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# Etude polyphasique de *Xanthomonas hortorum* à l'ère de la génomique : focus sur *X. hortorum* pv. *vitians*, l'agent causal de la bactériose foliaire de la laitue

Lucas Morinière

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**THÈSE de DOCTORAT DE L'UNIVERSITÉ DE LYON**  
opérée au sein de  
**l'Université Claude Bernard Lyon 1**

**École Doctorale N° 341**  
**Écosystèmes, Évolution, Modélisation, Microbiologie (E2M2)**

**Spécialité de doctorat : Écologie microbienne**  
**Discipline : Biologie**

Soutenue publiquement le 09/07/2021, par :

**Lucas MORINIÈRE**

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**ÉTUDE POLYPHASIQUE DE *XANTHOMONAS HORTORUM***  
**À L'ÈRE DE LA GÉNOMIQUE :**  
**FOCUS SUR *X. HORTORUM* PV. *VITIANS*, AGENT CAUSAL DE**  
**LA BACTÉRIOSE FOLIAIRE DE LA LAITUE**

---

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*« I never met a microbe I didn't like »*

Stanley Falkow (1934 – 2018)



**Étude polyphasique de *Xanthomonas hortorum* à l'ère de la génomique :  
focus sur *X. hortorum* pv. *vitians*, agent causal de la  
bactériose foliaire de la laitue**

par

**Lucas MORINIÈRE**

au sein du laboratoire

UMR CNRS 5557 INRAE 1418 Écologie Microbienne

Université Claude Bernard Lyon 1

43, Bd du 11 novembre 1918, 69622 Villeurbanne

## **Résumé**

Le genre *Xanthomonas* représente un réservoir majeur d'espèces bactériennes phytopathogènes. Parmi celles-ci, les différents pathovars de *X. hortorum* s'attaquent à une grande variété d'espèces végétales phylogénétiquement distantes. La bactériose foliaire de la laitue, causée par *X. hortorum* pv. *vitians*, est l'une des de ces pathologies dont les conséquences pour les cultures sont les plus importantes à l'échelle mondiale. Toutefois, les questions liées à l'étiologie de cette maladie infectieuse végétale nécessitaient une étude approfondie pour être élucidées. De plus, les aspects moléculaires et génétiques de l'interaction hôte-pathogène et de l'adaptation environnementale n'avaient jamais été investigués. À travers une approche mixte alliant les principes de la taxonomie polyphasique et le criblage par Tn-seq des gènes contribuant à la valeur sélective de *X. hortorum* pv. *vitians* en conditions *in vitro* et *in planta*, cette étude entend répondre à ces problématiques en se reposant sur les dernières évolutions dans le champ de la génomique. La taxonomie de *X. hortorum* a tout d'abord été remaniée en proposant sa combinaison avec *X. cynarae*. Ensuite, le génome essentiel de *X. hortorum* pv. *vitians* a été caractérisé et distingué entre gènes conservés et gènes accessoires « égoïstes » acquis par transferts horizontaux. Enfin, les gènes et fonctions importants pour la valeur sélective du pathogène lors des phases successives épiphytes et endophytes de l'interaction avec la laitue ont été identifiés. Ces travaux révèlent la complexité insoupçonnée de l'espèce *X. hortorum* et éclairent les bases génétiques de la plasticité écologique de *X. hortorum* pv. *vitians*.

**Mots-clés** : *Xanthomonas hortorum* ; bactériose de la laitue ; taxonomie ; Tn-seq ; génomique



**A polyphasic study of *Xanthomonas hortorum* in the genomic era:  
focus on *X. hortorum* pv. *vitians*, the causal agent of  
the bacterial leaf spot of lettuce**

by  
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at  
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University Claude Bernard Lyon 1  
43, Bd du 11 novembre 1918, 69622 Villeurbanne

**Abstract**

The genus *Xanthomonas* constitutes a major reservoir of phytopathogenic bacterial species. Among them, the pathovars of *X. hortorum* attacks a wide variety of phylogenetically distant botanical species. The bacterial leaf spot of lettuce caused by *X. hortorum* pv. *vitians* is one of the most destructive of these diseases at a worldwide scale. However, substantial interrogations remain regarding the etiology of this disease. Moreover, the molecular and genetic determinants sustaining the host-pathogen interaction and the environmental adaptation have never been investigated. Through a mixed approach combining the principles of polyphasic taxonomy and the genome-wide Tn-seq screening of genes contributing to the fitness of *X. hortorum* pv. *vitians* in *in vitro* and *in planta* conditions, this study aims at solving these issues using the latest developments in the field of genomics. First, investigations on the taxonomy of this phytopathogenic bacteria led to reconsider the genomic diversity existing within the species and to propose the combination of *X. hortorum* and *X. cynarae*. Then, the essential genome of *X. hortorum* pv. *vitians* was characterized and essential genes were discriminated between core and “selfish” accessory ones, the latter being acquired through horizontal gene transfer events. At last, a comprehensive overview of the genes and functions required for the fitness of *X. hortorum* pv. *vitians* during the successive epiphytic and endophytic phases of the lettuce infection was provided. This work reveals the unsuspected complexity of the *X. hortorum* species and enlighten the genetic bases of the ecological plasticity of *X. hortorum* pv. *vitians*.

**Keywords:** *Xanthomonas hortorum* ; bacterial leaf spot of lettuce ; taxonomy ; Tn-seq ; genomics



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# TABLE DES MATIÈRES

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<b>PROLOGUE</b>	p.21
<b>INTRODUCTION GÉNÉRALE</b>	p.25
<b>SYNTHÈSE BIBLIOGRAPHIQUE</b>	p.33
1. Évolutions passées et futures des concepts et définitions d'espèce procaryote	p.33
2. Processus historique de la reconnaissance taxonomique de <i>Xanthomonas hortorum</i> en tant qu'espèce unique	p.37
3. Étendue insoupçonnée du spectre d'hôte de <i>Xanthomonas hortorum</i>	p.40
4. Facteurs épidémiologiques et exploration du cycle de vie environnemental	p.46
5. Interactions avec l'hôte végétal et son système immunitaire	p.49
<b>CHAPITRE I : Taxonomie de <i>Xanthomonas hortorum</i> et diversité de <i>X. hortorum</i> pv. <i>vitians</i></b>	p.55
<b>Préambule</b>	p.55
<b>Abstract</b>	p.56
<b>Introduction</b>	p.56
<b>Experimental procedures</b>	p.57
Bacterial strains, isolation procedure and growth conditions	p.57
Pathogenicity tests	p.57
Multilocus sequence analysis	p.60
Genome sequencing, assembly, annotation and acquisition	p.60
Phylogenomic analysis	p.60
Overall Genome Relatedness Indices (OGRI) calculations	p.60
Phenotypic profiling	p.60
<b>Results</b>	p.61
Pathogenicity assays	p.61
Multilocus sequence analysis	p.61
Whole-genome based phylogenies	p.63
Pairwise overall genome relatedness indices comparisons	p.63
Phenotype	p.65
<b>Discussion</b>	p.65
<b>Taxonomy</b>	p.67
Emended description of <i>Xanthomonas hortorum</i> Vauterin <i>et al.</i> 1995	p.67
<i>X. hortorum</i> pv. <i>vitians</i> (Brown 1918) comb. nov.	p.67
<i>X. hortorum</i> pv. <i>cynarae</i> (Trébaol <i>et al.</i> 2000) comb. nov.	p.68
<i>X. hortorum</i> pv. <i>gardneri</i> (ex Sutic 1957) comb. nov.	p.68
<i>X. hortorum</i> pv. <i>hederae</i> (Arnaud 1920)Vauterin <i>et al.</i> 1995	p.68
<i>X. hortorum</i> pv. <i>pelargonii</i> (Brown 1923) Vauterin <i>et al.</i> 1995	p.68
<i>X. hortorum</i> pv. <i>carotae</i> (Kendrick 1934) Vauterin <i>et al.</i> 1995	p.68
<i>X. hortorum</i> pv. <i>taraxaci</i> (Niederhauser 1943) Vauterin <i>et al.</i> 1995	p.69
<b>References</b>	p.69

## CHAPITRE II : Caractérisation et conservation du génome essentiel *in vitro* de *X. hortorum* pv. *vitians* p.73

<b>Préambule</b>	p.73
<b>Abstract</b>	p.74
<b>Data summary</b>	p.74
<b>Impact statement</b>	p.75
<b>Introduction</b>	p.75
<b>Methods</b>	p.76
Bacterial strains, plasmids and growth conditions	p.76
Complete genome sequencing, assembly and annotation	p.76
Construction of the transposon library, DNA preparation and sequencing	p.76
Assessment of genome essentiality	p.77
Gene set enrichment analysis and cellular pathway mapping	p.77
Analysis of essential genes conservation at multiple taxonomic levels	p.77
<b>Results and discussion</b>	p.78
Complete genome sequencing of <i>X. hortorum</i> pv. <i>vitians</i> LM16734 and design of a highly saturated transposon mutant library	p.78
<i>In vitro</i> identification of essential and fitness-related genes	p.79
Analysis of essential genes of <i>X. hortorum</i> pv. <i>vitians</i> associated with core bacterial features	p.80
Conservation of essential genes among <i>Xanthomonadaceae</i> and investigations of specific essential features and mobile genetic elements	p.82
<b>Conclusions</b>	p.85
<b>References</b>	p.85

## CHAPITRE III : Bases moléculaires de l'adaptation de *X. hortorum* pv. *vitians* à son hôte végétal p.91

<b>Préambule</b>	p.91
<b>Abstract</b>	p.93
<b>Introduction</b>	p.94
<b>Methods</b>	p.96
Bacterial strains, growth conditions and Tn-seq library generation	p.96
Monitoring of bacterial population <i>in planta</i>	p.96
Transposon library inoculation in lettuce	p.96
Bacterial cells recovery from lettuce leaves	p.97
DNA extraction and library preparation	p.97
Sequencing read data pre-treatment	p.98
Assesment of gene fitness contribution in inoculum and <i>in planta</i>	p.98
Gene annotation, Gene Ontology term enrichment and KEGG pathway mapping	p.99

<b>Results &amp; Discussion</b>	<i>p.100</i>
Tn-seq experimental design validation and bottleneck effect assessment	<i>p.100</i>
Identification of 170 conditionally-essential genes <i>in planta</i> mostly involved in anabolism	<i>p.103</i>
The type II secretion system and carbohydrates and iron-siderophore scavenging systems ensure nutrition during lettuce infection	<i>p.109</i>
The LPS O-antigen is required to evade the plant immune response	<i>p.112</i>
Protection against the oxidative burst is a key feature for surviving the immune response	<i>p.114</i>
<b>Conclusion</b>	<i>p.116</i>
<b>References</b>	<i>p.117</i>
<b>DICUSSION GÉNÉRALE &amp; PERSPECTIVES</b>	<i>p.125</i>
1. Rappel du contexte et des problématiques	<i>p.125</i>
2. Histoire évolutive et émergence de <i>Xanthomonas hortorum</i>	<i>p.126</i>
3. Bases moléculaires de l'adaptation aux hôtes végétaux et à l'environnement	<i>p.132</i>
<b>CONCLUSION GÉNÉRALE</b>	<i>p.139</i>
<b>BIBLIOGRAPHIE</b>	<i>p.141</i>
<b>ANNEXES</b>	<i>p.159</i>
Chapitre I : Supplementary data	<i>p.159</i>
Chapitre II : Supplementary data	<i>p.163</i>
Chapitre III : Supplementary data	<i>p.181</i>



# LISTE DES ABBRÉVIATIONS

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ANI	= <i>Average Nucleotide Identity</i>
BLSL	= <i>Bacterial Leaf Spot of Lettuce</i>
CFBP	= <i>Collection Française des Bactéries associées aux Plantes</i>
COG	= <i>Cluster of Orthologous Groups</i>
cv.	= <i>cultivar</i>
CWDE	= <i>Cell Wall-Degrading Enzymes</i>
DAMP	= <i>Damage Associated Molecular Pattern</i>
DDH	= <i>DNA-DNA Hybridization</i>
DSF	= <i>Diffusible Signal Factor</i>
EPS	= <i>Exopolysaccharide</i>
ETI	= <i>Effector-Triggered Immunity</i>
FAO	= <i>Food and Agricultural Organization</i>
GO	= <i>Gene Ontology</i>
HR	= <i>Hypersensitive Response</i>
KEGG	= <i>Kyoto Encyclopedia of Genes and Genomes</i>
LMG	= <i>Laboratory of Microbiology, Gent</i>
LPS	= <i>Lipopolysaccharide</i>
MALDI-TOF	= <i>Matrix-Assisted Laser Desorption Ionization – Time Of Flight</i>
MAMP	= <i>Microbial-Associated Molecular Pattern</i>
MGE	= <i>Mobile Genetic Element</i>
MLSA	= <i>MultiLocus Sequence Analysis</i>
MLST	= <i>MultiLocus Sequence Typing</i>
MLVA	= <i>MultiLocus VNTR [Variable Number of Tandem Repeats] Analysis</i>
MTase	= <i>Methyltransferase</i>
NCPBP	= <i>National Collection of Plant-Pathogenic Bacteria</i>
OGM	= <i>Organisme Génétiquement Modifié</i>
OGRI	= <i>Overall Genome Relatedness Indices</i>
OLD	= <i>Overcoming Lysogenization Defect</i>
PRR	= <i>Pattern-Recognition Receptor</i>
PTI	= <i>Pattern-Triggered Immunity</i>
pv.	= <i>pathovar</i>
REase	= <i>Restriction Endonuclease</i>
ROS	= <i>Reactive Oxygen Species</i>
ST	= <i>Sequence Type</i>
subsp.	= <i>subspecies</i>
T2SS	= <i>Type II Secretion System</i>
T3E	= <i>Type III Effector</i>
T3SS	= <i>Type III Secretion System</i>
TALE	= <i>Transcription Activator-Like Effector</i>
TBDT	= <i>TonB-Dependent Transporter</i>
THG	= <i>Transfert Horizontal de Gène</i>
T <sub>m</sub>	= <i>melting temperature</i>
Tn-seq	= <i>Transposon Insertion Sequencing</i>



# LISTE DES TABLEAUX & FIGURES

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## PROLOGUE

**Figure 1.** *Women scientists in the lab* p.23

## INTRODUCTION GÉNÉRALE

**Figure 2.** Diversité au sein du genre *Xanthomonas* p.27

**Figure 3.** Symptômes de la bactériose foliaire de la laitue observés au cours des campagnes d'échantillonnage conduites entre 2016 et 2018 dans la région Auvergne-Rhône-Alpes p.29

**Figure 4.** Épisodes de bactériose foliaire de la laitue causée par *X. hortorum* pv. *vitians* à travers le monde rapportée dans la littérature scientifique p.30

## SYNTHÈSE BIBLIOGRAPHIQUE

**Figure 5.** Premières observations de symptômes causés par différents pathovars de *X. hortorum* p.38

**Tableau 1.** Synthèse exhaustive des spectres d'hôtes naturels et expérimentaux des souches de *Xanthomonas hortorum* p.42

**Figure 6.** Localisation histochimique de *X. hortorum* pv. *vitians* CFBP 498 avec un système rapporteur GUS p.50





# PROLOGUE

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Des invasions de criquets et autres plaies bibliques aux récentes dévastations des cultures d'oliviers du pourtour méditerranéen par la bactérie phytopathogène *Xylella fastidiosa* (Sicard et al., 2018), en passant par la tragique grande famine irlandaise de 1845-1852 causée par l'oomycète *Phytophthora infestans* (Turner, 2005) ou bien la destruction quasi-totale des vignobles français à la même époque par le phylloxéra de la vigne (*Daktulosphaira vitifoliae*) (Ordish, 1972), les épidémies végétales, appelées également épiphyties dans le champ scientifique, n'ont eu de cesse de marquer l'histoire et l'imaginaire collectif des civilisations humaines sédentaires et agricoles. Déjà dans l'Antiquité grecque, le philosophe et « écologue » précurseur Théophraste (371-288 av. J.C.), influencé par les travaux de son collègue Aristote (384-322 av. J.C.), préfigurait dans ses écrits les concepts de symbiose et de parasitisme concernant les végétaux, et tentait de classer les maladies végétales entre celles provenant de la plante elle-même et celles associées à une cause extérieure (Hughes, 1985). Autrefois pensées comme châtiments divins en réponse aux péchés des sociétés humaines, manifestations d'entités surnaturelles malveillantes ou catastrophes liées à la conjonction particulière des astres, la reconnaissance du lien causal entre épiphyties et invasions d'espèces phytopathogènes dans les cultures devra attendre l'essor des sciences physiques et biologiques aux XVII<sup>e</sup> et XVIII<sup>e</sup> siècles pour s'installer progressivement comme explication à ces phénomènes naturels (Lucas et al., 1992).

À la suite de l'invention du premier microscope et des premières descriptions de microorganismes par les savants Antoni Van Leeuwenhoek (1632-1723) et Robert Hooke (1635-1703) (Gest, 2004), la toute première description savante d'un microorganisme phytopathogène et de son caractère infectieux a été présentée en 1728 à l'Académie royale des Sciences par le Français Henri Louis Duhamel de Monceau (1700-1782) (Duhamel de Monceau, 1753). S'attachant à déterminer la cause d'une maladie du safran (*Crocus sativus*) observée dans les cultures de l'ancien comté du Gâtinais, celui-ci démontrait que cette maladie racinaire était causée par un champignon phytopathogène, plus tard identifié comme appartenant au genre *Rhizoctonia* par le mycologue suisse Augustin Pyramus De Candolle (1778-1841) (Harveson, 2018). Il démontrait également son caractère contagieux et transmissible de plante à plante, et proposait en conséquence des mesures de contrôle de la maladie bien en avance sur son temps. Peu considéré par ses pairs de l'époque et rapidement

tombé dans les oubliettes de l'Histoire, son travail précurseur pour la théorie microbienne n'en reste pas moins l'une des pierres angulaires de la fondation de la pathologie végétale en tant que discipline scientifique.

