acrodontium luzulae</i>	2	0	1	1	2	0	1	1	0	0	0	2	*
<i>Acrodontium metrosideri</i>	1	0	1	0	1	0	1	0	0	0	0	1	*
<i>Agaricomycetes</i> sp. I	1	0	0	1	1	0	1	0	0	0	1	0	Xu <i>et al.</i> (2014)
<i>Agaricomycetes</i> sp. II	1	0	1	0	0	1	1	0	0	1	0	0	
<i>Annulohyphoxylon multiforme</i>	1	1	0	0	0	1	0	1	0	0	1	0	*
<i>Arachnomyces peruvianus</i>	1	1	0	0	0	1	1	0	0	0	1	0	*
<i>Aspergillus conicus</i>	1	0	0	1	1	0	1	0	0	0	1	0	Borzykh and Zvereva (2015)
<i>Aspergillus inflatus</i>	1	0	0	1	1	0	1	0	0	0	1	0	*
<i>Aspergillus puulaauensis</i>	8	0	3	5	2	6	5	3	0	0	2	6	Letsiou <i>et al.</i> (2020)
<i>Aspergillus</i> sp. I	2	0	0	2	0	2	1	1	0	0	1	1	
<i>Aspergillus</i> sp. II	2	0	2	0	2	0	2	0	2	0	0	0	
<i>Aspergillus</i> sp. III	2	0	0	2	2	0	0	2	2	0	0	0	
<i>Aspergillus</i> sp. IV	1	1	0	0	0	1	1	0	0	0	1	0	Wei <i>et al.</i> (2018); Maamar <i>et al.</i> (2020)
<i>Aspergillus</i> sp. V	3	1	1	1	0	3	3	0	1	2	0	0	
<i>Aspergillus</i> sp. VI	1	0	1	0	0	1	1	0	0	0	0	1	
<i>Aspergillus</i> sp. VII	1	0	1	0	0	1	1	0	1	0	0	0	
<i>Beauveria</i> sp. I	1	0	0	1	1	0	1	0	0	0	0	1	
<i>Beauveria</i> sp. II	2	1	0	1	2	0	0	2	0	0	0	2	Slinkina <i>et al.</i> (2010)
<i>Bullera alba</i> *	1	0	1	0	1	0	1	0	0	0	0	1	*
<i>Calcarisporium cordycipitcola</i>	2	2	0	0	1	1	1	1	0	1	1	0	*
<i>Calcarisporium</i> sp. II	2	0	1	1	1	1	2	0	0	0	0	2	Silber <i>et al.</i> (2013)
<i>Calcarisporium</i> sp. I	3	1	1	1	0	3	3	0	0	0	0	3	

Chaetomiaceae sp.	1	0	1	0	1	0	1	0	0	0	1	0	Maamar <i>et al.</i> (2020)
<i>Chaetomium</i> sp.	1	0	0	1	1	0	1	0	0	1	0	0	
<i>Chondrostereum purpureum</i>	1	0	1	0	1	0	1	0	0	0	1	0	Marchese <i>et al.</i> (2021)
<i>Cladosporium aggregatocicatricatum</i>	1	0	0	1	1	0	1	0	0	1	0	0	Maamar <i>et al.</i> (2020); Álvarez-Barragán <i>et al.</i> (2021)
<i>Cladosporium halotolerans</i>	1	0	0	1	0	1	1	0	0	1	0	0	Dobretsov <i>et al.</i> (2021); Martorell <i>et al.</i> (2021)
<i>Cladosporium pulvericola</i>	1	0	0	1	1	0	0	1	0	0	1	0	*
<i>Cladosporium ramotenellum</i>	5	1	2	2	5	0	0	5	0	0	1	4	Patyshakuliyeva <i>et al.</i> (2019)
<i>Cladosporium</i> sp. I	12	6	4	2	10	2	7	5	2	0	2	8	
<i>Cladosporium</i> sp. II	35	9	8	18	28	7	21	14	13	5	1	16	
<i>Cladosporium</i> sp. III	23	9	6	8	18	5	14	9	0	0	0	23	
<i>Cladosporium</i> sp. IV	1	1	0	0	1	0	0	1	0	0	0	1	
<i>Cladosporium</i> sp. IX	2	0	0	2	1	1	2	0	0	2	0	0	Patyshakuliyeva <i>et al.</i> (2019);
<i>Cladosporium</i> sp. V	1	0	1	0	0	1	1	0	0	0	1	0	Maamar <i>et al.</i> (2020)
<i>Cladosporium</i> sp. VI	1	0	0	1	1	0	0	1	0	0	0	1	
<i>Cladosporium</i> sp. VII	1	1	0	0	1	0	1	0	0	1	0	0	
<i>Cladosporium</i> sp. VIII	1	0	0	1	0	1	1	0	0	0	0	1	
<i>Cladosporium</i> sp. X	2	0	1	1	1	1	2	0	0	0	0	2	
<i>Crocicreas coronatum</i>	1	0	1	0	1	0	1	0	0	0	1	0	*
<i>Crocicreas</i> sp.	1	0	0	1	1	0	1	0	0	0	1	0	Gnavi <i>et al.</i> (2014)
Cuniculitremaceae sp.	1	1	0	0	1	0	1	0	0	0	1	0	Angulo-Preckler <i>et al.</i> (2020)
<i>Debaryomyces hansenii</i>	1	0	0	1	1	0	1	0	0	0	1	0	Rivas-Garcia <i>et al.</i> (2019)
<i>Dioszegia hungarica</i>	1	0	0	1	1	0	1	0	0	0	1	0	*
<i>Emericellopsis</i> sp.	2	1	0	1	1	1	2	0	0	1	0	1	Zuccaro <i>et al.</i> (2004)
<i>Entyloma</i> sp.	1	0	1	0	1	0	1	0	1	0	0	0	Marchese <i>et al.</i> (2021)
Exobasidiomycetes sp. I	1	0	1	0	0	1	0	1	0	0	0	1	
Exobasidiomycetes sp. II	1	1	0	0	1	0	1	0	0	1	0	0	Singh <i>et al.</i> (2012)
Exobasidiomycetes sp. III	1	1	0	0	1	0	1	0	1	0	0	0	

