



HAL
open science

Degradation of hydroxycinnamic acids as signal of plant perception: regulation and role in the *Agrobacterium fabrum* ecology

Thibault Meyer

► **To cite this version:**

Thibault Meyer. Degradation of hydroxycinnamic acids as signal of plant perception: regulation and role in the *Agrobacterium fabrum* ecology. Ecology, environment. Université de Lyon, 2018. English. NNT: 2018LYSE1101 . tel-02917952

HAL Id: tel-02917952

<https://theses.hal.science/tel-02917952>

Submitted on 20 Aug 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



N°d'ordre NNT : 2018LYSE1101

THESE de DOCTORAT DE L'UNIVERSITE DE LYON

opérée au sein de

l'Université Claude Bernard Lyon 1

Ecole Doctorale N° 341

(Ecosystèmes Evolution Modélisation Microbiologie)

Spécialité de doctorat : Biologie

Discipline : Ecologie microbienne

Soutenue publiquement le 29/06/2018, par :

Thibault MEYER

La dégradation des acides hydroxycinnamiques comme signal de perception de la plante: Régulation et rôle dans l'écologie d'*Agrobacterium fabrum*

Devant le jury composé de :

DESSAUX Yves, Directeur de recherche, CNRS, I2BC, Gif-Sur-Yvette	Rapporteur
SARNIGUET Alain, Directeur de recherche, INRA, IGEPP, Le Rheu	Rapporteur
GUIDOT Alice, Chargé de recherche, INRA, LIPM, Castanet-Tolosan	Examinatrice
WISNIEWSKI-DYÉ Florence, Professeure, LEM, Université Lyon 1	Présidente du Jury
COMTE Gilles, Professeur, LEM, Université Lyon 1	Co-directeur de thèse
LAVIRE Céline, Maître de conférences, LEM, Université Lyon 1	Directrice de thèse
MORÉRA Solange, Directrice de recherche, CNRS, I2BC, Gif-Sur-Yvette	Invitée

UNIVERSITE CLAUDE BERNARD - LYON 1

Président de l'Université

Président du Conseil Académique

Vice-président du Conseil d'Administration

Vice-président du Conseil Formation et Vie Universitaire

Vice-président de la Commission Recherche

Directrice Générale des Services

M. le Professeur Frédéric FLEURY

M. le Professeur Hamda BEN HADID

M. le Professeur Didier REVEL

M. le Professeur Philippe CHEVALIER

M. Fabrice VALLÉE

Mme Dominique MARCHAND

COMPOSANTES SANTE

Faculté de Médecine Lyon Est – Claude Bernard

Faculté de Médecine et de Maïeutique Lyon Sud – Charles Mérieux

Faculté d'Odontologie

Institut des Sciences Pharmaceutiques et Biologiques

Institut des Sciences et Techniques de la Réadaptation

Département de formation et Centre de Recherche en Biologie Humaine

Directeur : M. le Professeur G.RODE

Directeur : Mme la Professeure C. BURILLON

Directeur : M. le Professeur D. BOURGEOIS

Directeur : Mme la Professeure C. VINCIGUERRA

Directeur : M. X. PERROT

Directeur : Mme la Professeure A-M. SCHOTT

COMPOSANTES ET DEPARTEMENTS DE SCIENCES ET TECHNOLOGIE

Faculté des Sciences et Technologies

Département Biologie

Département Chimie Biochimie

Département GEP

Département Informatique

Département Mathématiques

Département Mécanique

Département Physique

UFR Sciences et Techniques des Activités Physiques et Sportives

Observatoire des Sciences de l'Univers de Lyon

Polytech Lyon

Ecole Supérieure de Chimie Physique Electronique

Institut Universitaire de Technologie de Lyon 1

Ecole Supérieure du Professorat et de l'Education

Institut de Science Financière et d'Assurances

Directeur : M. F. DE MARCHI

Directeur : M. le Professeur F. THEVENARD

Directeur : Mme C. FELIX

Directeur : M. Hassan HAMMOURI

Directeur : M. le Professeur S. AKKOUCHE

Directeur : M. le Professeur G. TOMANOV

Directeur : M. le Professeur H. BEN HADID

Directeur : M. le Professeur J-C PLENET

Directeur : M. Y.VANPOULLE

Directeur : M. B. GUIDERDONI

Directeur : M. le Professeur E.PERRIN

Directeur : M. G. PIGNAULT

Directeur : M. le Professeur C. VITON

Directeur : M. le Professeur A. MOUGNIOTTE

Directeur : M. N. LEBOISNE

Tout obstacle renforce la détermination. Celui qui s'est fixé un but n'en change pas.

Léonard De vinci

Remerciements

Je tiens en premier lieu à remercier tous les membres du jury, Yves Dessaux, Alain Sarniguet, Alice Guidot et Florence Wisniewski-Dyé d'avoir bien voulu évaluer mes travaux de thèse.

Je remercie Yvan Moëgne-locco pour m'avoir accueilli au sein de son laboratoire.

Je remercie par avance ceux que j'aurais oublié et j'en suis désolé.

J'adresse mes remerciements à Céline Lavire et Xavier Nesme pour m'avoir accueilli dans leur équipe.

Je tiens à remercier tous les membres de mon comité de pilotage, William Nasser, Vincent Grossi, pour m'avoir aiguillé et pour avoir apporté un œil extérieur et expert sur mes travaux, et tout particulièrement, Florence pour tous les conseils donnés sur les divers projets.

Un énorme merci à Céline, pour ces 6 années d'encadrement. Je ne regrette pour rien au monde d'avoir continué mon parcours sous ton encadrement. J'admire ton audace scientifique qui nous a amené à mon sujet de M2 puis à cette thèse. Ce fut un immense plaisir d'être encadré par toi. Merci de m'avoir fait découvrir le monde de la recherche et partager ta passion pour *Agrobacterium*. Je te remercie aussi de m'avoir fait confiance et de m'avoir accordé autant d'autonomie. Tu as réussi à gérer mon excès d'enthousiasme et ma soif de manipulations dans certains moments, et à me motiver et me guider dans les moments plus compliqués. Merci pour tout ce que tu m'as enseigné et pour ces brainstormings scientifiques fructueux autour d'un repas, je pense que je te dois mon poids en pâte d'ailleurs. Pardon pour les heures de sommeils sacrifiées, pour le temps que tu as passé sur mes travaux et pas avec ta famille et pour mon orthographe médiocre. J'espère d'ailleurs avoir élevé un peu mon niveau de rédaction. Si ce n'est pas le cas, j'espère alors que tu es désormais capable de comprendre le stéphanois sans traducteur. C'est avec tristesse que je quitterai le laboratoire et je regretterai tes qualités scientifiques et humaines. En espérant collaborer avec ma « chef » dans le futur.

Gilles, je te remercie pour ton encadrement depuis le Master 2 et l'autonomie que tu m'as accordée. J'ai beaucoup apprécié que tu te sois toujours rendu disponible pour répondre à mes questions ou pour discuter de l'avancée de mes travaux. L'apport de ton point de vue "côté plante" et tes conseils pour la rédaction furent précieux. Tu as su m'aider à approfondir mes connaissances sur la métabolomique des plantes et à utiliser les bons termes. Je reste admiratif de tes compétences expertes en voie métabolique et structure des molécules. Merci de m'avoir fait profiter de tout cela.

Un grand merci, à Ludo, c'est toi qui m'a initié le premier aux rudiments du clonage et qui m'a transmis le « virus » de la bio. mol., et tu es aussi un peu à l'origine de ma venue dans l'équipe. J'ai eu la chance de profiter de tes enseignements et de ta pédagogie qui sont pour moi un modèle. Je te remercie encore pour tes conseils, tes suggestions, ton importante contribution à ces travaux et le temps passé à réfléchir sur l'acide férulique. Merci aussi pour m'avoir toujours encouragé à élever mon niveau d'humour (en 6 ans, je suis passé de gris à noir foncé). Je vais beaucoup regretter ta compagnie et nos discussions scientifiques. J'espère te revoir autour d'un bon repas car il est déjà 10h30, bientôt l'heure de manger, en plus ce midi en légume c'est pomme de terre et en viande, jambon cru (typiquement auvergnat).

Un immense merci à Solange et Armelle, pour cette collaboration fructueuse. Ce fut un plaisir d'interagir avec vous, de profiter de votre expertise en cristallographie et merci de m'avoir initié à la magie des structures protéiques. J'espère que cette collaboration perdurera et que d'autres articles viendront récompenser nos travaux.

Je remercie Lisa Sanchez pour m'avoir appris les rudiments de l'extraction des ARN végétaux infernaux.

Je tiens à remercier tous les membres de l'équipe 4 : Zahar, Daniel, Xavier pour l'excellence de tes saucissons, Franck pour ton côté rafraîchissant, Isabelle pour ton aide en métabo, David mon fabuleux collègue de bureau pense bien à faire des tests Elisa et oublie pas on n'embête pas un chien quand il mange. Vincent merci pour ton aide pour toutes ces cinétiques. Aurélie merci pour nos discussions scientifiques au sommet et pour m'avoir appris que certains repas sans viande pouvaient être bon. Solène, je te souhaite bon courage pour la dénit et s'il te plaît maintient le niveau d'humour dans le bureau après mon départ. Rosa que la force des gènes spécifiques soit avec toi, Lucas bon courage et ne fait pas trop le GusGus avec les plasmides. Merci à Cindy et Florence R pour leurs gentillesse durant leurs passages dans l'équipe. Sam, je te suis reconnaissant pour ta précieuse aide en stat et beaucoup de courage pour les prochains mois, merci à Ben pour nos fabuleuses aventures en congrès. Je remercie William même si tu es lyonnais, tu es quand même sympa de me laisser gagner à MPG. Merci à Khaoula pour m'avoir initié au parfum des huiles essentielles.

Je tiens à remercier tous les membres du CESN pour les répétitions, les conseils et votre excellent café.

Elise, je te remercie pour avoir si bien traité mes petits plants de tomates à toutes les périodes de l'année et pour ta gentillesse.

Je remercie Laurence L. pour m'avoir initié à la joie des plaques biologiques et pour m'avoir offert une cachette pour la rédaction.

Je remercie tous les anciens doctorants pour leur sympathie et leurs accueils : Marie, Guillaume, Antoine, Florian. Seb merci pour les bons moments dans le bureau, de m'avoir appris le secret des gels retard et pour m'avoir fait partager tes constructions et tes connaissances sur l'acide férulique. Un grand merci à Jordan V, j'espère que ta soif de science ne s'apaisera pas, que l'on continuera à discuter (science et autre) ensemble encore longtemps, en espérant collaborer un jour. Merci à Camille de ne pas m'avoir laissé à terre. Je remercie les fous du billard du midi : Jeanne, Xavier, Morgane, Yoann, Lise et Laura (la championne de la paëlla ou de la daube provençale). Merci au D4 avec qui je suis au labo depuis quelques temps déjà : Guillaume, Elodie et Zazou vous me faites de la peine mais je vous apprécie énormément. Et pour finir merci Quentin, je ne sais pas lequel de nous deux a eu le plus de courage de supporter l'autre.

Je tiens à remercier toutes les petites mains (stagiaires) qui m'ont aidé dans l'enfer des repiquages, la compréhension de la régulation, les aventures colorées et mon penchant pour les opines: Carole, Raphaël, Adrien, Alexandre, Joffrey, Maxime. Clémence un remerciement particulier pour ton sérieux mais aussi ta sympathie, ta forte capacité de travail et ta grande contribution à la revue.

Je remercie Kastunori Suzuki pour m'avoir aidé à approfondir mes connaissances sur la transformation par *Agrobacterium* d'autres organismes que les plantes et surtout pour m'avoir fait découvrir ton laboratoire et ce fabuleux pays qu'est le Japon où se mêle la rigueur et bonne humeur et dont les usages et coutumes me passionnent. Et surtout j'ai enfin trouvé un pays où l'on mangeait aussi bien qu'en France. J'espère te revoir et pourquoi pas enfin aller au restaurant en France ensemble...

Un grand merci, aux amis de St Mart et handballeurs anonymes pour certains, Kevin, Théo, Greg, Polak, Pitch, Rémi, Robin, Steven et côté féminin, Maïté, Claude, Morgane, Mathilde, Estelle, Amandine et Charlotte que de moments joyeux et arrosés à vos côtés. Ma vie en dehors du labo aurait été beaucoup plus triste sans vous. Ps : Kevin et Théo : je vous fais confiance pour mi-Juillet.

Merci à ceux qui m'ont épaulé, guidé, soutenu mentalement et aussi financièrement pendant tant d'années, mes parents, si j'en suis là c'est grâce en partie à vous. Merci à mes frères et sœurs, qui ont su me faire profiter : de leurs capacités de patience à la pêche et dans la vie (Olivier), de leur dureté mais de leur savoir (Romain), de leurs pouvoirs de super-marraine pour Cléa et de correctrice hors-pair (Marjorie) et de leurs capacités à continuer de jouer avec moi malgré mon manque de finesse (Manon).

Merci aussi à ma belle-famille, pour vos encouragements. Vous êtes tous super avec le cœur sur la main, quelle joie d'intégrer officiellement votre famille dans un futur proche. Un merci tout particulier à Maxime pour m'avoir permis de pouvoir souvent aller me défouler au Hand, l'esprit tranquille.

Et la future MEYER pour la fin, 9 ans déjà que tu partages mes joies, mes doutes et ma façon d'être. Julie, tu as eu à supporter mes longues journées, ma mauvaise humeur quand les manips ne fonctionnaient pas, mes journées entières de rédaction, l'incertitude de l'avenir mais malgré tout cela tu m'as toujours chéri et encouragé. Tu es une mère, une compagne fabuleuse et pleine de tendresse encore merci. Pour finir, merci à ma fille Cléa, qui m'a accompagné depuis presque le début de cette aventure de thèse. Ton trop plein d'énergie, ta bonne humeur, ton brin de folie et ta curiosité furent une bouffée d'air pure dans les moments de rush. Je te demande pardon pour les moments où je fus moins disponible pour toi. Cette thèse vous est dédiée....

La dégradation des acides hydroxycinnamiques comme signal de perception de la plante: régulation et rôle dans l'écologie d'*Agrobacterium fabrum*

Résumé

Les agrobactéries établissent des relations à long terme avec les plantes et ce, dans deux styles de vie différents, rhizosphérique et pathogène (galle du collet). Dans ce mode de vie, les bactéries modifient génétiquement leur hôte et se créent ainsi une niche écologique spécifique (tumeur). La transition entre les deux styles de vie est déclenchée par la perception de signaux végétaux, parmi lesquels des acides hydroxycinnamiques (HCAs) comme l'acide férulique. Or dans l'espèce *Agrobacterium fabrum*, des gènes spécifiques permettent la dégradation des HCAs.

Nous avons émis l'hypothèse que cette dégradation était un signal de proximité de la plante et influençait alors des fonctions importantes pour l'interaction avec celle-ci. Nous avons caractérisé la régulation de la dégradation des HCAs, évalué son rôle dans la valeur sélective d'*A. fabrum*, et suggéré son importance dans la transition entre les styles de vie rhizosphérique et pathogène. Nous avons montré que la dégradation des HCAs module le métabolisme carboné bactérien, notamment l'utilisation d'acides aminés et d'oligosaccharides de la famille du raffinose. Nous avons caractérisé la protéine MelB qui permet l'import de ces sucres, du mélibiose et du galactinol. Leur utilisation est importante pour la colonisation des plantes dès la germination. L'analyse de l'expression des gènes et du métabolisme bactérien en présence d'un composé signal de la plante, nous a révélé de nouveaux déterminants importants pour l'écologie de ce phytopathogène, notamment des facteurs de transcription. En outre, cette analyse a confirmé l'importance des échanges cellulaires et de déterminants impliqués dans la compétition bactérienne.

Mot clef : *Agrobacterium fabrum*, acides hydroxycinnamiques, interaction plantes-bactéries, oligosaccharides de la famille du raffinose (RFO), transition écologique, régulation transcriptionnelle.

Degradation of hydroxycinnamic acids as signal of plant perception: regulation and role in the *Agrobacterium fabrum* ecology.

Abstract

Agrobacterium establish long term interactions with plants, either in a rhizosphere or pathogenic lifestyle. Pathogenic agrobacteria are causing the crown gall disease by genetically modifying the plant cells host, thus creating a specific ecological niche (tumor). The transition from the rhizosphere to the pathogenic lifestyle is triggered by bacterial perception of plant-derived signals, including hydroxycinnamic acids (HCAs) such as ferulic acid. However, *A. fabrum* strains have species-specific genes that allow HCAs degradation.

We hypothesized that in *A. fabrum*, the degradation of the HCAs is perceived as a plant signal which influences important functions involved in the interaction with plants. We characterized the regulation of HCAs degradation, evaluated its role in the fitness of *A. fabrum*, and suggested its importance for the transition between the rhizosphere and pathogenic lifestyles. Then, we showed that the degradation of HCAs modulates carbon metabolism, such as the use of amino acids and sugars belonging to the raffinose family oligosaccharides (RFO). We have demonstrated that besides these sugars, the MelB protein allows the import melibiose and galactinol. Their use is important for plant colonization, since seed germination. The analyzes of gene expression and bacterial metabolism in the presence of a plant signal compound, revealed new determinants important for *A. fabrum* ecology, including transcription factors. In addition, it confirmed the importance of cellular exchanges and bacterial competition for *Agrobacterium* fitness in planta.

Key words: *Agrobacterium fabrum*, hydroxycinnamic acids (HCAs), plant-bacteria interaction, raffinose family oligosaccharides (RFOs), ecological transition, transcriptional regulation.

Sommaire

Introduction générale	1
------------------------------	----------

Synthèse bibliographique	6
---------------------------------	----------

Partie A : Interaction pathogénique plante-Agrobacterium : déterminant de l'interaction à long terme 7

Abstract	8
----------	---

Introduction	9
--------------	---

I. Tumor: development of an unfriendly environment 11

Young tumor and dual role of ethylene	11
---------------------------------------	----

Mature tumor: an unfriendly environment	13
---	----

II. Key genetic determinants for *Agrobacterium* persistence in the hostile tumor environment 15

How <i>Agrobacterium</i> face to hostile conditions	15
---	----

Oxidative stress	15
------------------	----

Osmoprotection	15
----------------	----

Oxygen as limiting factor	16
---------------------------	----

Resistance to plant toxic compounds	17
-------------------------------------	----

Nutrition in tumor	18
--------------------	----

How <i>Agrobacterium</i> excels tumor microbiota	20
--	----

Conclusion	24
------------	----

Partie B : Influence écologique des acides hydroxycinnamiques et de leur dégradation : aspects trophiques et de signalisation 25

I. Biosynthèse des HCAs et rôles chez les végétaux 25

II. Dégradation bactérienne des HCAs 27

II.1. La désacétylation β -oxydative dépendante du CoenzymeA	30
--	----

II.2. La désacétylation non β -oxydative dépendante du CoenzymeA	32
--	----

III. Régulation de la dégradation des HCAs 34

III.1. Régulation des premières étapes de dégradation : régulateurs MarR	34
--	----

III.1.1. Mécanisme de fixation des régulateurs MarR	36
---	----

III.1.2. Levée de la répression	36
---------------------------------	----

III.2. Régulation des gènes impliqués dans la dégradation de l'acide vanillique.	38
--	----

IV. Autres rôle écologique de la dégradation des HCAs 40

IV.1. HCAs et chimiotactisme	40
------------------------------	----

IV.2. HCAs et communication bactérienne	40
V. HCAs et influence global dans l'écologie de phytopathogènes	42
V.1. HCAs non dégradés en tant que signal : implication dans l'écologie de <i>Dickeya dadantii</i>	42
V.2. Rôle global de la dégradation des HCAs dans l'écologie de <i>Ralstonia solanacearum</i>	46
Conclusion synthèse bibliographique	48
<hr/>	
Chapitre 1 : Caractérisation de la régulation de la région SpG8-1b et importance écologique chez <i>Agrobacterium fabrum</i>	50
<hr/>	
Préambule du chapitre 1	51
Article 1 : Regulation of hydroxycinnamic acid degradation drives <i>Agrobacterium fabrum</i> lifestyles.	52
Abstract	52
Introduction	52
Results	53
Discussion	56
Materials and Methods	57
Literature Cited	59
Supplementary table and figure.	61
Conclusion du chapitre 1	67
<hr/>	
Chapitre 2 : Rôle signal des HCA dans l'interaction <i>Agrobacterium fabrum</i>–plante	68
<hr/>	
Préambule du chapitre 2	69
Article 2 : Ferulic acid degradation induces global modulation of metabolism on <i>Agrobacterium fabrum</i>	70
Abstract	70
Introduction	71
Materials and methods	73
Results and discussion	77
Supplemental figures	83
Conclusion du chapitre 2	87

Chapitre 3 : Système HCA-RFO : fonctionnement et rôle écologique.	88
--	-----------

Préambule du chapitre 3	89
Article 3: The plant defense signal galactinol is specifically used as a nutrient by the bacterial pathogen <i>Agrobacterium fabrum</i>	91
Abstract	91
Introduction	91
Results	92
Discussion	97
Experimental procedures	99
References	101
Supporting Information	103
Article 4 : Influence of ferulic acid on <i>Agrobacterium fabrum</i> transcriptome	107
Abstract	107
Introduction	109
Materials and methods	110
Results	113
Supplemental figures	121
Conclusion du chapitre 3	123

Discussion générale et Perspectives	125
--	------------

Signal perçu par <i>A. fabrum</i> : HCA ou HCA-CoA	126
Importance de l'utilisation des RFOs, du galactinol et du mélibiose	127
Etude de l'interaction <i>A. fabrum</i> -plante : aspects méthodologiques	128
<i>Etude de la valeur adaptative conférée par les déterminants génétiques</i>	128
<i>Importance de l'étude du métabolome de l'hôte végétale et des méthodes d'analyse de l'expression in situ</i>	130
HCAs et styles de vie d' <i>A. fabrum</i> : proposition d'un modèle	131

Conclusion	136
-------------------	------------

Références	137
-------------------	------------

Annexes	156
----------------	------------

Liste des abréviations

°C : degré Celsius

ADN : Acide Désoxyribonucléique

ADNc : ADN complémentaire

ADN-T : ADN de transfert

ARN: Acide RiboNucléique

Bv : Biovar

EDTA : acide Ethylène Diamine Tétra Acétique

E-GFP : *Enhanced-Green Fluorescent Protein*

G : Guanine

GABA : acide γ -aminobutyrique (pour *Gamma Aminobutyric Acid*)

GFP : *Green Fluorescent Protein*

GntR : *Gluconate Regulator*

HSL : HomoSérine Lactone

ISR : Résistance Systémique Induite (pour *Induced Systemic Resistance*)

HCA : Acide hydroxycinnamique (pour *HydroxyCinnamic Acid*)

Kb : Kilobase

LB : Luria Bertani ou (*Lysogenic Broth*)

LPG : Levure Peptone Glucose (*YPG : Yeast Peptone Glucose*)

MarR: *Multiple antibiotic resistance Regulator*

PadR: *Phenolic acid decarboxylase Regulator*

PAL : Phenylalanine Ammonia-lyase

Pb : Paire de bases

PCR : *Polymerase Chain Reaction*

pTi : plasmide Ti (pour *Tumor inducing*)

QS : *Quorum Sensing*

RFO: Oligosaccharide de la Famille du Raffinose (pour *Raffinose Oligosaccharide Family*)

RNAseq : *RNA sequencing*

RT-qPCR : Reverse Transcription quantitative PCR

T3SS : *Type 3 Secretion System*

T6SS : *Type 6 Secretion System*

TAL : *Tyrosine Ammonia-lyase*

wHTH : *wing Helix-Turn-Helix*

Liste des figures

Synthèse bibliographique

Partie A : Interaction pathogénique plante-*Agrobacterium* : déterminant de l'interaction à long terme

Figure 1. Chain reaction behind the development of an unfriendly environment. 14

Figure 2 : Model of gene cluster expression regulation depending on Iron availability 22

Partie B : Influence écologique des acides hydroxycinnamiques et de leur dégradation : aspects trophiques et de signalisation

Figure 1: Voie de biosynthèse des phénylpropanoïdes 26

Figure 2: Voies de dégradation des HCAs par désacétylation β -oxydative et non β -oxydative dépendante du CoenzymeA 29

Figure 3 : Organisation des clusters de dégradation des HCAs chez *A. fabrum*, *C. glutamicum*, *R. jostii*, *R. palustris*, *Sphingobium sp.*, *Acinetobacter sp.*, *R. solanacearum* et *P. putida*. 33

Figure 4 : Mécanisme de régulation par les régulateurs MarR. 37

Figure 5 : Implication des HCAs dans la pathogénie de *D. dadantii* 43

Figure 6 : Importance des HCAs dans le cycle de développement de la maladie du flétrissement bactérien induite par *R. solanacearum*. 45

Chapitre 1 : Caractérisation de la régulation de la région SpG8-1b et importance écologique chez *Agrobacterium fabrum*

Figure 1: Ferulic acid and <i>p</i> -coumaric acid degradation pathway and organization of the SpG8-1b region.	53
Figure 2: Abundance of HCA compounds in the wild-type and C58 Δ <i>hcaR</i> mutant strains.	54
Figure 3: HcaR regulation mechanism: DNA binding and identification of HcaR as an effector.	55
Figure 4: Induction of <i>virB</i> expression by HCA compounds in the C58 wild-type, and the C58 Δ SpG8-1b and C58 Δ <i>hcaR</i> mutant strains.	56
Figure 5: Effect of <i>hcaR</i> deletion on plant colonization and on the expression of HCA degradation genes in planta.	56
Figure S1: A palindromic region is required for HcaR binding to the <i>atnI416-1417</i> promoter region.	64
Figure S2: Feruloyl-CoA and <i>p</i> -coumaroyl-CoA are HcaR effectors.	64
Figure S3: Population levels of the wild-type and the C58 Δ <i>hcaR</i> strains in tumor and rhizosphere.	65
Figure S4: Induction of <i>virB</i> in the C58 and C58 Δ <i>hcaR</i> mutant strains in tumor lifestyle.	65
Figure S5: Quantification of <i>PatuI416</i> induction on tumor and tomato roots.	66

Chapitre 2 : Rôle signal des HCA dans l'interaction *Agrobacterium fabrum*–plante

Figure 1: Metabolic activities on 13 carbon sources in *A. fabrum* strains in presence of HCAs.

76

Figure 2: Influence of ferulic acid degradation on lag phase of *A. fabrum* C58 strains on different carbon sources.

78

Figure 3: Influence of ferulic acid degradation on the induction of D-melibiose and L-proline uptake genes.

79

Figure S1 : Trophic resources respiration differences under phenolic compounds pressure between *A. fabrum* C58 derivatives strains.

86

Chapitre 3 : Système HCA-RFO : fonctionnement et rôle écologique.

Article 3: The plant defense signal galactinol is specifically used as a nutrient by the bacterial pathogen *Agrobacterium fabrum*

Figure 1: RFOs synthesis (a) and degradation (b).

92

Figure 2: The *mel* operon structure and MelB involvement in galactinol, melibiose and RFOs consumption.

93

Figure 3: Ribbon representation of MelB structures and ligand-binding site.

95

Figure 4: MelB phylogeny and binding signature.

96

Figure 5: Expression of *mel* operon genes *in vitro* and in tomato root.

97

Figure 6: MelB confers a competitive advantage in tomato roots.	98
Figure S1: ITC and fluorescence K_D measurements of MelB towards RFOs and their derivatives.	103
Figure S2: DSC thermograms of MelB.	104
Article 4: Influence of ferulic acid on <i>Agrobacterium fabrum</i> transcriptome	
Figure1: Genomic organization of the SpG8-1b region and ferulic acid degradation pathway.	108
Figure 2: Expression of <i>mel</i> operon genes in presence of ferulic acid.	113
Figure 3: Expression of the SpG8-1b cluster and degradation of ferulic acid after 9 hours of growth in AT melibiose medium supplemented with ferulic acid.	114
Figure 4: Expression of genes involved in metabolites exchange induction in presence of ferulic acid.	115
Figure 5: Organization of T6SS in <i>A. fabrum</i> and expression with and whitout ferulic acid.	119

Discussion générale et Perspectives

Figure 1 : Modèles d'influence de la disponibilité en HCAs sur l'écologie d' <i>A. fabrum</i> .	133
--	-----

Listes des tableaux

Synthèse bibliographique

Partie B : Influence écologique des acides hydroxycinnamiques et de leur dégradation : aspects trophiques et de signalisation

Tableau 1 : Voies et gènes impliqués dans la dégradation des HCAs selon les espèces bactériennes. 31

Tableau 2 : Régulation transcriptionnelle des gènes de dégradation des HCAs selon les espèces bactériennes. 35

Chapitre 1 : Caractérisation de la régulation de la région SpG8-1b et importance écologique chez *Agrobacterium fabrum*

Table 1: Ferulic acid degradation effect on the SpG8-1b genes expression after 24 hours of incubation. 54

Table 2: Ratio of reporter gene induction in the presence/absence of different HCAs after 24 hours of incubation. 55

TableS1: Strains and plasmids used in this study. 61

TableS2: Primers used in this study. 62

TableS3: ferulic acid degradation effect on the SpG8-1b genes expression after 24 hours of incubation. 63

TableS4: *p*-coumaric acid degradation effect on the SpG8-1b genes expression after 24 hours of incubation. 63

Chapitre 2 : Rôle signal des HCA dans l'interaction *Agrobacterium fabrum*–plante

Supplementary table S1: Strains and plasmids used in this study. 83

Supplementary table S2: Carbon sources used by the *A. fabrum* wild-type strain. 84

Chapitre 3 : Système HCA-RFO : fonctionnement et rôle écologique.

Article 3: The plant defense signal galactinol is specifically used as a nutrient by the bacterial pathogen *Agrobacterium fabrum*

Table 1: Affinity measurement for MelB. K_D values were measured by intrinsic protein fluorescence titration (Fluorescence) and by isothermal titration microcalorimetry (ITC). 94

Table 2: Crystallographic data and refinement parameters. 94

Table S1: Strains and plasmids used in this study. 105

Table S2: Primers used in this study.. 106

Article 4: Influence of ferulic acid on *Agrobacterium fabrum* transcriptome

Table 1: List of genes up-regulated ($\log_2\text{foldchange}>2$) or down-regulated ($\log_2\text{foldchange}<2$) in presence of ferulic acid in the *A. fabrum* wild-type strain. 116

Supplementary table S1: Strains and plasmids used in this study. 121

Supplementary table S2: Primers used in this study. 122

Introduction générale

Les végétaux sont les principaux producteurs primaires de carbone organique, dont un pourcentage important (15-20%) provenant de la photosynthèse est retrouvé au niveau des racines (Nguyen, 2009). Dans ce contexte la rhizosphère, définie comme la zone du sol sous l'influence directe des racines et des tissus végétaux, est un lieu privilégié pour la croissance de nombreux microorganismes (Bais *et al.*, 2006; Faure *et al.*, 2009; van Dam and Bouwmeester, 2016). En effet, l'exsudation racinaire est à l'origine d'une grande diversité de nutriments que ce soient des oses, des acides organiques, des acides aminés ou des acides gras (Dennis *et al.*, 2010; Nelson, 2004, 2017). La présence de ces composés attire et favorise la croissance de certains microorganismes qui atteignent des effectifs de 10^8 - 10^9 bactéries par gramme de racine (Baetz and Martinoia, 2014; Mendes *et al.*, 2013). Les plantes, via des récepteurs végétaux spécifiques qui reconnaissent des motifs moléculaires typiques de certains microorganismes (notamment provenant de flagellines, facteurs d'élongation ou lipopolysaccharides) appelés MAMPs ou PAMPs (*Microbe* ou *Pathogen Associated Molecular Patterns*) peuvent percevoir les microorganismes environnants (Baetz and Martinoia, 2014; Lareen *et al.*, 2016; Newman *et al.*, 2013). La nature des exsudats produits varie alors en fonction des microorganismes perçus, ce qui va aboutir à une sélection par la plante de son microbiote rhizosphérique (Bais *et al.*, 2006; Lareen *et al.*, 2016; Zhalnina *et al.*, 2018). Dans le cas de la perception de pathogènes, la plante peut notamment exsuder des composés antimicrobiens comme par exemple les acides hydroxycinnamiques (HCAs) (Baker *et al.*, 1997; Lanoue *et al.*, 2010). Ces composés sont entre autres des précurseurs de la synthèse de nombreux métabolites secondaires ainsi que de la lignine, l'un des principaux constituants de la paroi végétale (Croteau *et al.*, 2000; Vogt, 2010). Ils sont libérés dans les zones de blessures de la plante, où ils sont présents à des concentrations élevées, généralement toxiques pour les bactéries (Croteau *et al.*, 2000). Les HCAs sont notamment toxiques chez *Pectobacterium carotovorum*, *Escherichia coli*, *Xanthomonas campestris* ou *Pseudomonas syringae* (Joshi *et al.*, 2016; Yamazaki *et al.*, 2012). Leur action antimicrobienne provient notamment de leur capacité à perturber l'intégrité des membranes bactériennes (Shi *et al.*, 2016). Par ailleurs, la production de ces composés peut également être stimulée par la présence de microorganismes commensaux ou bénéfiques, et va alors permettre à la plante de mieux lutter contre des phytopathogènes (Blum *et al.*, 2000; Staman *et al.*, 2001). Les différents microorganismes interagissant avec les végétaux ont développé des mécanismes pour détoxifier ces composés et pour certains, pour les utiliser comme ressources trophiques, comme c'est le cas chez certaines bactéries du genre *Agrobacterium*.

Le genre *Agrobacterium* est un complexe d'espèces appartenant à la famille des Rhizobiaceae. Il contient entre autre, les espèces *A. skirniwicense*, *A. nepotum*, *A. radiobacter*, *A. rubi*, *A. pusense*, *A. larrymoorei*, *A. viscosum* et l'espèce génomique G8, pour laquelle il a été proposé le nom d'*A. fabrum*. D'autres espèces génomiques, qui n'ont pas encore reçu de nom latin, font aussi partie du complexe d'espèces *Agrobacterium* (Gan and Savka, 2018; Mousavi *et al.*, 2014; Ramírez-Bahena *et al.*, 2014). Il s'agit des espèces génomiques nommées G1, G3, de G5 à G7, G9 et G13. Les agrobactéries sont principalement retrouvées au niveau du sol (Costechareyre *et al.*, 2010; Lamovšek *et al.*, 2014; Vogel *et al.*, 2003) et de la rhizosphère (Bai *et al.*, 2015; Bouri *et al.*, 2016; Lamovšek *et al.*, 2014). Elles ont comme particularité génomique d'avoir un chromosome linéaire en plus d'un chromosome circulaire, ce qui les différencie des autres genres appartenant aux *Rhizobiaceae*, qui possèdent deux chromosomes circulaires (Ramírez-Bahena *et al.*, 2014). Les agrobactéries peuvent héberger différents plasmides en plus de ces deux réplicons, notamment le plasmide At (pAt) et le plasmide Ti (pTi : *tumor inducing plasmid*). Ce dernier contient les principaux déterminants permettant aux agrobactéries qui le possèdent d'être phytopathogènes, responsables de la maladie appelée Galle du collet. Cette maladie se caractérise par la formation d'une tumeur au collet des plantes suite à l'intégration dans le génome des cellules végétales d'une région génomique du pTi nommée ADN-T. L'expression par la plante de gènes présents sur cet ADN-T va permettre la synthèse d'une part d'hormones végétales, qui vont induire la prolifération anarchique des cellules et la formation de la tumeur (Nester, 2014), et d'autre part d'opines, composés préférentiellement utilisables par les bactéries porteuses du plasmide Ti (Lang *et al.*, 2014). Cette interaction pathogène ne mène généralement pas à la mort de la plante. Il y a ainsi établissement d'une interaction pathogène à long terme entre *Agrobacterium* et la plante, dont la tumeur constitue une niche préférentielle pour les agrobactéries portant le plasmide Ti ("concept d'opine"), protégées physiquement à l'intérieur de celle-ci et nourries par les cellules végétales (Dessaux and Faure, 2018; Guyon *et al.*, 1980).

Ainsi les bactéries ne contenant pas le pTi présentent un mode de vie rhizosphérique non pathogène, et les bactéries porteuses du plasmide Ti présentent deux principaux styles de vie, l'un rhizosphérique et l'autre pathogène. Dans la rhizosphère, certaines molécules exsudées par la plante sont des composés chimioattractants qui vont guider les agrobactéries vers la racine, qu'elles soient pathogènes ou non (Antunez-Lamas *et al.*, 2009; Ashby *et al.*, 1988; Parke *et al.*, 1985; Yao and Allen, 2006). Par exemple, la perception d'acide férulique (un HCA) induit

la migration bactérienne vers la racine qui le produit (Ashby *et al.*, 1988; Guo *et al.*, 2017; Huang *et al.*, 2017). Indépendamment de la présence du plasmide Ti, les agrobactéries peuvent adhérer aux cellules végétales de la racine grâce à différents mécanismes codés par des gènes chromosomiques (Matthysse, 2014; Nester, 2014). Lorsqu'une plante est blessée, les composés relargués vont aussi guider les microorganismes vers la zone de blessure. Au niveau de cette blessure, les conditions suivantes sont rassemblées : abondance de sucres, pH acide et présence de composés phénoliques comme l'acétosyringone, l'hydroxyacétosyringone ou l'acide férulique. Ces trois conditions vont induire l'expression de gènes de virulence, les gènes *vir* portés par le plasmide Ti (Brencic *et al.*, 2004; Cangelosi *et al.*, 1990a; Stachel *et al.*, 1985; Yuan *et al.*, 2008). Cette induction permet l'initiation du programme de pathogénie et le début du style de vie pathogène (Joubert *et al.*, 2002). Les mécanismes qui engendrent la pathogénie et notamment ceux influençant l'expression des gènes *vir* sont ainsi dus à la fois à des déterminants chromosomiques (chimiotactisme, adhésion) et à des déterminants plasmidiques. L'expression de ces gènes de virulence est en plus finement contrôlée par des protéines codées par des gènes du pTi comme la *O*-déméthylase VirH2. Cette protéine induit la transformation de l'acide férulique en acide caféique, qui est un composé non inducteur des gènes *vir* (Brencic *et al.*, 2004). Ce mécanisme de régulation permet de limiter le coût métabolique de l'expression des gènes de virulence lors des stades tardifs de l'infection ou dans des conditions inappropriées, comme la rhizosphère qui peut aussi contenir des composés inducteurs (Brencic *et al.*, 2004; Kalogeraki *et al.*, 1999).

Au sein du genre *Agrobacterium*, une analyse de génomique comparative a permis la découverte de 7 régions spécifiques d'espèces présentes chez *Agrobacterium fabrum* (e.g présentes chez toutes les bactéries de cette espèce et absentes des bactéries des autres espèces du genre *Agrobacterium*) (Lassalle *et al.*, 2011). Ces régions spécifiques d'espèces seraient des déterminants importants dans la vie d'*A. fabrum* en interaction avec la plante. Parmi ces régions, la région SpG81-b qui porte les gènes *atu1415-atu1422* est la seule région spécifique d'espèce présente sur le chromosome circulaire. Ce cluster a été caractérisé comme permettant la dégradation des HCAs et leurs utilisations comme ressource carbonée (Campillo *et al.*, 2014). Cette région contient aussi le gène *atu1422 (hcaR)*, potentiel régulateur de type MarR (*Multiple antibiotic resistance Regulator*), non fonctionnellement caractérisé au début de cette thèse.

Ainsi les bactéries du genre *Agrobacterium* possèdent des gènes chromosomiques importants pour la colonisation rhizosphérique et, pour certaines souches, des gènes portés par le plasmide

Ti impliqués dans le déterminisme du style de vie pathogène. L'établissement efficace de ce dernier style de vie est néanmoins aussi conditionné par l'expression de gènes portés par les chromosomes, qui vont notamment médier les phénomènes de chimiotactisme et d'adhésion cellulaire, mais aussi la multiplication et la persistance des bactéries dans la tumeur. Par ailleurs, au sein de l'espèce *A. fabrum*, la région génomique SpG8-1b qui contient des gènes spécifiques d'espèces pourrait interférer avec l'expression des gènes impliqués dans la virulence, mais aussi être utile pour la colonisation d'environnements riches en HCAs, en les détoxifiants, en les utilisant comme ressource trophique, ou comme signal de perception de la plante.

Les objectifs de cette thèse sont d'une part de caractériser la régulation de la dégradation des HCAs par la région SpG8-1b et d'évaluer si celle-ci interfère avec la pathogénie, et d'autre part d'étudier l'influence de la perception d'HCAs et de leur dégradation sur l'écologie de cette bactérie dans les deux styles qu'elle adopte au contact de la plante.

Ainsi, dans une première partie de synthèse bibliographique composée de deux revues, nous allons faire état des connaissances concernant, d'une part les déterminants génétiques impliqués dans la survie d'*Agrobacterium* dans la tumeur, et d'autre part, la dégradation bactérienne des HCAs et leur influence sur l'écologie de microorganismes modèles. Dans une seconde partie expérimentale, seront présentés sous la forme de trois chapitres les résultats de ces travaux. Le premier chapitre portera sur la caractérisation moléculaire et écologique de la régulation de la dégradation des HCAs médiée par le régulateur HcaR. Le second chapitre concernera l'étude de l'influence de la perception des HCAs et de leurs intermédiaires de dégradation sur le métabolisme carboné d'*A. fabrum*. Enfin, le dernier chapitre décrit d'une part la caractérisation écologique et fonctionnelle de la protéine MelB, impliquée dans le transport de différents sucres comme le mélibiose et dont l'expression est régulée par la présence d'HCA, et d'autre part l'analyse du transcriptome d'*A. fabrum* en présence à la fois de mélibiose et d'acide férulique. A l'issue de cette partie expérimentale, nous intégrerons les résultats obtenus et discuterons de l'importance de la perception des HCAs dans la compréhension des interactions *Agrobacterium*-plante et plus globalement dans l'écologie d'*A. fabrum*.

Synthèse bibliographique

Partie A : Interaction pathogénique plante-*Agrobacterium* : déterminant de l'interaction à long terme

Le texte suivant est une version provisoire d'une revue qui décrit les conditions rencontrées par *Agrobacterium* dans la tumeur et les déterminants moléculaires impliqués dans l'interaction à long terme entre *Agrobacterium* et la plante. Cette revue, a été co-écrite avec Clémence Thiour-Mauprivez et initiée dans le cadre de son rapport bibliographique de M2R. Ma contribution principale a concerné la description des déterminants génétiques, celle de Clémence Thiour-Mauprivez concerne majoritairement la description de l'environnement tumorale.

Ecological conditions and molecular determinants involved in *Agrobacterium* lifestyle in tumor

Thibault Meyer ^{*1}, Clémence Thiour-Mauprivez ^{*1,2}, Isabelle Kerzaon¹, Gilles Comte¹, Ludovic Vial¹, Céline Lavire¹

¹ UMR Ecologie Microbienne, CNRS, INRA, VetAgro Sup, UCBL, Université de Lyon, F-69622, Villeurbanne, Lyon, France; ² BAE: Biocapteurs-Analyses-Environnement, Université de Perpignan Via Domitia, 52 Avenue Paul Alduy, Perpignan Cedex 66860, France

* Both authors contributed equally to this paper.

Correspondence should be sent to: Céline Lavire, Ecologie Microbienne, Université Lyon 1, 43 Boulevard du 11 novembre F-69622 Villeurbanne cedex, France, Tel.: (+33) 426237126 ; Fax: (+33) 4 72 43 12 23;

Keywords: *Agrobacterium tumefaciens* biology; tumor lifestyle; crown-gall; molecular determinants; competition; plant defense; microbial community

Abstract

The study of the pathogenic determinants in their natural niches allows a better understanding of disease persistence and dissemination. Bacteria belonging to the *Agrobacterium* genus are soil borne bacteria able to colonize rhizosphere and are well known as phytopathogens thanks to their capacity to provoke tumors (crown-gall disease) by transferring a DNA region (T-DNA) into a wide range of plant. *Agrobacterium* studies are mostly focused on T-DNA integration, bacterial and plant factors influencing the genetic transformation efficiency. Only a few researches have concerned tumor environment or bacterial determinants involved in the long-term persistence of bacteria inside tumor. Even if the opine concept explains a major part of the *Agrobacterium*-plant interaction, by their own it cannot explain the competitiveness of *Agrobacterium* in tumor. This review proposes to gather the current knowledge about the conditions encountered in the tumor environment together with the genetic determinants that allow *Agrobacterium* survival and efficient colonization of the tumor. We described how tumor is setting up and how *Agrobacterium* can maintain itself in this stressful, nutrient-rich but competitive environment.

Introduction

The persistence of bacterial phytopathogens in soil results from many factors including the survival in the natural environment, adaptation capacities to different lifestyles and for efficiently shift from one lifestyle to another (Wei *et al.*, 2015; Sokurenko *et al.*, 2006; Engering *et al.*, 2013; Duprey *et al.*, 2014). On the one hand, the natural environments for plant pathogens are mainly the soil, the rhizosphere or the phyllosphere (Wolf and Boer, 2015). Survival in the rhizosphere requires high adaptation capacity to this environment which implies abilities to resist to both abiotic and biotic stress and a high capacity to rapidly access to nutritional sources (Romanuk *et al.*, 2009; Wei *et al.*, 2015; Ji and Wilson, 2002). On the other hand, the pathogenic lifestyle generally involves bacterial growth inside and tight interactions within the host. The success of the pathogen thus depends on its ability to face the conditions encountered inside the host, including plant defense mechanisms, availability of nutritive resources, and interactions with the host microbiota (Brader *et al.*, 2017; Does and Rep, 2017; Spanu and Panstruga, 2017).

Agrobacterium genus regroups soil borne bacterial species that are able to live in plant rhizosphere and which can be pathogenic when they harbour the Ti plasmid (Tumor-inducing). The latter contains a DNA region transferable to plant cells (T-DNA) and able to be integrated in their genome. The T-DNA genes are expressed by the infected plant leading to hormone production which induces unscheduled proliferation of plant cells (Drummond, 1979; Nester, 2014). Plants are then developing tumor, and are considered as suffering of crown gall disease. Thanks to its capacity to transfer a DNA region, *Agrobacterium* is well-studied as a natural genetic engineering (Hwang *et al.*, 2015; Krenek *et al.*, 2015). T-DNA also contains genes encoding for opine synthesis. Opines are conjugates of amino-acids and sugar or organic acids and are used as nutrients specifically by agrobacteria harboring the Ti plasmid. There are about forty characterized opines, some of which are able to trigger the transfer of the Ti plasmid from one bacterium to another, enhancing pathogenicity and contributing to the persistence of pathogenic bacteria (Guyon *et al.*, 1980). These properties lend to opines a central role in the interaction between *Agrobacterium* and the infected plant (Dessaux and Faure, 2018; Guyon *et al.*, 1980). The “opine concept” allocates in

presence of opines a better competitiveness to bacteria able to degrade them than to unable one. In case of detrimental environmental condition, the presence of nutrient does not necessarily favor the bacteria able to use them. Tumor exploitation also implies to deal with plant defense mechanisms. Plant defense involves the action of chemical compounds such as salicylic acid (SA), jasmonic acid (JA) or ethylene that allow induction of pathogenesis-related proteins which in turn leads to antimicrobial compounds synthesis (Costet *et al.*, 1999; Durrant and Dong, 2004). Even though plants set up defense mechanisms against *Agrobacterium* (Deeken *et al.*, 2006; Pitzschke, 2013; Shih *et al.*, 2018), the bacterium is known to bypass those defenses to settle in tumor durably (Lee *et al.*, 2009; Nonaka *et al.*, 2017; Nonaka and Ezura, 2014; Someya *et al.*, 2013). It must be quoted that *Agrobacterium* is one of the only phytopathogens that does not kill its host, using him for its niche construction.

However, non-pathogenic *Agrobacterium* strains are also isolated from tumor (Bélanger *et al.*, 1995; Llop *et al.*, 2009; Nesme *et al.*, 1987; Shams *et al.*, 2012). These strains could be either mutants of the strain inducing the tumor or from environmental origin. Tumors have also been reported to carry other microorganisms. Recently Faist *et al* (2016) have analyzed the microbial community of natural tumors of *Vitis vinifera* and reported that besides *Agrobacterium*, tumor microbiota also contains *Pseudomonas*, *Sphingomonas*, *Erwinia* and *Bradyrhizobium* genera. Other studies have reported that tumor can harbor bacteria belonging to the *Corynebacterium* or the *Arthrobacter* genus or the plant symbiont *Ensifer meliloti* (Moore *et al.*, 1997; Nautiyal and Dion, 1990; Tremblay *et al.*, 1987). The tumoral environment can also shelter fungal species which are also opine-degrader with *Cylindrocarpon destructans*, *C. heteronema* and *Fusarium solani* (Beauchamp *et al.*, 1990). Hence, even in the opine rich tumor-environment and beyond its ability to use opine as nutrient, *Agrobacterium* need additional determinants to compete with other microorganisms and to durably settle in the specific niche they constructed.

The pathogeny program induction through *vir* and *chv* gene expression, as well as the process of genetic transformation that leads to tumor formation, have been extensively studied (Gelvin, 2017; Lacroix and Citovsky, 2013; Nester, 2014). Moreover, the transition between the rhizospheric and the tumor lifestyles that is a critical step in the crown gall disease establishment, has been recently

reviewed (see Barton *et al.* 2018; Platt *et al.* 2012b, 2012a, 2014). However, the comprehension of *Agrobacterium* pathogenicity and long-term association to plant requires a better understanding of the bacterial ability to durably settle in the tumor. Actually, this ability cannot be dissociated from knowledge of the conditions they encountered in the tumor environment together with the molecular traits that allow them to persist and efficiently exploit this environment. Here, we summarize knowledge about tumor settlement and changes it produces in plant, together with the molecular determinants that allow *Agrobacterium* to survive in, exploit tumor and compete with other microorganisms.

I. Tumor: development of an unfriendly environment

The tumor is changing during its development and according to the plant biotic and abiotic environment. From literature, and even though slight differences between publications are observed, a tumor is considered young up to one month (Deeken *et al.*, 2006; Efetova *et al.*, 2007). At the time of the writing, no time-course study of physico-chemical in tumor has been realized. We will describe condition and metabolites encountered in the tumor at two developmental stages, the young tumor (0 to 1 month) and the mature tumor (more than 1 month).

Young tumor and dual role of ethylene

The auxin/cytokinin ratio controls tumor proliferation and development (Skoog and Miller, 1957). Compared to native plant cells that contain genes responsible for the synthesis of the two hormones, transformed cells contain additive T-DNA encoded genes for auxin and cytokinin synthesis (Skoog and Miller, 1957). Recently, it has been identified up to four times more auxin and ten times more cytokinin in *A. thaliana* tumors infected by the model strain *Agrobacterium fabrum* C58 than in non-infected plant stems (Zhang *et al.*, 2015a). Higher cytokinin and auxin concentrations will enhance ACC synthase activity which allows ethylene synthesis by plant (Tanaka *et al.*, 2006). The latter also plays an important role for tumor setting up. Indeed, Aloni *et al.* (1998) have compared tumors induced by *A. tumefaciens* in wild-type tomato and in *never-ripe* mutants (respectively sensitive and non-sensitive to ethylene). Mutant plants have tinier tumors and more vascularized tissues than wild-type plants (Aloni *et al.*, 1998). Same authors have also shown that ethylene leads

to a xylem diameter reduction nearby tumor and that, by growing faster than normal cells, tumor cells were robbing plant shoots of water (Aloni *et al.*, 1998). Thus, ethylene allowed tumor vascularization and plays a central role in tumor setting up and its supply (Figure 1 A).

Ethylene also takes part in plant defense mechanisms called systemic acquired resistance (SAR) and local acquired resistance (LAR) by allowing the induction of Pathogenesis-Related proteins (Costet *et al.*, 1999; Durrant and Dong, 2004). Thereby, ethylene, despite its role in tumor formation, may have negative effects on the bacterium. For example, it is known that ethylene plays a role in the frequency decrease of T-DNA transfer to plant cells (Nonaka and Ezura, 2014). Veena *et al.* (2003) have shown by transcriptomic analysis that plant genes favoring plant defenses are under-expressed on the latest stages of infection by *Agrobacterium*. Thus, ethylene may be useful for tumor creation rather than defense protein induction.

In reaching a certain amount of tumor cells, the hydric advantage firstly conferred by ethylene through its influence on vascularization is offset by an increased water loss. In fact, massive proliferation of tumor cells engenders an epidermal rupture and an uncontrolled evaporation of water contained in tumor (Figure 1A). Here, ethylene also plays a crucial role in tumor drought tolerance, as proposed by Efetova *et al.* (2007). A model of its role is summarized in Figure 1A: cytokinin and auxin trigger ethylene synthesis; the one will trigger abscisic acid synthesis in tumor. Abscisic acid induces suberin polymerization in order to create a protective layer around tumor, avoiding its desiccation (Efetova *et al.*, 2007).

Plant cells response to the hydric stress involves an enhancement of fatty acid desaturase activity in order to synthesize α -linolenic acid and ensure lipid membrane integrity (Zhang *et al.*, 2015a). Fatty acid desaturase 3 (FAD3) plays an important role in tumor. It has been shown, by comparing FAD3 mutant of *A. thaliana* to wild-type ones, both infected by *Agrobacterium*, that, at low rates of humidity, *fad3-2* mutant tumors were tinier than in wild-type plants. This demonstrates that the α -linolenic acid, synthesized by FAD3, is able to maintain lipid membrane integrity of plant cells in drought conditions (Klinkenberg *et al.*, 2014).

Few information about the metabolite contents in the young tumor is available except that it contains high concentration of proline, hexose such as glucose and fructose, and α -aminoadipinic acid (Efetova *et al.*, 2007; Wobus and Weber, 1999).

Mature tumor: an unfriendly environment

As tumor grows, the hydric stress somehow leads to irreversible consequences: an epidermis rupture of tumor cells and plant cells nearby tumor is going to take place, followed by a stomata hypertrophy, making them non-functional (Schurr *et al.*, 1996). Plant, then, gives priority in stomatal closure and root hydric supply by activating abscisic acid pathway. However, stomatal closure implies the increase in the level of H₂O₂, belonging to the ROS (Reactive Oxygen Species) family, that make oxygen rate decreases (Zhang *et al.* 2001, Figure 2B). The hypoxic stress has consequences on plant metabolism. For example, FAD3 is not efficient any more. In these conditions, Stearoyl ACP Desaturase 6 (SAD6), which can be used in a hypoxic environment, maintains plant cell membrane integrity by catalyzing fatty acid biosynthesis first steps (Klinkenberg *et al.*, 2014). In accordance, in *A. thaliana* tumor genes involved in photosynthesis are under-expressed, whereas genes involved in glycolysis and fermentation are over-expressed (Deeken *et al.*, 2006). We can assume that, transformed plant cells go from an autotrophic metabolism to a heterotrophic one, and that oxygen availability is limited, at least in some part of the tumor.

Besides oxygen availability, nutrient availability is important for bacterial development inside tumor. Water and minerals are mainly conducted by xylem and sugars and other biosynthesized carbonated compounds by phloem. Vascularization first steps appear, one more time, essentials to tumor since they allow bacterial nutrition. Compared to young tumors that are supplied by hexoses, mature tumor use sucrose which is more easily transportable and assimilable in drought conditions (Deeken *et al.*, 2006; Singh and Maclachlan, 1983; Wobus and Weber, 1999). Thereby, bacteria living in tumor first receive hexoses, such as fructose and glucose, and then, as long as hydric stress becomes more important, plant gives them sucrose (Deeken *et al.*, 2006). In addition to sucrose, glucose and fructose, and compared to non-infected stem, *A. thaliana* old tumor contains high

concentrations of amino acids (glutamine, serine, asparagine, glutamic acid, threonine, proline, aspartic acid, alanine, valine, isoleucine, leucine, histidine and arginine), nitrogen sources (GABA, urea, α -amino adipic acid) together with high amounts of sulfate and phosphate.

Simoh *et al.* (2009) have analyzed the metabolites contained in five weeks tumors of *Brassica rapa*. They found glucose, sucrose, amino acids (threonine, valine, glutamic acid, alanine, leucine, isoleucine and arginine) and organic acids (indole-3 acetic acid, succinic acid, malic acid, formic acid, fumaric acid). The flavonoid quercetin and kaempferol, and phenylpropanoid derivatives (sinapoyl- and coumaroyl malate) that could limit bacterial growth were also detected in the tumor (del Valle *et al.*, 2016; Wang *et al.*, 2018). The metabolites of the tumor would differ according to the plant species, the pathogenic strain that induced them, and environmental conditions. In any cases, in addition to opines, many nutrient sources could be used by agrobacteria inside tumors.

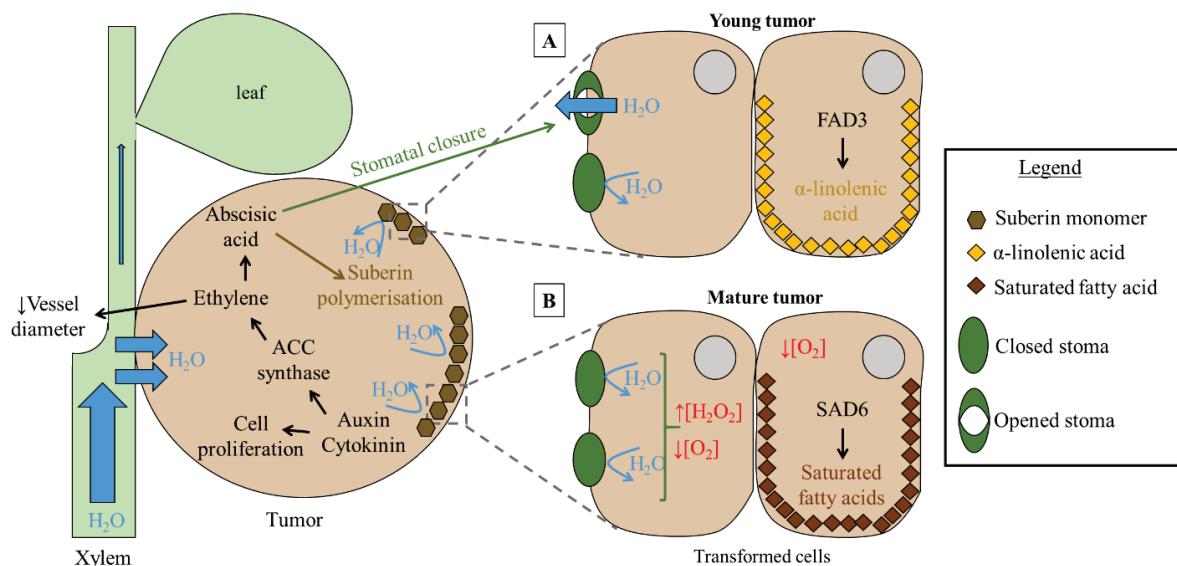


Figure 1. Chain reaction behind the development of an unfriendly environment. The T-DNA integration in the plant genome allows the production of auxin and cytokinin. High concentrations of these two phytohormones accelerate cell proliferation and tumor growth. ACC synthase expression is also induced and triggers ethylene production. Ethylene has two main roles in tumor: it reduces plant vessel diameter around tumor to ensure hydration and it triggers abscisic acid synthesis. The latter induces the polymerization of suberin that forms a protective layer. (A) In young tumors, FAD3 synthesizes α -linolenic acids to strengthen the transformed cell wall. (B) In mature tumor, hydric stress becomes more important and abscisic acid engenders the closure of many stomata. This phenomenon implies the utilization of H_2O_2 and decreases the oxygen rate. Under these conditions, SAD6 synthesizes saturated fatty acids to maintain the lipid membrane integrity of the transformed cells.

II. Key genetic determinants for *Agrobacterium* persistence in the hostile tumor environment

How *Agrobacterium* face to hostile conditions

Oxidative stress

One of the first response to pathogen perception by plant is the production of ROS, like superoxide (O_2^-), or hydrogen peroxide (H_2O_2) (Torres *et al.*, 2006). *Agrobacterium* is able to detoxify these toxic compounds due to proteins encoded by chromosomal genes. Firstly, this bacterium harbors three genes encoding superoxyde dismutase : *sodBI* (*atu0876*), *sodBII* (*atu4583*) and *sodBIII* (*atu4726*) (Saenkham *et al.*, 2007). The characterization of these three enzymes revealed that SodBI is responsible in the great majority of the transformation of O_2^- to O_2 and H_2O_2 , compared to the SodBII and SodBIII activities. The *sodBI* and the triple superoxyde dismutase mutant strains are less able to induce *vir* genes which is correlated to a reduced ability to provoke tumor formation than the wild-type strain (Saenkham *et al.*, 2007). Secondly, *A. tumefaciens* is able to degrade H_2O_2 to H_2O and O_2 thanks to a catalase called KatA (*atu4642* carried by its linear chromosome) ((Xu and Pan, 2000). A *katA* mutant strain was highly affected for its capacity to form tumors and it was suggested that, the infected plant does not activate hypersensitive response (HR) due to the expression of *Agrobacterium* catalase. This non-induction allows *Agrobacterium* to settle and to induce tumors in plant without risking plant tissue necrosis. This ability participates to the long term efficient interaction.

Osmoprotection

As described previously, tumor is a drought and ROS-rich environment. In order to maintain homeostasis, bacteria use two main strategies, i.e. accumulation of osmoprotectants and exopolysaccharides production (EPS). Osmoprotectants are compounds accumulated in the cell and protect the latter from different stresses: drought, dehydration and presence of oxygen radicals. *Agrobacterium* is able to uptake or synthesize a large range of compounds previously described to

be involved in osmoprotection (Bougouffa *et al.*, 2014; Kempf and Bremer, 1998; Panikulangara *et al.*, 2004). These compounds

include glycine betaine and choline (Boncompagni *et al.*, 1999), gamma-butyrobetaine (Nobile and Deshusses, 1986), proline (Haudecoeur *et al.*, 2009), sucrose and trehalose (Ampomah *et al.*, 2013), palatinose (De Costa *et al.*, 2003), melibiose, raffinose and stachyose (Meyer *et al.*, 2018b). The genes allowing the uptake of melibiose, raffinose and stachyose are expressed in mature tumors (González-Mula *et al.*, 2018), whereby they could allow bacterial osmoprotection. However, the role of those compounds in *Agrobacterium* osmoprotection has not been evaluated. A direct role of gamma-butyrobetaine, glycine betaine and choline in *Agrobacterium* osmoprotection has been only described *in vitro* but no study reported their role *in planta* (Boncompagni *et al.*, 1999; Nobile and Deshusses, 1986).

EPS synthesis is the second way to resist to environmental high or low osmolarity. *Agrobacterium* can synthesize diverse kind of EPS that are composed of β -1-2-glucan (Cangelosi *et al.*, 1990b), curdlan (Lassalle *et al.*, 2011; Liu *et al.*, 2016; Rondon *et al.*, 2004), succinoglycan (Tomlinson *et al.*, 2010), UPP (unipolar polysaccharide) cellulose and lipopolysaccharides. A role in osmoprotection has only been described for β -1-2-glucan that are produced in response to low-osmolarity conditions in different microorganisms including *Agrobacterium* (Cangelosi *et al.*, 1990b; Domínguez-Ferreras *et al.*, 2006; Ingram-Smith and Miller, 1998). However, some genes involved in EPS production (curdlan) are expressed in tumor (González-Mula *et al.*, 2018) where they could be useful as osmoprotectants. Alternatively, they could be useful for cell adhesion, as some of those EPS were shown to be involved in that process (For review see Matthyse 2014). It would be of interest to decipher the role of EPS inside tumor.

Oxygen as limiting factor

Tumors, as described previously, are heterogeneous environments in which oxygen can be a limiting factor. In low oxygen conditions, some *Agrobacterium* species are facultative denitrifiers (Baek and Shapleigh, 2005). A nitrate reductase (NapAB-Atu4408-Atu4409) allows them to reduce nitrate into nitrite (NO₂), the one is converted to nitric oxide (NO) by a nitrite reductase

(NirK-Atu4382). Transformation of NO to nitrous oxide (N₂O) is catalyzed by a NO reductase (NorB-Atu4388), which is accumulated due to the absence of the N₂O reductase (encoded by *nosZ* gene) (Kampschreur *et al.*, 2012).

The *norB* gene is expressed in a context of agroinfiltration in *A. thaliana* leaves and transcriptomic comparison between tumor and culture media suggest that partial denitrification occurs in tumor (Baek and Shapleigh, 2005; González-Mula *et al.*, 2018). These results indicate that, in one hand, denitrification can participate to *Agrobacterium* survival in tumor by giving energy in the absence of oxygen. In the other hand, *Agrobacterium* denitrification can also participate in bypassing plant defenses mediated by NO synthesis (Bellin *et al.*, 2013; Leitner *et al.*, 2009).

Resistance to plant toxic compounds

The host plant is responsible for the production of antibacterial compounds in the tumor including phenolic compounds (Cushnie and Lamb, 2005; Deeken *et al.*, 2006; Schwalm *et al.*, 2003; Shi *et al.*, 2016; Simoh *et al.*, 2009). The VirH2 protein is an *O*-demethylase which plays a role in the degradation of ferulic acid into caffeic acid, a less toxic phenolic compound. The pTi encoded gene *virH2* gene is highly expressed in tumor (Brencic *et al.*, 2004; González-Mula *et al.*, 2018). In *A. fabrum* species, it has been shown that the SpG8-1b genomic region is involved in the degradation of the following phenolic compounds: ferulic acid, caffeic acid and *p*-coumaric acid (Baude *et al.*, 2016; Campillo *et al.*, 2014; Lassalle *et al.*, 2011; Meyer *et al.*, 2018b). This region allows both to cope with toxic compound and use them for growth. Other non-characterized pathways annotated as involved in phenolic compound degradation are present in other *Agrobacterium* species, highlighting the importance of this function (Lassalle *et al.*, 2017).

Another mechanism to survive in presence of toxic compounds requires the action of efflux pumps. Palumbo *et al.* (1998) have shown that the protein encoded by *ifeBAR* is involved in flavonoid and isoflavonoid export. Moreover, the EmrBAR efflux pump is induced by indole and suggested to confer resistance to toxic compounds, as described in *E. meliloti* (Rossbach *et al.*, 2014; Santos *et al.*, 2014). Both systems could be useful for survival inside tumor, but *ifeBAR* and *emrBAR* were not upregulated in 3 weeks tumors of *A. thaliana* in comparison to culture media (González-Mula

et al., 2018). In this study *tetR/tetA*, encoding an efflux pump that allow resistance to tetracycline, was upregulated (González-Mula *et al.*, 2018). To date, plant compounds that are actually exported by TetR/A are not known (Lassalle *et al.*, 2011; Luo and Farrand, 1999).

Nutrition in tumor

Pathogenic agrobacteria are able to catabolize opines thanks to genes carried by their Ti plasmid. Opines are used as carbon, nitrogen but also phosphate or sulfur source for bacteria (Dessaux and Faure, 2018; Flores-Mireles *et al.*, 2012; Messens *et al.*, 1985). A special feature of Ti plasmid confers to the bacterium which harbor it, the possibility to catabolize only a specific type of opine according to the gene it contains (Christie and Gordon, 2014). Genes involved in the uptake of the different opines are well studied (Dessaux and Faure, 2018). Among them, the *nocT* (*atu6027*) gene encodes a periplasmic binding protein (PBP) which is described as involved in the binding of nopaline and pyronopaline (Lang *et al.*, 2014). The *ocd* (*atu6016*) gene encodes an ornithine cyclodeaminase which is involved in assimilation and nopaline degradation and assimilation (Sans *et al.*, 1987). *nocT* and *ocd* mutant strains are unable to use and to assimilate these two kinds of opines. In competition for tumor niche occupation, the wild-type strain outcompetes the two mutant strains, indicating that opines favor the survival of bacteria able to catabolize them in the competitive conditions of tumor.

Interestingly, after individual inoculation, the wild-type and the two *nocT* and *ocd* deletion mutants reach the same population level (Lang *et al.*, 2014). This finding implies that tumor provides other carbon sources that support the growth of the two deletion mutants. Accordingly, metabolomics studies and *Agrobacterium* transcriptome in tumor revealed a large diversity of available carbon sources together with the up-regulation of numerous and diverse genes involved in *A. fabrum* metabolism (Deeken *et al.*, 2006; González-Mula *et al.*, 2018; Simoh *et al.*, 2009). Annotation of the *A. fabrum* strain C58 genome predicts 190 ABC and 3 TRAP (Tripartite ATP-independent periplasmic) transporters (Goodner *et al.*, 2001; Wood *et al.*, 2001) putatively involved in the import of (plant) compounds, but a few of them have been characterized. Moreover, only few genes responsible for the degradation of carbon sources are described in *Agrobacterium*. Beyond opines

import and utilization genes, we proposed to make the state of knowledge of trophic resources that can support the growth of *Agrobacterium* in tumor.