Poussée par l'accélération de la survenue de nouvelles épiphyties d'origines extra-européennes sous l'influence de la mondialisation naissante des échanges et de l'agriculture, la pathologie végétale s'est affirmée à la fin du XIX<sup>e</sup> siècle comme une discipline scientifique à part entière, distincte de la botanique dont elle faisait alors partie (Sache et al., 2011). Historiquement considéré comme le « père de la pathologie végétale », le médecin et microbiologiste allemand Heinrich Anton de Bary (1831-1888) offrait une genèse officielle à la discipline dans les années 1860 en démontrant de manière implacable à ses nombreux contradicteurs que les champignons associés aux maladies végétales étaient bien la cause et non le résultat de ces maladies (Lucas et al., 1992). Prouvant par là même que le champignon phytopathogène *Phytophthora infestans* était bien l'agent causal du mildiou de la pomme de terre qui ravageait et affamait l'Europe depuis plusieurs années, il assurait à la pathologie végétale ses premières lettres de noblesse (Turner, 2005). De plus, il entraîna et instruisit plusieurs dizaines de disciples originaires de tout le continent, formant ainsi un premier contingent de mycologues et phytopathologistes amenés à diffuser la discipline dans les années suivantes à travers l'Europe (Lucas et al., 1992).

Ces mêmes décennies 1860-1870 furent également celles des multiples révolutions dans le champ des sciences biologiques portées, entre autres, par les travaux fondateurs de Charles Darwin (1809-1882) sur la théorie évolutionniste et la formulation du principe de sélection naturelle (Darwin, 1859), ainsi que par l'essor des écoles française et allemande de bactériologie sous la tutelle de leurs fondateurs respectifs Louis Pasteur (1822-1895) et Robert Koch (1843-1910) (Wainwright & Lederberg, 1992). Grâce aux avancées phénoménales permises par les découvertes de ces deux rivaux et pères fondateurs de la bactériologie, la pathologie végétale s'élargit aux bactéries et à leurs relations causales dans un grand nombre de maladies de plantes, notamment sous l'impulsion, entre autres, des travaux du phytopathologiste américain Erwin Frink Smith (1854-1927) au sein du *U.S. Department of Agriculture* (USDA) à partir de 1886 (Erwin Frink Smith, 1905). Celui-ci aura une influence historique considérable en formant toute une jeune génération de phytopathologistes, principalement féminines, qui contribueront à développer et populariser la discipline (**Fig. 1**) (Rossiter, 1974). L'une d'entre elles, Nellie Adalesa Brown (1876-1956), participera notamment en 1907 à la découverte d'*Agrobacterium tumefaciens*, l'agent causal

de la galle du collet (Erwin F. Smith & Townsend, 1907). Une dizaine d'années plus tard en 1918, elle publiera la toute première description de la bactérie responsable de la bactériose foliaire de la laitue (Brown, 1918), objet d'étude principal de la présente thèse.



**Figure 1.** *Women scientists in the lab*, environ 1920-1930, *National Photo Company Collection (Library of Congress)*. De gauche à droite : (debout) Nellie A. Brown, (assises) Lucia McCollock, Mary K. Bryan, Florence Hedges.

Ce retour à la genèse de la pathologie végétale replace le présent travail au sein de la longue histoire des femmes et des hommes qui, de tout temps, ont tenté de mieux comprendre leur environnement, leur champ, leur jardin, et de décrire minutieusement la multiplicité des interactions biologiques s'y déroulant. Car, comme le dit la populaire métaphore attribuée au philosophe médiéval français du XII<sup>e</sup> siècle Bernard de Chartres (env. 1080-1130), « [...] nous sommes comme des nains juchés sur les épaules de géants, de sorte que nous pouvons voir plus de choses qu'eux, et des choses plus éloignées qu'ils ne le pouvaient, non pas que nous jouissions d'une acuité particulière, ou par notre propre taille, mais parce que nous sommes portés vers le haut et exhaussés par leur taille gigantesque » (Dumez, 2009). Il y a fort à parier que cet homme de lettres n'a pas pu imaginer qu'il puisse également s'agir de géantes.



# INTRODUCTION GÉNÉRALE

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Ces deux dernières décennies, l'Organisation des Nations Unies (ONU) a engagé de nombreux programmes internationaux ayant pour objectifs l'éradication de la faim, de l'insécurité alimentaire et de la malnutrition à l'échelle mondiale d'ici à 2030 (*L'alimentation*, Nations Unies, <https://www.un.org/fr/sections/issues-depth/food/index.html>). Pourtant, les données de la FAO disponibles pour l'année 2019 montrent une accélération de la tendance observée depuis 2014, à savoir une augmentation lente mais constante du nombre de personnes souffrant de la faim à travers le monde (FAO, IFAD, UNICEF, WFP and WHO, 2020). À ce rythme, le nombre de personnes sous-alimentées pourrait atteindre 830 millions d'ici à 2030, contre 690 millions de personnes en 2019, soit déjà 8,9 % de la population mondiale. De manière générale, deux milliards d'individus à travers le monde (25,9 %) ont eu à souffrir de la faim au cours de l'année 2019 ou n'ont pas eu un accès régulier à une alimentation en quantité et qualité suffisantes (FAO, IFAD, UNICEF, WFP and WHO, 2020). Les raisons invoquées par les experts internationaux depuis plusieurs années : augmentation du nombre et de l'intensité des conflits, changements climatiques extrêmes, crises économiques majeures et événements imprévisibles aux conséquences désastreuses à l'instar de l'actuelle pandémie de COVID-19 ou des invasions sans précédents de criquets en Afrique de l'Est au cours des dernières années (FAO, IFAD, UNICEF, WFP and WHO, 2017, 2018, 2019, 2020). À cela s'ajoutent les inégalités économiques et sociales extrêmes qui conduisent à un accès différencié à une alimentation de base (Pereira & Oliveira, 2020), y compris au sein des pays riches (Pollard & Booth, 2019), et ce alors que la production globale de denrées alimentaires actuelle suffirait à nourrir correctement l'ensemble de la population mondiale (FAO, IFAD, UNICEF, WFP and WHO, 2020).

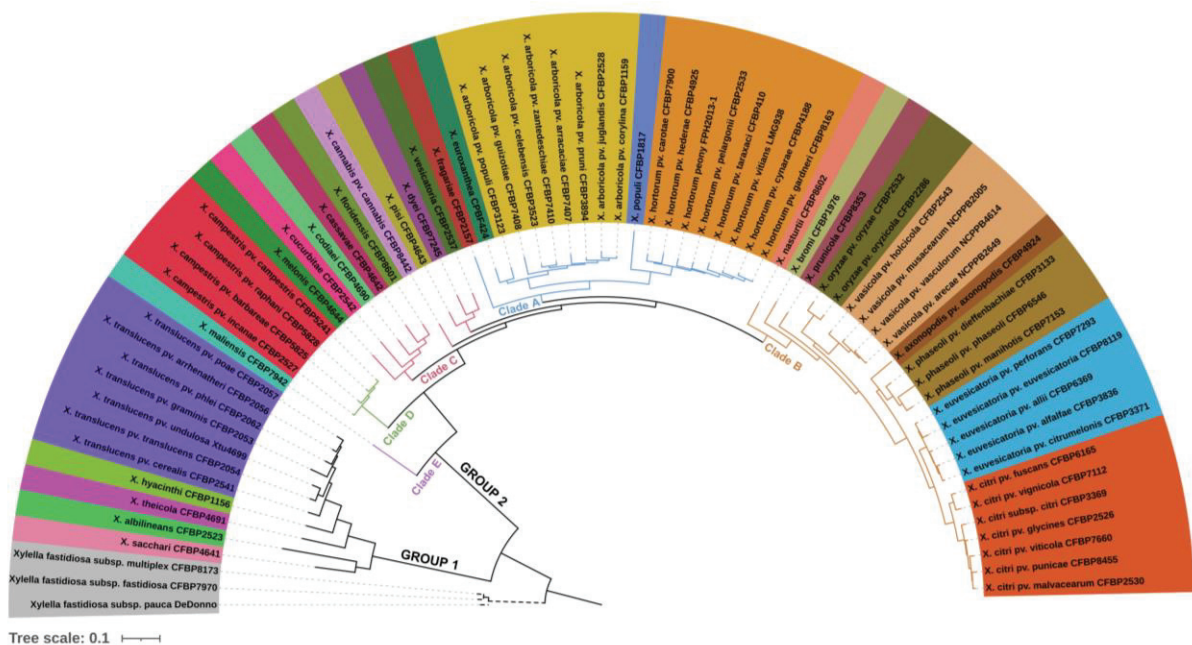
Ces causes sont souvent interconnectées les unes aux autres. Ainsi, les sécheresses participent à déclencher de nouveaux conflits territoriaux ou à aggraver ceux déjà existants (Ide, 2018), et les changements climatiques, tendant globalement à l'élévation des températures et des précipitations, favorisent la propagation des maladies végétales et des insectes ravageurs de cultures (Juroszek & Tiedemann, 2013; Velásquez et al., 2018). À titre d'exemple, une augmentation de la température et de la concentration en CO<sub>2</sub> atmosphérique en chambre de culture, pour aboutir à 30°C et 720 ppm de CO<sub>2</sub> contre 25°C et 420 ppm de CO<sub>2</sub> dans la condition contrôle, entraîne une augmentation de près de 25 % de la sévérité du

flétrissement bactérien et de la tache bactérienne du piment (*Capsicum* spp.) causées respectivement par les bactéries phytopathogènes *Ralstonia solanacearum* et *Xanthomonas vesicatoria* (Shin & Yun, 2010). Ces observations amènent les auteurs de l'étude à prédire un accroissement de la prévalence des pathologies végétales d'origine bactérienne dans les années à venir (Shin & Yun, 2010). En réalité, les effets pléiotropes des changements climatiques sur l'incidence des pathologies végétales microbiennes auront des conséquences très variables et difficiles à prédire (Velásquez et al., 2018). Mais déjà, les dégâts actuels causés par les agents phytopathogènes microbiens (champignons, bactéries, virus...) et les insectes ravageurs provoquent en moyenne des pertes de 20 à 30 % des récoltes mondiales des principales plantes cultivées comme le blé, le riz, le maïs, la pomme de terre ou le soja (Savary et al., 2019).

Face à ce constat, d'aucuns prônent l'augmentation du rendement, de l'efficacité de production et de la résistance des plantes face aux bioagresseurs, notamment par le développement des cultures massives d'organismes génétiquement modifiés (OGM) (Trivedi et al., 2016). D'autres soulignent, à juste titre, que cette course à l'efficacité ne fera que conduire inéluctablement à de nouvelles impasses et à de nouveaux problèmes (Hamant, 2020). Plutôt que produire plus, il nous faudrait donc produire mieux. Mais pour espérer produire mieux, il nous faudrait déjà en savoir plus, et mieux comprendre ce à quoi nous sommes confrontés. Pour ce qui relève du domaine de la pathologie végétale, nous pouvons dès lors nous inspirer de cet axiome célèbre du stratège chinois du VI<sup>e</sup> siècle avant notre ère, Sūn Zǐ (孫子) : « *Connais ton ennemi et connais-toi toi-même ; eussiez-vous cent guerres à soutenir, cent fois vous serez victorieux* » (Tzu, 2019). La métaphore guerrière semble d'ailleurs particulièrement prisée par les phytopathologistes, tant le vocabulaire de la discipline est riche en allégories martiales : les différents compartiments végétaux seraient des « champs de bataille » (Du et al., 2016; Yadeta & Thomma, 2013) où aurait lieu une « escalade » dans la « course aux armements » entre plantes hôtes et pathogènes (Boller & He, 2009; Wang et al., 2021), alimentée par le « trafic » pour faire pénétrer les « armes » que sont les effecteurs dans les cellules végétales « cibles » (Birch et al., 2006; Block et al., 2008), etc.

Même si près de 85 % des maladies végétales seraient causées par des « belligérants » issus du règne fongique (Pernezny et al., 2008), les bactéries ne sont pas en reste et nombreuses d'entre elles sont directement responsables de diverses maladies végétales aux spectres d'hôte et aux symptomatologies très variées (Vidaver & Lambrecht, 2004). Parmi les 10 bactéries phytopathogènes considérées comme les plus importantes au sein de la

communauté scientifique suite à une enquête d'opinion internationale (Mansfield et al., 2012), on retrouve en première position la vaste et diversifiée espèce génomique *Pseudomonas syringae*, en seconde le dévastateur complexe d'espèce *Ralstonia solanacearum*, en troisième l'espèce génomique au fort potentiel biotechnologique *Agrobacterium tumefaciens*, puis en quatrième, cinquième, et sixième positions plusieurs espèces du genre *Xanthomonas*, à savoir *X. oryzae*, *X. campestris* et *X. axonopodis*. La présence de trois espèces distinctes de *Xanthomonas* au sein de ce classement témoigne de l'importance qu'a ce genre bactérien pour les phytobactériologues, ainsi que des menaces majeures que ses membres font peser sur un pan considérable de la production agricole mondiale, autant en termes d'importance que de diversité des espèces végétales touchées (Jacques et al., 2016).



**Figure 2.** Diversité au sein du genre *Xanthomonas*. Phylogénie moléculaire construite avec PHYLOPHLAN v0.40 (cf. **Chapitre I**) à partir de tous les génomes disponibles des souches taxonomiquement pertinentes (types, pathotypes ou représentatives) des différentes espèces et pathovars. La division du groupe deux en 5 clades (A, B, C, D et E) est celle proposée par Timilsina *et al.* (Timilsina et al., 2020). Recension exhaustive des espèces, pathovars et génomes effectuée en avril 2021.

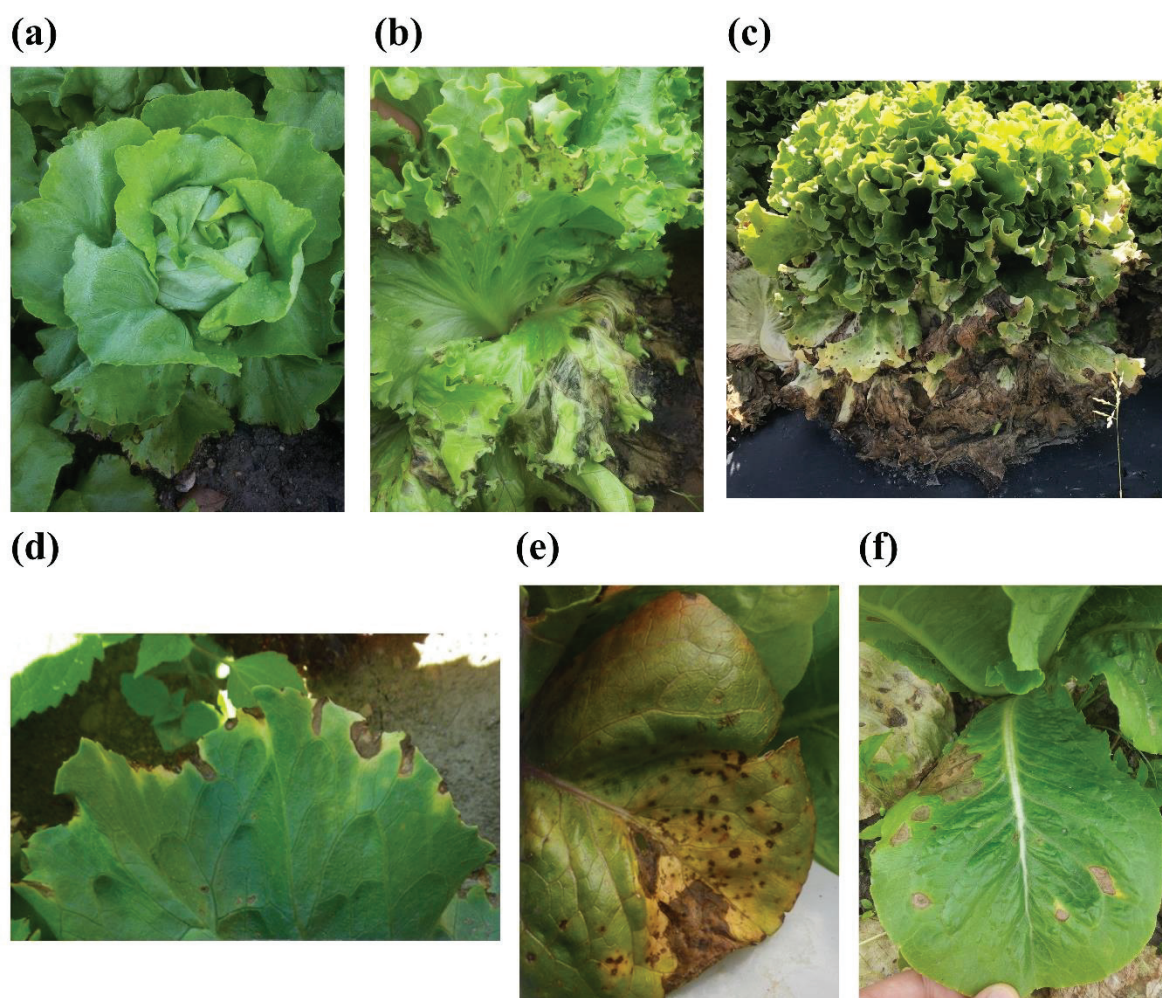
À l'heure actuelle, le genre *Xanthomonas* regroupe 30 espèces distinctes dont la nomenclature a été validée en accord avec les règles internationales de taxonomie bactérienne, en excluant les espèces synonymes (Constantin et al., 2016; Parte et al., 2020). Le genre est divisé en deux grands groupes, eux-mêmes subdivisés en plusieurs clades selon certains auteurs (Jacques et al., 2016; Timilsina et al., 2020) (**Fig. 2**). Ces bacilles Gram-négatifs issus



de la division des  $\gamma$ -protéobactéries sont notamment caractérisés par la possession d'un unique flagelle polaire et par une pigmentation jaune typique à laquelle on doit l'étymologie grecque du nom de genre ( $\xiανθός$ , prononcer *xanthos*, « jaune » ; et  $μονάς$ , pron. *monas*, « entité ») (Dye & Lelliott, 1974). Ces espèces sont également, pour bon nombre d'entre elles, subdivisées en de nombreux pathovars, une classification propre à la pathologie végétale servant à désigner « *une souche ou un groupe de souches présentant des caractéristiques identiques ou similaires, différenciées au niveau infraspécifique des autres souches de la même espèce ou sous-espèce sur la base d'une pathogénicité distincte sur une ou plusieurs plantes hôtes* » (Dye et al., 1980). Une recension des espèces végétales attaquées par les multiples pathovars de *Xanthomonas* spp. indique qu'environ 400 plantes au moins seraient concernées, dont 268 dicotylédones et 124 monocotylédones (Hayward, 1993). Cette somme ne tient toutefois pas compte des nombreuses autres espèces végétales qui pourraient être colonisées de manière asymptomatique par des souches non-pathogènes ou dont le caractère pathogène n'a jusqu'ici pu être vérifié (Essakhi et al., 2015; Triplett et al., 2015; Vauterin et al., 1996). Cette importante diversité végétale est à mettre en relation avec le haut degré de spécificité des souches de *Xanthomonas* vis-à-vis de leur(s) plante(s) hôte(s) (Jacques et al., 2016). La distinction en pathovars se faisant principalement sur la base de la symptomatologie observée sur une plante donnée, des spécificités tissulaires différentes peuvent également conduire à distinguer des souches proches attaquant une même plante en pathovars distincts, tels *X. oryzae* pvs. *oryzae* et *oryzicola*, infectant respectivement le tissu vasculaire et l'apoplasme des feuilles de riz (Niño-Liu et al., 2006).