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<i>Exobasidium</i> sp.	1	1	0	0	1	0	1	0	1	0	0	0	*
<i>Gibellulopsis nigrescens</i>	1	1	0	0	1	0	0	1	1	0	0	0	Garzoli et al. (2015)
Helotiales sp. I	2	1	0	1	2	0	2	0	0	2	0	0	
Helotiales sp. II	1	1	0	0	1	0	1	0	0	0	1	0	Gnavi et al. (2014); Rämä et al. (2014)
Helotiales sp. III	1	0	1	0	1	0	1	0	1	0	0	0	
Helotiales sp. IV	1	0	0	1	0	1	1	0	0	0	0	1	
Hypocreales sp II	1	1	0	0	0	1	1	0	1	0	0	0	Zuccaro et al. (2004); Silber et al. (2013); Rämä et al. (2014)
Hypocreales sp. I	1	1	0	0	0	1	1	0	0	0	0	1	
Hypocreales sp. III	1	0	1	0	1	0	0	1	0	0	1	0	
Hypocreales sp. IV	1	0	0	1	0	1	1	0	0	0	1	0	
<i>Keissleriella gloeospora</i>	1	0	0	1	1	0	0	1	0	0	1	0	*
<i>Lecanicillium</i> sp. I	1	1	0	0	0	1	1	0	0	0	0	1	
<i>Lecanicillium</i> sp. II	2	2	0	0	0	2	0	2	0	0	0	2	Huang et al. (2021)
<i>Lecanicillium</i> sp. III	2	0	2	0	1	1	1	1	0	0	0	2	
Leotiomyceta sp.	1	0	0	1	1	0	1	0	0	1	0	0	Navarri et al. (2017); Bovio et al. (2018)
<i>Leptobacillium leptobactrum</i>	1	0	0	1	1	0	1	0	0	0	0	1	*
<i>Metacordyceps chlamydosporia</i>	1	1	0	0	0	1	0	1	0	0	0	1	*
<i>Metarhizium</i> sp.	1	0	0	1	1	0	1	0	0	0	1	0	Kong et al. (2013)
<i>Microsphaeropsis arundinis</i>	2	0	0	2	1	1	2	0	0	0	2	0	Paulino et al. (2020)
<i>Microstroma bacarum</i>	1	0	0	1	0	1	1	0	0	1	0	0	*
<i>Mollisia</i> sp.	1	1	0	0	0	1	1	0	0	1	0	0	Bovio et al. (2018)
Mycosphaerellaceae sp.	1	1	0	0	0	1	1	0	0	0	1	0	
Mycosphaerellales sp. I	1	0	0	1	1	0	1	0	0	0	0	1	Suetrong et al. (2009)
Mycosphaerellales sp. II	2	2	0	0	1	1	2	0	1	0	0	1	
Mycosphaerellales sp. III	1	0	1	0	1	0	1	0	0	0	1	0	
<i>Neosetophoma cerealis</i>	1	0	1	0	0	1	0	1	1	0	0	0	*
<i>Oidiodendron</i> sp.	1	0	0	1	1	0	1	0	0	0	0	1	Navarri et al. (2017)
<i>Paraconiothyrium</i> sp.	1	0	0	1	0	1	1	0	0	0	1	0	Mohamed et al. (2010)

<i>Paradendryphiella salina</i>	1	0	0	1	1	0	0	1	0	1	0	0	Pilgaard <i>et al.</i> (2019)
<i>Paraphaeosphaeria</i> sp.	1	0	0	1	0	1	1	0	0	0	1	0	Tsuda <i>et al.</i> (2003)
<i>Parengyodontium album</i>	19	1	8	10	8	11	9	10	0	0	9	10	Dobretsov <i>et al.</i> (2021)
<i>Penicillium angulare</i>	1	1	0	0	1	0	0	1	0	0	0	1	*
<i>Penicillium brevicompactum</i>	7	2	4	1	6	1	3	4	0	1	2	4	Maamar <i>et al.</i> (2020)
<i>Penicillium crustosum</i>	1	0	1	0	1	0	1	0	0	0	1	0	Álvarez-Barragán <i>et al.</i> (2021)
<i>Penicillium decumbens</i>	1	0	1	0	1	0	1	0	0	0	0	1	Fuentes and Quiñones (2016)
<i>Penicillium expansum</i>	1	0	0	1	1	0	0	1	0	1	0	0	Kerzaon <i>et al.</i> (2009)
<i>Penicillium glabrum</i>	1	0	0	1	1	0	0	1	0	0	0	1	Maamar <i>et al.</i> (2020)
<i>Penicillium kongii</i>	1	0	0	1	1	0	1	0	0	0	0	1	Álvarez-Barragán <i>et al.</i> (2021)
<i>Penicillium pancosmium</i>	1	0	0	1	1	0	1	0	0	0	0	1	*
<i>Penicillium</i> sp. I	15	7	7	1	14	1	6	9	1	3	5	6	
<i>Penicillium</i> sp. II	2	1	1	0	2	0	1	1	2	0	0	0	
<i>Penicillium</i> sp. III	1	1	0	0	1	0	1	0	1	0	0	0	
<i>Penicillium</i> sp. IV	1	1	0	0	1	0	1	0	0	0	1	0	
<i>Penicillium</i> sp. IX	1	0	1	0	1	0	1	0	1	0	0	0	
<i>Penicillium</i> sp. V	5	3	0	2	2	3	2	3	4	0	1	0	
<i>Penicillium</i> sp. VI	1	0	0	1	0	1	1	0	1	0	0	0	
<i>Penicillium</i> sp. VII	2	0	0	2	0	2	0	2	0	0	2	0	
<i>Penicillium</i> sp. VIII	1	1	0	0	1	0	1	0	0	0	1	0	
<i>Penicillium</i> sp. X	1	0	1	0	1	0	0	1	0	0	0	1	Maamar <i>et al.</i> (2020); Álvarez-Barragán <i>et al.</i> (2021)
<i>Penicillium</i> sp. XI	1	1	0	0	1	0	1	0	0	0	0	1	
<i>Penicillium</i> sp. XII	1	0	1	0	1	0	1	0	0	0	0	1	
<i>Penicillium</i> sp. XIII	4	0	1	3	0	4	2	2	3	0	1	0	
<i>Penicillium</i> sp. XIV	1	0	1	0	1	0	0	1	1	0	0	0	
<i>Penicillium</i> sp. XIX	1	1	0	0	1	0	0	1	0	0	0	1	
<i>Penicillium</i> sp. XV	1	0	0	1	1	0	0	1	1	0	0	0	
<i>Penicillium</i> sp. XVI	2	0	2	0	2	0	0	2	0	0	2	0	
<i>Penicillium</i> sp. XVII	2	0	1	1	1	1	1	1	0	0	1	1	
<i>Penicillium</i> sp. XVIII	1	1	0	0	1	0	1	0	0	0	0	1	