Some pathogens use plant cell wall to feed inside their host (Reverchon and Nasser, 2013). *Agrobacterium*, was suggested to be able to degrade plant cell wall and released galacturonic acid due to the polygalacturonase action of PicA (Rong *et al.*, 1991). *picA* is overexpressed inside tumors (González-Mula *et al.*, 2018) but its exact role is unclear. *Agrobacterium* is able to import galacturonic acid thanks to the *gaaPQM* (*atu3135-atu3137*) operon which allows, in a lesser degree, the glucuronic acid uptake. Both of these organic acids are used for growth (Zhao and Binns, 2016). For more details about the galacturonic acid degradation see (Andberg *et al.*, 2012; Boer *et al.*, 2010; Taberman *et al.*, 2014a, 2014b; Zhao and Binns, 2016).

Glucose, xylose, fucose and arabinose are sugars usually found in the plant and numerous bacteria are able to use them as carbon sources (Bais *et al.*, 2006; Gunina and Kuzyakov, 2015; Lugtenberg *et al.*, 1999). The *gguABC-chvE* (*atu2345-atu2348*) and the *gxySBA* (*atu3574-atu3576*) clusters are two distinct ABC transporters allowing the D-glucose, D-xylose, D-fucose, D-galactose and L-arabinose uptake in *Agrobacterium* (Zhao and Binns, 2011, 2014). These two transporters are required for fucose, glucose and glucosamine utilization. Indeed, the double deletion of these two clusters abolishes the growth on these substrates. However, the presence of additive transporters was suggested for arabinose, xylose and galactose, as the double transporter deletion strain (deleted for *gguABC-chvE* and *gxySBA*) is still able to grow on these compounds. In the case of galactose, the *mel* operon (encoding an ABC transporter) was suggested as the additional transport system (Meyer *et al.*, 2018b). The *mel* operon expression is induced inside tumor (González-Mula *et al.*, 2018) and a deletion mutant of the PBP uptake gene (responsible of melibiose, galactinol, raffinose and stachyose uptake) is less competitive than the wild-type strain for tumor colonization (personal data). It is not known whether the imported compounds are used for osmoprotection and / or nutrition in that case.

Agrobacterium possesses genetic determinants that allow growth on plant compounds that have not yet been specifically reported in tumor. For example, Ampomah *et al.* (Ampomah *et al.*, 2013)

have demonstrated that *Agrobacterium* possesses a functional palatinose and trehalose uptake and degradation system. The *thuEFGKAB* operon (also known as *palREFGKAB* operon ; *atu3338-atu3343*) is composed of an ABC transporter (*thuFGK*) linked to a PBP (*thuE*) and two genes involved in a degradation process : *thuB* and *thuA* (Ampomah *et al.*, 2013). The deletion of *thuEFGK* abolishes the growth of *Agrobacterium* on palatinose (De Costa *et al.*, 2003) while the *thuAB* genes are required for *Agrobacterium* growth on maltitol, trehalose and isomeric forms of sucrose (palatinose, leucrose and trehalulose). The *thuEFGK* deletion mutant is not required for tumor formation but its role in the competitive colonization of the tumor have not yet been assayed.

González-Mula *et al.* (2018) have identified many genes annotated as responsible of carbon sources metabolism that are up-regulated in tumor in comparison with minimal media. Moreover, in a recent review, Dessaux and Faure underlined the ability of *Agrobacterium* to metabolize many carbon sources. From our study, *A. fabrum* C58 is metabolically active in presence of 87 different carbon sources (see chapter 2). All those data suggest that in addition to opines, *Agrobacterium* is using other plant compounds to sustain its growth inside tumor. Moreover, genes involved in their uptake and catabolism are awaiting functional characterization. In addition, for the few characterized genes, their roles are generally only tested *in vitro*, rarely *in planta* and even scarcely in competitive plant colonization. To our best knowledge, no study has reported a preference of *Agrobacterium* for opines rather than other sugars available in the tumor peculiar conditions which can be aerobic, microaerophilic or anaerobic. Thus, understanding *Agrobacterium* feeding inside tumor require additional efforts including functional genes characterization and ecological studies to decipher their role.

How *Agrobacterium* excels tumor microbiota

More than 150 different species were showed to compose tumor microbiota (Faist *et al.*, 2016) among which some are able to use opine as carbon source (Moore *et al.*, 1997; Nautiyal and Dion, 1990). For example, in tumor, some *Pseudomonas putida* strains are able to catabolize mannopine and some fungus catabolize mannopine and succinamopine (Nautiyal and Dion, 1990). Moreover,

Bell *et al.* (1990) have shown that *Pseudomonas* spp. take the advantage over *Agrobacterium* when co-cultured in a medium supplemented with octopine. Thus, *Pseudomonas* spp. probably use octopine more efficiently than *Agrobacterium* does. Genetic determinants that have been described in *Agrobacterium* could allow them to compete with tumor microbiota to durably settle in tumor.

For example, *Agrobacterium* genome contains a Type VI Secretion System (T6SS) (Bondage *et al.*, 2016; Lin *et al.*, 2013; Ma *et al.*, 2014; Wu *et al.*, 2012). T6SS is a molecular syringe that allows the injection of effectors, DNase and amidase for *A. fabrum* C58, into target cell in order to kill it (Bondage *et al.*, 2016; Ma *et al.*, 2014). This system is induced by acidic condition by the ChvG/I two component system and repressed by ExoR (Heckel *et al.*, 2014; Wu *et al.*, 2012). Ma *et al.* (2014) have demonstrated that, *in vitro* *P. aeruginosa* inhibits *Agrobacterium* growth thanks to a T6SS. However, after co-infiltration in leaves, *Agrobacterium* outcompete *P. aeruginosa* thanks to its T6SS (Ma *et al.*, 2014). Thus, *Agrobacterium* T6SS is efficient *in planta* but not *in vitro*. This suggests that T6SS genes have a distinct expression *in planta* and that this difference may be due to the action of plant compounds.

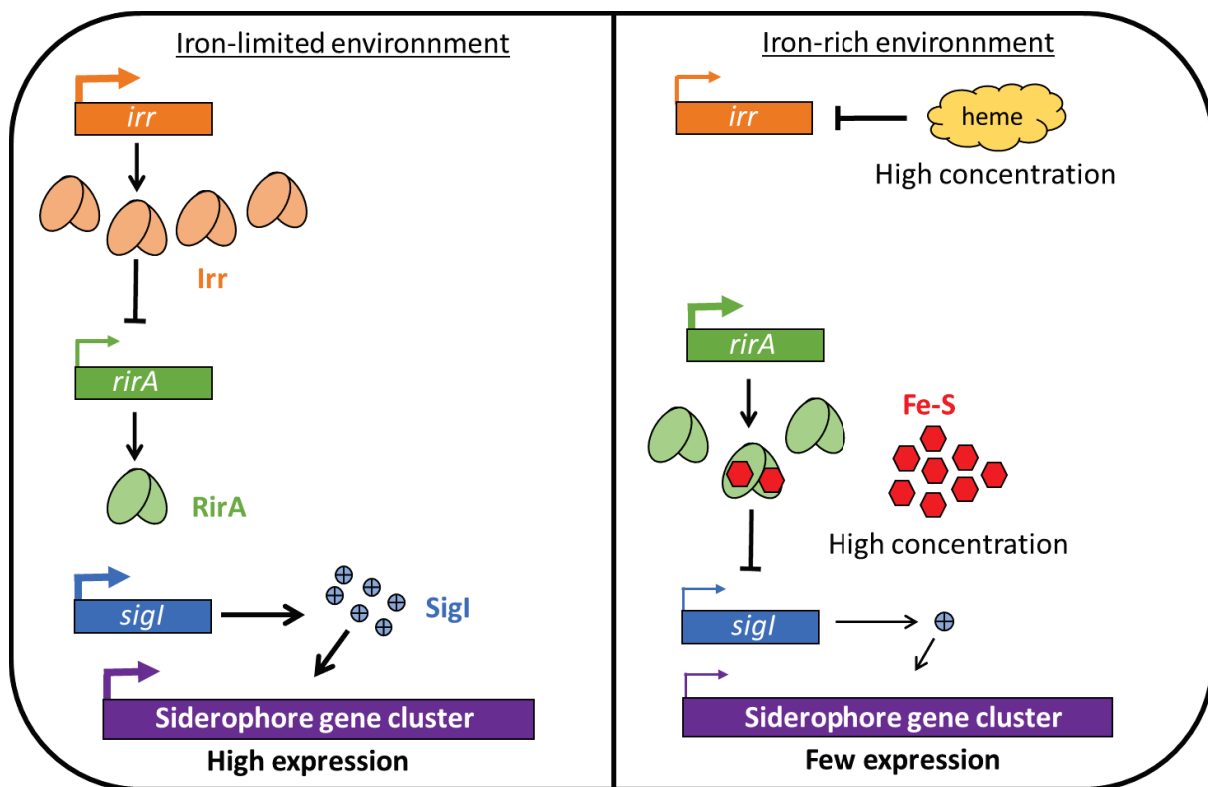


Figure 2: Model of gene cluster expression regulation depending on Iron availability

In an iron-limited environment, due to the weak concentration of heme and Fe-S, Irr protein is abundant and repress *rirA*. So SigI is not regulated by RirA and can induce siderophore synthesis. In an iron-rich environment, Irr is negatively regulated by the high concentration of heme. In these conditions, RirA is abundant and its binding to Fe-S allows the repression of SigI expression that leads to few expression of siderophore gene synthesis.

The ability to catch iron is important for competition in low-iron conditions and it is known that one plant defense mechanism against phytopathogen is iron scavenging (Aznar *et al.*, 2014; Niehus *et al.*, 2017). In this context, siderophores, the most widespread system in bacteria for iron acquisition are certainly important for tumor competitive colonization (Aznar *et al.*, 2014; Verbon *et al.*, 2017). The different species of *Agrobacterium* seem able to produce distinct siderophores, but the operon involved in siderophore synthesis has been characterized only in *A. fabrum* C58 (Rondon *et al.*, 2004). The deletion of this large gene cluster (nearly 50kb) abolishes the bacterial

capacity to survive in an iron limited-medium (Liu *et al.*, 2016; Rondon *et al.*, 2004). However, a tight control of siderophore gene expression is needed because this expression can be metabolically costly and can induce plant defense mechanisms (Verbon *et al.*, 2017; Harrison *et al.*, 2008; Miethke and Marahiel, 2007). Regulatory proteins controlling the siderophore cluster are RirA, Irr proteins and SigI, a sigma factor which is influenced by heme and Fe-S concentration (Hibbing and Fuqua, 2011; Qi *et al.*, 1999). The exact SigI mechanism of regulation and the structure of this siderophore remain unknown.

Moreover, it is known that bacteria can uptake iron thanks to ABC transporters. Among microorganisms that are highly adapted to plant environment, some possess multiple receptors allowing the catch of heterologous siderophores (Berendsen *et al.*, 2015; Lemanceau *et al.*, 2009; Loper and Buyer, 1991). The *Agrobacterium* genome contains 3 ABC transporters annotated as useful for iron uptake and up-regulated in tumor in comparison with culture media (González-Mula *et al.*, 2018). These transporters are encoded by the *atu2473–2476*, the *atu5311–atu5316* and the *atu0408–0406* genes. Neither information about the compound actually transported in *Agrobacterium* nor about the specificity of those transporters are available, but it is tempting to speculate that they should be important for heterologous siderophore import and long term persistence in tumor.

Conclusion

In conclusion, tumor is a hostile environment for agrobacteria that are submitted to plant defenses, hypoxic and drought conditions, as well as competition with other tumors inhabitants. Even if the opine concept explains a major part of the *Agrobacterium*-plant interaction, by their own it cannot explain the maintenance and the competitiveness of *Agrobacterium* in tumor. It is greatly influenced by survival to stress encountered, high metabolic capacities and efficient resource utilization which give a high capacity of colonization in order to outcompete organisms living in the same niche. However, the metabolites contained in tumor are sparsely described. To date, only one metabolomic study describe some tumor compounds and gave valuable but limited information. Indeed, it concerned the analysis of the 5 weeks old tumor content in only one plant species. Additional efforts are needed to describe the tumor content at different stages of the plant development and from different plants. Moreover, those analyses describe the metabolites that are present inside plant cells but that are not necessarily available for bacteria. Integrated analyses including plant metabolites and gene expression analyses for both partners would be essential to understand the different aspect of the long term-interaction inside tumor. Even if *Agrobacterium* has been subject to many functional studies, genetic characterizations are mostly focused on virulence genes and factors influencing the T-DNA integration and genetic transformation efficiency. There is a recent and growing interest in the characterization of genetic determinants allowing both the rhizosphere colonization, and the transition between the rhizosphere and pathogenic lifestyle. However, besides the characterization of new genetic clusters, an additional effort is needed not only to decipher the role of these clusters in bacterial virulence (ability to induce tumor) as it is often tested, but also to evaluate their role in the colonization and persistence both in the rhizosphere and the tumor. This would allow a better knowledge of key determinants of *Agrobacterium* ecology and thus, to get inside valuable information on disease persistence and dissemination.

Partie B : Influence écologique des acides hydroxycinnamiques et de leur dégradation : aspects trophiques et de signalisation

Dans la partie suivante, nous décrivons comment sont produits les HCAs, quels sont les déterminants génétiques et les mécanismes impliqués dans leur dégradation, et comment celle-ci est finement contrôlée. Nous décrivons l'importance de ces composés dans l'écologie de microorganismes phytopathogènes.

I. Biosynthèse des HCAs et rôles chez les végétaux

Les acides hydroxycinnamiques (HCAs) sont des composés phénoliques végétaux couramment retrouvés chez les plantes et qui regroupent les dérivés de l'acide cinnamique (Croteau *et al.*, 2000; Kumar and Pruthi, 2014; Zhao and Moghadasian, 2008). Du point de vue biosynthétiques, ils sont issus de la voie des phénylpropanoïdes décrite Figure 1 (Croteau *et al.*, 2000; Vogt, 2010). Cette voie débute par la conversion des acides aminés phénylalanine et tyrosine, respectivement en acide cinnamique et acide *p*-coumarique, par les enzymes Phénylalanine Ammonia-lyase (PAL) et Tyrosine Ammonia-lyase (TAL). L'enchaînement de réactions d'hydroxylation et de méthylation aboutit alors à la synthèse des dérivés de l'acide cinnamique qui sont les acides *p*-coumarique, caféique, 5-hydroxyfêrulique, fêrulique et sinapique. Outre cette série ubiquitaire d'HCAs, ces composés peuvent également être transformés en de nombreux autres métabolites par des voies biosynthétiques variées dont certaines peuvent être spécifiques d'un rôle ou d'une appartenance à un genre ou une famille. Ainsi, ces composés peuvent être activés sous la forme d'esters de Coenzyme A puis en monolignol par l'action respective de CoA ligase et de cinnamyl alcool deshydrogénase. De plus, l'un des dérivés des HCAs, le *p*-coumaroyl-CoA est aussi un précurseur de la synthèse des flavonoïdes. Ces derniers sont impliqués en outre dans la coloration des fleurs, la protection contre les stress, l'attraction des insectes. Certains flavonoïdes comme l'apigénine et la luteoline sont des molécules signal, intervenant dans l'interaction entre *Ensifer* et les légumineuses (Falcone Ferreyra *et al.*, 2012; Hassan and Mathesius, 2012). D'après Croteau *et al.* (Croteau *et al.*, 2000), les monolignols (en violet Figure 1) sont les unités qui sont polymérisées pour permettre la synthèse des lignines et d'autres structures végétale. La lignine joue un rôle primordial dans la rigidité de la paroi des végétaux et représente 40 % du carbone

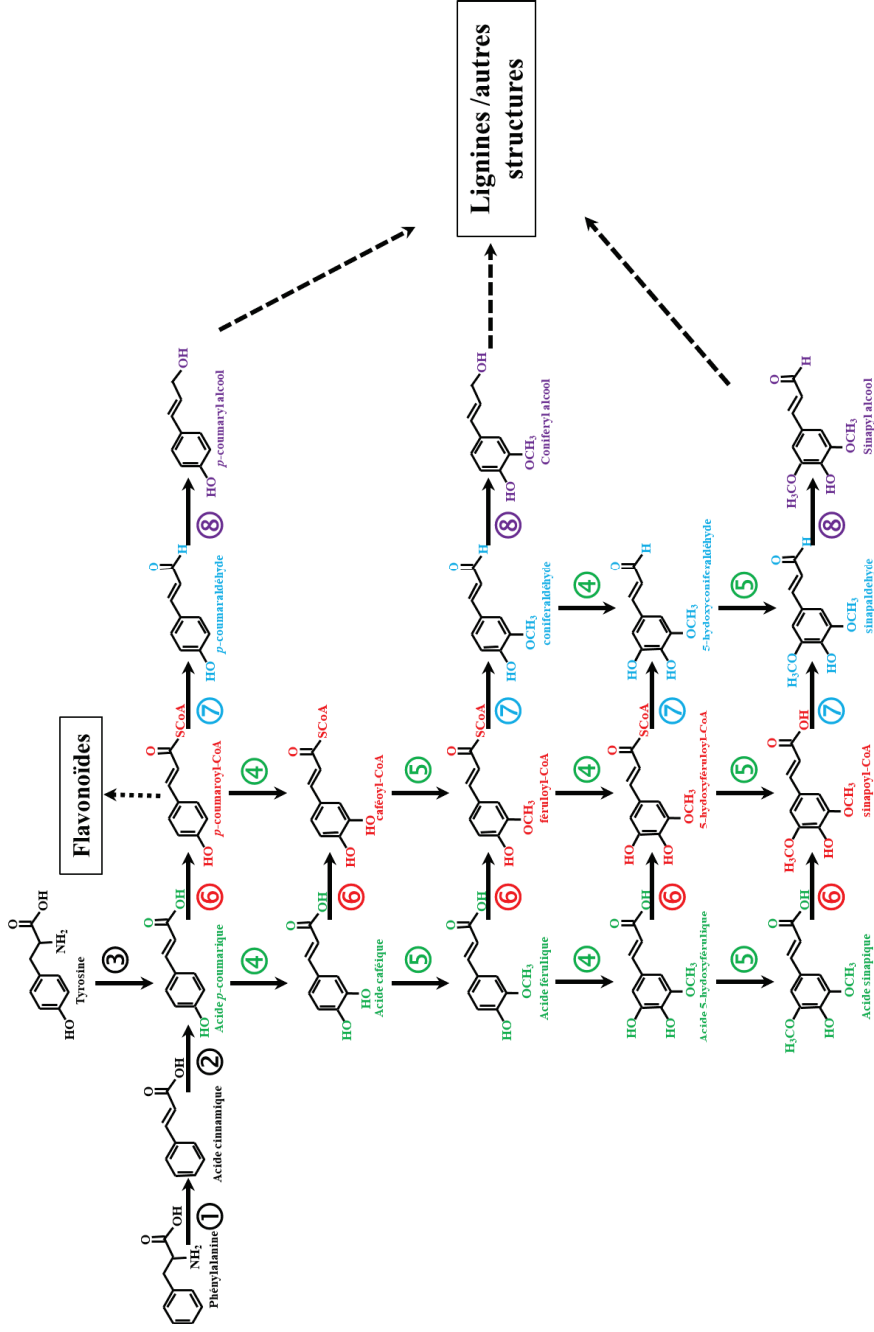


Figure 1: Voie de biosynthèse des phénylpropanoïdes

L'action de la PAL ① et d'une cinnamate-4-hydroxylase ③ ou d'une TAL ③: cinnamate-4-hydroxylase aboutit à la formation d'acide *p*-coumarique. Ce dernier est alors transformé via des hydroxylases ④ et des *O*-méthyltransférases ⑤ en différents HCAs (en vert). L'activation des HCAs via la fixation d'un CoA sur la chaîne insaturée par une CoA ligase ⑥ engendre la formation des intermédiaires indiqués en rouge. Ces composés sont ensuite modifiés par une cinnamoyl-CoA:NADPH oxydoréductase ⑦ en des dérivés aldéhydes des HCAs (en bleu). Ces aldéhydes sont transformés via l'action d'une cinnamyl alcool déshydrogénase ⑧ en monolignols (en violet) qui sont ensuite impliqués dans la synthèse des lignines et d'autres structures végétale. Adaptée d'après (Croteau *et al.*, 2000; Vogt, 2010).

présent sur Terre. A ce titre, elle joue un rôle important dans le cycle du carbone (Schlesinger, 1977). En plus d'être à la base de la synthèse de la lignine, les HCA sont aussi incorporés dans la paroi des végétaux où avec les lignines, ils jouent un rôle dans la rigidité structurale des tissus (Ishii, 1997).

Les HCAs et notamment leurs esters de CoA (fêruloyl-CoA, *p*-coumaroyl-CoA, sinapoyl-CoA) sont par ailleurs produits en grandes quantités pour renforcer les parois des végétaux en cas d'attaque de pathogène (Schmitt *et al.*, 1991). Ils sont relargués lors de la rupture des parois végétales et peuvent être exsudés au niveau de la rhizosphère (Bais *et al.*, 2006; Bakker *et al.*, 2013). Ils sont toxiques pour certaines bactéries, toutefois certaines sont capables de les détoxifier et/ou de s'en servir de ressource nutritive, et dans certains cas ce sont des molécules signal induisant des modifications d'expression de gènes (Joshi *et al.*, 2016; Leonard *et al.*, 2017; Yang *et al.*, 2008).

II. Dégradation bactérienne des HCAs

Les mécanismes de détoxification impliqués dans la résistance aux composés antimicrobiens impliquent généralement i) des modifications des protéines ou des structure ciblées, ii) la diminution de l'accumulation de ces composés via l'inhibition de perméase ou leur export via des pompes à efflux et iii) l'inactivation enzymatique du composé toxique (Blanco *et al.*, 2016; Li *et al.*, 2015a; Nikaido, 2009).

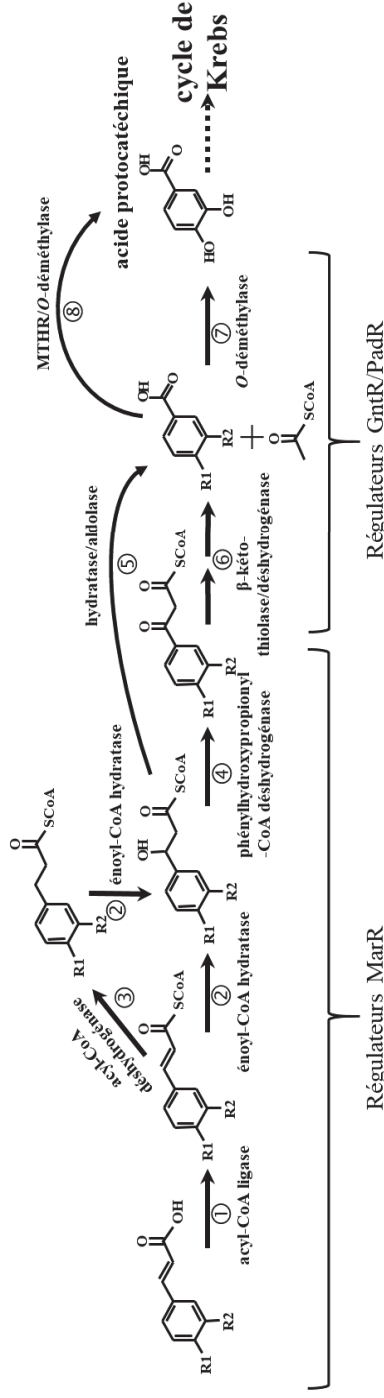
Dans le cas des HCAs, des pompes à efflux sont induites par ses composés mais l'export de ces derniers n'a jamais été testé (Ravirala *et al.* 2007; Brown *et al.* 2007). Le principal mécanisme de détoxification des HCAs est alors leur dégradation enzymatique. Cette dégradation conduit alors soit à la transformation d'une molécule en une autre molécule moins toxique, soit à la dégradation totale de ce composé et dans certains cas à son utilisation comme ressource énergétique et trophique. La transformation d'un HCA en un composé moins toxique peut être réalisée par une *O*-déméthylase comme chez *A. fabrum* C58, avec le produit du gène *virH2* qui transforme l'acide fêrulique en acide caféique (Brencic *et al.*, 2004). D'autres microorganismes comme *Enterobacter sp.*, *Bacillus sp.*, et *Lactobacillus plantarum*, sont capables de réaliser cette transformation via l'action d'une décarboxylase qui peut par exemple décarboxyler l'acide fêrulique en 4-vinylguaiacol pour transformer ce dernier en vanilline, qui est peu toxique

(Barthelmebs *et al.*, 2000; Gu *et al.*, 2011; Prim *et al.*, 2003). Cette transformation est par ailleurs intéressante d'un point de vue économique, car elle permet de transformer des composés végétaux contenant des HCAs en vanilline, produit commercialement valorisable (Kroon and Williamson, 1999; Plaggenborg *et al.*, 2006).

D'autres microorganismes sont non seulement capables de dégrader complètement les HCAs mais également de les utiliser comme ressource trophique (Campillo *et al.*, 2014; Karishma *et al.*, 2015; Lowe *et al.*, 2014; Overhage *et al.*, 1999). Les microorganismes peuplant la rhizosphère sont en présence d'autres ressources trophiques en plus des HCAs, notamment les acides aminés, les sucres et d'autres acides organiques. Il y a alors une certaine hiérarchie propre à chaque espèce bactérienne, où des sources de carbone vont être préférentiellement métabolisées au dépend d'autres. Chez *A. baylyi* il a été démontré une répression de l'expression des gènes de dégradation des HCAs (région génomique des gènes *hca*) en présence de succinate ou d'acétate (Fischer *et al.*, 2008). Les mécanismes et les gènes associés à ces régulations ne sont pas décrits, et il n'existe pas d'autre publication ayant évalué cette hiérarchie chez d'autres microorganismes. Au contraire, il a été récemment décrit que les microorganismes rhizosphériques préféraient consommer les acides organiques aromatiques (Zhalnina *et al.*, 2018) dont font partie les HCAs. C'est le cas chez *P. putida* CSV86 qui est capable de dégrader les HCAs et de les utiliser comme ressource énergétique. Lorsque cette souche est cultivée en présence d'acide férulique et de glucose, une croissance diauxique (deux phases exponentielles de croissance distinctes) est observée. Durant la première phase exponentielle, les gènes de dégradation de l'acide férulique sont activés alors que dans la seconde phase exponentielle de croissance le taux de glucose dans le milieu diminue (Karishma *et al.*, 2015). Les mêmes résultats ont été décrits en présence de vanilline ou d'acide vanillique et de glucose (Karishma *et al.*, 2015). Ainsi *P. putida* CSV86 présente une préférence trophique pour l'utilisation des HCAs par rapport au glucose (Karishma *et al.*, 2015).

Concernant, les voies métaboliques menant à la dégradation des HCAs (comme ressources trophiques), il existe deux voies métaboliques différentes. Toutes deux nécessitent une désacétylation dépendante du CoA, l'une étant β -oxydative et l'autre non β -oxydative. Ces deux voies mènent à des composés qui seront intégrés dans la voie des β -kétoadipates, permettant leur utilisation comme ressource trophique et énergétique via leur intégration dans le cycle de

Dégradation des HCAs par désacétylation β -oxydative



Dégradation des HCAs par désacétylation non β -oxydative

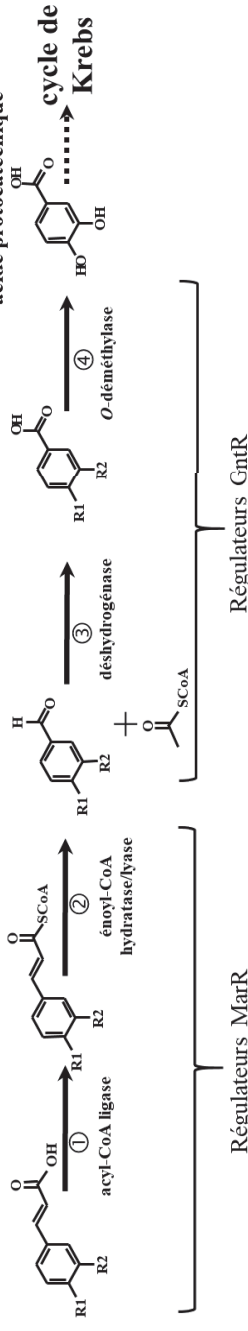


Figure 2: Voies de dégradation des HCAs par désacétylation β -oxydative et non β -oxydative dépendante du CoenzymeA

Les HCAs qui peuvent être dégradés sont l'acide férulique : R1 = OH ; R2 = OCH₃, l'acide caféique : R1 = OH ; R2 = OH et l'acide *p*-coumarique : R1 = OH. Les enzymes impliqués successivement sont indiqués. L'intégration de l'acide protocatéchique dans le cycle TCA est réalisée via la voie des β -kétoadipates. Les familles de régulateurs impliqués dans le contrôle de l'expression des différents gènes sont indiquées en dessous.

Krebs, assurant ainsi une croissance bactérienne (Harwood and Parales, 1996; MacLean *et al.*, 2006). Les différentes étapes de ces deux voies de dégradation sont présentées en Figure 2.

II.1. La désacétylation β -oxydative dépendante du Coenzyme A

La désacétylation β -oxydative dépendante du Coenzyme A est une voie analogue à celle du catabolisme des acide gras (Overhage *et al.*, 1999). Cette voie de dégradation a été proposée chez *Rhodotorula rubra* et *Aromatoleum aromaticum* (Huang *et al.*, 1993; Trautwein *et al.*, 2012) et caractérisée via de la mutagénèse et l'analyse des différents intermédiaires de dégradation produits chez *Corynebacterium glutamicum* ATCC 13032, *Rhodococcus jostii* RHA1, *Rhodopseudomonas palustris* CGA009 et *A. fabrum* (Campillo *et al.*, 2014; Hirakawa *et al.*, 2012; Kallscheuer *et al.*, 2016; Otani *et al.*, 2014, 2016). Le tableau 1 est une synthèse des informations concernant les enzymes impliquées et leurs fonctions selon les microorganismes. Mêmes si les enzymes de cette voie permettent de prendre en charge différents HCA, nous décrirons ci-dessous essentiellement les différentes étapes de la dégradation de l'acide férulique. La première étape d'activation, commune à toutes les bactéries, consiste en l'activation par un CoA au niveau de la chaîne latérale insaturée via l'action d'une acyl-CoA ligase (appelée aussi fatty acid-CoA ligase ou acyl-CoA synthase), formant le féruloyle-CoA. Chez *C. glutamicum*, au niveau de la chaîne insaturée du féruloyle-CoA, une déshydrogénation par une acyl-CoA-déshydrogénase, puis une hydratation de l'ester CoA ainsi formé par une énoyle-CoA hydratase, permettent la formation d'acide 4-hydroxy-3-méthoxyphényl- β -hydroxypropionique-CoA (HMPHP-CoA) (Kallscheuer *et al.*, 2016). Chez la majorité des autres bactéries, cette deuxième étape est en revanche réalisée par une seule enzyme, une énoyle-CoA hydratase qui catalyse la réaction d'hydratation de l'ester CoA et permet sa conversion en HMPHP-CoA. Le HMPHP-CoA est ensuite transformé en acide 4-hydroxy-3-méthoxyphényl- β -kétopropionique-CoA (HMPKP-CoA) par l'action d'une phényl-hydroxypropionyle-CoA déshydrogénase. Chez *A. fabrum*, *C. glutamicum* et *R. jostii*, le HMPKP-CoA est clivé en acide vanillique et en acétyl-CoA par une HMPKP-CoA β -kétotliolase/aminohydrolase (Campillo *et al.*, 2014; Hirakawa *et al.*, 2012; Kallscheuer *et al.*, 2016). Chez *R. palustris* CGA009, ces deux dernières étapes sont réalisées directement par une seule enzyme, une hydratase/aldolase (Otani *et al.*, 2014, 2016). L'acide vanillique est transformé en acide protocatéchique, par l'action d'une *O*-déméthylase codée par les gènes

Tableau 1 : Voies et gènes impliqués dans la dégradation des HCAs selon les espèces bactériennes.

Souches	Métabolites pris en charge	Voie de catabolisme	acyl-CoA ligase ①	énoyl-CoA hydratase/lyase② ou énoyl-CoA hydratase/aldolase⑤	acyl-CoA déshydrogénase ③	phénylhydroxypropionyl-CoA déshydrogénase ④	Vanillin déshydrogénase ④	β-kéto-thiolase/aminohydrolase ⑥	MTHR ⑦	O-déméthylase ⑧
<i>A. fabrum</i> C58	FA, CA, pCA	β-oxydative	<i>atu1416</i>	<i>atu1417</i>	Absent	<i>atu1415</i>	<i>vdh(atu4645) ?</i>	<i>atu1421</i>	<i>atu1418</i>	<i>atu1420</i>
<i>C. glutamicum</i> ATCC 13032	FA, CA, pCA	β-oxydative	<i>pdhA</i>	<i>pdhE</i>	<i>pdhD</i>	<i>pdhB</i>	<i>vdh</i>	<i>pdhC</i>	Absent	<i>vanAB</i>
<i>R. jostii</i> RHA1	FA, CA, pCA et dihydroferulique	β-oxydative	<i>coul</i>	<i>couM</i>	Absent	<i>couN</i>	Absent	<i>couO</i>	Absent	<i>vanAB</i>
<i>R. palustris</i> CGA009	FA, CA, pCA	β-oxydative	<i>couB</i>	<i>couA</i>	Absent	Absent	Absent	Absent	Absent	Non déterminée
<i>Sphingobium</i> sp.SYK-6	FA, CA, pCA	non β-oxydative	<i>ferA</i>	<i>ferB</i>	Absent	Absent	<i>ligV</i>	Absent	Absent	<i>ligM</i>
<i>P. putida</i>	FA, CA, pCA	non β-oxydative	<i>fcs</i>	<i>ech</i>	Absent	Absent	<i>vdh</i>	Absent	Absent	<i>vanAB</i>
<i>Acinetobacter</i> sp. ADP1	FA, CA, pCA	non β-oxydative	<i>hcaC</i>	<i>hcaA</i>	Absent	Absent	<i>hcaB</i>	Absent	Absent	<i>vanAB</i>
<i>P. fluorescens</i> BF13	FA, pCA	non β-oxydative	<i>fcs</i>	<i>ech</i>	Absent	Absent	<i>fcd? / vdh</i>	Absent	Absent	<i>vanAB</i>
<i>R. solanacearum</i>	FA, CA, pCA	non β-oxydative	<i>fcs</i>	<i>fca</i>	Absent	Absent	<i>vdh</i>	Absent	Absent	<i>vanAB</i>

Le signe « ? » indique les gènes pour lesquels, il y a une incertitude. FA= acide férulique, CA= acide caféique et pCA= acide p-coumarique.

vanAB. Chez *A. fabrum* cette étape est spécifique et nécessite l'action successive d'une méthylènetétrahydrofolate réductase (MTHR) et d'une *O*-déméthylase. L'acide protocatéchique est par la suite dégradé via la voie métabolique des β -kétoadipates (Harwood and Parales, 1996; MacLean *et al.*, 2006; Parke, 1995). La dégradation de l'acide *p*-coumarique, qui ne comporte pas de groupement méthyl, nécessite uniquement l'activité des enzymes impliquées dans les 4 premières étapes de dégradation de l'acide férulique, à savoir une acyl-CoA ligase, une énoyl-CoA hydratase, une phénylhydroxypropionyl-CoA déshydrogénase et une β -kéto-thiolase/aminohydrolase ou une hydratase/aldolase. Ces différentes étapes mènent à la formation respectivement de *p*-coumaroyl-CoA, de 4-hydroxy-phényl- β -hydroxypropionyl (HPHP)-CoA de 4-hydroxy-phényl- β -kétopropionyl-CoA (HPKP)-CoA, et d'acide *p*-hydroxybenzoïque (*p*-HB) (Campillo *et al.*, 2014; Hirakawa *et al.*, 2012; Otani *et al.*, 2014). Le *p*-HB est hydroxylé par une *p*-hydroxybenzoate hydroxylase ce qui engendre la formation d'acide protocatéchique, dégradé via la voie des β -kétoadipates.

II.2.La désacétylation non β -oxydative dépendante du CoenzymeA

La désacétylation non β -oxydative dépendante du CoenzymeA a été décrite chez *Sphingobium sp.* SYK-6, *P. putida*, *P. fluorescens* BF13, *Acinetobacter sp.* ADP1, et *Ralstonia solanacearum* (respectivement Kasai *et al.*, 2012; Venturi *et al.*, 1998; Calisti *et al.*, 2008; Kim *et al.*, 2016; Parke and Ornston, 2003). La première étape est aussi réalisée par une acyl-CoA ligase et aboutit à la formation d'un ester de CoA au niveau de la chaîne insaturée. Les hydroxycinnamoyl-CoA (caféoyl-CoA, féruloyl-CoA, *p*-coumaroyl-CoA) sont alors pris en charge par une enzyme ayant la double fonction d'hydratase et de lyase. L'action de cette enzyme produit la vanilline, qui n'est jamais produite dans la voie β -oxydative. La vanilline est ensuite transformée en acide vanillique, lui-même réduit en acide protocatéchique par l'action respective d'une phényl-hydroxypropionyl-CoA déshydrogénase et d'une *O*-déméthylase. Comme après la désacétylation β -oxydative, l'acide protocatéchique produit est utilisé comme ressource trophique via sa dégradation par la voie des β -kétoadipates.

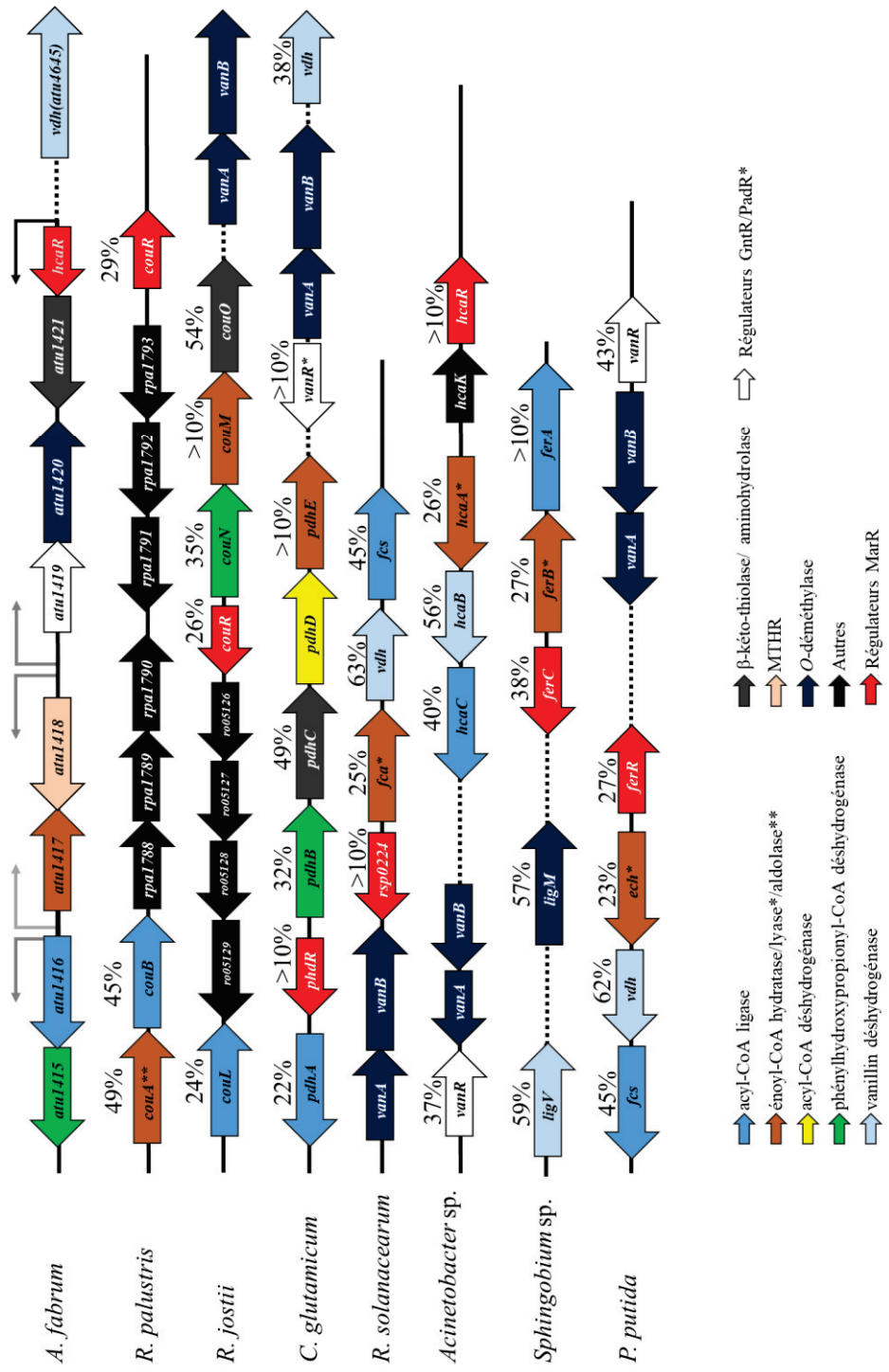


Figure 3 : Organisation des clusters de dégradation des HCAs chez *A. fabrum*, *C. glutamicum*, *R. jostii*, *R. palustris*, *Sphingobium sp.*, *Acinetobacter sp.*, *R. solanacearum* et *P. putida*. Les pourcentages indiquent l'identité en acide aminés en comparaison avec leurs homologues chez *A. fabrum*.

III. Régulation de la dégradation des HCAs

La régulation de la voie de dégradation des HCAs est impliquée dans la perception des changements environnementaux. D'après Perera et Grove (Perera and Grove, 2010), elle permet d'apporter une réponse appropriée en coordonnant l'expression des gènes adéquats à la situation, notamment en évitant une synthèse coûteuse d'enzymes en absence de ces composés. Les gènes régulés sont très généralement organisés en opéron, comme cela est indiqué en Figure 3, ce qui permet la coordination de ces gènes par un régulateur présent lui aussi dans la région génomique et qui très souvent s'autorégule (Deochand and Grove, 2017). Les gènes de dégradation ont des fonctions assez similaires (Tableau 2) mais diffèrent par leurs séquences en acide aminés chez ses bactéries (Figure 3). Nous observons les mêmes résultats concernant les régulateurs qui bien qu'ils diffèrent peu par les familles de régulateurs impliqués, diffèrent selon les espèces, notamment en termes de molécules perçues (ligand), ou par les séquences de fixation reconnues (Figure 3, Figure 4 et Tableau 2). Les régulateurs de la famille MarR (Deochand and Grove, 2017; Grove, 2017) contrôlent l'expression des gènes impliqués dans les premières étapes de la dégradation jusqu'à l'acide vanillique ou la vanilline et des régulateurs de type GntR (*Gluconate Regulator*) ou PadR (*Phenolic acid decarboxylase Regulator*) contrôlent l'expression des gènes impliqués dans la fin de la voie de catabolisme des HCAs (Figure 2).

III.1. Régulation des premières étapes de dégradation : régulateurs MarR

La famille des régulateurs MarR est un sous-groupe de facteurs de transcription qui contient environ 12 000 membres appartenant à des genres bactériens différents (Deochand and Grove, 2017; Grove, 2017). Le nombre moyen de régulateurs MarR par génome bactérien est de 7, cependant cette moyenne comprend de grandes disparités (Grove, 2017). En effet, les régulateurs de type MarR seraient presque absents chez des bactéries spécialistes d'une niche écologique comme *Helicobacter pylori*, inféodé à l'estomac, et le symbiote du puceron *Buchnera aphidicola* str. APS qui ne contiennent aucun régulateur MarR dans leur génome. Ces régulateurs sont retrouvés en plus grand nombre dans les génomes de bactéries capables d'adopter différents styles de vies tel que *A. fabrum* C58 qui contient 30 régulateurs de cette famille dans son génome et *Bradyrhizobium japonicum* USDA6 qui lui présente 39 régulateur MarR annoté dans son génome (Ellison and Miller, 2006) . Les régulateurs de type MarR sont

Tableau 2 : Régulation transcriptionnelle des gènes de dégradation des HCAs selon les espèces bactériennes

Souches	Composés inducteurs début de la voie	Régulateurs impliqués	Séquences reconnues par le régulateur MarR	Références
<i>Sphingobium sp.</i> SYK-6	féruoyl-CoA, <i>p</i> -coumaroyl-CoA, caféoyl-CoA et sinapoyl-CoA	FerC (MarR)	CGATGGCTTGCTCCCATCG ATGCTATGGCTTATAGCAT	(Kamimura <i>et al.</i> , 2017; Kasai <i>et al.</i> , 2012)
<i>P. putida</i>	Non déterminés	HcaR (MarR)/ VanR (GntR) ?	Non décrites	(Hughes <i>et al.</i> , 2017; Jiménez <i>et al.</i> , 2002; Plaggenborg <i>et al.</i> , 2001)
<i>Acinetobacter. sp.</i> ADP1	acide férulique, acide 3,4-dihydroxybenzoïque, acide <i>p</i> -coumarique et vanilline	HcaR (MarR)/ VanR(GntR)	GAATATCAGTTAAACTGATATTC	(Kim <i>et al.</i> , 2016; Parke and Ormston, 2003)
<i>P. fluorescens</i> BF13	féruoyl-CoA	FerC (MarR)/ VanR (GntR) ?	Non décrites	(Calisti <i>et al.</i> , 2008)
<i>C. glutamicum</i> ATCC 13032	Non déterminés	PhdR (MarR)/ VanR (PadR)	Non décrites	(Kallscheuer <i>et al.</i> , 2016; Morabbi Heravi <i>et al.</i> , 2015)
<i>R. jostii</i> RHA1	féruoyl-CoA, <i>p</i> -coumaroyl-CoA	CouR (MarR)	GATTGAGAAATGTCAATG CATTGACAAAGTTTCAAATG	(Otani <i>et al.</i> , 2014, 2016)
<i>R. palustris</i> CGA009	<i>p</i> -coumaroyl-CoA	CouR (MarR)	GTTATACTCTATAAC	(Hirakawa <i>et al.</i> , 2012)
<i>R. solanacearum</i>	Non déterminés	RSp0224 (MarR)?	GATATCAGGCCCGCTGATAIC ?	(Lowe <i>et al.</i> , 2014)
<i>A. fabrum</i> C58	féruoyl-CoA, <i>p</i> -coumaroyl-CoA	HcaR (MarR) /Atu1419 (GntR)?	TTACAAGGATATTATTTTCTTGTAAT	(Campillo <i>et al.</i> , 2014; Meyer <i>et al.</i> , 2018a)

Le signe « ? » indique les gènes à proximité du cluster de dégradation annotés comme des régulateurs mais dont la fonction n'a pas été validée expérimentalement.

décrits comme étant impliqués dans la répression de gènes codant pour i) des voies métaboliques parmi lesquelles les voies du catabolisme des HCAs (Perera and Grove, 2010), ii) la résistance au stress oxydatif, aux antibiotiques et aux métaux (Gundogdu *et al.*, 2015; Hao *et al.*, 2014), iii) pour des facteurs de virulence (Haque *et al.*, 2009). La partie suivante décrit les mécanismes de régulation et l'implication de ces régulateurs dans la régulation du catabolisme des HCAs.

III.1.1. Mécanisme de fixation des régulateurs MarR

Les protéines MarR agissent sous forme dimérique où chaque monomère possède un domaine wHTH qui permet la spécificité de reconnaissance et la fixation à certaines séquences nucléotidiques (Figure 4 et Tableau 2). Les séquences reconnues et décrites pour les régulateurs MarR de *Sphingomonas* sp. SYK-6, *Acinetobacter* sp. ADP1, *R. jostii* RHA1, *R. palustris* CGA009 sont indiquées dans le Tableau 2. Ce sont des séquences inversées, répétées (deux fois) et situées aux niveaux des régions promotrices des gènes cibles (Figure 4), en amont des premiers gènes de catabolisme des HCAs et du régulateur lui-même (séquence souvent imparfaite). Les séquences sont spécifiques de chaque régulateur MarR (Tableau 2), et leur taille varie de 20 à 30 pb. Cette fixation se fait en absence d'HCAs et aboutit à la répression de l'expression des gènes cibles (Figure 4). Différents mécanismes de régulation ont été proposés pour cette famille de régulateur. Il a été suggéré qu'ils se fixaient soit (i) en amont de l'ADN polymérase et empêchait ainsi son recrutement (Martin *et al.*, 1996), soit (ii) au niveau du site de fixation d'un activateur de la voie, entrant en compétition avec celui-ci (Galán *et al.*, 2003).

III.1.2. Levée de la répression

L'induction des voies de catabolisme des HCAs nécessite la levée de la fixation du répresseur MarR à l'ADN. Cette levée est produite par la fixation d'un ligand à ce régulateur. Plusieurs ligands peuvent être reconnus par une même protéine régulatrice. C'est le cas du régulateur HcaR d'*Acinetobacter* sp. qui fixe les acides férulique, 3,4-dihydroxybenzoïque, *p*-coumarique et la vanilline (Kim *et al.*, 2016). Les ligands sont dans ce cas les HCAs, autrement dit le substrat des enzymes de dégradation. Les molécules qui lèvent la répression peuvent aussi être des dérivés liés à un CoA, donc des intermédiaires de la dégradation. En effet, chez *Rhodococcus* sp., *R. palustris* et *P. fluorescens* BF13, les ligands sont le féruloïl-CoA et le *p*-coumaroïl-CoA

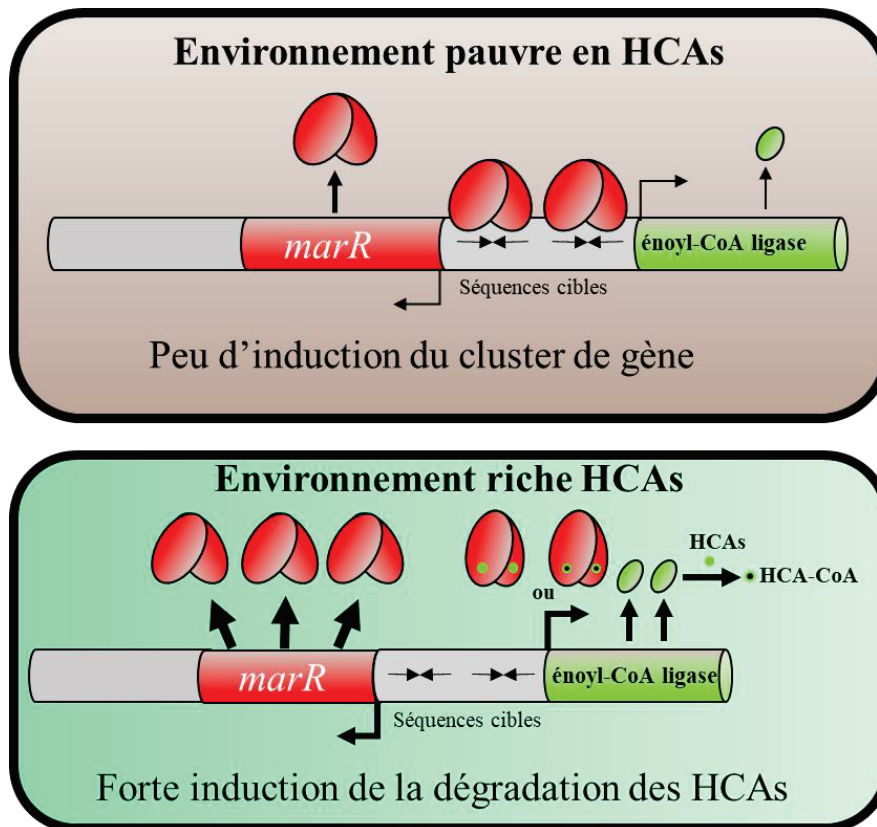


Figure 4 : Mécanisme de régulation par les régulateurs MarR.

Les gènes de dégradation sont retrouvés dans une même région génomique (cluster de gènes) dont le premier gène transcrit est l'énoyl-CoA ligase. Le gène codant pour le régulateur MarR et le premier gène impliqué dans le catabolisme des HCAs (énoyl-CoA ligase) sont en général divergents. Le régulateur MarR en absence d'HCA se fixe sous forme dimérique au niveau des séquences inversées répétées ($\rightarrow\leftarrow$) et chaque monomère fixe une partie de la région inversée répétée (\rightarrow ou \leftarrow). Dans ces conditions, il y a seulement un niveau basal de transcription du régulateur MarR et de l'énoyl-CoA ligase. En présence d'HCAs, la fixation du ligand par la protéine régulatrice, induit un changement de conformation du régulateur ce qui lève sa fixation à l'ADN. Le ligand suivant le régulateur MarR est soit un HCA ou un HCA-CoA dont la formation est catalysée par les quelques énoyl-CoA ligase produites en condition de répression. La levée de répression induit l'induction de la voie de catabolisme des HCAs. Lorsque le ligand est un HCA-CoA il y a alors création d'une boucle de rétroaction positive. La présence des ligands induisent alors l'expression de l'énoyl-CoA ligase ce qui induit encore plus de production des ligands, d'après (Grove, 2017; Perera and Grove, 2010).

(Otani *et al.*, 2016, 2014; Pan *et al.*, 2015; Calisti *et al.*, 2008; Hirakawa *et al.*, 2012). Dans le cas où les dérivés CoA sont les ligands du répresseur, il doit exister un niveau basal d'expression de l'énoyl-CoA ligase de la production de ce dérivé. En présence d'HCAs, le niveau basal d'expression du gène codant pour l'énoyl-CoA ligase est suffisant pour la production d'une quantité de ligand permettant la levée de la répression. La perception du ligand aboutit à la levée de la répression et ensuite à une boucle de rétroaction positive (Figure 4). La fixation des ligands (HCAs ou des dérivés) stabiliserait le régulateur MarR dans une conformation qui va diminuer son affinité pour la séquence nucléotidique reconnue induisant ainsi une levée de la répression (Kim *et al.*, 2016).

III.2. Régulation des gènes impliqués dans la dégradation de l'acide vanillique.

La régulation de la deuxième partie de la voie correspond à la dégradation de la vanilline ou de l'acide vanillique. En plus d'être produits via la dégradation des HCA, ces composés peuvent être exsudés au niveau de la rhizosphère. Ainsi une régulation indépendante des premières étapes de la voie permet d'optimiser la dégradation de la vanilline et l'acide vanillique indépendamment de leurs provenances. Le régulateur et les gènes *vanA* et *vanB* sont organisés en cluster et peuvent se retrouver dans une même région génomique que les gènes du début de la voie de dégradation (comme chez *A. fabrum* et *R. solanacearum*, Figure 3) ou être présent dans une autre région du génome (comme observé chez *C. glutamicum*, *Acinetobacter sp.* et *P. putida*, Figure 3). Les études concernant les régulateurs de l'expression des gènes de la fin de la voie de dégradation des HCAs sont peu nombreuses. Chez les bactéries possédant la voie de dégradation β -oxydative, des régulateurs appartenant à deux familles différentes (GntR et PadR) ont été proposés ou prédits pour contrôler l'expression des gènes de dégradation de l'acide vanillique, pour la voie de dégradation non β -oxydative il est retrouvé uniquement des régulateurs de la famille GntR. Aucun régulateur contrôlant l'expression du gène *vdh* (déshydrogénase ④, Figure 2) impliqué dans la dégradation non β -oxydative de la vanilline n'a été décrit à ce jour.

Chez *Acinetobacter sp.* un facteur de transcription nommé VanR (GntR) régule négativement l'expression des gènes *vanAB*, codant les protéines permettant la transformation (non- β -oxydative Tableau 1) de l'acide vanillique en acide protocatéchique (Figure 2, (Morawski *et al.*, 2000). Ce régulateur ainsi que les gènes *vanA* et *vanB* sont aussi présents chez *Caulobacter*

crenscentus qui pourtant ne dégrade pas les HCAs mais uniquement l'acide vanillique (Morawski *et al.*, 2000; Thanbichler *et al.*, 2007). VanR appartient à la famille des GntR, une famille de facteur de transcription décrit pour la première fois comme réprimant l'opéron gluconate (Hoskisson and Rigali, 2009; Suvorova *et al.*, 2015). Il y a actuellement plus de 40 000 GntR dans la base de donnée PFAM avec, comme pour les régulateurs de la famille MarR, une corrélation entre la diversité des styles de vie et le nombre de régulateur GntR (Finn *et al.*, 2016; Hoskisson and Rigali, 2009), (Vindal *et al.*, 2007). Ces protéines sont similaires entre elles au niveau de leur domaine N-terminal wHTH, mais plus variables pour la partie C-terminale impliquée dans la dimérisation et la fixation de ligands. Comme les régulateurs MarR, les régulateurs GntR agissent sous forme dimérique, et se fixent à l'ADN de façon symétrique au niveau des séquences d'opérateurs où chaque monomère reconnaît un site. Le ligand permettant d'abolir cette répression semble être l'acide vanillique chez *C. crescentus* et *Acinetobacter sp.* En effet, la présence d'acide vanillique active fortement l'expression des gènes *vanAB*. Cependant, aucune étude structurale n'a permis de démontrer une fixation directe de ce ligand par VanR (Morawski *et al.*, 2000; Thanbichler *et al.*, 2007). Pour *A. fabrum*, le régulateur Atu1419 de la famille GntR est annoté comme permettant la régulation de la dégradation des HCAs et donc potentiellement celle de l'acide vanillique.

Chez *C. glutamicum* le régulateur, aussi nommé VanR, appartient à la famille PadR. Les régulateurs de cette famille ont été décrits comme régulant l'expression de fonctions intervenant dans l'interaction avec l'hôte, dans la virulence et dans la résistance au stress (Brinkrolf *et al.*, 2006; Finn *et al.*, 2016). Ils sont caractérisés par la présence d'un domaine C-terminal permettant la dimérisation et d'un domaine N-terminal wHTH constitué de 80-90 résidus permettant la fixation à l'ADN. Dans le cas de VanR, il se fixe aux niveaux de séquences inversées répétées situées dans la région intergénique entre *vanR* et *vanAB*. Le ligand reconnu est l'acide vanillique et la fixation de ce composé induit un relâchement de l'ADN et la transcription des gènes *vanAB* (Morabbi Heravi *et al.*, 2015).

IV. Autres rôle écologique de la dégradation des HCAs

La dégradation des HCAs peut permettre non seulement leur transformation en une molécule moins toxique, mais aussi son utilisation comme ressource trophique. Mais, le rôle des HCAs dans les interactions plante-bactéries n'est pas seulement lié à leur toxicité et à la nutrition bactérienne. En effet, la distribution des HCAs est spatialement et temporellement hétérogène que ce soit au niveau de la rhizosphère ou le long de l'axe racinaire (Compant *et al.*, 2010; Walker *et al.*, 2003). Le gradient d'HCAs perçu peut alors influencer la perception par les bactéries des plantes. Ces composés peuvent alors être répulsifs ou attractifs pour les bactéries, et induire des phénomènes de chimiotactisme conduisant certaines bactéries vers la racine. Certains HCAs ont aussi un rôle dans la communication bactérienne.

IV.1. HCAs et chimiotactisme

L'effet chimioattractant des HCAs a été démontré chez de nombreuses bactéries telles que *A. fabrum*, *Bradyrhizobium japonicum*, et *P. putida* F1 (Ashby *et al.*, 1988; Guo *et al.*, 2017; Hughes *et al.*, 2017; Kape *et al.*, 1991; Parke *et al.*, 1985). Chez *B. japonicum*, le chimiotactisme est induit directement par les HCAs. Au contraire, chez *P. putida* F1, ce sont les intermédiaires de dégradation des HCAs qui induisent le chimiotactisme (Hughes *et al.*, 2017). En effet, le chimiotactisme en présence des acides férulique, coumarique et caféique est dans un premier temps réprimé par le régulateur HcaR (famille MarR), puis induit lorsque ces composés sont activés en féruloyl-CoA, *p*-coumaroyl-CoA et caféoyl-CoA respectivement (les premiers intermédiaires de dégradation des HCA). Ces résultats sont confirmés par le fait qu'un mutant du gène codant pour l'acyl-CoA ligase n'est plus attiré par les HCAs (Hughes *et al.*, 2017).

IV.2. HCAs et communication bactérienne

Le *quorum sensing* (QS) est un processus menant à la communication entre cellules bactériennes. Cette communication nécessite la synthèse et la reconnaissance de molécules de petites tailles qui sont appelées auto-inducteurs et qui peuvent être de natures différentes (Pereira *et al.*, 2013; Reading and Sperandio, 2006). La détection d'une concentration seuil de ces molécules est perçue par les bactéries comme un signal induisant alors une modification de l'expression génique. Les gènes régulés par le QS peuvent être impliqués dans diverses fonctions telles que la formation de biofilm, le transfert conjugatif, le swarming, la

luminescence et la sécrétion de facteurs de virulence (Papenfort and Bassler, 2016; Pereira *et al.*, 2013; Rutherford and Bassler, 2012; Whitehead *et al.*, 2001).

Dans ce contexte, Bodini *et al.* (2009) ont démontré à l'aide de gènes (fluorescent ou luminescent) rapporteurs de l'expression de gènes contrôlé par QS, qu'en présence d'acide *p*-coumarique et indépendamment de son effet toxique, l'activité de type QS diminuait fortement chez *A. fabrum*, *Chromobacterium violaceum* et *P. chlororaphis* (Bodini *et al.*, 2009). Chez les phytopathogènes *Pseudomonas aroidearum* et *P. carotovorum* ssp. *brasiliense*, le QS régule les gènes impliqués dans la formation de biofilm et dans l'expression de facteur de virulence. L'analyse par qRT-PCR de l'expression des gènes impliqués dans le QS couplée à l'analyse des molécules du QS produites en présence d'acide cinnamique, d'acide *p*-coumarique et de vanilline, a démontré que le QS et en conséquence la formation de biofilm était réduite chez ses souches (Joshi *et al.*, 2015, 2016). De plus, ils ont observé dans les mêmes études, une diminution de l'expression des gènes de virulence, corrélée à une réduction de la virulence sur disques foliaires de *Calla lily* et sur tubercules de pommes de terre en présence de concentrations non létales d'HCAs pures. Cependant les mécanismes d'actions de ces composés sur ces fonctions n'ont pas été décrits.

Selon les microorganismes, les HCAs peuvent aussi servir de précurseur pour la synthèse des molécules du QS. Schaefer *et al.* (2008) ont montré que *R. palustris*, *Bradyrhizobium* sp. et *Silicibacter pomeroyi* utilisaient l'acide *p*-coumarique comme substrat pour synthétiser des *p*-coumaroyl-HSL, molécule autoinductrice chez ces bactéries. En outre, parmi les gènes régulés par cette molécule plusieurs sont impliqués dans le chimiotactisme. Ainsi, il a été proposé que la perception des molécules de *p*-coumaroyl-HSL couplé à la mobilité permettait de guider les bactéries vers les racines grâce au gradient en acide *p*-coumarique (Palmer and Blackwell, 2008). Le même phénomène a été proposé chez *Bradyrhizobium* BTAi1 qui en plus de former du *p*-coumaroyl-HSL est capable sans apport exogène de former du cinnamoyl-HSL (Ahlgren *et al.*, 2011). La production dans un hôte hétérologue (*E. coli*) de l'enzyme permettant la production de *p*-coumaroyl-HSL permet aussi la synthèse des fatty-HSL à partir d'acide férulique et d'acide caféique. Ces composés peuvent interférer négativement avec le QS chez *A. fabrum* (Kang *et al.*, 2015, 2017). Il serait ainsi intéressant de tester si en condition naturelle

le *p*-coumaroyl-HSL produit par *R. palustris* module la communication d'autres genres bactériens différents au sein du microbiote de la plante.

V. HCAs et influence global dans l'écologie de phytopathogènes

L'incidence globale des HCAs sur l'écologie de certaines bactéries n'a actuellement pas été décrite. En effet, plusieurs travaux suggèrent que la présence des HCAs dans l'environnement peut avoir un effet important sur l'expression des gènes (et donc sur la physiologie bactérienne). Ainsi, la perception des HCAs module globalement le transcriptome d'*A. fabrum* et *C. glutamicum* et notamment des gènes impliqués respectivement dans la synthèse de sidérophores et dans la réponse aux stress (Baude *et al.*, 2016; Chen *et al.*, 2016, 2017).

Nous aborderons dans les deux sous-parties suivantes, l'influence des HCAs sur l'écologie de deux phytopathogènes *Dickeya dadantii* qui ne dégrade pas les HCAs et *R. solanacearum* qui est capable de les dégrader et de les utiliser comme ressource trophique.

V.1. HCAs non dégradés en tant que signal : implication dans l'écologie de *Dickeya dadantii*

Dickeya dadantii est une bactérie tellurique Gram-négative responsable de la maladie de la pourriture molle touchant plus de 35% des espèces végétales de la famille des angiospermes (Ma *et al.*, 2007). Cette maladie est la résultante de la dégradation de la pectine et de la macération des tissus végétaux. De nombreux facteurs sont responsables de son pouvoir pathogène, parmi lesquels la synthèse d'enzymes de type pectates lyase permettant de dégrader les parois végétales (Reverchon and Nasser, 2013). La bactérie est attirée dans un premier temps par chimioattraction par le jasmonate libéré au niveau des zones de blessure (Antunez-Lamas *et al.*, 2009, Figure 5). *D. dadantii* se retrouve alors dans l'espace intercellulaire et va sécréter des pectates lyases via des systèmes de sécrétion de type I et II (Figure 5A), engendrant ainsi une lyse des tissus végétaux (Delepelaire, 1998; Pineau Camille *et al.*, 2014). Il y alors relargage de nombreux composés de la paroi cellulaire (Figure 5B) parmi lesquels des HCAs et des DAMPs (*Damage-associated molecular patterns*). Couplés à la reconnaissance de motifs moléculaires associés aux pathogènes (PAMP), ces molécules induisent alors une réponse

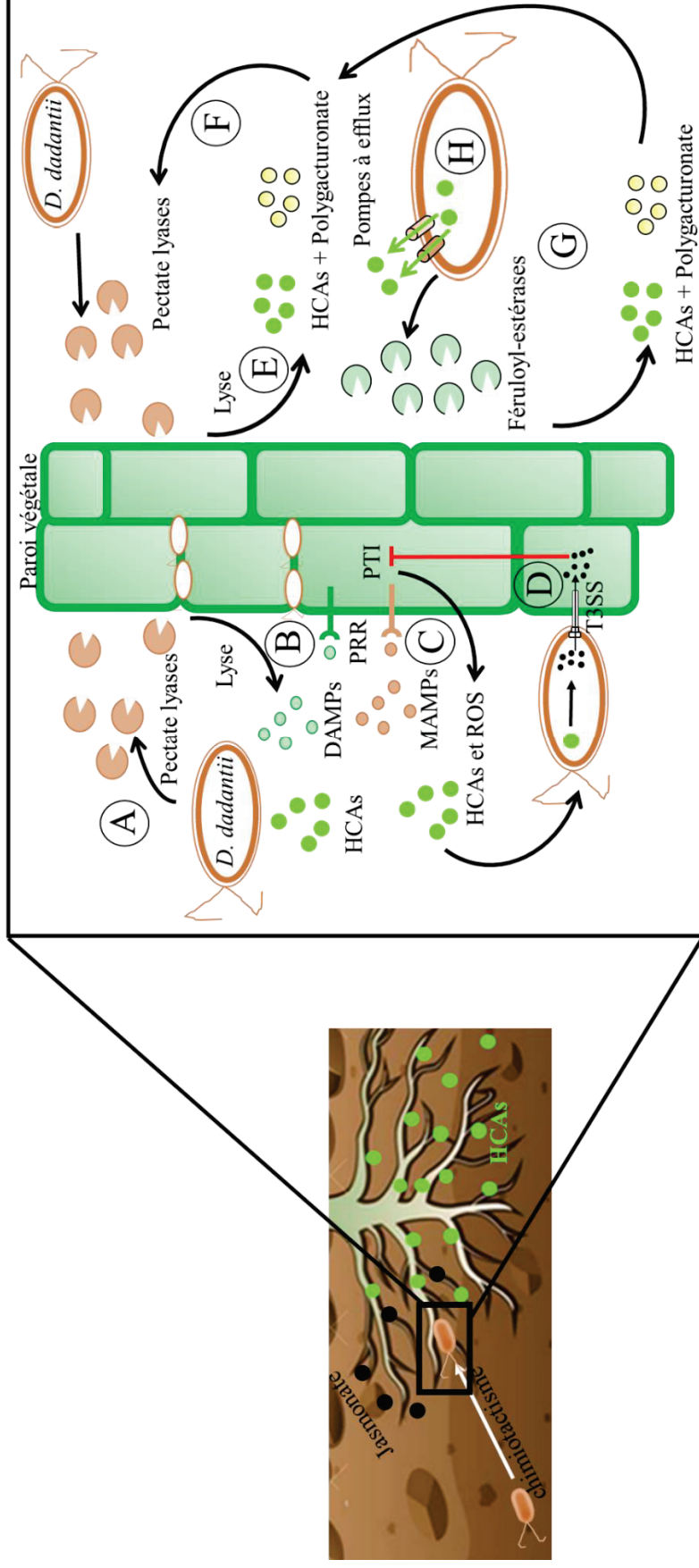


Figure 5 : Implication des HCAAs dans la pathogénie de *D. dadantii*

Le jasmonate attire par chimiotactisme *D. dadantii* vers la racine. (A) La synthèse de pectates lyases sécrétées par des systèmes de sécrétion de type 1 et 2 permet la lyse des parois végétales. (B) Il y a relargage de composé de type DAMPs et d'HCAAs mais aussi de composés bactériens de type MAMPs qui vont être reconnus par des récepteurs végétaux de type PRR. (C) Cette perception va induire une réaction de type PTI qui va engendrer la production de ROS et d'HCAAs. (H) Ces HCAAs peuvent être éfluxer de la cellule via deux pompes à éflux nommées AcrAB et EmrA. (D) Les HCAAs présents dans la bactérie vont provoquer aussi une induction du T3SS qui permet la réponse PTI. (E) D'autre part, le relargage d'HCAAs va induire des féruloyl-estérases qui vont induire la production d'HCAAs et de polygacturonate via la lyse cellulaire végétale. (F) Ces deux derniers composés ensemble sont inducteurs de leurs formations. (G) Il y a alors formation d'une boucle de rétroaction positive où les produits des réactions enzymatiques sont inducteurs de la synthèse des enzymes responsables de leurs formations. (Antunez-Lamas *et al.*, 2009; Bauer *et al.*, 1995; Hassan and Hugouvioux-Cotte-Pattat, 2011; Li *et al.*, 2009; Pérez-Bueno *et al.*, 2016; Ravirala *et al.*, 2007; Reverchon *et al.*, 2016; Yang *et al.*, 2008).

immunitaire de type PAMP/DAMP-triggered immunity (PTI) (Reverchon *et al.* 2016 et Figure 5C). Il y a alors production par la plante de ROS mais aussi de métabolites secondaires dont des HCAs comme l'acide férulique (Pérez-Bueno *et al.*, 2016). Ces HCAs sont toxiques pour *D. dadantii* qui n'est pas capable de le dégrader (Hassan and Hugouvieux-Cotte-Pattat, 2011). Cependant, elle possède deux pompes à efflux nommées AcrAB et EmrAB qui lui confèrent une résistance à une large gamme de molécules antimicrobiennes dont l'acide férulique (Ravirala *et al.*, 2007). Ces pompes à efflux sont induites par des composés phénoliques, parmi lesquels l'acide cinnamique (Figure 5H). Les auteurs n'ont pas étudié l'induction par l'acide férulique, mais une fois induite ces pompes pourraient permettre l'efflux de ce composé toxique.