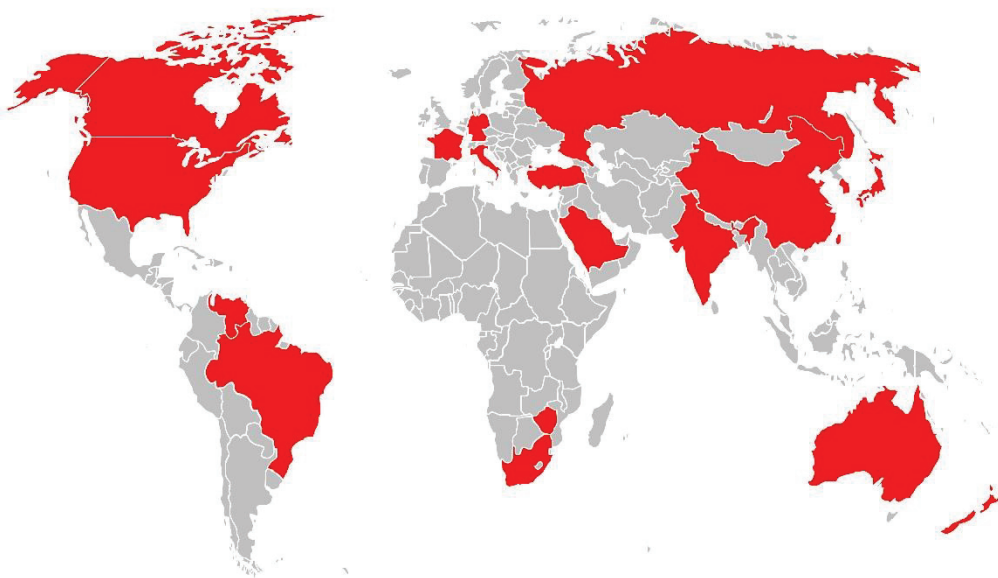
Si certaines espèces pathogènes de *Xanthomonas* sont considérées comme des organismes modèles largement étudiés à travers le monde en raison de l'importance économique des végétaux concernés, telles les espèces précitées *X. campestris* et *X. oryzae*, respectivement pathogènes des *Brassicaceae* et du riz, d'autres restent, pour des raisons variables et parfois contingentes, bien moins connues à ce jour. Ainsi, l'espèce *Xanthomonas hortorum* a jusqu'ici fait l'objet d'assez peu d'études comparativement à d'autres espèces du même genre. Originellement proposée en 1995 (Vauterin et al., 1995), nous en avons redéfini les contours au cours des travaux menés au début de cette thèse (cf. **Chapitre I**) pour y regrouper désormais sept pathovars aux spectres d'hôtes distincts (pvs. *hederae*, *pelargonii*, *carotae*, *taraxaci*, *vitians*, *cynarae* et *gardneri*). De plus, l'intérêt porté à *X. hortorum* est très inégal entre les pathovars, même si le fait que 16 des 35 génomes déposés dans la base de données du *National Center for Biotechnology Information* (NCBI) l'ont été au cours de

l'année 2020 (consultée en avril 2021) témoigne d'un regain d'intérêt récent pour cette espèce. À titre d'exemple, si les aspects relatifs à l'épidémiologie et au cycle de vie de *X. hortorum* pv. *vitians* ont été assez bien documentés au cours des trois dernières décennies (cf. **Synthèse bibliographique**), aucune étude génomique ne vient éclairer la dimension des interactions moléculaires avec l'hôte végétal. Des études de ce type ont en revanche été réalisées, à divers degrés, pour *X. hortorum* pvs. *carotae*, *cynarae* et *gardneri* (cf. **Synthèse bibliographique**). Enfin, et à l'exception des travaux présentés ici, nous ne disposons tout simplement pas d'informations génomiques concernant *X. hortorum* pvs. *taraxaci* et *hederae*.



**Figure 3.** Symptômes de la bactériose foliaire de la laitue observés au cours des campagnes d'échantillonnage conduites entre 2016 et 2018 dans la région Auvergne-Rhône-Alpes. **(a & d)** Symptômes précoces en marge du limbe foliaire sur laitue pommée, **(b)** larges zones nécrotiques humides sur laitue batavia, **(c)** destruction totale des feuilles périphériques sur laitue feuille de chêne, **(e & f)** tâches nécrotiques individualisées et confluentes avec halos chlorotiques sur **(e)** laitue rougette et **(f)** laitue romaine. Photos réalisées par l'auteur.

Dans le cadre de cette thèse, nous avons choisi comme modèle d'étude principal *Xanthomonas hortorum* pv. *vitians* (anciennement '*X. campestris* pv. *vitians*'), responsable d'une maladie appelée bactériose foliaire de la laitue (*bacterial leaf spot of lettuce*). Cette bactériose principalement observée sur l'ensemble des variétés de laitues cultivées (*Lactuca sativa*) se caractérise par l'apparition de petites lésions humides angulaires d'aspect brun-noir sur les feuilles périphériques de la plante de part et d'autre de la nervure centrale ou en marge du limbe foliaire. Très rapidement après l'apparition de ces premiers symptômes, ces lésions deviennent nécrotiques et sèches et s'entourent d'un halo chlorotique jaune (**Fig. 3**). Enfin, elles s'étendent pour confluer et former de larges nécroses qui peuvent couvrir l'ensemble des feuilles attaquées, puis se propager progressivement des feuilles périphériques vers la pomme de la plante. Bien que ne présentant pas de risque létal pour les plantes infectées, cette pathologie provoque des pertes économiques importantes pour les producteurs en diminuant la quantité et la qualité des récoltes et en augmentant les pertes post-récolte pendant les phases de stockage et de distribution (Bull & Koike, 2005; Koike & Gilbertson, 2017).



**Figure 4.** Épisodes de bactériose foliaire de la laitue causée par *X. hortorum* pv. *vitians* à travers le monde rapportée dans la littérature scientifique. Carte réalisée par l'auteur.

Présentant un caractère sporadique, cette maladie a été rapportée dans toutes les régions du monde dans la littérature scientifique (**Fig. 4**) – en Amérique du Nord (Fayette, Raid, et al., 2016; Stefanova & Hernández, 1999; Toussaint, 1999), en Amérique du Sud (Daboin & Tortolero, 1993; Tebaldi et al., 2015), en Europe (Morinière et al., 2020; Zoina & Volpe, 1992), en Afrique (Wallis & Joubert, 1972), au Moyen-Orient (Al-Saleh & Ibrahim, 2008; Ozyilmaz & Benlioglu, 2018), en Asie (Myung et al., 2010; Tsuchiya et al., 1981), et en

Océanie (Boesewinkel, 1977; Harrison, 1963) – et est devenue un problème économique important dans toutes les régions de production depuis une quinzaine d’années, notamment sous les climats chauds et humides, favorables au développement de la maladie (Koike & Gilbertson, 2017). Ainsi, des pertes quasi-totales des récoltes de laitues ont pu être observées dans certaines zones de production lors d’épisodes épiphytiques particulièrement sévères en Amérique du Nord (Lu & Raid, 2013; Toussaint, 1999). Qui plus est, des solutions pratiques et efficaces pour contrôler cette maladie dans les champs font toujours cruellement défaut (Fayette, Roberts, et al., 2016; Yigit, 2011), même si des progrès certains ont été réalisés ces dernières années dans l’identification de ressources génétiques de laitues permettant de produire des cultivars plus résistants (Hayes et al., 2014; Sandoya et al., 2019; Wang et al., 2016). Tant qu’elles ne pourront pas s’appuyer sur des études moléculaires caractérisant avec précision l’identité et la diversité des populations de pathogène ainsi que les interactions moléculaires que celles-ci entretiennent avec les hôtes végétaux au cours de l’infection, la réussite et la généralisation de l’application de ces recherches de moyens de contrôle reste par trop incertaine.

Dans ce contexte, il nous est donc apparu nécessaire de revenir aux fondamentaux de l’étude de cette pathologie et de son agent causal afin d’instaurer de nouvelles bases solides sur lesquelles pourront se reposer les recherches appliquées ultérieures. Nos objectifs étaient donc les suivants :

- Résoudre formellement la taxonomie des souches bactériennes associées à la bactériose foliaire de la laitue et caractériser l’étendue de la diversité génétique de ces populations à l’échelle régionale et mondiale.
- Développer une approche moléculaire à haut débit permettant d’étudier pour la première fois la structure et le contenu d’un génome modèle de ce pathogène dans des conditions *in vitro*.
- Identifier grâce à cette approche moléculaire les déterminants génétiques impliqués dans la survie et la pathogénie au cours de l’interaction hôte-pathogène en conditions *in vivo*.

Pour cela, nous avons constitué une collection de souches associées à la bactériose foliaire de la laitue par la réalisation de trois campagnes d’échantillonnage dans les champs de laitue de la région Auvergne-Rhône-Alpes pendant les saisons de production 2016 à 2018 et par l’acquisition de souches de collection. Tout d’abord, nous avons préalablement caractérisé la diversité des populations pathogènes par une approche de *Multi Locus Sequence Analysis*

(MLSA) ainsi que par des tests de pathogénicité en conditions contrôlées. Puis, nous avons conduit une étude de taxonomie polyphasique intégrant analyses génomiques, phylogénétiques et phénotypiques sur un nombre restreint de souches représentatives de cette diversité ainsi que sur la totalité des souches taxonomiquement pertinentes des pathovars et espèces proches. L'ensemble de ces travaux, menés en collaboration avec l'Institut de Recherche en Horticulture et Semences (IRHS) de l'INRAE d'Angers et le Département de Pathologie Végétale de l'Université de l'Etat de Pennsylvanie aux Etats-Unis, a été concrétisé sous la forme d'une publication en juillet 2020 dans le journal *Systematic and Applied Microbiology*, qui constitue le premier chapitre de cette thèse.

Dans un second temps, nous avons développé une approche de mutagenèse transpositionnelle à haut débit, appelée *Transposon Insertion Sequencing* (Tn-seq), sur une souche pathogène modèle pour laquelle nous avons séquencé un génome complet et de haute qualité. L'analyse de la librairie de mutants ainsi obtenue en conditions *in vitro* a permis de définir les contours du génome essentiel de cet organisme, c'est-à-dire l'ensemble des gènes considérés comme indispensables à la structure et au métabolisme de base. Grâce à l'utilisation de multiples méthodes de génomique comparative et de phylogénie moléculaire, nous avons pu opérer une réflexion critique sur le concept même de génome essentiel et discuter de la présence de potentiels « gènes égoïstes » (*selfish genes*) acquis par transferts horizontaux de gènes. Cette première étude moléculaire de *X. hortorum* pv. *vitians*, réalisée en collaboration avec le laboratoire Microbiologie, Adaptation, Pathogénie (MAP) de l'Université de Lyon, a fait l'objet d'une seconde publication en mars 2021 dans le journal *Microbial Genomics*, qui est présentée ici en tant que second chapitre de thèse.

Enfin, l'inoculation de la librairie de Tn-seq *in planta* dans des conditions expérimentales préalablement calibrées a permis de dessiner pour la toute première fois un tableau global des déterminants génétiques ayant un impact sur la valeur sélective (*fitness*) de ce pathogène au cours de son interaction avec la laitue. Aidés une nouvelle fois par des outils de génomique comparative et des analyses d'enrichissement fonctionnel, nous avons pu obtenir de cette approche innovante des informations sur les processus cellulaires et métaboliques mis à contribution par le pathogène pour subvenir à ses besoins trophiques et échapper aux défenses du système immunitaire végétal au cours des multiples phases de l'interaction hôte-pathogène. Ces résultats sont ici présentés dans le troisième chapitre sous forme d'un projet d'article qui sera proposé prochainement au journal *mSystems* pour publication.

# SYNTHÈSE BIBLIOGRAPHIQUE

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## 1. Évolutions passées et futures des concepts et définitions d'espèce procaryote

« *I look at the term species, as one arbitrarily given for the sake of convenience to a set of individuals closely resembling each other [...] »*

Charles Darwin, *On the Origins of Species*, 1859, p. 52

« *[Taxonomy] is the most subjective branch of any biological discipline, and in many ways is more of an art than a science. »*

S. T. Cowan, *Sense and Nonsense in Bacterial Taxonomy*, 1971

Que l'on soit botaniste, zoologiste ou microbiologiste, tout biologiste est, à un moment donné, amené à se poser la question suivante : qu'est-ce qu'une espèce ? Pour commencer à ébaucher une réponse à cette question complexe, il convient tout d'abord de distinguer le *concept* d'espèce de la *définition* d'une espèce (Rosselló-Móra, 2012).

Le *concept* d'espèce représente l'idée selon laquelle l'observation de schémas récurrents dans la nature des êtres vivants permettrait de circonscrire des unités fondamentales de la biodiversité, concept qui s'appliquerait ainsi idéalement de manière universelle à l'ensemble des formes vivantes quel que soit leur règne (Hey, 2001). Un premier problème se pose déjà en ce que la plupart des concepts d'espèces théorisés l'ont été spécifiquement pour les organismes eucaryotes, et ne sont donc pas adaptés aux procaryotes (Rosselló-Móra & Amann, 2001), tel le concept d'*espèce biologique* d'Ernst Mayr qui repose principalement sur les notions d'interfécondité et d'isolement reproductif (Mayr, 2000). De fait, la pertinence même d'avoir un concept d'espèce chez les procaryotes est un sujet de controverse récurrent et ancien chez les microbiologistes (Cowan, 1955; Vos, 2011). D'autre part, *définir* une espèce procaryote consiste à déterminer de manière pragmatique quel ensemble de caractères choisir et quelles valeurs leur appliquer pour dessiner les limites de l'espèce (Rosselló-Móra, 2012). Cette problématique a donc toujours été intrinsèquement liée aux évolutions des techniques et méthodes utilisées pour classifier les microorganismes (Rosselló-Móra & Amann, 2001).

Tout au long du premier siècle d'existence de la bactériologie (~ 1860–1960), une espèce procaryote était considérée comme un groupe *monothétique*, c'est-à-dire basé sur un ensemble unique de caractères considéré à la fois comme nécessaire et suffisant (Rosselló-Móra & Amann, 2001). Les groupes étaient déterminés à partir d'un certain nombre de traits phénotypiques et morphologiques subjectivement choisis et dont la combinaison était considérée comme exclusive à l'espèce étudiée (Goodfellow et al., 1997). Cette pratique s'est notamment formalisée avec la publication en 1923 de la première édition du *Bergey's Manual of Determinative Bacteriology* (Bergey et al., 1923), et culmina avec la publication de la 7<sup>ème</sup> édition en 1957 (Bergey et al., 1957). L'objectif était de compiler selon une structure logique et hiérarchique l'ensemble des caractéristiques phénotypiques, morphologiques et pathogéniques des espèces afin de permettre leur identification en suivant une méthode analytique, à la manière des clés de détermination botanique ou fongique. Les défauts et limites de cette vision de la taxonomie procaryote ont été largement discutés (Cowan, 1970; Goodfellow et al., 1997; Rosselló-Móra & Amann, 2001). À titre d'exemple, l'importante hétérogénéité phénotypique de l'espèce *Pseudomonas stutzeri* combinée au manque de communication internationale entre chercheurs a conduit cette espèce à être désignée simultanément sous plusieurs noms pendant toute la première moitié du XX<sup>e</sup> siècle (Rossello et al., 1991; Van Niel & Allen, 1952).