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<i>Penicillium</i> sp. XX	2	2	0	0	0	2	1	1	1	0	1	0	
<i>Penicillium</i> sp. XXI	1	0	0	1	1	0	0	1	1	0	0	0	
<i>Penicillium</i> <i>spathulatum</i>	1	1	0	0	1	0	1	0	0	0	0	1	*
<i>Penicillium</i> <i>ubiquetum</i>	2	0	0	2	1	1	1	1	0	0	0	2	Hoang et al. (2019)
<i>Peniophora</i> <i>rufomarginata</i>	1	0	0	1	0	1	1	0	0	1	1	0	*
<i>Peroneutypa</i> sp.	1	0	0	1	1	0	1	0	0	0	1	0	Dayarathne (2020)
Phaeosphaeriacea sp.	1	0	0	1	0	1	0	1	0	1	0	0	Elsebai et al. (2009)
<i>Phlebia</i> <i>radiata</i>	1	0	0	1	1	0	1	0	0	1	1	0	*
<i>Phlebia</i> sp.	1	0	0	1	0	1	1	0	0	0	0	1	Kamei et al. (2008)
<i>Phlebiopsis</i> <i>ravenelii</i>	1	1	0	0	1	0	1	0	0	0	0	0	*
<i>Phyllozoma</i> sp.	1	0	1	0	1	0	1	0	0	0	1	0	*
Pleosporales sp. I	1	0	1	0	1	0	0	1	0	0	1	0	Pilgaard et al. (2019); Paulino et al. (2020)
Pleosporales sp. II	1	0	0	1	0	1	1	0	0	0	1	0	
<i>Ramularia</i> sp. I	1	1	0	0	0	1	1	0	0	0	0	1	
<i>Ramularia</i> sp. II	1	0	1	0	1	0	1	0	0	0	0	1	Gnavi et al. (2017)
<i>Ramularia</i> sp. III	1	0	0	1	1	0	1	0	0	0	0	1	
<i>Roussoella</i> sp.	1	0	0	1	1	0	1	0	0	0	1	0	Ferreira et al. (2015)
<i>Sclerotinia</i> <i>spermophila</i>	1	0	0	1	1	0	0	1	0	0	0	0	*
<i>Sistotrema</i> sp.	1	0	1	0	0	1	1	0	1	0	0	0	Rédou et al. (2015)
Sordariomyceta sp.	1	1	0	0	1	0	0	1	0	1	0	0	Zuccaro et al. (2004); Slinkina et al. (2010); Silber et al. (2013); Rämä et al. (2014); Maamar et al. (2020); Dobretsov et al. (2021); Huang et al. (2021)
Sordariomycetes sp. I	1	0	0	1	0	1	0	1	0	0	1	0	
Sordariomycetes sp. II	1	0	1	0	0	1	1	0	0	0	0	1	
Sporidiobolaceae sp.	2	1	1	0	2	0	2	0	2	0	0	0	Vitale et al. (2020); Li et al. (2021)

<i>Sporobolomyces ruberrimus</i>	2	1	0	1	1	1	1	1	1	1	0	0	1	Vitale et al. (2020)
<i>Sporobolomyces</i> sp. I	1	0	0	1	0	1	0	1	1	0	0	0	0	Vitale et al. (2020); Li et al. (2021)
<i>Sporobolomyces</i> sp. II	1	0	0	1	1	0	1	0	0	0	0	0	1	
<i>Stachybotrys chartarum</i>	5	0	0	5	2	3	3	2	5	0	0	0	0	Yang et al. (2019)
<i>Stereum</i> sp.	1	0	0	1	1	0	1	0	0	0	0	1	0	Yamada et al. (2018)
<i>Tilletiopsis washingtonensis</i>	1	1	0	0	0	1	1	0	0	0	0	0	1	Duarte et al. (2016)
<i>Trametes versicolor</i>	2	0	1	1	2	0	2	0	0	0	0	2	0	Rédou et al. (2015)
Tremellomycetes sp.	1	1	0	0	1	0	1	0	0	0	0	1	0	Angulo-Preckler et al. (2020)
<i>Trichoderma</i> sp.	1	0	0	1	1	0	1	0	0	0	0	1	0	Ruiz et al. (2007)
Ustilaginaceae sp.	1	0	1	0	1	0	1	0	0	1	0	0	0	Jones et al. (2009)
<i>Verticillium</i> sp.	1	1	0	0	1	0	1	0	1	0	0	0	0	Almeida et al. (2010)
<i>Wallemia</i> sp. I	1	0	0	1	1	0	1	0	0	0	0	0	1	
<i>Wallemia</i> sp. II	1	1	0	0	1	0	1	0	0	0	0	1	0	Oleinikova et al. (2010)
* taxa newly observed from marine environment														

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Annexe 3 : Qualitative activities of fungal strains isolated from *P. palmata*. Indications of location (*endo* = endophyte, *epi* = epiphyte, *epi/endo* = non-determined location), incubation temperature, incubation medium (*Palm* = “*P. palmata*” medium, *Wick* = Wickerham medium) and sampling season for each isolate is presented.