Indépendamment de l'efflux, l'acide cinnamique (de même que l'acide *o*-coumarique) induit l'expression du système de sécrétion de type III (T3SS) de *D. dadantii* (Yang *et al.*, 2008), ce qui lui permet d'interférer avec la réponse immunitaire de type PTI (Bauer *et al.*, 1995, Figure 5D). Le T3SS, une nano-seringue ou injectisome (Büttner and He, 2009, 3; Puhar and Sansonetti, 2014), permet à *D. dadantii* d'injecter une protéine effectrice nommée DspE directement dans le cytosol de son hôte supprimant ainsi la réponse de type PTI (Bauer *et al.*, 1995; Glasner *et al.*, 2011). Par ailleurs, la présence d'acide férulique induit l'expression de deux féruloylestérase qui sont impliquées dans le clivage de la liaison entre l'acide férulique et la pectine dans les parois végétales et donc dans la macération des tissus végétaux (Hassan and Hugouvieux-Cotte-Pattat, 2011, Figure 5G). En outre, il a été démontré dans cette même étude que la présence simultanée d'acide férulique et polygacturonate (relargué durant la macération) était responsable d'une expression plus forte de certaines pectates lyases (Figure 5E). L'action de ces enzymes engendre alors une augmentation de la concentration en acide férulique et en polygacturonate et donc une suractivation des pectates lyases (Hassan and Hugouvieux-Cotte-Pattat, 2011 et Figure 5F).

Ces résultats soulignent que même chez une bactérie incapable de les dégrader, les HCAs peuvent être utilisés comme signaux modifiant l'expression de gènes importants dans l'écologie de ce pathogène et dans son interaction avec la plante. De manière notable, chez *D. dadantii*, il semble exister une spécificité de reconnaissance des HCAs.

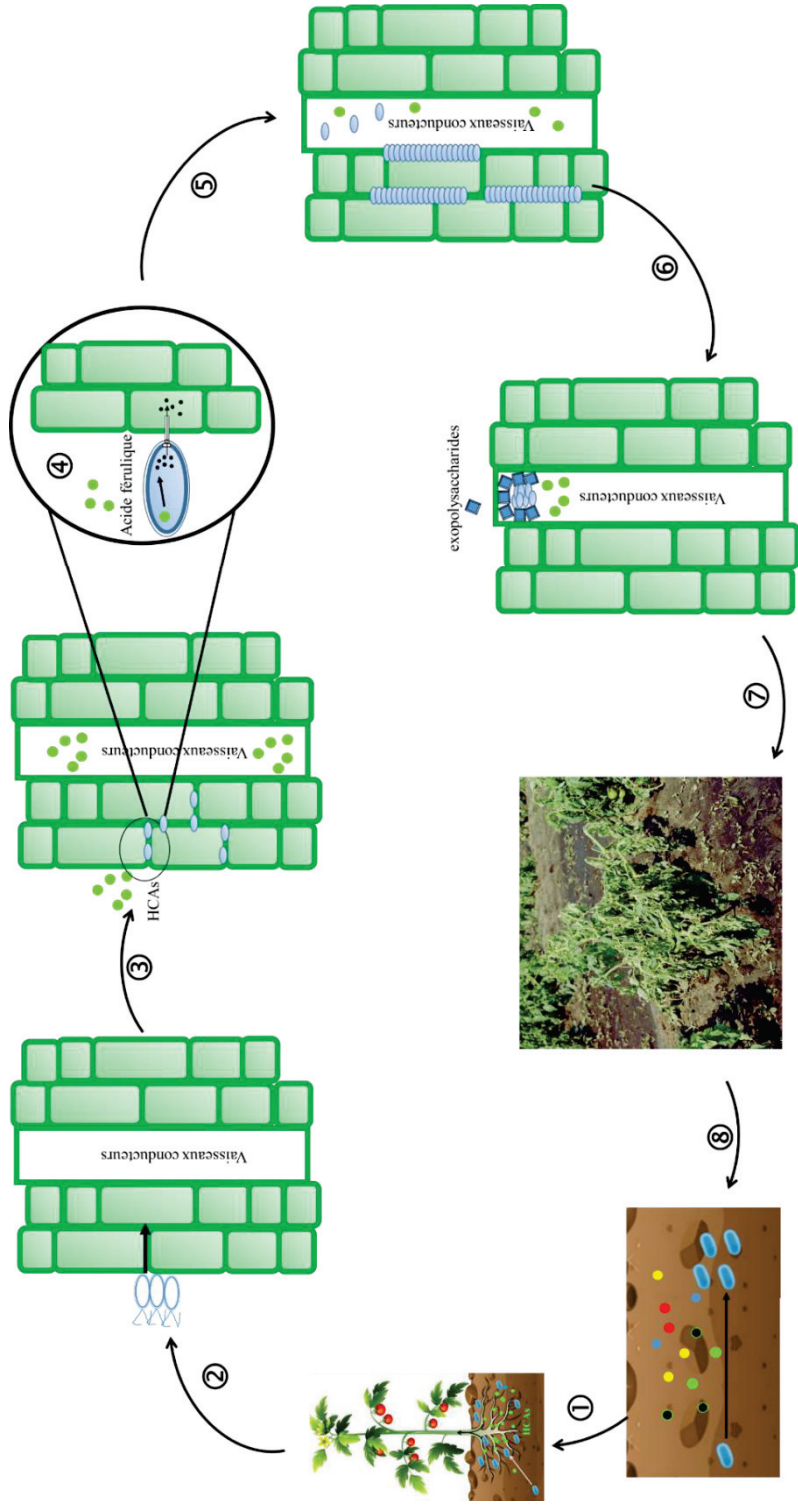


Figure 6 : Importance des HCAs dans le cycle de développement de la maladie du flétrissement bactérien induite par *R. solanacearum*.

① *R. solanacearum* est attirée par les exsudats racinaires et potentiellement par les HCAs. Elle utilise pour sa survie dans la rhizosphère, les HCAs comme ressources trophiques.
 ② Elle colonise ensuite les espaces intercellulaires ce qui induit sa reconnaissance par la plante et une réponse de type PTI. ③ La réponse PTI induit le relargage de composé de défense parmi lesquels les HCAs qui peuvent être dégradé et utilisé comme ressources nutritives pour coloniser l'hôte. ④ Le T3SS est induit par l'acide férulique ce qui mène à l'inhibition de la réponse de type PTI. ⑤ L'augmentation de la population de *R. solanacearum* aboutit à la formation de biofilm et des cellules isolées se détachent pour envahir les vaisseaux conducteurs. ⑥ Les HCAs et d'autres sources de carbones supportent la croissance bactérienne et d'important biofilm via la production d'exopolysaccharides sont formés. ⑦ La production excessive d'exopolysaccharides mène à la mort de la plante. ⑧ La présence d'HCAs dans les débris végétaux peut permettre la survie du pathogène.

V.2. Rôle global de la dégradation des HCAs dans l'écologie de *Ralstonia solanacearum*

R. solanacearum est une bactérie reconnue comme un phytopathogène majeur responsable de la maladie du flétrissement bactérien sur plus de 200 espèces végétales, notamment la tomate (Salanoubat *et al.*, 2002). Les différentes étapes de la pathogénie et l'implication des HCAs dans le cycle de pathogénie, sont décrits ci-dessous et dans le modèle en Figure 6.

R. solanacearum est tout d'abord attirée par certains composés de la plante produits au niveau des racines. Il a été démontré que les souches qui étaient le plus attirées par les exsudats de *Solanum lycopersicum* (tomate) et de *Oriza sativa* (riz) étaient les plus virulentes (Yao and Allen, 2006). Même si des HCAs purs n'ont pas été testés il a été émis, l'hypothèse que les HCAs pouvaient jouer un rôle de chimioattractants (Lowe *et al.*, 2014). Les gènes de synthèse des HCAs (Figure 1) sont surexprimés fortement chez les plants de tomates appartenant aux variétés les plus résistantes à ce pathogène. Ces résultats suggèrent que les HCAs auraient un rôle dans la protection de la plante contre *R. solanacearum*. Cependant, cette bactérie possède deux moyens de détoxification de ces composés antimicrobiens. En effet, elle possède tout d'abord deux pompes à efflux, nommées AcrA et DinF. Lorsque ces dernières sont mutées, une sensibilité plus forte à de nombreux composés antimicrobiens est observée, parmi lesquels l'acide caféique (Brown *et al.*, 2007). De plus, cette bactérie contient un région génomique (Tableau 1) lui permettant de détoxifier les HCAs via la voie de désacétylation non β -oxydative dépendante du CoenzymeA (Lowe *et al.*, 2014). Cette voie, en plus de permettre la détoxification permet à la bactérie de croître en présence d'HCA. Il a été montré que cette capacité conférait un avantage adaptatif dans la colonisation de l'hôte végétale. En effet, un mutant du gène codant pour la féruloyl-synthase (mutant *fcs*-incapable de dégrader les HCAs) est moins compétitif que la souche sauvage au niveau de racines et de tiges de tomates (Lowe *et al.*, 2014).

Après l'adhésion aux racines de la plante, *R. solanacearum* pénètre dans les espaces intercellulaires (Figure 6 ②) où elle va se développer jusqu'à former un biofilm (Hikichi, 2016). La formation de biofilm dépendant de la densité cellulaire, elle n'est possible que lorsqu'un certain seuil de molécule signal du QS de type (R)-methyl-3-hydroxy-palmitate (3-OH-PAME) et/ou (R)-methyl-3-hydroxymyristate (3-OH-MAME) est atteint (Kai *et al.*, 2015). A ce stade,

l'action du T3SS et de ses effecteurs est essentielle au pouvoir pathogène car il permet de moduler négativement l'immunité de la plante (Angot *et al.*, 2006; Jones and Dangl, 2006). Ce système n'est pas exprimé de façon constitutive et Zhang *et al.* (2017) ont démontré *in vitro* que la présence d'acide férulique induisait l'expression des gènes du T3SS (figure 6 ③). Les acides cinnamique, *p*-coumarique et caféique ne sont en revanche pas inducteurs. L'induction du T3SS a été étudiée *in planta* et il a été démontré que l'apport exogène d'acide férulique induisait ce système, mais uniquement en condition hydroponique (Zhang *et al.*, 2017). L'induction par l'acide férulique est due à la surexpression des gènes *prhA-hrpG*, qui induisent alors l'expression du régulateur HrpB. Ce régulateur induit l'expression des gènes *hrp* et donc le T3SS (Zhang *et al.*, 2015b). Aucune étude ne s'est intéressée à l'impact de la dégradation de l'acide férulique sur l'induction du T3SS. En effet, les souches utilisées dans le cadre de cette étude sont capables de dégrader les HCAs (Lowe *et al.*, 2014) ce qui pourrait influencer l'induction du T3SS, en diminuant la quantité du composé inducteur, et donc l'expression des gènes cibles. En fonction, des conditions environnementales et des autres composés de la plante, si les gènes de dégradation des HCAs ne sont pas induits, comme c'est peut-être le cas dans les conditions hydroponiques testées par Zhang *et al.* (2017), il pourrait subsister suffisamment d'HCAs pour induire l'expression du T3SS. Après l'étape de colonisation de l'espace intercellulaire via la formation de biofilm, des cellules vont se détacher et aller coloniser les vaisseaux du xylème. Dans ces vaisseaux, les HCAs sont accumulés en réponse à un pathogène (Mandal and Mitra, 2008; Khokhani *et al.*, 2017). La dégradation des HCAs à ce stade est importante pour l'efficacité de la colonisation de la tige de la plante (Lowe *et al.*, 2014; Khokhani *et al.*, 2017). La production bactérienne d'une grande quantité d'exopolysaccharides au niveau des vaisseaux du xylème de la plante, va provoquer alors une obstruction des vaisseaux et va mener au flétrissement et à la mort des plantes (Genin and Denny, 2012). Les gènes impliqués dans la dégradation des HCAs pourraient probablement permettre à la bactérie de survivre sur les débris végétaux dans le sol une fois que la plante est morte, mais cette hypothèse n'a pas encore été évaluée. Ainsi, les HCAs jouent un rôle primordial dans l'écologie de *R. solanacearum* dès les premières étapes de la pathogénie jusqu'aux étapes de colonisation de l'hôte et potentiellement dans le sol après avoir tué son hôte.

Conclusion synthèse bibliographique

Une très grande part des études sur *Agrobacterium* concerne la caractérisation de gènes de virulence, l'analyse de leur expression et la transformation végétale. Il existe un intérêt grandissant concernant les déterminants génétiques impliqués dans la transition entre les styles de vie rhizosphérique et pathogène (pour revue Barton *et al.* 2018). Comparativement, il existe peu d'études sur les déterminants génétiques permettant la persistance d'*Agrobacterium* dans chacun des deux environnements qu'elle colonise : la rhizosphère et la tumeur. Une revue récente s'est focalisée sur les déterminants génétiques importants pour la colonisation rhizosphérique (Dessaux and Faure 2018). Cette synthèse décrit donc les conditions rencontrées par *Agrobacterium* dans la tumeur (Partie A) et synthétise les connaissances relatives aux déterminants génétiques impliqués dans la résistance aux stress, l'acquisition de ressource nutritive et la compétition avec le microbiote tumoral. La construction et l'exploitation par *A. fabrum* de la niche tumorale résulte de l'expression de ces déterminants génétiques. D'autres gènes non caractérisés fonctionnellement sont exprimés dans cet environnement (González-Mula *et al.* 2018) et pourraient permettre la survie et le maintien dans la tumeur. Des progrès sont donc à réaliser dans la caractérisation fonctionnelle des gènes, et l'implication de ces gènes dans l'écologie d'*Agrobacterium fabrum* devrait être analysée plus systématiquement que ce soit dans la tumeur et/ou la rhizosphère.

L'adaptation d'*Agrobacterium* à ces environnements contrastés (rhizosphère et tumeur) et la transition d'un style de vie à un autre nécessite la perception de signaux végétaux et leur intégration pour permettre l'expression des gènes au moment adéquat. De nombreux signaux sont impliqués dans l'induction de la virulence et il a été récemment décrit que certains pouvaient être impliqués dans la transition entre le style de vie rhizosphérique et pathogénique (Subramoni *et al.* 2014; Barton *et al.* 2018). Dans ce contexte, les HCAs sont décrits comme des composés végétaux impliqués dans l'induction des gènes de virulence (Kalogeraki *et al.* 1999; Joubert *et al.* 2002; Bhattacharya *et al.* 2010). Cependant, ils sont présents en grande quantité dans l'environnement rhizosphérique et nous avons vu dans la partie B que ce sont des composés toxiques pour les microorganismes. Certains ont développé des mécanismes pour les dégrader et/ou utiliser comme ressources trophiques via deux voies métaboliques que nous avons décrites. L'expression des premiers gènes impliqués dans ces voies de catabolisme est

régulée par des régulateurs de type MarR. Nous avons souligné l'importance du premier intermédiaire de dégradation des HCAs dans la régulation pour certains régulateurs MarR. La dégradation des HCAs peut aussi être régulée via des phénomènes de répression catabolique au contact d'autres sources carbonées mais la présence d'HCAs semble aussi modifier globalement le métabolisme bactérien. Cependant, aucune étude ne l'a encore clairement démontré. En outre, le rôle des HCAs n'est pas seulement trophique. En effet, la perception de ces composés est impliquée notamment dans le chimiotactisme vers la plante et dans la communication bactérienne. Nous avons développé deux exemples suggérant un rôle signal des HCAs, dans l'interaction entre des phytopathogènes et des végétaux et montrant leur importance dans l'écologie de ces deux bactéries à plusieurs stades de leur cycle de vie.

Cette synthèse bibliographique souligne d'une part l'importance des HCAs dans les interactions plantes-bactéries et probablement chez *Agrobacterium*, et d'autre part le besoin d'étudier le rôle des déterminants génétiques dans les deux styles de vie de la bactérie en interaction avec la plante. Elle génère différentes interrogations : (i) Le féruloïl-CoA influence-t-il l'expression de différentes fonctions non directement liées à la dégradation des HCAs ? (ii) Est-ce que la dégradation des HCAs influence le métabolisme bactérien d'une manière générale ? (iii) Quel rôle joue la régulation par les régulateurs MarR dans l'expression des fonctions modulées et plus globalement dans les interactions plante-bactérie ? Dans ce travail de thèse, nous avons donc étudié l'effet global de la perception et de la dégradation des HCAs sur l'écologie d'*A. fabrum*. Nous avons caractérisé fonctionnellement les gènes impliqués et étudié leur rôle dans les deux styles de vie de la bactérie.

**Chapitre 1 : Caractérisation
de la régulation de la région
SpG8-1b et importance
écologique chez
*Agrobacterium fabrum***

Préambule du chapitre 1

Le dialogue moléculaire entre les plantes et les microorganismes est fortement influencé par les composés produits par l'un ou l'autre des partenaires. La rhizosphère est un lieu privilégié pour la communication plante-bactéries car l'exsudation de métabolites de nature et de quantité diverses va influencer les nombreux microorganismes qui y vivent (Faure *et al.*, 2009; Steinauer *et al.*, 2016; Zhang *et al.*, 2014). Nous avons vu dans le chapitre précédent que les HCAs ont un rôle important dans l'interaction entre les bactéries et les plantes. Ces derniers, qui ont généralement une action antimicrobienne, peuvent être dégradés et alors servir de ressources trophiques pour certains microorganismes en interaction avec un hôte végétal.

Chez *A. fabrum*, les HCAs sont connus comme étant impliqués dans les deux modes de vie (rhizosphérique et pathogène). En effet, ils ont un rôle chimioattractant dans le cadre du style de vie rhizosphérique et pour les souches portant le plasmide Ti, certains HCAs comme l'acide férulique sont inducteurs des gènes de virulence (Ashby *et al.*, 1988; Joubert *et al.*, 2002). Or, une voie de dégradation des HCAs qui est spécifique de cette espèce permet l'utilisation de ces composés comme ressources trophiques (Campillo *et al.*, 2014). Chez cette espèce, cette voie pourrait interférer avec l'induction des gènes *vir* en dégradant certains inducteurs. L'implication de cette voie dans l'écologie d'*A. fabrum* et sa régulation n'est pour le moment pas décrite. Nous avons émis les hypothèses que la protéine HcaR, annotée comme un répresseur de la famille MarR, était impliquée dans la régulation de la dégradation des HCAs et que cette régulation était importante dans l'interaction entre *A. fabrum* et la plante. Nous avons caractérisé fonctionnellement et biochimiquement ce régulateur, étudié son expression et son implication dans la valeur adaptative de la bactérie lors de son interaction avec la plante que ce soit dans le style de vie rhizosphérique ou pathogène.

Ces travaux ont été publiés en 2018, dans le journal *Molecular Plant-Microbe Interactions* (MPMI). Pour cette publication, j'ai réalisé l'analyse des interactions entre HcaR-régions promotrices-ligands par gel retard, les tests de compétitions entre les différentes souches, la construction du vecteur pOT1eM, l'étude de l'expression des gènes *vir* in vitro et in planta, l'analyse statistiques des données., l'analyse des interactions entre HcaR-régions promotrices-ligands par gel retard et les tests de compétitions entre les différentes souches. J'ai aussi effectué les observations par microscopie confocale (rendue possible par la construction du vecteur pOT1eM) et rédigé la première version de cet article.

Regulation of Hydroxycinnamic Acid Degradation Drives *Agrobacterium fabrum* Lifestyles

Thibault Meyer,¹ Sébastien Renoud,¹ Armelle Vigouroux,² Aurélie Miomandre,¹ Vincent Gaillard,¹ Isabelle Kerzaon,¹ Claire Prigent-Combaret,¹ Gilles Comte,¹ Solange Moréra,² Ludovic Vial,¹ and Céline Lavire^{1,†}

¹Université de Lyon, F-69622, Lyon, France; Université Lyon 1, Villeurbanne, France; CNRS, UMR5557, Ecologie Microbienne, Villeurbanne, France; and INRA, UMR1418, Villeurbanne, France; and ²Institute for Integrative Biology of the Cell (I2BC), CNRS CEA Univ. Paris-Sud, Université Paris-Saclay, Avenue de la Terrasse, Gif-sur-Yvette 91198, France

Accepted 16 February 2018.

Regulatory factors are key components for the transition between different lifestyles to ensure rapid and appropriate gene expression upon perceiving environmental cues. *Agrobacterium fabrum* C58 (formerly called *A. tumefaciens* C58) has two contrasting lifestyles: it can interact with plants as either a rhizosphere inhabitant (rhizospheric lifestyle) or a pathogen that creates its own ecological niche in a plant tumor via its tumor-inducing plasmid (pathogenic lifestyle). Hydroxycinnamic acids are known to play an important role in the pathogenic lifestyle of *Agrobacterium* spp. but can be degraded in *A. fabrum* species. We investigated the molecular and ecological mechanisms involved in the regulation of *A. fabrum* species-specific genes responsible for hydroxycinnamic acid degradation. We characterized the effectors (feruloyl-CoA and *p*-coumaroyl-CoA) and the DNA targets of the MarR transcriptional repressor, which we named HcaR, which regulates hydroxycinnamic acid degradation. Using an *hcaR*-deleted strain, we further revealed that hydroxycinnamic acid degradation interfere with virulence gene expression. The HcaR deletion mutant shows a contrasting competitive colonization ability, being less abundant than the wild-type strain in tumors but more abundant in the rhizosphere. This supports the view that *A. fabrum* C58 HcaR regulation through ferulic and *p*-coumaric acid perception is important for the transition between lifestyles.

Bacteria can adopt different lifestyles and adapt to multiple habitats because of specific pathways whose expression is tightly controlled (Brooks et al. 2011; Cases et al. 2003; White

et al. 2011). Indeed, a given pathway can be beneficial in one lifestyle but deleterious in another (Andersson and Hughes 2010; Price et al. 2013). The transition between lifestyles involves a coordinated modification of gene expression upon signal perception to express the appropriate gene at the right time (Duprey et al. 2014; Valentini et al. 2018). However, the presence of a regulatory system selected for its effectiveness in one environment can have different consequences (either beneficial or deleterious) in environments submitted to different constraints (Schick et al. 2015). Therefore, one may wonder whether regulatory systems are mainly optimized for bacteria to adapt to different lifestyles or are specialized for one of them.

Agrobacterium fabrum (one of the species of the *A. tumefaciens* complex) strain C58 is a suitable model to study the relative fitness cost of a regulator in contrasting environments. This bacterium has two lifestyles: (i) as a nonpathogen rhizosphere colonizer or (ii) as a pathogen that causes crown-gall disease by creating its own ecological niche in a plant tumor via its tumor-inducing plasmid (pTi) (Nester 2015). Hydroxycinnamic acids (HCA) play an important role in the pathogenic lifestyle of *Agrobacterium* spp. (Guo et al. 2017; Joubert et al. 2002). Briefly, a wounded plant releases amino acids, sugars, and phenolic compounds such as acetosyringone and HCA that contribute to guiding *Agrobacterium* spp. to the wounded site by chemotaxis (Ashby et al. 1988; Guo et al. 2017). Moreover, HCA trigger the expression of pTi-encoded virulence (*vir*) genes responsible for T-DNA transfer to the plant host cell nuclei and its insertion into the plant genome which, in turn, results in crown gall formation (Bhattacharya et al. 2010; Bolton et al. 1986; Kalogeraki et al. 1999). Among *vir* genes, *virH2* encodes an O-demethyl transferase, thought to detoxicate HCA-like ferulic acid and to be involved in the negative control of *vir* gene induction (Brenic et al. 2004). However, the circular chromosome of *A. fabrum* contains a species-specific gene cluster named SpG81-b (i.e., a region present in bacteria of this species but absent from strains of other *Agrobacterium* spp.) also involved in HCA degradation (Lassalle et al. 2011). The SpG81-b region is composed of eight genes from *atu1415* to *atu1422* (*hcaR*); it enables bacteria to utilize HCA as a carbon source (Fig. 1) (Campillo et al. 2014). In addition, HCA degradation induces the transcription of the SpG81-b region, probably through HCA degradation intermediates (Baude et al. 2016).

The uncharacterized regulatory protein HcaR (Atu1422), annotated as a multiple antibiotic resistance regulator (MarR), could be involved in the repression of SpG81-b gene cluster expression in the absence of HCA (Lassalle et al. 2011). This protein belongs to the MarR family, among which regulatory

[†]Corresponding author: C. Lavire; E-mail: celine.lavire@univ-lyon1.fr

Funding: T. Meyer, A. Vigouroux, L. Vial, S. Moréra, and C. Lavire were supported by the CNRS (Mission pour l'interdisciplinarité, Agromics 2014–2016). Part of this work was initiated by the EcoGenome project of the French Agence Nationale de la Recherche (grant ANR-BLAN-08-0090) and by the French national program EC2CO-Biofect/ECodyn/Dril/MicrobiEn (IBAD). T. Meyer received a doctoral grant from the French Ministère de l'Éducation Nationale, de l'Enseignement Supérieur et de la Recherche. This work benefited from the I2BC microcalorimetry platform, supported by FRISBI ANR-10-INSB-05-01, and from the DTAMB, the "Centre Technologique des Microstructures" and "Serre and chambres climatiques" platforms, supported by the FR BioEnviS Research Federation.

*The e-Xtra logo stands for "electronic extra" and indicates that five supplementary figures and four supplementary tables are published online.

© 2018 The American Phytopathological Society

proteins involved in the repression of HCA degradation genes have been described recently in other soil microorganisms (Kim et al. 2016; Otani et al. 2016). These repressors can interact with different ligands: HCA (ferulic and *p*-coumaric acids) or CoA-activated HCA (*p*-coumaroyl-CoA and feruloyl-CoA). At the onset of the study of HcaR of *A. fabrum* C58, we had no clue whether SpG8-1b genes were expressed in interaction with plants (in the rhizosphere or in tumors). However, we suspected that regulation of HCA degradation from the SpG8-1b region would be more important for bacterial competitiveness in the tumor than in the rhizosphere environment; thus, we investigated the molecular and ecological roles of HcaR using biochemical and in planta approaches. The present article describes how HcaR regulates SpG8-1b gene expression and how HCA degradation intermediates can release HcaR from DNA binding. We also demonstrate that, in contrast to rhizosphere colonization, repression of HCA degradation by HcaR is important for providing *A. fabrum* a competitive advantage in colonizing tumors and is required to induce *vir* genes upon ferulic acid perception. HcaR regulation could be decisive for the transition between the rhizospheric and pathogenic lifestyles.

RESULTS

HCA degradation: Comparison between the C58 wild-type and C58Δ*hcaR* strains.

Degradation of different HCA in the C58 and C58Δ*hcaR* strains was compared by measuring the remaining HCA in the growth culture supernatant after 8 h by high-performance liquid chromatography (HPLC) (Fig. 2). Acetosyringone and acetovanillone were not degraded by either strain. Ferulic acid, *p*-coumaric acid, caffeic acid, and 3,4-dimethoxycinnamic acid were more degraded in the mutant than in the wild type (10, 6.5, 1, and 2% left, as compared with 59, 22, 63, and 100% left in the wild type, respectively). Interestingly, some HCA such as cinnamic acid, 4-methoxycinnamic acid, *o*-coumaric acid, and *m*-coumaric acid were only degraded by the C58Δ*hcaR* strain

(11, 21, 32, and 21% left, as compared with 97, 98, 97, and 98% left in the wild type, respectively).

Ferulic acid and *p*-coumaric acid modulate the expression of SpG8-1b genes.

Plasmid reporter gene fusions were constructed for the transcription units of the SpG8-1b region (Fig. 1) and introduced into the wild-type strain in order to evaluate gene expression in response to HCA. Compared with the fluorescence level measured in the absence of ferulic acid (Table 1), the fluorescence level measured in the presence of ferulic acid was significantly higher (i.e., 2.36-, 1.68-, 1.29-, and 1.10-fold for *atu1416*, *atu1417*, *atu1418*, and *hcaR* reporter fusions, respectively). Leaving aside *atu1418*, which is not involved in *p*-coumaric acid degradation (Fig. 1), similar results were obtained with *p*-coumaric acid (Table 2; Supplementary Table S4). Acetovanillone, caffeic acid, 3,4-dimethoxycinnamic acid, cinnamic acid, *m*-coumaric acid, *o*-coumaric acid, and 4-methoxycinnamic acid had no inductive effect on *atu1416*, *atu1417*, *atu1418*, or *hcaR* reporter fusions (Table 2). Overall, these results indicate that only ferulic and *p*-coumaric acids induced the expression of SpG8-1b HCA-degradation genes.

The MarR transcriptional regulator HcaR is a repressor of SpG8-1b gene expression.

The plasmid reporter fusions of SpG8-1b gene expression were introduced into the C58Δ*hcaR* strain; then, the fluorescence level was measured in the absence or in the presence of ferulic acid. In the C58Δ*hcaR* mutant strain, the *atu1416*, *atu1417*, and *hcaR* genes were constitutively expressed (a high fluorescence level was measured even in the absence of ferulic acid). Moreover the fluorescence level measured for the *atu1416*, *atu1417*, and *hcaR* reporter fusions were two to six times higher in the C58Δ*hcaR* mutant strain than in the wild-type strain in either the absence or presence of ferulic acid (Table 1). In contrast, the fold changes between C58Δ*hcaR* and the wild-type strains were similar for the *atu1418* gene fusion and the empty pOT1e vector (Table 1). These results show that

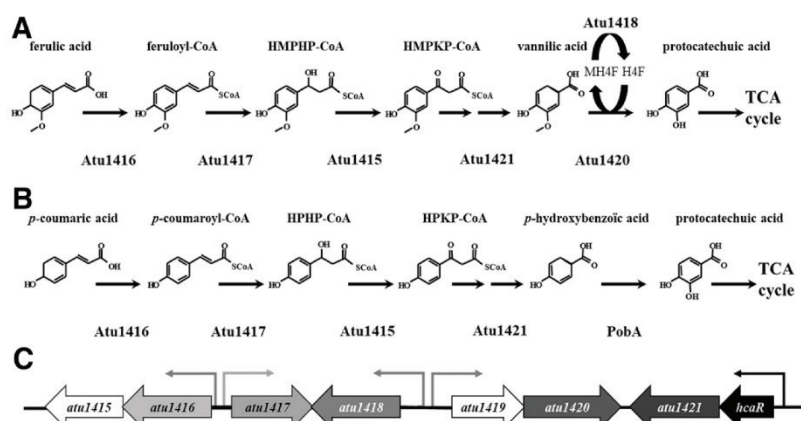


Fig. 1. Ferulic acid and *p*-coumaric acid degradation pathway and organization of the SpG8-1b region. **A**, Ferulic acid degradation pathway. A coenzyme A is added to ferulic acid by Atu1416, a feruloyl-CoA synthase. Feruloyl-CoA is then converted by enoyl-CoA hydratase Atu1417 into 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl (HMPHP)-CoA which, in turn, is transformed into vanillic acid by Atu1415, a phenylhydroxypropionyl-CoA dehydrogenase, and then Atu1421, a 4-hydroxy-3-methoxyphenyl-β-ketopropionyl-CoA (HMPKP)-CoA β-keto-thiolase. Atu1418 and Atu1420 degrade vanillic acid into protocatechuic acid. The *pca* genes, described by Parke (1995, 1997), allow the integration of this latter molecule into the TCA cycle. **B**, *p*-Coumaric acid degradation pathway. The *pca* genes, described by Parke (1995, 1997), allow the integration of this latter molecule into the TCA cycle. **C**, Organization of the SpG8-1b region. The *atu1415-atu1416*, *atu1419-atu1420*, and *hcaR-atu1421* genes are all in the same transcription unit. Promoters are shown with small arrows indicating the direction of gene transcription.

HcaR is a transcriptional repressor that regulates *atu1416*, *atu1417*, and its own transcription.

DNA-binding target and affinity.

In order to characterize the DNA-binding target of HcaR, we purified HcaR, which appeared as a dimer in solution according to gel filtration measurements. Using the intergenic regions (approximately 300 bp) of *atu1416-1417*, *atu1418-1419*, *hcaR*, and *atu2645* (gene outside the SpG8-1b region) as DNA probes for binding assays (Fig. 3A), we showed that HcaR bound to the *atu1416-1417* and *hcaR* DNA probes but not to the *atu2645* and *atu1418-1419* DNA probes. These results demonstrate that HcaR acts as a transcriptional repressor by targeting the *atu1416-1417* and *hcaR* intergenic regions.

To further characterize bound DNA, *in silico* comparison of the promoter regions of *atu1416-1417* and *hcaR* was performed. We identified a 25-bp conserved region containing 16 identical base pairs (Fig. 3B). The region contained two inverted repeated sequences overlapping the -35 box regulatory element of *atu1416-1417* whereas, in the *hcaR* promoter sequence, the inverted repeated sequence imperfectly overlapped the -10 and -35 regulatory elements (Fig. 3B). In order to verify that HcaR binds to the conserved DNA region, we also performed gel-shift assays using two 170-bp probes located either downstream (P_{Down}) or upstream (P_{Up}) of the conserved region (Supplementary Fig. S1). Purified HcaR bound to the probe containing the 25-bp motif whereas P_{Down} and P_{Up} did not.

The mean K_D value between HcaR and the conserved DNA region used for gel-shift experiments determined by isothermal titration microcalorimetry (ITC) was $49 \pm 9 \mu\text{M}$. ITC data revealed a 2:1 binding stoichiometry (one HcaR dimer binds one DNA molecule, as given from $n = 0.53 \pm 0.15$), and demonstrated a positive enthalpy change of $16.180 \pm 555.4 \text{ cal/mol}$ upon DNA binding (Fig. 3D).

HcaR effectors are feruloyl-CoA and *p*-coumaroyl-CoA.

To identify whether ferulic acid or ferulic acid intermediate degradation compounds were the effectors of HcaR, the plasmid reporter fusions of SpG8-1b gene expression were introduced into the *C58Δatu1415*, *C58Δatu1416*, and *C58Δatu1417* mutant strains, and fluorescence levels were measured in the presence or absence of ferulic acid (Supplementary Table S3). In *C58Δatu1416*, which did not degrade

ferulic acid (Fig. 1), supplementation with ferulic acid had no effect on gene induction (basal fluorescence), indicating that this compound was not the effector. In the *C58Δatu1415* strain, the degradation process stopped at the 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA stage (Fig. 1), and the fluorescence level of the *atu1416* and *atu1417* reporter fusions was greater in the presence of ferulic acid, suggesting that feruloyl-CoA or 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA could be the effectors. In the *C58Δatu1417* strain, in which feruloyl-CoA accumulates (Fig. 1), expression of the *atu1416*, *atu1417*, and *hcaR* reporter gene fusions increased by 5.1-, 3.7-, and 1.4-fold, respectively, as compared with expression in the absence of ferulic acid. In addition, this induction level was greater than in C58. Overall, these results show that feruloyl-CoA was responsible for the induction of *atu1416*, *atu1417*, and *hcaR* gene expression.

Using gel-shift assays, we verified the ability of feruloyl-CoA to relieve the interaction between HcaR and the conserved intergenic region. As expected, ferulic acid had no effect on protein-DNA binding, unlike feruloyl-CoA (Fig. 3C). Similar results were obtained with *p*-coumaroyl-CoA (Supplementary Fig. S2). Therefore, feruloyl-CoA and *p*-coumaroyl-CoA are the effectors of HcaR and they activate protein release from DNA.

Table 1. Ferulic acid degradation effect on the SpG8-1b gene expression after 24 h of incubation

Genes	C58	C58Δ <i>hcaR</i>	Fold change ^a
<i>Patu1416</i>			
0 μM	6.12 ± 0.37	40.53 ± 1.73	6.62
750 μM	14.43 ± 0.48	45.06 ± 2.89	3.12
<i>Patu1417</i>			
0 μM	4.23 ± 0.32	18.39 ± 0.61	4.35
750 μM	7.13 ± 0.45	18.64 ± 0.82	2.61
<i>Patu1418</i>			
0 μM	13.01 ± 0.49	10.19 ± 0.35	0.78
750 μM	16.8 ± 1.32	13.5 ± 0.56	0.80
<i>PhcaR</i>			
0 μM	6.41 ± 0.43	14.73 ± 0.63	2.30
750 μM	7.06 ± 0.40	14.00 ± 0.73	1.98
empty pOT1c			
0 μM	5.25 ± 0.32	4.06 ± 0.15	0.77
750 μM	5.41 ± 0.34	4.07 ± 0.19	0.75

^a Numbers in bold indicate ???

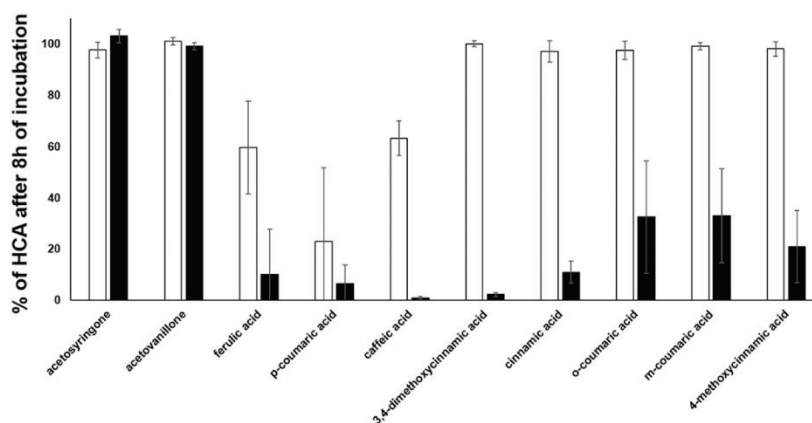


Fig. 2. Abundance of hydroxycinnamic acid (HCA) compounds in the wild-type and *C58ΔhcaR* mutant strains. The abundance of different HCA potentially degraded by the SpG8-1b pathway were measured in culture supernatant in both strains. The presence of the supplemented compounds was quantified by high-performance liquid chromatography after 8 h of incubation and compared with their concentration prior to incubation ($T_0 = 100\%$). Compound degradation is represented in white for the wild-type strain and in black for the *C58ΔhcaR* strain.

hcaR deletion modifies *virB* gene expression.

HCA are considered to be inducers of *vir* genes; therefore, we compared the ability of strains C58, C58ΔSpG8-1b (deleted for *hcaR* and all genes involved in HCA degradation—no HCA degradation), and C58Δ*hcaR* (*hcaR*-deleted—constitutive HCA degradation) to induce *virB* gene expression, using a plasmid reporter fusion (*virB-egfp* transcriptional fusion) (Fig. 4). Acetosyringone, which is not degraded by C58 (Fig. 2), induced fluorescence in the strains, which shows that HcaR is not directly involved in *vir* gene expression. In strain C58, a significant fluorescence level was detected in the presence of ferulic acid, confirming that this compound induced *virB* gene expression (Fig. 4). In the C58ΔSpG8-1b mutant, the fluorescence level in the presence of ferulic acid was higher than in the wild type. In the C58Δ*hcaR* mutant, however, no fluorescence

signal was measured (Fig. 4), probably due to the rapid degradation of ferulic acid (Fig. 2).

Implications of HcaR regulation in *A. fabrum* lifestyles.

The C58 and C58Δ*hcaR* strains were individually inoculated into plant stems. Both strains induced tumors and colonized the stems similarly (Supplementary Fig. S3), even though expression of *vir* genes differed between the two strains. Indeed, fewer bacteria were detected based on enhanced green fluorescent protein (eGFP) fluorescence in C58Δ*hcaR* (Supplementary Fig. S4). In a tomato tumor generated after inoculation with the C58Δ*hcaR* mutant strain containing an *atu1416* plasmid reporter fusion, all the bacteria within the tumor expressed HCA degradation genes (constitutive expression: yellow cells, Fig. 5A; Supplementary Fig. S5). In contrast, in strain C58, among

Table 2. Ratio of reporter gene induction in the presence or absence of different hydroxycinnamic acids after 24 h of incubation^a

Gene	Acetovanillone	<i>p</i> -Coumaric acid	Caffeic acid	3,4-Dimethoxycinnamic acid	Cinnamic acid	<i>m</i> -Coumaric acid	<i>o</i> -Coumaric acid	4-Methoxycinnamic acid
<i>Patu1416</i>	0.99	5.70	0.85	0.92	0.93	1.13	1.07	1.08
<i>Patu1417</i>	1.12	3.50	0.80	0.89	0.94	1.13	0.99	1.16
<i>Patu1418</i>	1.02	0.90	0.91	0.84	0.93	1.02	0.86	1.02
<i>PhcaR</i>	1.18	1.68	0.89	0.89	1.02	1.06	1.02	1.15
Empty pOT1e	1.13	1.04	0.91	0.80	0.95	1.12	1.00	1.09

^a Numbers in bold indicate ???

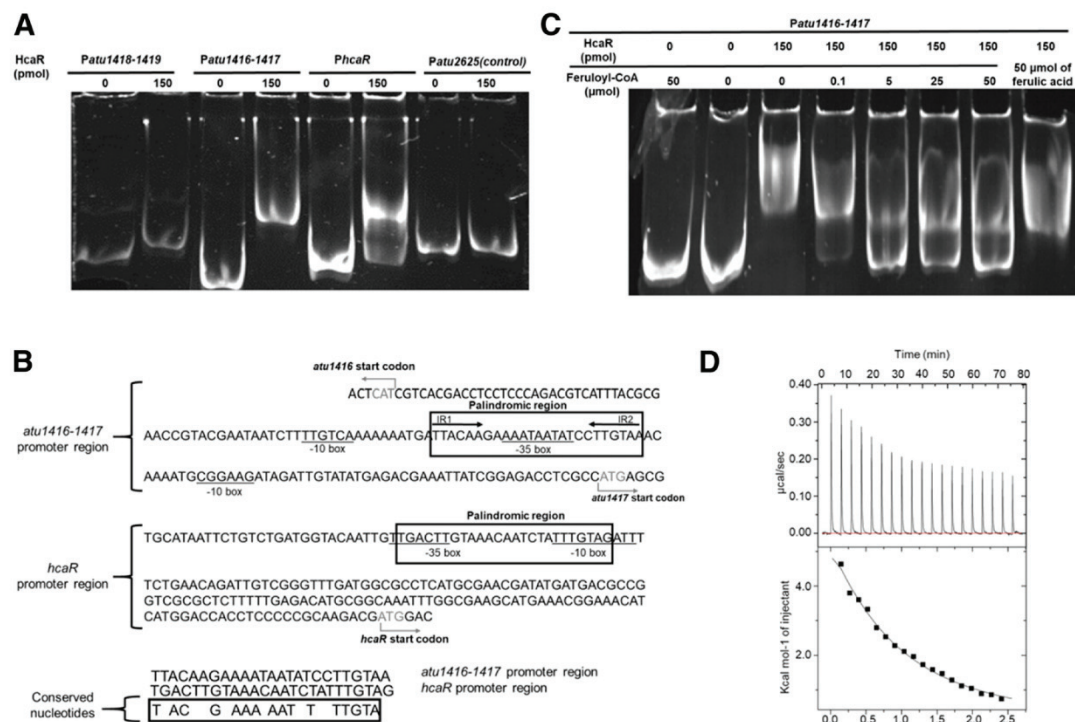


Fig. 3. HcaR regulation mechanism: DNA binding and identification of HcaR as an effector. **A**, Gel-shift analysis of HcaR binding to the promoter regions of SpG8-1b. **B**, In silico analysis of the *atu1416-1417* and *hcaR* promoter regions: -10 and -35 boxes are highlighted, palindromic regions are framed, and inverted repeat sequences are indicated by arrows. **C**, Gel-shift analysis of HcaR binding to the Patu1416-1417 region, with feruloyl-CoA at different concentrations (0.1 to 50 μM) or with 50 μM ferulic acid (last well). **D**, The top graph of the HcaR microcalorimetry experiment shows heat differences following injection of the DNA ligand (top panel) and integrated heats of injection with the best fit (solid line) to a single binding model using MicroCal Origin (lower panel).

the cells present inside the tumor (red or yellow cells), only a few expressed the HCA degradation genes (yellow cells, Fig. 5A). The ability of the two strains to efficiently colonize the tumor was tested in a 1:1 competitive experiment. *hcaR* deletion had no deleterious effects in vitro (measured competitive index [CI] = 1.18, not significantly different from 1, $P = 0.96$) but, in the tumor tissue, *C58ΔhcaR* was less abundant than *C58* (CI = 0.66, significantly lower from 1, $P = 4.7 \times 10^{-6}$) (Fig. 5C).

The *C58* and *C58ΔhcaR* strains were individually inoculated on tomato seed. Both strains grew similarly in the rhizosphere. In the plants inoculated with either the *C58* or the *C58ΔhcaR* strain containing an *atu1416* fusion reporter plasmid, most cells expressed HCA degradation genes (yellow-green, Fig. 5C). However, 15 days after seed coinoculation (1-1), *C58ΔhcaR*

was more abundant in the tomato rhizosphere than *C58* (CI = 4.18, significantly different from 1, $P = 8.7 \times 10^{-6}$). These data reveal differences in the expression of HCA degradation genes and opposite effects of HcaR regulation in the two plant environments; namely, the tumor and the rhizosphere.

DISCUSSION

In this study, we addressed the molecular mechanism of the species-specific *A. fabrum* HcaR regulator and its ecological role in the two contrasting lifestyles of *Agrobacterium*. Indeed, HCA is degraded through the expression of the species-specific chromosomal gene cluster and is also involved in the initiation of the plasmid-encoded pathogenicity program.

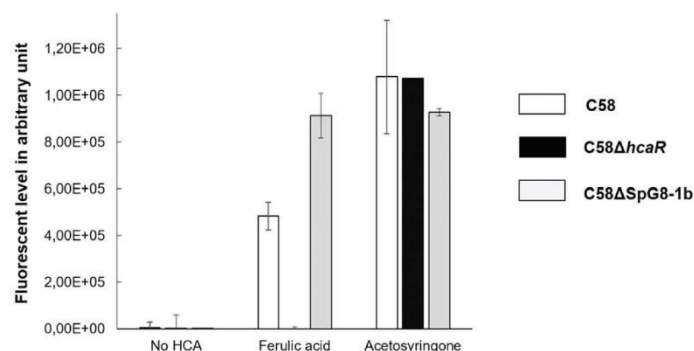


Fig. 4. Induction of *virB* expression by hydroxycinnamic acid (HCA) compounds in the *C58* wild-type and the *C58ΔSpG8-1b* and *C58ΔhcaR* mutant strains. Induction of *virB* in *C58* (white squares), *C58ΔSpG8-1b* (gray squares), or *C58ΔhcaR* (black squares) cultured in culture medium supplemented with 750 μ M ferulic acid or acetosyringone was monitored by measuring fluorescence. Fluorescence levels (in arbitrary units) were corrected by dividing fluorescence values by values of the optical density at 600 nm, and then subtracting the fluorescence value of the empty pOT1e vector. Acetosyringone, which is not degraded by the SpG8-1b pathway, was used as a positive control of *virB* induction.

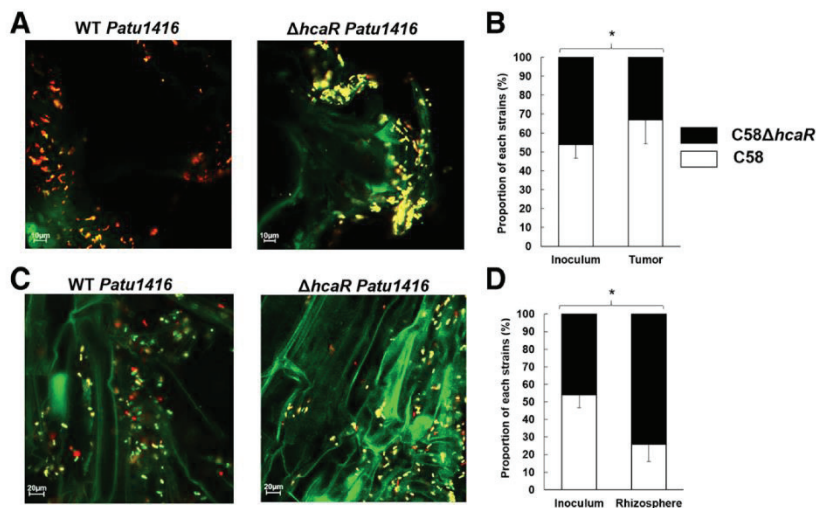


Fig. 5. Effect of *hcaR* deletion on plant colonization and on the expression of hydroxycinnamic acid (HCA) degradation genes in planta. Expression of Patu1416 reporter **A** and **C**, fusions **B** and **D**, fitness were compared between *C58* (wild type [WT], white) and *C58ΔhcaR* (mutant, black) in a tomato tumor (upper part) and in the rhizosphere (lower part). Representative pictures from three biological replicates and two independent experiments are shown (**A** and **C**). Red fluorescence from M-cherry indicates the presence of bacteria, while green fluorescence corresponds to the area of Patu1416 induction. Yellow fluorescence shows bacteria that were both active and able to express Patu1416. Plant autofluorescence is represented in green and the scale in white. The proportion of each simultaneously inoculated strain was calculated in the tumor and the rhizosphere after 3 and 2 weeks of growth, respectively (**B** and **D**).

We characterized HcaR as a transcriptional repressor of the MarR family (Deochand and Grove 2017; Grove 2017; Perera and Grove 2010), which is dimeric in solution and binds to a 25-nucleotide palindromic sequence overlapping the -10 or -35 box. DNA-HcaR binding represses its own transcription and that of the first genes involved in the HCA degradation pathway. This type of regulation is consistent with regulation mechanisms triggered by regulators of the MarR family, whereby the repressor prevents gene transcription by binding to DNA sequences in promoter regions until an effector releases it from DNA to allow gene transcription (Grove 2017). For the MarR regulator of *Acinetobacter* sp. ADP1, which regulates HCA degradation genes, the following ligands can abolish its DNA repression: ferulic acid, *p*-coumaric acid, 3,4-dihydroxybenzoate, and vanillin (Kim et al. 2016; Parke and Ornston 2003). In contrast, we identified only two effectors of HcaR in *A. fabrum* C58, which are different because they are CoA derivatives of ferulic acid and of *p*-coumaric acid.

Hence, the effectors (feruloyl-CoA or *p*-coumaroyl-CoA) that enhance the expression of the degradation pathway are the products of the first step of this pathway (feruloyl-CoA synthase, *Atu1416*). This involves a low basal expression of *atuh1416* to create a positive feedback loop in the presence of HCA. The same ligands have been associated with CouR and FerR regulators from *Rhodococcus* sp. and *Rhodospseudomonas palustris*, respectively (Hirakawa et al. 2012; Otani et al. 2014; Pan et al. 2015). Nonetheless, the DNA-binding motif recognized by *A. fabrum* C58 HcaR differs from any other MarR regulator (Calisti et al. 2008; Hirakawa et al. 2012; Kim et al. 2016; Otani et al. 2016). Although we showed that HcaR is activated by two HCA derivatives, this protein can regulate the degradation of numerous HCA (Fig. 2). Indeed, HCA that are not able to induce SpG8-1b gene expression (Table 2) can be degraded by proteins of the HCA degradation pathway (*C58ΔhcaR*; Fig. 2). That is why we named it HcaR. Interestingly, the HCA degradation pathway is indirectly induced by ferulic acid and *p*-coumaric acid, which are the major constituents of plant cell walls. Moreover, ferulic acid released by wounded plant cells is one of the chemical signals that induces the *Agrobacterium* pathogenic program (Brenic et al. 2004; Joubert et al. 2002; McCullen and Binns 2006). The presence of this compound leads to the expression of *vir* genes (Nester 2015). Additionally, a pTi-encoded HCA degradation protein (VirH2) transforms ferulic acid into caffeic acid. This has been suggested to reduce the metabolic cost of over-expressing the pathogenic machinery by controlling *vir* gene expression, because caffeic acids do not induce *vir* gene expression (Brenic et al. 2004). Thus, HCA degradation gene regulation through HcaR could interfere with pathogenesis-related gene expression.

In the present study, the *virB* gene was poorly expressed in the *C58ΔhcaR* mutant both in vitro in the presence of ferulic acid (Fig. 4) and in planta. In contrast, *vir* genes are expressed in strain C58 and in the *C58ΔSpG8-1b* mutant, which contains neither HcaR nor the degradation genes (Fig. 4). These observations imply that HcaR is not directly involved in *vir* gene activation. Hence, a rapid degradation of the *vir* gene inducer (ferulic acid) due to constitutive expression of HCA degradation genes in the *C58ΔhcaR* mutant does influence the expression of *vir* genes. Thus, it is tempting to speculate that HcaR might play an important role in the pathogenic lifestyle. According to the usual method to test *Agrobacterium* pathogenicity (i.e., inoculation of pure culture on injured tomato stems) (Vaudequin-Dransart et al. 1995), HcaR is not crucial for pathogenicity. Indeed, both the wild type and the *C58ΔhcaR* mutant were able to form tumors. Nonetheless, several key steps of *Agrobacterium* pathogenicity (for example chemotaxis

and attachment) (Heindl et al. 2014; Merritt et al. 2007) are bypassed by our inoculation method and one cannot exclude that HcaR would play a role in a more natural context.

Regulation by a MarR regulator has been proposed to confer a fitness advantage to bacteria in the absence of HCA in order to avoid synthesis of unnecessary enzymes (Grove 2017). HCA are not uniformly distributed in plant tissues, nor are they consistently available for bacteria (Mandal et al. 2009; Simoh et al. 2009). Because this system induces SpG8-1b gene expression after HCA detection, it could be advantageous for bacteria to avoid the metabolic cost of constitutively expressing HCA degradation genes in plant environments with a low HCA content. In a pathogenic context, our findings are consistent with this hypothesis. In tumor tissue, HCA are linked to malate and formed a stock of hydroxycinnamoyl-malate (Simoh et al. 2009). Hence, strain C58 can adapt its metabolism via HcaR regulation according to HCA availability, as shown by in planta expression analyses (Figs. 5A and C). A fitness study of *hcaR*-deleted *C58ΔhcaR* demonstrated that this regulation mechanism was advantageous because C58 outcompeted the mutant strain. The disadvantage of the *C58ΔhcaR* mutant strain could be linked to a metabolic cost associated with the constitutive expression of HCA degradation genes in a low-HCA environment.

Analyses of the importance of HcaR regulation in a rhizospheric context gave opposite results, because the *C58ΔhcaR* mutant outcompeted strain C58 in the rhizosphere (Fig. 5D). Similarly, a competitive advantage conferred by HCA degradation during colonization of the tomato rhizosphere has been reported in *Ralstonia solanacearum* (Lowe et al. 2015). In our case, because the rhizosphere is rich in free HCA (Mandal et al. 2009), it seemed important for bacteria to achieve competitive rhizosphere colonization not only to degrade HCA, as both strains did, but also to rapidly assimilate a broad range of HCA, as the *C58ΔhcaR* mutant did.

In summary, we showed that HcaR regulation influences expression of *vir* genes that are important and influences the execution of the program necessary for pathogenesis. Moreover, it seems relevant to repress HCA degradation genes in the pathogenic context and to rapidly express them in the rhizospheric context. Feruloyl-CoA and *p*-coumaroyl-CoA are the HcaR effectors that allow a positive feedback loop and induce HCA degradation gene expression. Thus, more than being involved in the pathogenicity itself, HcaR regulation through ferulic acid perception may be important for the transition between *A. fabrum* rhizospheric and pathogenic lifestyles.

MATERIALS AND METHODS

Bacterial strains and growth conditions.

The bacteria and plasmids used for this study are listed in Supplementary Table S1. *Escherichia coli* were grown routinely, with shaking (150 rpm), at 37°C in Luria-Bertani (LB) medium. Growth media were supplemented with appropriate antibiotics (tetracycline, 10 µg/ml; gentamicin, 15 µg/ml; and ampicillin, 100 µg/ml) when necessary. The *A. fabrum* strains were grown with shaking (160 rpm), at 28°C in yeast peptone glucose (YPG)-rich medium or in AT minimal medium supplemented with 10 mM succinate and 10 mM ammonium sulfate (Campillo et al. 2012). AT minimal medium was supplemented with 750 µM HCA or phenolic compounds as required, and with the appropriate antibiotic (gentamicin, 20 µg/ml). Phenolic compounds (i.e., acetosyringone, acetovanillone, ferulic acid, *p*-coumaric acid, caffeic acid, 4-dimethoxycinnamic acid, cinnamic acid, 4-methoxycinnamic acid, *o*-coumaric acid, and *m*-coumaric acid) were obtained from Sigma-Aldrich (St. Louis). HCA-CoA derivatives were obtained from TransMIT PlantMetaChem (Giessen, Germany).

1A medium was used to select *A. fabrum* strains after in planta inoculation (Shams et al. 2012).

Construction of the deletion mutant and transcriptional fusions.

The C58 Δ *hcaR* strain was constructed by recombination with a suicide vector (pJQ200sk vector, Quandt and Hynes 1993) according to a strategy previously described by Lassalle et al. (2011). The vector containing the recombinant region (amplified by polymerase chain reaction [PCR]), flanking downstream and upstream of the *hcaR* gene, was introduced into *A. fabrum* C58 by electroporation. Single-crossover integration was selected by gentamycin resistance on YPG medium plates. Gentamycin-resistant colonies were spread on YPG plates containing 10% sucrose to obtain plasmid excision and double-crossover events. *hcaR* deletion was confirmed by PCR analysis and DNA sequencing (GenoScreen, Lille, France).

eGFP transcriptional fusions were generated as follows. The promoter regions of the *virB* and SpG8-1b genes (namely, *PvirB*, *Patu1415*, *Patu1416*, *Patu1417*, *Patu1418*, and *PhcaR*) were amplified with specific primers listed in Supplementary Table S2 and cloned into pGEM-T Easy vectors prior to subcloning of the *Clal-Sall* fragment into the reporter plasmid pOT1e (Allaway et al. 2001) digested with the same enzymes. pOT1eM was obtained by cloning *m-cherry* under Tac promoter control (constitutive expression) into the pOT1e vector digested with *Clal* and *Sall* (Habbadi et al. in press). The previously described promoter regions were ligated to the pOT1eM vector in the *SpeI* restriction site. Constructions were confirmed by PCR using pOT1eF and pOT1eR primers and by sequencing. Reporter constructions were introduced into *A. fabrum* C58 wild-type and mutant strains by electroporation, and gentamycin-resistant colonies were selected.

Measurement of promoter activity.

Expression of the different genes involved in HCA degradation was measured in *A. fabrum* with a pOT1e plasmid harboring an eGFP transcriptional fusion (Allaway et al. 2001). Fluorescence was quantified in microplates filled with 200 μ l of AT medium and supplemented with phenolic compounds at a final concentration of 750 μ M. Microplate wells were inoculated with overnight cultures to obtain an optical density at 600 nm (OD_{600nm}) equal to 0.2. Microplate readings were performed with a Xenius spectrofluorometer (SAFAS-Monaco, Monaco, Monaco), after 14 or 24 h of incubation at 28°C. The following parameters were used: absorbance at 600 nm, fluorescence excitation at 488 nm, and emission at 510 nm. Results were normalized by dividing them by the OD_{600nm} values and then they were compared with values from the empty pOT1e vector. Fluorescence levels were compared using the Wilcoxon rank sum test adjusted with Bonferroni method ($P = 0.05$), and computed with the “vegan” package in the R v3.1.3 statistical software environment (R Core Team 2014).

Cloning, expression, and purification of HcaR.

The *hcaR* gene was PCR amplified with the primer pair (petHcaRFXhoI-petHcaRFXbaI). The product was digested with *XbaI-XhoI* and ligated into the pET29a plasmid digested with the same enzymes. *E. coli* C41 strain was transformed by the recombinant plasmid. For HcaR purification, cells were grown at 37°C in LB medium with kanamycin until they reached an OD_{600nm} value of 0.6, then supplemented with 0.5 mM isopropyl- β -D-thiogalactoside to induce HcaR production. After centrifugation, cells were suspended in 50 mM Tris-HCl (pH 8.0) and 300 mM NaCl, and disrupted by sonication. After centrifugation at 25,000 \times g for 30 min at 4°C, each supernatant was loaded onto a HiTrap talon column (GE

Healthcare, Uppsala, Sweden). Protein elution was performed with 50 mM Tris-HCl (pH 8.0), 300 mM imidazole, and 150 mM NaCl. The fractions containing HcaR were loaded onto a gel filtration column (HiLoad 26/60 Superdex 200 prep grade; GE Healthcare) equilibrated with 50 mM Tris-HCl (pH 8.0) and 150 mM NaCl. The protein fractions were pooled, concentrated, and stored at -80°C.

Gel-shift assay. HcaR binding was determined by gel-shift assays. The intergenic regions of *atu1416-1417*, *atu1418-1419*, and *hcaR* were amplified with specific primer pairs. The *atu2645* intergenic region was used as a nonspecific control probe. Gel-shift assays were performed at 28°C in a 10- μ l reaction mixture containing 0.3 pmol of the DNA probe, 2 μ l of 5 \times binding buffer (Invitrogen, Carlsbad, CA), 150 pmol of HcaR protein, and 0 to 50 μ M aromatic compounds. HcaR-free controls were included. After incubation for 30 min at room temperature, 2 μ l of EMSA gel-loading solution (Invitrogen) was added, and the samples were separated by electrophoresis on 6% polyacrylamide (29:1 ratio) gels in 0.5 \times Tris-boric acid-EDTA (TBE: 90 mM Tris-boric acid and 2 mM EDTA) buffer at 150 V and 4°C for 1.5 h. Gels were then stained with SYBR Green EMSA nucleic acid gel stain (Invitrogen) for 20 min in 0.5 \times TBE, and DNA was visualized under UV light.

K_D measurements by microcalorimetry. ITC experiments were performed at 20°C with an ITC200 isothermal titration calorimeter from MicroCal (GE Healthcare). Protein concentration, in terms of the monomer in the microcalorimeter cell (0.2 ml), was 50 μ M. Nineteen 2- μ l injections of DNA fragment solution at 500 μ M were performed at 180-s intervals, with stirring at 1,000 rpm. Experimental data were fitted to theoretical titration curves with the software program supplied by MicroCal (Origin). This program uses the relation between the heat generated by each injection and ΔH (enthalpy changes in Kcal mol⁻¹), K_a (the association binding constant in M⁻¹), n (the number of binding sites), total protein concentration, and free and total ligand concentrations.

HPLC analyses. The chemical reagents (solvent and standards) were purchased from VWR (Leuven, Belgium) and Interchim (Montluçon, France). HPLC analyses were used to monitor HCA degradation, as previously described (Campillo et al. 2014), using an Agilent 1200 series HPLC system (Agilent Technologies, Santa Clara, CA) coupled to a UV-visible Diode Array Detector (Agilent Technologies) equipped with a Kromasil 100-5C₁₈ column (250 nm by 4.6 mm, 5 μ m; AkzoNobel, Bohus, Sweden). The HCA elution gradient (MeOH/H₂O) was performed according to Campillo et al. (2014). UV-vis spectra and the retention times of commercial standards for all HCA were also analyzed as controls. After 8 h of incubation, the percentage of degraded HCA was calculated by dividing the quantity of HCA measured in culture supernatant by the quantity present in the initial culture medium ($T_0 = 100\%$ of the HCA). Standard deviations were obtained from three technical and two biological replicates.

Plant inoculation and quantification of bacterial colonization. For rhizospheric studies, tomato seed (*Solanum lycopersicum*) were sterilized according to Vacheron et al. (2016). Seed were scarified, inoculated with 10 μ l of overnight culture (10⁶ CFU/ml) of the appropriate strains, and grown in a climatic chamber with 18 h of light and 6 h of darkness on plates containing 0.8% agar plant cell culture supplemented with a nutrient solution (Plant-Prod 15-15-30 High K; Master Plant-Prod Inc., Brampton, ON Canada) at 1.5 g/liter. For in planta pathogenic studies, an incision was made with a scalpel blade in the stems of 3-week-old tomato plants cultivated with a photoperiod of 18 h of light and 6 h of darkness in a greenhouse, and then the plants were inoculated with 10 μ l of overnight culture (10⁸ CFU/ml) of the appropriate strains.

To determine bacterial colonization levels, roots and tumors were first ground 2 and 3 weeks after inoculation, respectively. Serial dilutions were plated onto 1A medium (tumor) or YPG medium (roots) using a spiral plater (EasySpiral; Interscience, Saint-Nom-la-Bretèche, France). After 48 h of incubation at 28°C, colonies were counted using a Scan1200 camera coupled with a computer system for CFU determination. Colonization results (six plants per strain, with enumeration of four Petri dishes for each plant) were log transformed and compared with the Mann-Whitney-Wilcoxon test ($P = 0.05$), using the R v3.1.3 statistical software environment (R Core Team 2014).