Avec l'apparition des premières technologies computationnelles et la découverte du rôle de l'ADN dans l'encodage de l'information génétique, le concept d'espèce procaryote a évolué vers une forme *polythétique* (ou *phénétique*), considérée comme plus objective car basée sur l'accumulation statistique de caractères non exclusifs, indépendants et covariants, donnant naissance à la taxonomie *numérique* (Sneath & Sokal, 1973). Le développement des différentes techniques d'hybridation ADN-ADN (*DNA-DNA hybridization*, DDH) à partir des années 1960 a également profondément transformé les pratiques de la taxonomie procaryote (Rosselló-Móra, 2006). Ces techniques permettent de déterminer la similarité de contenu en bases azotées entre deux génomes en mesurant la stabilité de l'hétéroduplex issu de la dénaturation et de l'hybridation des ADN double-brins (Grimont, 1988). De multiples observations empiriques ont montré à l'époque que les « espèces phénotypiques » (*taxospecies*) déjà décrites correspondaient à des « espèces génomiques » (*genospecies*) présentant des valeurs d'hybridation ADN-ADN entre 60 et 80 % et des valeurs de  $\Delta T_m$  généralement inférieures à 7°C (Grimont, 1988; Johnson, 1973). En conséquence, un comité *ad hoc* a alors recommandé de manière non contraignante l'utilisation de valeurs

d'hybridation ADN-ADN supérieures à 70 % et de  $\Delta T_m$  inférieures à 5°C comme limites permettant de former des espèces procaryotes robustes (Wayne et al., 1987). Cette nouvelle vision ne faisait toutefois pas table rase du système précédent, le comité préconisant également de conserver l'utilisation de propriétés phénotypiques comme critère nécessaire à la distinction entre deux espèces génomiques (Wayne et al., 1987).

Enfin, la volonté de proposer un concept d'espèce intégratif prenant en compte l'ensemble des dimensions biologiques des organismes, en recoupant des informations de nature différentes (écologique, moléculaire, phénotypique, évolutive...) obtenues notamment grâce aux développements des technologies de séquençage et de génotypage basé sur la PCR, a conduit à la naissance de la taxonomie *polyphasique* (Colwell, 1970; Vandamme et al., 1996). Avec l'avènement des technologies de séquençage de génome entiers et leur traitement à haut débit par voie computationnelle à partir de la fin des années 1990, les hybridations ADN-ADN ont progressivement été remplacées par l'utilisation d'indices de similarité globale entre génomes (*Overall Genome Relatedness Indices*, OGRI) comme les scores ANI (*Average Nucleotide Identity*) ou les hybridations ADN-ADN *in silico* (*isDDH*) (Meier-Kolthoff et al., 2013; Richter & Rosselló-Móra, 2009). De plus, les reconstructions phylogénétiques jusqu'ici basées seulement sur les séquences 16S ou 23S sont devenues plus résolutes en se basant sur la concaténation d'un petit nombre de gènes de ménage (*Multi Locus Sequence Analysis*, MLSA) (Glaeser & Kämpfer, 2015) ou même sur un grand nombre de gènes orthologues issus des génomes (Philippe et al., 2005). De manière similaire, la compilation de tests phénotypiques individuels a été majoritairement remplacée par l'utilisation de techniques de profilage phénotypique à haut débit comme les microplaques Biolog (Bochner, 2009), ou plus récemment le dispositif de protéotypage MALDI-TOF (*Matrix-Assisted Laser Desorption Ionisation – Time of Flight*) (Welker & Moore, 2011). Un avantage considérable de toutes ces nouvelles méthodes est que leur standardisation permet de compiler leurs résultats dans des bases de données publiques et cumulatives, facilitant ainsi considérablement les comparaisons et l'échange d'information (Rosselló-Móra, 2012).

Actuellement, il existe un consensus majoritaire entendant qu'une espèce procaryote peut être comprise comme « *une catégorie qui circonscrit des populations d'individus monophylétiques [c'est-à-dire partageant un dernier ancêtre commun exclusif], génétiquement et phénotypiquement homogènes, et qui peuvent être clairement distinguées d'autres entités similaires sur la base de paramètres standardisés* » (Rosselló-Móra & Amann, 2015). Toutefois, il est intéressant de noter que si les concepts et les méthodes ont considérablement



évolué depuis les premiers pas de la taxonomie procaryote, cela s'est fait de manière graduelle et continue, sans véritable rupture de paradigme. Ainsi, la valeur limite de 70 % d'hybridation ADN-ADN a été choisie pour faire correspondre au mieux les espèces génomiques aux espèces phénotypiques déjà décrites, et les valeurs d'OGRI sélectionnées comme limites l'ont été afin de servir de substituts plus robustes mais équivalents aux hybridations ADN-ADN (Richter & Rosselló-Móra, 2009). On peut également noter que d'autres modèles conceptuels existent et peuvent être considérés de manière complémentaire pour appréhender la réalité complexe des espèces bactériennes. A titre d'exemple, le modèle de l'écotype stable (*stable ecotype*) développé par Cohan et ses collaborateurs (Cohan, 2002; Koepfel et al., 2008) permet de renforcer les dimensions écologique et évolutive du concept d'espèce bactérienne en le définissant par le prisme de la divergence adaptative et de l'occupation d'une niche écologique particulière (Vos, 2011).

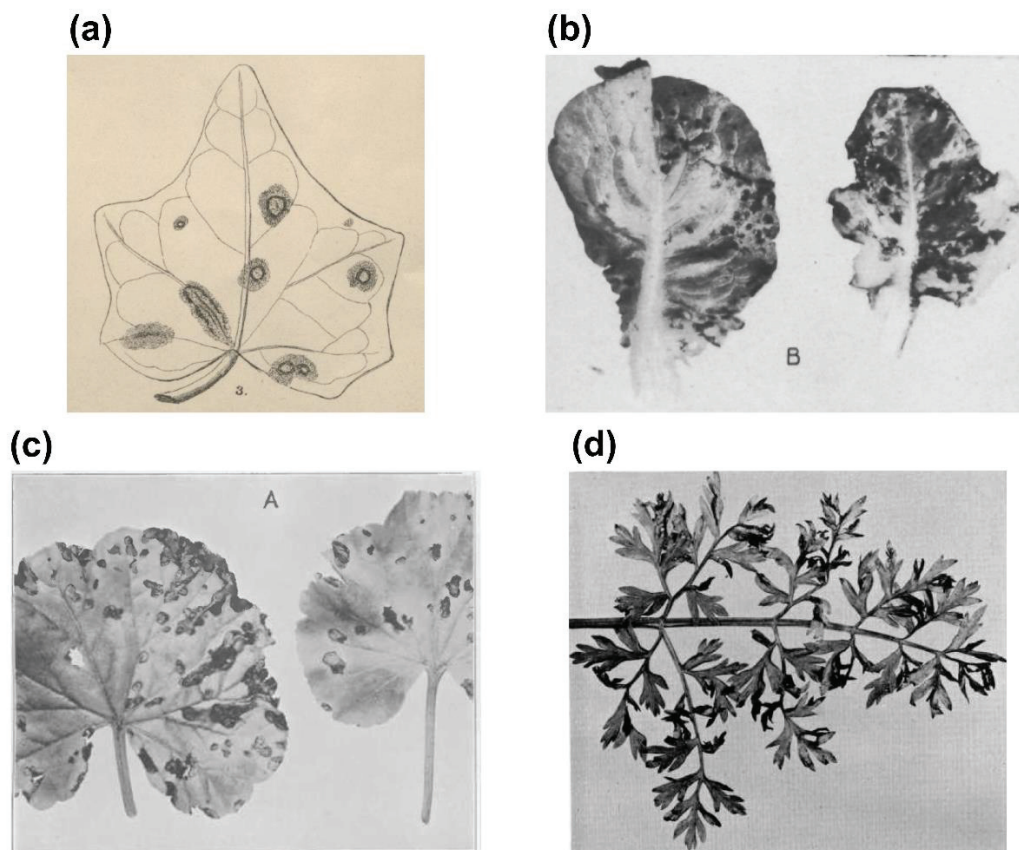
Comme nous venons de le présenter, la taxonomie procaryote est une discipline en constante mutation qui tente perpétuellement d'intégrer les révolutions conceptuelles de la microbiologie. Les perspectives qui se dessinent donc pour le futur de la taxonomie procaryote incluent (i) une nécessaire prise en compte des microorganismes non cultivables (Konstantinidis et al., 2017), jusqu'ici globalement laissés de côté par la discipline, (ii) le possible remplacement des souches types par des génomes types en tant que matériel de référence (Konstantinidis et al., 2020), et (iii) l'automatisation partielle des systèmes de classification et de nomenclature permettant l'intégration exponentielle de la diversité connue des microorganismes procaryotes au sein d'un cadre taxonomique unique (Marakeby et al., 2014).

## 2. Processus historique de la reconnaissance taxonomique de *Xanthomonas hortorum* en tant qu'espèce unique

L'histoire longue et complexe de la taxonomie des différents membres de l'espèce *Xanthomonas hortorum* (Vauterin *et al.* 1995) Morinière *et al.* 2020 est à mettre en relation avec l'évolution de la taxonomie du genre *Xanthomonas* Dowson 1939 lui-même. Autrefois, de nouvelles espèces bactériennes étaient généralement décrites à chaque fois qu'une nouvelle bactérie phytopathogène était isolée sur un nouvel hôte végétal, ou même lorsqu'une bactérie isolée était déjà décrite mais présentait un spectre d'hôte différent (Young *et al.*, 1978). Par la suite, une révision majeure du Code International de Nomenclature des Procaryotes (*International Code of Nomenclature for Prokaryotes*, ICNP) a eu lieu en 1975 (Lapage *et al.*, 1975) et s'est concrétisée sous la forme de la publication en 1980 des premières Listes de Noms Bactériens Approuvés (*Approved Lists of Bacterial Names*) (Skerman *et al.*, 1980). La transposition de ces changements aux bactéries pathogènes de plantes (Young *et al.*, 1978) a conduit à l'adoption en 1980 par la communauté des phytopathologistes des Standards Internationaux pour Nommer les Pathovars de Bactéries Phytopathogènes (*International Standards for Naming Pathovars of Phytopathogenic Bacteria*, communément appelés « les Standards ») (Dye *et al.*, 1980), qui ont notamment introduit le rang de *pathovar* déjà présenté plus haut (cf. **Introduction générale**).

Les premiers rapports dans la littérature scientifique faisant état de maladies causées par des pathovars de *X. hortorum* remontent au tournant du XX<sup>e</sup> siècle (**Fig. 5**). On retrouve des descriptions détaillées d'une maladie du lierre (*Hedera helix*) en 1894 en Allemagne (Lindau, 1894), d'une maladie des géraniums (*Pelargonium* spp.) et d'une maladie foliaire de la laitue dans l'État du Massachusetts aux États-Unis en 1898 et 1907, respectivement (Stone, 1907; Stone & Smith, 1898). Si des bactéries étaient déjà reconnues comme étant la cause de ces maladies, celles-ci n'ont à l'époque pas fait l'objet d'identifications précises, et n'ont été ni classées ni nommées. La première description taxonomique officielle d'un *X. hortorum* a été celle de l'agent causal de la bactériose foliaire de la laitue, baptisé *Bacterium vitians* en 1918 par Nellie A. Brown (Brown, 1918). Dans les années suivantes, *Bacterium hederæ* et *Bacterium pelargonii* ont été isolées et décrites comme étant responsables respectivement des maladies du lierre et des géraniums (Arnaud, 1920; Brown, 1923). Ces trois bactéries ont été rapidement reclassifiées dans le genre *Phytomonas*, introduit dans la première édition du *Bergey's* (Bergey *et al.*, 1923). Une décennie plus tard, *Phytomonas carotæ* (originellement

proposée en tant que '*Pseudomonas carotae*') a été caractérisée à partir d'une maladie observée sur les carottes cultivées (*Daucus carota* subsp. *sativus*) (Kendrick, 1934).



**Figure 5.** Premières observations de symptômes causés par différents pathovars de *X. hortorum* sur (a) feuille de lierre, adapté de Lindau, 1894, (b) feuilles de laitue, adapté de Brown, 1918, (c) feuilles de géranium, adapté de Brown, 1923 et (d) feuilles de carotte, adapté de Kendrick, 1934.

Un changement important a eu lieu avec la création du genre *Xanthomonas* en 1939 (Dowson, 1939), conduisant à l'intégration des 4 espèces précédemment mentionnées dans ce genre en tant que *X. vitians*, *X. hederæ*, *X. pelargonii* et *X. carotæ* (Dowson, 1939; Starr & Burkholder, 1942). En parallèle, la bactérie responsable d'une maladie du pissenlit kazakh (*Taraxacum kok-shagyz*) dont la présence avait été rapportée en 1936 en URSS (Sigrianski, 1936) a été également observée aux États-Unis en 1943 et nommée *X. taraxaci* (Niederhauser, 1943). Tous ces pathogènes étaient considérés comme des espèces individuelles de *Xanthomonas* jusqu'à la refonte en profondeur du genre supervisée en 1978 par D. W. Dye, à la suite de l'adoption des Standards (Dye, 1978). Un grand nombre d'espèces de *Xanthomonas* n'étant pas distinguable entre elles par d'autres critères que par leur spectre d'hôte, elles ont alors été abaissées au rang de pathovars et incluses par défaut dans l'espèce polytypique *X. campestris* (Pammel 1895) Dowson 1939 (Dye, 1978).

Finalement, le genre *Xanthomonas* a de nouveau été refondu en profondeur au début des années 1990 sur la base de résultats d'hybridation ADN-ADN (Hildebrand et al., 1990; Palleroni et al., 1993; Vauterin et al., 1995). Vauterin et ses collaborateurs (Vauterin et al., 1995) ont notamment proposé la combinaison de *Xanthomonas campestris* pvs. *vitians*, *hederae*, *pelargonii*, *carotae* et *taraxaci* au sein d'une nouvelle espèce, *X. hortorum* Vauterin et al. 1995. La souche pathotype de *X. campestris* pv. *hederae* CFBP 4925<sup>T</sup> (= LMG 733<sup>T</sup> = NCPPB 939<sup>T</sup>) a été choisie en tant que souche type de l'espèce *X. hortorum* (Vauterin et al., 1995). Cependant, la dénomination '*X. hortorum* pv. *vitians*' (Brown 1918) Vauterin et al. 1995 a été refusée l'année suivante par le Comité de Taxonomie des Bactéries Pathogènes de Plante (*Committe on the Taxonomy of Plant-Pathogenic Bacteria*, CTPPB) en raison des incertitudes sur l'identité de la souche pathotype CFBP 2538 (= LMG 937) et de l'absence de protologue pour ce taxon (Young et al., 1996). Cette situation a abouti à la conservation par défaut de *X. campestris* pv. *vitians* (Brown 1918) Dye 1978b en tant que seule nomenclature officielle pour ce pathogène.

De manière intéressante, les historiques taxonomiques des pathogènes causant les maladies de la grasse de l'artichaut (*Cynara scolymus*) et de la gale bactérienne de la tomate (*Solanum lycopersicum*) et du poivron (*Capsicum annuum*) ont connu un processus similaire jusqu'à leur reconnaissance en tant que pathovars de *X. hortorum*. Ces deux maladies ont été rapportées pour la première fois dans les années 1950 (Ridé, 1956; Šutic, 1957) et rapidement supposées comme étant causées par des souches de *Xanthomonas*, mais sans descriptions officielles (Dye, 1966; Ridé, 1956). Ces deux bactéries ont donc dû attendre le début du XXI<sup>e</sup> siècle pour être officiellement décrites en tant que *X. cynarae* sur artichaut (Trébaol et al., 2000) et *X. gardneri* sur tomate et poivron (Jones et al., 2004). Même si des données phylogénétiques ont suggéré dès la fin des années 2000 la forte proximité génétique existante entre *X. hortorum*, *X. cynarae* et *X. gardneri* (Parkinson et al., 2009; Young et al., 2008), la question de leur légitimité en tant qu'espèce n'a finalement été résolue que récemment (cf. **Chapitre I**).

### 3. Étendue insoupçonnée du spectre d'hôte de *Xanthomonas hortorum*

Afin de décrire précisément et de comprendre le spectre d'hôte de *Xanthomonas hortorum*, il convient tout d'abord de distinguer les notions de spectre d'hôte *naturel* et de spectre d'hôte *expérimental* chez les bactéries phytopathogènes. Le spectre d'hôte *naturel* comprend toutes les plantes sauvages ou domestiquées véritablement infectées par le pathogène dans les écosystèmes naturels ou agricoles (Bull & Koike, 2015). La validation de ces observations est souvent fondamentalement dépendante de la vérification des postulats de Koch, qui impliquent l'isolement d'un microorganisme à partir d'un hôte malade, la survenue de la même pathologie lors de la réinfection sur un hôte sain en conditions contrôlées, et le ré-isolement du microorganisme à partir de ce même hôte (Bos, 1981). Ce spectre d'hôte naturel est également le critère principal permettant de distinguer les pathovars des espèces bactériennes phytopathogènes (Dye et al., 1980). En revanche, le spectre d'hôte *expérimental* englobe toutes les espèces végétales qui présentent des lésions après inoculation du pathogène en conditions artificielles (Bull & Koike, 2015). Son étendue est donc grandement dépendante du nombre de plantes testées et de la procédure d'inoculation utilisée. Une infiltration directe d'une suspension concentrée de pathogène dans les tissus végétaux peut amener à observer des lésions nécrotiques là où la pulvérisation de la même suspension, mieux à même de mimer des conditions d'infections proches de ce qui se passe dans la nature, ne conduira pas à l'apparition de symptômes (Toussaint et al., 2012). Bien que sa mise en place soit plus complexe et chronophage, il faudrait donc systématiquement privilégier l'inoculation par pulvérisation dans les études de spectre d'hôte, dans la mesure où cela permet de vérifier non seulement la capacité du pathogène à produire une nécrose, mais également à coloniser et pénétrer naturellement dans les plantes. Quoi qu'il en soit, étudier le spectre d'hôte expérimental d'une bactérie phytopathogène nous offre des informations d'une grande valeur sur le potentiel du pathogène à s'adapter à de nouveaux hôtes végétaux, c'est-à-dire à effectuer un « saut d'hôte » (*host jump*) (Jacques et al., 2016).

Chaque pathovar de *X. hortorum* possède son propre spectre d'hôte naturel (**Tab. 1**). Qui plus est, de nombreuses souches de *X. hortorum* non assignées à des pathovars ont été isolées à partir d'un grand nombre de plantes ces dernières décennies et sont exhaustivement rapportées ci-dessous. Afin d'assurer l'homogénéité de la taxonomie botanique utilisée dans cette section, les nomenclatures binominales de toutes les espèces végétales citées ont été systématiquement vérifiées sur la base de données *World Flora Online* (<http://www.worldfloraonline.org>, accédée en février 2021).