Assig nation	MMS code	Cellulase	Xylanase	Location	Temperature (°C)	Medium	Season
<i>Acremonium longisporum</i>	1705	-	-	endo	12	Palm	summer
<i>Acremonium</i> sp. I	1809	-	-	epi	12	Palm	winter
<i>Acremonium</i> sp. I	1773	-	-	epi	27	Palm	winter
<i>Acremonium</i> sp. I	1960	+	++	epi/endo	27	Palm	spring
<i>Acremonium</i> sp. I	1815	++	+	epi/endo	12	Palm	winter
<i>Acremonium</i> sp. I	1802	-	-	epi/endo	12	Palm	winter
<i>Acremonium</i> sp. I	1781	-	-	epi/endo	27	Palm	winter
<i>Acremonium</i> sp. I	1798	-	-	epi/endo	27	Wick	winter
<i>Acremonium</i> sp. II	1777	++	++	epi/endo	27	Wick	winter
<i>Acremonium</i> sp. III	1774	-	-	endo	27	Wick	winter
<i>Acrodontium luzulae</i>	1931	-	+	epi	12	Wick	spring
<i>Acrodontium luzulae</i>	1876	-	-	epi/endo	12	Palm	spring
<i>Acrodontium metrosideri</i>	1941	-	-	epi	12	Wick	spring
Agaricomycetes sp. I	1835	++	+	epi/endo	12	Wick	winter
Agaricomycetes sp. II	1753	+	-	epi	27	Wick	autumn
<i>Annulohyphoxylon multiforme</i>	1856	+	-	endo	27	Palm	winter
<i>Arachnomyces peruvianus</i>	1853	+	+	endo	27	Wick	winter
<i>Aspergillus conicus</i>	1842	-	-	epi/endo	12	Wick	winter
<i>Aspergillus inflatus</i>	1838	+	-	epi/endo	12	Wick	winter
<i>Aspergillus puulaaensis</i>	1946	+	++	epi	27	Wick	spring

<i>Aspergillus puulaauensis</i>	1869	++	+	epi	27	Palm	spring
<i>Aspergillus puulaauensis</i>	1772	+	+	epi	27	Palm	winter
<i>Aspergillus puulaauensis</i>	1788	++	+	epi/endo	27	Wick	winter
<i>Aspergillus puulaauensis</i>	1952	+	+	epi/endo	12	Wick	spring
<i>Aspergillus puulaauensis</i>	1963	+	+	epi/endo	12	Wick	spring
<i>Aspergillus puulaauensis</i>	1968	+	+	epi/endo	27	Palm	spring
<i>Aspergillus puulaauensis</i>	1971	+	+	epi/endo	27	Wick	spring
<i>Aspergillus</i> sp. I	1785	+	+	epi/endo	27	Palm	winter
<i>Aspergillus</i> sp. I	1957	+	+	epi/endo	27	Wick	spring
<i>Aspergillus</i> sp. II	1699	++	+	epi	12	Wick	summer
<i>Aspergillus</i> sp. II	1683	+	+	epi	12	Wick	summer
<i>Aspergillus</i> sp. III	1733	++	++	epi/endo	12	Palm	summer
<i>Aspergillus</i> sp. III	1735	++	+	epi/endo	12	Palm	summer
<i>Aspergillus</i> sp. IV	1776	-	-	endo	27	Wick	winter
<i>Aspergillus</i> sp. V	1704	-	-	endo	27	Wick	summer
<i>Aspergillus</i> sp. V	1749	-	-	epi	27	Wick	autumn
<i>Aspergillus</i> sp. V	1752	-	-	epi/endo	27	Wick	autumn
<i>Aspergillus</i> sp. VI	1932	-	-	epi	27	Wick	spring
<i>Aspergillus</i> sp. VII	1726	-	+	epi	27	Wick	summer
<i>Beauveria</i> sp. I	1881	-	-	epi/endo	12	Wick	spring
<i>Beauveria</i> sp. II	1988	-	-	endo	12	Palm	spring
<i>Beauveria</i> sp. II	1895	-	++	epi/endo	12	Palm	spring
<i>Calcarisporium cordycipiticola</i>	1747	++	+	endo	27	Palm	autumn

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<i>Calcarisporium cordycipiticola</i>	1806	++	-	endo	12	Wick	winter
<i>Calcarisporium</i> sp. I	1864	+	+	epi	27	Wick	spring
<i>Calcarisporium</i> sp. I	1981	-	+	endo	27	Wick	spring
<i>Calcarisporium</i> sp. I	1894	-	-	epi/endo	27	Wick	spring
<i>Calcarisporium</i> sp. II	1934	-	+	epi	27	Wick	spring
<i>Calcarisporium</i> sp. II	1955	-	+	epi/endo	12	Wick	spring
Chaetomiaceae sp.	1820	++	+	epi	12	Wick	winter
<i>Chaetomium</i> sp.	1741	+	+	epi/endo	12	Wick	autumn
<i>Chondrostereum purpureum</i>	1808	+	+	epi	12	Wick	winter
<i>Cladosporium aggregatocaticratum</i>	1739	+	-	epi/endo	12	Wick	autumn
<i>Cladosporium halotolerans</i>	1754	-	-	epi/endo	27	Wick	autumn
<i>Cladosporium pulvericola</i>	1800	++	+	epi/endo	12	Palm	winter
<i>Cladosporium ramotenellum</i>	1855	++	++	endo	12	Palm	winter
<i>Cladosporium ramotenellum</i>	1950	+	+	epi	12	Palm	spring
<i>Cladosporium ramotenellum</i>	1949	-	-	epi	12	Palm	spring
<i>Cladosporium ramotenellum</i>	1959	++	++	epi/endo	12	Palm	spring
<i>Cladosporium ramotenellum</i>	1966	+	++	epi/endo	12	Palm	spring
<i>Cladosporium</i> sp. I	1980	++	++	endo	27	Wick	spring
<i>Cladosporium</i> sp. I	1913	++	+	endo	12	Wick	spring
<i>Cladosporium</i> sp. I	1696	+	-	endo	12	Wick	summer
<i>Cladosporium</i> sp. I	1901	-	-	endo	12	Wick	spring
<i>Cladosporium</i> sp. I	1995	-	-	endo	12	Palm	spring
<i>Cladosporium</i> sp. I	1998	-	-	endo	27	Palm	spring