CI were determined using the *hcaR* kanamycin-sensitive mutant and a kanamycin-resistant derivative of the C58 (p*Tiatu6148*:Km wild-type strain) Lang et al. 2013). Strains were mixed in 1:1 volumes (10^8 CFU/ml each), 10 μ l was inoculated into wounded tomato stems or seed, and 100 μ l was used to inoculate YPG medium for in vitro competition assays. The input ratio of the initial inoculum was determined by plating the initial inoculum with a spiral plater (EasySpiral; Interscience) onto YPG agar medium. In vitro competition assays were stopped after 1 day of incubation and in planta competition assays (tomato roots) 2 weeks after inoculation. Serial dilutions of the culture medium (in vitro output) or ground roots (in planta output) were plated on YPG medium. Tumor competition assays (in planta output) were stopped 3 weeks after inoculation; tumors were ground and serial dilutions were plated onto 1A medium. For each condition (initial inoculum, in vitro output, and in planta output), 100 colonies were transferred onto YPG medium containing kanamycin and neomycin (each at 25 μ g/ml), in order to determine the relative proportions of the two strains. Three independent assays were performed with eight plants each for in planta competition assays and four technical replicates for in vitro competition assays. CI were calculated as described by Bäumler et al. (1997). Briefly, the C58 Δ *hcaR*/C58 ratio of each in vitro or in planta output sample was divided by the corresponding input ratio in the initial inoculum. Each CI condition was analyzed using the Mann-Whitney-Wilcoxon test ($P = 0.05$) to test whether CI values differed according to each condition (in vitro, tumor, and tomato rhizosphere).

Confocal microscopy analyses. A confocal laser-scanning microscope (LSM 800 Meta Confocal Microscope; Zeiss, Oberkochen, Germany) was used to visualize the expression of the *Patu1416* and *Pvirb* e-gfp-transcriptional fusions harbored by the C58 and C58 Δ *hcaR* strains on tomato roots and tumors. *Patu1416* and *Pvirb* expression were analyzed at 14 and 2 days postinoculation, respectively. Thin tomato root sections and young tumor tissue were mounted between a slide and a coverslip in commercial mounting fluid (Aqua Poly/Mount; Polysciences, Inc., Warrington, PA). eGFP and M-cherry were excited with an argon laser at 488 and 584 nm, respectively, and fluorescence was monitored at 528 nm and 607 nm. M-cherry (constitutive expression) reported the presence of bacteria and eGFP reported gene expression. Images were recorded using LSM 800 software (Zeiss). For each image, the arithmetic mean intensity of M-cherry and eGFP were obtained using LSM 800 software. The arithmetic means eGFP fluorescence intensity was corrected by that of M-cherry. Comparison of the expression was performed by the Mann-Whitney-Wilcoxon test ($P = 0.05$). Standard deviation was obtained from three biological replicates and two independent experiments.

ACKNOWLEDGEMENTS

We thank L. Loiseau (PARMIC platform of UMR 5557, Ecologie Microbienne, INRA, UMR 1418) for technical assistance and A. Buchwalter for reading the manuscript and providing suggestions.

LITERATURE CITED

- Allaway, D., Schofield, N. A., Leonard, M. E., Gilardoni, L., Finan, T. M., and Poole, P. S. 2001. Use of differential fluorescence induction and optical trapping to isolate environmentally induced genes. *Environ. Microbiol.* 3:397-406.
- Andersson, D. I., and Hughes, D. 2010. Antibiotic resistance and its cost: Is it possible to reverse resistance? *Nat. Rev. Microbiol.* 8:260-271.
- Ashby, A. M., Watson, M. D., Loake, G. J., and Shaw, C. H. 1988. Ti plasmid-specified chemotaxis of *Agrobacterium tumefaciens* C58C1 toward *vir*-inducing phenolic compounds and soluble factors from monocotyledonous and dicotyledonous plants. *J. Bacteriol.* 170: 4181-4187.
- Bäumler, A. J., Tsolis, R. M., Valentine, P. J., Ficht, T. A., and Heffron, F. 1997. Synergistic effect of mutations in *invA* and *lpfC* on the ability of *Salmonella typhimurium* to cause murine typhoid. *Infect. Immun.* 65: 2254-2259.
- Bhattacharya, A., Sood, P., and Citovsky, V. 2010. The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. *Mol. Plant Pathol.* 11:705-719.
- Bolton, G. W., Nester, E. W., and Gordon, M. P. 1986. Plant phenolic compounds induce expression of the *Agrobacterium tumefaciens* loci needed for virulence. *Science* 232:983-985.
- Brenic, A., Eberhard, A., and Winans, S. C. 2004. Signal quenching, detoxification and mineralization of *vir* gene-inducing phenolics by the VirH2 protein of *Agrobacterium tumefaciens*. *Mol. Microbiol.* 51: 1103-1115.
- Brooks, A. N., Turkarslan, S., Beer, K. D., Lo, F. Y., and Baliga, N. S. 2011. Adaptation of cells to new environments. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 3:544-561.
- Calisti, C., Ficca, A. G., Barghini, P., and Ruzzi, M. 2008. Regulation of ferulic catabolic genes in *Pseudomonas fluorescens* BF13: Involvement of a MarR family regulator. *Appl. Microbiol. Biotechnol.* 80:475-483.
- Campillo, T., Lavire, C., Shams, M., Pothier, J. F., and Pulawska, J. 2012. Detection and identification methods and new tests as developed and used in the framework of Cost873 for bacteria pathogenic to stone fruits and nuts tumorigenic *Agrobacterium* spp. *J. Plant Pathol.* 94: S1.97-S1.104.
- Campillo, T., Renoud, S., Kerzaon, I., Vial, L., Baude, J., Gaillard, V., Bellvert, F., Chamignon, C., Comte, G., Nesme, X., Lavire, C., and Hommais, F. 2014. Analysis of hydroxycinnamic acid degradation in *Agrobacterium fabrum* reveals a coenzyme A-dependent, beta-oxidative deacetylation pathway. *Appl. Environ. Microbiol.* 80:3341-3349.
- Cases, I., de Lorenzo, V., and Ouzounis, C. A. 2003. Transcription regulation and environmental adaptation in bacteria. *Trends Microbiol.* 11:248-253.
- Deochand, D. K., and Grove, A. 2017. MarR family transcription factors: Dynamic variations on a common scaffold. *Crit. Rev. Biochem. Mol. Biol.* 52:595-613.
- Duprey, A., Reverchon, S., and Nasser, W. 2014. Bacterial virulence and Fis: Adapting regulatory networks to the host environment. *Trends Microbiol.* 22:92-99.
- Grove, A. 2017. Regulation of metabolic pathways by MarR family transcription factors. *Comput. Struct. Biotechnol. J.* 15:366-371.
- Guo, M., Huang, Z., and Yang, J. 2017. Is there any crosstalk between the chemotaxis and virulence induction signaling in *Agrobacterium tumefaciens*? *Biotechnol. Adv.* 35:505-511.
- Habbadi, K., Meyer, T., Vial, L., Gaillard, V., Benkirane, R., Benbouazza, A., Kerzaon, I., Achbani, E. H., and Lavire, C. Essential oils of *Origanum compactum* and *Thymus vulgaris* exert a protective effect against the phytopathogen *Allorhizobium vitis*. *Environ. Sci. Pollut. Res. Int.* (In press). doi.org/10.1007/s11356-017-1008-9
- Heindl, J. E., Wang, Y., Heckel, B. C., Mohari, B., Feirer, N., and Fuqua, C. 2014. Mechanisms and regulation of surface interactions and biofilm formation in *Agrobacterium*. *Front. Plant Sci.* 5:176.
- Hirakawa, H., Schaefer, A. L., Greenberg, E. P., and Harwood, C. S. 2012. Anaerobic *p*-coumarate degradation by *Rhodospseudomonas palustris* and identification of CouR, a MarR repressor protein that binds *p*-coumaroyl coenzyme A. *J. Bacteriol.* 194:1960-1967.
- Joubert, P., Beaupère, D., Lelièvre, P., Wadouachi, A., Sangwan, R. S., and Sangwan-Norreel, B. S. 2002. Effects of phenolic compounds on *Agrobacterium vir* genes and gene transfer induction—A plausible molecular mechanism of phenol binding protein activation. *Plant Sci.* 162:733-743.
- Kalogeraki, V. S., Zhu, J., Eberhard, A., Madsen, E. L., and Winans, S. C. 1999. The phenolic *vir* gene inducer ferulic acid is O-demethylated by the VirH2 protein of an *Agrobacterium tumefaciens* Ti plasmid. *Mol. Microbiol.* 34:512-522.

- Kim, Y., Joachimiak, G., Bigelow, L., Babnigg, G., and Joachimiak, A. 2016. How aromatic compounds block DNA binding of HcaR catabolite regulator. *J. Biol. Chem.* 291:13243-13256.
- Lang, J., Planamente, S., Mondy, S., Dessaux, Y., Moréra, S., and Faure, D. 2013. Concerted transfer of the virulence Ti plasmid and companion At plasmid in the *Agrobacterium tumefaciens*-induced plant tumour. *Mol. Microbiol.* 90:1178-1189.
- Lassalle, F., Campillo, T., Vial, L., Baude, J., Costechareyre, D., Chapulliot, D., Shams, M., Abrouk, D., Lavire, C., Oger-Desfeux, C., Hommais, F., Guéguen, L., Daubin, V., Muller, D., and Nesme, X. 2011. Genomic species are ecological species as revealed by comparative genomics in *Agrobacterium tumefaciens*. *Genome Biol. Evol.* 3:762-781.
- Lowe, T. M., Ailloud, F., and Allen, C. 2015. Hydroxycinnamic acid degradation, a broadly conserved trait, protects *Ralstonia solanacearum* from chemical plant defenses and contributes to root colonization and virulence. *Mol. Plant-Microbe Interact.* 28:286-297.
- Mandal, S., Mitra, A., and Mallick, N. 2009. Time course study on accumulation of cell wall-bound phenolics and activities of defense enzymes in tomato roots in relation to *Fusarium* wilt. *World J. Microbiol. Biotechnol.* 25:795-802.
- McCullen, C. A., and Binns, A. N. 2006. *Agrobacterium tumefaciens* and plant cell interactions and activities required for interkingdom macromolecular transfer. *Annu. Rev. Cell Dev. Biol.* 22:101-127.
- Merritt, P. M., Danhorn, T., and Fuqua, C. 2007. Motility and chemotaxis in *Agrobacterium tumefaciens* surface attachment and biofilm formation. *J. Bacteriol.* 189:8005-8014.
- Nester, E. W. 2015. *Agrobacterium*: Nature's genetic engineer. *Front. Plant Sci.* 5:730.
- Otani, H., Lee, Y.-E., Casabon, I., and Eltis, L. D. 2014. Characterization of *p*-hydroxycinnamate catabolism in a soil Actinobacterium. *J. Bacteriol.* 196:4293-4303.
- Otani, H., Stogios, P. J., Xu, X., Nocek, B., Li, S.-N., Savchenko, A., and Eltis, L. D. 2016. The activity of CouR, a MarR family transcriptional regulator, is modulated through a novel molecular mechanism. *Nucleic Acids Res.* 44:595-607.
- Pan, C., Hu, Y. L., Jiang, X. N., and Gai, Y. 2015. Cloning, expression, crystallization and crystallographic analysis of CouR from *Rhodospseudomonas palustris*. *Acta Crystallogr. Sect. F Struct. Biol. Commun.* 71: 1416-1420.
- Parke, D. 1995. Supraoperonic clustering of *pca* genes for catabolism of the phenolic compound protocatechuate in *Agrobacterium tumefaciens*. *J. Bacteriol.* 177:3808-3817.
- Parke, D. 1997. Acquisition, reorganization, and merger of genes: Novel management of the β -ketoacid pathway in *Agrobacterium tumefaciens*. *FEMS Microbiol. Lett.* 146:3-12.
- Parke, D., and Ornston, L. N. 2003. Hydroxycinnamate (*hca*) catabolic genes from *Acinetobacter* sp. strain ADP1 are repressed by HcaR and are induced by hydroxycinnamoyl-coenzyme A thioesters. *Appl. Environ. Microbiol.* 69:5398-5409.
- Perera, I. C., and Grove, A. 2010. Molecular mechanisms of ligand-mediated attenuation of DNA binding by MarR family transcriptional regulators. *J. Mol. Cell Biol.* 2:243-254.
- Price, M. N., Deuschbauer, A. M., Skerker, J. M., Wetmore, K. M., Ruths, T., Mar, J. S., Kuehl, J. V., Shao, W., and Arkin, A. P. 2013. Indirect and suboptimal control of gene expression is widespread in bacteria. *Mol. Syst. Biol.* 9:660.
- Quandt, J., and Hynes, M. F. 1993. Versatile suicide vectors which allow direct selection for gene replacement in gram-negative bacteria. *Gene* 127:15-21.
- R Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
- Schick, A., Bailey, S. F., and Kassen, R. 2015. Evolution of fitness trade-offs in locally adapted populations of *Pseudomonas fluorescens*. *Am. Nat.* 186 (Suppl 1):S48-S59.
- Simoh, S., Quintana, N., Kim, H. K., Choi, Y. H., and Verpoorte, R. 2009. Metabolic changes in *Agrobacterium tumefaciens*-infected *Brassica rapa*. *J. Plant Physiol.* 166:1005-1014.
- Vacheron, J., Moëgne-Loccoz, Y., Dubost, A., Gonçalves-Martins, M., Muller, D., and Prigent-Combaret, C. 2016. Fluorescent pseudomonas strains with only few plant-beneficial properties are favored in the maize rhizosphere. *Front. Plant Sci.* 7:1212.
- Valentini, M., Gonzalez, D., Mavridou, D. A., and Filloux, A. 2018. Lifestyle transitions and adaptive pathogenesis of *Pseudomonas aeruginosa*. *Curr. Opin. Microbiol.* 41:15-20.
- Vaudequin-Dransart, V., Petit, A., Poncet, C., Ponsonnet, C., Nesme, X., Jones, J. B., Bouzar, H., Chilton, W. S., and Dessaux, Y. 1995. Novel Ti plasmids in *Agrobacterium* strains isolated from fig tree and chrysanthemum tumors and their opinelike molecules. *Mol. Plant-Microbe Interact.* 8:311-321.
- White, A. P., Sibley, K. A., Sibley, C. D., Wasmuth, J. D., Schaefer, R., Surette, M. G., Edge, T. A., and Neumann, N. F. 2011. Intergenic sequence comparison of *Escherichia coli* isolates reveals lifestyle adaptations but not host specificity. *Appl. Environ. Microbiol.* 77: 7620-7632.

Proof Only

Supplementary table and figure.

TableS1: Strains and plasmids used in this study

	Relevant genotype and description	Reference or source
<i>Escherichia coli</i>		
JM109	<i>endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ Δ(lac-proAB) e14- [F' traD36 proAB+ lacIq lacZΔM15] hsdR17(rK-mK+)</i>	NEB catalog
C41	<i>F⁻ ompT gal dcm hsdS_B(r_B⁻ m_B⁻)(DE3)pLysS (Cm^r)</i>	NEB catalog
<i>Agrobacterium fabrum</i>		
C58	Wild-type	CFBP 1903
C58Δ <i>atu1415</i>	C58 deleted of <i>atu1415</i> gene	Campillo <i>et al.</i> (2014)
C58Δ <i>atu1416</i>	C58 deleted of <i>atu1416</i> gene	Campillo <i>et al.</i> (2014)
C58Δ <i>atu1417</i>	C58 deleted of <i>atu1417</i> gene	Campillo <i>et al.</i> (2014)
C58Δ <i>atu1418</i>	C58 deleted of <i>atu1418</i> gene	Campillo <i>et al.</i> (2014)
C58Δ <i>hcaR</i>	C58 deleted of <i>hcaR</i> gene	This study
C58 pTi <i>atu6148</i> :Km	C58 disrupted for <i>atu6148</i> gene ; Km ^R	Lang <i>et al.</i> (2013)
C58ΔSpG8-1b	C58 deleted of SpG8-1b genes cluster ; Km ^R	Lassalle <i>et al.</i> (2012)
Plasmids		
pJQ200SK	Suicide vector; P15A <i>sacB</i> ; Gm ^R	Quandt & Hynes (1993)
pOT1e	Promoter-probe vector based on pBBR1MCS-5 replicon; contains promoterless eGFP and MCS between two transcriptional terminators; Gm ^R	Allaway <i>et al.</i> (2001)
pOT1eM	pOT1e derivative harboring <i>Ptac-m-cherry</i> inserted in Clal-Sall site	Unpublished data
pET-29(a)	<i>E. coli</i> expression vector (6His-tag), Km ^R	Novagen
<i>Patu1416</i> -eGFP	Upstream region <i>atu1416</i> inserted in site Clal-Sall in pOT1e; Gm ^R	This study
<i>Patu1417</i> -eGFP	Upstream region <i>atu1417</i> inserted in site Clal-Sall in pOT1e; Gm ^R	This study
<i>Patu1418</i> -eGFP	Upstream region <i>atu1418</i> inserted in site Clal-Sall in pOT1e; Gm ^R	This study
<i>Patu1419</i> -eGFP	Upstream region <i>atu1419</i> inserted in site Clal-Sall in pOT1e; Gm ^R	This study
<i>lPhcaR</i> -eGFP	Upstream region <i>hcaR</i> inserted in site Clal-Sall in pOT1e; Gm ^R	This study
pOT1eM- <i>Patu1416</i> -eGFP	Upstream region <i>atu1416</i> inserted in site PstI in pOT1em; Gm ^R	This study
pET-29(a)- <i>hcaR</i>	<i>hcaR</i> inserted in site XbaI-XhoI in pET-29(a), His-tag fusion, Km ^R	This study

TableS2: Primers used in this study

Target	Primer	Primer sequence (5'-3')	Primer length
Transcriptional fusion			
<i>atu1416</i> promoter	1416FClal	ATCGATTGTGGATATGTTCCGCCAAG	500 bp
	1416R	GAACGACACCAGTGAAGGAGA	
<i>atu1417</i> promoter	1417FClal	ATCGATACCGAAAGGTCATGATCGAG	517 bp
	1417R	CAACGAAACGGTCACTGATG	
<i>atu1418</i> promoter	1418FClal	ATCGATGCCAGCCAGGATGTTGCTAG	734 bp
	1418R	TTTCCCTGAGTTTCGATCACC	
<i>hcaR</i> promoter	1422FClal 1422R	ATCGATGCGAGAGGCTCCAGATAGGT AAGTGCGGTGAAGAAATCAT	569 bp
<i>virB</i> promoter	VirBFClal VirBR	ATCGATTCCATCATCATTGGAGGAAGT CACGTGGAGGCCAGTAAGAT	718 bp
Deletion of <i>hcaR</i>			
<i>hcaR</i> upstream region	<i>hcaR</i> UpF	GGGCCTTTGCTATTGTGAAA	1003 bp
	<i>hcaR</i> UpR	ACAGGCCCGTGAATATTGA	
<i>hcaR</i> downstream region	<i>hcaR</i> DwF	GTATGCACATCGATGGCAATAAGTTCAT CAATATTCACGGCCTGTCATCGTCTTGC	1023 bp
	<i>hcaR</i> DwR	GGGGGAGGT	
deletion verification	ExtF	CCCCGATTTGAAGTATTCAGG	2381 bp
	ExtR	GCCACAAGCAGAAGGAAATC CGCTCATCATATTTCTCGAA TCTTCTAGAAAATAATTTGTTAACTTTA AGAAGGAGATATACATATGGACGTCAA	
<i>hcaR</i> overexpression	pET <i>hcaR</i> FXbal pET <i>hcaR</i> RXhol	TATCGACCG CTCCTCGAGTCAGTGGTGGTGGTGGT GGTGATATTCACGGCCTGTTCTC	528 bp
gel shift assays			
<i>atu1416-atu1417</i>	14161417F	ATTC AATTTCTGCGGATAGG	326 bp
	14161417R	CTGTTGAGGCCAACAAGG	
<i>atu1418-1419</i>	14181419F	CCATGTGTCGCAGCATCC	370 bp
	14181419R	CACCGGAATTATCCCAAAAA	
<i>hcaR</i>	<i>hcaR</i> F	ATGTCGATCTGTGCAACC	375 bp
	<i>hcaR</i> R	GATATTGACGTCCATCGTCT	
<i>atu2645</i>	2645F	CGACAGAGGCCGGTTATTTG	378 bp
	2645R	CAACCATCATGATCAGGGCG	
<i>atu1416-456</i>	-267F	GGCAATAGCCTCAACGAAAC	170 bp
	-267R	TGCGGAAGATAGATTGTATATGAG	
<i>atu1416-59</i>	-59F	TTTGACAAAAGATTATTCGTACGG	170 bp
	-59R	CTATCCGCAGAAATTGAATGAG	

TableS3: ferulic acid degradation effect on the SpG8-1b genes expression after 24 hours of incubation

	C58Δatu1416	C58Δatu1415	C58Δatu1417	C58Δatu1418
Patu1416				
0 μM	4.67±0.24	4.16±0.44	4.67±0.36	5.7±0.23
750 μM	3.85±0.14	5.33±0.43	24.23±0.92	12.07±0.57
Fold change	0.825	1.28	5.187	2.116
P-value	0.002	0.002	0.002	0.002
Patu1417				
0 μM	3.84±0.36	3.27±0.23	2.87±0.45	5.5±0.26
750 μM	3.45±0.20	4.07±0.47	10.64±0.43	6.35±0.16
Fold change	0.899	1.242	3.702	1.156
P-value	0.026	0.004	0.004	0.002
Patu1418				
0 μM	11.22±0.30	10.1±0.60	10.43±0.29	11.54±0.22
750 μM	9.94±0.27	9.2±0.23	9.08±0.29	16.02±1.06
Fold change	0.886	0.911	0.871	1.388
P-value	0.002	0.041	0.002	0.002
PhcaR				
0 μM	6.13±0.26	5.43±0.22	5.07±0.34	6.82±0.24
750 μM	5.46±0.30	5.63±0.41	7.36±0.57	7.19±0.26
Fold change	0.891	1.037	1.450	1.055
P-value	0.002	0.180	0.002	0.065
empty pOT1e				
0 μM	4.67±0.18	4.11±0.48	3.96±0.31	5.57±0.27
750 μM	4.88±0.21	4.45±0.55	4.36±0.40	4.81±0.39
Fold change	1.045	1.083	1.101	0.864
P-value	0.093	0.485	0.132	0.009

TableS4: p-coumaric acid degradation effect on the SpG8-1b genes expression after 24 hours of incubation

	C58	C58Δatu1422	C58Δatu1416	C58Δatu1415	C58Δatu1417	C58Δatu1418
Patu1416						
0 μM	4.59±0.3	22.53±0.72	4.55±0.04	4.72±0.49	6.98±0.17	7.68±0.09
750 μM	26.15±4.09	22.99±0.82	4.95±0.05	23.48±0.97	28.52±1.07	21.32±1.88
Fold change	5.70	1.02	1.09	4.97	4.09	2.78
P-value	0.002	0.029	0.029	0.029	0.029	0.029
Patu1417						
0 μM	3.49±0.13	12.75±0.54	4.6±0.13	4.06±0.32	5.79±0.22	6.2±0.13
750 μM	12.21±1.73	12.59±0.24	4.73±0.22	12.72±0.33	24.06±0.16	12.49±0.75
Fold change	3.50	0.99	1.03	3.13	4.16	1.98
P-value	0.002	0.886	0.486	0.029	0.029	0.029
Patu1418						
0 μM	9.71±0.26	6.32±0.15	10.5±0.24	9.23±0.2	13.89±0.13	15.44±0.28
750 μM	8.76±0.51	5.72±0.01	10.24±0.05	9.28±0.25	10.63±0.03	13.83±0.75
Fold change	0.90	0.905063291	0.98	1.01	0.77	0.90
P-value	0.005	0.029	0.200	0.686	0.029	0.029
Patu1422						
0 μM	4.96±0.21	7.9±0.11	7.12±0.28	8.29±0.25	7.6±0.05	8.21±0.17
750 μM	8.3±0.28	7.92±0.29	7.33±0.1	8.91±0.19	11.98±0.27	9.93±0.07
Fold change	1.68	1.00	1.03	1.07	1.58	1.21
P-value	0.002	0.886	0.343	0.686	0.029	0.029
empty pOT1e						
0 μM	3.67±0.13	6.57±0.06	3.04±0.05	4.34±0.05	6.86±0.26	6.41±0.06
750 μM	3.8±0.12	6.34±0.12	3.02±0.08	4.37±0.08	6.33±0.11	6.31±0.05
Fold change	1.04	0.96	1.01	1.01	0.92	0.98
P-value	0.127	0.029	0.486	0.886	0.029	0.486

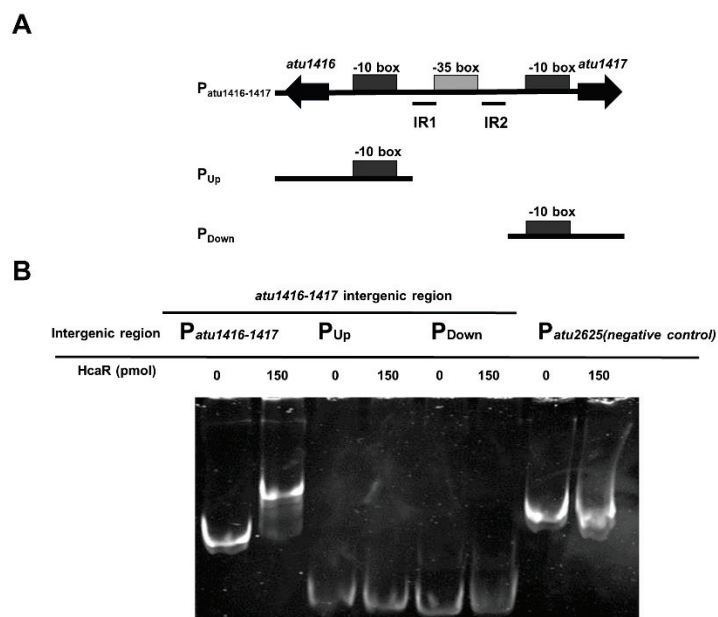


Figure S1: A palindromic region is required for HcaR binding to the *atu1416-1417* promoter region

(A) Localization of shorter DNA probes in the *atu1416-1417* promoter region for gel shift assessment of the HcaR binding site. (B) Effect of the presence of a palindromic region on HcaR DNA binding.

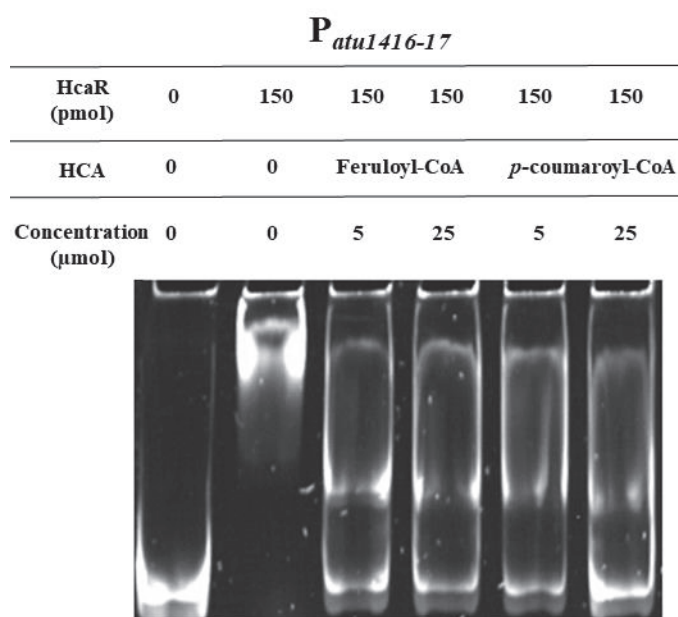


Figure S2: Feruloyl-CoA and *p*-coumaroyl-CoA are HcaR effectors

Gel shift analysis of HcaR binding to the *Patu1416-1417* region, in presence of feruloyl-CoA or *p*-coumaroyl-CoA at different concentrations (25 and 50 μM).

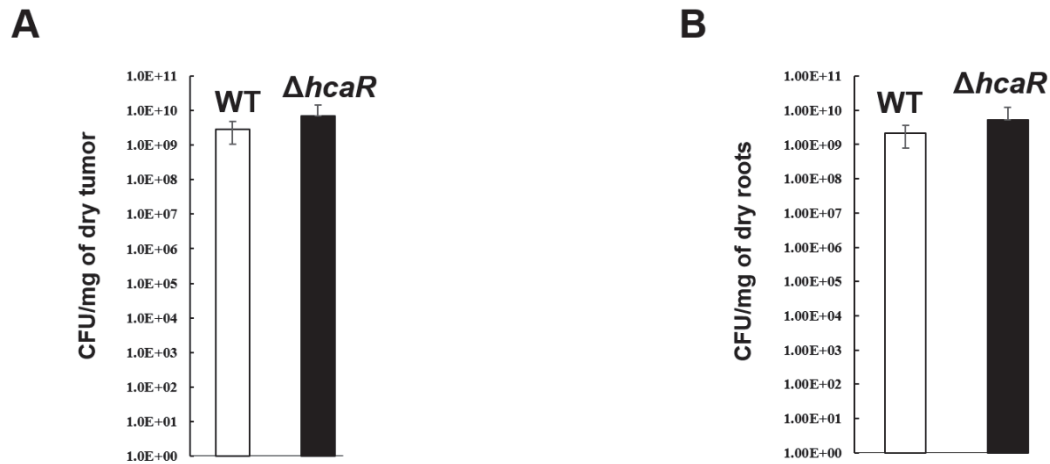


Figure S3: Population levels of the wild-type and the $C58\Delta hcaR$ strains in tumor and rhizosphere

The population level was compared between the C58 (white color) and the $C58\Delta hcaR$ (black color) strains in a tomato tumor (A) and rhizosphere (B). In the tumor and in the rhizosphere, the population level was similar for both strains (Mann-Whitney-Wilcoxon test, $P = 0.22$).

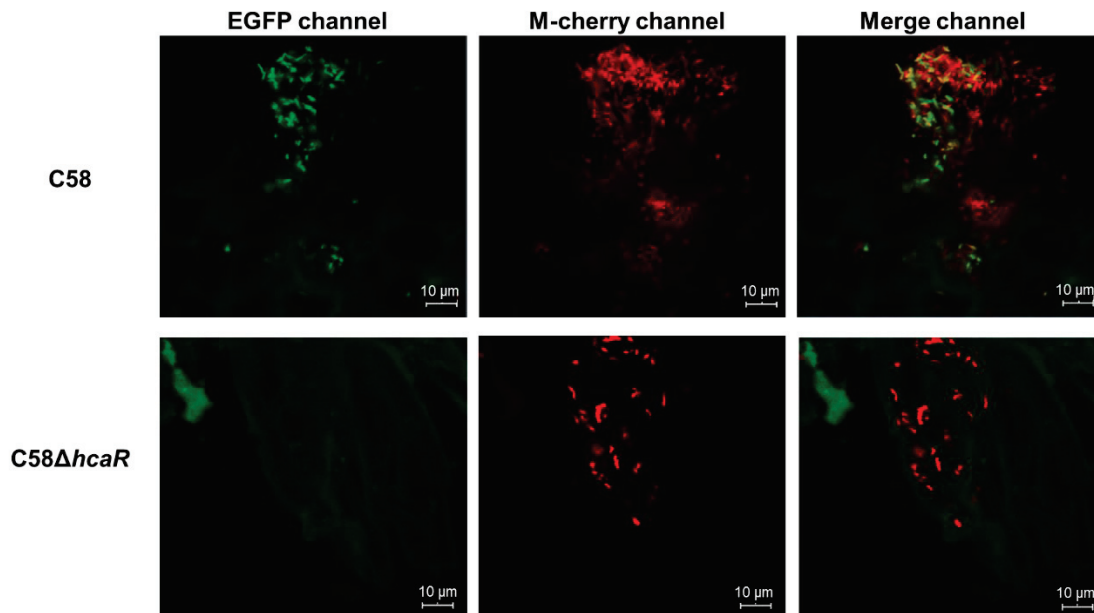


Figure S4: Induction of $virB$ in the C58 and $C58\Delta hcaR$ mutant strains in tumor lifestyle.

Expression of $PvirB$ reporter fusions (A, B) were compared between C58 (wild-type, white) and $C58\Delta hcaR$ (mutant, black) in a tomato tumor. Representative pictures from five biological replicates and three independent experiments are shown. Red fluorescence from M-cherry indicates the presence of bacteria, while green fluorescence corresponds to the area of $PvirB$ induction. Yellow fluorescence shows bacteria that were both active and able to express $PvirB$. The scale is represented in white. Expression of the virulence gene ($virB$) was higher in C58 than in $C58\Delta hcaR$ in the tumor lifestyle (A, B, Mann-Whitney-Wilcoxon test, $P = 0.022$).

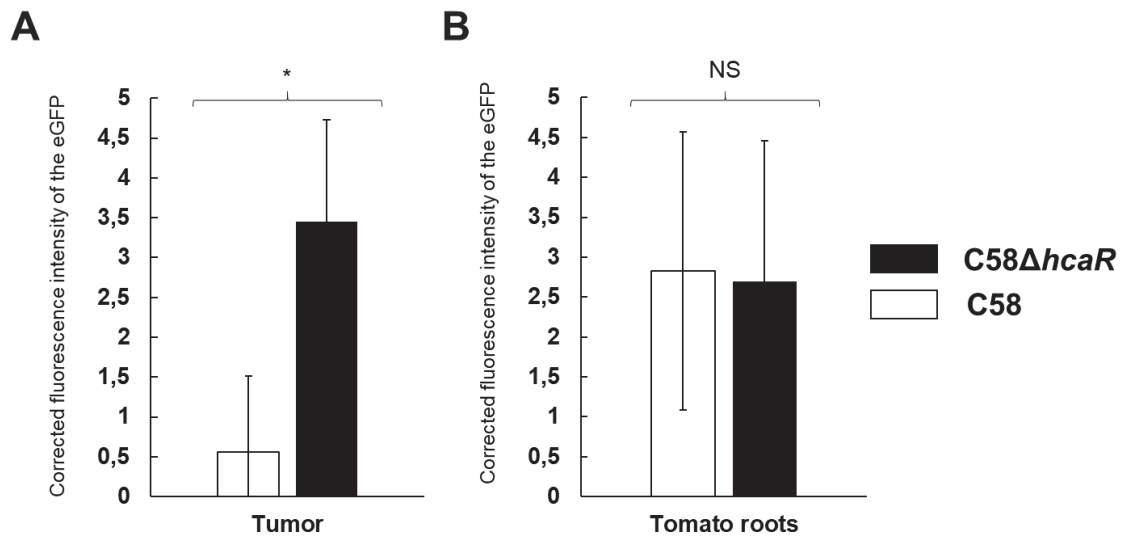


Figure S5: Quantification of *Patu1416* induction on tumor and tomato roots.

The corrected fluorescence intensity of the eGFP was used for comparison and quantification of *Patu1416* induction in tumor (A) and in roots (B) between the wild-type (white bars) and the C58ΔhcaR strains (black bars). *Patu1416* induction was significantly higher in the C58ΔhcaR than in C58 in tumor (A, Mann-Whitney-Wilcoxon test, $P = 0.02$). No significant difference of *Patu1416* induction was quantified between both strains in rhizosphere (B, $P = 0.93$).

Conclusion du chapitre 1

Dans ce chapitre nous avons caractérisé d'un point de vue mécanistique et écologique la régulation de la dégradation des HCAs par HcaR, un régulateur de type MarR. La protéine HcaR se fixe au niveau de séquences inversées répétées situées dans les régions promotrices des premiers gènes de l'îlot SpG8-1b de dégradation des HCAs (*atu1416-1417*) et en amont de sa propre région promotrice, ce qui réprime leur transcription. Les effecteurs de ce régulateur sont le féruloïl-CoA et le *p*-coumaroïl-CoA. Leur fixation à HcaR permet de lever la répression et l'induction de l'expression des gènes de dégradation des HCAs. Dans la rhizosphère de tomates, les gènes de la région sont exprimés fortement alors que dans la tumeur leur expression est plus limitée. Nous avons démontré que cette régulation interférait avec l'expression des gènes *vir* impliqués dans la virulence d'*A. fabrum*. Nous avons par ailleurs observé un effet contrasté de la délétion de ce régulateur au niveau des capacités de colonisation d'*A. fabrum*. En effet, la souche délétée de ce gène est plus abondante que la souche sauvage dans la rhizosphère de tomate et moins abondante dans la tumeur. Comme cela a été suggéré par Grove pour les régulateurs MarR (Grove, 2017), la régulation de la dégradation des HCAs semble optimisée chez *A. fabrum* pour permettre l'expression du cluster de dégradation au moment adéquat. Ce système de régulation pourrait être impliqué chez *A. fabrum* dans la transition entre ses deux styles de vie. Au vue de l'importance de ces composés en tant que signal dans l'écologie de certains pathogènes comme *D. dadantii* et *R. solanacearum*, et comme cette dégradation semble peu propice à la croissance d'*A. fabrum*, nous avons émis l'hypothèse que la perception et la dégradation des HCAs pourrait influencer d'autres fonctions impliquées dans l'écologie d'*A. fabrum*, et notamment interférer avec le métabolisme carboné de celle-ci.

**Chapitre 2 : Rôle signal des
HCA dans l'interaction
Agrobacterium fabrum–plante**

Préambule du chapitre 2

La rhizosphère contient une multitude de ressources trophiques (acides organiques, acides aminés, sucres, composés phénoliques ...) qui sont produites via l'exsudation racinaire, les microorganismes qui la peuplent et la perte de cellules composant la coiffe racinaire (Dennis *et al.*, 2010). Ces exsudats sont impliqués dans la communication moléculaire avec les organismes bénéfiques ou commensaux (Raguso *et al.*, 2015; van Dam and Bouwmeester, 2016) et permettent aussi à certaines plantes de lutter contre des pathogènes (Baetz and Martinoia, 2014; De Coninck *et al.*, 2015). Dans le cadre de l'interaction plante-*Agrobacterium fabrum*, nous avons vu que des HCAs produits par la plante peuvent être dégradés par la bactérie, et influencent son écologie. Dans la rhizosphère, cette dégradation est induite par le féruloïl-CoA ou le *p*-coumaroyl-CoA alors que dans la tumeur l'absence de ces inducteurs provoque une répression de l'expression des gènes de dégradation. La perception des HCAs est aussi à l'origine d'une modulation de l'expression de nombreux gènes, comme le soulignent les comparaisons de transcriptome en présence et en absence d'HCAs chez *A. fabrum* (Baude *et al.* 2016), et comme cela a aussi été observé chez *Corynebacterium* (Chen *et al.*, 2017). Parmi, les gènes identifiés comme cibles de cette modulation, certains permettent probablement la dégradation ou le transport de ressources trophiques (analyse personnelle d'après les données de transcriptomique et les annotations). La présence des HCAs est à l'origine de la modulation d'un grand nombre de fonctions et peut influencer la nutrition bactérienne, notamment chez certaines bactéries interagissant avec les plantes (cf Synthèse bibliographique Partie B) (Fischer *et al.*, 2008; Karishma *et al.*, 2015; Mohan and Phale, 2017).

Nous avons ainsi émis l'hypothèse que la dégradation des HCAs par la région SpG8-1b était perçue comme un signal de la proximité de la plante et induisait une adaptation à cet environnement. Cette reconnaissance induirait une modulation du métabolisme carboné et notamment influencerait l'utilisation de ressources trophiques importantes pour *A. fabrum*. Afin de tester cette hypothèse, nous avons étudié globalement l'effet de la dégradation des HCAs sur le métabolisme carboné de cette bactérie.

Ces travaux font l'objet d'un article en construction intitulé « Hydroxycinnamic acids degradation induces a metabolism shift in *Agrobacterium fabrum* ». J'ai réalisé l'ensemble des expérimentations présentées dans cet article.

Article 2 : Ferulic acid degradation induces global modulation of metabolism on *Agrobacterium fabrum*

Thibault Meyer ¹, Ludovic Vial ¹, Gilles Comte ¹, Céline Lavire ¹

¹ Université de Lyon, F-69622, Lyon, France ; Université Lyon 1, Villeurbanne, France ; CNRS, UMR5557, Ecologie Microbienne, Villeurbanne, France ; INRA, UMR1418, Villeurbanne, France

Correspondence should be sent to: Céline Lavire, Ecologie Microbienne, Université Lyon 1, 43 Boulevard du 11 novembre F-69622 Villeurbanne cedex, France, Tel.: (+33) 426237126 ; Fax: (+33) 4 72 43 12 23; E-mail: celine.lavire@univ-lyon1.fr

Keywords: *Agrobacterium fabrum*; hydroxycinnamic acids perception; plant signal; modulation of carbon metabolism; fitness; crown-gall; rhizosphere; transcriptional modulation; species-specific genes

Abstract

Agrobacterium fabrum are soil α -proteobacteria often isolated from plants. Even though agrobacteria are mostly commensal, they become pathogenic when they harbor the Ti (Tumor inducing) plasmid, inciting the crown gall disease. Genomic comparison permitted to identify 7 genomic regions specific of this species (i.e absent from other *Agrobacterium* species) that are likely involved in plant-bacterium interactions. Among those regions, we previously characterized one cluster of genes involved in hydroxycinnamic acids (HCAs) degradation and demonstrated that HCA degradation genes are expressed during plant colonization. HCAs like ferulic and *p*-coumaric acids are plant compounds involved in plant-bacterium interactions either as toxics, or as nutritive resources, chemoattractants or virulence inducers.

We hypothesized that HCA degradation could be a way for *A. fabrum* to sense plant environment and to adapt its metabolism accordingly. By using deletion mutants unable to degrade HCA and phenotypic microarrays (Biolog™), we showed that the feruloyl-CoA and *p*-coumaroyl-CoA (activated form of ferulic and *p*-coumaric acids) influences the metabolic activities and the growth on carbon sources for specific substrates belonging to sugars, organic

acids or amino acids. We demonstrated that the transcriptional repression of uptake genes explains the lack of growth on certain metabolites belonging to two different metabolic pathways and important for *A. fabrum* fitness in interaction with plants. Taken together our results highlight importance of HCA perception for *A. fabrum*-plant interaction.

Introduction

Hydroxycinnamic acids (HCAs) includes the cinnamic acid (Croteau *et al.* 2000; Vogt 2010) and its hydroxylated derivatives, for example ferulic acid, *p*-coumaric acid, caffeic acid, and sinapic acid. They are highly produced by plants, can be incorporated in plant cell wall whereby they play an important role to ensure the rigidity of plant stem. HCAs are also exudated in the rhizosphere where they can influence microbial community (Mandal *et al.* 2010; Zhalnina *et al.* 2018; Badri *et al.* 2013). They are first known as antimicrobial compounds which disrupt the membrane polarity like it has been shown for *Escherichia coli*, *Cronobacter sakazakii*, *Pectobacterium carotovorum*, *Pseudomonas syringae* and *Xanthomonas campestris* (Joshi *et al.* 2016; Yamazaki *et al.* 2012; Shi *et al.* 2016). Nonetheless, these compounds can be perceived as chemoattractants for bacteria, guiding them to plant roots (Ashby *et al.* 1988; Kape *et al.* 1991; Hughes *et al.* 2017). Moreover, some soil born microorganisms are able to degrade them either for detoxification or for their use as trophic resources (Barthelmebs *et al.* 2000; Hirakawa *et al.* 2012; Jiménez *et al.* 2002; Parke and Ornston 2003; Calisti *et al.* 2008; Kamimura *et al.* 2017). The detection of HCAs has been reported to influence the expression of genes of ecological importance for bacteria in interaction with plants. Those functions includes siderophore synthesis (Baude *et al.* 2016), type 3 secretion system (Yamazaki *et al.* 2012; Li *et al.* 2009, 2015; Zhang *et al.* 2017), and bacterial communication (Joshi *et al.* 2016, 2016; Schaefer *et al.* 2008; Kang *et al.* 2015, 2017). In the two phytopathogen, *Dickeya dadantii* (Hassan and Hugouvieux-Cotte-Pattat 2011; Yang *et al.* 2008) and *Ralstonia solanacearum* HCAs are involved in the colonization of the host and in virulence genes induction (Lowe *et al.* 2014; Khokhani *et al.* 2017).

Agrobacterium is a soil phytopathogen adopting two lifestyles in interaction with plant, a rhizospheric and a pathogenic lifestyle. The latter is developed by agrobacteria harboring the Ti (tumor-inducing) plasmid, that are thus able to transfer a pTi gene region called T-DNA to plant cell genome. Its integration to plant nucleus and the expression of transferred T-DNA by plant cells lead to the formation of a tumor, the crown-gall disease's symptoms (Nester 2014;

Gelvin 2017). HCAs are important compounds for the *Agrobacterium*'s ecology as they are involved in both lifestyles of the bacteria (Meyer *et al.* 2018a). First, agrobacteria are attracted by HCAs like ferulic acid that are present in the roots (Ashby *et al.* 1988; Meyer *et al.* 2018a). Second, HCAs are also released by wounded plant together with sugars. The perception of those compounds guided *A. fabrum* to specific injured plant area where the bacteria attach (Barton *et al.* 2018; Nester 2014; Matthysse 2014). In this situation, the presence of acidic conditions, sugars and phenolic compound, like ferulic acid, induces *vir* genes expression (Ti plasmid encoded), that lead to the T-DNA transfer to plant cell. Among *Agrobacterium*, *A. fabrum* contains the species-specific region called SpG8-1b. In this region, 8 genes (*atul415-hcaR*) are involved in the degradation of some HCAs (ferulic acid, *p*-coumaric acid and caffeic acid) and their use as carbon source (Lassalle *et al.* 2011; Campillo *et al.* 2014). However, HCAs degradation seem few energetic and not efficient to support *A. fabrum* growth (Campillo *et al.* 2014; Baude *et al.* 2016). The regulation of HCAs degradation pathway is suggested to be important for *A. fabrum* transition between lifestyles (Meyer *et al.* 2018a), noticeably as it can interfere with virulence genes expression. The regulation of this pathway has been described recently (Meyer *et al.* 2018a). Upon binding to the promoter region of the regulated genes, HcaR is the repressor of the first's genes of the HCAs degradation pathway. The ligation of feruloyl-CoA (or *p*-coumaroyl-CoA) to HcaR releases it from the promoter sequence and allows gene transcription. Perception of HCAs modulates the expression of numerous ABC transporters (Baude *et al.* 2016). This suggest that, through their influence on the uptake of other compounds, HCAs would influence the bacterial metabolism. It is not known whether the effect on the expression of ABC transporters genes is due to the degradation of the HCAs (role of intermediate degradation compounds) or the perception of HCAs. In *Pseudomonas putida*, the chemoattraction to HCAs requires the perception of their activated forms feruloyl-CoA and *p*-coumaroyl-CoA (Hughes *et al.* 2017). In a similar way, those two compounds, that are the effectors leading to HCA degradation genes expression in *A. fabrum*, could be a signal driving the expression of ABC transporter's gene, thus playing a role in the control of bacterial metabolism.

To test this hypothesis, we investigated the modulation of carbon sources utilization by *A. fabrum* in presence of different HCAs. By comparing the wild type strain (transiently producing the putative effector HCA-CoA) to mutant strains unable to degrade HCAs, (either or not producing the putative effector), we demonstrated that the perception of the first degradation

intermediates feruloyl-CoA or *p*-coumaroyl-CoA induces a reduction of both metabolic activity and growth on some plant metabolites. Some of the metabolites are important for the interaction with plants and we suggested that HCA degradation could be a way for *A. fabrum* to sense plant environment and to adapt its metabolism accordingly.

Materials and methods

Bacterial strains, plasmids, and culture conditions.

The bacterial strains and plasmids used in this study are presented in Supplementary Table S1. *Escherichia coli* strains were grown at 37°C in Luria-Bertani medium. *A. fabrum* strains and its derivatives were routinely cultivated at 28°C in YPG (per liter: 5g of yeast extract, 5 g of bacto peptone, 10g of glucose and pH adjusted to 7.2) rich medium. The AT minimal medium supplemented with 10 mM of carbon sources and 10 mM ammonium sulfate (pH adjusted to 7.2) was used for growth analyses and promoter activities assays. When necessary, gentamicin was used at 25 µg/mL for *A. fabrum* strains and 15 µg/mL for *E. coli* strains.

Metabolic activity using Phenotype Microarray (PM) in presence of HCA

Cells growing at 28°C during 2 days in YPG plates (Agar 15g/L; yeast extract 5 g/L; peptone 5 g/L; glucose 10 g/L; pH=7.2) were resuspended in IFO fluid during two hours in order to lowered residual metabolic activities. Then, according to the manufacturer instructions, each strains were adjusted to a transmittance of 42% using a turbidimeter (Biolog, Inc., Hayward, CA, USA). To that end we used IF-0 fluid containing 0,0017% of either DMSO (Solvent) or a 500 µM solution of HCAs dissolved in DMSO. 100 µL of this suspension were pipetted into each well of the PM1 and PM2A plates (Biolog, Inc., Hayward, CA, USA). The microplate was incubated at 28°C during 72h and the conversion of tetrazolium salt to a purple compound was monitored by the Omnilog™ Sytem in order to obtain respiration curves. Purple conversion indicates that the carbon source is used for metabolism (Bochner 2009). 3 biological replicates were performed for each condition.

For each carbon source and each condition, areas under the curves (AUC) were calculated using the opm package (Vehkala *et al.* 2015) and the R software. The AUC value of the well A1 containing the negative control (Water) was subtracted to all the other carbon sources values. Wells for which AUC values are null or negative in all the condition were suppressed from the analysis (never used in any condition and for any strain). AUC values were then presented in a

heatmap realized with the pheatmap package in the R software with the following parameters: Euclidean clustering distance for rows and correlation clustering distance for columns. Each conditions were compared using Tukey test (P -value=0.05) provided in the “vegan” package in R Core Team, 2014.

Construction of transcriptional reporter fusion

The promoter of *livH* (*atu2427-atu2424*) were PCR amplified using primers *livHF*(CGGGGGATCCACTAGGCCATTTCGATCTTCGCTA) and *livHR*(TTCTTCCTCCACTAGATGTCTTCCCCCGGGTAT). The In fusion technology (Takara Clontech, Kyoto, Japan) was used in order to clone the DNA fragments in the SpeI digested-pOT1eM (Meyer *et al.* 2018a) resulting in the *Plivh* plasmid. The latter was verified by PCR using pOT1eFor and pOT1eRev primers (Allaway *et al.* 2001). *Plivh* was transferred to *A. fabrum* strains by electroporation and the plasmid presence was confirmed by PCR.

Measurement of promoter activity

After overnight culture, the cells were harvested by centrifugation and washed in sterile distilled water. The optical density at 600 nm was adjusted to 0.1 in 96-well plates (Cellstar[®], Greiner Bio-One, Germany) containing 200 μ l of AT medium supplemented with nitrogen and the different carbon sources with or without phenolic compounds.

The OD_{600nm} and levels of intensity of the e-GFP fluorescence was quantified in microplate with a TECAN apparatus (Tecan Spark[™] 15M, Männedorf, Switzerland). Measurements were carried out after 24h after inoculation at 28°C under shaking at 750 rpm. E-GFP fluorescence was measured with the following parameter: excitation wavelength at 488nm and emission wavelength at 510nm. All the conditions were performed in quadruplicate with three independent biological replicates. For each strain derivatives, the fluorescence values were corrected by dividing by the OD_{600nm} and by subtracting by the corresponding fluorescence value of the empty pOT1e plasmid. Different conditions were compared using the R v3.1.3 statistical software environment and the Mann-Whitney-Wilcoxon test (P -value=0.05).

Growth in presence of HCA

A Microbiology Bioscreen C Reader (Labsystems, Finland) was used to compare the growth on different carbon sources in the presence or in the absence of 500 μ M of phenolic compounds. Overnight cells were washed and the optical density at 600 nm was adjusted at 0.05 in 200 μ l

of AT medium supplemented with nitrogen and the appropriate carbon source, in Bioscreen honeycomb 100-well sterile plates. The microplates were incubated during 4.5 days at 28°C with shaking at medium amplitude. Cells growth was measured every 20 min. All the conditions were performed in five technical replicates and in three biological replicates. Lag phases were calculated with the opm package (R software) and comparisons were carried with the Tukey test (P-value=0.05).

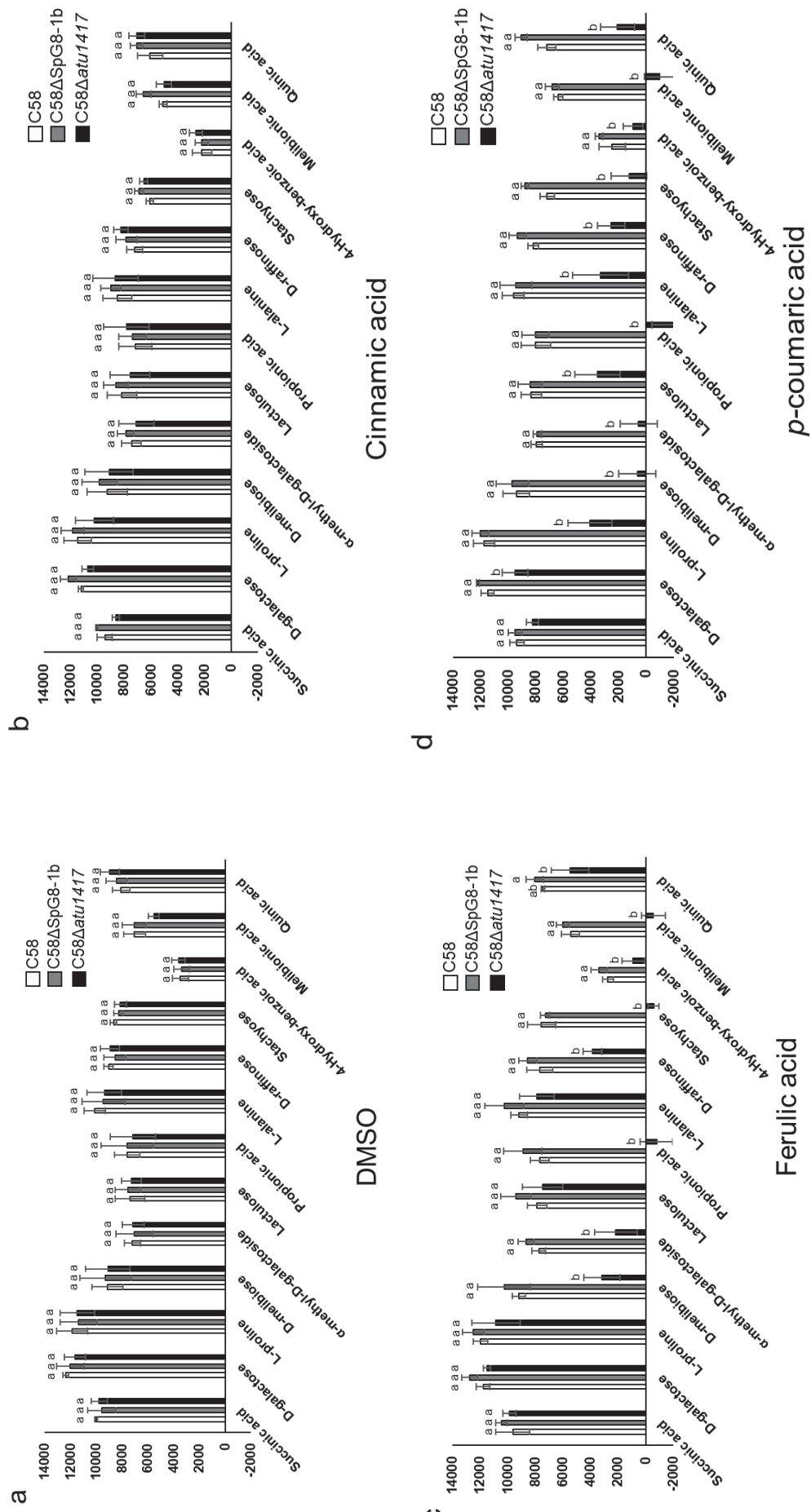


Figure 1: Metabolic activities on 13 carbon sources in *A. fabrum* strains in presence of HCAs. The metabolic activities of C58, C58Δ*atu1417* and C58Δ*SpG8-1b* strains were measured in presence of DMSO (a) and the following HCAs, cinnamic acid (b), ferulic acid (c) and *p*-coumaric acid (d). Letters indicate significant differences between strains (Tukey test, P-value=0.05).

Results and discussion

A. fabrum C58 metabolic activities

Metabolic activity on 190 carbon sources were assayed using phenotypic microarrays (PM1 and PM2A) and Omnilog plate reader. Among 190 sources, the C58 strain metabolize 87 compounds listed in supplementary Table S2, including 44 sugars and polyols, 8 glycosides, 18 carboxylic acids, 12 amino acids and 5 metabolites of others class compound. Among these trophic resources, 43 have been already described to be used by *A. fabrum* (Parke 2000; Meyer *et al.* 2018b; González-Mula *et al.* 2018; Dessaux and Faure 2018). The three strains used in this study differ in their ability to degrade ferulic and *p*-coumaric acids: the wild-type C58 strain transiently produce feruloyl-CoA and *p*-coumaroyl-CoA to completely degrade HCA, C58 Δ *atu1417* is unable to degrade HCA but non-transiently produce feruloyl-CoA and *p*-coumaroyl-CoA, and C58 Δ SpG8-1b is unable both to produce feruloyl-CoA and *p*-coumaroyl-CoA, and to degrade HCAs. In our standard condition (0.0017% solvent - in absence of HCAs), all the strains have the same metabolic abilities on the 87 carbon sources they use (For example: succinic acid, Figure 1a and figure S1).

Feruloyl-CoA influences metabolic activities on some carbon sources

We compared the effects of ferulic, *p*-coumaric and cinnamic acids on metabolic activity of the three strains. Results for all conditions are presented by a heatmap in figure S1 using the area under the curve (AUC) for the 87 metabolized carbon sources and for each conditions. Independently of the strain, the presence of cinnamic acid seems to influence some metabolic activities, for instance in presence of D-glucosamine (Figure S1). The influence of cinnamic acid will be the subject of subsequent analyses. On the contrary, in presence of ferulic and *p*-coumaric acid, the C58 Δ *atu1417* strain shows a weak metabolic activity for 9 carbon sources compared to the C58 and the C58 Δ SpG8-1b strains (figure 1). The carbon sources are sugars (D-melibiose, D-raffinose, D-stachyose), glycoside (α -methyl-D-galactoside), organic acids (melibionic acid, 4-hydroxybenzoic acid, propionic acid, quinic acid) and amino acids (L-alanine). In presence of cinnamic acid and in standard conditions, the three strains have the same metabolic activity on the latter compounds (Figure 1). Metabolic abilities seem to be reduced in presence of ferulic acid in the C58 Δ *atu1417* for lactulose and L-proline, but the reduction of metabolic activity is only significant with *p*-coumaric acid (Figure 1). The result on C58 Δ *atu1417* might be explained by a toxic effect of accumulating CoA derivatives, as

suggested in a mutant strain of *hcaA* encoding an enoyl-CoA hydratase (homologous to Atu1417) in *A. baylyi* (Parke and Ornston 2004). However, no toxic effect has been reported in all the other homologous gene in the following species: *P. putida*, *P. fluorescens*, *Rhodopseudomonas palustris*, *R. solanacaerum*, *Rhodococcus jostii*, *Sphingobium paucimobilis* and *Corynebacterium glutamicum* respectively (Jiménez *et al.* 2002; Calisti *et al.* 2008; Otani *et al.* 2014; Lowe *et al.* 2014; Hirakawa *et al.* 2012; Kasai *et al.* 2012; Kallscheuer *et al.* 2016). The toxic effect of feruloyl-CoA accumulation seems thus unlikely. Moreover, in this study the metabolic activity in presence of both ferulic and *p*-coumaric acid is similar in the three strains for the majority of carbon sources. This shows that the *C58Δatu1417* strain has the same ability to metabolize all the carbon sources as C58 and the *C58ΔSpG8-1b* strain, except the latter 11 sources. Hence the weaker metabolic activity in presence of ferulic and *p*-coumaric acids on the 11 compounds is not due to a reduced growth ability in the mutant strain, but is specifically linked to the non transient presence of feruloyl-CoA.

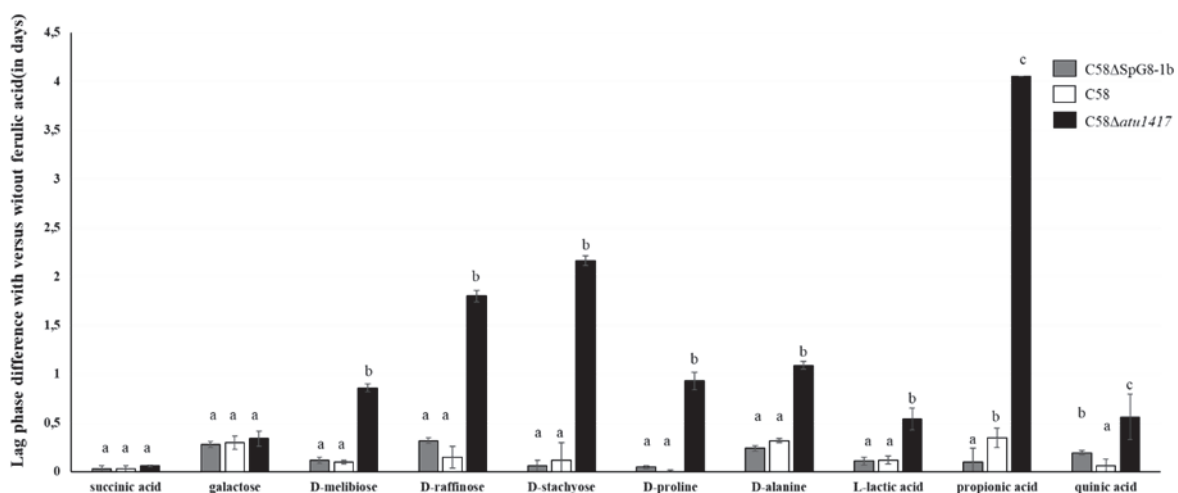


Figure 2: Influence of ferulic acid degradation on lag phase of *A. fabrum* C58 strains on different carbon sources. The lag phase was calculated after 4.5 of growth on different carbon sources for the C58, C58Δ*atu1417* and C58Δ*SpG8-1b* strains in presence or absence of ferulic acid. The lag phase presented corresponds to the lag phase value in presence of this compound corrected by subtraction by the lag phase value in absence of ferulic acid. Letters above indicates significant differences between strains (Tukey test, P-value=0.05).

The feruloyl-CoA negatively modulate the growth on some carbon sources

We tested if the growth of the three strains is influenced in the same way as metabolic activities in the presence or in the absence of ferulic acid. We used galactose and succinic acid as control. The lag phase differences between the conditions with and without ferulic acid are presented in figure 2. For all strains, the presence of ferulic acid has no significant effect on the growth on galactose and succinic acid. The C58 and C58Δ*SpG8-1b* strains have similar lag phases except

for quinic and propionic acids. On the contrary, *C58 Δ atul1417* has longer lag phases in all carbon sources but galactose and succinic acid. For propionic acid we even observed no growth in presence of ferulic acid. This is in line with the results of metabolic activities. In addition, even though it was only a trend using the biolog PM1 and PM2A biolog plates, the growth of *C58 Δ atul1417* mutant in L-alanine and L-proline is significantly delayed by ferulic acid. Hence, in the *C58 Δ atul1417* strain (non transient presence of feruloyl-CoA), the ferulic acid activation into feruloyl-CoA represses the use of 8 different compounds as nutritive resources for growth. In presence of propionic acid, the growth-delay is even significantly reduced in the wild-type strain (transient presence of feruloyl-CoA) compared to the *C58 Δ SpG8-1b* which do not produces feruloyl-CoA. This suggest that, in addition to the *C58 Δ atul1417* strain, the presence of ferulic acid could modify the metabolism of the wild-type strain. However, in our condition this is tricky to visualized due to the low amount of ferulic acid in the batch culture medium and the transient production of feruloyl-CoA by the wild-type strain.

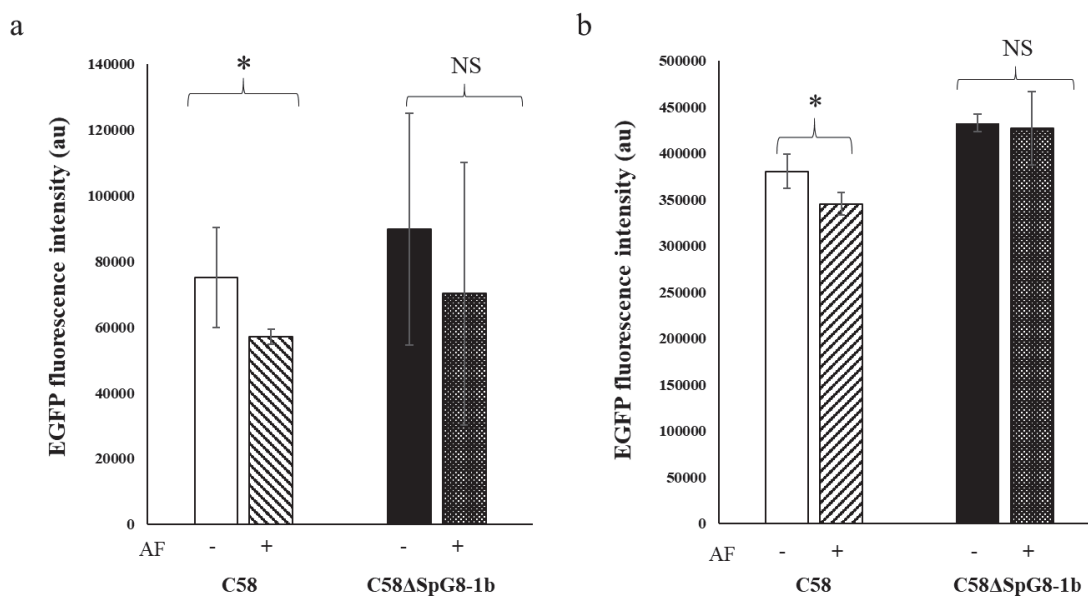


Figure 3: Influence of ferulic acid degradation on the induction of D-melibiose and L-proline uptake genes.

P_{mel} transcriptional fusion expression is measured in D-melibiose in the C58 and C58 Δ SpG8-1b strains (a) and P_{livH} transcriptional fusion expression is quantified in L-proline in the C58 and C58 Δ SpG8-1b strains (b) in absence (-) or presence of ferulic acid. Standard error is calculated from 4 technicals and 3 biologicals replicates. Asterisks indicate significant differences between the condition with and without ferulic acid and NS indicates not significant differences (Mann-whitney test, P-value: 0.05).

Feruloyl-CoA modulates the transcription of L-proline and RFOs degradation genes

A delay in growth could be linked to a repression of genes transcription or a repression of enzymatic activity. We study the expression of L-proline and D-melibiose catabolism genes to determine if the growth delay observed on those compounds is due to a transcriptional repression. The D-melibiose degradation genes were monitored with the P_{mel} transcriptional fusion (Meyer *et al.* 2018b). Previous studies revealed that *braDEF-livH* (*atu2422-atu2427*) cluster is involved in L-proline uptake (Chevrot *et al.* 2006; Haudecoeur *et al.* 2009). To get access to the impact of ferulic acid degradation on L-proline uptake, we constructed the $pOT1e-P_{LivH}$ reporter fusion. The two plasmidic transcriptional fusion were introduced into C58 and C58 Δ SpG8-1b strains. The C58 Δ *atu1417* strain cannot be used in L-Proline and D-melibiose as sole carbon source because of the growth delay in presence of ferulic acid. The analyse of P_{LivH} expression indicates that the fusion is induced by L-proline (data not shown). Expressions of P_{mel} and P_{LivH} were measured in the wild-type and SpG8-1b deletion mutant strains (Figure3). In the SpG8-1b deletion mutant, unable to use HCAs as trophic source (Lassalle *et al.* 2011) and to activate HCA into HCA-CoA (Campillo *et al.* 2014; Meyer *et al.* 2018a), the ferulic acid has no significant effect on D-melibiose and L-proline uptake genes. On the contrary, in the wild-type strain, which transiently produce feruloyl-CoA in presence of ferulic acid, the fluorescence expression was significantly lower in presence of ferulic acid than in absence of this compounds on D-melibiose and L-proline. These results indicate that the presence of feruloyl-CoA is responsible for the transcriptional repression of D-melibiose and L-proline degradation genes.