*Xanthomonas hortorum* pv. *hederae* est principalement connu pour être un pathogène de l'espèce du lierre commun (*H. helix*) (Arnaud, 1920; Trantas et al., 2016), mais a également été isolé à partir d'autres espèces de lierre symptomatiques (**Tab. 1**). Certaines souches semblent être aussi pathogènes de diverses espèces de la famille des *Araliaceae* dans les écosystèmes naturels (**Tab. 1**). Des inoculations artificielles ont aussi conduit à l'apparition de lésions sur le Faux aralia (*Pterandra elegantissima*) et le lierre du Japon (*H. rhombea*) (Suzuki et al., 2002), et selon des sources indirectes sur le lierre de Colchide (*H. colchica*) (Leyns et al., 1984). Aucun rapport ne fait cependant état d'isolement de souches de *X. hortorum* pv. *hederae* à partir de ces trois espèces en conditions naturelles.

Les souches de *X. hortorum* pv. *pelargonii* sont spontanément retrouvées sur toute une gamme d'espèces végétales symptomatiques appartenant aux genres *Geranium* et *Pelargonium*, eux-mêmes regroupés dans la famille des *Geraniaceae* (**Tab. 1**). Il a également été observé que certaines souches pouvaient causer des symptômes légers sur des poinsettias (*Euphorbia pulcherrima*) inoculés en serre avec une suspension bactérienne concentrée (Rockey et al., 2015).

**Tableau 1.** Synthèse exhaustive des spectres d'hôtes naturels et expérimentaux des souches de *Xanthomonas hortorum* (Vauterin *et al.* 1995) Morinière *et al.* 2020. La taxonomie botanique des espèces végétales atteintes est succinctement présentée pour chacune d'entre elles<sup>a</sup>, ainsi que le type de maladie provoquée en conditions naturelles.

Clades	Ordre	Famille	Genre	Espèce <sup>b</sup>	Pathovar	Spectre d'hôte <sup>c</sup>	Maladie <sup>d</sup>	Commentaires	Références		
Eudicots	Asterales	Asteraceae	<i>Lactuca</i>	<i>sativa</i>	<i>vitiens</i>	N	BLS		Brown, 1918; Morinière <i>et al.</i> , 2020		
				<i>serriola</i>	<i>vitiens</i>	N	BLS	Une seule souche isolée	Morinière <i>et al.</i> , 2020; Toussaint <i>et al.</i> , 2012		
			<i>Taraxacum</i>	<i>biennis</i>	<i>vitiens</i>	E				Toussaint <i>et al.</i> , 2012	
				<i>officinale</i>	<i>vitiens</i>	E				Morinière <i>et al.</i> , 2020; Toussaint <i>et al.</i> , 2012	
			<i>Sonchus</i>	<i>kok-saghyz</i>	<i>taraxaci</i>	N				Niederhauser, 1943	
				<i>oleraceus</i>	<i>vitiens</i>	E				Toussaint <i>et al.</i> , 2012	
				<i>asper</i>	<i>vitiens</i>	E				Toussaint <i>et al.</i> , 2012	
			<i>Cichorium</i>	<i>intybus</i>	non-assigné					Zacaroni <i>et al.</i> , 2012	
			<i>Artemisia</i>	<i>biennis</i>	<i>vitiens</i>	E				Toussaint <i>et al.</i> , 2012	
			<i>Matricaria</i>	<i>annua</i>	non classé						Ssekiwoko <i>et al.</i> , 2009
				<i>discoidea</i>	<i>vitiens</i>	E					Toussaint <i>et al.</i> , 2012
			<i>Atractium</i>	<i>minus</i>	<i>vitiens</i>	E				Toussaint <i>et al.</i> , 2012	
			<i>Cynara</i>	<i>scolymus</i>	<i>cynarae</i>	N					Trebaol <i>et al.</i> , 2000
					<i>gardneri</i>	E					Timilsina <i>et al.</i> , 2019
				<i>Gnaphalium</i>	<i>uliginosum</i>	<i>vitiens</i>	E				Toussaint <i>et al.</i> , 2012
				<i>Ambrosia</i>	<i>artemisiifolia</i>	<i>vitiens</i>	E				Toussaint <i>et al.</i> , 2012
				<i>Galinsoga</i>	<i>quadriradiata</i>	<i>vitiens</i>	E				Toussaint <i>et al.</i> , 2012
				<i>Senecio</i>	<i>vulgaris</i>	<i>vitiens</i>	E				Toussaint <i>et al.</i> , 2012
				<i>Catendula</i>	<i>officinalis</i>	non-assigné					Parkinson <i>et al.</i> , 2009
				<i>Atractium</i>	<i>lappa</i>	" <i>X. campestris</i> pv. <i>nigromaculans</i> " <sup>a</sup>	N				Dehghan-Niri & Rahimian, 2016; Parkinson <i>et al.</i> , 2009
Apiales	Araliaceae	<i>Hedera</i>	<i>helix</i>	<i>hederae</i>	N				Arnaud, 1920; Trantas <i>et al.</i> , 2016		
			<i>canariensis</i>	<i>hederae</i>	N				Suzuki <i>et al.</i> , 2002		
			<i>nepalensis</i> (var. <i>sinensis</i> )	<i>hederae</i>	N				Zhang <i>et al.</i> , 2015		
			<i>rhombea</i>	<i>hederae</i>	E				Suzuki 2002		
			<i>colchica</i>	<i>hederae</i>	E				Leyns <i>et al.</i> , 1984		
		<i>Schefflera</i>	<i>atinophylla</i>	<i>hederae</i>	N					Chase, 1984; Norman <i>et al.</i> , 1999; Tolba, 2017	
			<i>arboricola</i>	<i>hederae</i>	N					Chase, 1984; Norman <i>et al.</i> , 1999	
		<i>Fatsia</i>	<i>japonica</i>	<i>hederae</i>	N				Chase, 1984		
		<i>Polyscias</i>	spp.	<i>hederae</i>	N				Norman <i>et al.</i> , 1999		
		<i>Plerandra</i>	<i>elegantissima</i>	<i>hederae</i>	E				Chase, 1984		
		<i>Apiaceae</i>	<i>Daucus</i>	<i>carotae</i>	N					du Toit <i>et al.</i> , 2014; Kendrick, 1934; Myung <i>et al.</i> , 2014	
		<i>Solanaceae</i>	<i>Nicotiana</i>	<i>tabacum</i>	E					Toussaint <i>et al.</i> , 2012	
	<i>Solanum</i>	<i>lycopersicum</i>	E/N?					Al-Saleh <i>et al.</i> , 2011; Morinière <i>et al.</i> , 2020; Sahin & Miller, 1998			
		<i>americanum</i>	E					Aratijo <i>et al.</i> , 2015			





Les spectres d'hôte naturel et artificiel de *X. hortorum* pv. *vitians* ont été assez largement documentés dans la littérature. Ce pathogène attaque toutes les variétés de laitue cultivée (*L. sativa*), et selon toute vraisemblance l'espèce sauvage ancestrale, la laitue scariote (*L. serriola*) (Morinière et al., 2020; Toussaint et al., 2012). Une expérience de détermination du spectre d'hôte expérimental de grande ampleur a également démontré que ce pathovar pouvait, à divers degrés, infecter et survivre de manière épiphyte sur plusieurs plantes sauvages de la famille des *Asteraceae* (**Tab. 1**). Certaines souches sont aussi faiblement virulentes sur tomate (*S. lycopersicum*) et poivron (*C. annuum*) dans des expériences d'inoculations conduites en serre (Al-Saleh et al., 2011; Morinière et al., 2020; Sahin & Miller, 1998). De manière intéressante, nous avons démontré dans le premier chapitre qu'une seule souche de *X. hortorum* pv. *vitians*, la CFBP 7999 (= ICMP 7383), isolée sur un pied de tomate présentant des symptômes caractéristiques de la gale bactérienne en Nouvelle-Zélande, avait la capacité de provoquer des symptômes sévères sur tomates réinoculées en serre. Ces observations laissent supposer qu'un récent saut d'hôte de la laitue vers la tomate a eu lieu chez cette souche.

Le seul hôte naturel recensé de *X. hortorum* pv. *cynarae* est l'artichaut (*C. scolymus*) (Trébaol et al., 2000), mais ce pathovar peut également causer l'apparition de symptômes foliaires sur feuilles de poivron infiltrées (Timilsina et al., 2019). Les souches de *X. hortorum* pv. *gardneri* peuvent être isolées sur tomate ou poivron cultivés dans les agroécosystèmes (Jones et al., 2004), mais il semblerait également que certaines souches ne soient pathogènes que sur l'une de ces deux espèces de *Solanaceae* quand d'autres le sont sur les deux (Potnis et al., 2015). Enfin, l'inoculation de *X. hortorum* pv. *gardneri* sur feuilles d'artichaut scarifiées cause une nécrose limitée (Timilsina et al., 2019).

Au regard de la littérature disponible, il semble que les spectres d'hôte des pathovars *X. hortorum* pv. *carotae* et *taraxaci* n'ont jamais été réellement investigués depuis leurs descriptions originales. En conséquence, les seuls hôtes connus pour ces pathovars sont leurs hôtes d'isolement respectifs, à savoir la carotte sauvage (*D. carota*) et sa sous-espèce cultivée (*D. carota* subsp. *sativus*) pour *X. hortorum* pv. *carotae* (du Toit et al., 2005; Kendrick, 1934), et le pissenlit Kazakh (*T. kok-saghyz*) pour *X. hortorum* pv. *taraxaci* (Niederhauser, 1943).

Comme annoncé plus haut, de nombreuses souches appartenant à l'espèce *X. hortorum* mais n'ayant pas été classées dans un pathovar ont été isolées sur une grande variété d'autres plantes (**Tab. 1**). Ainsi, des souches ont été isolées sur plusieurs espèces de pivoine (*Paeonia* spp.) aux États-Unis (Klass et al., 2019; Oliver et al., 2012) et sur l'armoïse annuelle

(*Artemisia annua*) en Ouganda (Ssekiwoko et al., 2009). Une maladie inconnue observée sur des espèces de lavande (*Lavandula dentata*, *L. angustifolia* et *L. × intermedia*) a tout d'abord été attribuée à *X. campestris* (Koike, 1995) avant que les souches soient identifiées comme appartenant à *X. hortorum* sur la base de données de séquence (Roberts & Parkinson, 2014; Rotondo et al., 2020). Des tests de similarité phénotypique ont également permis de mettre en évidence que des souches associées à une maladie de l'hortensia à feuille de chêne (*Hydrangea quercifolia*) observée aux États-Unis appartiendraient probablement à *X. hortorum* (Uddin et al., 1996). Les postulats de Koch ont été vérifiés pour toutes ces espèces végétales, indiquant sans ambiguïté que celles-ci sont donc des hôtes naturels de souches non-classées de *X. hortorum*. La grande bardane (*Arctium lappa*) est aussi probablement un hôte naturel de certaines souches de *X. hortorum* (Dehghan-Niri & Rahimian, 2016), qui seraient alors synonymes du taxon insuffisamment décrit *X. campestris* pv. *nigromaculans* (Takimoto, 1927).

En plus des plantes pour lesquelles les informations disponibles sont suffisantes pour affirmer qu'elles sont selon toute vraisemblance des hôtes naturels de *X. hortorum*, d'autres rapports associent des souches de *X. hortorum* à des espèces végétales inattendues. Ainsi, de nombreuses souches ont été isolées à partir de lots de graines de diverses plantes de la famille des *Poaceae* en Russie (Egorova et al., 2014) — à savoir le blé (*Triticum* sp.), l'orge (*Hordeum vulgare*), le seigle (*Secale cereale*) et l'avoine (*Avena sativa*). L'infiltration d'inoculum dans les feuilles de ces plantes induit la formation de lésions vasculaires ou nécrotiques locales (Egorova, 2015). Toutefois, aucune maladie des *Poaceae* n'a jamais été associée à *X. hortorum* dans les agroécosystèmes. Pour finir, deux études de MLSA ont rapporté l'isolement de souches uniques de *X. hortorum* à partir de symptômes observés sur feuilles d'olivier (*Olea europaea*) (Young et al., 2010), d'avocat (*Persea americana*) et de souci officinal (*Calendula officinalis*) (Parkinson et al., 2009). Étant donné l'absence de vérification des postulats de Koch dans tous ces rapports, ces espèces ne peuvent pas être considérées comme des hôtes naturels de *X. hortorum* en l'état et il faudrait déterminer si ces souches sont commensales ou pathogènes.

#### 4. Facteurs épidémiologiques et exploration du cycle de vie environnemental

Même si les facteurs épidémiologiques des pathologies causées par *X. hortorum* n'ont pas été étudiés chez tous les pathovars de l'espèce, les informations disponibles pour certains d'entre eux permettent de brosser un portrait global des paramètres favorisant le développement, la transmission et la dissémination de ces pathogènes dans les agroécosystèmes. Tout d'abord, il a été documenté que le développement au champ des maladies causées par *X. hortorum* était dépendant des conditions climatiques. Des températures modérées (20 à 25°C), des épisodes de pluie et surtout une humidité atmosphérique élevée semblent être particulièrement associées à des épisodes épiphytiques importants (Dougherty, 1974; du Toit et al., 2005; J. B. Jones et al., 1998; Kendrick, 1934; Robinson et al., 2006; Toussaint, 1999). La température optimale d'infection de *X. hortorum* pv. *vitians* sur laitues cultivées en serre a ainsi été déterminée à 22,7°C (Robinson et al., 2006). Des épisodes de froid pourraient également sensibiliser les plantes et favoriser l'infection par ces pathogènes (Brown, 1918; Pennisi & Pane, 1990). L'infection par des souches pathogènes de *X. hortorum* favoriserait également la surinfection par d'autres types de phytopathogènes, notamment eucaryotes comme des champignons ou des oomycètes (Toussaint, 1999). Il a également été rapporté qu'un effet synergétique aggravant l'infection du lierre par *X. hortorum* pv. *hederae* pouvait être observé en cas de co-infection avec l'espèce polyvalente *Pantoea agglomerans* (ex-*Erwinia herbicola*) (Burkholder & Guterman, 1932; Dutkiewicz et al., 2016). Lors des campagnes d'échantillonnage et d'isolement de *X. hortorum* pv. *vitians*, nous avons d'ailleurs constaté au moyen de séquençages 16S que des souches de *Pantoea* étaient très fréquemment retrouvées sur les feuilles de laitue infectées (*données non-publiées*).

À l'instar de la plupart des espèces de *Xanthomonas* (An et al., 2020), les *X. hortorum* passent vraisemblablement une partie importante de leur cycle de vie en dehors de l'interaction pathogène avec la plante hôte. Ainsi, *X. hortorum* pvs. *vitians*, *carotae* et *gardneri* peuvent être des contaminants des graines, qui constitueraient alors des sources principales d'inoculum pour ces pathogènes (Koike & Gilbertson, 2017; Mtui et al., 2010; Potnis et al., 2015; Scott & Dung, 2020). La contamination des graines peut s'opérer de deux manières : (i) par contamination de surface suite à la colonisation des organes reproductifs de la plante par des bactéries épiphytes et (ii) par contamination interne via l'infection systémique du système vasculaire et le transfert direct dans les graines (Barak et al., 2002).

Dans le cas de *X. hortorum* pv. *vitians*, il semblerait que la contamination se fasse par voie externe (Barak et al., 2002), tandis que la contamination des graines de carotte par *X. hortorum* pv. *carotae* pourrait survenir à la fois par voie externe et par voie vasculaire (du Toit et al., 2005). Chez *X. hortorum* pv. *carotae* où cette transmission a été abondamment documentée, les niveaux d'infestation des graines peuvent atteindre jusqu'à  $10^8$  UFC/g de graine (Scott & Dung, 2020). De plus, des graines de laitue artificiellement infectées par *X. hortorum* pv. *vitians* donnent des plantes contaminées (Barak et al., 2002; Sahin & Miller, 1997). Toutefois, si la contamination des lots de graines commerciales de carotte semble fréquente (du Toit et al., 2005; Scott & Dung, 2020), les lots de graines de laitue vendus dans le commerce ne sont généralement pas ou peu contaminés selon les rares données disponibles (Koike & Gilbertson, 2017; Zoina & Volpe, 1992). D'après ce qui a pu être observé chez d'autres espèces de *Xanthomonas*, les bactéries contaminantes des graines pourraient survivre pendant des périodes de plusieurs mois, voire plusieurs années (Bashan et al., 1982; Murty & Devadath, 1984), faisant ainsi des graines des réservoirs de long terme pour ces pathogènes. Compte tenu de la mondialisation du commerce des graines, celles-ci participent aussi probablement à la dissémination des pathogènes à l'échelle de la planète et contribuent à modifier la composition des populations endémiques de pathogènes par l'introduction de clones qui n'étaient jusqu'alors pas présents sur certains territoires.

Parmi les autres réservoirs environnementaux identifiés, les débris végétaux contaminés enfouis dans le sol lors du broyage des plants non récoltés, les adventices et les eaux d'irrigation peuvent faire office de réservoirs à court et moyen termes. *Xanthomonas hortorum* pv. *vitians* a été ainsi retrouvée dans des réseaux d'irrigation et sur les feuilles de plusieurs espèces d'adventices lors d'échantillonnages menés sur plusieurs saisons de production en Floride (Fayette et al., 2017). Ce pathogène possède en effet la capacité de survivre de manière épiphyte sur une gamme importante d'adventices pendant au moins 4 à 5 semaines (Barak et al., 2001; Fayette et al., 2017; Toussaint et al., 2012). Des expériences de plein champ en Californie ont aussi permis de mettre en évidence que ce pathogène pouvait survivre à la période hivernale sous des climats tempérés en association avec des débris végétaux enfouis pendant une période allant jusqu'à 5 mois (Barak et al., 2001). Toutefois, une expérience similaire réalisée en Floride a montré que les populations de *X. hortorum* pv. *vitians* ne survivaient pas plus d'un mois dans des débris enfouis (Fayette et al., 2017), suggérant que ce type de réservoir serait différentiellement important selon les régions et les conditions environnementales et pédoclimatiques.