<i>Cladosporium</i> sp. I	1680	+	-	epi	12	Wick	summer
<i>Cladosporium</i> sp. I	1832	+	-	epi	12	Palm	winter
<i>Cladosporium</i> sp. I	1858	-	-	epi	12	Palm	spring
<i>Cladosporium</i> sp. I	1868	-	-	epi	12	Wick	spring
<i>Cladosporium</i> sp. I	1836	-	+	epi/endo	12	Palm	winter
<i>Cladosporium</i> sp. I	1870	+	-	epi/endo	12	Wick	spring
<i>Cladosporium</i> sp. II	1896	++	+	endo	12	Wick	spring
<i>Cladosporium</i> sp. II	1912	++	+	endo	12	Wick	spring
<i>Cladosporium</i> sp. II	1897	++	-	endo	27	Wick	spring
<i>Cladosporium</i> sp. II	1689	+	-	endo	12	Wick	summer
<i>Cladosporium</i> sp. II	1692	+	-	endo	12	Wick	summer
<i>Cladosporium</i> sp. II	1695	+	-	endo	12	Palm	summer
<i>Cladosporium</i> sp. II	1899	-	-	endo	12	Palm	spring
<i>Cladosporium</i> sp. II	1902	-	-	endo	12	Wick	spring
<i>Cladosporium</i> sp. II	1907	-	-	endo	27	Wick	spring
<i>Cladosporium</i> sp. II	1681	+	-	epi	12	Wick	summer
<i>Cladosporium</i> sp. II	1693	+	-	epi	12	Palm	summer
<i>Cladosporium</i> sp. II	1716	+	-	epi	12	Wick	summer
<i>Cladosporium</i> sp. II	1720	+	-	epi	12	Palm	summer
<i>Cladosporium</i> sp. II	1729	-	-	epi	12	Palm	summer
<i>Cladosporium</i> sp. II	1859	-	-	epi	12	Palm	spring
<i>Cladosporium</i> sp. II	1863	-	-	epi	12	Wick	spring
<i>Cladosporium</i> sp. II	1865	-	-	epi	12	Palm	spring

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<i>Cladosporium</i> sp. II	1738	++	++	epi/endo	12	Palm	autumn
<i>Cladosporium</i> sp. II	1746	-	++	epi/endo	27	Palm	autumn
<i>Cladosporium</i> sp. II	1687	+	+	epi/endo	12	Palm	summer
<i>Cladosporium</i> sp. II	1682	+	-	epi/endo	12	Wick	summer
<i>Cladosporium</i> sp. II	1686	+	-	epi/endo	12	Palm	summer
<i>Cladosporium</i> sp. II	1713	+	-	epi/endo	27	Wick	summer
<i>Cladosporium</i> sp. II	1833	+	-	epi/endo	27	Wick	winter
<i>Cladosporium</i> sp. II	1725	-	-	epi/endo	12	Wick	summer
<i>Cladosporium</i> sp. II	1742	-	-	epi/endo	12	Wick	autumn
<i>Cladosporium</i> sp. II	1751	-	-	epi/endo	12	Palm	autumn
<i>Cladosporium</i> sp. II	1872	-	-	epi/endo	12	Wick	spring
<i>Cladosporium</i> sp. II	1879	-	-	epi/endo	12	Wick	spring
<i>Cladosporium</i> sp. II	1882	-	-	epi/endo	12	Wick	spring
<i>Cladosporium</i> sp. II	1884	-	-	epi/endo	12	Wick	spring
<i>Cladosporium</i> sp. II	1887	-	-	epi/endo	12	Palm	spring
<i>Cladosporium</i> sp. II	1892	-	-	epi/endo	12	Wick	spring
<i>Cladosporium</i> sp. II	1744	-	-	epi/endo	27	Palm	autumn
<i>Cladosporium</i> sp. II	1874	-	-	epi/endo	27	Wick	spring
<i>Cladosporium</i> sp. III	1978	+	++	endo	12	Wick	spring
<i>Cladosporium</i> sp. III	1991	+	++	endo	12	Wick	spring
<i>Cladosporium</i> sp. III	1984	+	++	endo	27	Wick	spring
<i>Cladosporium</i> sp. III	1992	-	++	endo	27	Wick	spring
<i>Cladosporium</i> sp. III	1987	+	+	endo	12	Palm	spring

<i>Cladosporium</i> sp. III	1979	+	-	endo	12	Wick	spring
<i>Cladosporium</i> sp. III	1909	-	-	endo	12	Palm	spring
<i>Cladosporium</i> sp. III	1994	-	-	endo	12	Palm	spring
<i>Cladosporium</i> sp. III	1997	-	-	endo	27	Palm	spring
<i>Cladosporium</i> sp. III	1948	++	+	epi	12	Palm	spring
<i>Cladosporium</i> sp. III	1923	+	+	epi	12	Wick	spring
<i>Cladosporium</i> sp. III	1939	+	+	epi	12	Wick	spring
<i>Cladosporium</i> sp. III	1927	-	+	epi	12	Palm	spring
<i>Cladosporium</i> sp. III	1933	++	-	epi	27	Wick	spring
<i>Cladosporium</i> sp. III	1930	-	-	epi	12	Wick	spring
<i>Cladosporium</i> sp. III	1936	-	-	epi	12	Palm	spring
<i>Cladosporium</i> sp. III	1937	-	-	epi	12	Palm	spring
<i>Cladosporium</i> sp. III	1972	+	++	epi/endo	27	Wick	spring
<i>Cladosporium</i> sp. III	1953	-	++	epi/endo	12	Wick	spring
<i>Cladosporium</i> sp. III	1961	+	+	epi/endo	12	Wick	spring
<i>Cladosporium</i> sp. III	1969	+	+	epi/endo	12	Wick	spring
<i>Cladosporium</i> sp. III	1973	-	+	epi/endo	12	Palm	spring
<i>Cladosporium</i> sp. III	1871	-	-	epi/endo	12	Wick	spring
<i>Cladosporium</i> sp. IV	1993	-	-	endo	12	Palm	spring
<i>Cladosporium</i> sp. V	1775	-	-	epi	27	Wick	winter
<i>Cladosporium</i> sp. VI	1975	+	++	epi/endo	12	Palm	spring
<i>Cladosporium</i> sp. VII	1771	-	+	endo	12	Wick	autumn
<i>Cladosporium</i> sp. VIII	1956	++	++	epi/endo	27	Wick	spring