Feruloyl-CoA is linked to the transcriptional repression of 4-hydroxybenzoic metabolism

The 4-hydroxybenzoic acid is known to be present in plant: it is an antimicrobial compounds accumulated in the phloeme during ISR (Induced systemic resistance)(Smith-Becker *et al.* 1998; Barker and Frost 2001; Jung *et al.* 2016). It is the last compound produced by the degradation of *p*-coumaric acid thanks to the enzymes from the SpG8-1b cluster (Campillo *et al.* 2014). The 4-hydroxybenzoic acid is converted by PobA to protocatechic acid, which is degraded by PcaDCHGB of the β -ketoadipate pathway (Parke 1995). The 4-hydroxybenzoic acid degradation is regulated by the PcaQ activator/repressor (Parke 1993, 1996). Baude *et al.* (2016) have shown that the *pcaQ* and *pobA* genes transcription is repressed in presence of ferulic acid (Baude *et al.* 2016). From our study, the repression of those genes seems to be

linked to the presence of feruloyl-CoA and would be responsible of the reduced metabolism and the growth-delay observed in presence of ferulic acid. It would be of interest to compare *pcaQ* and *pobA* genes expression of C58 and the C58 Δ SpG8-1b in presence of 4-hydroxybenzoic acid and quinic acid. Indeed, quinic acid is a plant compound, which can be converted to protocatechic acid by *Agrobacterium* (Parke 2000). As for the 4-hydroxybenzoic acid, the growth delay might be linked to the repression of *pcaQ*. However, the genes involved in quinic acid degradation are not characterized. The expression of the genes involved in the catabolism of quinic acid and the other carbon sources is thus still to be studied.

Importance for *A. fabrum* ecology

Interestingly, among the compounds that are not use in the C58 Δ *atu1417* (figure 1 and 2) in presence of ferulic acid and *p*-coumaric acid, five compounds are linked to the raffinose family oligosaccharides (D-melibiose, D-raffinose, D-stachyose, α -methyl-D-galactoside and melibionnic acid ; Sengupta *et al.* 2015). The five compounds are uptaken by *A. fabrum* thanks to the MelB periplasmic binding protein (PBP) associated to the MelCDE ABC-transporters and used as carbon sources upon degradation by the putative MelA and MelF α -galactosidase (Meyer *et al.* 2018b). RFO are described to be osmoprotectants and the PBP MelB involved in their uptake leads to a competitive advantage in the rhizosphere from the early step of colonization of the plant (Meyer *et al.* 2018b). Moreover, galactinol which is also uptaken by MelB has been shown involved in plant defenses (Kim *et al.* 2008; Cho *et al.* 2010).

We demonstrated that L-Proline uptake is negatively influenced by ferulic acid and *p*-coumaric acid degradation. L-Proline is suggested as an osmoprotectant (Chevrot *et al.* 2006; Haudecoeur *et al.* 2009) and can be used as carbon source (supplementary Table S2). The PBP LivH and its associate ABC transporters uptake L-proline and GABA (γ -aminobutyric acid) but with a better affinity to L-Proline. In presence of ferulic or coumaric acid, the GABA import could also be reduced. Yet, GABA is important for *Agrobacterium* ecology. Indeed, the reduction of GABA uptake leads to high virulence of the strain and reduce pTi dissemination (Haudecoeur *et al.* 2009; Chevrot *et al.* 2006; Lang *et al.* 2016).

For L-Proline, D-melibiose, RFOs and 4-hydroxybenzoic acid, the activation of ferulic (and coumaric) acids into feruloyl-CoA (coumaroyl-CoA) leads to a reduced expression of genes involved in their import, and/or degradation in the wild-type strain. For the remaining compounds, we could not analyse the genes expression but a significant impact of the non

transiently presence of HCAs-CoA was observed on the metabolism and growth of the C58 Δ *atu1417* mutant strain. This phenomon could be important for the wild-type C58 strain in interaction with plant, as HCAs could be continuously present at least in some part of the plant. For example, in the rhizosphere, the *atu1416* responsible of HCAs-CoA formation is highly induced indicating the presence of ferulic and/or *p*-coumaric acid (Meyer *et al.* 2018a). In this context, the wild-type strain can be in constant presence of HCAs, thus continuously containing CoA derivatives and repressing metabolism of RFOs, L-proline and other carbon sources. In the view of the importance of RFOs and L-Proline for *A. fabrum*, deciphering the regulatory system involved in this regulation can provided key information for the understanding of *A. fabrum* ecology.

Supplemental figures

Supplementary table S1: Strains and plasmids used in this study.

	Relevant characteristics	Reference or source
<i>Escherichia coli</i>		
JM109	<i>endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ Δ(lac-proAB) e14- [F' traD36 proAB+ lacIq lacZΔM15] hsdR17(rK-mK+)</i>	NEB catalog
Stellar	<i>F-, endA1, supE44, thi-1, recA1, relA1, gyrA96, phaA, ΦS0Δ lacZΔ M15, Δ(lacZLA - argF) U169, Δ(mvr - hsdRMS - mcrBC), ΔmcrA, λ-</i>	Takara Clontech
<i>Agrobacterium fabrum</i>		
C58	Wild-type	CFBP 1903
C58Δ <i>atu1417</i>	C58 deleted of <i>atu1417</i> gene	(Campillo <i>et al.</i> 2014)
C58ΔSpG8-1b	C58 deleted of SpG8-1b gene cluster; Km ^R	(Lassalle <i>et al.</i> 2011)
Plasmids		
pOT1e	Promoter-probe vector based on pBBR1MCS-5 replicon; contains promoterless eGFP and MCS between two transcriptional terminators; Gm ^R	(Allaway <i>et al.</i> 2001)
pOT1eM	pOT1e derivative harboring <i>Ptac-m-cherry</i> inserted in ClaI-Sall site	(Meyer <i>et al.</i> 2018a)
<i>Patu1416</i>	Upstream region <i>atu1416</i> inserted in ClaI-Sall site in pOT1e; Gm ^R	(Meyer <i>et al.</i> 2018a)
pOT1e- <i>P_{mel}</i>	Upstream region <i>atu4660-atu4665</i> inserted in ClaI-Sall site in pOT1e; Gm ^R	(Meyer <i>et al.</i> 2018b)
pOT1e- <i>P_{ivH}</i>	Upstream region <i>ivH (atu2427-atu2424)</i> inserted in ClaI-Sall site in pOT1e; Gm ^R	This study

Supplementary table S2: Carbon sources used by the *A. fabrum* wild-type strain.

Classe	Compounds
amino acids	<p>L-proline L-asparagine L-glutamine L-serine L-threonine L-alanine Alanylglycine D-glucosamine L-histidine L-hydroxyproline L-glutamic acid L-aspartic acid</p>
carboxylic acids	<p>Succinic Acid D-glucuronic acid D-gluconic acid L-lactic acid Sodium formate D,L-malic acid Acetic acid Fumaric acid Bromo-succinic acid Propionic acid Mono-methyl succinate L-malic acid 4-hydroxy-benzoic acid β-hydroxy-butyric acid γ-hydroxy-butyric acid Melibionic acid Quinic acid L-pyroglutamic acid</p>
glycosides	<p>α-methyl-D-galactoside Dextrin Pectin Amygdalin Arbutin α-methyl-D-glucoside β-methyl-D-xylopyranoside D-Salicin</p>
sugars and polyols	<p>Adonitol myo-inositol D-arabitol L-arabitol D-lactitol Maltitol Xylitol L-arabinose N-acetyl-D-glucosamine D-galactose D-trehalose D-mannose Dulcitol (galactitol)</p>

	D-sorbitol
	Glycerol
	L-fucose
	D-xylose
	D-mannitol
	D-glucose-6-phosphate
	D-ribose
	L-rhamnose
	D-fructose
	D-glucose
	D-maltose
	D-melibiose
	α -D-lactose
	Lactulose
	Sucrose
	α -D-glucose-1-phosphate
	D-fructose-6-phosphate
	β -methyl-D-glucoside
	Maltotriose
	D-cellobiose
	D-arabinose
	D-fucose
	3-O- β -D-galactopyranosyl-D-
	arabinose
	β -gentiobiose
	Palatinose
	D-raffinose
	L-Sorbose
	Stachyose
	D-tagatose
	Turanose
	Dihydroxyacetone
others	
	L-galactonic acid- γ -lactone
	Uridine
	D-ribo-1,4-lactone
	D-galactonic acid- γ -lactone
	Ethanolamine

Compounds which are already described to be used by *A. fabrum* are indicated in bold (Parke 2000; Meyer *et al.* 2018b; González-Mula *et al.* 2018; Dessaux and Faure 2018).

Supplementary figure S1

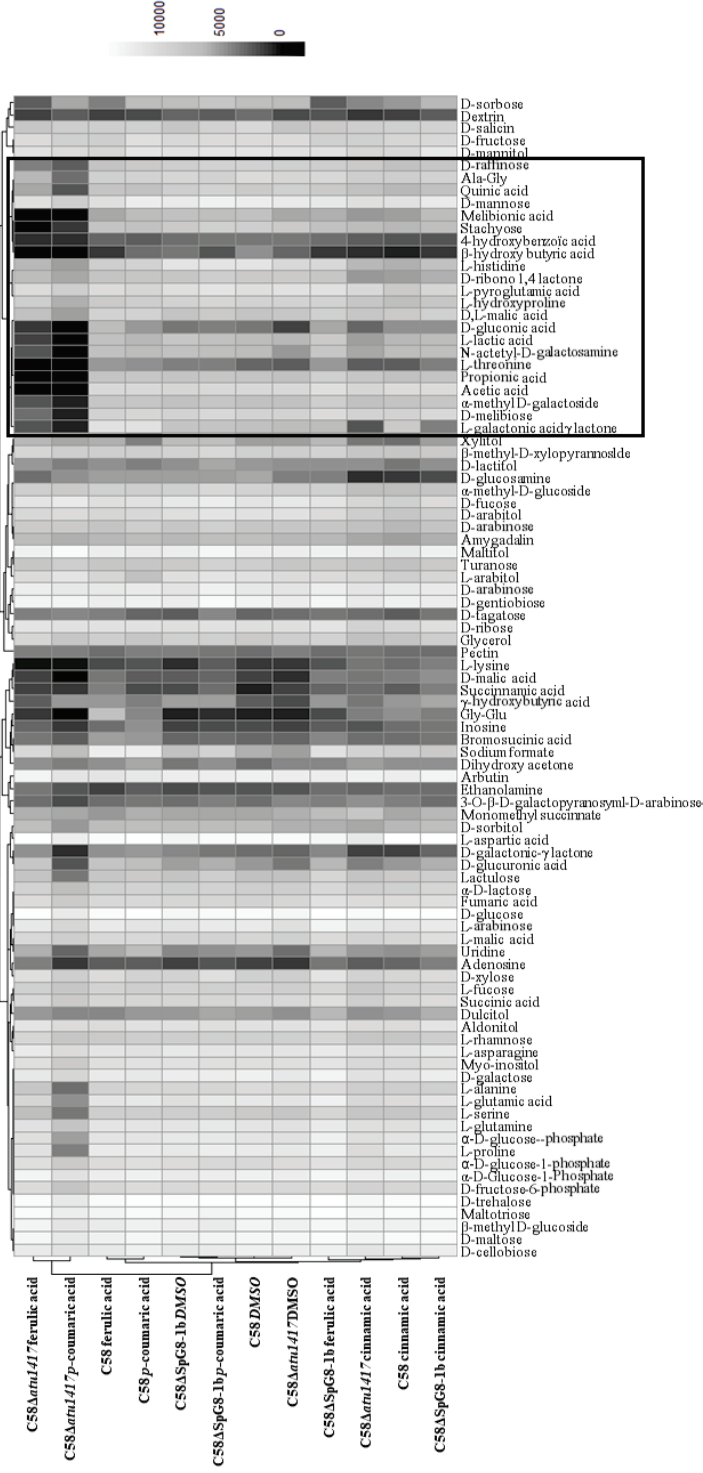


Figure S1 : Trophic resources respiration differences under phenolic compounds pressure between *A. fabrum* C58 derivatives strains. Phenotypic microarrays (PM) are used for measured carbon respiration differences between the C58 wild-type strain, the C58Δatn1417 mutant strain which accumulate *p*-coumaroyl-CoA and feruloyl-CoA and the C58ΔSpG8-1b mutant unable to degrade phenolic compounds. The heatmap represents normalized means values of the area under the curve (AUC) for each conditions from three replicates. The black color indicates few metabolism activity of the trophic resource.

Conclusion du chapitre 2

Nous avons étudié les conséquences de la perception des HCAs sur le métabolisme carboné d'*A. fabrum*. L'utilisation de puces phénotypiques (Biolog) et l'analyse de la croissance bactérienne en présence d'HCAs a permis de montrer que la présence de certains intermédiaires de dégradation (féruloyl-CoA et *p*-coumaroyl-CoA) modifiait l'assimilation (activité métabolique et croissance) de ressources carbonées par *A. fabrum*. Nous avons découvert que cette modulation lors de la dégradation des HCAs était corrélée à une baisse de la transcription des gènes impliqués dans la dégradation (i) d'une famille de composés, les oligosaccharides de la famille du raffinose (RFO); (ii) d'un acide aminé, la proline et (iii) de l'acide 4-hydroxybenzoïque. La diminution de l'utilisation de ces composés comme ressources trophiques pourrait conférer un avantage adaptatif à *A. fabrum* en interaction avec la plante. En effet, parmi ces composés, la proline et les RFOs sont connus comme étant des osmoprotectants (Bougouffa *et al.*, 2014). Leur accumulation intracellulaire pourrait permettre à la bactérie de résister aux conditions hostiles de la tumeur (Synthèse bibliographique - Partie B). Par ailleurs la proline a un rôle dans le contrôle de la virulence et du transfert du plasmide Ti, notamment à travers son interaction avec le GABA. Enfin, les RFOs sont des molécules abondantes dans les graines (de Souza Vidigal *et al.*, 2016; Sengupta *et al.*, 2015) et leur catabolisme pourrait permettre la colonisation de celles-ci.

La modulation du métabolisme par la dégradation des acides férulique et coumarique interroge sur le rôle et l'importance du système HCAs-RFO dans l'écologie d'*A. fabrum*.

Chapitre 3 : Système HCA- RFO : fonctionnement et rôle écologique.

Préambule du chapitre 3

La capacité d'un microorganisme à s'établir en premier dans une niche comme la rhizosphère lui donne un avantage vis-à-vis des espèces s'établissant plus tardivement, ce qu'on appelle le « priority effect » (Fukami *et al.*, 2005; Stuble and Souza, 2016). La première étape de colonisation peut commencer dès la germination des graines, où de nombreux composés sont relargués et peuvent avoir différentes actions sur les microorganismes du sol (Bais *et al.*, 2006; van Dam and Bouwmeester, 2016). Certains sont (i) chimioattractants (Zhang *et al.*, 2014), (ii) peuvent avoir une action antimicrobienne (Baetz and Martinoia, 2014; Lanoue *et al.*, 2010) et (iii) peuvent servir de ressources trophiques aux microorganismes ayant la capacité de les dégrader (Gunina and Kuzyakov, 2015; Moe, 2013). La présence chez un microorganisme de capacités métaboliques diverses est avantageuse pour son installation dans cet environnement compétitif, notamment vis-à-vis de microorganismes d'espèces différentes (Cray *et al.*, 2013). Dans le cas des microorganismes phytopathogènes, la colonisation et la survie dans la rhizosphère est une étape clef avant le passage à un style de vie pathogène. La compréhension des déterminants impliqués dans le style de vie rhizosphérique permet de mieux appréhender le cycle de vie d'un pathogène (Brader *et al.*, 2017), or chez *A. fabrum* très peu d'études portent sur les déterminants génétiques importants dans ce contexte.

Nous avons démontré dans le cadre du chapitre 2 que chez *A. fabrum* la dégradation des HCAs dans la rhizosphère donnait un avantage compétitif à la bactérie. Cette dégradation module négativement l'expression de gènes probablement impliqués dans la dégradation des oligosaccharides de la famille du raffinose (RFO). Ces sucres sont abondants dans les graines de nombreuses plantes et sont relargués au moment de la germination (Andersen *et al.*, 2005; Nelson, 2004; Sengupta *et al.*, 2015). De plus, ils sont décrits comme étant important dans la résistance à des stress abiotiques et biotiques chez les végétaux (Ibáñez *et al.*, 2013; Kim *et al.*, 2008; Nishizawa *et al.*, 2008; Taji *et al.*, 2002). Leur synthèse est liée à la fixation d'un résidu galactosyl au niveau d'une molécule de saccharose en position α -1,6. Le précurseur de ce résidu galactosyl provient du galactinol. Ce dernier est synthétisé par une galactinol synthase et

confère aussi des capacités de résistances aux stress chez les plantes. Il est par ailleurs décrit comme étant accumulé en réponse à l'inoculation bactérienne et permet la résistance à un pathogène via la résistance systémique induite (ISR) (Cho *et al.*, 2010; Kim *et al.*, 2008). Chez *E. meliloti*, ces composés, hormis le galactinol, ont été décrits comme servant de ressources trophiques à ce symbiote (Bringham *et al.*, 2001; Gage and Long, 1998). *A. fabrum* possède un opéron nommé *mel* très proche (>70% d'identité) de celui d'*E. meliloti* : le gène *atu4661* (*melB*) code potentiellement pour une periplasmic binding protein (PBP), les gènes *atu4660* et *atu4665* sont putativement deux α -galactosidases et les gènes *atu4662-4664* coderaient pour le transporteur ABC. Chez *E. meliloti* cet opéron permet l'import et le clivage de RFOs en molécules de degré de polymérisation plus faible (verbascose>stachyose>raffinose>mélibiose). Dans un premier temps, nous avons caractérisé la PBP MelB et étudié le rôle de cette protéine dans les phases précoces de colonisation de la rhizosphère. Ces travaux ont été publiés dans le journal Journal of Biological Chemistry (JBC) en 2018 et sont le fruit d'une collaboration avec Armelle Vigouroux et Solange Moréra (I2BC, Gif-Sur-Yvette). Concernant ma contribution, j'ai réalisé la mutation de la PBP MelB, la construction et les analyses de la fusion transcriptionnelle *in vitro* et *in planta*, j'ai effectué les tests de compétition et participé à l'analyse phylogénétique de MelB. Dans un second temps, pour connaître l'influence de la perception d'acide férulique sur le transcriptome d'*A. fabrum*, nous avons comparé le transcriptome de bactéries cultivées en mélibiose (composé des plantes et de la rhizosphère) en absence ou en présence d'acide férulique. Ces travaux font l'objet d'une publication en construction nommée « Impact of ferulic acid perception on *Agrobacterium fabrum* transcriptome ».



The plant defense signal galactinol is specifically used as a nutrient by the bacterial pathogen *Agrobacterium fabrum*

Received for publication, January 13, 2018, and in revised form, March 27, 2018. Published, Papers in Press, March 30, 2018, DOI 10.1074/jbc.RA118.001856

Thibault Meyer^{†1,2}, Armelle Vigouroux^{5,1}, Magali Aumont-Nicaise⁵, Gilles Comte[‡], Ludovic Vial[‡], Céline Lavire^{‡3}, and Solange Moréra^{§4}

From the [†]UMR Ecologie Microbienne, CNRS, INRA, VetAgro Sup, UCBL, Université de Lyon, F-69622, Villeurbanne, Lyon, France and [§]CNRS CEA Université Paris Sud, Université Paris-Saclay, Institute for Integrative Biology of the Cell (I2BC), Avenue de la Terrasse, 91198 Gif-sur-Yvette, France

Edited by Joseph M. Jez

The bacterial plant pathogen *Agrobacterium fabrum* uses periplasmic-binding proteins (PBPs) along with ABC transporters to import a wide variety of plant molecules as nutrients. Nonetheless, how *A. fabrum* acquires plant metabolites is incompletely understood. Using genetic approaches and affinity measurements, we identified here the PBP MelB and its transporter as being responsible for the uptake of the raffinose family of oligosaccharides (RFO), which are the most widespread D-galactose-containing oligosaccharides in higher plants. We also found that the RFO precursor galactinol, recently described as a plant defense molecule, is imported into *Agrobacterium* via MelB with nanomolar range affinity. Structural analyses and binding mode comparisons of the X-ray structures of MelB in complex with raffinose, stachyose, galactinol, galactose, and melibiose (a raffinose degradation product) revealed how MelB recognizes the nonreducing end galactose common to all these ligands and that MelB has a strong preference for a two-unit sugar ligand. Of note, MelB conferred a competitive advantage to *A. fabrum* in colonizing the rhizosphere of tomato plants. Our integrative work highlights the structural and functional characteristics of melibiose and galactinol assimilation by *A. fabrum*, leading to a competitive advantage for these bacteria in the rhizosphere. We propose that the PBP MelB, which is highly conserved among both symbionts and pathogens from *Rhizobiaceae* family, is a major trait in these bacteria required for early steps of plant colonization.

The plant–rhizospheric microbial population interaction is dynamic and largely influenced by root exudates, with either beneficial or harmful consequences for plant growth develop-

ment and health (1). The germinating seeds in contact with the surrounding soil and microorganisms have strong influences on the rhizosphere composition and favor fast-growing microorganisms able to exploit carbon released, to resist to antimicrobial compounds, and to outcompete other surrounding bacteria (2–4). Raffinose and stachyose from the raffinose family of oligosaccharides (RFO)⁵ accumulate in plant seeds as energy-storage metabolites, and are released during plant germination (2, 5). The precursor of RFO synthesis, namely galactinol, a D-galactose bound to an inositol, is produced by the plant enzyme galactinol synthase (Gals) (5, 6) (Fig. 1). Galactinol plays an important role in plant health, being involved in plant resistance against abiotic (drought and temperature) (7, 8) and biotic stresses (9–11). Indeed, this molecule, which accumulates in plants in response to bacterial inoculation, is involved in the induced systemic resistance to phytopathogens (9). Raffinose and stachyose are synthesized from sucrose by the subsequent addition of activated galactose moieties donated by galactinol using plant raffinose and stachyose synthases, respectively (Fig. 1). Therefore, RFOs are α -(1,6)-galactosyl extensions of sucrose.

Periplasmic-binding proteins (PBPs) associated with their ATP-binding cassette (ABC) transporter are essential for transport (12). A PBP-mediated transport system is responsible for RFO uptake from seed exudates into bacterial cells as previously shown in *Ensifer meliloti* 1021 (13, 14). RFOs, which are degraded by α -galactosidases in this latter strain, are used as nutrients, and their assimilation may be involved in bacterial survival in plant rhizosphere (13, 14). In more detail, raffinose and stachyose can be degraded into melibiose and fructose, and raffinose and galactose, respectively (15, 16). Bacterial assimilation of RFOs and melibiose was associated with trophic advantage in plant–bacteria interaction (14, 17), whereas nothing was known for galactinol. *Agrobacteria* are telluric and rhizosphere bacteria, commonly isolated from roots of numerous plants as commensal bacteria. They can also be pathogenic with the presence of the tumor-inducing plasmid (18, 19). They are then able to create their own ecological niche after plant cell transformation that leads to tumor formation in a wide range of

This work was supported by CNRS (Mission pour l'interdisciplinarité, Agromics 2014–2016) (to T. M., A. V., S. M., and C. L.). The authors declare that they have no conflicts of interest with the contents of this article.

This article contains Figs. S1 and S2 and Tables S1 and S2.

The atomic coordinates and structure factors (codes 6EPY, 6EQ1, 6EQ8, 6EQO, and 6EPZ) have been deposited in the Protein Data Bank (<http://www.pdb.org/>).

¹ This work was submitted to fulfill the requirements for a doctorate of biology at ED341-E2M2 from Université de Lyon, granted from the French Ministère de l'Éducation Nationale, de l'Enseignement Supérieur et de la Recherche.

² These authors contributed equally to this work.

³ To whom correspondence may be addressed. E-mail: celine.lavire@univ-lyon1.fr.

⁴ To whom correspondence may be addressed. E-mail: solange.morera@i2bc.paris-saclay.fr.

⁵ The abbreviations used are: RFOs, raffinose family of oligosaccharides; PBP, periplasmic-binding protein; YPG medium, yeast peptone glucose medium; ITC, isothermal titration microcalorimetry; RMSD, root mean square deviation; dpi, days post inoculation; eGFP, enhanced GFP.

7930 J. Biol. Chem. (2018) 293(21) 7930–7941

© 2018 Meyer et al. Published under exclusive license by The American Society for Biochemistry and Molecular Biology, Inc.

ASBMB

Downloaded from <http://www.jbc.org/> at CNRS on May 25, 2018

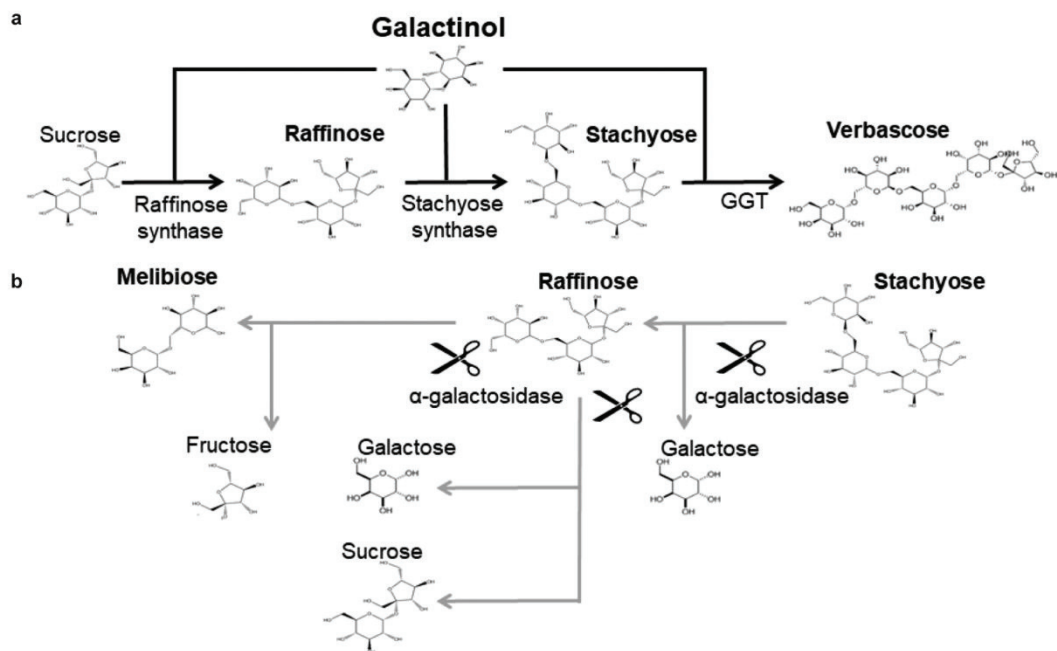


Figure 1. RFO synthesis and degradation. Reactions for synthesis (a) are represented with black arrows whereas those for degradation (b) are with gray arrows. The first step of RFOs biosynthesis starts with the formation of galactinol from UDP-galactose and inositol catalyzed by the plant enzyme galactinol synthase. Raffinose, stachyose, and verbascose are synthesized from sucrose by the subsequent addition of activated galactose moieties donated by galactinol using plant raffinose and stachyose synthases and galactan:galactan galactosyltransferase (GGT), respectively. Cleavage, shown by scissors, of raffinose by an α -galactosidase leads to the formation of either melibiose and fructose or sucrose and galactose. For RFOs of higher degree of polymerization (DP), an α -galactosidase activity results in RFOs of lower DP and galactose.

plants (18). An *in silico* analysis of α -galactosidases distribution in bacteria indicated that *Agrobacterium fabrum* C58 strain contains an operon putatively involved in RFO transport and degradation (20–22). This operon that we named *mel* is similar to the *agp* operon of *E. meliloti* (13, 14), and encodes the PBP MelB (Atu4661) which shares 73% sequence identity with the PBP AgpA, its associated ABC transporter (Atu4662–Atu4664), and two α -galactosidases (Atu4660 and Atu4665). All these latter proteins display between 74 and 86% sequence identity with their *E. meliloti* corresponding homologues (Fig. 2a).

We hypothesized that this *mel* operon was responsible for the transport and assimilation of α -galactosides in *A. fabrum*. Here, we focused on its transport function, and investigated the genetic and molecular role of the PBP MelB through an integrative approach using a defective mutant *in cellulose* and *in planta*, crystallography, and affinity measurements. We showed that MelB was the PBP responsible for RFO, melibiose, and galactinol import into *A. fabrum* C58, displaying the highest affinity for galactinol in nanomolar range and preferring to bind a 2-unit ligand. We structurally characterized the binding mode of MelB for its different ligands. Overall, our work highlights how the capacity of agrobacteria to assimilate plant α -galactosides confers on them an advantage in colonizing efficiently the plant tomato rhizosphere, explaining why the PBP MelB is highly conserved among symbionts and pathogen rhizobiales.

Results

The PBP MelB is responsible for galactinol, melibiose, and RFOs (raffinose and stachyose) uptake

The growth profiles of *A. fabrum* C58 wildtype (WT) and C58 Δ *melB*-defective mutant for MelB were compared in rich (YPG) and minimal medium containing RFOs, their derivatives or succinate (as control) as the sole source of carbon. The C58 Δ *melB* mutant has the same growth rate as the WT strain in minimum medium with succinate and in rich medium. However, the C58 Δ *melB* mutant did not grow on galactinol, melibiose, raffinose, and stachyose, in contrast to WT (Fig. 2b). Therefore, MelB associated to its ABC transporter is the transport system responsible for the uptake and is necessary for the assimilation of these four molecules in pure culture.

MelB exhibits a high affinity for galactinol

Binding of galactinol, melibiose, raffinose, and stachyose to the purified recombinant mature protein MelB was explored using tryptophan fluorescence spectroscopy (MelB possesses 16 tryptophans) and isothermal titration microcalorimetry (ITC). Intrinsic protein fluorescence titration experiments yielded apparent dissociation constant K_D values of 10 ± 1 nM and 72 ± 4 nM with galactinol and melibiose, respectively, showing that MelB is very efficient for galactinol binding.

Galactinol and melibiose bound to MelB

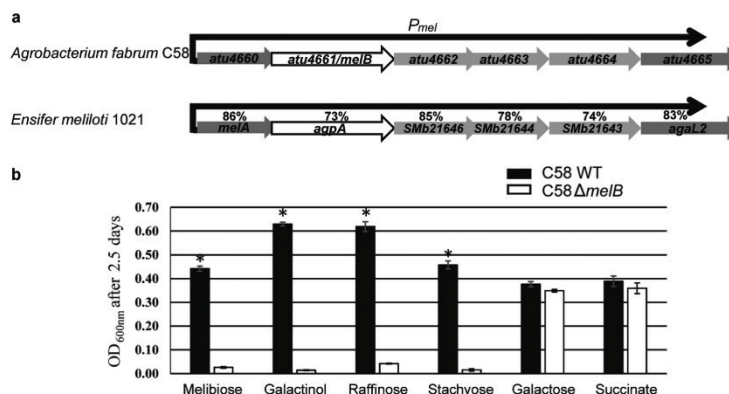


Figure 2. The *mel* operon structure and MelB involvement in galactinol, melibiose, and RFO consumption. *a*, *atu4660*–*atu4665* genes belong to the same transcription unit (operon prediction by Westover *et al.* (22) that we called the *mel* operon). The *mel* promoter P_{mel} indicates the gene transcription direction. Both *atu4660* and *atu4665* genes are annotated as two α -galactosidases, *atu4661* as the PBP MelB and *atu4662*–*atu4664* genes as the associated ABC transporter. Comparison is between the *mel* operon genes and their homologues in *E. meliloti* 1021 (13, 14), the percentages of sequence identity between each homologous protein are indicated; for example, the PBPs AgpA and MelB share 73% sequence identity. *b*, 2.5 days growth (A at 600 nm) of *A. fabrum* C58 WT strain (in white) and the C58 Δ melB mutant (in black) in AT minimal medium supplemented with different carbon sources. Standard deviations were calculated from five technical and two biological replicates. Asterisks indicated significant differences (Mann-Whitney *p* value = 0.05).

Reducing the ligand size to a monosaccharide (galactose) or increasing it resulted in a substantial affinity reduction compared with galactinol: K_D values of 35-fold higher for the raffinose, and over 1000-fold higher for both galactose and stachyose, respectively (Table 1 and Fig. S1). The K_D values were slightly higher using ITC than those determined by auto-fluorescence, but this increased K_D was consistent (Table 1 and Fig. S1). Because MelB was not stable at high concentration during the time course of ITC experiment, we were not able to measure an interpretable signal for the stachyose binding. The ITC data confirmed the 1:1 binding stoichiometry for all ligands and revealed a high enthalpy of binding for galactinol and melibiose meaning that both ligands use the same binding mechanism mainly involving polar interactions. In contrast, the binding mode of raffinose was characterized by an unfavorable enthalpy contribution and a strong entropy term indicating that hydrophobic interactions may play a predominant role and/or a displacement of water molecules occurs upon ligand binding. The galactose interaction adopts an intermediate behavior with a high entropy term, accompanied by a weakly favorable enthalpy of binding, suggesting that polar bonds are less important for galactose alone compared with a 2-unit ligand, resulting in a lower enthalpy, thus a weaker affinity. MelB is specific for α -(1,6)-galactosides (RFOs). Indeed, no interaction could be measured with glucose, sucrose, cellobiose, lactose, and α -(1,6)-glucosides.

Thermal denaturation experiments revealed a contribution of more than 3 °C for two ligands for protein stability (Fig. S2). Indeed, adding galactinol or melibiose led to a melting temperature (T_m) of over 46 °C compared with the 43 °C for the unliganded protein in agreement with the measured K_D values. Galactose or stachyose binding did not stabilize MelB whereas the raffinose binding produced a slight effect with a T_m of 45 °C.

MelB is a PBP from cluster C

The mature MelB expression plasmid was a synthetic gene lacking the first 18 signal sequence residues that serve for localization to bacterial periplasm. The numbering used for the description of residues corresponds to the mature protein of 677 amino acids. Because MelB is the biggest PBP so far and shares low sequence identity (around 20%) compared with PBPs with known three-dimensional structures, we first solved the structure of selenated MelB in complex with raffinose at 2 Å resolution by single wavelength anomalous dispersion method. The asymmetric unit is composed of four similar MelB–raffinose complexes (average root mean square deviation (RMSD) value of 0.4 Å). By the molecular replacement method, we then solved the structure of MelB in complex with galactinol, melibiose, galactose, and stachyose at 2.2, 1.8, 2.5, and 2.1 Å resolution, respectively (Table 2). The asymmetric unit of the galactinol and melibiose complexes also contains four very similar molecules with RMSD values between monomers ranging from 0.2 to 0.4 Å whereas that of the galactose and stachyose complexes possesses two identical molecules. Moreover, the five ligand-bound structures are very similar with an average RMSD value of 0.4 Å. They all adopt a closed conformation. MelB fold is monomeric, composed of two lobes, each formed by a central β -sheet flanked by α -helices (Fig. 3a). The biggest lobe (lobe 1) consists of residues 8–354 and 619–678 and the smallest (lobe 2) comprises the residues 364–610. Two short segments (Fig. 3a) define the hinge region connecting the two lobes. MelB possesses a typical fold of cluster C within the PBP structural classification (12) as SSM-EBI (<http://www.ebi.ac.uk/msd-srv/ssm>)⁶ reports: RMSD between MelB and similar PBP structures binding oligopeptide are over 2.6 Å for 450 C α

⁶ Please note that the JBC is not responsible for the long-term archiving and maintenance of this site or any other third party hosted site.

Table 1
Affinity measurement for MelB K_D values were measured by intrinsic protein fluorescence titration (Fluorescence) and by isothermal titration microcalorimetry (ITC).

	Fluorescence		ITC					
	K_D	R^2	K_D	n	Enthalpy (ΔH)	Entropy (ΔS)	Entropic contribution ($-T\Delta S$)	Free enthalpy (ΔG)
	μM		μM		cal/mol	cal/mol/deg	cal/mol	cal/mol
Galactinol	0.010 ± 0.001	0.99	0.12 ± 0.03	0.83	-6908	8	-2356	-9264
Melibiose	0.072 ± 0.004	0.99	0.76 ± 0.11	0.94	-6412	6.1	-1794	-8206
Raffinose	0.347 ± 0.42	0.99	2.9 ± 0.5	0.97	2115	32.5	-9527	-7412
Galactose	13.8 ± 3	0.99	24 ± 2	1	-797	18.4	-5393	-6190
Stachyose	24.6 ± 2.4	0.99	Not determined					

Table 2
Crystallographic data and refinement parameters

Values for the highest resolution shell are in parentheses.

	MelB				
	Raffinose (SeMet)	Galactinol	Melibiose	Galactose	Stachyose
PDB code	6EPY	6EQ8	6EPZ	6EQ0	6EQ1
Space group	C2	C2	C2	C2	C2
Cell parameters (Å, °)	$a = 354.3$ $b = 74.3$ $c = 108.2$ $\beta = 105.5$	$a = 355.3$ $b = 73.7$ $c = 108.1$ $\beta = 105.5$	$a = 351.6$ $b = 73.7$ $c = 107.6$ $\beta = 105.4$	$a = 107.8$ $b = 73.9$ $c = 171.1$ $\beta = 92.5$	$a = 108.2$ $b = 74$ $c = 171.4$ $\beta = 92.4$
Resolution (Å)	50–2 (2.17–2)	50–2.2 (2.3–2.2)	48–1.8 (1.9–1.8)	50–2.5 (2.59–2.5)	50–2.1 (2.2–2.1)
No. of observed reflections	1,143,584 (175,894)	725,141 (106,000)	1,633,125 (250,861)	314,407 (48,297)	541,503 (82,382)
No. of unique reflections	333,472 (51,505)	137,636 (20,717)	246,319 (38,629)	49,700 (7682)	79,391 (12,494)
R_{sym} (%)	7 (52.8)	17.1 (112.5)	10 (77.2)	18.2 (100)	15.4 (200)
Completeness (%)	99.8 (94.4)	98.7 (92.6)	99.4 (97.1)	99.2 (95.7)	99.6 (97.9)
I/σ	10.8 (2)	7.5 (1.5)	11.4 (1.9)	9 (1.6)	8.12 (0.7)
$CC_{1/2}$	99.8 (80.1)	99.3 (57.9)	99.8 (80)	99.1 (51.2)	99.7 (50.1)
R_{cryst} (%)	17.5	18.5	17.7	19.4	19.1
R_{free} (%)	19.9	21.7	19.5	24.2	22.5
RMS bond deviation (Å)	0.01	0.01	0.01	0.01	0.01
RMS angle deviation (°)	1.0	1.09	1	1.16	1.13
Average B (Å ²)					
protein	44.3	45.6	33.7	55.9	52.6
ligand	41.2	36.6	30.5	40.5	44.7
solvent	51.7	49.6	39.2	55	52.2

 $CC_{1/2}$ = percentage of correlation between intensities from random half-dataset (40).

atoms. Nonetheless, a detailed structural comparison is irrelevant because MelB presents a distinct ligand-binding site.

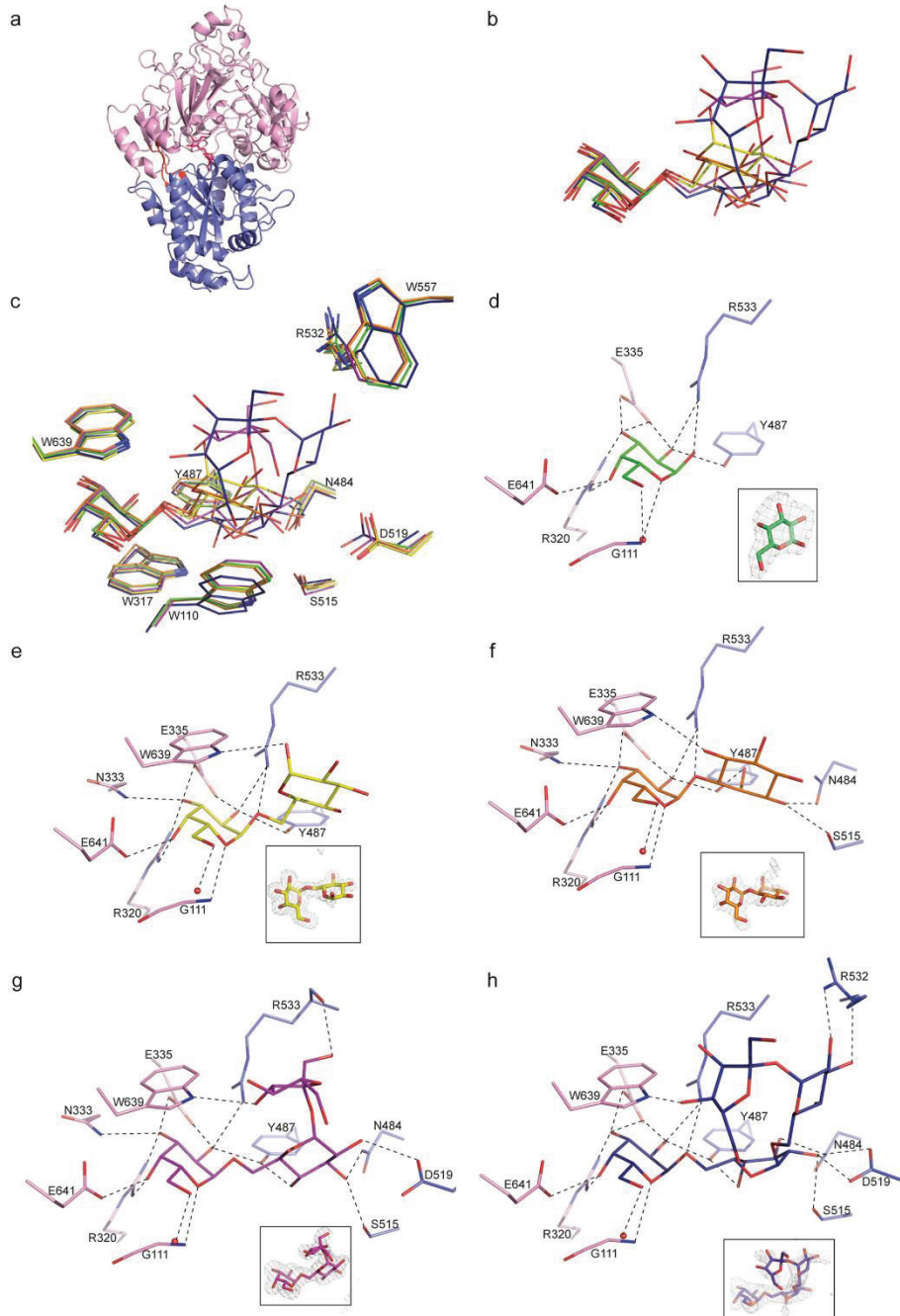
Ligand-binding site of MelB

All ligands are bound between the two closed lobes of MelB. The size and the volume of the ligand-binding site constrain the conformation of bound RFOs. Indeed, raffinose and stachyose bind in a very compact form (Fig. 3*b*), and the addition of a galactosyl moiety at the nonreducing end of stachyose corresponding to verbasose will abolish its binding, because the pocket is not large enough to accommodate a pentasaccharide. All ligands are well defined in their electron density maps except the fructose moiety of the stachyose likely responsible for its low affinity (Fig. 3, *c–h*). They share a buried nonreducing end galactosyl unit wedged between two aromatic residues (Trp³¹⁷ and Trp⁶³⁹) which superimposes very well with the bound galactose alone (Fig. 3, *c–h*). These galactoses at position 1 make 10 similar protein contacts involving the main chain amino group of Gly¹¹¹ and the side chains of Arg³²⁰, Asn³³³, Glu³³⁵, and Glu⁶⁴¹ from lobe 1 and both side chains of Tyr⁴⁸⁷ and Arg⁵³³ from lobe 2. The O6 atom of this pyranose interacts with a conserved water molecule observed in each complexed structure (except in molecule B of MelB-galactose complex), which in turn makes hydrogen bonds with the amino group of Gly¹¹² and the side chain of Asp¹¹⁴. In both structures of MelB

in complex with galactose and stachyose, the galactose at position 1 does not interact with Asn³³³ but because of two hydrogen bonds with the side chain of Glu³³⁵, it conserves 10 interactions with MelB. Modeling a glucosyl unit at the nonreducing end (position 1) creates steric hindrance between the equatorial C4-OH and the Trp⁶³⁹ indol explaining the specificity of MelB for a galactosyl unit at position 1 as shown by the affinity measurement.

In contrast to position 1, at position 2, the glucosyl moieties of melibiose and raffinose, the inositol moiety of galactinol and the galactose moiety of stachyose do not superimpose and can shift up to 3.5 Å to allow, for example, the fructose or the sucrose accommodation of raffinose and stachyose in the ligand-binding site, respectively (Fig. 3, *b* and *c*). A conformational change less than 1 Å for Trp¹¹⁰ and Tyr⁴⁸⁷ side chains is observed to accommodate a glucosyl unit at position 2 (Fig. 3*c*). More arrangements from amino acids of lobe 2 can occur to accommodate the glucosyl units at positions 3 and 4 (Fig. 3*c*). For example, Trp⁵⁵⁷ is pushed away by more than 1 Å compared with the other liganded structures to find a room for the fructose at position 4. All units at position 2 have in common the stacking onto the aromatic indol of Trp¹¹⁰, with the optimum one for the inositol because of the shorter link (one carbon shorter) between the two subunits of galactinol compared with melibiose and RFOs. The melibiose's glucose and the inositol

Galactinol and melibiose bound to MelB



Downloaded from <http://www.jbc.org/> at CNRS on May 25, 2018

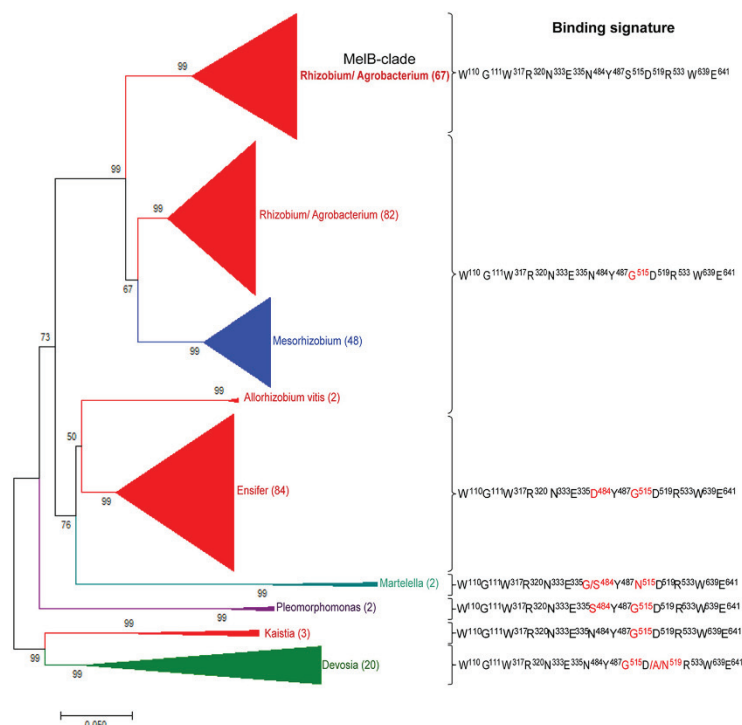


Figure 4. MelB phylogeny and binding signature. For each protein clade, the residues, which are identical to (*black*) and different from (*red*) those involved in the galactinol/melibiose/raffinose binding of *A. fabrum* C58 MelB are indicated. Number in bracket represents the number of MelB-relative PBPs per clade.

share only one protein contact: an oxygen interacts with the NH of the Trp⁶³⁹ side chain (Fig. 3, *e* and *f*). The inositol makes three additional hydrogen bonds with Asn⁴⁸⁴, Tyr⁴⁸⁷, and Ser⁵¹⁵ from lobe 2 whereas the rest of the oxygen of the melibiose's glucose interacts with the protein side chains via water molecules only. In contrast, the glucose moiety of raffinose and the galactose moiety of stachyose at position 2 make an additional H-bond compared with inositol involving the side chains of Asp⁵¹⁹ (Fig. 3, *g* and *h*). The fructose at position 3 in raffinose interacts with the main chain of Arg⁵³³ from lobe 2 and the NH of the Trp⁶³⁹ indol from lobe 1 (Fig. 3*g*). In contrast, Arg⁵³² side chain makes two H-bonds with the glucose moiety at position 3 in stachyose whereas only one is present between the fructose moiety at position 4 and the Trp⁶³⁹ side chain (Fig. 3*h*).

MelB is highly conserved among rhizobiales

Searching for MelB conservation in the bacterial kingdom (protein database at NCBI), and subsequent phylogenetic anal-

ysis revealed >310 PBPs above 65% sequence identity (Fig. 4). Galactinol-, melibiose-, and raffinose-binding signatures share 10 amino acids Trp¹¹⁰-Gly¹¹¹-Trp³¹⁷-Arg³²⁰-Asn³³³-Glu³³⁵-Tyr⁴⁸⁷-Arg⁵³³-Trp⁶³⁹-Glu⁶⁴¹. Stachyose binding shares only nine of these latter because Asn³³³ does not interact with stachyose. Two additional residues (Asn⁴⁸⁴ and Ser⁵¹⁵) are involved in galactinol, raffinose, and stachyose binding compared with melibiose binding. One additional residue Asp⁵¹⁹ belongs to the raffinose and stachyose signatures. A last additional residue Arg⁵³² defines the stachyose-binding signature composed of 13 residues in total. Members of the MelB subgroup (67 PBPs) display more than 90% identity sequence with the conserved binding signature. They all belong to *Rhizobium* and *Agrobacterium* genera. Outside the MelB cluster, the signature slightly degenerates for galactinol and raffinose binding. Nonetheless, modeling indicates that their binding would not be affected. Remark-

Figure 3. Ribbon representation of MelB structures and ligand-binding site. *a*, raffinose in magenta is located in the cleft between lobes 1 and 2 shown in slate and in pink, respectively, and the hinge region is in red. *b*, superposition of the bound galactose, melibiose, galactinol, raffinose, and stachyose shown in green, yellow, orange, magenta, and blue sticks, respectively, in the binding site of MelB. *c*, same figure as in *b* showing the stacking between ligands and tryptophan (Trp⁶³⁹, Trp³¹⁷, and Trp¹¹⁰). Except Trp⁶³⁹ and Trp³¹⁷, all the other labeled amino acids mainly from lobe 2 can move up to 1 Å upon ligand binding. *d-h*, galactose (*d*), melibiose (*e*), galactinol (*f*), raffinose (*g*), and stachyose (*h*) bound to the binding site of MelB are shown in the same code color as in *b*. Hydrogen bonds between MelB and each ligand are shown as dashed lines in black (distances are up to 3.2 Å). A water molecule forming a hydrogen bond with each ligand is shown as a red circle. Each ligand is shown in its annealing Fo-Fc omit map contoured at 4 σ .

Galactinol and melibiose bound to MelB

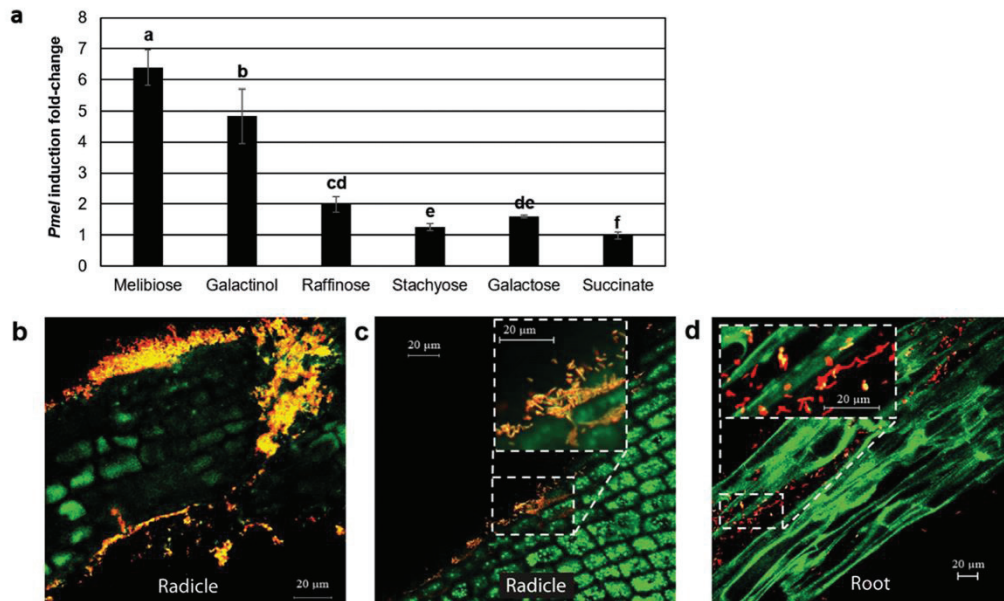


Figure 5. Expression of *mel* operon genes in cellulose and in tomato radicle and root. *a*, comparison of *mel* operon gene expression in AT minimal medium supplemented with different carbon sources. Standard deviations were obtained from four technical and two biological replicates. Letters above histograms indicate significant different -fold change values (Tukey's test, p value = 0.05). *b-d*, bacterial *mel* operon gene expression at two early stages of plant growth. Gene expression was monitored using the pOT1eM- P_{mel} transcriptional reporter fusion by confocal microscopy at 2 (*b* and *c*) and 14 (*d*) dpi, corresponding to radicle emergence and root elongation stages, respectively. Representative pictures from five plants per stages are shown. Red fluorescence from *M*-cherry indicates the presence of bacteria, whereas yellow fluorescence shows bacteria that were both active and able to express P_{mel} -*egfp*. Plant auto fluorescence, represented in green allows distinguishing different types of plant cells, small compact cells from the radicle and elongated cells from the growing root. The scale is represented in white. Most cells expressed P_{mel} -*egfp* at 2 dpi, whereas at 14 dpi, few cells only expressed the transcriptional fusion.

ably, all these PBPs belong to soil- and plant-interacting genera.

Galactinol and melibiose are inducers of *mel* operon genes

We constructed the C58 pOT1e- P_{mel} reporter fusion strain to study *in cellulose* gene expression of *mel* operon in the presence of commercial compounds. Compared with succinate, slight but significant inductions were observed with raffinose, galactose, and stachyose (2-, 1.6-, and 1.25-fold change values, respectively) (Fig. 5*a*). In contrast, galactinol and melibiose are efficient inducers with 4.8- and 6.5-fold change values, respectively.

mel operon genes are expressed in early plant colonization

At two early stages of plant colonization, *in planta* expression of *mel* operon has been studied in the WT strain harboring the pOT1eM- P_{mel} plasmid reporter fusion (*m-cherry* constitutive expression and *egfp* inducible expression). 48 h after seed imbibition and inoculation, most bacteria in contact with the radicle cells expressed *mel* operon, as shown by the yellow cells in Fig. 5, *b* and *c*. 14 days after seed imbibition and inoculation, among bacterial cells present on plant roots (red and yellow cells), some of them were still expressing RFO uptake and degradation genes (yellow cells in Fig. 5*d*). Thus, *mel* operon was more expressed at the beginning of plant colonization.

The PBP MelB confers a competitive advantage in colonizing tomato rhizosphere

The colonization of plant rhizosphere by *A. fabrum* C58 WT and C58 Δ *melB* mutant was evaluated at 2 days post inoculation (dpi) and 14 dpi (Fig. 6). When tomato seeds were inoculated with each strain individually, the bacterial colonization level did not significantly differ at 2 dpi (Mann-Whitney p value of 0.09) whereas at 14 dpi, this was slightly higher for the WT strain (Mann-Whitney p value $<2.2e-16e$) (Fig. 6*a*). When *A. fabrum* C58 WT and C58 Δ *melB* mutant were co-inoculated, a slight and a drastic reduced fitness was observed for the C58 Δ *melB* mutant at 2 and 14 dpi, respectively (Fig. 6*b*), revealing a selective advantage conferred by galactinol/melibiose/RFO exploitation under a competitive challenge.

Discussion

This work reveals the molecular and ecological roles, and structural basis of the PBP MelB, encoded by the linear chromosome of *A. fabrum* C58.

At the beginning of the study, we made the straightforward assumption that MelB was behaving like its homologous PBP AgpA from *E. meliloti* described as an α -galactoside transporter (13). Our gene expression analyses and growth assays experiments showed that similarly to what has been reported for expression of *agp* operon genes in *E. meliloti*, α -galactosides

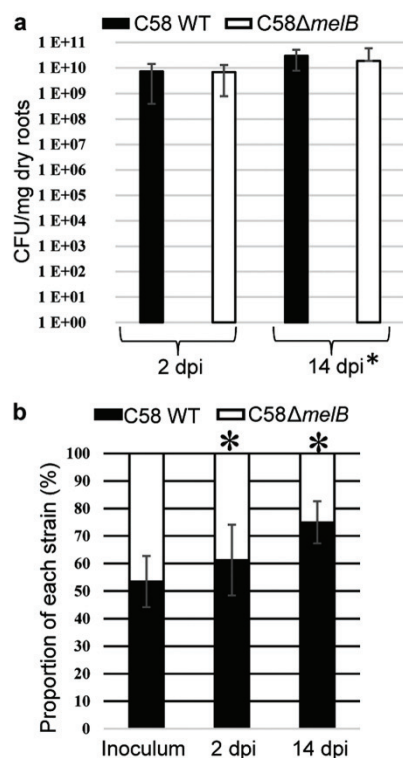


Figure 6. MelB confers a competitive advantage in tomato roots. *a*, *A. fabrum* bacterial concentration (cfu/mg dry roots) in tomato roots (at 2 and 14 dpi) infected with either *A. fabrum* C58 WT or C58Δ*melB* mutant. Standard deviations were calculated from three technical and five biological replicates. *b*, proportion of *A. fabrum* genotypes (%) in inoculum and tomato root at 2 and 14 dpi infected with a mixture (1:1 ratio) of *A. fabrum* C58 WT and C58Δ*melB* mutant. Standard deviations were calculated from 4 biological replicates and 10 independent assays. Asterisks indicate significant differences (Mann-Whitney test).

induce expression of *mel* operon genes in *A. fabrum*, and are used as carbon sources after being imported by MelB. Indeed, in contrast to the WT strain, a MelB defective mutant is unable to grow on α -galactosides. Moreover, using two different biophysical methods, we demonstrated that MelB can bind melibiose, raffinose, and stachyose. Because of the small volume cavity of the ligand binding, stachyose displays a weak affinity for MelB (micromolar range) compared with melibiose and raffinose (nanomolar range). The accommodation of stachyose requires drastic conformational constraints on the ligand when bound to the protein. Indeed, only small local rearrangements of few protein side chains (Trp¹¹⁰ and Trp⁵⁵⁷) forming the binding site can occur. With an affinity in the micromolar range, MelB also binds galactose, which is the sugar common to all α -galactosides present at the nonreducing end. Nonetheless, the low affinity of MelB for galactose prevents galactose from competing with melibiose and raffinose. From our results, MelB can appear as an alternative galactose transporter suspected by

Kemner *et al.* (23), which allowed a *chvE-gguABC* defective mutant to grow on galactose (23, 24). Conversely, the presence of the ChvE-GguABC sugar transporter can explain why *melB* defective mutant was still able to grow on galactose.

An unexpected outcome of our study was that galactinol, which is the precursor of α -galactosides production in plants, was uptaken into agrobacteria via the MelB-mediated transport system. Moreover, MelB displays a preference for galactinol with high affinity (nanomolar range) indicating that this molecule must be efficiently imported into *A. fabrum*, in line with the gene expression results. Remarkably, MelB recognizes similarly the nonreducing end galactose common to all tested α -galactosides and galactinol. Overall, using genetic, structural, and affinity data, this work demonstrates that the MelB-mediated transport system contributes to the import of α -galactosides with a strong preference for a 2-unit ligand (melibiose) and mainly contributes to that of galactinol. To our knowledge, this is the first description of a bacterial PBP allowing galactinol import.

The imported sugars are used to sustain bacterial growth. Galactinol- and α -galactoside-rich environments would facilitate the settlement of bacteria capable to assimilate these plant compounds efficiently. From *in planta* competition assays on tomato between the *A. fabrum* WT and *melB* defective mutant, we showed that MelB confers a marked selective advantage in colonizing tomato rhizosphere, and since early time. This observation correlates with the presence of melibiose and raffinose in the plant rhizosphere, as they are highly abundant in the seeds (25) and known to be released during seed germination (14). Hence, the competitive advantage of the WT in the tomato rhizosphere could be because of a trophic advantage of the strain during seed germination. A germinating seed can indeed be considered as a new environment to be colonized. The community composition of the mature plant is influenced by historical contingency (timing and order of arrival) of the seed community members and their ability to efficiently settle in that environment (26), as the first establishing species are known to affect the ability of potential immigrants to establish. This is called the priority effect (27). Thus, the ability of bacteria to compete and settle in germinating seed environment by growing on galactinol or α -galactosides released at this time could have long effect on their ability to persist and colonize plant rhizosphere. This is consistent with our findings that at 14 dpi, the competitive advantage of the WT strain is even higher than the one measured at 2 dpi.

Besides the trophic advantage linked to the *mel* operon, this operon could be associated to another aspect of bacterial plant interactions, linked to plant defense signaling and protection against pathogens. Indeed galactinol is a plant compound involved in plant defense (9–11). For example, *Pseudomonas chlororaphis* O6-mediated induced systemic resistance was shown associated with an elevation of galactinol content within plants, which conferred disease resistance against pathogen attack (9). The disease resistance was associated with induction of the expression of a set of pathogen-responsive genes (9, 10). Moreover, in *Arabidopsis*, deletion of enzymes that decrease the galactinol and/or raffinose content has been shown to increase plant resistance against the phytopathogenic nema-

Galactinol and melibiose bound to MelB

tode *Heterodera schachtii* (11). Similarly, *Agrobacterium mel*-mediated activity could modify the level of plant galactinol and/or raffinose, which could either drive bacterial recognition by the plant or reduce plant defense signaling through the fall of galactinol content. *Agrobacterium* is known to be able to bypass and overcome plant defenses (28). It would thus be of interest to study the involvement of the *mel* operon in that situation.

In this study, we defined the galactinol-/melibiose-/raffinose-binding signature and found out that this is strictly conserved in MelB homologues in *Agrobacterium* and *Rhizobium*, which share a high sequence identity over 90% with MelB. Moreover, phylogenetic and structural data showed that this wide occurrence is extended among *Rhizobiaceae*, all plant-interacting genera (*Mesorhizobium*, *Allorhizobium*, *Ensifer*, *Martellella*, *Pleomorphomonas*, *Kaistia*, and *Devosia*). Therefore, whatever advantage it gives, it is tempting to speculate that galactinol/melibiose (and to a lesser extent raffinose) may be associated with a selective pressure toward the acquisition of binding, transport, and degradation functions in microorganisms, making the PBP MelB a major trait in the first step of tomato colonization and likely of other plant species.

Experimental procedures

Bacterial culture conditions

Bacteria and plasmids used in this study are shown in Table S1. *Escherichia coli* strains were grown at 37 °C in LB medium supplemented when it was necessary with appropriate antibiotics (tetracycline 10 µg/ml, gentamicin 15 µg/ml, ampicillin 100 µg/ml). *A. fabrum* C58 strain and its derivatives were cultivated at 28 °C in YPG (yeast extract, 5 g per liter, peptone, 5 g per liter, glucose, 10 g per liter, and pH adjusted to 7.2) rich medium supplemented when required with neomycin (25 µg/ml), kanamycin (25 µg/ml), and/or gentamicin (20 µg/ml). In growth assays, AT minimal medium supplemented with 10 mM ammonium sulfate and 10 mM carbon sources was used. 200 µl were inoculated in Bioscreen honeycomb 100-well sterile plates and incubated in a Bioscreen C Reader (Labsystems, Helsinki, Finland) at 28 °C during 5 days. Cell growth was measured every 20 min. Analyses were performed in five technical replicates and in three biological replicates.

Construction of *melB* defective mutant in *A. fabrum* C58 and transcriptional fusion

The *A. fabrum* C58Δ*melB* defective mutant was constructed as described previously (29) without marker exchange. Briefly, the recombinant region containing the upstream and downstream region flanking the *melB* gene (amplified by PCR using primers listed in Table S2) was inserted into pJQ200sk vector (30) leading to a nonpolar mutant. The resulting plasmid was introduced into *A. fabrum* C58 by electroporation. Bacteria were spread on YPG medium plates containing gentamicin (20 µg/ml) for the first selection and gentamicin-resistant colonies were spread on YPG plates supplemented with 5% sucrose for the second selection. The deletion of *melB* was verified by sequencing (GenoScreen, Lille, France).

The C58 pOT1e-*P_{mel}* transcriptional reporter fusion strain was obtained as follows: the promoter region of *atu4660-atu4665* genes named *P_{mel}* was PCR amplified (using primer

listed in Table S2) and the PCR fragment obtained was ligated into ClaI-SalI digested vector pOT1e. pOT1eM-*P_{mel}* plasmid was obtained by cloning *P_{mel}* into SpeI digested pOT1eM vector as described previously (31). Transcriptional reporter constructions were introduced into *A. fabrum* by electroporation.

Cloning, expression, and purification of mature MelB

The mature MelB expression plasmid was chemically synthesized using codon optimization for the expression in *E. coli* and inserted into pET-9a plasmid using NdeI and BamHI restriction enzyme (GenScript, Piscataway, NJ). *E. coli* BL21 competent cells transformed with pET9a-MelB were grown in LB media at 37 °C until A_{600} of 0.6. 0.5 mM of isopropyl β-D-thiogalactopyranoside (IPTG) was added to the culture for overnight expression at 20 °C. The cells were pelleted by centrifugation at 4000 × *g* for 15 min at 4 °C, resuspended in 50 mM Tris-HCl, pH 8, 300 mM NaCl, and 20 mM imidazole, and disrupted by sonication. After centrifugation at 25,000 × *g* for 30 min, the filtered supernatant was injected on a nickel affinity column (HiTrap 5 ml, GE Healthcare). After a washing step of 6% 50 mM Tris-HCl, pH 8, 300 mM NaCl, and 300 mM imidazole (Buffer B), the protein was eluted with Buffer B and injected on a gel filtration Superdex 200 26/60 (GE Healthcare) using 50 mM Tris-HCl, pH 8, and 150 mM NaCl. The protein fractions were pooled, concentrated at 10.7 mg/ml, and stored at –80 °C.

Expression and purification of mature seleniated MelB

The *E. coli* BL21 cells transformed with the plasmid pET9a-MelB were grown overnight at 28 °C in M9 media supplemented with 0.4% glucose; 2 mM MgSO₄; 1 µM CaCl₂; 100 mg/liter of lysine, threonine, and phenylalanine; and 50 mg/liter of leucine, valine, isoleucine, and methionine. The pelleted cells were resuspended in fresh M9 media (same as above) with 100 mg/liter of selenomethionine instead of methionine for 1 h at 37 °C before inducing the expression with 0.5 mM isopropyl β-D-thiogalactopyranoside overnight at 20 °C. The cells were centrifuged at 4000 × *g* for 15 min at 4 °C. The purification protocol was the same as described above.

Crystallization and data collection of MelB

Crystallization conditions for seleniated MelB in the presence of 2 mM raffinose were screened using Qiagen kits (Valencia, CA) with a Cartesian NanoDrop robot (Genomic Solutions). The crystals were manually reproduced in hanging drops experiments by mixing equal volumes of protein solution and the precipitant solution 25% PEG 4000, 0.2 M NaCl, 0.1 M Mes, pH 6.5, and 0.2 M CaCl₂. For the four other complexes, a similar condition without CaCl₂ and 0.6 M NaCl was used. Crystals were transferred to a cryoprotectant solution (mother liquor supplemented with 25% PEG 400) and flash-frozen in liquid nitrogen. X-ray diffraction data sets were collected at 100 K on the Proxima 1 or 2 beamlines (SOLEIL synchrotron, Saint-Aubin, France). Data processing was performed using the XDS package (32) (Table 2).

Structure determination and refinement of MelB

The crystal structure of the MelB-raffinose complex was determined by SAD method from selenomethionine-labeled

protein and refined at 2 Å resolution. Solvent content analysis using CCP4 (Collaborative Computational Project, Number 4) indicated the presence of four monomers in the asymmetric unit. The positions of 12 over 15 selenium atoms per monomer were found using SHELX suite program (33). The phases were calculated using PHASER (34) and density modification was performed by PARROT (CCP4 suite). An iterative process of manual building in COOT combined with phase calculation with a partial model was used as input, allowed the modeling of the complete polypeptide chain. The structures of all other liganded MelB were solved using the SeMet-MelB monomer as a search model. Refinement of each structure was performed with BUSTER-2.10 (34), NCS restraints, and TLS group. Because of the strong anisotropy of the crystals of MelB-stachyose, the DEBYE and STARANISO programs developed by Global Phasing Ltd. were applied to the data scaled with AIMLESS using the STARANISO server (<http://staraniso.globalphasing.org>).⁶ These programs perform an anisotropic cut-off of merge intensity data on the basis of an analysis of local $I/s(I)$; compute Bayesian estimates of structure amplitudes, taking into account their anisotropic fall-off; and apply an anisotropic correction to the data. The corrected anisotropic amplitudes were used for further refinement of the MelB-stachyose structure with BUSTER-2.10. Inspection of the density maps and manual rebuilding were performed using COOT (35). The three-dimensional models of stachyose and galactinol were generated with the ProDRG webserver (36), whereas those of melibiose and raffinose were found in the Protein Data Bank. Refinement details of each structure are shown in Table 2. Molecular graphics images were generated using PyMOL.