Les *X. hortorum* peuvent se propager au sein d'une parcelle ou entre parcelles par projection de débris aérosolisés issus du broyage des plants non récoltés (du Toit et al., 2005), ou par projection de microgouttelettes de pluie ou d'eau d'irrigation (Potnis et al., 2015; Wellman-Desbiens, 1998). Des expériences portant sur la propagation de *X. hortorum* pv. *vitians* en serre ont permis de montrer que les aérosols formés lors de l'impact de gouttes d'eau d'irrigation sur des feuilles de laitue infectées pouvaient projeter la bactérie jusqu'à une dizaine de mètres et propager la maladie à l'ensemble de la serre à partir d'un seul foyer infectieux en quelques jours (Wellman-Desbiens, 1998). La dissémination pourrait aussi être favorisée par les manipulations des plantes lors du travail au champ, en provoquant des blessures par exemple (Potnis et al., 2015). Les étapes de transport et de transplantation des plantules joueraient également un rôle important dans la dissémination de proche en proche de ces pathogènes (Simonton et al., 2021).

Enfin, la détection de souches de *Xanthomonas* métaboliquement actives sous forme d'aérosols dans les eaux de nuage (Failor et al., 2017; Vaïtilingom et al., 2012) et dans les masses d'air de basse atmosphère (Els et al., 2019) suggèrent un rôle potentiel de ces compartiments environnementaux comme réservoirs alternatifs et comme sources de dissémination sur de grandes distances, comme cela a été démontré pour la bactérie phytopathogène *Pseudomonas syringae* (Morris et al., 2008). De manière générale, les eaux de nuages ont été proposées comme étant des réservoirs importants du microbiote phyllosphérique des plantes, y compris pour des bactéries phytopathogènes (Vaïtilingom et al., 2012). Certaines souches des espèces *X. translucens* et *X. campestris* sont également *Ice Nucleation Active* (INA), c'est-à-dire qu'elles possèdent la capacité à induire la cristallisation de l'eau liquide à des températures inférieures à 0°C, un trait écologique important pour l'adaptation aux conditions environnementales extrêmes des masses d'eau de l'atmosphère (Failor et al., 2017; Joly et al., 2013). Une rapide recherche d'orthologues du gène *inaX*, acteur majoritaire de ce phénotype INA chez *X. translucens* (Zhao & Orser, 1990), dans les génomes de *X. hortorum* permet d'ailleurs de mettre en évidence la présence d'orthologues conservés (identité de séquence protéique > 70 %) chez tous les pathovars de *X. hortorum*. De plus, nous avons recherché la présence de séquences spécifiques de *X. hortorum* pv. *vitians* dans des bases de données métagénomiques environnementales grâce au programme MEESEEQS développé par Aurélie PETICCA au cours de son stage de Master 1 (*données non-publiées*). La présence de *X. hortorum* pv. *vitians* a notamment été détectée dans des échantillons de poussières atmosphériques collectés à Beijing, en Chine, lors d'épisodes

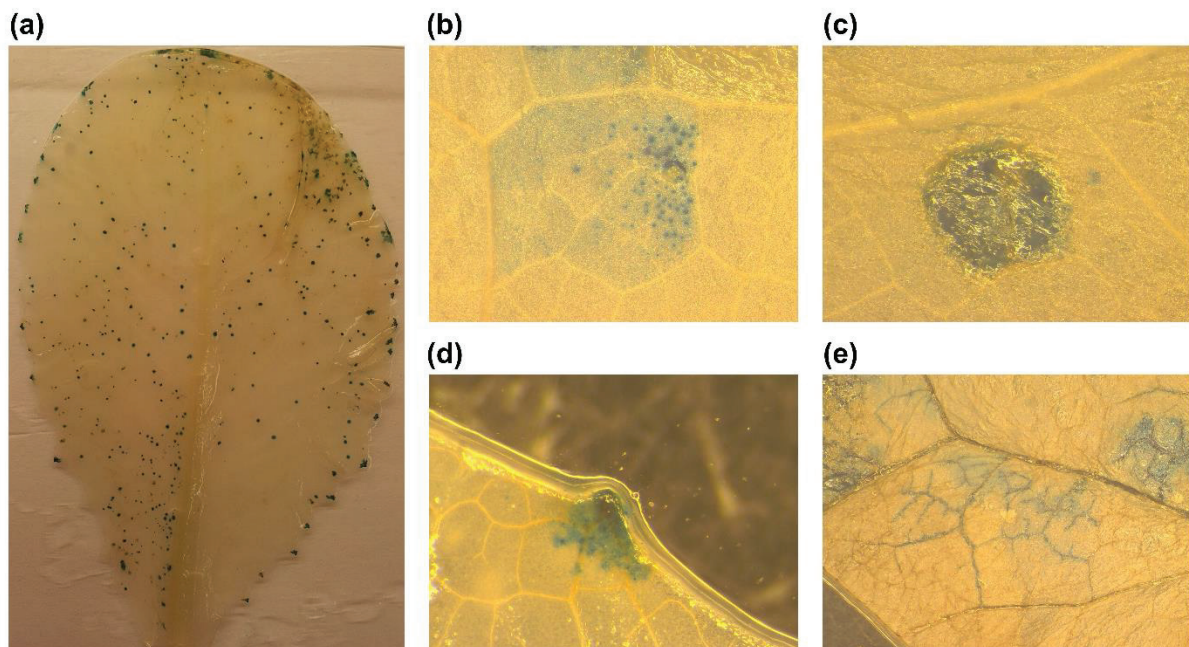
sévères de *smogs* en 2014 (Cao et al., 2014). Il serait donc intéressant d'étudier plus en profondeur la présence de *X. hortorum* dans ces compartiments environnementaux afin de mieux comprendre l'ensemble du cycle de vie non-infectieux et les mécanismes de dissémination de ces pathogènes.

## 5. Interactions avec l'hôte végétal et son système immunitaire

Le cycle infectieux des bactéries du genre *Xanthomonas* peut être divisé en une première étape de vie épiphyte suivie par une étape de colonisation endophyte (An et al., 2020). Lors de leur arrivée sur un nouvel hôte végétal, elles vont s'installer à la surface des feuilles et pouvoir s'y maintenir pendant de longues périodes (Gent et al., 2005; Rigano et al., 2007; Zarei et al., 2018), ce qui en fait des acteurs importants du microbiote phyllosphérique (Vorholt, 2012). Lors de cette phase, les *Xanthomonas* doivent survivre dans des conditions environnementales défavorables et résister à de nombreux stress, notamment du fait de l'exposition aux radiations lumineuses, des variations importantes de température et d'humidité et d'un accès limité à des ressources nutritives (Lindow & Brandl, 2003; Vacher et al., 2016). La formation de biofilms, la sécrétion d'exopolysaccharides comme le xanthane et l'expression de gènes d'adhésines contribuent à l'adaptation aux conditions de vie épiphyte (An et al., 2020). La sécrétion d'enzymes extracellulaires dégradant les polymères de la paroi végétale (celluloses, hémicelluloses, pectines...) par le système de sécrétion de type II et l'expression de systèmes d'acquisition et d'assimilation des carbohydrates joueraient aussi un rôle important dans la nutrition à ce stade (Blanvillain et al., 2007; Boulanger et al., 2014; Déjean et al., 2013).

Ensuite, les bactéries se dirigent vers les points d'entrée dans la feuille par une mobilité active probablement contrôlée par des phénomènes de chimiotactisme (An et al., 2020; Moreira et al., 2015). En fonction de leurs types tissulaires (Ryan et al., 2011), les *Xanthomonas* se dirigent préférentiellement vers les stomates ou les hydathodes et s'y agrègent afin de pénétrer dans les tissus et d'entamer la phase endophyte de leur cycle infectieux. Les *Xanthomonas* vasculaires colonisent en général les hydathodes, qui sont des structures poreuses spécialisées situées à la marge du limbe foliaire et impliquées notamment dans l'exsudation d'eau issue du parenchyme aquifère et des vaisseaux du xylème (Cerutti et al., 2017). Les *Xanthomonas* non-vasculaires, eux, s'installent dans les espaces intercellulaires du mésophylle, dans un compartiment appelé apoplasme, via la pénétration des stomates

(Zhang et al., 2009). Certains pathovars de *X. hortorum* sont considérés comme des pathogènes vasculaires et d'autres comme non vasculaires (Jacques et al., 2016), mais leurs voies de pénétration dans les plantes n'ont pas été caractérisées à proprement parler. A titre d'exemple, la diffusion de *Xanthomonas hortorum* pvs. *pelargonii* et *vitians* dans le système vasculaire des plantes hôtes a été étudiée, mais seulement par injection directe d'inoculum dans les vaisseaux (Barak et al., 2002; Barel et al., 2015). Des expériences préliminaires menées avec un système rapporteur GUS au cours de l'année de Master 2 indiquent que *X. hortorum* pv. *vitians* pénétrerait à la fois par les stomates et les hydathodes de laitues, et aurait donc une stratégie de colonisation mixte impliquant à la fois le réseau vasculaire et l'apoplasme (Rapport M2 Lucas Morinière, 2017, non-publié) (**Fig. 6**).



**Figure 6.** Localisation histochimique de *X. hortorum* pv. *vitians* CFBP 498 avec un système rapporteur GUS. **(a)** Observation de multiples points d'infection sur feuille de laitue à 14 jours post-inoculation (JPI). **(b)** Colonisation de stomates à 14 JPI. **(c)** Nécrose avancée du mésophylle à 21 JPI. **(d)** Colonisation d'une hydathode en bordure du limbe foliaire à 14 JPI. **(e)** Propagation systémique dans le réseau vasculaire à 21 JPI.

Tout au long de ce processus infectieux, les *Xanthomonas* sont confrontées à la réponse du système immunitaire végétal inné et tentent de contrecarrer ses effets. La première ligne du système immunitaire inné des plantes est la ***Pattern-Triggered Immunity*** (PTI), qui consiste en la reconnaissance de motifs moléculaires microbiens conservés appelés *Microbe-Associated Molecular Patterns* (MAMPs) ou de constituants endommagés de l'organisme

végétal lui-même appelés *Damage-Associated Molecular Patterns* (DAMPs) (Boller & Felix, 2009). Cette reconnaissance est médiée par des récepteurs de surface spécifiques enchâssés dans la membrane plasmique des cellules végétales et regroupés sous l'appellation de *Pattern Recognition Receptors* (PRRs) (Boller & Felix, 2009). La terminologie de MAMPs et de DAMPs a remplacé les anciens « éliciteurs » du système immunitaire végétal conceptualisés quelques décennies plus tôt (Darvill & Albersheim, 1984). Parmi les MAMPs les plus connus, on retrouve chez les bactéries le peptide flg22 issu du flagelle, le peptide elf18 dérivé du facteur d'élongation EF-Tu, certaines parties du lipopolysaccharide (LPS) de surface et des constituants du peptidoglycane ; chez les champignons, le chitosan et la chitine de la paroi cellulaire ainsi que certaines xylanases (Boller & Felix, 2009). Les DAMPs, quant à eux, peuvent être différents composants des parois cellulaires végétales issus notamment de la dégradation par les enzymes de dégradation de la paroi (*Cell Wall Degrading Enzymes*, CWDEs) sécrétés par les bactéries ou les champignons, des peptides et protéines apoplasmiques, des nucléotides, des sucres ou des acides aminés extracellulaires (Hou et al., 2019). Certains composés organiques volatiles (*Volatile Organic Compounds*, VOCs), principalement connus pour leur rôle dans la signalisation plante-plante en réponse aux herbivores (Meents & Mithöfer, 2020), pourraient également jouer un rôle dans l'immunité face aux phytopathogènes microbiens (Tilocca et al., 2020). La reconnaissance de ces motifs par les PRRs, qui sont principalement des *Leucine-Rich Repeat – Receptor-Like Kinases* (LRR-RLK) ou des *Receptor-Like Proteins* (RLP) contenant des motifs conservés LRR ou des motifs extracellulaires de lysine (LysM), entraîne des cascades de signalisation impliquant les différentes hormones végétales (acide salicylique, jasmonate et éthylène) et qui vont enclencher en réponse les différents mécanismes immunitaires de la plante (Glazebrook, 2005; Islam et al., 2021; Pieterse et al., 2009). Ces réponses immunitaires, dont l'activation se fait de manière séquentielle au cours du temps, impliquent entre autres la fermeture des stomates (Zeng et al., 2010), des flux d'ions importants, une production intense d'espèces réactives de l'oxygène (ROS), des modifications transcriptionnelles importantes, des phosphorylations des protéines et une déposition de callose (Boller & Felix, 2009).

Afin de prévenir cette réponse immunitaire, les *Xanthomonas* vont injecter directement dans les cellules végétales des effecteurs qui vont aller perturber les cascades de signalisation immunitaire, notamment en sécrétant des effecteurs via les systèmes de sécrétion de type III (SST3) et de type VI (SST6) (Ryan et al., 2011; Timilsina et al., 2020). C'est là qu'entre en jeu pour les plantes la deuxième ligne de défense immunitaire innée, appelée



**Effector-Triggered Immunity** (ETI) (Boller & Felix, 2009). La reconnaissance hautement spécifique de ces effecteurs par des protéines de résistance encodées par les gènes *R* va permettre de restaurer la signalisation cellulaire et d'accentuer même la réponse immunitaire en déclenchant notamment la réponse hypersensible (*Hypersensitive Response*, HR), qui se caractérise notamment par une rapide apoptose cellulaire programmée et localisée (Balint-Kurti, 2019). La plupart des gènes *R* encodent la synthèse de protéines cytoplasmiques de la classe des *Nucleotide-Binding – Leucine Rich Repeat* (NB-LRR) (Boller & Felix, 2009). L'étude de ces mécanismes d'interaction gène pour gène hautement spécifiques entre effecteurs microbiens et protéines *R* végétales a donné naissance au modèle d'évolution en zig-zag du système immunitaire végétal (Jones & Dangl, 2006). Selon ce modèle, au cours de leur longue coévolution, les microorganismes phytopathogènes acquièrent de nouveaux effecteurs permettant de contrer l'action des protéines *R*, puis les plantes acquièrent de nouvelles protéines *R* permettant de reconnaître ces effecteurs, et ainsi de suite (Jones & Dangl, 2006). Cependant, les avancées conceptuelles dans le domaine de la modélisation ont conduit récemment à remettre en question ce modèle en zig-zag pour lui préférer une approche de modèle en réseau, mieux à même de décrire précisément l'importante complexité de la réponse immunitaire végétale (Katagiri, 2018).

Peu d'études se sont pour l'instant intéressées à la dimension moléculaire des interactions hôte-pathogène chez l'espèce *X. hortorum*. Il semblerait que tous les pathovars possèdent un système de sécrétion de type III similaire à celui existant chez *X. campestris* pv. *campestris* (Potnis et al., 2011; Timilsina et al., 2019). Le cluster *hrp* encodant ce SST3 contient notamment un gène *hrpW* codant une protéine fusionnée pectate lyase-harpin (Potnis et al., 2011) et qui n'est retrouvée que chez un nombre restreint de *Xanthomonas* (White et al., 2009). À ce jour, seuls les génomes de *X. hortorum* pvs. *gardneri* et *carotae* ont été explorés à la recherche d'effecteurs de type III (Kimbrel et al., 2011; Potnis et al., 2011; Schwartz et al., 2015). Le gène *avrHah1* identifié chez *X. hortorum* pv. *gardneri* est celui qui a fait l'objet du plus grand intérêt dans la mesure où il encode un *Transcription Activator-Like Effector* (TALE) de la famille AvrBs3/PthA (Schornack et al., 2008). De manière générale, les TALEs sont des effecteurs de type III particuliers qui, une fois injectés dans le cytoplasme des cellules végétales, auront la capacité de pénétrer le noyau des cellules afin de moduler directement l'expression des gènes de l'hôte (Xu et al., 2017). Le TALE AvrHah1 contrôle notamment l'expression de deux gènes de modification de la pectine, indiquant que son rôle pourrait être de provoquer une modification des parois végétales afin de promouvoir

l'adsorption d'eau dans l'apoplasme, augmenter les dommages tissulaires et faciliter la sortie des bactéries pathogènes de l'apoplasme vers la surface foliaire en fin de phase infectieuse (Schwartz et al., 2017). Quoi qu'il en soit, les répertoires d'effecteurs de type III, appelés aussi « effectomes », sont considérés comme les déterminants principaux de la spécificité d'hôte chez les *Xanthomonas* (Hajri et al., 2009). L'étude des répertoires d'effecteurs des différents pathovars de *X. hortorum* permise par les avancements dans les domaines du séquençage et de la génomique (Arroyo-Velez et al., 2020) offrirait donc des possibilités intéressantes pour comprendre la grande spécificité d'hôte de chacun des pathovars.



# CHAPITRE I :

## Taxonomie de *Xanthomonas hortorum* et diversité de *X. hortorum* pv. *vitians*

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### Préambule

La taxonomie représente le point de départ de toute étude scientifique en biologie. Articulés dans un système cohérent, les noms sont alors des vecteurs de sens qui renseignent sur les caractéristiques principales d'un organisme, sur ses relations avec les autres formes de vie similaires et sa place dans le système de classification hiérarchique unifié (Cowan, 1971). Dans le domaine de la pathologie végétale, la connaissance de la taxonomie et de l'identité des microorganismes phytopathogènes est donc indispensable pour tous les acteurs d'une filière de production végétale à l'élaboration de stratégies de diagnostic et de contrôle raisonnées (Bull & Koike, 2015). Comme l'ont très justement écrit Morris et ses collaborateurs à ce propos, « *les noms font partie d'un processus dialectique au sein duquel ils fixent le cadre génétique et fonctionnel, qui détermine à son tour la robustesse des outils d'identification, qui eux-mêmes délimitent le cadre.* » (Morris et al., 2017).