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<i>Cladosporium</i> sp. IX	1740	-	+	epi/endo	12	Wick	autumn
<i>Cladosporium</i> sp. IX	1743	-	-	epi/endo	27	Wick	autumn
<i>Cladosporium</i> sp. X	1944	+	+	epi	12	Wick	spring
<i>Cladosporium</i> sp. X	1964	+	+	epi/endo	27	Wick	spring
<i>Crocicreas coronatum</i>	1827	-	+	epi	12	Wick	winter
<i>Crocicreas</i> sp.	1814	-	-	epi/endo	12	Wick	winter
Cuniculitremaeae sp.	1854	-	-	endo	12	Wick	winter
<i>Emericellopsis</i> sp.	1915	+	-	endo	27	Wick	spring
<i>Emericellopsis</i> sp.	1750	+	+	epi/endo	12	Wick	autumn
<i>Entyloma</i> sp.	1706	-	-	epi	12	Wick	summer
Exobasidiomycetes sp. I	1951	-	-	epi	27	Palm	spring
Exobasidiomycetes sp. II	1768	-	-	endo	12	Wick	autumn
Exobasidiomycetes sp. III	1688	-	-	endo	12	Wick	summer
<i>Exobasidium</i> sp.	1703	-	-	endo	12	Wick	summer
<i>Gibellulopsis nigrescens</i>	1694	+	++	endo	12	Palm	summer
Helotiales sp. I	1769	-	-	endo	12	Wick	autumn
Helotiales sp. I	1767	-	-	epi/endo	12	Wick	autumn
Helotiales sp. II	1805	-	-	endo	12	Wick	winter
Helotiales sp. III	1684	-	-	epi	12	Wick	summer
Helotiales sp. IV	1965	-	+	epi/endo	27	Wick	spring
Hypocreales sp. I	1908	++	+	endo	27	Wick	spring
Hypocreales sp. II	1736	++	+	endo	27	Wick	summer
Hypocreales sp. III	1831	-	-	epi	12	Palm	winter

Hypocreales sp. IV	1845	-	-	epi/endo	27	Wick	winter
<i>Keissleriella gloeospora</i>	1846	-	-	epi/endo	12	Palm	winter
<i>Lecanicillium</i> sp. I	1914	-	-	endo	27	Wick	spring
<i>Lecanicillium</i> sp. II	1989	-	-	endo	27	Palm	spring
<i>Lecanicillium</i> sp. II	1990	-	-	endo	27	Palm	spring
<i>Lecanicillium</i> sp. III	1928	-	-	epi	12	Palm	spring
<i>Lecanicillium</i> sp. III	1935	-	-	epi	27	Wick	spring
Leotiomyceta sp.	1766	-	+	epi/endo	12	Wick	autumn
<i>Leptobacillium leptobactrum</i>	1873	+	-	epi/endo	12	Wick	spring
<i>Metacordyceps chlamydosporia</i>	1900	-	-	endo	27	Palm	spring
<i>Metarhizium</i> sp.	1839	-	-	epi/endo	12	Wick	winter
<i>Microsphaeropsis arundinis</i>	1834	-	++	epi/endo	12	Wick	winter
<i>Microsphaeropsis arundinis</i>	1779	-	-	epi/endo	27	Wick	winter
<i>Mollisia</i> sp.	1748	-	-	endo	27	Wick	autumn
Mycosphaerellales sp. I	1891	-	+	epi/endo	12	Wick	spring
Mycosphaerellales sp. II	1734	-	-	endo	12	Wick	summer
Mycosphaerellales sp. II	1898	-	-	endo	27	Wick	spring
Mycosphaerellales sp. III	1825	-	+	epi	12	Wick	winter
<i>Neosetophoma cerealis</i>	1701	-	-	epi	27	Palm	summer
<i>Oidiodendron</i> sp.	1954	-	-	epi/endo	12	Wick	spring
<i>Paraconiothyrium</i> sp.	1844	-	-	epi/endo	27	Wick	winter
<i>Paradendryphiella salina</i>	1761	-	++	epi/endo	12	Palm	autumn
<i>Paraphaeosphaeria</i> sp.	1787	-	-	epi/endo	27	Wick	winter

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<i>Parengyodontium album</i>	1982	+	+	endo	27	Palm	spring
<i>Parengyodontium album</i>	1947	+	++	epi	12	Palm	spring
<i>Parengyodontium album</i>	1929	+	++	epi	27	Palm	spring
<i>Parengyodontium album</i>	1938	+	-	epi	12	Palm	spring
<i>Parengyodontium album</i>	1926	+	-	epi	27	Wick	spring
<i>Parengyodontium album</i>	1792	-	-	epi	27	Palm	winter
<i>Parengyodontium album</i>	1793	-	-	epi	27	Wick	winter
<i>Parengyodontium album</i>	1794	-	-	epi	27	Wick	winter
<i>Parengyodontium album</i>	1795	-	-	epi	27	Palm	winter
<i>Parengyodontium album</i>	1967	+	++	epi/endo	12	Palm	spring
<i>Parengyodontium album</i>	1977	+	++	epi/endo	27	Palm	spring
<i>Parengyodontium album</i>	1958	+	+	epi/endo	27	Wick	spring
<i>Parengyodontium album</i>	1812	++	-	epi/endo	12	Palm	winter
<i>Parengyodontium album</i>	1811	-	-	epi/endo	12	Wick	winter
<i>Parengyodontium album</i>	1813	-	-	epi/endo	12	Palm	winter
<i>Parengyodontium album</i>	1962	-	-	epi/endo	12	Wick	spring
<i>Parengyodontium album</i>	1970	-	-	epi/endo	12	Wick	spring
<i>Parengyodontium album</i>	1780	-	-	epi/endo	27	Wick	winter
<i>Parengyodontium album</i>	1799	-	-	epi/endo	27	Wick	winter
<i>Penicillium angulare</i>	1996	+	+	endo	12	Palm	spring
<i>Penicillium brevicompactum</i>	1910	++	++	endo	12	Wick	spring
<i>Penicillium brevicompactum</i>	1904	+	+	endo	12	Wick	spring
<i>Penicillium brevicompactum</i>	1791	+	++	epi	12	Palm	winter