Fluorescence titration measurements of MelB

Each ligand bound to MelB was monitored by autofluorescence by exciting the protein at a wavelength of 295 nm and monitoring the quenching of fluorescence emission of tryptophans at 335 nm. All experiments were performed at 22 °C in 96-well plates (1/2 Area Plate-96F, PerkinElmer Life Sciences) using Tecan Infinite M1000 (Tecan, Männedorf, Switzerland) in 25 mM Tris-HCl, pH 8.0, and 150 mM NaCl with a fixed amount of proteins (1 μM) and increasing concentrations of ligand. Each ligand has no emission signal at 335 nm. The data were analyzed using Origin[®] 7 software and fitted to the following equation.

$$f = \Delta \text{Fluorescence}_{\text{max}} \times \text{abs}(x) / (K_D + \text{abs}(x)) \quad (\text{Eq. 1})$$

Isothermal titration microcalorimetry measurements of MelB

Isothermal titration microcalorimetry experiments were performed with an ITC200 isothermal titration calorimeter from MicroCal (GE Healthcare). The experiments were carried out at 20 °C. Protein concentration in the microcalorimeter cell (0.2 ml) varied from 10 to 300 μM. Nineteen injections of 2 μl of ligand solution (raffinose, stachyose, melibiose, galactose, and galactinol) concentration from 0.1 to 2.8 mM were performed at intervals of 180 s while stirring at 500 rpm. The experimental data were fitted to theoretical titration curves with software supplied by MicroCal (ORIGIN[®]). This software uses the rela-

tionship between the heat generated by each injection and ΔH (enthalpy change in kcal mol⁻¹), K_d (the association binding constant in mol⁻¹), n (the number of binding sites), total protein concentration, and free and total ligand concentrations (37).

Differential scanning calorimetry

Thermal stability of the WT and liganded MelB (13 μM and 50 μM for protein and ligand, respectively) was studied by differential scanning calorimetry (DSC) on a MicroCal model VP-DSC in a standard buffer. Each measurement was preceded by a baseline scan with the standard buffer. All solutions were degassed just before loading into the calorimeter. Scans were performed at 1 K·min⁻¹ between 20 and 90 °C. The heat capacity of the buffer was subtracted from that of the protein sample before analysis. Thermodynamic parameters were determined by fitting the data to the following equation,

$$\Delta C_p(T) = \frac{K_d(T) \Delta H_{\text{cal}} \Delta H_{\text{vH}}}{[1 + K_d(T)]^2 RT^2} \quad (\text{Eq. 2})$$

where K_d is the equilibrium constant for a two-state process, ΔH_{vH} is the enthalpy calculated on the basis of a two-state process, and ΔH_{cal} is the measured enthalpy.

Phylogenetic analysis

Sequences were analyzed using BlastP from NCBI (<https://blast.ncbi.nlm.nih.gov/>) and MicroScope (<https://www.genoscope.cns.fr/>).⁶ Alignments of MelB and related sequences were conducted using ClustalW software. Relationship tree was built using Mega software, version 7. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Poisson correction method and are in units of the number of amino acid substitutions per site.

Measurement of mel operon gene expression

Expression of *mel* operon genes was measured in the C58 pOT1e-*P_{mel}* strain. Quantification of fluorescence was carried out in a microplate filled with 200 μl of AT medium, supplemented with different carbon sources at a final concentration of 10 mM. Microplate wells were inoculated with overnight cultures to obtain an A_{600} of 0.2. A TECAN apparatus (Tecan SparkTM 15 M, Männedorf, Switzerland) was used to read microplates after 24 h of incubation at 28 °C. The following parameters were used: absorbance at 600 nm, fluorescence excitation at 488 nm, and emission at 510 nm. Results were normalized by the A_{600} and -fold change values were obtained by dividing the fluorescence by the corresponding value obtained from the empty pOT1e vector. The fluorescence level comparison was carried out using the Tukey's test (p value = 0.05) and computed with the "vegan" package in the R v3.1.3 statistical software environment (R Core Team, 2014).

Plant inoculation

For bacterial colonization, competition assays, and confocal observation studies, tomato seeds (*Solanum lycopersicum*

Galactinol and melibiose bound to MelB

"Marmande") were sterilized as described (38). Seeds were plated on 0.8% agar plant cell culture supplemented with 1.5 g/liter of the Plant-Prod 15-15-30 High K nutrient solution (Master Plant-Prod Inc., Brampton, Ontario, Canada). They were inoculated with 10 μ l of overnight culture (10^6 cfu/ml) of a single strain (*A. fabrum* C58 pTi_{at}6148:K_m derivative of the WT strain (39) or C58 Δ melB mutant), or with a mixture of both at 1:1 ratio (competition). Petri dishes were placed 2 days in the dark and then in a climatic chamber at 24 °C with 18/8 h for light/dark and 65% of humidity. To determine bacterial colonization level, roots were ground at 2 and 14 dpi. Serial dilutions of crushed roots were plated on the YPG medium and colonies were counted after 2 days of incubation at 28 °C. Significant difference in the population level resulting from five plants per strain, with enumeration of three Petri dishes for each plant, was evaluated with Mann-Whitney test (p value = 0.05) performed with the R v3.1.3 statistical software environment.

In competition experiments, the colonized bacteria were nonselectively recovered at 2 and 14 dpi. To that end, crushed roots were first plated with a spiral plater (EasySpiral[®], Interscience, Saint-Nom-la-Bretèche, France) on YPG medium without antibiotics to enable a biologically unbiased recovery of both C58 pTi_{at}6148:K_m WT strain and C58 Δ melB mutant (kanamycin sensitive). Two hundred individual colonies were then plated in YPG medium with kanamycin/neomycin to determine the relative proportions of C58 pTi_{at}6148:K_m and C58 Δ melB mutant strains (output ratio). The determination of the initial strains ratio of the inoculum was realized using the same protocol. The experimental assays were performed with 10 independent assays and repeated four times. The proportions of WT strains between the initial strain ratio and *in planta* output ratios were compared with the test of equal or given proportions (p value = 0.05).

Confocal microscopy analyses

Visualization of reporter bacterial cells harboring pOT1eM-P_{mel} on tomato radicles and roots was performed using a confocal laser scanning microscope (LSM 800 Meta Confocal Microscope, Zeiss, Oberkochen, Germany). In the reporter strain, M-Cherry (*red color*) is constitutively expressed and enhanced GFP (eGFP) (*green color*) is expressed under P_{mel} control. The red color indicates the bacteria presence (red cells) whereas eGFP reports the induction of P_{mel} shown as yellow-green cells. At 2 and 14 dpi, tomato radicles and roots were mounted between a slide and a coverslip in a commercial mounting fluid (Aqua Poly/Mount, Polysciences, Inc., Warrington, PA). The eGFP and the M-cherry were excited with argon laser at 488 nm and 584 nm, respectively, and fluorescence was captured at 528 nm and 607 nm. Analyses of images (five plants per condition) were performed thanks to LSM 800 software (Zeiss, Oberkochen, Germany).

Coordinates

The atomic coordinates and structure factors have been deposited at the Protein Data Bank (PDB) under accession codes 6EPY (seleniated MelB with raffinose), 6EQ1 (MelB with

stachyose), 6EQ8 (MelB with galactinol), 6EPZ (MelB with melibiose) and 6EQ0 (MelB with galactose).

Author contributions—T. M., L. V., and C. L. performed all the microbiology work. A. V. and S. M. performed all the crystallography work. A. V. performed the fluorescence assays. A. V. and M. A. N. performed the microcalorimetry experiments. A. V., T. M., C. L., and S. M. performed the phylogenetic analysis. S. M. and C. L. wrote the manuscript. All the authors discussed the results and contributed to the writing of the manuscript.

Acknowledgments—This work has benefited from the I2BC crystallization and microcalorimetry platforms supported by FRISBI ANR-10-INSB-05-01 as well as from the "Centre Technologique des Microstructures" and the "Serre et chambres climatiques" platforms, supported by the FR BioEnviS Research Federation. We acknowledge SOLEIL for provision of synchrotron radiation facilities (proposals ID 20130869, 20140774, and 20160782) in using Proxima beamlines. We thank Andrew Saurin for critical reading of the manuscript.

References

1. Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M. (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* **57**, 233–266 CrossRef Medline
2. Nelson, E. B. (2004) Microbial dynamics and interactions in the sphere. *Annu. Rev. Phytopathol.* **42**, 271–309 CrossRef Medline
3. Nelson, E. B. (2018) The seed microbiome: Origins, interactions, and impacts. *Plant Soil* **422**, 7–34 CrossRef
4. Barret, M., Briand, M., Bonneau, S., Prévieux, A., Valière, S., Bouchez, O., Hunault, G., Simonneau, P., and Jacques, M.-A. (2015) Emergence shapes the structure of the seed microbiota. *Appl. Environ. Microbiol.* **81**, 1257–1266 CrossRef Medline
5. Sengupta, S., Mukherjee, S., Basak, P., and Majumder, A. L. (2015) Significance of galactinol and raffinose family oligosaccharide synthesis in plants. *Front. Plant Sci.* **6**, 656 CrossRef Medline
6. Nishizawa, A., Yabuta, Y., and Shigeoka, S. (2008) Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiol.* **147**, 1251–1263 CrossRef Medline
7. Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., Yamaguchi-Shinozaki, K., and Shinozaki, K. (2002) Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. *Plant J.* **29**, 417–426 CrossRef Medline
8. Ibáñez, C., Collada, C., Casado, R., González-Melendi, P., Aragoncillo, C., and Allona, I. (2013) Winter induction of the galactinol synthase gene is associated with endodormancy in chestnut trees. *Trees* **27**, 1309–1316 CrossRef
9. Kim, M. S., Cho, S. M., Kang, E. Y., Im, Y. J., Hwangbo, H., Kim, Y. C., Ryu, C.-M., Yang, K. Y., Chung, G. C., and Cho, B. H. (2008) Galactinol is a signaling component of the induced systemic resistance caused by *Pseudomonas chlororaphis* O6 root colonization. *Mol. Plant Microbe Interact.* **21**, 1643–1653 CrossRef Medline
10. Cho, S. M., Kang, E. Y., Kim, M. S., Yoo, S. J., Im, Y. J., Kim, Y. C., Yang, K. Y., Kim, K. Y., Kim, K. S., Choi, Y. S., and Cho, B. H. (2010) Jasmonate-dependent expression of a galactinol synthase gene is involved in priming of systemic fungal resistance in *Arabidopsis thaliana*. *Botany* **88**, 452–461 CrossRef
11. Siddique, S., Endres, S., Sobczak, M., Radakovic, Z. S., Fragner, L., Grunler, F. M. W., Weckwerth, W., Tenhaken, R., and Bohlmann, H. (2014) Myo-inositol oxygenase is important for the removal of excess myo-inositol from syncytia induced by *Heterodera schachtii* in *Arabidopsis* roots. *New Phytol.* **201**, 476–485 CrossRef Medline
12. Berntsson, R. P.-A., Smits, S. H. J., Schmitt, L., Slotboom, D.-J., and Poolman, B. (2010) A structural classification of substrate-binding proteins. *FEBS Lett.* **584**, 2606–2617 CrossRef Medline

13. Gage, D. J., and Long, S. R. (1998) α -Galactoside uptake in *Rhizobium meliloti*: Isolation and characterization of *agpA*, a gene encoding a periplasmic binding protein required for melibiose and raffinose utilization. *J. Bacteriol.* **180**, 5739–5748 [Medline](#)
14. Bringhurst, R. M., Cardon, Z. G., and Gage, D. J. (2001) Galactosides in the rhizosphere: Utilization by *Sinorhizobium meliloti* and development of a biosensor. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4540–4545 [CrossRef](#) [Medline](#)
15. Liljeström, P. L., and Liljeström, P. (1987) Nucleotide sequence of the *melA* gene, Coding for α -galactosidase in *Escherichia coli* K-12. *Nucleic Acids Res.* **15**, 2213–2220 [CrossRef](#) [Medline](#)
16. Charaoui-Boukerzaza, S., and Hugouvieux-Cotte-Pattat, N. (2013) A family 3 glycosyl hydrolase of *Dickeya dadantii* 3937 is involved in the cleavage of aromatic glucosides. *Microbiology.* **159**, 2395–2404 [CrossRef](#) [Medline](#)
17. Liu, Y., Chen, L., Wu, G., Feng, H., Zhang, G., Shen, Q., and Zhang, R. (2017) Identification of root-secreted compounds involved in the communication between cucumber, the beneficial *Bacillus amyloliquefaciens*, and the soil-borne pathogen *Fusarium oxysporum*. *Mol. Plant Microbe Interact.* **30**, 53–62 [CrossRef](#) [Medline](#)
18. Nester, E. W. (2014) *Agrobacterium*: nature's genetic engineer. *Front. Plant Sci.* **5**, 730 [CrossRef](#) [Medline](#)
19. Abarca-Grau, A. M., Penyalver, R., López, M. M., and Marco-Noales, E. (2011) Pathogenic and non-pathogenic *Agrobacterium tumefaciens*, *A. rhizogenes* and *A. vitis* strains form biofilms on abiotic as well as on root surfaces: Biofilms formed by *Agrobacterium* spp. *Plant Pathol.* **60**, 416–425 [CrossRef](#)
20. Hall, B. G., Pikis, A., and Thompson, J. (2009) Evolution and biochemistry of family 4 glycosidases: Implications for assigning enzyme function in sequence annotations. *Mol. Biol. Evol.* **26**, 2487–2497 [CrossRef](#) [Medline](#)
21. Wood, D. W., Setubal, J. C., Kaul, R., Monks, D. E., Kitajima, J. P., Okura, V. K., Zhou, Y., Chen, L., Wood, G. E., Almeida, N. F., Jr., Woo, L., Chen, Y., Paulsen, I. T., Eisen, J. A., Karp, P. D., et al. (2001) The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. *Science.* **294**, 2317–2323 [CrossRef](#) [Medline](#)
22. Westover, B. P., Buhler, J. D., Sonnenburg, J. L., and Gordon, J. I. (2005) Operon prediction without a training set. *Bioinformatics* **21**, 880–888 [CrossRef](#)
23. Kemner, J. M., Liang, X., and Nester, E. W. (1997) The *Agrobacterium tumefaciens* virulence gene *chvE* is part of a putative ABC-type sugar transport operon. *J. Bacteriol.* **179**, 2452–2458 [CrossRef](#) [Medline](#)
24. Cornish, A., Greenwood, J. A., and Jones, C. W. (1989) Binding-protein-dependent sugar transport by *Agrobacterium radiobacter* and *A. tumefaciens* grown in continuous culture. *J. Gen. Microbiol.* **135**, 3001–3013 [CrossRef](#) [Medline](#)
25. Andersen, K. E., Bjerregaard, C., Møller, P., Sørensen, J. C., and Sørensen, H. (2005) Compositional variations for α -galactosides in different species of leguminosae, brassicaceae, and barley: A chemotaxonomic study based on chemometrics and high-performance capillary electrophoresis. *J. Agric. Food Chem.* **53**, 5809–5817 [CrossRef](#) [Medline](#)
26. Kristin, A., and Miranda, H. (2013) The root microbiota—a fingerprint in the soil? *Plant Soil* **370**, 671–686 [CrossRef](#)
27. Fukami, T., Martijn Bezemer, T., Mortimer, S. R., and van der Putten, W. H. (2005) Species divergence and trait convergence in experimental plant community assembly. *Ecol. Lett.* **8**, 1283–1290 [CrossRef](#)
28. Veena Jiang, H., Doerge, R. W., and Gelvin, S. B. (2003) Transfer of T-DNA and Vir proteins to plant cells by *Agrobacterium tumefaciens* induces expression of host genes involved in mediating transformation and suppresses host defense gene expression. *Plant J.* **35**, 219–236 [CrossRef](#)
29. Lassalle, F., Campillo, T., Vial, L., Baude, J., Costechareyre, D., Chapulliot, D., Shams, M., Abrouk, D., Lavire, C., Oger-Desfeux, C., Hommais, F., Gueguen, L., Daubin, V., Muller, D., and Nesme, X. (2011) Genomic species are ecological species as revealed by comparative genomics in *Agrobacterium tumefaciens*. *Genome Biol. Evol.* **3**, 762–781 [CrossRef](#)
30. Quandt, J., and Hynes, M. F. (1993) Versatile suicide vectors which allow direct selection for gene replacement in gram-negative bacteria. *Gene* **127**, 15–21 [CrossRef](#) [Medline](#)
31. Meyer, T., Renoud, S., Vigouroux, A., Miomandre, A., Gaillard, V., Kerzaon, I., Prigent-Combaret, C., Comte, G., Moréra, S., Vial, L., and Lavire, C. (2018) Regulation of hydroxycinnamic acid degradation drives *Agrobacterium fabrum* lifestyles. *Mol. Plant Microbe Interact.* [CrossRef](#) [Medline](#)
32. Kabsch, W. (2010) XDS. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 125–132 [CrossRef](#) [Medline](#)
33. Sheldrick, G. M. (2008) A short history of SHELX. *Acta Crystallogr. A.* **64**, 112–122 [CrossRef](#) [Medline](#)
34. McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C., and Read, R. J. (2007) Phaser crystallographic software. *J Appl. Crystallogr.* **40**, 658–674 [CrossRef](#) [Medline](#)
35. Emsley, P., and Cowtan, K. (2004) Coot: Model-building tools for molecular graphics. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126–2132 [CrossRef](#) [Medline](#)
36. Schüttelkopf, A. W., and van Aalten, D. M. F. (2004) PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 1355–1363 [CrossRef](#) [Medline](#)
37. Wiseman, T., Williston, S., Brandts, J. F., and Lin, L. N. (1989) Rapid measurement of binding constants and heats of binding using a new titration calorimeter. *Anal. Biochem.* **179**, 131–137 [CrossRef](#) [Medline](#)
38. Vacheron, J., Moëgne-Loccoz, Y., Dubost, A., Gonçalves-Martins, M., Muller, D., and Prigent-Combaret, C. (2016) Fluorescent *Pseudomonas* strains with only few plant-beneficial properties are favored in the maize rhizosphere. *Front. Plant Sci.* **7**, 1212 [CrossRef](#) [Medline](#)
39. Lang, J., Planamente, S., Mondy, S., Dessaux, Y., Moréra, S., and Faure, D. (2013) Concerted transfer of the virulence Ti plasmid and companion At plasmid in the *Agrobacterium tumefaciens*-induced plant tumour. *Mol. Microbiol.* **90**, 1178–1189 [CrossRef](#) [Medline](#)
40. Karplus, P. A., and Diederichs, K. (2012) Linking crystallographic model and data quality. *Science.* **336**, 1030–1033 [CrossRef](#) [Medline](#)

Supporting Information

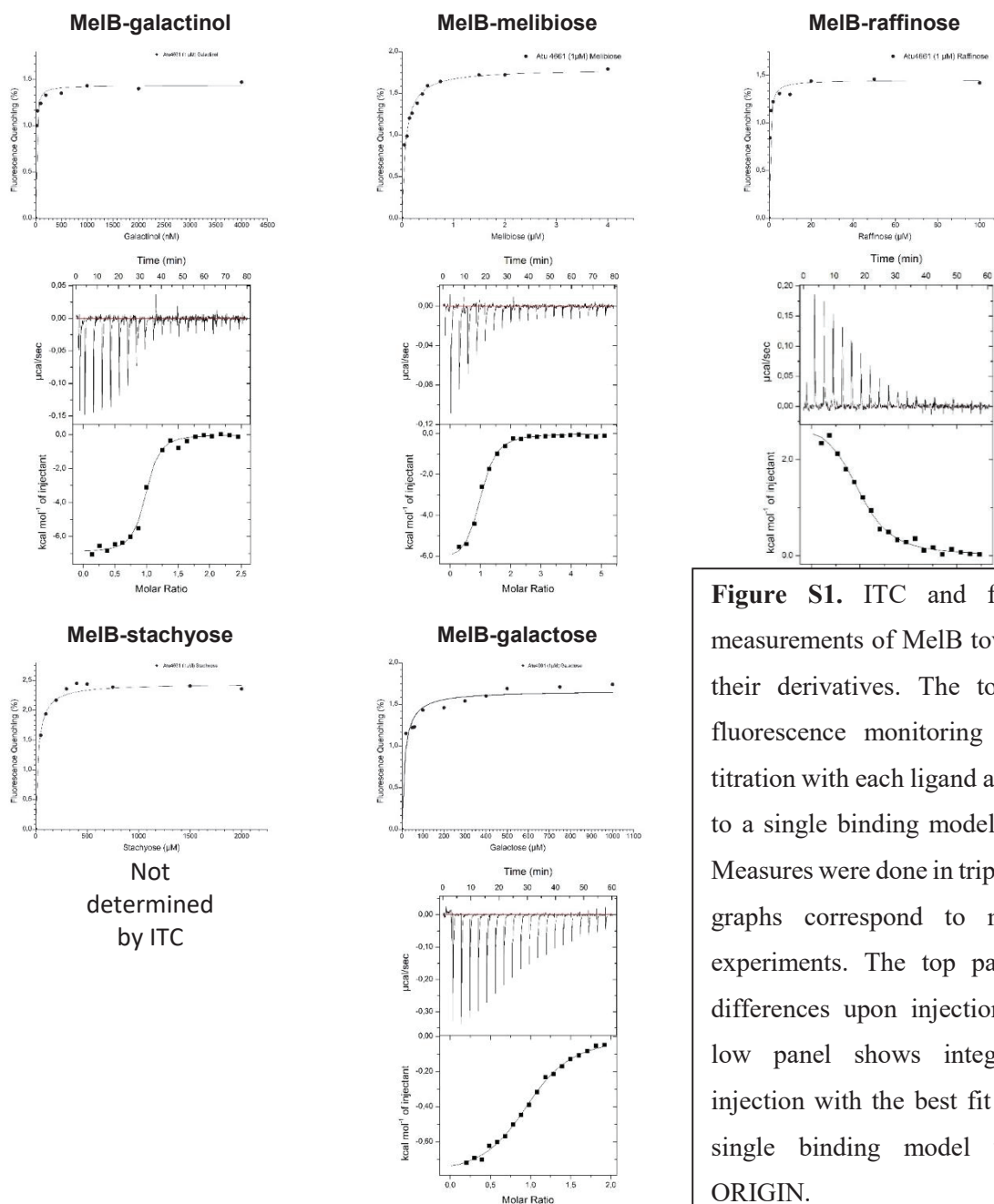
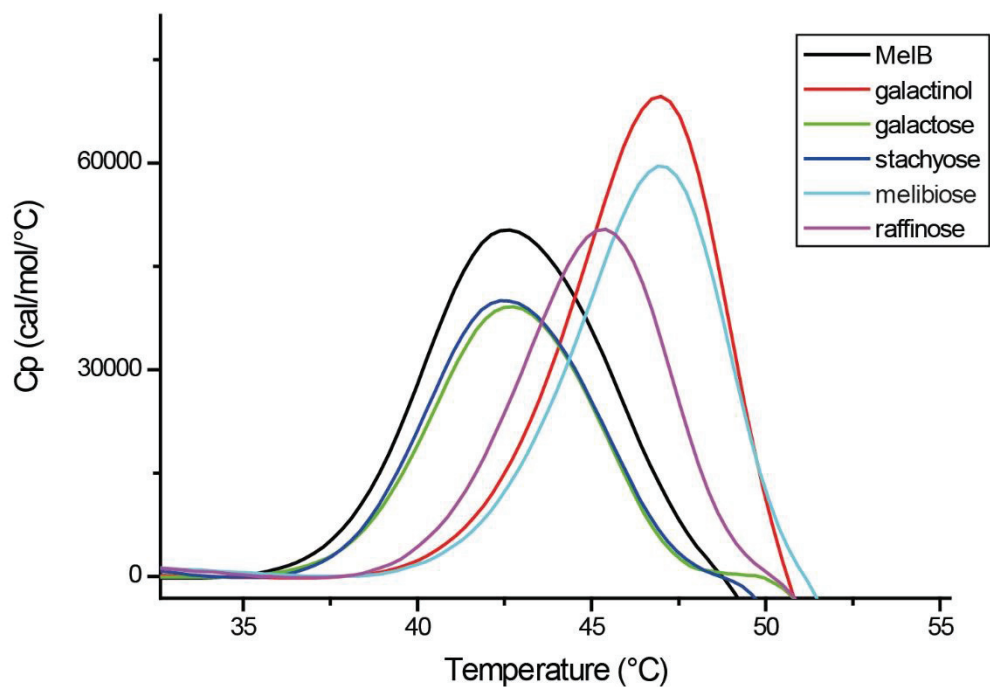


Figure S1. ITC and fluorescence K_D measurements of MelB towards RFOs and their derivatives. The top graphs show fluorescence monitoring of MelB upon titration with each ligand and fit (solid line) to a single binding model using Origin 7. Measures were done in triplicate. The lower graphs correspond to microcalorimetry experiments. The top panel shows heat differences upon injection of ligand and low panel shows integrated heats of injection with the best fit (solid line) to a single binding model using Microcal ORIGIN.



	T_m (°C)	ΔH (Kcal/mol/°C)
MelB	43.5	323
+ galactinol	46.5	362
+ galactose	43.3	225
+ stachyose	43.2	236
+ melibiose	46.6	314
+ raffinose	45.1	265

Figure S2. DSC thermograms of MelB

Table S1. Strains and plasmids used in this study

Strains	Relevant genotype and description	Reference or source
<i>Escherichia coli</i>		
JM109	<i>endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ Δ(lac-proAB) e14- [F' traD36 proAB+ lacIq lacZΔM15] hsdR17(rK-mK+)</i>	NEB catalog
BL21	$F^- ompT gal dcm lon hsdS_B(r_B^- m_B^-) [malB^+]_{K-12}(\lambda^S)$	NEB catalog
Stellar	<i>F⁻, endA1, supE44, thi-1, recA1, relA1, gyrA96, phoA, Φ80d lacZΔ M15, Δ(lacZYA - argF) U169, Δ(mrr - hsdRMS - mcrBC), ΔmcrA, λ-</i>	Takara Clontech
<i>Agrobacterium fabrum</i>		
C58	Wild-type	CFBP 1903
C58Δ <i>melB</i> (<i>atu4661</i>)	C58 deleted of <i>melB</i> (<i>atu4661</i>) gene	This study
C58 p <i>Tiatu6148</i> :Km	C58 disrupted for <i>atu6148</i> gene ; Km ^R	(1)
Plasmids		
pJQ200SK	Suicide vector; P15A <i>sacB</i> ; Gm ^R	(2)
pOT1e	Promoter-probe vector based on pBBR1MCS-5 replicon; contains promoterless eGFP and MCS between two transcriptional terminators; Gm ^R	(3)
pOT1eM	pOT1e derivative harboring <i>Ptac-m-cherry</i> inserted in ClaI-SalI site	(4)
pET-9(a)	<i>E. coli</i> expression vector (N-term T7-tag fusion), Km ^R	Novagen
pOT1e- <i>Pmel</i>	Upstream region <i>atu4660-atu4665</i> inserted in ClaI-SalI site in pOT1e; Gm ^R	This study
pOT1eM- <i>Pmel</i>	Upstream region <i>atu4660-atu4665</i> inserted in SpeI site in pOT1eM; Gm ^R	This study
pET-9(a)- <i>melB</i>	<i>melB</i> inserted in NdeI-BamHI site in pET-29(a) (N-term T7-tag fusion), Km ^R	This study

1. Lang, J., Planamente, S., Mondy, S., Dessaux, Y., Moréra, S., and Faure, D. (2013) Concerted transfer of the virulence Ti plasmid and companion At plasmid in the *Agrobacterium tumefaciens*-induced plant tumour. Mol. Microbiol. 90, 1178–1189

2. Quandt, J., and Hynes, M. F. (1993) Versatile suicide vectors which allow direct selection for gene replacement in gram-negative bacteria. Gene. 127, 15–21

3. Allaway, D., Schofield, N. A., Leonard, M. E., Gilardoni, L., Finan, T. M., and Poole, P. S. (2001) Use of differential fluorescence induction and optical trapping to isolate environmentally induced genes. Environ. Microbiol. 3, 397–406

4. Meyer, T., Renoud, S., Vigouroux, A., Miomandre, A., Gaillard, V., Kerzaon, I., Prigent-Combaret, C., Comte, G., Moréra, S., Vial, L., and Lavire, C. (2018) Regulation of hydroxycinnamic acid degradation drives *Agrobacterium fabrum* lifestyles. Mol. Plant-Microbe Interact. MPMI. 10.1094/MPMI-10-17-0236-R

Table S2. Primers used in this study

Primer			
Target	Primer name	Primer sequence (5'-3')	Reference
<i>Transcriptional fusion</i>			
<i>mel</i> promoter insertion into pOT1e	<i>PmelF</i>	ATCGATACCACATAATGGCGGTTCTC	This study
	<i>PmelR</i>	CGAGATTGTGCTGGCTGATA	
<i>mel</i> promoter insertion into pOT1eM	<i>PmelF'</i>	CGGGGGATCCACTAGACCACATAATGGCGGTTCTC	This study
	<i>PmelR'</i>	TTCTTCCTCCACTAGCGAGATTGTGCTGGCTGATA	
<i>Deletion of melB</i>			
<i>melB</i> flanking upstream region	<i>UpmelBF</i>	GAATTCCTGCAGCCCCAACCACCAGACATGGTTC	This study
	<i>UpmelBR</i>	GGATATGCTTCAGTTTTGCATCGTCATTCCCTCCC	
<i>melB</i> flanking downstream region	<i>DwmelBF</i>	AACTGAAGCATATCCGGGA	This study
	<i>DwmelBR</i>	ACTAGTGGATCCCCCTCTCGCCACTCAGCATGT	

Article 4 : Influence of ferulic acid on *Agrobacterium fabrum* transcriptome

Thibault Meyer ¹, Vincent Gaillard ¹, Gilles Comte ¹, Ludovic Vial ¹, Céline Lavire ¹

¹ Université de Lyon, F-69622, Lyon, France ; Université Lyon 1, Villeurbanne, France ; CNRS, UMR5557, Ecologie Microbienne, Villeurbanne, France ; INRA, UMR1418, Villeurbanne, France

Correspondence should be sent to: Céline Lavire, Ecologie Microbienne, Université Lyon 1, 43 Boulevard du 11 novembre F-69622 Villeurbanne cedex, France, Tel.: (+33) 426237126 ; Fax: (+33) 4 72 43 12 23; E-mail: celine.lavire@univ-lyon1.fr

Keywords: *Agrobacterium tumefaciens*; hydroxycinnamic acids; plant signal; carbon metabolism; fitness; crown-gall; rhizosphere; species-specific genes

Abstract

The plant pathogen *A. fabrum* is known to respond to multiple plant signals in the context of pathogenic lifestyle. Usually, *A. fabrum* recovers from soil and rhizosphere and possess numerous molecular traits which can be involved in the microbial competitive colonization in these environments, for example its ability to exploit diverse trophic resources, to resist to toxic compounds, to synthesize siderophore, and to kill bacterial species sharing the same ecological niche by a type VI secretion system. However, few information is available about plant signal involved in the induction of the expression of these molecular traits. In this context, we studied the influence of the presence of ferulic acid on transcriptome in presence of an important trophic source for *A. fabrum* in the rhizosphere, the melibiose. By comparing transcriptome in the presence or in the absence of ferulic acid, we shown that rapidly after ferulic acid perception, 14 % of bacterial genes are up- or down-regulated. Among these genes, we identify genes of the type VI secretion system, genes involved in transport processes either import or exports of compounds together with regulatory genes. These findings suggest that ferulic acid is perceived as a plant signal modulating presumptive important molecular traits for the rhizosphere colonization.

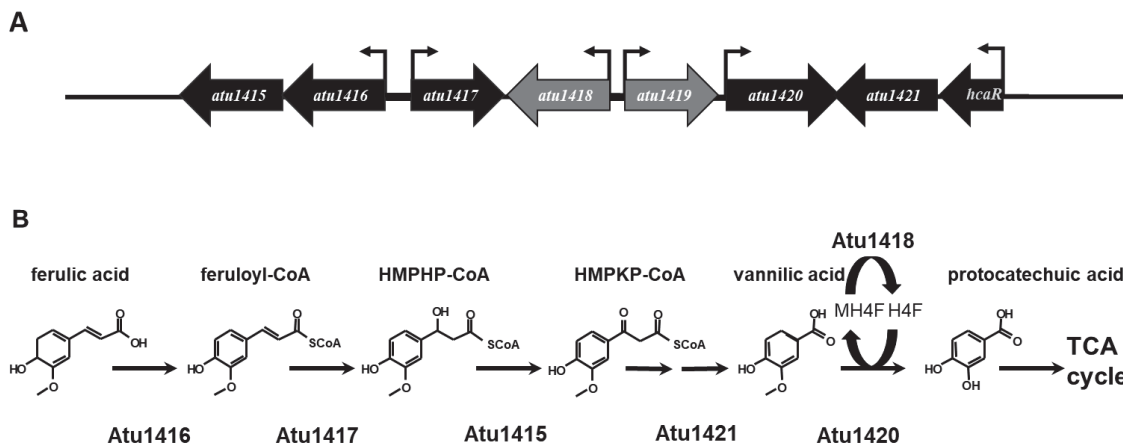


Figure1: Genomic organization of the SpG8-1b region and ferulic acid degradation pathway.

(A) Organization of the SpG8-1b genomic region which contains the *atu1415-atu1416*, *atu1419-atu1420*, and *hcaR-atu1421*. Arrows indicate the promoter regions and the direction of gene transcription. (B) Ferulic acid degradation pathway: ferulic acid is activated by Atu1416, a feruloyl-CoA synthase into Feruloyl-CoA. The latter is then converted by enoyl-CoA hydratase Atu1417 into 4-hydroxy-3-methoxyphenyl- β -hydroxypropionyl (HMPHP)-CoA, which is in turn transformed into vanillic acid by Atu1415, a phenylhydroxypropionyl-CoA dehydrogenase, and then Atu1421, a 4-hydroxy-3-methoxyphenyl- β -ketopropionyl-CoA (HMPKP)-CoA β -keto-thiolase. Atu1418 and Atu1420 degrade vanillic acid into protocatechuic acid. The *pca* genes, described by (Parke, 1995, 1997), allow the integration of this latter molecule into the TCA cycle.

Introduction

Agrobacterium fabrum is well known as facultative phytopathogenic bacteria able to cause crown gall disease when it harbors the tumor-inducing plasmid (Christie and Gordon 2014; Nester 2014; Wood *et al.* 2001). Numerous plant compounds including GABA, indole, opine, salicylic acid have been described as plant signal influencing the genes expression of *A. fabrum* in the tumor lifestyle (Lee *et al.* 2015; Lang *et al.* 2016; Wetzel *et al.* 2014; Yuan *et al.* 2007). However, Agrobacteria are usually soil or rhizospheric inhabitant bacteria (Krimi *et al.* 2002; Lundberg *et al.* 2012; Bulgarelli *et al.* 2012; Bouri *et al.* 2016) and possess numerous molecular determinants to survive in these competitive environments including siderophore gene synthesis, resistance genes to osmotic stress, type VI secretion system (Rondon *et al.* 2004; Boncompagni *et al.* 1999; Ma *et al.* 2014). In the context of rhizosphere, a rich carbon sources environment due to exudation and plant cell death, *A. fabrum* reveals a huge metabolic capacities of adaptation (Dessaux and Faure 2018). Nonetheless, only few studies report the involvement of plant signals in the induction of the expression of molecular determinants in the rhizosphere contrary to the pathogenic lifestyle.

Previously, we characterized two genes cluster important for the rhizosphere lifestyle of *A. fabrum* C58 : the SpG8-1b region involved in hydroxycinnamic acids (HCAs) degradation and the *mel* operon involved in specific sugars catabolism (Meyer *et al.* 2018a, 2018b). The *mel* operon is encoded by the linear chromosome and contains two α -galactosidases (Atu4660 and Atu4665) and an uptake system with a periplasmic binding protein (MelB encoded by *atu4661*) coupling to an ABC transporter (Atu4662–Atu4664). The MelB protein is responsible of the specific uptake of the raffinose family of oligosaccharides (RFO) which are widespread carbon sources in plant seed and rhizosphere (Andersen *et al.* 2005; Sengupta *et al.* 2015; Gage and Long 1998). Interestingly, this protein also allows the very efficient uptake of melibiose and galactinol which are respectively product of RFO degradation and precursor of RFO synthesis (Sengupta *et al.* 2015). Moreover, galactinol is a plant defense signal and contributes to resistance to multiples stress in plant (Ibáñez *et al.* 2013; Nishizawa *et al.* 2008; Cho *et al.* 2010; Kim *et al.* 2008). Degradation of these compounds is required for *A. fabrum* efficient competitive colonization of tomato plant at the germination stage (Meyer *et al.* 2018b).

A second important genes cluster for the rhizosphere colonization is the SpG8-1b region of *A. fabrum*. It is encoded by the circular chromosome and allows the uptake and the use as carbon

source of some HCAs like ferulic acid or *p*-coumaric acid (Figure 1, Campillo *et al.* 2014; Meyer *et al.* 2018a), toxic plant compounds for many microorganisms living in the rhizosphere (Hassan and Hugouvieux-Cotte-Pattat 2011; Borges *et al.* 2013; Joshi *et al.* 2015). Besides to be important for the induction of the pathogeny (Bolton *et al.* 1986; Kalogeraki *et al.* 1999), HCAs are degraded by *A. fabrum* thanks to SpG8-1b genes in the rhizosphere of tomato plants (Meyer *et al.* 2018a). Expression of genes encoded by this cluster are repressed by the transcriptional regulator HcaR in the absence of HCAs. In rich HCA-environment, the activation of ferulic acid or *p*-coumaric acid respectively to feruloyl-CoA or *p*-coumaroyl-CoA, releases the DNA-binding of HcaR and leads to the de-repression of the SpG8-1b genes transcription. Thus, the SpG8-1b genes are expressed upon ferulic acid (and coumaric acid) perception. Additionally, the *hcaR* deletion mutant which constitutively express the firsts SpG8-1b genes outcompeted the wild type strain in competition in tomato rhizosphere (Meyer *et al.* 2018a). This suggest that a rapid and constitutive expression of HCA degradation genes would be beneficial for rhizosphere colonization. HCA degradation genes are lately expressed in the presence of succinate, which seems to repress the SpG8-1b genes expression (Campillo *et al.* 2014; Meyer *et al.* 2018a). Our unpublished results (Meyer *et al.* in prep-article 2) revealed that HCAs are more rapidly used in the presence of melibiose than succinate. However, HCAs-CoA derivatives could be responsible of a slightly longer lag phase of growth in presence of melibiose. Moreover, the feruloyl-CoA was shown responsible for the transcriptional repression of some genes (Meyer *et al.* in prep-article 2).

To decipher the influence of HCA perception and degradation in presence of one carbon source important for the rhizosphere colonization, we analyzed the transcriptome of *A. fabrum* C58 with melibiose in the absence and in the presence of ferulic acid. We demonstrated that ferulic acid perception induces modulation of gene cluster expression important for *A. fabrum* ecology. These results underline the signal role of ferulic acid in the adaptation of *A. fabrum* to the rhizosphere.

Materials and methods

Bacterial strains, plasmids, and culture conditions.

The bacterial strains and plasmids are listed in the Supplementary Table S1. *A. fabrum* C58 was cultivated at 28°C in solid YPG (Yeast Peptone Glucose)-rich medium or liquid AT minimal medium supplemented with 10 mM of D-melibiose and 10 mM ammonium sulfate (pH adjusted

to 7.2), with or without ferulic acid (500 μ M). *Escherichia coli* strains were cultivated at 37°C in LB medium. When required, gentamicin was used at 15 μ g/mL and 25 μ g/mL for *E. coli* and *A. fabrum* strains respectively.

Construction and measurement of transcriptional reporter fusions

The presumptive promoters of putative differentially expressed genes were PCR amplified using primers listed in table S2. DNA fragments were cloned using Infusion technology (Takara Clontech, Kyoto, Japan)(Raman and Martin 2014) in the SpeI digested-pOT1eM (Meyer *et al.* 2018a) resulting in plasmids listed in table S1. Plasmids were transferred to *A. fabrum* strains by electroporation and the presence of plasmids were confirmed by PCR using pOT1eFor and pOT1eRev primers (Allaway *et al.* 2001).

According to previously described method (Meyer *et al.* 2018a), expressions of the different genes studied were measured in the *A. fabrum* strains harboring promoter transcriptional fusions after 9 hours of growth in AT medium with or without ferulic acid (500 μ M) with a TECAN apparatus (Tecan Spark™ 15M, Männedorf, Switzerland).The fluorescence values were normalized by subtracting the fluorescence value of the empty pOT1eM vector in the same condition. Statistical differences were tested with the Mann-Whitney-Wilcoxon test (*P-value*=0.05) using the R v3.1.3 statistical software environment.

RNA extraction

Strain were grown overnight in AT medium containing D-melibiose as carbon source. The bacterial cells were adjusted to an OD_{600nm} of 0.1 in AT medium with D-melibiose and supplemented or not with 500 μ M of ferulic acid. The degradation of ferulic acid was measured by HPLC-UV method according to Campillo *et al.* (2014) and OD_{600nm} was monitored. All the culture conditions were realized in triplicate.

After 9 h of growth, 10-mL of bacterial cells were blocked with 2ml of STOP solution (5% of phenol acid in absolute ethanol), centrifuged and kept at -80°C until RNA extraction.

Extraction of total RNAs was realized using Nucleospin RNA isolation kit following the manufacturer's protocol (Macherey Nagel Co., Diiren, Germany). The DNA contamination was deleted with two additional steps of DNase digestion with the Turbo DNafree kit according to manufacturer recommendation (Ambion, Austin, TX). DNA contamination was checked by

PCR on *atu1417* gene using primer pair *atu1417F* and *atu1418R* (see table S2). Samples were analyzed by arrays in Bioanalyser 2100 and stored at -80°C.

cDNA libraries constructions and statistical analyses were performed at the MGX-Montpellier GenomiX platform (Montpellier, France). 6 RNA libraries were constructed corresponding to the triplicate of the two treatments (with and without ferulic acid). The RNA-Seq libraries were constructed with the TruSeq Stranded mRNA Sample Preparation Kit from Illumina according to the manufacturer instructions (Illumina Inc., San Diego, CA). Libraries were checked after PCR cycles with quantification of DNA on Fragment Analyzer with the kit Standard Sensitivity NGS and by qPCR (ROCHE Light Cycler 480). Sequencing libraries were denatured with NaOH and diluted to 8 pM in Illumina's HT1 buffer for loading onto a single lane of the flow cell. The 50 nt single read were sequenced on HiSeq2500 (Illumina, San Diego, CA).

RNAseq data analysis

Image analyses were carried out with HiSeq Control Software (HCS) and the base-calling with the Real-Time Analysis component (RTA) from Illumina (San Diego, CA). Demultiplexing was realized using *bcl2fastq* and quality checks were performed using respectively Sequencing Analysis Viewer and FastQC. For contaminant screening, FastQ Screen was used. Fastq sequences passing Illumina filters (CAF) were mapped on the genome of *A. fabrum* (GCF 000092025.1 ASM9202v1 version) using BWA (Burrows-Wheeler Aligner) software and were converted to reads count per gene using HTSeq-count. Differential genes expression was tested by the R software and the DESeq method (Anders and Huber 2010) using only genes counts superior to 15 counts (cumulate between the two compared conditions) and after normalization by the method of Relative Log Expression implemented in the EdgeR package (Robinson *et al.* 2010; McCarthy *et al.* 2012). Genes with significant adjusted P-value (adjusted P-value < 0.05 according to the Benjamini-Hochberg FDR method (Benjamini and Hochberg 1995) and a log2Fold-change value superior to 2 were identified as differentially expressed between the two conditions (with and without ferulic acid) The transcriptomic data were deposited in the NCBI database.

Results

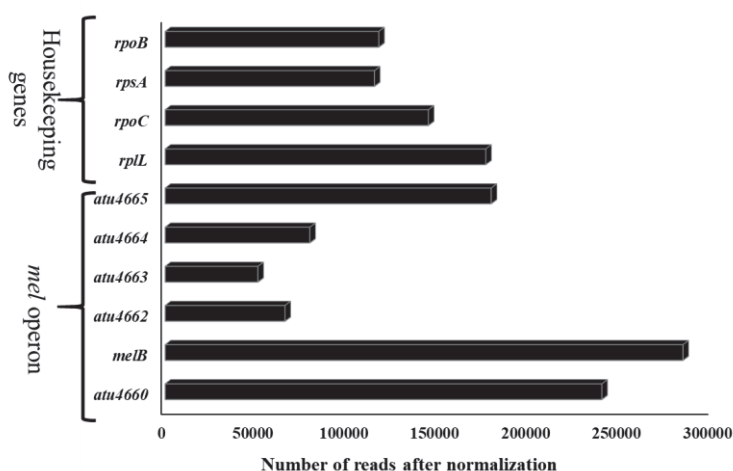
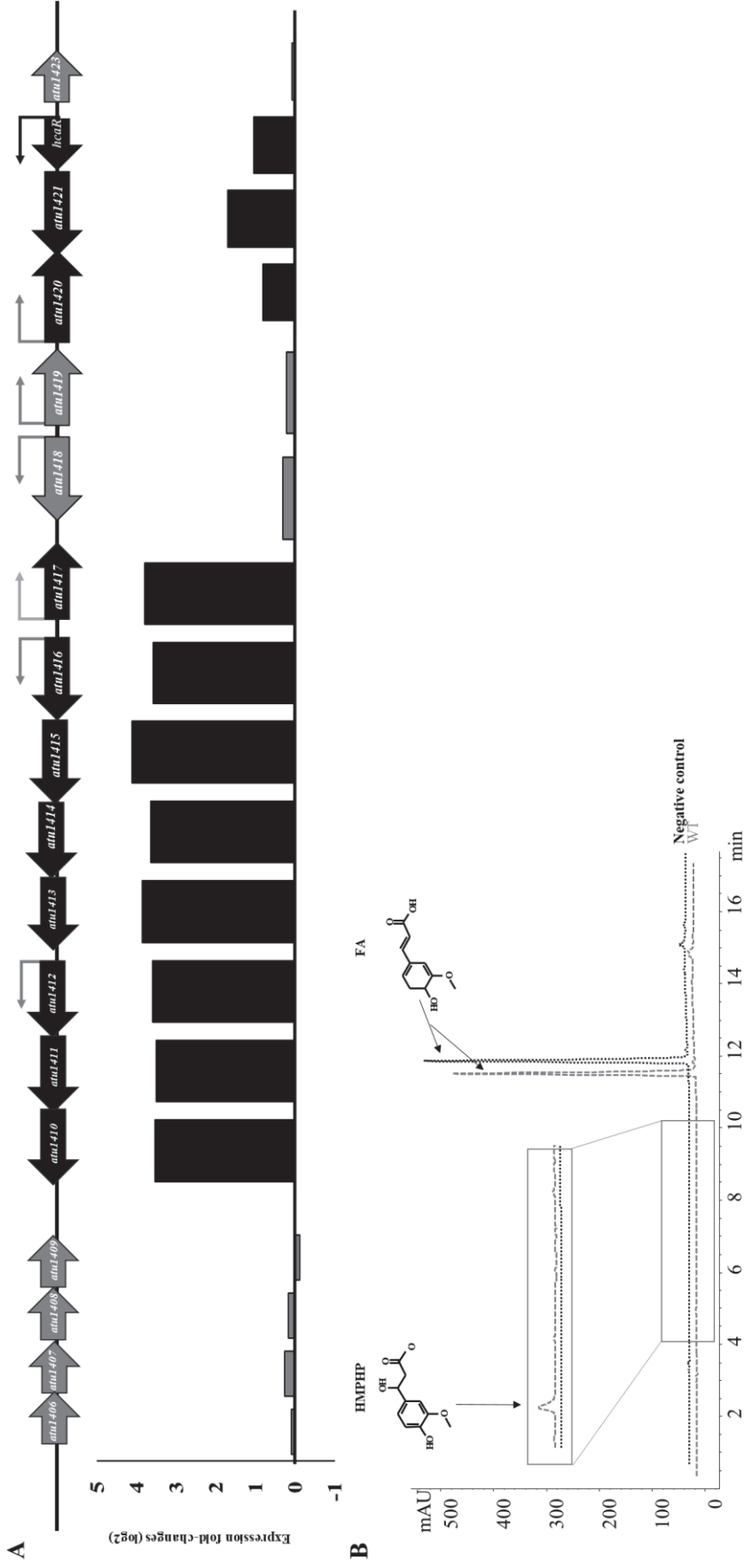


Figure 2: Expression of *mel* operon genes in presence of ferulic acid. The number of reads of the RNA seq experiment is given for the *mel* operon genes and four representative housekeeping genes. It shows high expression of the *mel* operon genes after 9 hours of growth in AT melibiose medium.

Gene of the *mel* operon are highly expressed with and without ferulic acid

The expression of the *mel* operon genes was quantified by a RNAseq experiment after 9 h of growth in AT melibiose medium in presence or in the absence of ferulic acid. We demonstrated that the genes encoding the α -galactosidases (*atu4660* and *atu4665*), *melB* and genes encoding the ABC transporter (*atu4662*–*atu4664*) are strongly expressed in melibiose and at the same level as many housekeeping genes known to be highly transcribed (Figure 2). There is no significant difference in *mel* genes expression between conditions with and without ferulic acid (p -value < 0.05 according to the Benjamini-Hochberg FDR method). The induction of *mel* operon gene expression in presence of melibiose is consistent with our previous study (Meyer *et al.*, 2018). However, from Meyer *et al.* (in prep-Chapitre2-article2) a reduction of *mel* genes expression occurred in the presence of ferulic acid. This is not observed in our analysis, probably due to differences in the experimental procedure. Indeed, in our study the *mel* operon genes is already and highly expressed before adding ferulic acid because the strains are precultured in AT melibiose medium. On the contrary Meyer *et al.* (in prep-Chapitre2-article2) used strains precultured in a rich medium before adding both melibiose and ferulic acid, which involves that the *mel* operon was not transcribed before ferulic acid addition.



The presence of ferulic acid influences *A. fabrum* transcriptome

The time of transcriptomic analysis, corresponds to the beginning of ferulic acid degradation. The degradation of ferulic acid was analyzed by HPLC by measuring ferulic acid and intermediates degradation compounds present in the culture medium at the time of RNA extraction (Figure 3b; Campillo *et al.* 2014). At this time, compared to the non-inoculated medium, only 5 % of ferulic acid was degraded. However, the detection of small amounts of HPMHP indicates that the degradation process has begun. The absence of HMPKP and vanillic acid shows that only the first steps of the degradation occurred in the cell, i.e. activation into feruloyl-CoA and production of HMPHP-CoA. Consistently, from the RNAseq sequencing results genes involved in the first step of the degradation of ferulic acid, i.e. *atu1415*, *atu1416* and *atu1417*, are significantly induced in presence of ferulic acid (Figure 3A log₂ FC respectively: 4.14, 3.59 and 3.81). The genes involved in the second step of the degradation process (*atu1418*, *atu1420*, *atu1421*) are not significantly induced (Figure 3A log₂ FC < 1.7). Hence the conditions of transcriptomic analyses are as follows: exponential growth (data not shown), beginning of ferulic acid degradation, presence of at least ferulic acid and feruloyl-CoA inside the cell. In these conditions, the expression of 14% of the genome was modified. Among the 718 genes for which the expression was modified, 454 genes were up-regulated and 264 down-regulated. Among them, we identified 32 genes for which the absolute log₂ FC ≥ 2 (Table 1). Five genes encoding hypothetical protein and one gene encoding superoxide dismutase (*sodbIII*) were not organized in cluster, thus not analyzed in this study. Excluding the 8 genes involved in HCAs degradation, we focused the analysis on the genes organized in cluster and constructed one transcriptional fusion for each cluster of interest.

Table 1: List of genes up-regulated ($\log_2\text{foldchange}>2$) or down-regulated ($\log_2\text{foldchange}<2$) in presence of ferulic acid in the *A. fabrum* wild-type strain.

Gene ID	Gene name	$\log_2\text{FoldChange(FC)}$	P-value adjusted	Cluster
1133123	<i>slyA</i>	2,88	6,7E-10	SlyA
1133124	<i>atu1086</i>	2,97	1,7E-20	
1133125	<i>atu1087</i>	2,14	1,8E-08	
1133448	<i>atu1410</i>	3,55	8,7E-58	HCAs degradation
1133449	<i>atu1411</i>	3,51	2,3E-64	
1133450	<i>atu1412</i>	3,61	2,3E-64	
1133451	<i>atu1413</i>	3,87	7,1E-54	
1133452	<i>atu1414</i>	3,65	9,5E-67	
1133453	<i>atu1415</i>	4,14	7,9E-77	
1133454	<i>atu1416</i>	3,59	1,8E-41	
1133455	<i>atu1417</i>	3,81	9,7E-70	
1133539	<i>atu1501</i>	-2,00	2,3E-04	
1134042	<i>atu2004</i>	-2,05	1,9E-08	undefined
1134067	<i>atu2029</i>	-4,07	2,6E-81	Transcriptional factor
1134068	<i>atu2030</i>	-2,57	9,0E-34	
1135459	<i>atu3585</i>	-2,04	1,5E-10	undefined
1135913	<i>atu4039</i>	-2,06	8,9E-26	undefined
1136206	<i>impL</i>	2,00	1,0E-23	Type 6 secretion system
1136207	<i>impK</i>	2,13	7,6E-26	
1136208	<i>impJ</i>	2,15	2,0E-26	
1136209	<i>impI</i>	2,13	4,5E-24	
1136210	<i>impH</i>	2,26	8,8E-27	
1136211	<i>impG</i>	2,25	2,8E-22	
1136212	<i>impF</i>	2,40	8,9E-26	
1136213	<i>impE</i>	2,32	3,7E-28	
1136214	<i>impD</i>	2,41	8,5E-33	
1136215	<i>impC</i>	2,42	3,4E-12	
1136216	<i>impB</i>	2,17	2,3E-28	
1136352	<i>emrA</i>	3,00	6,5E-51	Efflux pump
1136353	<i>emrB</i>	3,06	1,7E-51	
1136600	<i>sodBIII</i>	-2,14	1,5E-05	ROS resistance
5729694	<i>atu8019</i>	-2,36	1,7E-09	undefined

In red are indicated genes down-regulated in presence of ferulic acid and in green genes which are up-regulated.

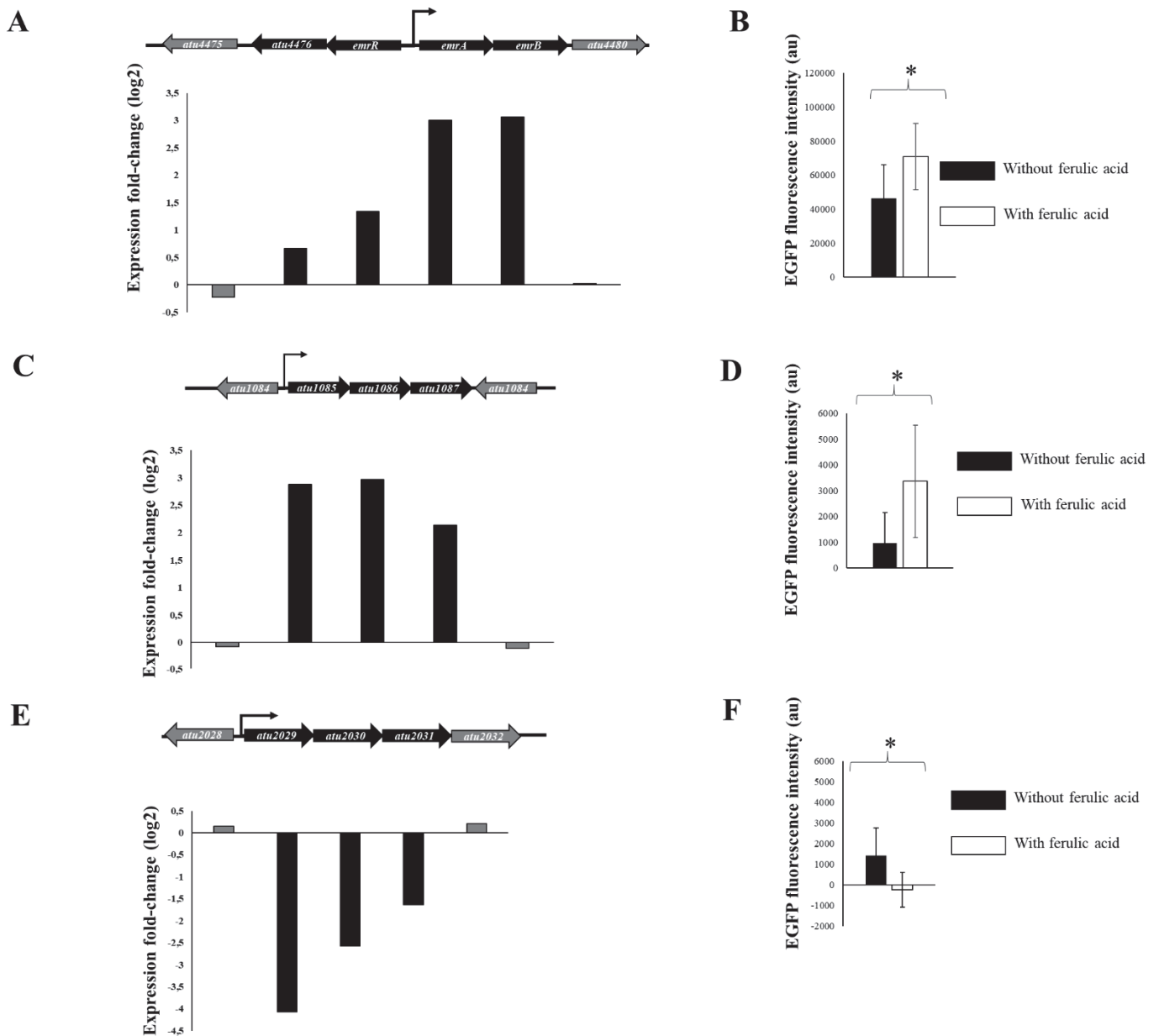


Figure 4: Expression of genes involved in metabolites exchange induction in presence of ferulic acid.

(A and B) the *emrBAR* cluster encoding an efflux pump (*emrA* and *emrB*) and a regulatory protein (*emrR*) was up-regulated in presence of ferulic acid. (C and D) The *atu1085-1087* cluster encoding the *slyA* gene (*atu1085*), a MarR transcriptional regulator; a putative transporter composed of a MFS permease (*atu1086*) and a secretion protein (*atu1087*), is down-regulated by ferulic acid. (E and F) The *atu2029-2031* genes cluster containing a putative export lipoprotein (*atu2029*) associated to a sigma factor (*atu2030*) and an antisigma factor (*atu2031*), is up-regulated by ferulic acid. (B,D and F) Analyses of promoter activities for the *emr* gene cluster (B), the *slyA* cluster (D) and the *atu2029-2031* gene cluster (F).

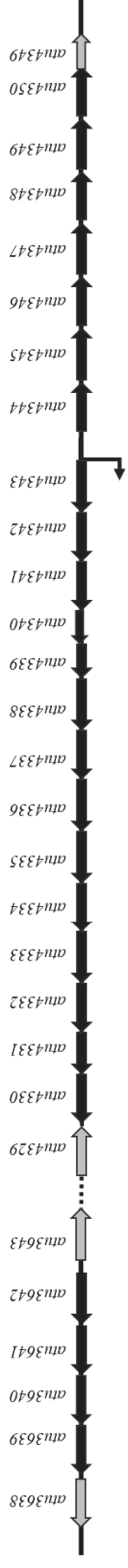
Genes expression involved in metabolites transport are influenced by the presence of ferulic acid

The *emrBAR* and *slyA* operon genes are upregulated in the presence of ferulic acid (Figure 4A, 4B). The fluorescence level of P_{emr} transcriptional fusion is significantly higher in presence of ferulic acid (Figure 4B), which confirmed results obtained by RNAseq experiment (Figure 4A and C). The *emrBAR* cluster has been described as an efflux pump in *Ensifer meliloti* 1021 (Rossbach *et al.* 2014; Santos *et al.* 2014). The *emrBAR* genes of *E. meliloti* 1021 respectively share 84%, 86% and 67% of amino acid similarities with the *emrBAR* genes of *A. fabrum* C58. Lee *et al.* (Lee *et al.* 2015) have shown that this efflux pump is induced in presence of indole and proposed that this pump allows the antibiotics resistance. The induction of *emrBAR* gene cluster expression by ferulic acid (Figure 4A and B) could be useful for *A. fabrum* during the interaction with plant, to detoxify plants compounds as already reported for IfeBAR efflux pump in other *Agrobacterium tumefaciens* species (Palumbo *et al.* 1998).

The fluorescence level of P_{atu1085} transcriptional fusion is significantly higher in presence of ferulic acid (Figure 4D), which confirmed that the genes of the *slyA* cluster (*atu1085-1087*) are induced in presence of this compound. This cluster contains two genes *atu1086* and *atu1087* encoding proteins (respectively MFS permease and secretion protein) putatively involved in the export of toxic compounds. SlyA, a MarR type regulator, shares 49% of amino acid similarity with its homologous protein in *Dickeya dadantii*. In the latter bacteria, SlyA is involved in the resistance to antibiotic and to oxidative stress and is necessary for the full pathogeny (Haque *et al.*, 2009). MarR regulator are known to repress the expression of the genes present in the same operon (Grove 2017) and are more expressed in response to the ligand which abolishes its repression. Accordingly, in the tumor, *slyA* and *atu1087* are significantly expressed in comparison with culture medium (González-Mula *et al.*, 2018).

The *atu2029-atu2031* genomic region is the only cluster which is significantly down-regulated in presence of ferulic acid (Figure 4E). We validated RNAseq results with the P_{atu2029} transcriptional fusion which is significantly repressed in a medium containing ferulic acid (Figure 4F). *atu2029* encodes a putative export lipoprotein overexpressed in the tumor, but whose exact role is unknown (González-Mula *et al.*, 2018). The cluster also contains a putative extracytoplasmic sigma factor (*atu2030*) and its cognate antisigma factor (*atu2031*). The genes that are regulated by these transcriptional factors are not described. It is tempting to speculate

A



B



C

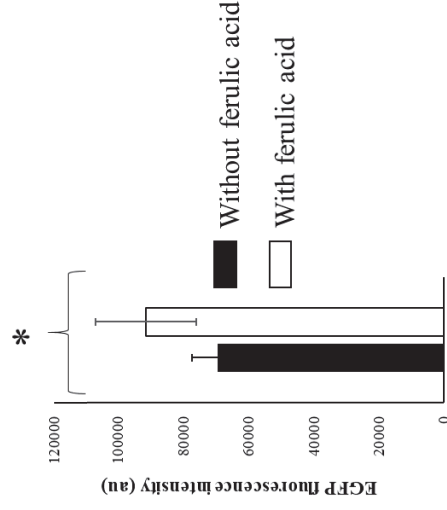


Figure 5: Organization of T6SS in *A. fabrum* and expression with and without ferulic acid.

(A) The T6SS of *A. fabrum* contains the *vrg2* operon (*atu3639-atu3642*), the *imp* (*atu4330-atu4343*) and *hcp* (*atu4344-atu4352*) gene cluster (Ma *et al.* 2014; Wu *et al.* 2012). The small arrow indicates the localization and the direction of the transcriptional fusion *Pimp*. (B) Log₂ fold change in the expression of the T6SS genes. Genes in black are significantly up-regulated, genes in grey are outside the cluster and not regulated in presence of ferulic acid. (C) Confirmation of RNAseq results by *Pimp* transcriptional fusion analyses without and with ferulic acid.

that the Atu2030 protein is involved in the activation of genes responsible for the degradation of carbon sources whose expression was reduced in presence of feruloyl-CoA (Chapitre2-article2). Analysis of the phenotype of the *atu2031* deletion mutants would allow to test this hypothesis.

Type VI secretion system is induced by ferulic acid

The presence of ferulic acid induces the type VI secretion system (T6SS) expression (figure 5A). All the genes of the *imp* operons and the 7 genes of the *hcp* operon are significantly up-regulated in response to ferulic acid (figure 4B). The *vgrG2* gene cluster is up-regulated but in lesser extent than the *imp* and *hcp* operons. Analyses of transcriptional fusion confirm RNAseq results (Figure 5C). The type VI secretion system, through a molecular machine, allows especially the delivery of toxin into others prokaryotic cells. Ma *et al.* (2014) described that this system allows to kill and to outcompete *Pseudomonas aeruginosa* but only *in planta* after leaves infiltration. It was suggested that the plant environment is a key determinant for the efficient expression of this system. Our results provide further evidence that ferulic acid can be one of the plant compounds whose perception leads to the type VI secretion system expression. Further investigations are necessary to determine if the presence of ferulic acid or feruloyl-CoA is indeed responsible for the *in planta* induction of the type VI secretion system. At first, comparing the expression of the *imp* operon in presence of ferulic acid in the wild-type strain and in a mutant unable to produce feruloyl-CoA will reveal the compound responsible for the over-expression of *imp* genes.

In conclusion, the presence of ferulic acid or of their first intermediates of degradation is a signal leading to the modulation of the expression of numerous genes encoding functions that can be determinant for the *A. fabrum* adaptation to the plants including i) exchange across membranes, ii) competition iii) resistance to toxics and iv) regulation factors. In future works we will decipher the distinct role of ferulic acid and of its intermediates degradation compounds (especially feruloyl-Coa) in the induction of the genes clusters and we will analyze the importance of the regulated functions. This may allow us to improve knowledge about the importance of HCAs in the *A. fabrum* ecology.

Supplemental figures

Supplementary table S1: Strains and plasmids used in this study

	Relevant characteristics	Reference or source
<i>Escherichia coli</i>		
JM109	<i>endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ Δ(lac-proAB) e14-[F' traD36 proAB+ lacIq lacZΔM15] hsdR17(rK-mK+)</i>	NEB catalog
Stellar	<i>F⁻, endA1, supE44, thi-1, recA1, relA1, gyrA96, phoA, Φ80d lacZΔ M15, Δ (lacZYA - argF) U169, Δ (mrr - hsdRMS - mcrBC), ΔmcrA, λ⁻</i>	Takara Clontech
<i>Agrobacterium fabrum</i>		
C58	Wild-type	CFBP 1903
Plasmids		
pOT1e	Promoter-probe vector based on pBBR1MCS-5 replicon; contains promoterless eGFP and MCS between two transcriptional terminators; Gm ^R	(Allaway <i>et al.</i> , 2001)
pOT1eM	pOT1e derivative harboring <i>Ptac-m-cherry</i> inserted in ClaI-SalI site	(Meyer <i>et al.</i> , 2018a)
<i>Patu1416</i>	Upstream region <i>atu1416</i> inserted in ClaI-SalI site in pOT1e; Gm ^R	(Meyer <i>et al.</i> , 2018a)
pOT1eM- <i>Patu1085</i>	Upstream region <i>atu1085</i> inserted in SpeI site in pOT1eM; GmR	This study
pOT1eM- <i>Patu4343</i>	Upstream region <i>atu4343</i> inserted in SpeI site in pOT1eM; GmR	This study
pOT1eM- <i>P_{emr}</i>	Upstream region <i>atu4478-atu4479</i> inserted in SpeI site in pOT1eM; GmR	This study
pOT1eM- <i>Patu2029-atu2031</i>	Upstream region <i>atu2029-atu2031</i> inserted in SpeI site in pOT1eM; GmR	This study

Supplementary table S2: Primers used in this study

Target	Primer	Primer sequence (5'-3')	Primer length
<i>Transcriptional fusion</i>			
<i>atu1085</i> promoter	<i>atu1085F</i>	CGGGGGATCCACTAGGGGGTGATCTTCTCACAGC	376 bp
	<i>atu1085R</i>	TTCTTCTCCACTAGTCCAGAAGCTGCTCCTTGTC	
<i>atu4343</i> promoter	<i>atu4343F</i>	CGGGGGATCCACTAGCCAACGAGGCGATTGAGATC	330 bp
	<i>atu4343R</i>	TTCTTCTCCACTAGCGTTCTTGAATCGTTGCGGA	
<i>emr</i> promoter	<i>emrF</i>	CGGGGGATCCACTAGGACAGACCGTTTCCGTTTCC	222 bp
	<i>emrR</i>	TTCTTCTCCACTAGTTCTGGGCCGACATGAGATT	
<i>atu2029-atu2031</i> promoter	<i>atu2029F</i>	CGGGGGATCCACTAGGCGCGTCTGAATAGTTTTCC	254 bp
	<i>atu2029R</i>	TTCTTCTCCACTAGTTGTGCGTTTTGACCGTTTC	
RNAseq experiments			
<i>atu1417</i> gene	<i>atu1417F</i>	CCGGACACGACGCCTGA	
	<i>atu1417R</i>	ATGGGTTGGTCGAGTTTTTG	
RNA sequencing	TruSeq Adapter	Universal AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	
Replicate1	TruSeq Adapter WT1	GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTGCTTG	
Replicate2	TruSeq Adapter WT2	GATCGGAAGAGCACACGTCTGAACTCCAGTCACTGACCAATCTCGTATGCCGTCTTCTGCTTG	
Replicate3	TruSeq Adapter WT3	GATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTATCTCGTATGCCGTCTTCTGCTTG	
Ferulic replicate1	TruSeq Adapter WTferulicacid1	GATCGGAAGAGCACACGTCTGAACTCCAGTCACGCCAATATCTCGTATGCCGTCTTCTGCTTG	
Ferulic replicate2	TruSeq Adapter WTferulicacid2	GATCGGAAGAGCACACGTCTGAACTCCAGTCACCAGATCATCTCGTATGCCGTCTTCTGCTTG	
Ferulic replicate3	TruSeq Adapter WTferulicacid3	GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTTGAATCTCGTATGCCGTCTTCTGCTTG	

Conclusion du chapitre 3

Dans ce chapitre, nous avons analysé l'importance dans la vie rhizosphérique d'*A. fabrum* de l'import et de la dégradation d'oses de la famille des RFOs, ainsi que des molécules liées à leur synthèse ou dégradation. La région génomique (*atu4660-atu4665*) est annotée comme permettant le transport et l'utilisation uniquement des RFOs (raffinose, stachyose, verbascose). Nous avons focalisé notre étude sur le décryptage de la fonction, de l'expression et de la spécificité de la PBP MelB (Atu4661).