Lorsque nous avons commencé notre étude, trois dénominations différentes étaient encore utilisées pour décrire les populations de souches responsables de la bactériose foliaire de la laitue (*X. campestris* pv. *vitians*, *X. axonopodis* pv. *vitians* et *X. hortorum* pv. *vitians*). Sans connaître la diversité génétique et la réalité taxonomique derrière cette situation confuse, comment envisager une étude sérieuse des stratégies de diagnostic et de lutte applicables contre cette pathologie végétale ? En collaboration avec la SERAIL, la CFBP et l'IRHS de l'INRAE d'Angers, nous avons cherché à déterminer la diversité génétique des populations de pathogènes associées à la bactériose foliaire de la laitue à l'échelle régionale, et dans une certaine mesure nationale et internationale. Ces premiers travaux nous ont amené à réaliser qu'il était indispensable de résoudre le statut taxonomique de l'espèce *X. hortorum* dans son ensemble, notamment en raison de sa très forte proximité avec l'espèce *X. cynarae*. Nous avons alors étendu notre collaboration en nous associant avec le Département de Pathologie Végétale de l'Université de Pennsylvanie afin de réaliser une étude de taxonomie polyphasique ambitieuse qui a fait l'objet d'une première publication dans le journal *Systematic and Applied Microbiology* en juillet 2020 et de 3 présentations orales dans des congrès nationaux et une dans un congrès international entre 2016 et 2020.



# Clarifying the taxonomy of the causal agent of bacterial leaf spot of lettuce through a polyphasic approach reveals that *Xanthomonas cynarae* Trébaol *et al.* 2000 emend. Timilsina *et al.* 2019 is a later heterotypic synonym of *Xanthomonas hortorum* Vauterin *et al.* 1995

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## ABSTRACT

Assessment of the taxonomy and diversity of *Xanthomonas* strains causing bacterial leaf spot of lettuce (BLSL), commonly referred to as *Xanthomonas campestris* pv. *vitiens*, has been a long-lasting issue which held back the global efforts made to understand this pathogen. In order to provide a sound basis essential to its study, we conducted a polyphasic approach on strains obtained through sampling campaigns or acquired from collections. Results of a multilocus sequence analysis crossed with phenotypic assays revealed that the pathotype strain does not match the description of the nomenclature provided by Brown in 1918. However, strain LMG 938 = CFBP 8686 does fit this description. Therefore, we propose that it replaces LMG 937 = CFBP 2538 as pathotype strain of *X. campestris* pv. *vitiens*.

Then, whole-genome based phylogenies and overall genome relatedness indices calculated on taxonomically relevant strains exhibited the intermediate position of *X. campestris* pv. *vitiens* between closely related species *Xanthomonas hortorum* and *Xanthomonas cynarae*. Phenotypic profiles characterized using Biolog microplates did not reveal stable diagnostic traits legitimizing their distinction. Therefore, we propose that *X. cynarae* Trébaol *et al.* 2000 emend. Timilsina *et al.* 2019 is a later heterotypic synonym of *X. hortorum*, to reclassify *X. campestris* pv. *vitiens* as *X. hortorum* pv. *vitiens* comb. nov. and to transfer *X. cynarae* pathovars in *X. hortorum* as *X. hortorum* pv. *cynarae* comb. nov. and *X. hortorum* pv. *gardneri* comb. nov. An emended description of *X. hortorum* is provided, making this extended species a promising model for the study of *Xanthomonas* quick adaptation to different hosts.

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## Introduction

Bacterial leaf spot of lettuce (BLSL) is a foliar disease occurring on all types of the cultivated lettuce *Lactuca sativa* L. Its presence has been reported around the world where lettuce is grown [1,39,65,74,81] and has become a preoccupying threat for lettuce producers over the last decades in the absence of efficient means to control the disease [10,20,25]. Typical symptoms of BLSL starts

with small water-soaked lesions on the outer leaves which later become necrotic and surrounded by a chlorotic halo, before coalescing into large necrotic patches under humid and warm conditions [11]. Although not directly lethal for the plant, this damage will reduce the quality and yield, sometimes dramatically, and have been suggested to increase the susceptibility to more severe fungal pathogens [65]. All major commercial lettuce types can be infected, even though relative resistances in some types or cultivars have been observed [9,25]. It has also been reported that on lettuce seed crops, symptoms can develop on stems and flower bracts as brown to black longitudinal lesions [32,53]. The pathogen is thought to be seedborne [32,69,81] and able to move systematically within the stems of lettuce plants [4]. It can also remain viable for months in

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buried plant debris or surface irrigation water, and was shown to maintain high epiphytic populations on a wide variety on weeds [5,18,66].

The bacterium responsible was first isolated in 1916 in South Carolina and named *Bacterium vitians* Brown 1918 [7] before being reclassified as *Xanthomonas vitians* Dowson 1943 at the creation of the genus [14]. Following the revision of the *International Code of Nomenclature of Bacteria* (1980), which created stricter rules for naming bacterial species, it was integrated into the polytypic species *X. campestris* [77]. A new infraspecies designation, the “pathovar”, used to describe a group of organisms within a species with a particular host range or causing distinct disease symptoms, was introduced upon this occasion. The species epithet *vitians* was used as the new pathovar epithet to maintain continuity between literature published before and after this change. Finally, DNA-DNA hybridization experiments showed that strains of *X. campestris* pv. *vitians* (Brown 1918) Dye 1978 clustered into two different species [72]. The pathotype strain CFBP 2538<sup>PT</sup> (=NCPBP 976<sup>PT</sup> = LMG 937<sup>PT</sup>) was grouped with *X. axonopodis* Vauterin et al. 1995 as *X. axonopodis* pv. *vitians*. It is important to note that CFBP 2538<sup>PT</sup> is not pathogenic on lettuce but weakly pathogenic on tomato and pepper [26,53,54]. Another commonly used representative strain of BLSL, LMG 938 (=CFBP 8686 = NCPBP 2248), was included into the newly formed species *X. hortorum* Vauterin et al. 1995 with four other pathovars (*hederae*, *pelargonii*, *taraxaci*, *carotae*). Additional studies on the genetic diversity of strains associated with BLSL demonstrated that strains that cause BLSL were all similar to LMG 938 and genetically distant from CFBP 2538<sup>PT</sup> [3,53]. However, the name “*X. hortorum* pv. *vitians* Vauterin et al. 1995” was not valid according to the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (ISPP-CTPPB, the governing body of pathovar nomenclature) because there was no description given for the new taxon based on a proposed pathotype strain [79]. As a result, the pathogen is still referred to as *X. campestris* pv. *vitians* type A referring to the pathotype strain CFBP 2538<sup>PT</sup> only, or *X. campestris* pv. *vitians* type B for all the BLSL-causing strains including LMG 938 [72]. The lack of a valid pathovar name for this taxon within *X. hortorum* has generated confusion and is a source of mistakes and misunderstandings [41,43,57,76]. This is compounded by the fact that *X. campestris* pv. *vitians* CFBP 2538<sup>PT</sup> does not fit the description of the pathovar provided by Brown 1918 and therefore is unsuitable to serve as the pathotype. According to standard 11 of the *International Standards for Naming Pathovars of Phytopathogenic Bacteria* [15], ‘If a pathotype or neopathotype strain has become unsuitable due to changes in its characters or for other reasons, then the matter should be referred to the Taxonomy Committee, which may decide to take action leading to replacement of the strain.’ Thus, we sent a letter to the ISPP-CTPPB on October the 29th, 2019 to request replacement of this pathotype strain and to propose strain LMG 938<sup>neoPT</sup> = CFBP 8686<sup>neoPT</sup> = NCPBP 2248<sup>neoPT</sup> as a neopathotype strain of *X. campestris* pv. *vitians*. In this manuscript we make two proposals that will resolve the nomenclatural issues regarding the BLSL pathogens in *X. hortorum* and that will maintain the priority (Brown 1918) for the pathogen causing BLSL. First, we propose the replacement of the pathotype strain of *X. campestris* pv. *vitians*, then the transfer of this pathovar in *X. hortorum* as *X. hortorum* pv. *vitians* comb. nov.

Another layer of complexity to the taxonomy of *X. hortorum* pathovars is that on one hand they are genetically heterogeneous and that on the other hand some of them are highly genetically related to *X. cynarae* pv. *cynarae* (pathogenic on artichoke) and *X. cynarae* pv. *gardneri* (pathogenic on tomato and pepper) [29,68,78]. These pathovar names result from the recent proposal of synonymy between *X. cynarae* and *X. gardneri* [64]. Thus, *X. hortorum* is a paraphyletic species and a comprehensive taxonomic study of *X.*

*hortorum* pathovars including their nearest phylogenetic neighbors is needed to resolve their classification.

The present study aims at resolving formally the taxonomy of the causal agent of BLSL and its close relatives within the comprehensive framework of the global structure of the genus *Xanthomonas*. In order to investigate the genetic diversity of strains associated with BLSL, we conducted sampling campaigns in the Rhône-Alpes region, France, and completed our set with historical collection strains from various origins. Then, an extensive polyphasic approach was conducted based on pathogenicity assays, multilocus sequence analysis (MLSA) on three housekeeping genes, *de novo* whole-genome sequencing, phylogenomic tree reconstruction, overall genome relatedness indices (OGRIs) calculations and standardized biochemical phenotypic profiling. The results obtained support our proposals to replace the pathotype strain of *X. campestris* pv. *vitians*, to transfer this pathovar in *X. hortorum* as *X. hortorum* pv. *vitians* comb. nov. and to propose the synonymy between *X. hortorum* and *X. cynarae*, reclassifying former pathovars of *X. cynarae* as *X. hortorum* pv. *cynarae* comb. nov. and *X. hortorum* pv. *gardneri* comb. nov.

## Experimental procedures

### Bacterial strains, isolation procedure and growth conditions

All the strains used in this study and their related information are listed in Table 1. The collection consists of 55 strains of *X. campestris* pv. *vitians* including the pathotype strain, the type, pathotype or representative strains of the four pathovars of *X. hortorum* (pvs. *pelargonii*, *hederae*, *carotae* and *taraxaci*), four strains of *X. cynarae* including the type strain and the pathotype strain of *X. cynarae* pv. *gardneri*. Strains were either obtained from the CIRM-CFBP (International Center for Microbial Resources-French Collection of Plant Associated Bacteria, Angers, France), the BCCM/LMG (Belgian Co-ordinated Collections of Micro-organisms/Laboratory of Microbiology, Ghent, Belgium) or collected during two sampling campaigns in summers 2016 and 2017 in the Rhône-Alpes region, France. Diseased lettuce and weed samples were harvested and grinded in sterile deionized water, then plated on semi-selective MMG medium [67] supplemented with cycloheximide at 50 µg/mL. After 4 days of incubation at 28 °C, typical colonies of *Xanthomonas* were isolated and identified by MLSA as described below. For long-term storage, strains were mixed in 1/10<sup>th</sup> tryptic soy broth (TSB) containing 30% glycerol and stored at -80 °C. Bacterial cultures were made in 1/10<sup>th</sup> TSB or on 1/10<sup>th</sup> tryptic soy agar (TSA) plates and cultivated 24–48 h at 28 °C.

### Pathogenicity tests

Pathogenicity of all bacterial strains was tested on leaf lettuce cv. Météore and oakleaf lettuce cv. Kirinia. Plants were grown in a greenhouse in 8-cm pots containing TS3 mold (Klasmann-Deilmann, Geeste, Germany) during 3–4 weeks. Overnight bacterial cultures (0.8–1.0 OD<sub>600nm</sub>) were spectrophotometrically adjusted to 0.2 OD<sub>600nm</sub> in sterile deionized water, corresponding to approximately 10<sup>8</sup> CFU/mL with Tween 80 added at 0.08%. Fifty mL of the resulting suspensions were inoculated with a hand sprayer until run-off on 8 plants per strain per cultivar. Eight plants sprayed with sterile deionized water supplemented with Tween 80 at 0.08% served as a negative control. Plants were incubated in a Fitoclima 10.000 EH environmental chamber (Aralab, Rio de Mouro, Portugal) at 25 °C with at least 90% relative humidity and an 18 h photoperiod. After 48 h, relative humidity was adjusted to 70% until the end of the experiment. Disease severity was measured every 2–3 days on each plant for three weeks using the 5-point scale disease

**Table 1**  
Bacterial strains used in this study.

Proposed nomenclature <sup>a</sup>	Strain no.	Other collection no.	Former nomenclature <sup>b</sup>	Host of isolation	Geographic origin	Year of isolation	Pathogenicity <sup>y</sup>		Reference
							Lettuce cv. Kirinia	Lettuce cv. Météore	
<i>X. citri</i> <sup>c</sup>	CFBP 2538 <sup>c</sup>	ATCC 19320 <sup>e</sup>	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Dye 1978	<i>Lactuca</i> sp.	United States	1917	-	-	[5]
		ICMP 336							
		LMC 937							
<i>X. hortorum</i> pv. <i>hederae</i>	CFBP 5858 <sup>f</sup>	CFBP 4925 <sup>t</sup>	<i>X. axonopodis</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995	<i>Hedera helix</i>	United States	1944	-	-	[63]
		LMC 733							
		NCPPB 939							
		ICMP 453							
<i>X. hortorum</i> pv. <i>carotae</i> <sup>e</sup>	CFBP 7900 <sup>+</sup>	M081 <sup>+</sup>	<i>X. axonopodis</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995	<i>Daucus carota</i>	United States	2011	-	-	[24]
		ICMP 16775 <sup>t</sup>							
<i>X. hortorum</i> pv. <i>cynarae</i>	CFBP 4188 <sup>g</sup>	ICMP 16775 <sup>t</sup>	<i>X. cynarae</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995	<i>Cynara scolymus</i>	Bretagne, France	1996	-	-	[60]
		ICMP 16775 <sup>t</sup>							
<i>X. hortorum</i> pv. <i>gardneri</i>	CFBP 8163 <sup>g</sup>	ATCC 19865 <sup>t</sup>	<i>X. gardneri</i> (ex Šutič 1957) Jones et al. 2006	<i>Solanum lycopersicum</i>	Yugoslavia	1953	-	+	[23]
		NCPPB 881							
<i>X. hortorum</i> pv. <i>pelargonii</i>	CFBP 8588	JS749-3	<i>X. gardneri</i> (ex Šutič 1957) Timilsina et al. 2019	<i>Solanum lycopersicum</i>	La Réunion, France	1997	-	+	[56]
		LMC 7314 <sup>t</sup>							
<i>X. hortorum</i> pv. <i>taraxaci</i>	CFBP 2533 <sup>g</sup>	NCPPB 2985	<i>X. gardneri</i> (ex Šutič 1957) Timilsina et al. 2019	<i>Pelargonium peltatum</i>	New Zealand	1974	-	-	[63]
		ICMP 4321							
<i>X. hortorum</i> pv. <i>vitians</i> <sup>c</sup>	LM 16389	ATCC 19318 <sup>t</sup>	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Dye 1978	<i>Taraxacum kok-saghyz</i>	United States	1942	-	-	[63]
		LMC 870							
<i>X. hortorum</i> pv. <i>vitians</i> <sup>c</sup>	LMC 938 <sup>no</sup>	NCPPB 940	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Dye 1978	<i>Taraxacum</i> sp.	Isère, France	2016	-	-	This study
		CFBP 8644							
<i>X. hortorum</i> pv. <i>vitians</i> <sup>c</sup>	CFBP 3976	CFBP 8686	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995 <sup>+</sup>	<i>Lactuca sativa</i>	Zimbabwe	1966	+	+	[63]
		NCPPB 2248 <sup>t</sup>							
<i>X. hortorum</i> pv. <i>vitians</i> <sup>c</sup>	CFBP 498	NCPPB 232	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995 <sup>+</sup>	<i>Lactuca</i> sp.	United States	1949	+	+	This study
		NCPPB 969							
<i>X. hortorum</i> pv. <i>vitians</i> <sup>c</sup>	CFBP 499	NCPPB 969	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995 <sup>+</sup>	<i>Lactuca scariola</i>	United States	1961	+	+	This study
		NCPPB 992							
<i>X. hortorum</i> pv. <i>vitians</i> <sup>c</sup>	CFBP 500	CFBP 3971	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995 <sup>+</sup>	<i>Lactuca</i> sp.	United States	1949	+	+	This study
		CFBP 3971							
<i>X. hortorum</i> pv. <i>vitians</i> <sup>c</sup>	CFBP 3973	CFBP 3973	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995 <sup>+</sup>	<i>Lactuca sativa</i>	France	1994	+	+	This study
		CFBP 3973							
<i>X. hortorum</i> pv. <i>vitians</i> <sup>c</sup>	CFBP 3975	CFBP 3975	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995 <sup>+</sup>	<i>Lactuca sativa</i>	France	1994	+	+	This study
		CFBP 3975							
<i>X. hortorum</i> pv. <i>vitians</i> <sup>c</sup>	CFBP 3976	CFBP 3976	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995 <sup>+</sup>	<i>Lactuca sativa</i>	France	1994	+	+	This study
		CFBP 3976							
<i>X. hortorum</i> pv. <i>vitians</i> <sup>c</sup>	CFBP 3978	CFBP 3978	<i>X. campestris</i> pv. <i>vitians</i> (Brown 1918) Vauterin et al. 1995 <sup>+</sup>	<i>Lactuca sativa</i>	France	1994	+	+	This study
		CFBP 3978							

Table 1 (Continued)