<i>Penicillium brevicompactum</i>	1861	+	++	epi	12	Palm	spring
<i>Penicillium brevicompactum</i>	1860	+	+	epi	12	Palm	spring
<i>Penicillium brevicompactum</i>	1790	-	-	epi	12	Palm	winter
<i>Penicillium brevicompactum</i>	1745	+	+	epi/endo	27	Wick	autumn
<i>Penicillium crustosum</i>	1796	+	++	epi	12	Wick	winter
<i>Penicillium decumbens</i>	1942	-	-	epi	12	Wick	spring
<i>Penicillium expansum</i>	1762	+	+	epi/endo	12	Palm	autumn
<i>Penicillium glabrum</i>	1974	+	+	epi/endo	12	Palm	spring
<i>Penicillium kongii</i>	1883	-	-	epi/endo	12	Wick	spring
<i>Penicillium pancosmium</i>	1890	-	+	epi/endo	12	Wick	spring
<i>Penicillium</i> sp. I	1917	+	++	endo	12	Palm	spring
<i>Penicillium</i> sp. I	1770	-	++	endo	12	Wick	autumn
<i>Penicillium</i> sp. I	1919	-	++	endo	12	Palm	spring
<i>Penicillium</i> sp. I	1916	+	+	endo	12	Palm	spring
<i>Penicillium</i> sp. I	1918	+	+	endo	12	Palm	spring
<i>Penicillium</i> sp. I	1697	+	+	endo	27	Wick	summer
<i>Penicillium</i> sp. I	1851	-	-	endo	12	Wick	winter
<i>Penicillium</i> sp. I	1758	-	+	epi	12	Palm	autumn
<i>Penicillium</i> sp. I	1759	-	+	epi	12	Wick	autumn
<i>Penicillium</i> sp. I	1819	-	+	epi	12	Palm	winter
<i>Penicillium</i> sp. I	1823	-	+	epi	12	Palm	winter
<i>Penicillium</i> sp. I	1821	-	-	epi	12	Wick	winter
<i>Penicillium</i> sp. I	1824	-	-	epi	12	Palm	winter

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<i>Penicillium</i> sp. I	1866	-	-	epi	12	Palm	spring
<i>Penicillium</i> sp. I	1880	-	+	epi/endo	12	Wick	spring
<i>Penicillium</i> sp. II	1715	+	+	endo	12	Palm	summer
<i>Penicillium</i> sp. II	1732	-	-	epi	12	Wick	summer
<i>Penicillium</i> sp. III	1728	-	-	endo	12	Wick	summer
<i>Penicillium</i> sp. IV	1849	++	+	endo	12	Wick	winter
<i>Penicillium</i> sp. V	1721	+	+	endo	12	Palm	summer
<i>Penicillium</i> sp. V	1707	+	+	endo	27	Palm	summer
<i>Penicillium</i> sp. V	1708	+	+	endo	27	Wick	summer
<i>Penicillium</i> sp. V	1782	++	++	epi/endo	27	Palm	winter
<i>Penicillium</i> sp. V	1719	+	++	epi/endo	12	Wick	summer
<i>Penicillium</i> sp. VI	1714	+	-	epi/endo	27	Wick	summer
<i>Penicillium</i> sp. VII	1783	+	++	epi/endo	27	Palm	winter
<i>Penicillium</i> sp. VII	1786	+	++	epi/endo	27	Palm	winter
<i>Penicillium</i> sp. VIII	1803	-	-	endo	12	Wick	winter
<i>Penicillium</i> sp. IX	1724	++	+	epi	12	Wick	summer
<i>Penicillium</i> sp. X	1862	-	++	epi	12	Palm	spring
<i>Penicillium</i> sp. XI	1905	+	-	endo	12	Wick	spring
<i>Penicillium</i> sp. XII	1924	+	+	epi	12	Wick	spring
<i>Penicillium</i> sp. XIII	1711	+	+	epi	27	Palm	summer
<i>Penicillium</i> sp. XIII	1784	+	++	epi/endo	27	Palm	winter
<i>Penicillium</i> sp. XIII	1709	+	+	epi/endo	27	Wick	summer
<i>Penicillium</i> sp. XIII	1710	+	+	epi/endo	27	Wick	summer

<i>Penicillium</i> sp. XIV	1717	+	+	epi	12	Palm	summer
<i>Penicillium</i> sp. XV	1731	++	+	epi/endo	12	Palm	summer
<i>Penicillium</i> sp. XVI	1828	+	+	epi	12	Palm	winter
<i>Penicillium</i> sp. XVI	1829	-	+	epi	12	Palm	winter
<i>Penicillium</i> sp. XVII	1830	+	+	epi	12	Palm	winter
<i>Penicillium</i> sp. XVII	1875	+	+	epi/endo	27	Wick	spring
<i>Penicillium</i> sp. XVIII	1906	+	++	endo	12	Wick	spring
<i>Penicillium</i> sp. XIX	1986	+	+	endo	12	Palm	spring
<i>Penicillium</i> sp. XX	1712	+	+	endo	27	Palm	summer
<i>Penicillium</i> sp. XX	1852	+	+	endo	27	Wick	winter
<i>Penicillium</i> sp. XXI	1718	+	+	epi/endo	12	Palm	summer
<i>Penicillium spathulatum</i>	1911	-	++	endo	12	Wick	spring
<i>Penicillium ubiquetum</i>	1888	+	-	epi/endo	12	Palm	spring
<i>Penicillium ubiquetum</i>	1893	+	-	epi/endo	27	Wick	spring
<i>Peniophora rufomarginata</i>	1756	-	-	epi/endo	27	Wick	autumn
<i>Peroneutypa</i> sp.	1817	+	++	epi/endo	12	Wick	winter
Phaeosphaeriacea sp.	1763	-	-	epi/endo	27	Palm	autumn
<i>Phlebia radiata</i>	1765	-	-	epi/endo	12	Wick	autumn
<i>Phlebia</i> sp.	1885	-	-	epi/endo	27	Wick	spring
<i>Phlebiopsis ravenelii</i>	1850	-	-	endo	12	Wick	winter
Pleosporales sp. I	1822	+	+	epi	12	Palm	winter
Pleosporales sp. II	1843	-	+	epi/endo	27	Wick	winter
<i>Ramularia</i> sp. I	1983	-	+	endo	27	Wick	spring