Nous avons démontré via l'utilisation d'une fusion transcriptionnelle rapportrice de l'expression des gènes de dégradation des RFOs, que ces gènes sont induits fortement par le mélibiose et le galactinol qui ne sont pas des RFOs. Cet opéron est induit plus faiblement par le raffinose, le galactose et le stachyose. Les PBPs ont un rôle crucial dans le transport de composés (Berntsson *et al.*, 2010) car elles permettent la reconnaissance spécifique de certaines molécules et leurs prises en charge par des transporteurs. En combinant des approches génétiques et biochimiques nous avons caractérisé la fonction du gène *melB*. Ce dernier permet bien la fixation des RFOs et est nécessaire à leur transport. En outre, cette protéine permet de transporter avec une plus forte affinité le mélibiose et le galactinol. L'analyse de la structure établie par cristallographie aux rayons X montre que MelB reconnaît ces ligands au niveau du groupement galactose terminal qui est commun à tous les ligands. De plus, MelB fixe préférentiellement des ligands possédant 2 unités, comme le mélibiose et le galactinol. Contrairement à son annotation, elle est donc plus spécifique de composés n'appartenant pas aux RFOs. Cette étude est la première description du transport et de l'utilisation du galactinol par une bactérie. Le fait de dégrader le galactinol, composé impliqué dans les défenses végétales, pourrait interférer dans la reconnaissance par la plante d'*A. fabrum*. Ainsi, ces travaux ont permis de caractériser la structure et la fonction d'une PBP qui permet d'assimiler préférentiellement le mélibiose et un composé impliqué dans les défenses végétales, le galactinol. La présence de ce gène confère à *A. fabrum* un avantage compétitif dans la rhizosphère de tomate dès les premiers stades de colonisation. Cette protéine est retrouvée chez de nombreux autres pathogènes et symbiontes appartenant à la famille des *Rhizobioaceae* avec une très forte

similarité ce qui suggère un rôle crucial de ce gène pour la colonisation rhizosphérique chez d'autres microorganismes en interaction avec les plantes.

Dans un second temps, la comparaison des transcriptomes d'*A. fabrum* en présence de mélibiose et en présence ou en absence d'acide férulique (un HCA), montre une modulation de l'expression de 14% des gènes présents dans le génome d'*A. fabrum*. La présence d'acide férulique, induit la surexpression de gènes impliqués dans le système de sécrétion de type VI (T6SS). Ces gènes sont importants dans la compétition avec d'autres microorganismes *in planta* (Ma *et al.*, 2014). Les gènes *emrBA* et *atu1086* surexprimés en présence d'acide férulique pourraient permettre de résister à des composés antimicrobiens végétaux. Deux probables facteurs de transcription sont aussi modulés, l'un étant surexprimé (*slyA*) et l'autre sous-exprimé en présence d'HCAs (facteur sigma *atu2029*-antisigma *atu2031*). Ces facteurs de transcription pourraient avoir un rôle dans le contrôle de l'expression du métabolisme carboné lors de la dégradation des HCAs (Chapitre2-article 2).

Les résultats de ce chapitre soulignent que la perception du signal HCA induit une modification de l'expression de fonctions liées à l'utilisation de ressources et à la gestion des échanges avec la plante ou avec les autres microorganismes (osmoprotection, galactinol, pompe à efflux, perméase, protéine de sécrétion et T6SS). Il semble important de réguler finement l'expression de ces fonctions au contact de la plante, régulation qui pourrait faire intervenir les facteurs de transcription SlyA et Atu2029 après l'intégration du signal HCAs.

Discussion générale et Perspectives

Signal perçu par *A. fabrum* : HCA ou HCA-CoA

Nous avons vu que les HCAs modulaient des fonctions telles que le chimiotactisme (Ashby *et al.*, 1988) ou encore le QS (Joshi *et al.*, 2015, 2016; Schaefer *et al.*, 2008) et étaient fortement impliqués dans l'écologie de bactéries phytopathogènes, qu'elles soient ou non capables de les dégrader (Synthèse bibliographique, Partie B). Ces études se sont cependant intéressées à l'effet global de la présence d'HCAs et ne portaient pas sur la caractérisation des mécanismes à l'origine de cette influence ou des molécules concernées. En particulier, pour les bactéries capables de les dégrader, l'influence des intermédiaires de dégradation n'est généralement pas évaluée (Synthèse bibliographique, Partie B). La seule étude traitant ce sujet concernait le chimiotactisme chez *P. putida*, médié par le féruloyl-CoA (Hughes *et al.* 2017). Chez *A. fabrum*, les HCAs interviennent dans les deux modes de vie de la bactérie, notamment l'acide férulique, qui est un composé chimioattractant (Ashby *et al.* 1988). Il serait intéressant de tester, comme chez *P. putida*, l'effet des composés intermédiaires de dégradation des HCAs dans le chimiotactisme d'*A. fabrum* vers les HCA, en particulier de tester l'importance des dérivés CoA. Dans le cadre de la pathogénie, le plasmide Ti se retrouve dans les agrobactéries quelles que soient leur espèce. Or la région SpG8-1b comportant les gènes de dégradation des HCAs est spécifique à l'espèce *A. fabrum*. Il est ainsi logique que la molécule inductrice des gènes *vir* soit l'acide férulique et non ses intermédiaires de dégradation. Nous avons démontré que le mutant délété des gènes de dégradation de l'acide férulique présente une induction plus forte des gènes *vir* que la souche sauvage en présence d'acide férulique (Meyer *et al.*, 2018a). Les mêmes résultats ont été obtenus avec le mutant du gène *virH2* qui ne transforme plus l'acide férulique en acide caféique (Kalogeraki *et al.*, 1999). Nous avons donc confirmé que la perception de l'acide férulique et non de ses dérivés, induisait les gènes de virulence chez *A. fabrum*.

La répression du métabolisme carboné est en revanche liée aux dérivés HCAs-CoA et non aux HCAs en tant que tels. En effet, contrairement à la souche sauvage, chez le mutant de la région SpG8-1b qui est capable de percevoir l'acide férulique mais pas de le dégrader (Meyer *et al.*, 2018a), il n'y a pas de diminution de l'expression des gènes de dégradations de la D-proline ni de l'opéron *mel* (dégradation des RFOs) en présence d'acide férulique (Chapitre 2 - article 2). Dans le cadre de l'article 2 en préparation, nous n'avons pas encore testé si les dérivés CoA de l'acide *p*-coumarique ont la même influence que le féruloyl-CoA. Il serait par ailleurs intéressant de

vérifier si l'expression des gènes influencée par le féruloyl-CoA n'est pas modifiée en présence d'acide cinnamique, ce dernier n'étant ni activé en cinnamoyl-CoA ni dégradé dans les souches concernées par cette étude.

La présence d'HCAs influence globalement le transcriptome d'*A. fabrum* (Baude *et al.*, 2016). L'expression de certains déterminants génétiques pourrait être influencée par l'acide férulique et d'autres par le féruloyl-CoA. Dans le mutant C58 Δ *atu1417*, nous attendons des modifications d'expressions plus importantes pour les souches induites par un HCA-CoA alors que dans un mutant du gène *atu1416*, qui ne produira plus de féruloyl-CoA, nous attendons une expression réduite. Cependant le mutant C58 Δ *atu1417* ne croît pas en présence de mélibiose. Pour déterminer si le féruloyl-CoA est à l'origine des modifications d'expression observées, nous ne pouvons pas donc pas l'utiliser dans les conditions des expériences de RNAseq. Une alternative pour analyser le phénotype du mutant du gène *atu1417*, serait de trouver une source de carbone qui soit utilisée en même temps que les HCAs, et qui ne soit pas réprimée par ceux-ci. Avec une telle source de carbone, nous pourrions distinguer parmi les gènes dont l'expression est modifiée par l'acide férulique, ceux qui dépendent de l'acide férulique de ceux influencés par l'HCA-CoA. Nous testerons dans un premier temps le galactose, proche des RFOs, ou l'une des sources de carbone non réprimées par l'acide férulique.

Importance de l'utilisation des RFOs, du galactinol et du mélibiose

Il a été démontré chez *E. meliloti*, qu'une protéine nommée AgpA permettait l'import des RFOs (raffinose et verbascose) (Gage and Long, 1998). Dans l'article 3, nous avons décrit comment la PBP MelB fixe les RFOs et nous avons étudié ses capacités de fixation d'autres composés de la voie de biosynthèse des RFOs (cf Figure 1 Chapitre3-article 3). Ceci nous a permis de démontrer que cette protéine est certes impliquée dans l'import des RFOs mais qu'elle est plus probablement dédiée à importer le galactinol et le mélibiose, composés pour lesquels, elle a la plus grande affinité (Meyer *et al.*, 2018b- Chapitre3-article 3). Cet import est utile pour la colonisation rhizosphérique. Ceci semble relié à un avantage trophique mais nous ne pouvons pas exclure le fait que l'import de ces composés, connus comme étant des osmoprotectants, permette aussi de mieux résister aux stress oxydatifs rencontrés dans la rhizosphère (Nishizawa *et al.*, 2008, Figure 1 ⑥ page 151). L'opéron *mel* contient deux α -galactosidases non caractérisées jusque-là. La présence de ces deux

galactosidases dans un même cluster est étonnante. Elles pourraient agir à des pH différents, avoir des substrats différents et/ou être régulées différemment. La comparaison du phénotype de la souche sauvage à celui de simples mutants des α -galactosidases et du mutant des deux α -galactosidases permettrait de connaître le rôle et la spécificité de chacune des α -galactosidases. En utilisant ces mutants nous pourrions par ailleurs tester le rôle osmoprotecteur des RFOs suggéré dans la synthèse bibliographique (Partie A) et l'article 2 (Chapitre 2 – article 2). De plus, il serait intéressant de tester si l'import ou la dégradation du galactinol, qui est connu comme étant un composé impliqué dans les défenses végétales (Cho *et al.*, 2010; Kim *et al.*, 2008) peut interférer dans la reconnaissance par la plante d'*A. fabrum*. Ceci n'a jamais été testé et l'article 4 est la première description de l'utilisation du galactinol par un microorganisme. Cependant, le rôle de l'utilisation de ces composés, n'est sûrement pas restreint à la rhizosphère et pourrait aussi être important dans le maintien dans la tumeur. En effet, une récente étude a démontré que l'une des deux α -galactosidases et les gènes d'import sont exprimés dans la tumeur (González-Mula *et al.*, 2018). Ceci sous-entend que les RFOs et/ou le galactinol et le mélibiose sont présents et dégradés *in tumora*.

Etude de l'interaction *A. fabrum*-plante : aspects méthodologiques

Etude de la valeur adaptative conférée par les déterminants génétiques

Chez *Agrobacterium*, la caractérisation des gènes est souvent réalisée uniquement du point de vue fonctionnel. Lorsque les aspects de l'interaction avec la plante sont pris en compte, les analyses sont souvent restreintes à l'étude du rôle des fonctions étudiées dans la virulence. Nous avons dans ces travaux, non seulement étudié la virulence mais aussi la capacité des souches mutées à se multiplier et à se maintenir au sein de la rhizosphère et de la tumeur, en compétition ou non avec la souche sauvage. Nous avons ainsi montré que la dégradation des RFOs est importante pour la colonisation rhizosphérique dès les premiers stades de germination (Chapitre 3 - article 3). Or, comme souligné dans la synthèse bibliographique, il existe encore peu d'informations sur les déterminants génétiques importants dans la rhizosphère. Ces expériences d'analyse de valeur adaptative de gènes dans la colonisation des plantes, nous ont également permis de proposer que la répression des gènes de dégradation des HCAs était importante pour la transition entre le style de vie rhizosphérique et pathogène, afin d'éviter la dégradation des HCAs inducteurs des gènes de

virulence (Meyer *et al.*, 2018a-Chapitre1-article 1). Cependant, la méthode de compétition que nous utilisons actuellement implique le marquage de l'une de ces deux souches avec une cassette de résistance à un antibiotique et nécessite le repiquage individuel de milliers de clones afin d'établir l'abondance relative des deux souches. Cette technique étant chronophage, nous développons actuellement une nouvelle méthode d'analyse à l'aide de marquage colorés qui nous permettra de réduire le temps de réalisation des expériences de compétition, et permettra un recours plus systématique à l'analyse de la valeur adaptative des gènes lors de leur caractérisation fonctionnelle. Elle nous permettra par ailleurs, d'étudier l'avantage adaptatif des gènes d'intérêts à de nombreux stades du développement de la tumeur ou de la racine, ce qui n'a pu être fait dans le cadre de cette thèse pour l'opéron *mel* ou les gènes de dégradation des HCAs. L'exsudation est hétérogène en terme de quantité et de diversité de composés selon la région et l'âge de la racine considérée (Aulakh *et al.*, 2001; Chaparro *et al.*, 2014; Ofek *et al.*, 2011). De même au niveau de la tumeur, il existe des différences à la fois structurales et biochimiques selon son stade de développement (Synthèse bibliographique – Partie A). Il serait ainsi intéressant de tester comment évoluent les effectifs des souches sauvages et mutées en ne considérant plus la tumeur ou les racines comme un ensemble mais en séparant les différentes parties racinaires (racines pivot et latérale) ou tumorales. Ceci permettra de prendre en compte la dynamique spatio-temporelle dans ces deux contextes (rhizosphère et tumeur) pour l'analyse de l'importance des HCAs dans l'écologie d'*A. fabrum* C58.

Actuellement, les études qui portent sur l'implication de certains gènes dans la pathogénie ne sont que peu représentatives des conditions naturelles. En effet, la mesure du pouvoir pathogène telle qu'elle est classiquement réalisée implique l'inoculation à forte densité d'une souche d'*A. fabrum* directement dans une tige de tomate préalablement blessée (Vaudequin-Dransart *et al.*, 1995). Cette méthode permet d'étudier la capacité d'une souche à induire des tumeurs et à se maintenir dans cet environnement. Cependant, cette méthode ne permet pas d'analyser l'importance de certaines étapes indispensables pour la pathogénie dans un contexte plus naturel, telles que le chimiotactisme vers la zone de blessure, et l'adhésion aux cellules végétales (Barton *et al.*, 2018). Ainsi, l'étape clef pour la compréhension du cycle de vie d'*Agrobacterium* à savoir la transition entre le style de vie rhizosphérique et pathogène n'est pas prise en compte. Cette problématique est commune à d'autres modèles d'interaction phytopathogènes comme *R. solanacearum*, chez qui le régulateur

EfpR n'a pour le moment pas de rôle écologique précis dû aux limites des méthodes d'inoculation actuellement utilisées (Perrier *et al.*, 2016). La mise en place de nouvelles méthodes permettant d'étudier les déterminants génétiques impliqués dans cette transition pourrait aider à la compréhension du cycle de vie de ces pathogènes.

Importance de l'étude du métabolome de l'hôte végétale et des méthodes d'analyse de l'expression in situ

Un système rapporteur avec un gène fluorescent permet de quantifier *in vitro* l'expression d'un gène et peut être utilisé comme biosenseur indiquant la présence de certains métabolites (Bringhurst *et al.*, 2001; Pini *et al.*, 2017). Cette méthode peut aussi être utilisée pour étudier l'expression des gènes d'intérêt *in situ* notamment pour les interactions bactéries-eucaryotes (Uliczka *et al.*, 2011; Verplaetse *et al.*, 2015). L'utilisation de cet outil peut cependant être limitée dans le cas de la localisation précise de l'expression *in planta*. En effet, une absence de fluorescence peut provenir d'une indisponibilité de composés inducteurs ou de l'absence de bactérie dans la zone considérée. Pour pallier ce problème, nous avons amélioré le vecteur original (pOT1e, (Allaway *et al.*, 2001) afin de construire un nouvel outil plasmidique de suivi de l'expression génique, utilisable au sein de plusieurs genres bactériens (*Agrobacterium*, *Allorhizobium*- annexe 2, *Pseudomonas*,...). Ce dernier permet à la fois la localisation des bactéries actives (expression constitutive de la *m-cherry*) et l'analyse de l'expression du gène d'intérêt (*eGFP* sous contrôle du promoteur du gène d'intérêt). Ce vecteur fluorescent a été utilisé dans des études par microscopie confocale pour analyser l'expression *in planta* de différents gènes d'intérêts dans le cadre des articles 1 et 3 (Chapitre 1 – article 1 et Chapitre 3 – article 3). Nous avons ainsi mis en évidence une faible expression des gènes de dégradation des HCA dans la tumeur, et émis l'hypothèse d'une faible disponibilité des HCA dans ce contexte. Il serait cependant nécessaire d'étudier plus précisément les métabolites de la tumeur dans nos conditions (cf Synthèse bibliographique – Partie A), car la présence d'autres composés comme le succinate pourrait être à l'origine de la non expression des gènes analysés (modèle en Figure 1 A). En effet, le succinate est connu pour réprimer l'utilisation des HCAs, donc lorsqu'il y a du succinate et même en présence d'HCA, il n'y aura pas d'expression de la fusion et donc pas de fluorescence (Baude *et al.*, 2016; Campillo *et al.*, 2014). De même, dans la rhizosphère, environnement très hétérogène (Aulakh *et al.*, 2001; Chaparro *et al.*, 2014; Ofek *et al.*, 2011), nous avons observé que l'expression des gènes de dégradation des

HCA diffère selon la région racinaire considérée et l'âge de la racine (donnée non montrée). Ceci pourrait indiquer une différence de profils métaboliques selon les racines (pivot ou latérale et jeune ou âgée), et par exemple la présence simultanée de succinate et d'acide férulique dans certaines zones racinaires uniquement. Nous sommes actuellement limités par la méthode utilisée pour valider cette hypothèse, d'une part car elle est destructive pour l'échantillon végétal et ne permet donc pas de réaliser un suivi cinétique de l'expression bactérienne, et d'autre part car elle ne permet pas de suivre l'expression de plusieurs gènes dans une même bactérie. Nous pouvons envisager différentes améliorations pour les études ultérieures. Nous pourrions tout d'abord utiliser un système de micro-fluidique couplé à de la microscopie confocale (Massalha *et al.*, 2017), qui est une méthode donnant accès à une meilleure résolution et à une meilleure sensibilité lors de l'observation en cinétique de l'ensemble du système racinaire sans être destructive. D'autre part, nous envisageons de perfectionner le vecteur fluorescent afin qu'ils puissent exprimer trois marqueurs fluorescents différents (un constitutif et deux sous le contrôle des promoteurs de gènes d'intérêts). Ainsi, en plus de la colonisation bactérienne, nous pourrions étudier simultanément l'expression au cours du temps de deux gènes bactériens comme la dégradation des HCAs et l'import des RFOs, ou la dégradation des HCAs et celle de l'acide succinique. L'expression de 3 gènes rapporteurs peut être coûteuse pour la bactérie, mais dans notre cas les gènes étudiés semblent avoir des expressions mutuellement exclusives, ce qui pourrait permettre l'utilisation d'un tel système. Ceci pourra améliorer la compréhension du fonctionnement du système HCAs-RFOs d'un point de vue spatio-temporelle et en condition plus naturelle.

HCAs et styles de vie d'*A. fabrum* : proposition d'un modèle

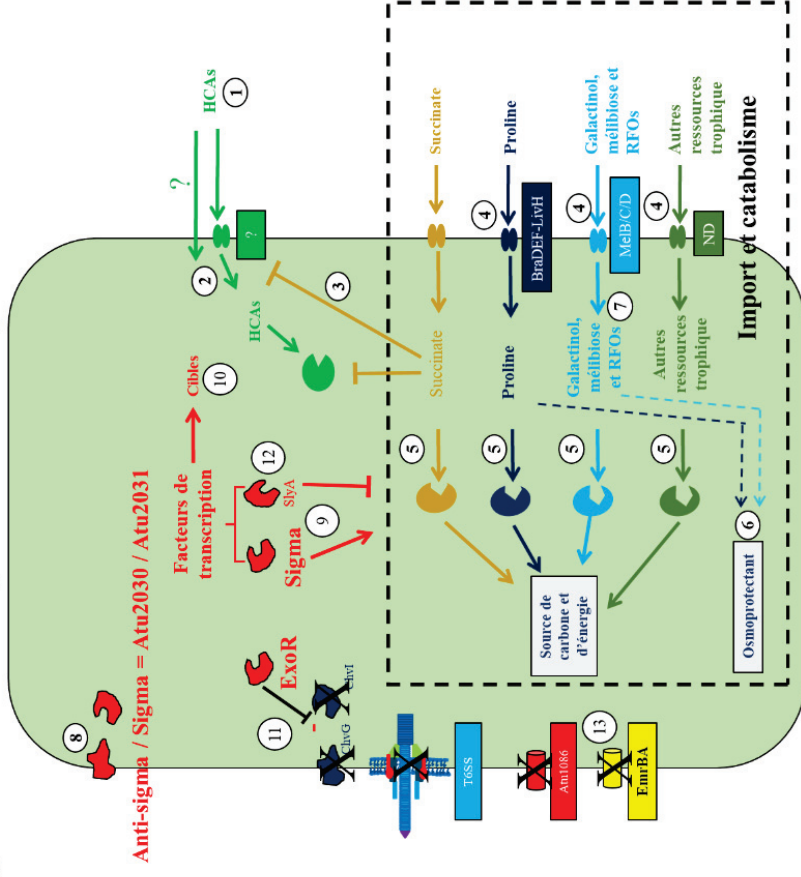
Lassalle *et al.* (2011) ont proposé que les régions spécifiques d'espèces d'*A. fabrum* lui confèrent un avantage écologique spécifique en lien avec la plante. Nous avons émis les hypothèses que la dégradation des HCAs chez *A. fabrum* était perçue comme un signal de proximité de la plante et que la régulation de cette dégradation était importante dans les deux styles de vies d'*A. fabrum*. De manière intéressante, nous avons montré que la région SpG8-1b en plus de permettre la détoxification des HCAs (Campillo *et al.*, 2014) et le contrôle de l'expression des gènes virulence (Meyer *et al.*, 2018a), avait une influence sur l'expression d'autres déterminants génétique (Chapitre 2 - article 2 et Chapitre 3 – article 4). Nous avons notamment utilisé une méthode globale

pour caractériser l'influence de la perception des HCAs et de leurs dérivés sur le métabolisme carboné (biolog en présence d'HCA). L'extension de l'utilisation de puces phénotypiques à l'analyse du métabolisme azoté (ou phosphaté) ou de la résistance aux composés toxiques (puces phénotypiques biolog® existantes) pourrait permettre d'appréhender le rôle des HCAs plus globalement au niveau du métabolisme et en intégrant les notions de résistances à des toxiques. Nous pourrions alors dresser un inventaire plus complet des phénotypes modulés par les HCAs. En effet, en combinant l'analyse du métabolisme carboné et du transcriptome, nous avons observé que la perception des HCAs modulait l'expression de certains déterminants génétiques impliqués dans au moins l'une des étapes du cycle de vie d'*A. fabrum*. En synthétisant les différents résultats de la bibliographie et ceux présentés dans ces travaux de thèse, nous avons modélisé l'implication des HCAs dans différentes fonctions clefs de l'écologie d'*A. fabrum* (Figure 1).

Dans le contexte rhizosphérique, nous avons démontré que les gènes de dégradation des RFOs sont peu exprimés deux semaines post-inoculation (Chapitre 3 - article 3), alors que les gènes de dégradation des HCAs sont exprimés fortement (Chapitre 1 - article 1). Ces observations sont cohérentes avec notre hypothèse selon laquelle la dégradation des RFOs est réprimée par la présence des dérivés CoA (fêruloyl-CoA et le *p*-coumaroyl-CoA Chapitre 2 - article 2 ; Figure 1(11)). L'analyse du contenu phytochimique des racines de tomates de 15 jours permettrait de connaître si certaines zones de la rhizosphère de tomates à ce stade de développement sont effectivement riches en HCAs et contiennent des RFOs, du mélibiose et/ou du galactinol (Chapitre 2 - article 2, Figure 1 B (11)).

Dans un environnement riche en HCAs, l'expression de différents gènes est activée (Chapitre 3- article 4). Il s'agit par exemple de l'expression du T6SS, impliqué dans la compétition inter-bactérienne (Figure 1 B (14)). Ce système est induit par un pH acide (pH=5.5), via le système ChvG/I et réprimé en condition non acide par ExoR (Heckel *et al.*, 2014; Wu *et al.*, 2012). Dans nos conditions (données de RNA seq non montrées), nous avons observé une tendance cohérente avec ces observations : la transcription du système *chVG/I* est légèrement surexprimée et celle de *exoR* est légèrement sous régulée. Nous étudierons, par RTq-PCR (*Reverse Transcription quantitative PCR*) ou par fusion transcriptionnelle si ces deux déterminants sont réellement influencés par les HCAs. L'expression du T6SS est importante dans la colonisation notamment de la rhizosphère par les microorganismes

A Environnement pauvre en HCAs ou en présence de succinate



B Environnement riche en HCAs disponibles

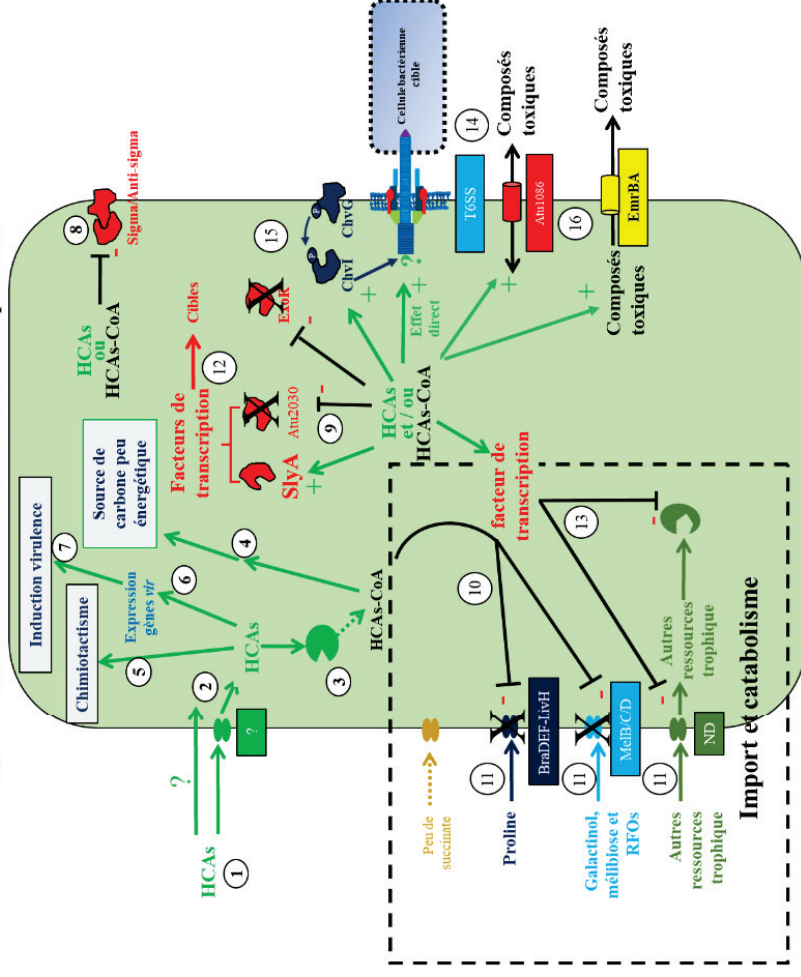


Figure 1 : Modèles d'influence de la disponibilité en HCAs sur l'écologie d'*A. fabrum*.

(A) Modèle en milieu pauvre en HCA (1) ou en présence de succinate qui réprime l'import (2) et/ou la dégradation de ces composés (3). Dans ces conditions, la proline, le galactinol, le mélbiose, les RFOs et d'autres ressources trophiques (6) et/ou peuvent influencer les réactions de défenses de la plante comme c'est le cas du galactinol (7). L'import de certains composés peut aussi permettre l'osmoprotection bactérienne (6) et/ou peuvent influencer les réactions de défenses de la plante comme c'est le cas du galactinol (7). Les facteurs sigma et antisigma sont fortement exprimés, le facteur sigma existe sous forme libre (8) et va contrôler (induire) l'expression de gènes cibles pour le moment inconnus (9). Les facteurs de transcription que ce soit SlyA ou le facteur sigma pourraient réguler l'import et le métabolisme de certaines ressources trophiques (10). Dans cet environnement le T6SS est réprimé potentiellement via la répression du système ChvG/I par ExoR (11) ; les gènes codant pour la synthèse de la pompe à efflux *emrBA*, du facteur de transcription *slyA* (*atu1085*) et de la perméase *atu1086* sont peu exprimés (12).

(B) En environnement riche en HCAs (1), ces derniers pénètrent directement dans les cellules ou via un transporteur non décrit (2). Ils sont ensuite activés en HCAs-CoA (3) puis dégradés via la région SpG8-1b afin d'être utilisés comme une source de carbone, mais de manière peu efficace (4). Les HCAs non dégradés vont attirer les bactéries vers la plante par chimiotactisme (5) et dans un contexte de pathogénie (c'est-à-dire en présence de certains sucres, et à pH acide) ils sont inducteurs des gènes *vir* (6) ce qui induit l'expression du programme de pathogénie (7). Les HCAs ou leurs dérivés inhibent l'expression du facteur sigma qui est piégé par le facteur antisigma (8). Au contraire, le facteur de transcription SlyA est surexprimé (9). Les HCAs-CoA inhibent la transcription des gènes d'import de certaines ressources trophiques (10), alors que pour d'autres ressources dont l'import et/ou l'utilisation est inhibée, il n'est pas connu si cela est en réponse aux HCAs ou HCA-CoA (12). Quel que soit le signal, l'effet est probablement indirect via l'influence qu'ils ont sur certains facteurs de transcription (11). SlyA et le facteur sigma sont des facteurs de transcription candidats (12). Dans cet environnement riche en HCAs, ces composés ou leurs catabolites induisent l'expression du T6SS qui permet de tuer d'autres cellules bactériennes cibles (13). Cette induction peut être due à une inhibition de ExoR ou à une activation du système ChvG/I (14). La présence ou l'activation des HCAs induits aussi le système EmrBA qui permettrait alors l'efflux de composés toxiques hors de la cellule bactérienne (15).

qui le possèdent et l'expriment (Bernal *et al.*, 2018; Marchi *et al.*, 2013). L'induction de ce système doit donc conférer un avantage adaptatif à *A. fabrum* dans un environnement riche en HCAs. Dans ces mêmes conditions, les régions génomiques *atu1085-1087(slyA)*, et *emrBAR* sont surexprimées (Chapitre3-article 4). Celles-ci contiennent des gènes codant pour des fonctions impliquées dans les échanges cellulaires : les gènes *emrBA* et *atu1086* sont probablement impliqués respectivement dans l'export de composés toxiques et dans l'import ou l'export de composés d'origine végétale. Ces régions génomiques seraient alors induites par un signal de proximité du végétal (acide férulique ou autres HCAs et dérivés) et permettrait de mieux résister à la présence de composés toxiques végétaux (Figure 1 B ⑩). Il serait intéressant d'évaluer si ces déterminants génétiques sont bien impliqués dans la compétitivité d'*A. fabrum* dans un environnement riche en HCAs et / ou en composés toxiques.

Nous avons identifié des gènes d'import et de dégradation de certaines ressources trophiques végétales comme les RFOs ou la proline, dont la transcription est régulée négativement par les HCAs (Figure 1 B ⑪). L'importance de l'utilisation de ces sources de carbone est sûrement dépendante de l'environnement considéré, elles ne sont peut-être pas utiles dans le contexte d'un environnement riche en HCA, et il est commun que les pathogènes soient versatiles d'un point de vue métabolique (Peyraud *et al.*, 2016). Les déterminants génétiques impliqués dans le catabolisme de autres composés dont l'utilisation est impactée par les HCAs, ne sont pas connus chez *A. fabrum* (Synthèse bibliographique – Partie A). Nous avons par ailleurs identifié trois facteurs de transcription (*slyA* et *atu2030-2031*). Il est possible que ces facteurs de transcription soient impliqués dans la modulation de l'utilisation des ressources carbonées découvertes dans l'article 2 (Figure 1 B ⑧⑨⑫). Les mutants de ces trois gènes ont été construits, nous allons analyser leur capacité à croître sur les ressources trophiques identifiées, puis leurs transcriptomes en présence d'acide férulique afin de connaître leurs gènes cibles dans ces conditions (Figure 1 B ⑫).

Les environnements riches en HCAs peuvent correspondre à certaines régions de la rhizosphère comme vu plus haut, ou aux zones de blessures de la plante où ses composés vont être relargués. Ils seront alors initiateurs de la transition entre le style de vie rhizosphérique et pathogénique. En revanche les tumeurs matures contiennent moins d'HCAs disponibles (Chapitre 1 - article 1). Ceci est cohérent avec l'analyse du transcriptome d'*A. fabrum* dans la tumeur mûre, qui démontre que le T6SS n'est pas activé contrairement à l'opéron *mel* comme dans notre modèle dans un environnement pauvre en HCAs (González-Mula *et al.*, 2018). Nous avons pour notre

part observé que l'opéron *mel* était très fortement exprimé au niveau de la racicule d'une graine germée, un jour post-inoculation, ce qui pourrait indiquer que dans cet environnement comme dans la tumeur mûre, peu d'HCAs sont disponibles (Figure 1 A ④⑤) et Chapitre 3- article 3). Comme les gènes de dégradation des HCAs ne sont pas exprimés en présence de succinate (Baude *et al.*, 2016; Campillo *et al.*, 2014), la présence de ce composé pourrait aussi expliquer une absence de répression de l'opéron *mel* sur la graine en germination malgré la présence d'acide férulique (Figure 1 A ③). Là encore l'analyse des métabolites libérés par la graine en germination est importante pour valider l'une ou l'autre des explications et compléter notre modèle. Dans ce modèle (Figure 1 A), nous proposons que les facteurs de transcription identifiés dans l'article 4 (Chapitre 3) sont impliqués dans l'activation de l'utilisation comme ressources trophiques de plusieurs composés (Figure 1 A ④⑤⑥) dont la proline, le mélibiose, et le galactinol, ce qui permettrait de mieux coloniser les plantes dès le stade de la germination (composés décrits dans le Chapitre 3-article 3). Nous supposons que les fonctions surexprimées en présence d'acide férulique ne sont pas activées dans la tumeur mûre ni sur la graine en germination (Figure 1 A ⑪⑫).

Conclusion

L'ensemble de ces travaux a permis de démontrer que de nombreuses fonctions d'intérêt écologique sont influencées par la perception et/ou la dégradation des HCAs, qui sont ainsi effectivement des composés végétaux jouant un rôle signal chez *A. fabrum*. Cependant, la compréhension globale du rôle de ce signal dans l'écologie de la bactérie nécessite d'intégrer un niveau de complexité supérieur. En effet, ce signal est probablement seulement actif dans des conditions particulières liées notamment à la disponibilité des différents métabolites, aux conditions physico-chimiques et à la présence compétitive d'autres microorganismes. Par ailleurs, les fonctions dont l'expression est influencée par les HCAs, et qui sont déjà caractérisées, sont importantes dans l'écologie d'*A. fabrum*. Ces résultats soulignent l'importance des mécanismes de régulation (HcaR, SlyA, facteur sigma), et des échanges cellulaires que ce soit avec l'environnement (*melB*, *emrAB* et *atu1086*) ou avec d'autres microorganismes en compétition (T6SS). L'opéron *mel* que nous avons caractérisé dans cette thèse s'est aussi révélé important pour la colonisation et pourrait en outre jouer un rôle dans l'induction des défenses de plantes. Ainsi, l'étude du rôle des déterminants génétiques dont l'expression est modulée en présence d'HCAs semble un moyen prometteur pour découvrir de nouvelles fonctions intervenant dans l'adaptation d'*Agrobacterium* à la plante et ainsi permettre d'améliorer la compréhension de l'écologie de cette bactérie. Cette démarche pourrait être appliquée à l'étude d'autres bactéries en interaction avec les plantes, qu'elles soient phyto-bénéfiques ou phytopathogènes.

Références

- Abarca-Grau, A. M., Penyalver, R., López, M. M., and Marco-Noales, E. 2011. Pathogenic and non-pathogenic *Agrobacterium tumefaciens*, *A. rhizogenes* and *A. vitis* strains form biofilms on abiotic as well as on root surfaces: Biofilms formed by *Agrobacterium* spp. *Plant Pathol.* 60:416–425
- Ahlgren, N. A., Harwood, C. S., Schaefer, A. L., Giraud, E., and Greenberg, E. P. 2011. Aryl-homoserine lactone quorum sensing in stem-nodulating photosynthetic bradyrhizobia. *Proc. Natl. Acad. Sci. U. S. A.* 108:7183–7188
- Allaway, D., Schofield, N. A., Leonard, M. E., Gilardoni, L., Finan, T. M., and Poole, P. S. 2001. Use of differential fluorescence induction and optical trapping to isolate environmentally induced genes. *Environ. Microbiol.* 3:397–406
- Aloni, R., Wolf, A., Feigenbaum, P., Avni, A., and Klee, H. J. 1998. The never ripe mutant provides evidence that tumor-induced ethylene controls the morphogenesis of *Agrobacterium tumefaciens*-induced crown Galls on tomato stems. *Plant Physiol.* 117:841–849
- Ampomah, O. Y., Avetisyan, A., Hansen, E., Svenson, J., Huser, T., Jensen, J. B., and Bhuvaneshwari, T. V. 2013. The *thuEFGKAB* operon of *Rhizobia* and *Agrobacterium tumefaciens* codes for transport of trehalose, maltitol, and isomers of sucrose and their assimilation through the formation of their 3-Keto derivatives. *J. Bacteriol.* 195:3797–3807
- Andberg, M., Maaheimo, H., Boer, H., Penttilä, M., Koivula, A., and Richard, P. 2012. Characterization of a novel *Agrobacterium tumefaciens* galactarolactone cycloisomerase enzyme for direct conversion of D-galactarolactone to 3-deoxy-2-keto-L-threo-hexarate. *J. Biol. Chem.* 287:17662–17671
- Anders, S., and Huber, W. 2010. Differential expression analysis for sequence count data. *Genome Biol.* 11:R106
- Andersen, K. E., Bjerregaard, C., Møller, P., Sørensen, J. C., and Sørensen, H. 2005. Compositional variations for alpha-galactosides in different species of leguminosae, brassicaceae, and barley: a chemotaxonomic study based on chemometrics and high-performance capillary electrophoresis. *J. Agric. Food Chem.* 53:5809–5817
- Angot, A., Peeters, N., Lechner, E., Vailleau, F., Baud, C., Gentzbittel, L., Sartorel, E., Genschik, P., Boucher, C., and Genin, S. 2006. *Ralstonia solanacearum* requires F-box-like domain-containing type III effectors to promote disease on several host plants. *Proc. Natl. Acad. Sci. U. S. A.* 103:14620–14625
- Antunez-Lamas, M., Cabrera, E., Lopez-Solanilla, E., Solano, R., González-Melendi, P., Chico, J. M., Toth, I., Birch, P., Pritchard, L., Prichard, L., Liu, H., and Rodriguez-Palenzuela, P. 2009. Bacterial chemoattraction towards jasmonate plays a role in the entry of *Dickeya dadantii* through wounded tissues. *Mol. Microbiol.* 74:662–671
- Ashby, A. M., Watson, M. D., Loake, G. J., and Shaw, C. H. 1988. Ti plasmid-specified chemotaxis of *Agrobacterium tumefaciens* C58C1 toward *vir*-inducing phenolic compounds and soluble factors from monocotyledonous and dicotyledonous plants. *J. Bacteriol.* 170:4181–4187
- Aulakh, M. S., Wassmann, R., Bueno, C., Kreuzwieser, J., and Rennenberg, H. 2001. Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. *Plant Biol.* 3:139–148
- Aznar, A., Chen, N. W. G., Rigault, M., Riache, N., Joseph, D., Desmaële, D., Mouille, G., Boutet, S., Soubigou-Taconnat, L., Renou, J.-P., Thomine, S., Expert, D., and Dellagi, A. 2014. Scavenging iron: a novel mechanism of plant immunity activation by microbial siderophores. *Plant Physiol.* 164:2167–2183
- Badri, D. V., Chaparro, J. M., Zhang, R., Shen, Q., and Vivanco, J. M. 2013. Application of natural blends of phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *J. Biol. Chem.* 288:4502–4512
- Baek, S.-H., and Shapleigh, J. P. 2005. Expression of nitrite and nitric oxide reductases in free-living and plant-associated *Agrobacterium tumefaciens* C58 cells. *Appl. Environ. Microbiol.* 71:4427–4436
- Baetz, U., and Martinoia, E. 2014. Root exudates: the hidden part of plant defense. *Trends Plant Sci.* 19:90–98
- Bai, Y., Müller, D. B., Srinivas, G., Garrido-Oter, R., Potthoff, E., Rott, M., Dombrowski, N., Münch, P. C., Spaepen, S., Remus-Emsermann, M., Hüttel, B., McHardy, A. C., Vorholt, J. A., and Schulze-Lefert, P. 2015. Functional overlap of the *Arabidopsis* leaf and root microbiota. *Nature.* 528:364–369
- Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57:233–266
- Baker, B., Zambryski, P., Staskawicz, B., and DineshKumar, S. P. 1997. Signaling in plant-microbe interactions. *Science.* 276:726–733

- Bakker, P. A. H. M., Berendsen, R. L., Doornbos, R. F., Wintermans, P. C. A., and Pieterse, C. M. J. 2013. The rhizosphere revisited: root microbiomics. *Front. Plant Sci.* 4
- Barret, M., Briand, M., Bonneau, S., Prèveaux, A., Valière, S., Bouchez, O., Hunault, G., Simoneau, P., and Jacquesa, M.-A. 2015. Emergence shapes the structure of the seed microbiota. *Appl. Environ. Microbiol.* 81:1257–1266
- Barker, J. L., and Frost, J. W. 2001. Microbial synthesis of *p*-hydroxybenzoic acid from glucose. *Biotechnol. Bioeng.* 76:376–390
- Barthelmebs, L., Divies, C., and Cavin, J.-F. 2000. Knockout of the *p*-coumarate decarboxylase gene from *Lactobacillus plantarum* reveals the existence of two other inducible enzymatic activities involved in phenolic acid metabolism. *Appl. Environ. Microbiol.* 66:3368–3375
- Barton, I. S., Fuqua, C., and Platt, T. G. 2018. Ecological and evolutionary dynamics of a model facultative pathogen: *Agrobacterium* and crown gall disease of plants. *Environ. Microbiol.* 20:16–29
- Baude, J., Vial, L., Villard, C., Campillo, T., Lavire, C., Nesme, X., and Hommais, F. 2016. Coordinated regulation of species-specific hydroxycinnamic acid degradation and siderophore biosynthesis pathways in *Agrobacterium fabrum*. *Appl. Environ. Microbiol.* 82:3515–3524
- Bauer, D. W., Wei, Z. M., Beer, S. V., and Collmer, A. 1995. *Erwinia chrysanthemi* harpinEch: an elicitor of the hypersensitive response that contributes to soft-rot pathogenesis. *Mol. Plant-Microbe Interact. MPMI.* 8:484–491
- Beauchamp, C. J., Chilton, W. S., Dion, P., and Antoun, H. 1990. Fungal catabolism of crown Gall opines. *Appl. Environ. Microbiol.* 56:150–155
- Bélanger, C., Canfield, M. L., Moore, L. W., and Dion, P. 1995. Genetic analysis of nonpathogenic *Agrobacterium tumefaciens* mutants arising in crown gall tumors. *J. Bacteriol.* 177:3752–3757
- Bell, C. R., Cummings, N. E., Canfield, M. L., and Moore, L. W. 1990. Competition of octopine-catabolizing *Pseudomonas spp.* and octopine-type *Agrobacterium tumefaciens* for octopine in chemostats. *Appl. Environ. Microbiol.* 56:2840–2846
- Bellin, D., Asai, S., Delledonne, M., and Yoshioka, H. 2013. Nitric oxide as a mediator for defense responses. *Mol. Plant-Microbe Interact. MPMI.* 26:271–277
- Benjamini, Y., and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B Methodol.* 57:289–300
- Berendsen, R. L., van Verk, M. C., Stringlis, I. A., Zamioudis, C., Tommassen, J., Pieterse, C. M. J., and Bakker, P. A. H. M. 2015. Unearthing the genomes of plant-beneficial *Pseudomonas* model strains WCS358, WCS374 and WCS417. *BMC Genomics.* 16:539
- Bernal, P., Llamas, M. A., and Filloux, A. 2018. Type VI secretion systems in plant-associated bacteria. *Environ. Microbiol.* 20:1–15
- Berntsson, R. P.-A., Smits, S. H. J., Schmitt, L., Slotboom, D.-J., and Poolman, B. 2010. A structural classification of substrate-binding proteins. *FEBS Lett.* 584:2606–2617
- Bhattacharya, A., Sood, P., and Citovsky, V. 2010. The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. *Mol. Plant Pathol.* 11:705–719
- Blanco, P., Hernando-Amado, S., Reales-Calderon, J. A., Corona, F., Lira, F., Alcalde-Rico, M., Bernardini, A., Sanchez, M. B., and Martinez, J. L. 2016. Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. 4:14
- Bochner, B. R. 2009. Global phenotypic characterization of bacteria. *FEMS Microbiol. Rev.* 33:191–205
- Bodini, S. F., Manfredini, S., Epp, M., Valentini, S., and Santori, F. 2009. Quorum sensing inhibition activity of garlic extract and *p*-coumaric acid. *Lett. Appl. Microbiol.* 49:551–555
- Boer, H., Maaheimo, H., Koivula, A., Penttilä, M., and Richard, P. 2010. Identification in *Agrobacterium tumefaciens* of the D-galacturonic acid dehydrogenase gene. *Appl. Microbiol. Biotechnol.* 86:901–909
- Bolton, G. W., Nester, E. W., and Gordon, M. P. 1986. Plant phenolic compounds induce expression of the *Agrobacterium tumefaciens* loci needed for virulence. *Science.* 232:983–985

- Boncompagni, E., Østerås, M., Poggi, M.-C., and le Rudulier, D. 1999. Occurrence of choline and glycine betaine uptake and metabolism in the family *Rhizobiaceae* and their roles in osmoprotection. *Appl. Environ. Microbiol.* 65:2072–2077
- Bondage, D. D., Lin, J.-S., Ma, L.-S., Kuo, C.-H., and Lai, E.-M. 2016. VgrG C terminus confers the type VI effector transport specificity and is required for binding with PAAR and adaptor–effector complex. *Proc. Natl. Acad. Sci.* :201600428
- Borges, A., Ferreira, C., Saavedra, M. J., and Simoes, M. 2013. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microb. Drug Resist.* 19:256–265
- Bougouffa, S., Radovanovic, A., Essack, M., and Bajic, V. B. 2014. DEOP: a database on osmoprotectants and associated pathways. Database. 2014
- Bouri, M., Chattaoui, M., Gharsa, H. B., McClean, A., Kluepfel, D., Nesme, X., and Rhouma, A. 2016. Analysis of *Agrobacterium* populations isolated from tunisian soils: genetic structure, avirulent-virulent ratios and characterization of tumorigenic strains. *J. Plant Pathol.* 98:265–274
- Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L.-J., and Sessitsch, A. 2017. Ecology and genomic insights into plant-pathogenic and plant-nonpathogenic endophytes. *Annu. Rev. Phytopathol.* 55:61–83
- Brencic, A., Eberhard, A., and Winans, S. C. 2004. Signal quenching, detoxification and mineralization of *vir* gene-inducing phenolics by the VirH2 protein of *Agrobacterium tumefaciens*. *Mol. Microbiol.* 51:1103–1115
- Bringhurst, R. M., Cardon, Z. G., and Gage, D. J. 2001. Galactosides in the rhizosphere: Utilization by *Sinorhizobium meliloti* and development of a biosensor. *Proc. Natl. Acad. Sci. U. S. A.* 98:4540–4545
- Brinkrolf, K., Brune, I., and Tauch, A. 2006. Transcriptional regulation of catabolic pathways for aromatic compounds in *Corynebacterium glutamicum*. *Genet. Mol. Res. GMR.* 5:773–789
- Brown, D. G., Swanson, J. K., and Allen, C. 2007. Two host-induced *Ralstonia solanacearum* genes, *acrA* and *dinF*, encode multidrug efflux pumps and contribute to bacterial wilt virulence. *Appl. Environ. Microbiol.* 73:2777–2786
- Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F. O., Amann, R., Eickhorst, T., and Schulze-Lefert, P. 2012. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature.* 488:91–95
- Büttner, D., and He, S. Y. 2009. Type III Protein Secretion in Plant Pathogenic Bacteria. *Plant Physiol.* 150:1656–1664
- Calisti, C., Ficca, A. G., Barghini, P., and Ruzzi, M. 2008. Regulation of ferulic catabolic genes in *Pseudomonas fluorescens* BF13: involvement of a MarR family regulator. *Appl. Microbiol. Biotechnol.* 80:475–483
- Campillo, T., Lavire, C., Shams, M., Pothier, J. F., and Pulawska, J. 2012. Detection and identification methods and new tests as developed and used in the framework of Cost873 for bacteria pathogenic to stone fruits and nuts. *J. Plant Pathol.*
- Campillo, T., Renoud, S., Kerzaon, I., Vial, L., Baude, J., Gaillard, V., Bellvert, F., Chamignon, C., Comte, G., Nesme, X., Lavire, C., and Hommais, F. 2014. Analysis of hydroxycinnamic acid degradation in *Agrobacterium fabrum* reveals a coenzyme A-dependent, beta-oxidative deacetylation pathway. *Appl. Environ. Microbiol.* 80:3341–3349
- Cangelosi, G. A., Ankenbauer, R. G., and Nester, E. W. 1990a. Sugars induce the *Agrobacterium* virulence genes through a periplasmic binding protein and a transmembrane signal protein. *Proc. Natl. Acad. Sci. U. S. A.* 87:6708–6712
- Cangelosi, G. A., Martinetti, G., and Nester, E. W. 1990b. Osmosensitivity phenotypes of *Agrobacterium tumefaciens* mutants that lack periplasmic beta-1,2-glucan. *J. Bacteriol.* 172:2172–2174
- Charaoui-Boukerzaza, S., and Hugouvieux-Cotte-Pattat, N. 2013. A family 3 glycosyl hydrolase of *Dickeya dadantii* 3937 is involved in the cleavage of aromatic glucosides. *Microbiology.* 159:2395–2404
- Chaparro, J. M., Badri, D. V., and Vivanco, J. M. 2014. Rhizosphere microbiome assemblage is affected by plant development. *ISME J.* 8:790–803
- Chatterjee, A., and O'Brian, M. R. 2018. Rapid evolution of a bacterial iron acquisition system. *Mol. Microbiol.*

- Chen, C., Pan, J., Yang, X., Guo, C., Ding, W., Si, M., Zhang, Y., Shen, X., and Wang, Y. 2016. Global transcriptomic analysis of the response of *Corynebacterium glutamicum* to Vanillin. PLoS ONE. 11
- Chen, C., Pan, J., Yang, X., Xiao, H., Zhang, Y., Si, M., Shen, X., and Wang, Y. 2017. Global transcriptomic analysis of the response of *Corynebacterium glutamicum* to ferulic acid. Arch. Microbiol. 199:325–334
- Chevrot, R., Rosen, R., Haudecoeur, E., Cirou, A., Shelp, B. J., Ron, E., and Faure, D. 2006. GABA controls the level of quorum-sensing signal in *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. U. S. A. 103:7460–7464
- Cho, S. M., Kang, E. Y., Kim, M. S., Yoo, S. J., Im, Y. J., Kim, Y. C., Yang, K. Y., Kim, K. Y., Kim, K. S., Choi, Y. S., and Cho, B. H. 2010. Jasmonate-dependent expression of a galactinol synthase gene is involved in priming of systemic fungal resistance in *Arabidopsis thaliana*. Botany. 88:452–461
- Christie, P. J., and Gordon, J. E. 2014. The *Agrobacterium* Ti Plasmids. Microbiol. Spectr. 2
- Compant, S., van der Heijden, M. G. A., and Sessitsch, A. 2010. Climate change effects on beneficial plant-microorganism interactions. FEMS Microbiol. Ecol. 73:197–214
- Cornish, A., Greenwood, J. A., and Jones, C. W. 1989. Binding-protein-dependent sugar transport by *Agrobacterium radiobacter* and *A. tumefaciens* grown in continuous culture. J. Gen. Microbiol. 135:3001–3013
- Costechareyre, D., Rhouma, A., Lavire, C., Portier, P., Chapulliot, D., Bertolla, F., Boubaker, A., Dessaux, Y., and Nesme, X. 2010. Rapid and efficient identification of *Agrobacterium* species by *recA* allele analysis: *Agrobacterium recA* diversity. Microb. Ecol. 60:862–872
- Costet, L., Cordelier, S., Dorey, S., Baillieul, F., Fritig, B., and Kauffmann, S. 1999. Relationship between Localized Acquired Resistance (LAR) and the Hypersensitive Response (HR): HR is necessary for LAR to occur and salicylic acid is not sufficient to trigger LAR. Mol. Plant. Microbe Interact. 12:655–662
- Cray, J. A., Bell, A. N. W., Bhaganna, P., Mswaka, A. Y., Timson, D. J., and Hallsworth, J. E. 2013. The biology of habitat dominance; can microbes behave as weeds? Microb. Biotechnol. 6:453–492
- Crosa, J. H., and Walsh, C. T. 2002. Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. Microbiol. Mol. Biol. Rev. MMBR. 66:223–249
- Croteau, R., Kutchan, T. M., and Lewis, N. G. 2000. Natural products (secondary metabolites).
- Cushnie, T. P. T., and Lamb, A. J. 2005. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents. 26:343–356
- De Coninck, B., Timmermans, P., Vos, C., Cammue, B. P. A., and Kazan, K. 2015. What lies beneath: belowground defense strategies in plants. Trends Plant Sci. 20:91–101
- De Costa, D. M., Suzuki, K., and Yoshida, K. 2003. Structural and functional analysis of a putative gene cluster for palatinose transport on the linear chromosome of *Agrobacterium tumefaciens* MAFF301001. J. Bacteriol. 185:2369–2373
- De Souza Vidigal, D., Willems, L., van Arkel, J., Dekkers, B. J. W., Hillhorst, H. W. M., and Bentsink, L. 2016. Galactinol as marker for seed longevity. Plant Sci. 246, 112–118.
- Deeken, R., Engelmann, J. C., Efetova, M., Czirjak, T., Müller, T., Kaiser, W. M., Tietz, O., Krischke, M., Mueller, M. J., Palme, K., Dandekar, T., and Hedrich, R. 2006. An integrated view of gene expression and solute profiles of *Arabidopsis* tumors: a genome-wide approach. Plant Cell. 18:3617–3634
- Del Valle, P., García-Armesto, M. R., de Arriaga, D., González-Donquiles, C., Rodríguez-Fernández, P., and Rúa, J. 2016. Antimicrobial activity of kaempferol and resveratrol in binary combinations with parabens or propyl gallate against *Enterococcus faecalis*. Food Control 61, 213–220.
- Delepelaire, P. 1998. *Erwinia* metalloprotease permease: aspects of secretion pathway and secretion functions. Methods Enzymol. 292:67–81
- Dennis, P. G., Miller, A. J., and Hirsch, P. R. 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol. Ecol. 72:313–327
- Deochand, D. K., and Grove, A. 2017. MarR family transcription factors: dynamic variations on a common scaffold. Crit. Rev. Biochem. Mol. Biol. :1–19

- Dessaux, Y., and Faure, D. 2018. Niche construction and exploitation by *Agrobacterium* How to survive and face competition in soil and plant habitats. pages 1–32 in: SpringerLink, Current Topics in Microbiology and Immunology. Springer, Berlin, Heidelberg.
- Does, H. C. van der, and Rep, M. 2017. Adaptation to the host environment by plant-pathogenic Fungi. *Annu. Rev. Phytopathol.* 55, 427–450.
- Domínguez-Ferreras, A., Pérez-Arnedo, R., Becker, A., Olivares, J., Soto, M. J., and Sanjuán, J. 2006. Transcriptome profiling reveals the importance of plasmid pSymB for osmoadaptation of *Sinorhizobium meliloti*. *J. Bacteriol.* 188, 7617–7625.
- Drummond, M. 1979. Crown gall disease. *Nature.* 281:281343a0
- Duprey, A., Reverchon, S., and Nasser, W. 2014. Bacterial virulence and Fis: adapting regulatory networks to the host environment. *Trends Microbiol.* 22:92–99
- Durrant, W. E., and Dong, X. 2004. Systemic acquired resistance. *Annu. Rev. Phytopathol.* 42:185–209
- Efetova, M., Zeier, J., Riederer, M., Lee, C.-W., Stingl, N., Mueller, M., Hartung, W., Hedrich, R., and Deeken, R. 2007. A central role of abscisic acid in drought stress protection of *Agrobacterium*-induced tumors on *Arabidopsis*. *Plant Physiol.* 145:853–862
- Ellison, D. W., and Miller, V. L. 2006. Regulation of virulence by members of the MarR/SlyA family. *Curr. Opin. Microbiol.* 9, 153–159.
- Emsley, P., and Cowtan, K. 2004. Coot: model-building tools for molecular graphics. *Acta Crystallogr Biol Crystallogr.* 60:2126–2132
- Engering, A., Hogerwerf, L., and Slingenberg, J. 2013. Pathogen–host–environment interplay and disease emergence. *Emerg. Microbes Infect.* 2:e5
- Faist, H., Keller, A., Hentschel, U., and Deeken, R. 2016. Grapevine (*Vitis vinifera*) crown Galls host distinct microbiota. *Appl. Environ. Microbiol.* 82, 5542–5552.
- Falcone Ferreyra, M. L., Rius, S. P., and Casati, P. 2012. Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Front. Plant Sci.* 3
- Faure, D., Vereecke, D., and Leveau, J. H. J. 2009. Molecular communication in the rhizosphere. *Plant Soil.* 321:279–303
- Finn, R. D., Coggill, P., Eberhardt, R. Y., Eddy, S. R., Mistry, J., Mitchell, A. L., Potter, S. C., Punta, M., Qureshi, M., Sangrador-Vegas, A., Salazar, G. A., Tate, J., and Bateman, A. 2016. The Pfam protein families database: towards a more sustainable future. *Nucleic Acids Res.* 44:D279–285
- Fischer, R., Bleichrodt, F. S., and Gerischer, U. C. 2008. Aromatic degradative pathways in *Acinetobacter baylyi* underlie carbon catabolite repression. *Microbiology.* 154:3095–3103
- Flores-Mireles, A. L., Eberhard, A., and Winans, S. C. 2012. *Agrobacterium tumefaciens* can obtain sulphur from an opine that is synthesized by octopine synthase using S-methylmethionine as a substrate. *Mol. Microbiol.* 84, 845–856.
- Fukami, T., Martijn Bezemer, T., Mortimer, S. R., and van der Putten, W. H. 2005. Species divergence and trait convergence in experimental plant community assembly. *Ecol. Lett.* 8:1283–1290
- Gage, D. J., and Long, S. R. 1998. alpha-Galactoside uptake in *Rhizobium meliloti*: isolation and characterization of *agpA*, a gene encoding a periplasmic binding protein required for melibiose and raffinose utilization. *J. Bacteriol.* 180:5739–5748
- Galán, B., Kolb, A., Sanz, J. M., García, J. L., and Prieto, M. A. 2003. Molecular determinants of the *hpa* regulatory system of *Escherichia coli*: the HpaR repressor. *Nucleic Acids Res.* 31:6598–6609
- Gan, H. M., and Savka, M. A. 2018. One More Decade of *Agrobacterium* Taxonomy. *Curr. Top. Microbiol. Immunol.*
- Gelvin, S. B. 2017. Integration of *Agrobacterium* T-DNA into the plant genome. *Annu. Rev. Genet.* 51, 195–217.
- Genin, S., and Denny, T. P. 2012. Pathogenomics of the *Ralstonia solanacearum* species complex. *Annu. Rev. Phytopathol.* 50:67–89

- Glasner, J. D., Yang, C.-H., Reverchon, S., Hugouvieux-Cotte-Pattat, N., Condemine, G., Bohin, J.-P., Van Gijsegem, F., Yang, S., Franza, T., Expert, D., Plunkett, G., San Francisco, M. J., Charkowski, A. O., Py, B., Bell, K., Rauscher, L., Rodriguez-Palenzuela, P., Toussaint, A., Holeva, M. C., He, S. Y., Douet, V., Boccara, M., Blanco, C., Toth, I., Anderson, B. D., Biehl, B. S., Mau, B., Flynn, S. M., Barras, F., Lindeberg, M., Birch, P. R. J., Tsuyumu, S., Shi, X., Hibbing, M., Yap, M.-N., Carpentier, M., Dassa, E., Umehara, M., Kim, J. F., Rusch, M., Soni, P., Mayhew, G. F., Fouts, D. E., Gill, S. R., Blattner, F. R., Keen, N. T., and Perna, N. T. 2011. Genome sequence of the plant-pathogenic bacterium *Dickeya dadantii* 3937. *J. Bacteriol.* 193:2076–2077
- González-Mula, A., Lang, J., Grandclément, C., Naquin, D., Ahmar, M., Soulère, L., Queneau, Y., Dessaux, Y., and Faure, D. 2018. Lifestyle of the biotroph *Agrobacterium tumefaciens* in the ecological niche constructed on its host plant. *New Phytol.*
- Goodner, B., Hinkle, G., Gattung, S., Miller, N., Blanchard, M., Quorollo, B., Goldman, B. S., Cao, Y., Askenazi, M., Halling, C., Mullin, L., Houmiel, K., Gordon, J., Vaudin, M., Iartchouk, O., Epp, A., Liu, F., Wollam, C., Allinger, M., Doughty, D., Scott, C., Lappas, C., Markelz, B., Flanagan, C., Crowell, C., Gurson, J., Lomo, C., Sear, C., Strub, G., Cielo, C., and Slater, S. 2001. Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. *Science.* 294:2323–2328
- Grove, A. 2017. Regulation of Metabolic Pathways by MarR Family Transcription Factors. *Comput. Struct. Biotechnol. J.* 15:366–371
- Gu, W., Yang, J., Lou, Z., Liang, L., Sun, Y., Huang, J., Li, X., Cao, Y., Meng, Z., and Zhang, K.-Q. 2011. Structural basis of enzymatic activity for the ferulic acid decarboxylase (FADase) from *Enterobacter* sp. Px6-4. *PLoS One.* 6:e16262
- Gundogdu, O., da Silva, D. T., Mohammad, B., Elmi, A., Mills, D. C., Wren, B. W., and Dorrell, N. 2015. The *Campylobacter jejuni* MarR-like transcriptional regulators RrpA and RrpB both influence bacterial responses to oxidative and aerobic stresses. *Food Microbiol.* :724
- Gunina, A., and Kuzyakov, Y. 2015. Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. *Soil Biol. Amp Biochem.* 90:87–100
- Guo, M., Huang, Z., and Yang, J. 2017. Is there any crosstalk between the chemotaxis and virulence induction signaling in *Agrobacterium tumefaciens*? *Biotechnol. Adv.*
- Guyon, P., Chilton, M. D., Petit, A., and Tempé, J. 1980. Agropine in “null-type” crown gall tumors: Evidence for generality of the opine concept. *Proc. Natl. Acad. Sci. U. S. A.* 77:2693–2697
- Hall, B. G., Pikis, A., and Thompson, J. 2009. Evolution and biochemistry of family 4 glycosidases: implications for assigning enzyme function in sequence annotations. *Mol. Biol. Evol.* 26:2487–2497
- Hao, Z., Lou, H., Zhu, R., Zhu, J., Zhang, D., Zhao, B. S., Zeng, S., Chen, X., Chan, J., He, C., and Chen, P. R. 2014. The multiple antibiotic resistance regulator MarR is a copper sensor in *Escherichia coli*. *Nat. Chem. Biol.* 10:21–28
- Haque, M. M., Kabir, M. S., Aini, L. Q., Hirata, H., and Tsuyumu, S. 2009. SlyA, a MarR family transcriptional regulator, is essential for virulence in *Dickeya dadantii* 3937. *J. Bacteriol.* 191:5409–5418
- Harrison, F., Paul, J., Massey, R. C., and Buckling, A. 2008. Interspecific competition and siderophore-mediated cooperation in *Pseudomonas aeruginosa*. *ISME J.* 2:49–55
- Harwood, C. S., and Parales, R. E. 1996. The beta-ketoadipate pathway and the biology of self-identity. *Annu. Rev. Microbiol.* 50:553–590
- Hassan, S., and Hugouvieux-Cotte-Pattat, N. 2011. Identification of two feruloyl esterases in *Dickeya dadantii* 3937 and induction of the major feruloyl esterase and of pectate lyases by ferulic acid. *J. Bacteriol.* 193:963–970
- Hassan, S., and Mathesius, U. 2012. The role of flavonoids in root–rhizosphere signalling: opportunities and challenges for improving plant–microbe interactions. *J. Exp. Bot.* 63:3429–3444
- Haudecoeur, E., Planamente, S., Cirou, A., Tannières, M., Shelp, B. J., Moréra, S., and Faure, D. 2009. Proline antagonizes GABA-induced quenching of quorum-sensing in *Agrobacterium tumefaciens*. *Proc. Natl. Acad. Sci. U. S. A.* 106:14587–14592
- Heckel, B. C., Tomlinson, A. D., Morton, E. R., Choi, J.-H., and Fuqua, C. 2014. *Agrobacterium tumefaciens* *exoR* controls acid response genes and impacts exopolysaccharide synthesis, horizontal gene transfer, and virulence gene expression. *J. Bacteriol.* 196, 3221–3233.