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<i>Ramularia</i> sp. II	1867	-	-	epi	12	Wick	spring
<i>Ramularia</i> sp. III	1878	-	-	epi/endo	12	Wick	spring
<i>Rousoella</i> sp.	1837	++	+	epi/endo	12	Wick	winter
<i>Sclerotinia spermophila</i>	1847	+	+	epi/endo	12	Palm	winter
<i>Sistotrema</i> sp.	1690	-	+	epi	27	Wick	summer
Sordariomyceta sp.	1764	-	-	endo	12	Palm	autumn
Sordariomycetes sp. I	1848	-	-	epi/endo	27	Palm	winter
Sordariomycetes sp. II	1857	-	+	epi	27	Wick	spring
<i>Stachybotrys chartarum</i>	1685	+	++	epi/endo	12	Wick	summer
<i>Stachybotrys chartarum</i>	1679	+	++	epi/endo	27	Wick	summer
<i>Stachybotrys chartarum</i>	1702	+	++	epi/endo	27	Palm	summer
<i>Stachybotrys chartarum</i>	1678	+	-	epi/endo	27	Wick	summer
<i>Stachybotrys chartarum</i>	1700	-	-	epi/endo	12	Palm	summer
<i>Stereum</i> sp.	1840	-	-	epi/endo	12	Wick	winter
<i>Tilletiopsis washingtonensis</i>	1985	-	-	endo	27	Wick	spring
<i>Trametes versicolor</i>	1826	-	+	epi	12	Wick	winter
<i>Trametes versicolor</i>	1797	-	+	epi/endo	12	Wick	winter
<i>Trichoderma</i> sp.	1816	-	-	epi/endo	12	Wick	winter
Ustilaginaceae sp.	1755	-	-	epi	12	Wick	autumn
<i>Verticillium</i> sp.	1723	-	-	endo	12	Wick	summer
<i>Wallemia</i> sp. I	1877	-	-	epi/endo	12	Wick	spring
<i>Wallemia</i> sp. II	1807	-	-	endo	12	Wick	winter
unknown	1757	-	+	endo	12	Wick	autumn

unknown	1903	-	-	endo	12	Wick	spring
unknown	1943	+	++	epi	12	Wick	spring
unknown	1920	+	+	epi	12	Wick	spring
unknown	1921	+	+	epi	12	Wick	spring
unknown	1922	+	+	epi	12	Wick	spring
unknown	1925	+	+	epi	12	Wick	spring
unknown	1945	+	+	epi	27	Wick	spring
unknown	1976	+	+	epi/endo	27	Palm	spring
unknown	1801	++	-	epi/endo	12	Palm	winter
unknown	1727	-	-	epi/endo	27	Palm	summer
unknown	1760	-	-	epi/endo	27	Wick	autumn
unknwon	1778	+	+	epi/endo	27	Wick	winter

Titre : Etude de la communauté fongique associée à l'algue rouge *Palmaria palmata* pour la production d'enzymes de dégradation pariétale à visée biotechnologique

Mots clés : algues rouges, champignons, xylanases, cellulases, R-phycoérythrine

Résumé : La communauté fongique associée à l'algue rouge *Palmaria palmata* a été étudiée, en particulier pour sa production de xylanases et de cellulases, enzymes utilisées dans l'extraction de composés d'intérêt comme la R-phycoérythrine (R-PE). Dans un premier temps, des récoltes régulières de *P. palmata* ont été effectuées pendant un an afin de réaliser un vaste travail d'isolement et d'identification de souches fongiques. Il s'agit de la première étude globale de diversité fongique chez *P. palmata*. 308 souches fongiques ont été isolées, réparties dans 147 taxons appartenant majoritairement aux Ascomycètes. Ensuite, un criblage enzymatique a été réalisé permettant d'évaluer qualitativement les activités xylanolytiques et cellulolytiques de chaque champignon isolé. Il en résulte que sur les 308 souches fongiques, la majorité d'entre-elles est caractérisée par au moins l'une de ces deux activités enzymatiques. Suite à ce criblage, six souches ont été sélectionnées pour une analyse quantitative de leur double activité xylanolytique et cellulolytique.

Ces souches appartiennent aux genres *Cladosporium*, *Penicillium* et *Aspergillus* majoritairement observés chez *P. palmata*. La quantification de ces activités enzymatiques a été réalisée et des tests préliminaires de dégradation de thalle de *P. palmata* ont été effectués afin d'extraire la R-PE. Ces tests de dégradation ont été réalisés à partir d'extraits enzymatiques bruts. Les souches MMS1733 (*Aspergillus* sp.), MMS1906 (*Penicillium* sp.) et MMS1910 (*Penicillium brevicompactum*) ont semblé particulièrement intéressantes pour leur production d'enzymes permettant l'extraction de R-PE. Parmi les pistes de recherches futures, la purification des enzymes issues de ces souches, leur caractérisation plus fine ainsi que leur utilisation potentielle à grande échelle pourraient aboutir à des résultats particulièrement prometteurs dans le domaine des biotechnologies marines.

Title : Study of the fungal community associated to the red macroalga *Palmaria palmata* for the production of cell wall degrading enzymes of biotechnological interest

Keywords : red macroalgae, fungi, xylanases, cellulases, R-phycoerythrin

Abstract: The fungal community associated with the red alga *Palmaria palmata* was studied, in particular for its production of xylanases and cellulases which are used in the extraction of compounds of interest such as the R-phycoerythrin (R-PE). Initially, over a period of one year, *P. palmata* was regularly harvested in order to isolate and identify strains from algal thalli. Up to now, it is the first global study dealing with fungal diversity from *P. palmata*. 308 fungal strains were isolated, among 147 taxa mostly belonging to the Ascomycetes. Next, an enzymatic screening was performed on fungal isolates to assess qualitatively their xylanolytic and cellulolytic activities. Among the 308 fungal strains, the majority were characterised by at least one of these two enzymatic activities. Six isolates were selected from this screening for a quantitative analysis of their double xylanolytic and cellulolytic activity.

These strains belonged to the genera *Cladosporium*, *Penicillium* and *Aspergillus* mainly observed in *P. palmata*. Quantification of enzymatic activities was performed as well as preliminary tests of *P. palmata* thalli degradation for R-PE extraction. These degradation tests were realized by using crude enzymatic extracts. Strains MMS1733 (*Aspergillus* sp.), MMS1906 (*Penicillium* sp.) and MMS1910 (*Penicillium brevicompactum*) seemed particularly interesting for R-PE extraction. Among the perspectives, the purification of enzymes from these strains, their detailed characterisation and their potential large-scale use could lead to particularly promising results in the field of marine biotechnology.