- Hibbing, M. E., and Fuqua, C. 2011. Antiparallel and Interlinked Control of Cellular Iron Levels by the Irr and RirA Regulators of *Agrobacterium tumefaciens*. *J. Bacteriol.* 193:3461–3472
- Hikichi, Y. 2016. Interactions between plant pathogenic bacteria and host plants during the establishment of susceptibility. *J. Gen. Plant Pathol.* 82:326–331
- Hirakawa, H., Schaefer, A. L., Greenberg, E. P., and Harwood, C. S. 2012. Anaerobic *p*-coumarate degradation by *Rhodopseudomonas palustris* and identification of CouR, a MarR repressor protein that binds *p*-coumaroyl Coenzyme A. *J. Bacteriol.* 194:1960–1967
- Hoskisson, P. A., and Rigali, S. 2009. Chapter 1: Variation in form and function the helix-turn-helix regulators of the GntR superfamily. *Adv. Appl. Microbiol.* 69:1–22
- Hu, X., Zhao, J., DeGrado, W. F., and Binns, A. N. 2013. *Agrobacterium tumefaciens* recognizes its host environment using ChvE to bind diverse plant sugars as virulence signals. *Proc. Natl. Acad. Sci. U. S. A.* 110:678–683
- Huang, Z., Dostal, L., and Rosazza, J. P. 1993. Mechanisms of ferulic acid conversions to vanillic acid and guaiacol by *Rhodotorula rubra*. *J. Biol. Chem.* 268:23954–23958
- Huang, Z., Zhou, Q., Sun, P., Yang, J., and Guo, M. 2017. Two *Agrobacterium tumefaciens* CheW proteins are incorporated into one chemosensory pathway with different efficiencies. *Mol. Plant. Microbe Interact.* 31, 460–470.
- Hughes, J. G., Zhang, X., Parales, J. V., Ditty, J. L., and Parales, R. E. 2017. *Pseudomonas putida* F1 uses energy taxis to sense hydroxycinnamic acids. *Microbiol. Read. Engl.* 163:1490–1501
- Hwang, H.-H., Gelvin, S. B., and Lai, E.-M. 2015. Editorial: “*Agrobacterium* biology and its application to transgenic plant production.” *Front. Plant Sci.* 6:265
- Ibáñez, C., Collada, C., Casado, R., González-Melendi, P., Aragoncillo, C., and Allona, I. 2013. Winter induction of the galactinol synthase gene is associated with endodormancy in chestnut trees. *Trees.* 27:1309–1316
- Ingram-Smith, C., and Miller, K. J. (1998). Effects of ionic and osmotic strength on the Ggucosyltransferase of *Rhizobium meliloti* responsible for cyclic β -(1,2)-glucan biosynthesis. *Appl. Environ. Microbiol.* 64, 1290–1297.
- Ishii, T. 1997. Structure and functions of feruloylated polysaccharides. *Plant Sci.* 127:111–127
- Ji, P., and Wilson, M. 2002. Assessment of the importance of similarity in carbon source utilization profiles between the biological control agent and the pathogen in biological control of bacterial speck of tomato. *Appl. Environ. Microbiol.* 68:4383–4389
- Jiménez, J. I., Miñambres, B., García, J. L., and Díaz, E. 2002. Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* KT2440. *Environ. Microbiol.* 4:824–841
- Jones, J. D. G., and Dangl, J. L. 2006. The plant immune system. *Nature.* 444:323–329
- Joshi, J. R., Burdman, S., Lipsky, A., Yariv, S., and Yedidia, I. 2016. Plant phenolic acids affect the virulence of *Pectobacterium aroidearum* and *P. carotovorum* ssp. *brasiliense* via quorum sensing regulation. *Mol. Plant Pathol.* 17:487–500
- Joshi, J. R., Burdman, S., Lipsky, A., and Yedidia, I. 2015. Effects of plant antimicrobial phenolic compounds on virulence of the genus *Pectobacterium*. *Res. Microbiol.* 166:535–545
- Joubert, P., Beaupère, D., Lelièvre, P., Wadouachi, A., Sangwan, R. S., and Sangwan-Norreel, B. S. 2002. Effects of phenolic compounds on *Agrobacterium vir* genes and gene transfer induction—a plausible molecular mechanism of phenol binding protein activation. *Plant Sci.* 162:733–743
- Jung, D.-H., Kim, E.-J., Jung, E., Kazlauskas, R. J., Choi, K.-Y., and Kim, B.-G. 2016. Production of *p*-hydroxybenzoic acid from *p*-coumaric acid by *Burkholderia glumae* BGR1. *Biotechnol. Bioeng.* 113, 1493–1503.
- Kabsch, W. 2010. XDS. *Acta Crystallogr. D Biol. Crystallogr.* 66:125–32
- Kai, K., Ohnishi, H., Shimatani, M., Ishikawa, S., Mori, Y., Kiba, A., Ohnishi, K., Tabuchi, M., and Hikichi, Y. 2015. Methyl 3-hydroxymyristate, a diffusible signal mediating *phc* quorum sensing in *Ralstonia solanacearum*. *Chembiochem Eur. J. Chem. Biol.* 16:2309–2318

- Kallscheuer, N., Vogt, M., Kappelmann, J., Krumbach, K., Noack, S., Bott, M., and Marienhagen, J. 2016. Identification of the *phd* gene cluster responsible for phenylpropanoid utilization in *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 100:1871–1881
- Kalogeraki, V. S., Zhu, J., Eberhard, A., Madsen, E. L., and Winans, S. C. 1999. The phenolic *vir* gene inducer ferulic acid is *O*-demethylated by the VirH2 protein of an *Agrobacterium tumefaciens* Ti plasmid. *Mol. Microbiol.* 34:512–522
- Kamimura, N., Takahashi, K., Mori, K., Araki, T., Fujita, M., Higuchi, Y., and Masai, E. 2017. Bacterial catabolism of lignin-derived aromatics: New findings in a recent decade: Update on bacterial lignin catabolism. *Environ. Microbiol. Rep.* 9:679–705
- Kampschreur, M. J., Kleerebezem, R., Picioreanu, C., Bakken, L., Bergaust, L., de Vries, S., et al. 2012. Metabolic modeling of denitrification in *Agrobacterium tumefaciens*: a tool to study inhibiting and activating compounds for the denitrification pathway. *Front. Microbiol.* 3, 370.
- Kang, S.-Y., Kim, B.-M., Heo, K. T., Jang, J.-H., Kim, W.-G., and Hong, Y.-S. 2017. Production of bacterial quorum sensing antagonists, caffeoyl- and feruloyl-HSL, by an artificial biosynthetic pathway. *J. Microbiol. Biotechnol.* 27:2104–2111
- Kang, S.-Y., Lee, J. K., Jang, J.-H., Hwang, B. Y., and Hong, Y.-S. 2015. Production of phenylacetyl-homoserine lactone analogs by artificial biosynthetic pathway in *Escherichia coli*. *Microb. Cell Factories.* 14:191
- Kape, R., Parniske, M., and Werner, D. 1991. Chemotaxis and *nod* gene activity of *Bradyrhizobium japonicum* in response to hydroxycinnamic acids and isoflavonoids. *Appl. Environ. Microbiol.* 57:316–319
- Karishma, M., Trivedi, V. D., Choudhary, A., Mhatre, A., Kambli, P., Desai, J., and Phale, P. S. 2015. Analysis of preference for carbon source utilization among three strains of aromatic compounds degrading *Pseudomonas*. *FEMS Microbiol. Lett.* 362
- Karplus, P. A., and Diederichs, K. 2012. Linking crystallographic model and data quality. *Science.* 336:1030–1033
- Kasai, D., Kamimura, N., Tani, K., Umeda, S., Abe, T., Fukuda, M., and Masai, E. 2012. Characterization of FerC, a MarR-type transcriptional regulator, involved in transcriptional regulation of the ferulate catabolic operon in *Sphingobium* sp strain SYK-6. *Fems Microbiol. Lett.* 332:68–75
- Kemner, J. M., Liang, X., and Nester, E. W. 1997. The *Agrobacterium tumefaciens* virulence gene *chvE* is part of a putative ABC-type sugar transport operon. *J. Bacteriol.* 179:2452–2458
- Kempf, B., and Bremer, E. 1998. Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Arch. Microbiol.* 170:319–330
- Khokhani, D., Lowe-Power, T. M., Tran, T. M., and Allen, C. 2017. A single regulator mediates strategic switching between attachment/spread and growth/virulence in the plant pathogen *Ralstonia solanacearum*. *mBio* 8, e00895-17.
- Kim, M. S., Cho, S. M., Kang, E. Y., Im, Y. J., Hwangbo, H., Kim, Y. C., Ryu, C.-M., Yang, K. Y., Chung, G. C., and Cho, B. H. 2008. Galactinol is a signaling component of the induced systemic resistance caused by *Pseudomonas chlororaphis* O6 root colonization. *Mol. Plant-Microbe Interact.* MPMI. 21:1643–1653
- Kim, Y., Joachimiak, G., Bigelow, L., Babnigg, G., and Joachimiak, A. 2016. How aromatic compounds block dna binding of HcaR catabolite regulator. *J. Biol. Chem.* 291:13243–13256
- Klinkenberg, J., Faist, H., Saupe, S., Lambertz, S., Krischke, M., Stingl, N., Fekete, A., Mueller, M. J., Feussner, I., Hedrich, R., and Deeken, R. 2014. Two fatty acid desaturases, stearyl-acyl carrier protein δ 9-desaturase6 and fatty acid desaturase3, are involved in drought and hypoxia stress signaling in *Arabidopsis* crown galls. *Plant Physiol.* 164:570–583
- Kong, C. H., Li, H. B., Hu, F., Xu, X. H., and Wang, P. 2006. Allelochemicals released by rice roots and residues in soil. *Plant Soil.* 288:47–56
- Krenek, P., Samajova, O., Luptovciak, I., Duskocilova, A., Komis, G., and Samaj, J. 2015. Transient plant transformation mediated by *Agrobacterium tumefaciens*: Principles, methods and applications. *Biotechnol. Adv.* 33:1024–1042
- Krimi, Z., Petit, A., Mougél, C., Dessaux, Y., and Nesme, X. 2002. Seasonal fluctuations and long-term persistence of pathogenic populations of *Agrobacterium* spp. in soils. *Appl. Environ. Microbiol.* 68:3358–3365

- Kristin, A., and Miranda, H. 2013. The root microbiota—a fingerprint in the soil? *Plant Soil*. 370:671–686
- Kroon, P. A., and Williamson, G. 1999. Hydroxycinnamates in plants and food: current and future perspectives. *J. Sci. Food Agric.* 79:355–361
- Kumar, N., and Pruthi, V. 2014. Potential applications of ferulic acid from natural sources. *Biotechnol. Rep.* 4:86–93
- Lacroix, B., and Citovsky, V. 2013. The roles of bacterial and host plant factors in *Agrobacterium*-mediated genetic transformation. *Int. J. Dev. Biol.* 57:467–481
- Lamovšek, J., Stare, B. G., and Urek, G. 2014. Isolation of non-pathogenic *Agrobacterium* spp. biovar 1 from agricultural soils in Slovenia. *Phytopathol. Mediterr.* 53:130–139
- Lang, J., Gonzalez-Mula, A., Tacconat, L., Clement, G., and Faure, D. 2016. The plant GABA signaling downregulates horizontal transfer of the *Agrobacterium tumefaciens* virulence plasmid. *New Phytol.* 210:974–983
- Lang, J., Planamente, S., Mondy, S., Dessaux, Y., Moréra, S., and Faure, D. 2013. Concerted transfer of the virulence Ti plasmid and companion At plasmid in the *Agrobacterium tumefaciens*-induced plant tumour. *Mol. Microbiol.* 90:1178–1189
- Lang, J., Vigouroux, A., El Sahili, A., Kwasiborski, A., Aumont-Nicaise, M., Dessaux, Y., Shykoff, J. A., Moréra, S., and Faure, D. 2017. Fitness costs restrict niche expansion by generalist niche-constructing pathogens. *ISME J.* 11:374–385
- Lanoue, A., Burlat, V., Henkes, G. J., Koch, I., Schurr, U., and Röse, U. S. R. 2010. De novo biosynthesis of defense root exudates in response to *Fusarium* attack in barley. *New Phytol.* 185:577–588
- Lareen, A., Burton, F., and Schäfer, P. 2016. Plant root-microbe communication in shaping root microbiomes. *Plant Mol. Biol.* 90:575–587
- Lassalle, F., Campillo, T., Vial, L., Baude, J., Costechareyre, D., Chapulliot, D., Shams, M., Abrouk, D., Lavire, C., Oger-Desfeux, C., Hommais, F., Gueguen, L., Daubin, V., Muller, D., and Nesme, X. 2011. Genomic species are ecological species as revealed by comparative genomics in *Agrobacterium tumefaciens*. *Genome Biol. Evol.* 3:762–781
- Lassalle, F., Planel, R., Penel, S., Chapulliot, D., Barbe, V., Dubost, A., Calteau, A., Vallenet, D., Mornico, D., Bigot, T., Guéguen, L., Vial, L., Muller, D., Daubin, V., and Nesme, X. 2017. Ancestral genome estimation reveals the history of ecological diversification in *Agrobacterium*. *Genome Biol. Evol.* 9:3413–3431
- Lee, C.-W., Efetova, M., Engelmann, J. C., Kramell, R., Wasternack, C., Ludwig-Müller, J., Hedrich, R., and Deeken, R. 2009. *Agrobacterium tumefaciens* promotes tumor induction by modulating pathogen defense in *Arabidopsis thaliana*. *Plant Cell*. 21:2948–2962
- Lee, J.-H., Kim, Y.-G., Baek, K.-H., Cho, M. H., and Lee, J. 2015. The multifaceted roles of the interspecies signalling molecule indole in *Agrobacterium tumefaciens*. *Environ. Microbiol.* 17:1234–1244
- Leitner, M., Vandelle, E., Gaupels, F., Bellin, D., and Delledonne, M. 2009. NO signals in the haze: nitric oxide signalling in plant defence. *Curr. Opin. Plant Biol.* 12:451–458
- Lemanceau, P., Expert, D., Gaymard, F., Bakker, P. A. H. M., and Briat, J.-F. 2009. Chapter 12 role of iron in plant–microbe interactions. Pages 491–549 in: *Advances in Botanical Research, Advances in Botanical Research*. Academic Press.
- Leonard, S., Hommais, F., Nasser, W., and Reverchon, S. 2017. Plant-phytopathogen interactions: bacterial responses to environmental and plant stimuli. *Environ. Microbiol.* 19:1689–1716
- Li, X.-Z., Plésiat, P., and Nikaido, H. 2015a. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clin. Microbiol. Rev.* 28:337–418
- Li, Y., Hutchins, W., Wu, X., Liang, C., Zhang, C., Yuan, X., Khokhani, D., Chen, X., Che, Y., Wang, Q., and Yang, C.-H. 2015. Derivative of plant phenolic compound inhibits the type III secretion system of *Dickeya dadantii* via HrpX/HrpY two-component signal transduction and Rsm systems. *Mol. Plant Pathol.* 16:150–163
- Li, Y., Peng, Q., Selimi, D., Wang, Q., Charkowski, A. O., Chen, X., and Yang, C.-H. 2009. The plant phenolic compound *p*-coumaric acid represses gene expression in the *Dickeya dadantii* type III secretion system. *Appl. Environ. Microbiol.* 75:1223–1228

- Liljestoöm, P. L., and Liljestrom, P. 1987. Nucleotide sequence of the *mela* gene, Coding for α -galactosidase in *Escherichia coli* K-12. *Nucleic Acids Res.* 15:2213–2220
- Lin, J.-S., Ma, L.-S., and Lai, E.-M. 2013. Systematic dissection of the *Agrobacterium* Type VI secretion system reveals machinery and secreted components for subcomplex formation. *PLOS ONE.* 8:e67647
- Liu, Y., Chen, L., Wu, G., Feng, H., Zhang, G., Shen, Q., and Zhang, R. 2017. Identification of root-secreted compounds involved in the communication between cucumber, the beneficial *Bacillus amyloliquefaciens*, and the soil-borne pathogen *Fusarium oxysporum*. *Mol. Plant-Microbe Interact.* MPMI. 30:53–62
- Liu, Z., Xie, Y., Zhang, X., Hu, X., Li, Y., Ding, X., Xia, L., and Hu, S. 2016. Efficient construction of large genomic deletion in *Agrobacterium tumefaciens* by combination of Cre/loxP system and triple recombineering. *Curr. Microbiol.* 72:465–472
- Llop, P., Murillo, J., Lastra, B., and López, M. M. 2009. Recovery of nonpathogenic mutant bacteria from tumors caused by several *Agrobacterium tumefaciens* strains: a frequent event? *Appl. Environ. Microbiol.* 75:6504–6514
- Loper, J. E., and Buyer, J. S. 1991. Siderophores in microbial interactions on plant surfaces. *Mol. Plant-Microbe Interact.* MPMI USA.
- Lowe, T. M., Ailloud, F., and Allen, C. 2014. Hydroxycinnamic acid degradation, a broadly conserved trait, protects *Ralstonia solanacearum* from chemical plant defenses and contributes to root colonization and virulence. *Mol. Plant. Microbe Interact.* 28:286–297
- Lugtenberg, B. J., Kravchenko, L. V., and Simons, M. 1999. Tomato seed and root exudate sugars: composition, utilization by *Pseudomonas* biocontrol strains and role in rhizosphere colonization. *Environ. Microbiol.* 1:439–446
- Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Del Rio, T. G., Edgar, R. C., Eickhorst, T., Ley, R. E., Hugenholtz, P., Tringe, S. G., and Dangl, J. L. 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature.* 488:86–90
- Luo, Z. Q., and Farrand, S. K. 1999. Cloning and characterization of a tetracycline resistance determinant present in *Agrobacterium tumefaciens* C58. *J. Bacteriol.* 181:618–626
- Ma, B., Hibbing, M. E., Kim, H.-S., Reedy, R. M., Yedidia, I., Breuer, J., Breuer, J., Glasner, J. D., Perna, N. T., Kelman, A., and Charkowski, A. O. 2007. Host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya*. *Phytopathology.* 97:1150–1163
- Ma, L.-S., Hachani, A., Lin, J.-S., Filloux, A., and Lai, E.-M. 2014. *Agrobacterium tumefaciens* deploys a superfamily of type VI secretion DNase effectors as weapons for interbacterial competition in planta. *Cell Host Microbe.* 16:94–104
- MacLean, A. M., MacPherson, G., Aneja, P., and Finan, T. M. 2006. Characterization of the beta-ketoadipate pathway in *Sinorhizobium meliloti*. *Appl. Environ. Microbiol.* 72:5403–5413
- Mandal, S., and Mitra, A. 2008. Accumulation of cell wall-bound phenolic metabolites and their upliftment in hairy root cultures of tomato (*Lycopersicon esculentum* Mill.). *Biotechnol. Lett.* 30:1253–1258
- Mandal, S., Mitra, A., and Mallick, N. 2009. Time course study on accumulation of cell wall-bound phenolics and activities of defense enzymes in tomato roots in relation to *Fusarium* wilt. *World J. Microbiol. Biotechnol.* 25:795–802
- Marchi, M., Boutin, M., Gazengel, K., Rispe, C., Gauthier, J.-P., Guillerm-Erckelboudt, A.-Y., Lebreton, L., Barret, M., Daval, S., and Sarniguet, A. 2013. Genomic analysis of the biocontrol strain *Pseudomonas fluorescens* Pf29Arp with evidence of T3SS and T6SS gene expression on plant roots. *Environ. Microbiol. Rep.* 5:393–403
- Martin, R. G., Jair, K. W., Wolf, R. E., and Rosner, J. L. 1996. Autoactivation of the *marRAB* multiple antibiotic resistance operon by the MarA transcriptional activator in *Escherichia coli*. *J. Bacteriol.* 178:2216–2223
- Massalha, H., Korenblum, E., Malitsky, S., Shapiro, O. H., and Aharoni, A. 2017. Live imaging of root–bacteria interactions in a microfluidics setup. *Proc. Natl. Acad. Sci.* :201618584
- Matthysse, A. G. 2014. Attachment of *Agrobacterium* to plant surfaces. *Plant-Microbe Interact.* 5:252
- McCarthy, D. J., Chen, Y., and Smyth, G. K. 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res.* 40:4288–4297

- McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C., and Read, R. J. 2007. Phaser crystallographic software. *J Appl Crystallogr.* 40:658–674
- Meena, K. K., Sorty, A. M., Bitla, U. M., Choudhary, K., Gupta, P., Pareek, A., Singh, D. P., Prabha, R., Sahu, P. K., Gupta, V. K., Singh, H. B., Krishanani, K. K., and Minhas, P. S. 2017. Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. *Front. Plant Sci.* 8
- Mendes, R., Garbeva, P., and Raaijmakers, J. M. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.* 37:634–663
- Messens, E., Lenaerts, A., Montagu, M. V., and Hedges, R. W. 1985. Genetic basis for opine secretion from crown gall tumour cells. *Mol. Gen. Genet. MGG.* 199:344–348
- Meyer, T., Renoud, S., Vigouroux, A., Miomandre, A., Gaillard, V., Kerzaon, I., Prigent-Combaret, C., Comte, G., Morera, S., Vial, L., and Lavire, C. 2018a. Regulation of hydroxycinnamic acid degradation drives *Agrobacterium fabrum* lifestyles. *Mol. Plant-Microbe Interact. MPMI.*
- Meyer, T., Vigouroux, A., Aumont-Niçaise, M., Comte, G., Vial, L., Lavire, C., and Morera, S. 2018b. The plant defense signal galactinol is specifically used as a nutrient by the bacterial pathogen *Agrobacterium fabrum*. *J. Biol. Chem.*
- Miethke, M., and Marahiel, M. A. 2007. Siderophore-based iron acquisition and pathogen control. *Microbiol. Mol. Biol. Rev. MMBR.* 71:413–451
- Moe, L. A. 2013. Amino acids in the rhizosphere: From plants to microbes. *Am. J. Bot.* 100:1692–1705
- Mohan, K., and Phale, P. S. 2017. Carbon source-dependent inducible metabolism of veratryl alcohol and ferulic acid in *Pseudomonas putida* CSV86. *Appl. Environ. Microbiol.* 83:e03326-16
- Moore, L. W., Chilton, W. S., and Canfield, M. L. 1997. Diversity of opines and opine-catabolizing bacteria isolated from naturally occurring crown Gall tumors. *Appl. Environ. Microbiol.* 63:201–207
- Morabbi Heravi, K., Lange, J., Watzlawick, H., Kalinowski, J., and Altenbuchner, J. 2015. Transcriptional regulation of the vanillate utilization genes (*vanABK* Operon) of *Corynebacterium glutamicum* by VanR, a PadR-like repressor. *J. Bacteriol.* 197:959–972
- Morawski, B., Segura, A., and Ornston, L. N. 2000. Repression of *Acinetobacter* vanillate demethylase synthesis by VanR, a member of the GntR family of transcriptional regulators. *FEMS Microbiol. Lett.* 187:65–68
- Morton, E. R., Platt, T. G., Fuqua, C., and Bever, J. D. 2014. Non-additive costs and interactions alter the competitive dynamics of co-occurring ecologically distinct plasmids. *Proc. Biol. Sci.* 281:20132173
- Mousavi, S. A., Österman, J., Wahlberg, N., Nesme, X., Lavire, C., Vial, L., Paulin, L., de Lajudie, P., and Lindström, K. 2014. Phylogeny of the *Rhizobium-Allorhizobium-Agrobacterium* clade supports the delineation of *Neorhizobium* gen. nov. *Syst. Appl. Microbiol.* 37:208–215
- Nautiyal, C. S., and Dion, P. 1990. Characterization of the opine-utilizing microflora associated with samples of soil and plants. *Appl. Environ. Microbiol.* 56:2576–2579
- Nelson, E. B. 2004. Microbial dynamics and interactions in the spermosphere. *Annu. Rev. Phytopathol.* 42:271–309
- Nelson, E. B. 2017. The seed microbiome: Origins, interactions, and impacts. *Plant Soil.* :1–28
- Nesme, X., Michel, M.-F., and Digat, B. 1987. Population heterogeneity of *Agrobacterium tumefaciens* in Galls of *Populus* L. from a single nursery. *Appl. Environ. Microbiol.* 53:655–659
- Nester, E. W. 2014. *Agrobacterium*: nature's genetic engineer. *Front. Plant Sci.* 5:730
- Newman, M.-A., Sundelin, T., Nielsen, J. T., and Erbs, G. 2013. MAMP (microbe-associated molecular pattern) triggered immunity in plants. *Front. Plant Sci.* 4:139
- Ngok-Ngam, P., Ruangkiattikul, N., Mahavihakanont, A., Virgem, S. S., Sukchawalit, R., and Mongkolsuk, S. 2009. Roles of *Agrobacterium tumefaciens* RirA in iron regulation, oxidative stress response, and virulence. *J. Bacteriol.* 191:2083–2090
- Nguyen, C. 2009. Rhizodeposition of Organic C by Plant: Mechanisms and controls. Pages 97–123 in: *Sustainable Agriculture*, Springer, Dordrecht.

- Niehus, R., Picot, A., Oliveira, N. M., Mitri, S., and Foster, K. R. 2017. The evolution of siderophore production as a competitive trait. *Evol. Int. J. Org. Evol.* 71:1443–1455
- Nikaido, H. 2009. Multidrug Resistance in Bacteria. *Annu. Rev. Biochem.* 78:119–146
- Nishizawa, A., Yabuta, Y., and Shigeoka, S. 2008. Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiol.* 147:1251–1263
- Nobile, S., and Deshusses, J. 1986. Transport of gamma-butyrobetaine in an *Agrobacterium* species isolated from soil. *J. Bacteriol.* 168:780–784
- Nonaka, S., and Ezura, H. 2014. Plant-*Agrobacterium* interaction mediated by ethylene and super-*Agrobacterium* conferring efficient gene transfer. *Front. Plant Sci.* 5:681
- Nonaka, S., Someya, T., Zhou, S., Takayama, M., Nakamura, K., and Ezura, H. 2017. An *Agrobacterium tumefaciens* strain with gamma-aminobutyric acid transaminase activity shows an enhanced genetic transformation ability in plants. *Sci. Rep.* 7:42649
- Ofek, M., Hadar, Y., and Minz, D. 2011. Colonization of cucumber seeds by bacteria during germination. *Environ. Microbiol.* 13:2794–2807
- Otani, H., Lee, Y.-E., Casabon, I., and Eltis, L. D. 2014. Characterization of *p*-hydroxycinnamate catabolism in a soil *Actinobacterium*. *J. Bacteriol.* 196:4293–4303
- Otani, H., Stogios, P. J., Xu, X., Nocek, B., Li, S.-N., Savchenko, A., and Eltis, L. D. 2016. The activity of CouR, a MarR family transcriptional regulator, is modulated through a novel molecular mechanism. *Nucleic Acids Res.* 44:595–607
- Overhage, J., Priefert, H., and Steinbüchel, A. 1999. Biochemical and genetic analyses of ferulic acid catabolism in *Pseudomonas* sp. Strain HR199. *Appl. Environ. Microbiol.* 65:4837–4847
- Palmer, A. G., and Blackwell, H. E. 2008. Deciphering a protolanguage for bacteria-host communication. *Nat. Chem. Biol.* 4:452–454
- Palumbo, J. D., Kado, C. I., and Phillips, D. A. 1998. An isoflavonoid-inducible efflux pump in *Agrobacterium tumefaciens* is involved in competitive colonization of roots. *J. Bacteriol.* 180:3107–3113
- Pan, C., Hu, Y., Jiang, X., and Gai, Y. 2015. Cloning, expression, crystallization and crystallographic analysis of CouR from *Rhodospseudomonas palustris*. *Acta Crystallogr. Sect. F-Struct. Biol. Commun.* 71:1416–1420
- Panikulangara, T. J., Eggers-Schumacher, G., Wunderlich, M., Stransky, H., and Schöffl, F. 2004. Galactinol synthase1. A novel heat shock factor target gene responsible for heat-induced synthesis of raffinose family oligosaccharides in *Arabidopsis*. *Plant Physiol.* 136:3148–3158
- Papenfort, K., and Bassler, B. 2016. Quorum-sensing signal-response systems in gram-negative bacteria. *Nat. Rev. Microbiol.* 14:576–588
- Parke, D. 1997. Acquisition, reorganization, and merger of genes: Novel management of the beta-ketoadipate pathway in *Agrobacterium tumefaciens*. *Fems Microbiol. Lett.* 146:3–12
- Parke, D. 1996. Characterization of PcaQ, a LysR-type transcriptional activator required for catabolism of phenolic compounds, from *Agrobacterium tumefaciens*. *J. Bacteriol.* 178:266–272
- Parke, D. 1993. Positive regulation of phenolic catabolism in *Agrobacterium tumefaciens* by the *pcaQ* gene in response to beta-carboxy-cis,cis-muconate. *J. Bacteriol.* 175:3529–3535
- Parke, D. 2000. Positive selection for mutations affecting bioconversion of aromatic compounds in *Agrobacterium tumefaciens*: analysis of spontaneous mutations in the protocatechuate 3,4-dioxygenase gene. *J. Bacteriol.* 182:6145–6153
- Parke, D. 1995. Supraoperonic clustering of *pca* genes for catabolism of the phenolic compound protocatechuate in *Agrobacterium tumefaciens*. *J. Bacteriol.* 177:3808–3817
- Parke, D., and Ornston, L. N. 2003. Hydroxycinnamate (*hca*) catabolic genes from *Acinetobacter* sp. strain ADP1 are repressed by HcaR and are induced by hydroxycinnamoyl-coenzyme A thioesters. *Appl. Environ. Microbiol.* 69:5398–5409
- Parke, D., and Ornston, L. N. 2004. Toxicity caused by hydroxycinnamoyl-Coenzyme A thioester accumulation in mutants of *Acinetobacter* sp. strain ADP1. *Appl. Environ. Microbiol.* 70:2974–2983

- Parke, D., Rivelli, M., and Ornston, L. N. 1985. Chemotaxis to aromatic and hydroaromatic acids: comparison of *Bradyrhizobium japonicum* and *Rhizobium trifolii*. *J. Bacteriol.* 163:417–422
- Pereira, C. S., Thompson, J. A., and Xavier, K. B. 2013. AI-2-mediated signalling in bacteria. *FEMS Microbiol. Rev.* 37:156–181
- Perera, I. C., and Grove, A. 2010. Molecular mechanisms of ligand-mediated attenuation of DNA binding by MarR family transcriptional regulators. *J. Mol. Cell Biol.* 2:243–254
- Perera, I. C., Lee, Y.-H., Wilkinson, S. P., and Grove, A. 2009. Mechanism for attenuation of DNA binding by MarR family transcriptional regulators by small molecule ligands. *J. Mol. Biol.* 390:1019–1029
- Pérez-Bueno, M. L., Granum, E., Pineda, M., Flors, V., Rodríguez-Palenzuela, P., López-Solanilla, E., and Barón, M. 2016. Temporal and spatial resolution of activated plant defense responses in leaves of *Nicotiana benthamiana* infected with *Dickeya dadantii*. *Front. Plant Sci.* 6
- Perrier, A., Peyraud, R., Rengel, D., Barlet, X., Lucasson, E., Gouzy, J., Peeters, N., Genin, S., and Guidot, A. 2016. Enhanced in planta fitness through adaptive mutations in EfpR, a dual regulator of virulence and metabolic functions in the plant pathogen *Ralstonia solanacearum*. *PLoS Pathog.* 12
- Peyraud, R., Cottret, L., Marmiesse, L., Gouzy, J., and Genin, S. 2016. A resource allocation trade-off between virulence and proliferation drives metabolic versatility in the plant pathogen *Ralstonia solanacearum*. *PLoS Pathog.* 12
- Pineau Camille, Guschinskaya Natalia, Robert Xavier, Gouet Patrice, Ballut Lionel, and Shevchik Vladimir E. 2014. Substrate recognition by the bacterial type II secretion system: more than a simple interaction. *Mol. Microbiol.* 94:126–140
- Pini, F., East, A. K., Appia-Ayme, C., Tomek, J., Karunakaran, R., Mendoza-Suarez, M., Edwards, A., Terpolilli, J., Rowoth, J., Downie, J. A., and Poole, P. 2017. Lux bacterial biosensors for in vivo spatiotemporal mapping of root secretion. *Plant Physiol.* :pp.01302.2016
- Pitzschke, A. 2013. *Agrobacterium* infection and plant defense—transformation success hangs by a thread. *Front. Plant Sci.* 4
- Plaggenborg, R., Overhage, J., Loos, A., Archer, J. A. C., Lessard, P., Sinskey, A. J., Steinbüchel, A., and Priefert, H. 2006. Potential of *Rhodococcus* strains for biotechnological vanillin production from ferulic acid and eugenol. *Appl. Microbiol. Biotechnol.* 72:745–755
- Plaggenborg, R., Steinbüchel, A., and Priefert, H. 2001. The coenzyme A-dependent, non- β -oxidation pathway and not direct deacetylation is the major route for ferulic acid degradation in *Delftia acidovorans*. *FEMS Microbiol. Lett.* 205:9–16
- Platt, T. G., Bever, J. D., and Fuqua, C. 2012a. A cooperative virulence plasmid imposes a high fitness cost under conditions that induce pathogenesis. *Proc. Biol. Sci.* 279:1691–1699
- Platt, T. G., Fuqua, C., and Bever, J. D. 2012b. Resource and competitive dynamics shape the benefits of public goods cooperation in a plant pathogen. *Evol. Int. J. Org. Evol.* 66:1953–1965
- Platt, T. G., Morton, E. R., Barton, I. S., Bever, J. D., and Fuqua, C. 2014. Ecological dynamics and complex interactions of *Agrobacterium* megaplasmids. *Front. Plant Sci.* 5:635
- Prim, N., Pastor, F. I. J., and Diaz, P. 2003. Biochemical studies on cloned *Bacillus* sp. BP-7 phenolic acid decarboxylase PadA. *Appl. Microbiol. Biotechnol.* 63:51–56
- Puhar, A., and Sansonetti, P. J. 2014. Type III secretion system. *Curr. Biol.* 24:R784–R791
- Qi, Z., Hamza, I., and O’Brian, M. R. 1999. Heme is an effector molecule for iron-dependent degradation of the bacterial iron response regulator (Irr) protein. *Proc. Natl. Acad. Sci. U. S. A.* 96:13056
- Quandt, J., and Hynes, M. F. 1993. Versatile suicide vectors which allow direct selection for gene replacement in gram-negative bacteria. *Gene.* 127:15–21
- Raguso, R. A., Agrawal, A. A., Douglas, A. E., Jander, G., Kessler, A., Poveda, K., and Thaler, J. S. 2015. The raison d’être of chemical ecology. *Ecology.* 96:617–630
- Raman, M., and Martin, K. 2014. One solution for cloning and mutagenesis: In-Fusion® HD Cloning Plus. *Nat. Methods.* 11

- Ramírez-Bahena, M. H., Vial, L., Lassalle, F., Diel, B., Chapulliot, D., Daubin, V., Nesme, X., and Muller, D. 2014. Single acquisition of protelomerase gave rise to speciation of a large and diverse clade within the *Agrobacterium/Rhizobium* supercluster characterized by the presence of a linear chromid. *Mol. Phylogenet. Evol.* 73:202–207
- Ravirala, R. S., Barabote, R. D., Wheeler, D. M., Reverchon, S., Tatum, O., Malouf, J., Liu, H., Pritchard, L., Hedley, P. E., Birch, P. R. J., Toth, I. K., Payton, P., and San Francisco, M. J. D. 2007. Efflux pump gene expression in *Erwinia chrysanthemi* is induced by exposure to phenolic acids. *Mol. Plant-Microbe Interact. MPMI.* 20:313–320
- Reading, N. C., and Sperandio, V. 2006. Quorum sensing: the many languages of bacteria. *FEMS Microbiol. Lett.* 254:1–11
- Reverchon, S., Muskhelivili, G., and Nasser, W. 2016. Chapter Three - Virulence program of a bacterial plant pathogen: the *Dickeya* model. Pages 51–92 in: *Progress in Molecular Biology and Translational Science, Host-Microbe Interactions*. M. San Francisco and B. San Francisco, eds. Academic Press.
- Reverchon, S., and Nasser, W. 2013. *Dickeya* ecology, environment sensing and regulation of virulence programme. *Environ. Microbiol. Rep.* 5:622–636
- Robinson, M. D., McCarthy, D. J., and Smyth, G. K. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinforma. Oxf. Engl.* 26:139–140
- Romanuk, T. N., Zhou, Y., Brose, U., Berlow, E. L., Williams, R. J., and Martinez, N. D. 2009. Predicting invasion success in complex ecological networks. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364:1743–1754
- Rondon, M. R., Ballering, K. S., and Thomas, M. G. 2004. Identification and analysis of a siderophore biosynthetic gene cluster from *Agrobacterium tumefaciens* C58. *Microbiol. Read. Engl.* 150:3857–3866
- Rong, L. J., Karcher, S. J., and Gelvin, S. B. 1991. Genetic and molecular analyses of *picA*, a plant-inducible locus on the *Agrobacterium tumefaciens* chromosome. *J. Bacteriol.* 173:5110–5120
- Rossbach, S., Kunze, K., Albert, S., Zehner, S., and Göttfert, M. 2014. The *Sinorhizobium meliloti* EmrAB efflux system is regulated by flavonoids through a TetR-like regulator (EmrR). *Mol. Plant-Microbe Interact. MPMI.* 27:379–387
- Royer, M., Larbat, R., Le Bot, J., Adamowicz, S., Nicot, P. C., and Robin, C. 2016. Tomato response traits to pathogenic *Pseudomonas* species: Does nitrogen limitation matter? *Plant Sci.* 244:57–67
- Rutherford, S. T., and Bassler, B. L. 2012. Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harb. Perspect. Med.* 2
- Saenkham, P., Eiamphungporn, W., Farrand, S. K., Vattanaviboon, P., and Mongkolsuk, S. 2007. Multiple superoxide dismutases in *Agrobacterium tumefaciens*: functional analysis, gene regulation, and influence on tumorigenesis. *J. Bacteriol.* 189:8807–8817
- Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottier, P., Camus, J. C., Cattolico, L., Chandler, M., Choisine, N., Claudel-Renard, C., Cunnac, S., Demange, N., Gaspin, C., Lavie, M., Moisan, A., Robert, C., Saurin, W., Schiex, T., Siguier, P., Thébault, P., Whalen, M., Wincker, P., Levy, M., Weissenbach, J., and Boucher, C. A. 2002. Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature.* 415:497–502
- Sans, N., Schröder, G., and Schröder, J. 1987. The Noc region of Ti plasmid C58 codes for arginase and ornithine cyclodeaminase. *Eur. J. Biochem.* 167:81–87
- Santos, M. R., Marques, A. T., Becker, J. D., and Moreira, L. M. 2014. The *Sinorhizobium meliloti* EmrR regulator is required for efficient colonization of *Medicago sativa* root nodules. *Mol. Plant-Microbe Interact. MPMI.* 27:388–399
- Sardesai, N., Lee, L.-Y., Chen, H., Yi, H., Olbricht, G. R., Stirnberg, A., Jeffries, J., Xiong, K., Doerge, R. W., and Gelvin, S. B. 2013. Cytokinins secreted by *Agrobacterium* promote transformation by repressing a plant *myb* transcription factor. *Sci. Signal.* 6:ra100
- Schaefer, A. L., Greenberg, E. P., Oliver, C. M., Oda, Y., Huang, J. J., Bittan-Banin, G., Peres, C. M., Schmidt, S., Juhászová, K., Sufirin, J. R., and Harwood, C. S. 2008. A new class of homoserine lactone quorum-sensing signals. *Nature.* 454:595–599
- Schlesinger, W. H. 1977. Carbon Balance in Terrestrial Detritus. *Annu. Rev. Ecol. Syst.* 8:51–81

- Schmitt, D., Pakusch, A. E., and Matern, U. 1991. Molecular cloning, induction and taxonomic distribution of caffeoyl-CoA 3-O-methyltransferase, an enzyme involved in disease resistance. *J. Biol. Chem.* 266:17416–17423
- Schurr, U., Schuberth, B., Aloni, R., Pradel, K. S., Schmundt, D., Jähne, B., and Ullrich, C. I. 1996. Structural and functional evidence for xylem-mediated water transport and high transpiration in *Agrobacterium tumefaciens*-induced tumors of *Ricinus communis**. *Bot. Acta.* 109:405–411
- Schüttelkopf, A. W., Aalten, D. M. F., and van Aalten, D. M. F. 2004. PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallogr. D Biol. Crystallogr.* 60:1355–63
- Schwalm, K., Aloni, R., Langhans, M., Heller, W., Stich, S., and Ullrich, C. I. 2003. Flavonoid-related regulation of auxin accumulation in *Agrobacterium tumefaciens*-induced plant tumors. *Planta.* 218:163–178
- Sengupta, S., Mukherjee, S., Basak, P., and Majumder, A. L. 2015. Significance of galactinol and raffinose family oligosaccharide synthesis in plants. *Front. Plant Sci.* 6:656
- Shams, M., Campillo, T., Lavire, C., Muller, D., Nesme, X., and Vial, L. 2012. Rapid and efficient methods to isolate, type strains and determine species of *Agrobacterium* spp. in pure culture and complex environments.
- Sheldrick, G. M. 2008. A short history of SHELX. *Acta Crystallogr. A.* 64:112–22
- Shi, C., Zhang, X., Sun, Y., Yang, M., Song, K., Zheng, Z., Chen, Y., Liu, X., Jia, Z., Dong, R., Cui, L., and Xia, X. 2016. Antimicrobial activity of ferulic acid against *Cronobacter sakazakii* and possible mechanism of action. *Foodborne Pathog. Dis.* 13:196–204
- Shih, P.-Y., Chou, S.-J., Müller, C., Halkier, B. A., Deeken, R., and Lai, E.-M. 2018. Differential roles of glucosinolates and camalexin at different stages of *Agrobacterium*-mediated transformation: GS in *Agrobacterium*-mediated transformation. *Mol. Plant Pathol.*
- Siddique, S., Endres, S., Sobczak, M., Radakovic, Z. S., Fragner, L., Grundler, F. M. W., Weckwerth, W., Tenhaken, R., and Bohlmann, H. 2014. Myo-inositol oxygenase is important for the removal of excess myo-inositol from syncytia induced by *Heterodera schachtii* in *Arabidopsis* roots. *New Phytol.* 201:476–485
- Simoh, S., Quintana, N., Kim, H. K., Choi, Y. H., and Verpoorte, R. 2009. Metabolic changes in *Agrobacterium tumefaciens*-infected *Brassica rapa*. *J. Plant Physiol.* 166:1005–1014
- Singh, R., and Maclachlan, G. 1983. Transport and metabolism of sucrose versus hexoses in relation to growth in etiolated pea stem. *Plant Physiol.* 71:531–535
- Skoog, F., and Miller, C. O. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symp. Soc. Exp. Biol.* 11:118–130
- Smith-Becker, J., Marois, E., Huguette, E. J., Midland, S. L., Sims, J. J., and Keen, N. T. 1998. Accumulation of salicylic acid and 4-hydroxybenzoic acid in phloem fluids of cucumber during systemic acquired resistance is preceded by a transient increase in phenylalanine ammonia-lyase activity in petioles and stems. *Plant Physiol.* 116:231–238
- Sokurenko, E. V., Gomulkiewicz, R., and Dykhuizen, D. E. 2006. Source–sink dynamics of virulence evolution. *Nat. Rev. Microbiol.* 4:548–555
- Somers, E., Vanderleyden, J., and Srinivasan, M. 2004. Rhizosphere bacterial signalling: a love parade beneath our feet. *Crit. Rev. Microbiol.* 30:205–240
- Someya, T., Nonaka, S., Nakamura, K., and Ezura, H. 2013. Increased 1-aminocyclopropane-1-carboxylate deaminase activity enhances *Agrobacterium tumefaciens*-mediated gene delivery into plant cells. *MicrobiologyOpen.* 2:873–880
- Spanu, P. D., and Panstruga, R. 2017. Editorial: Biotrophic Plant-Microbe Interactions. *Front. Plant Sci.* 8
- Stachel, S. E., Messens, E., Montagu, M. V., and Zambryski, P. 1985. Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. *Nat. USA.*
- Steinauer, K., Chatzinotas, A., and Eisenhauer, N. 2016. Root exudate cocktails: the link between plant diversity and soil microorganisms? *Ecol. Evol.* 6:7387–7396
- Stuble, K. L., and Souza, L. 2016. Priority effects: natives, but not exotics, pay to arrive late. *J. Ecol.* 104:987–993
- Subramoni, S., Nathoo, N., Klimov, E., and Yuan, Z.-C. 2014. *Agrobacterium tumefaciens* responses to plant-derived signaling molecules. *Front. Plant Sci.* 5

- Suvorova, I. A., Korostelev, Y. D., and Gelfand, M. S. 2015. GntR family of bacterial transcription factors and their DNA binding motifs: structure, positioning and co-evolution. *PLoS ONE*. 10:e0132618
- Taberman, H., Andberg, M., Parkkinen, T., Jänis, J., Penttilä, M., Hakulinen, N., Koivula, A., and Rouvinen, J. 2014a. Structure and function of a decarboxylating *Agrobacterium tumefaciens* keto-deoxy-d-galactarate dehydratase. *Biochemistry (Mosc.)*. 53:8052–8060
- Taberman, H., Andberg, M., Parkkinen, T., Richard, P., Hakulinen, N., Koivula, A., and Rouvinen, J. 2014b. Purification, crystallization and preliminary X-ray diffraction analysis of a novel keto-deoxy-D-galactarate (KDG) dehydratase from *Agrobacterium tumefaciens*. *Acta Crystallogr. Sect. F Struct. Biol. Commun.* 70:49–52
- Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., Yamaguchi-Shinozaki, K., and Shinozaki, K. 2002. Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. *Plant J. Cell Mol. Biol.* 29:417–426
- Tanaka, Y., Sano, T., Tamaoki, M., Nakajima, N., Kondo, N., and Hasezawa, S. 2006. Cytokinin and auxin inhibit abscisic acid-induced stomatal closure by enhancing ethylene production in *Arabidopsis*. *J. Exp. Bot.* 57:2259–2266
- Thanbichler, M., Iniesta, A. A., and Shapiro, L. 2007. A comprehensive set of plasmids for vanillate- and xylose-inducible gene expression in *Caulobacter crescentus*. *Nucleic Acids Res.* 35:e137
- Tomlinson, A. D., Ramey-Hartung, B., Day, T. W., Merritt, P. M., and Fuqua, C. 2010. *Agrobacterium tumefaciens* ExoR represses succinoglycan biosynthesis and is required for biofilm formation and motility. *Microbiology*. 156:2670–2681
- Torres, M. A., Jones, J. D. G., and Dangl, J. L. 2006. Reactive oxygen species signaling in response to pathogens. *Plant Physiol.* 141:373–378
- Trautwein, K., Wilkes, H., and Rabus, R. 2012. Proteogenomic evidence for β -oxidation of plant-derived 3-phenylpropanoids in “*Aromatoleum aromaticum*” EbN1. *Proteomics*. 12:1402–1413
- Tremblay, G., Gagliardo, R., Chilton, W. S., and Dion, P. 1987. Diversity among opine-utilizing bacteria: identification of coryneform isolates. *Appl. Environ. Microbiol.* 53:1519–1524
- Uliczka, F., Pisano, F., Kochut, A., Opitz, W., Herbst, K., Stolz, T., and Dersch, P. 2011. Monitoring of gene expression in bacteria during infections using an adaptable set of bioluminescent, fluorescent and colorigenic fusion vectors. *PLOS ONE*. 6:e20425
- Vacheron, J., Moënne-Loccoz, Y., Dubost, A., Gonçalves-Martins, M., Muller, D., and Prigent-Combaret, C. 2016. Fluorescent *Pseudomonas* strains with only few plant-beneficial properties are favored in the maize rhizosphere. *Front. Plant Sci.* 7:1212
- Valentini, M., Gonzalez, D., Mavridou, D. A., and Filloux, A. 2017. Lifestyle transitions and adaptive pathogenesis of *Pseudomonas aeruginosa*. *Curr. Opin. Microbiol.* 41:15–20
- Vaudequin-Dransart, V., Petit, A., Poncet, C., Ponsonnet, C., Nesme, X., Jones, J. B., Bouzar, H., Chilton, W. S., and Dessaux, Y. 1995. Novel Ti plasmids in *Agrobacterium* strains isolated from fig tree and chrysanthemum tumors and their opinelike molecules. *Mol. Plant-Microbe Interact. MPMI*. 8:311–321
- Veena, Jiang, H., Doerge, R. W., and Gelvin, S. B. 2003. Transfer of T-DNA and Vir proteins to plant cells by *Agrobacterium tumefaciens* induces expression of host genes involved in mediating transformation and suppresses host defense gene expression. *Plant J. Cell Mol. Biol.* 35:219–236
- Vehkala, M., Shubin, M., Connor, T. R., Thomson, N. R., and Corander, J. 2015. Novel R pipeline for analyzing biolog phenotypic microarray data. *PLoS ONE*. 10:e0118392
- Velmourougane, K., Saxena, G., and Prasanna, R. 2017. Plant-microbe interactions in the rhizosphere: mechanisms and their ecological benefits. Pages 193–219 in: *Plant-Microbe Interactions in Agro-Ecological Perspectives*, Springer, Singapore.
- Venturi, V., Zennaro, F., Degrassi, G., Okeke, B. C., and Bruschi, C. V. 1998. Genetics of ferulic acid bioconversion to protocatechuic acid in plant-growth-promoting *Pseudomonas putida* WCS358. *Microbiology*. 144:965–973
- Verbon, E. H., Trapet, P. L., Stringlis, I. A., Kruijs, S., Bakker, P. A. H. M., and Pieterse, C. M. J. 2017. Iron and immunity. *Annu. Rev. Phytopathol.* 55:355–375

- Verplaetse, E., Slamti, L., Gohar, M., and Lereclus, D. 2015. Cell differentiation in a *Bacillus thuringiensis* population during planktonic growth, biofilm formation, and host infection. *mBio*. 6:e00138-15
- Vindal, V., Ranjan, S., and Ranjan, A. 2007. In silico analysis and characterization of GntR family of regulators from *Mycobacterium tuberculosis*. *Tuberc. Edinb. Scotl.* 87:242–247
- Vogel, J., Normand, P., Thioulouse, J., Nesme, X., and Grundmann, G. L. 2003. Relationship between spatial and genetic distance in *Agrobacterium* spp. in 1 cubic centimeter of soil. *Appl. Environ. Microbiol.* 69:1482–1487
- Vogt, T. 2010. Phenylpropanoid Biosynthesis. *Mol. Plant.* 3:2–20
- Walker, T. S., Bais, H. P., Grotewold, E., and Vivanco, J. M. 2003. Root exudation and rhizosphere biology. *Plant Physiol.* 132:44–51
- Wang, S., Yao, J., Zhou, B., Yang, J., Chaudry, M. T., Wang, M., Xiao, F., Li, Y., and Yin, W. 2018. Bacteriostatic effect of quercetin as an antibiotic alternative *in vivo* and its antibacterial mechanism *in vitro*. *J. Food Prot.* 81:68–78
- Wei, Z., Yang, T., Friman, V.-P., Xu, Y., Shen, Q., and Jousset, A. 2015. Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. *Nat. Commun.* 6
- Westover, B. P., Buhler, J. D., Sonnenburg, J. L., and Gordon, J. I. 2005. Operon prediction without a training set. *Bioinforma. Oxf. Engl.* 21:880–888
- Wetzel, M. E., Kim, K.-S., Miller, M., Olsen, G. J., and Farrand, S. K. 2014. Quorum-dependent mannopine-inducible conjugative transfer of an *Agrobacterium* opine-catabolic plasmid. *J. Bacteriol.* 196:1031–1044
- Whitehead, N. A., Barnard, A. M., Slater, H., Simpson, N. J., and Salmond, G. P. 2001. Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol. Rev.* 25:365–404
- Wichelecki, D. J., Vetting, M. W., Chou, L., Al-Obaidi, N., Bouvier, J. T., Almo, S. C., and Gerlt, J. A. 2015. ATP-binding Cassette (ABC) transport system solute-binding protein-guided identification of novel d-altritol and galactitol catabolic pathways in *Agrobacterium tumefaciens* C58. *J. Biol. Chem.* 290:28963–28976
- Wiseman, T., Williston, S., Brandts, J. F., and Lin, L. N. 1989. Rapid measurement of binding constants and heats of binding using a new titration calorimeter. *Anal Biochem.* 179:131–137
- Wobus, U., and Weber, H. 1999. Sugars as signal molecules in plant seed development. *Biol. Chem.* 380:937–944
- Wolf, J. van der, and Boer, S. H. D. 2015. Phytopathogenic bacteria. Pages 65–77 in: *Principles of Plant-Microbe Interactions*, Springer, Cham.
- Wood, D. W., Setubal, J. C., Kaul, R., Monks, D. E., Kitajima, J. P., Okura, V. K., Zhou, Y., Chen, L., Wood, G. E., Almeida, N. F., Woo, L., Chen, Y., Paulsen, I. T., Eisen, J. A., Karp, P. D., Bovee, D., Chapman, P., Clendinning, J., Deatherage, G., Gillet, W., Grant, C., Kutuyavin, T., Levy, R., Li, M.-J., McClelland, E., Palmieri, A., Raymond, C., Rouse, G., Saenphimmachak, C., Wu, Z., Romero, P., Gordon, D., Zhang, S., Yoo, H., Tao, Y., Biddle, P., Jung, M., Krespan, W., Perry, M., Gordon-Kamm, B., Liao, L., Kim, S., Hendrick, C., Zhao, Z.-Y., Dolan, M., Chumley, F., Tingey, S. V., Tomb, J.-F., Gordon, M. P., Olson, M. V., and Nester, E. W. 2001. The Genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. *Science.* 294:2317–2323
- Wu, C.-F., Lin, J.-S., Shaw, G.-C., and Lai, E.-M. 2012. Acid-induced type VI secretion system is regulated by ExoR-ChvG/ChvI signaling cascade in *Agrobacterium tumefaciens*. *PLOS Pathog.* 8:e1002938
- Xu, X. Q., and Pan, S. Q. 2000. An *Agrobacterium* catalase is a virulence factor involved in tumorigenesis. *Mol. Microbiol.* 35:407–414
- Yamazaki, A., Li, J., Zeng, Q., Khokhani, D., Hutchins, W. C., Yost, A. C., Biddle, E., Toone, E. J., Chen, X., and Yang, C.-H. 2012. Derivatives of plant phenolic compound affect the type III secretion system of *Pseudomonas aeruginosa* via a GacS-GacA two-component signal transduction system. *Antimicrob. Agents Chemother.* 56:36–43
- Yang, S., Peng, Q., Francisco, M. S., Wang, Y., Zeng, Q., and Yang, C.-H. 2008. Type III Secretion System genes of *Dickeya dadantii* 3937 are induced by plant phenolic acids. *PLOS ONE.* 3:e2973
- Yao, J., and Allen, C. 2006. Chemotaxis is required for virulence and competitive fitness of the bacterial wilt pathogen *Ralstonia solanacearum*. *J. Bacteriol.* 188:3697–3708

- Yuan, Z.-C., Edlind, M. P., Liu, P., Saenkham, P., Banta, L. M., Wise, A. A., Ronzone, E., Binns, A. N., Kerr, K., and Nester, E. W. 2007. The plant signal salicylic acid shuts down expression of the *vir* regulon and activates quorum-quenching genes in *Agrobacterium*. *Proc. Natl. Acad. Sci. U. S. A.* 104:11790–11795
- Yuan, Z.-C., Haudecoeur, E., Faure, D., Kerr, K. F., and Nester, E. W. 2008. Comparative transcriptome analysis of *Agrobacterium tumefaciens* in response to plant signal salicylic acid, indole-3-acetic acid and γ -amino butyric acid reveals signalling cross-talk and *Agrobacterium*–plant co-evolution. *Cell. Microbiol.* 10:2339–2354
- Zanker, H., von Lintig, J., and Schröder, J. 1992. Opine transport genes in the octopine (*occ*) and nopaline (*noc*) catabolic regions in Ti plasmids of *Agrobacterium tumefaciens*. *J. Bacteriol.* 174:841–849
- Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., da Rocha, U. N., Shi, S., Cho, H., Karaoz, U., Loqué, D., Bowen, B. P., Firestone, M. K., Northen, T. R., and Brodie, E. L. 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nat. Microbiol.* 3:470–480
- Zhang, N., Wang, D., Liu, Y., Li, S., Shen, Q., and Zhang, R. 2014. Effects of different plant root exudates and their organic acid components on chemotaxis, biofilm formation and colonization by beneficial rhizosphere-associated bacterial strains. *Plant Soil.* 374:689–700
- Zhang, X., Zhang, L., Dong, F., Gao, J., Galbraith, D. W., and Song, C. P. 2001. Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba*. *Plant Physiol.* 126:1438–1448
- Zhang, Y., Lee, C.-W., Wehner, N., Imdahl, F., Svetlana, V., Weiste, C., Dröge-Laser, W., and Deeken, R. 2015a. Regulation of oncogene expression in T-DNA-transformed host plant cells. *PLoS Pathog.* 11:e1004620
- Zhang, Y., Li, J., Zhang, W., Wang, R., Qiu, Q., Luo, F., Hikichi, Y., Ohnishi, K., and Ding, W. 2017. Ferulic acid, but not all hydroxycinnamic acids, is a novel T3SS inducer of *Ralstonia solanacearum* and promotes its infection process in host plants under hydroponic condition. *Front. Plant Sci.* 8
- Zhang, Y., Luo, F., Wu, D., Hikichi, Y., Kiba, A., Igarashi, Y., Ding, W., and Ohnishi, K. 2015b. PrhN, a putative *marR* family transcriptional regulator, is involved in positive regulation of type III secretion system and full virulence of *Ralstonia solanacearum*. *Front. Microbiol.* 6:357
- Zhao, J., and Binns, A. N. 2011. Characterization of the *mmsAB-araD1* (*gguABC*) genes of *Agrobacterium tumefaciens*. *J. Bacteriol.* 193:6586–6596
- Zhao, J., and Binns, A. N. 2014. GxySBA ABC transporter of *Agrobacterium tumefaciens* and its role in sugar utilization and *vir* gene expression. *J. Bacteriol.* 196:3150–3159
- Zhao, J., and Binns, A. N. 2016. Involvement of *Agrobacterium tumefaciens* galacturonate Tripartite ATP-Independent Periplasmic (TRAP) transporter GaaPQM in virulence gene expression. *Appl. Environ. Microbiol.* 82:1136–1146
- Zhao, Z., and Moghadasian, M. H. 2008. Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. *Food Chem.* 109:691–702

Annexes

Annexe 1. Publication n°1 “Genomic characterization of *Ensifer aridi*, a proposed new species of nitrogen-fixing rhizobium recovered from Asian, African and American deserts”

Ces travaux ont fait l’objet d’une publication en 2017 dans le journal **BMC Genomics**. Cette publication traite de la description, à partir de différents isolats provenant de déserts basés sur trois continents différents, d’une nouvelle espèce nommée *Ensifer aridii*. La caractérisation de cette espèce est basée sur des analyses génomiques menant à la découverte de fonctions importantes dans l’adaptation à différents stress (alcalin, sécheresse, forte salinité et forte température) mais est aussi basée sur la description de fonctions métaboliques communes. J’ai contribué à ces travaux en réalisant une partie des expériences de phénotypages métaboliques par l’analyse de la croissance sur de nombreux substrats en utilisant des puces phénotypiques (Biolog) et en confirmant ces résultats au niveau de la croissance bactérienne sur certaines ressources carbonées. J’ai aussi participé à l’analyse des profils plasmidiques des différentes souches (Gel eckhardt).

RESEARCH ARTICLE

Open Access



Genomic characterization of *Ensifer aridi*, a proposed new species of nitrogen-fixing rhizobium recovered from Asian, African and American deserts

Antoine Le Quéré^{1,2*} , Nisha Tak³, Hukam Singh Gehlot³, Celine Lavire^{4,5}, Thibault Meyer^{4,5}, David Chapulliot^{4,5}, Sonam Rathi³, Ilham Sakrouhi¹, Guadalupe Rocha⁶, Marine Rohmer⁷, Dany Severac⁷, Abdelkarim Filali-Maltouf¹ and Jose-Antonio Munive⁶

Abstract

Background: Nitrogen fixing bacteria isolated from hot arid areas in Asia, Africa and America but from diverse leguminous plants have been recently identified as belonging to a possible new species of *Ensifer* (*Sinorhizobium*). In this study, 6 strains belonging to this new clade were compared with *Ensifer* species at the genome-wide level. Their capacities to utilize various carbon sources and to establish a symbiotic interaction with several leguminous plants were examined.

Results: Draft genomes of selected strains isolated from Morocco (Merzouga desert), Mexico (Baja California) as well as from India (Thar desert) were produced. Genome based species delineation tools demonstrated that they belong to a new species of *Ensifer*. Comparison of its core genome with those of *E. meliloti*, *E. medicae* and *E. fredii* enabled the identification of a species conserved gene set. Predicted functions of associated proteins and pathway reconstruction revealed notably the presence of transport systems for octopine/nopaline and inositol phosphates. Phenotypic characterization of this new desert rhizobium species showed that it was capable to utilize malonate, to grow at 48 °C or under high pH while NaCl tolerance levels were comparable to other *Ensifer* species. Analysis of accessory genomes and plasmid profiling demonstrated the presence of large plasmids that varied in size from strain to strain. As symbiotic functions were found in the accessory genomes, the differences in symbiotic interactions between strains may be well related to the difference in plasmid content that could explain the different legumes with which they can develop the symbiosis.

Conclusions: The genomic analysis performed here confirms that the selected rhizobial strains isolated from desert regions in three continents belong to a new species. As until now only recovered from such harsh environment, we propose to name it *Ensifer aridi*. The presented genomic data offers a good basis to explore adaptations and functionalities that enable them to adapt to alkalinity, low water potential, salt and high temperature stresses. Finally, given the original phylogeographic distribution and the different hosts with which it can develop a beneficial symbiotic interaction, *Ensifer aridi* may provide new biotechnological opportunities for degraded land restoration initiatives in the future.

Keywords: Rhizobium- legume symbiosis, *Ensifer*, Desert, Adaptation, Comparative genomics

* Correspondence: antoine.le-quere@ird.fr

¹Laboratoire de Microbiologie et Biologie Moléculaire, Université Mohammed V, Av Ibn Batouta BP 1014, Rabat, Morocco

²IRD, Laboratoire des Symbioses Tropicales et Méditerranéennes UMR113,

IRD/INRA/CIRAD/Montpellier SupAgro/Université de Montpellier, TA A82/J,

Campus International de Baillarguet, 34398 Montpellier, Cedex 5, France

Full list of author information is available at the end of the article



© The Author(s). 2017 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

Annexe 2. Publication n°2 “Essential oils of *Origanum compactum* and *Thymus vulgaris* exert a protective effect against the phytopathogen *Allorhizobium vitis*”

Ces travaux ont fait l’objet d’une publication publiée en 2017 dans le journal **Environmental Science and Pollution Research**.

Dans cette publication, il est décrit comment différentes huiles essentielles en particulier celles extraites à partir de *Origanum compactum* (Origan) et *Thymus vulgaris* (Thym) peuvent être utilisées pour leurs capacités antimicrobiennes afin de lutter contre le phytopathogène *Allorhizobium vitis*. J’ai contribué à cette étude en construisant le vecteur fluorescent (pOT1eM) et en réalisant les observations en microscopie confocale permettant de visualiser l’effet sur l’intégrité membranaire bactérienne des huiles essentielles. J’ai aussi réalisé les tests de détermination des concentrations minimales inhibitrices (CMI) pour le carvacrol et le thymol qui sont des composés majoritaires des deux huiles essentielles analysées.



Essential oils of *Origanum compactum* and *Thymus vulgaris* exert a protective effect against the phytopathogen *Allorhizobium vitis*

Khaoula Habbadi^{1,2,3} · Thibault Meyer³ · Ludovic Vial³ · Vincent Gaillard³ · Rachid Benkirane² · Abdellatif Benbouazza¹ · Isabelle Kerzaon³ · El Hassan Achbani¹ · Céline Lavire³

Received: 10 February 2017 / Accepted: 11 December 2017
© Springer-Verlag GmbH Germany, part of Springer Nature 2017

Abstract

Allorhizobium (Agrobacterium) vitis is a host-specific pathogenic bacterium that causes grapevine crown gall disease, affecting vine growth and production worldwide. The antibacterial activities of different aromatic plant essential oils were tested in vitro and in planta against *A. vitis*. Among the essential oils tested, those of *Origanum compactum* and *Thymus vulgaris* showed the most significant in vitro antibacterial activities, with a MIC of 0.156 and 0.312 mg/mL, respectively. A synergistic effect of these two essential oils (1:1) was observed and confirmed by the checkerboard test. Carvacrol (61.8%) and thymol (47.8%) are, respectively, the major compounds in the essential oils of *O. compactum* and *T. vulgaris* and they have been shown to be largely responsible for the antibacterial activities of their corresponding essential oils. Results obtained in vitro were reinforced by an in planta pathogenicity test. A mixture of *O. compactum* and *T. vulgaris* essential oils (1:1), inoculated into the injured stem of a tomato plant and a grapevine at 0.312 mg/mL as a preventive treatment, reduced both the number of plants developing gall symptoms and the size of the tumors.

Keywords Crown gall · *Allorhizobium vitis* · Biocontrol · Essential oils · *Origanum compactum* · *Thymus vulgaris* · *Agrobacterium*

Introduction

Crown gall is one of the most important plant bacterial diseases, caused by bacteria belonging to the genus *Agrobacterium* (Sawada et al. 1993). This disease could be

considered akin to a “plant cancer,” where the bacterium hijacks the plant’s cellular-level machinery to serve its own growth and tumor development (Zupan and Zambryski 1995). The tumorigenicity of agrobacteria is determined by the presence of a large tumor-inducing (Ti) plasmid. Part of the plasmid, the T-DNA (transferred DNA), is transferred and integrated into the plant cell genome, leading to gall development. In addition to the genes involved in tumorigenesis, the T-DNA also contains genes responsible for the biosynthesis of opines, specific carbon, and nitrogen sources for *Agrobacterium* development (Akiyoshi et al. 1984; Lacroix and Citovsky 2013).

The grapevine is very sensitive to this disease (Burr et al. 1998) which, in this case, is mostly caused by *Agrobacterium vitis* (Ophel and Kerr 1990), recently renamed *Allorhizobium vitis* (Mousavi et al. 2014, 2015). Whereas older vines usually survive the infection, although they show stress symptoms, young vines that develop a tumor at their graft union often die (Süle and Burr 1998). In both cases, the damage could threaten the harvest and the grape quality in the absence of appropriate crop management (Burr et al. 1998). *A. vitis* can survive in the plant tissues and remain asymptomatic until

Responsible editor: Philippe Garrigues

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11356-017-1008-9) contains supplementary material, which is available to authorized users.

✉ El Hassan Achbani
achbani105@gmail.com

✉ Céline Lavire
celine.lavire@univ-lyon1.fr

¹ Laboratoire de recherche et de protection des plantes, URPP-INRA-Meknes, Meknes, Morocco

² Laboratoire de Botanique, Biotechnologie, et Protection des Plantes, Faculté des Sciences, Kenitra, Morocco

³ Université de Lyon, Université Lyon 1, CNRS, UMR 5557, Ecologie Microbienne, INRA, UMR1418, 69622 Villeurbanne CEDEX, France

Résumé

Les agrobactéries établissent des relations à long terme avec les plantes et ce, dans deux styles de vie différents, rhizosphérique et pathogène (galle du collet). Dans ce mode de vie, les bactéries modifient génétiquement leur hôte et se créent ainsi une niche écologique spécifique (tumeur). La transition entre les deux styles de vie est déclenchée par la perception de signaux végétaux, parmi lesquels des acides hydroxycinnamiques (HCAs) comme l'acide férulique. Or dans l'espèce *Agrobacterium fabrum*, des gènes spécifiques permettent la dégradation des HCAs.

Nous avons émis l'hypothèse que cette dégradation était un signal de proximité de la plante et influençait alors des fonctions importantes pour l'interaction avec celle-ci. Nous avons caractérisé la régulation de la dégradation des HCAs, évalué son rôle dans la valeur sélective d'*A. fabrum*, et suggéré son importance dans la transition entre les styles de vie rhizosphérique et pathogène. Nous avons montré que la dégradation des HCAs module le métabolisme carboné bactérien, notamment l'utilisation d'acides aminés et d'oligosaccharides de la famille du raffinose. Nous avons caractérisé la protéine MelB qui permet l'import de ces sucres, du mélibiose et du galactinol. Leur utilisation est importante pour la colonisation des plantes dès la germination. L'analyse de l'expression des gènes et du métabolisme bactérien en présence d'un composé signal de la plante, nous a révélé de nouveaux déterminants importants pour l'écologie de ce phytopathogène, notamment des facteurs de transcription. En outre, cette analyse a confirmé l'importance des échanges cellulaires et de déterminants impliqués dans la compétition bactérienne.

Mot clef : *Agrobacterium fabrum*, acides hydroxycinnamiques, interaction plantes-bactéries, oligosaccharides de la famille du raffinose (RFO), transition écologique, régulation transcriptionnelle.