Proposed nomenclature <sup>a</sup>	Strain no.	Other collection no. Former nomenclature <sup>b</sup>	Host of isolation	Geographic origin		Year of isolation		Pathogenicity <sup>c</sup>		Reference
				Isère, France	Jura, France	1995	1995	+	+	
CFBP 3980			<i>Lactuca sativa</i>	Isère, France		1995	1995	+	+	This study
CFBP 3983			<i>Lactuca sativa</i>	Jura, France		1995	1995	+	+	This study
CFBP 3984			<i>Lactuca sativa</i>	Vaucluse, France		1994	1994	+	+	This study
CFBP 3985			<i>Lactuca sativa</i>	Rhône, France		1995	1995	+	+	This study
CFBP 3986			<i>Lactuca sativa</i>	France		1994	1994	+	+	This study
CFBP 3987			<i>Lactuca sativa</i>	France		1994	1994	+	+	This study
CFBP 3990			<i>Lactuca sativa</i>	France		1995	1995	+	+	This study
CFBP 3993			<i>Lactuca sativa</i>	Loiret, France		1995	1995	+	+	This study
CFBP 3995			<i>Lactuca sativa</i>	Isère, France		1996	1996	+	+	This study
CFBP 3996			<i>Lactuca sativa</i>	Isère, France		1996	1996	+	+	This study
CFBP 7999		ICMP 7383	<i>X. gardneri</i> (ex <i>Sutii</i> <i>Solanum lycopersicum</i> 1957) Jones et al. 2006	New Zealand		1980	1980	+	+	[56]
LM 16382			<i>Lactuca sativa</i> cv. Escale	Isère, France		2016	2016	+	+	This study
LM 16383			<i>Lactuca sativa</i> cv. Escale	Isère, France		2016	2016	+	+	This study
LM 16384			<i>Lactuca sativa</i> cv. Escale	Isère, France		2016	2016	+	+	This study
LM 16386			<i>Lactuca sativa</i> cv. Escale	Isère, France		2016	2016	+	+	This study
LM 16387			<i>Lactuca sativa</i> cv. Minestronne	Isère, France		2016	2016	+	+	This study
LM 16388		CFBP 8640	<i>Lactuca sativa</i> cv. Minestronne	Isère, France		2016	2016	+	+	This study
LM 16734		CFBP 8638	<i>Lactuca sativa</i> cv. Parrinice	Savoie, France		2016	2016	+	+	This study
LM 16735		CFBP 8639	<i>Lactuca sativa</i> cv. Almagro	Savoie, France		2016	2016	+	+	This study
LM 16736			<i>Lactuca sativa</i> cv. Almagro	Savoie, France		2016	2016	+	+	This study
LM 16011 A		CFBP 8641	<i>Lactuca sativa</i>	Ain, France		2016	2016	+	+	This study
LM 16012			<i>Lactuca sativa</i>	Ain, France		2016	2016	+	+	This study
LM 16013			<i>Lactuca sativa</i>	Ain, France		2016	2016	+	+	This study
LM 16014			<i>Lactuca sativa</i>	Ain, France		2016	2016	+	+	This study
LM 16691			<i>Lactuca sativa</i> cv. Funride	Rhône, France		2016	2016	+	+	This study
LM 17421		CFBP 8642	<i>Lactuca sativa</i> cv. Almagro	Loire, France		2017	2017	+	+	This study
LM 17422			<i>Lactuca sativa</i> cv. Celesti	Loire, France		2017	2017	+	+	This study
LM 17423			<i>Lactuca sativa</i> cv. Almagro	Loire, France		2017	2017	+	+	This study
LM 17691			<i>Lactuca sativa</i> cv. Olana	Rhône, France		2017	2017	+	+	This study
LM 17692			<i>Lactuca sativa</i> cv. Olana	Rhône, France		2017	2017	+	+	This study
LM 17694			<i>Lactuca sativa</i>	Rhône, France		2017	2017	+	+	This study
LM 17695			<i>Lactuca sativa</i> cv. Kisheri	Rhône, France		2017	2017	+	+	This study
LM 17696			<i>Lactuca sativa</i> cv. Oseka	Rhône, France		2017	2017	+	+	This study
LM 17697			<i>Lactuca sativa</i> cv. Analota	Rhône, France		2017	2017	+	+	This study
LM 17381			<i>Taraxacum</i> sp.	Isère, France		2017	2017	+	+	This study
LM 17382			<i>Lactuca sativa</i> cv. Impression	Isère, France		2017	2017	+	+	This study
LM 17384			<i>Lactuca sativa</i> cv. Lilybel	Isère, France		2017	2017	+	+	This study
LM 17385			<i>Lactuca sativa</i> cv. Tourbillon	Isère, France		2017	2017	+	+	This study
LM 17388			<i>Lactuca sativa</i> cv. Tourbillon	Isère, France		2017	2017	+	+	This study
LM 173810			<i>Lactuca sativa</i> cv. Juliana	Isère, France		2017	2017	+	+	This study
LM 173811			<i>Lactuca sativa</i> cv. Kisheri	Isère, France		2017	2017	+	+	This study
LM 173812			<i>Lactuca sativa</i> cv. Kisheri	Isère, France		2017	2017	+	+	This study
LM 17011			<i>Lactuca sativa</i>	Ain, France		2017	2017	+	+	This study
LM 17012			<i>Lactuca sativa</i>	Ain, France		2017	2017	+	+	This study
LM 17013			<i>Lactuca sativa</i>	Ain, France		2017	2017	+	+	This study
LM 17014			<i>Lactuca sativa</i>	Ain, France		2017	2017	+	+	This study
LM 18071			<i>Lactuca sativa</i>	Ardèche, France		2018	2018	+	+	This study
LM 18072			<i>Lactuca sativa</i>	Ardèche, France		2018	2018	+	+	This study

<sup>a</sup> Proposed nomenclature in accordance with the *International Code of Nomenclature of Prokaryotes* and the *International Standards for Naming Pathovars of Phytopathogenic Bacteria*.

<sup>b</sup> Two different nomenclatures may be displayed in order to respect validly published names and assure continuity through literature by adding the ones sometimes still used by researchers.

<sup>c</sup> Cancellation of CFBP 2538 as the pathotype strain of the *vitians* pathovar and proposal of LMG 938 as the neopathotype were submitted by letter to the Committee on the Taxonomy of Plant Pathogenic Bacteria.



index described by Bull and Koike [11]. Following the same procedure, a subset of strains was assayed for pathogenicity on tomato cv. Marmande, including the three strains of *X. cynarae* pv. *gardneri*, reference strain of *X. hortorum* pv. *carotae*, pathotype strain of *X. hortorum* pv. *taraxaci*, and seven strains of *X. campestris* pv. *vitians*.

#### Multilocus sequence analysis

Multilocus sequence analysis using three housekeeping genes (*gyrB*, *rpoD* and *gapA*) was performed on all the strains studied. Loci *gyrB* (DNA gyrase  $\beta$  subunit) and *rpoD* (RNA polymerase  $\sigma 70$  factor) were chosen among the 7 genes used in a sequence-based study of *X. arboricola* [56], and *gapA* (glyceraldehyde-3-phosphate dehydrogenase A) was added because of its efficiency to discriminate *X. campestris* pv. *vitians* strains as demonstrated in a previous study [19]. Primer sequences, annealing temperatures and resulting fragment lengths after trimming are displayed in Table S1. Colony-PCRs were performed in a total volume of 50  $\mu$ L adjusted with ultra-pure water and consisting of 1X reaction buffer, 1.5 mM of  $MgCl_2$ , 0.2 mM of each dNTP, 5% of DMSO, 0.2 mM of each primer and 5 U/mL of Taq'Ozyme (Ozyme, Montigny-Le-Bretonneux, France). PCR amplifications were performed in a Biometra Tone thermocycler (Analytik Jena, Jena, Germany) using the following program: 5 min of initial denaturation at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at appropriate annealing temperature, 1 min at 72 °C, and a final extension for 5 min at 72 °C. PCR products were then purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany). DNA quality and concentrations were assessed with a Nanophotometer NP80 (Implen, Munich, Germany) before Sanger sequencing of one strand at Genoscreen (Lille, France) or GATC Biotech (Konstanz, Germany) with primers XgyrB1F (*gyrB*), rpoDXSoR6 (*rpoD*) or gap1F (*gapA*). Sequences of *gyrB* and *rpoD* loci of the CFBP strains were provided by the CIRM-CFBP. Sequences were aligned, trimmed and concatenated following the alphabetical order using CLUSTAL $\Omega$  [60], resulting in a 2331 bp sequence. The best-fitted nucleotide substitution model was evaluated in MEGA 7.0.26 based both on the corrected Akaike information criterion (AICc) and the maximized log likelihood (ln L). Maximum likelihood phylogeny was constructed in MEGA 7.0.26 [33] using the Generalized Time Reversible substitution model (GTR) with a gamma distribution of rate variation among sites (shape parameter=4) and acceptance of invariant sites (I). Branch support was assessed with 500 bootstrap replicates. *X. populi* CFBP 1817<sup>T</sup> was chosen as an outgroup. All the sequences generated were deposited in the National Center for Biotechnology Information GenBank under accession numbers MK610462 to MK610526 for *gapA*, MK610527 to MK610591 for *gyrB* and MK610592 to MK610656 for *rpoD*. The full concatenated alignment is provided along with the supplementary material.

#### Genome sequencing, assembly, annotation and acquisition

Genomic DNAs were extracted and purified from 25 ml 1/10<sup>th</sup> TSB overnight cultures using the standard phenol-chloroform method [55]. The genomes of five strains of *X. campestris* pv. *vitians* type B (LM 16388, LM 16735, CFBP 498, CFBP 499 and CFBP 3978), pathotype strain *X. campestris* pv. *vitians* type A CFBP 2538<sup>PT</sup>, pathotype strains of *X. hortorum* pv. *taraxaci* CFBP 410<sup>PT</sup>, *X. hortorum* pv. *pelargonii* CFBP 2533<sup>PT</sup> and *X. cynarae* pv. *gardneri* CFBP 8163<sup>PT</sup> were sequenced with Illumina technology in HiSeq paired-end 2  $\times$  150 bp at GATC Biotech (Konstanz, Germany). Additionally, historical representative strain of *X. campestris* pv. *vitians* type B LMG 938 was sequenced in Illumina MiSeq 2  $\times$  250 bp at Penn State University. Paired-end reads were assembled in contigs using UNICYCLER v0.4.2 [73] with a minimum contig size of 200 bp, then

annotated with PROKKA v1.14 [58]. Additionally, 46 genome assemblies representing the taxonomic diversity of *Xanthomonas* clade II as reported by Jacques et al. [28] with the emended propositions of Constantin et al. [13] were acquired from the NCBI Genbank database. Genome characteristics, GenBank accession numbers and quality statistics are listed in Table S2.

#### Phylogenomic analysis

Phylogenomic tree reconstruction was performed on all the genomes of type, pathotype and representative strains of *Xanthomonas* clade II using PHYLOPHLAN v1.10 [59]. Type strain of *Stenotrophomonas maltophilia* CFBP 3035<sup>T</sup> was selected as an outgroup. A core of ~350 single-copy full-length predicted proteins from the PhyloPhlan database were retrieved in the genomes with USEARCH v8.0.1623 [16], then aligned and concatenated with MUSCLE v3.8.31 [17]. Finally, a phylogenomic tree was built with FASTTREE 2.1.10 SSE3 [44] and robustness was locally assessed by the Shimodaira-Hasegawa test (SH) using 1,000 resamples. Additionally, a phylogeny was also constructed on all the available genomes of *X. campestris* pv. *vitians* type B, *X. hortorum* and *X. cynarae* with *X. populi* as an outgroup following the same procedure.

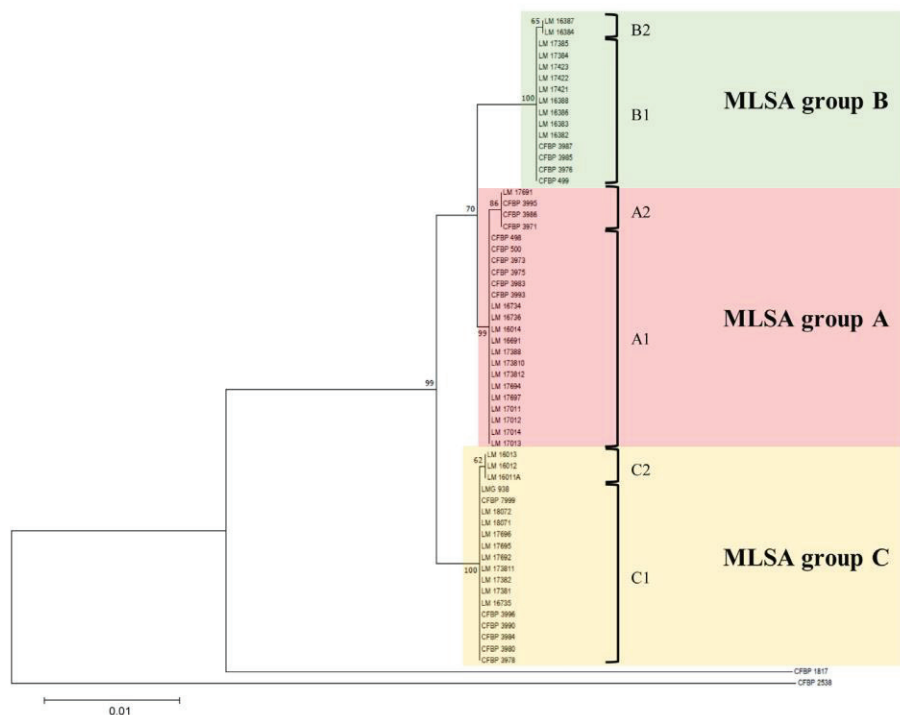
#### Overall Genome Relatedness Indices (OGRI) calculation

All OGRI values were calculated on a subset containing the genomes of all the type, pathotype or representative strains of *X. campestris* pv. *vitians*, *X. hortorum*, *X. cynarae*, and type strains of *X. populi* and *X. citri*. Pairwise MUMMER-driven average nucleotide identities (ANIm) and tetranucleotide frequencies (Tetra) were calculated using the web server JSPECIESWS v3.0.20 (<http://jspecies.ribohost.com/jspeciesws>) [47] whereas average nucleotide identities based on BLAST+ alignments (ANiB) were obtained with ORTHOANI v0.93.1 [34]. *In silico* DNA-DNA hybridization values (isDDH) [36] were determined at the web server of ggdc v2.1 (Genome-to-Genome Distance Calculator) available at the DSMZ website (<http://ggdc.dsmz.de/ggdc.php>).

#### Phenotypic profiling

Phenotypes of *Xanthomonas* strains were characterized using Biolog GEN III (carbon sources and chemical resistances), PM02A (additional carbon sources) and PM03B (nitrogen sources) microplates (Biolog, Hayward, United States). Experiments were repeated in triplicates for GEN III microplates and duplicates for PM02A and PM03B microplates. All strains were subcultured from frozen stock cultures on TSA 1/10<sup>th</sup> at 28 °C for 72 h, then spread on TSA plates for 20–24 h at 28 °C. For GEN III microplates, tubes of fluid IF-A were inoculated with sterile swabs to obtain transmittance values ranging from 92 to 96%. For PM02A and PM03B microplates, inoculation fluid IF-0 1 $\times$  was prepared by addition of manufacturer's Dye Mix A at 1.2%. Finally, a 50 $\times$  stock solution composed of sodium succinate 1 M and ferric citrate 100  $\mu$ M was prepared and sterilized by filtration on 0.2  $\mu$ m filters, then added in the inoculation fluid only for PM03B microplates at a 1x final concentration. Fluids were then inoculated following the same procedure as for the GEN III microplates to obtain to transmittance values of 85%. Plates were subsequently filled with 100  $\mu$ L per well and incubated 5 days at 25 °C in an OmniLog system. Raw data were imported in R and analyzed using the opm package [70]. The maximum heights of the curves (parameter A) were discretized using empirical cutoffs of 75 and 125 omnilog arbitrary units for weak and positive reaction respectively.

Some of the phenotypic features reported in Brown's first description of the pathogen responsible for bacterial leaf spot of lettuce [7] were also tested to check the authenticity of the patho-



**Fig. 1.** Multilocus sequence analysis (MLSA) phylogeny of bacterial leaf spot of lettuce related strains based on the concatenation of partial sequences of housekeeping genes *gapA*, *gyrB* and *rpoD*. MLSA groups A, B and C are highlighted in red, green and yellow respectively. Brackets indicates the different sequence types (STs) observed on the concatenated dataset (named A1, A2, B1, B2, C1 and C2). Bootstrap values are indicated in percentages and were calculated on 500 replicates. Tree scale on the bottom-left corner reflects nucleotide substitution per site. *X. populii* type strain CFBP 1817<sup>T</sup> and pathotype strain *X. campestris* pv. *vitians* CFBP 2538<sup>PT</sup> were chosen as outgroups.

type strain. Gram staining were performed by staining with crystal violet for 1 min followed by Lugol for 1 min, then decolorized with 70% ethanol for 20 s and finally stained with safranin for 1 min. Starch hydrolysis was recorded after 5 days of growth on starch agar plates containing 3 g/L of beef extract, 10 g/L of soluble starch and 12 g/L of bacteriological agar by flooding the plates with Lugol's iodine solution. Gelatin hydrolysis was assayed every day for 7 days in stab-inoculated 15-mL Falcon tubes filled with 5 mL of 5 g/L of bacteriological peptone, 3 g/L of beef extract and 120 g/L of gelatin. Motility, hydrogen sulfide and indole production were tested in stab-inoculated 15-mL Falcon tubes filled with 5 mL of Sulfate Indole Motility (SIM) medium made of 20 g/L of tryptone, 6.1 g/L of bacteriological peptone, 0.13 g/L of anhydrous sodium thiosulfate, 0.2 g/L of hexahydrate ferrous ammonium sulfate and 3.5 g/L of bacteriological agar. Indole production was assayed 5 days after inoculation using James reagent (Biomérieux, Marcy-l'Etoile, France). Litmus milk was prepared with 100 g/L of skimmed milk powder, 0.5 g/L of sodium sulfite and 0.2% (w/v) of litmus and 10 mL were dispensed in 15-mL Falcon tubes. Reactions in the medium were recorded after 7 days.

## Results

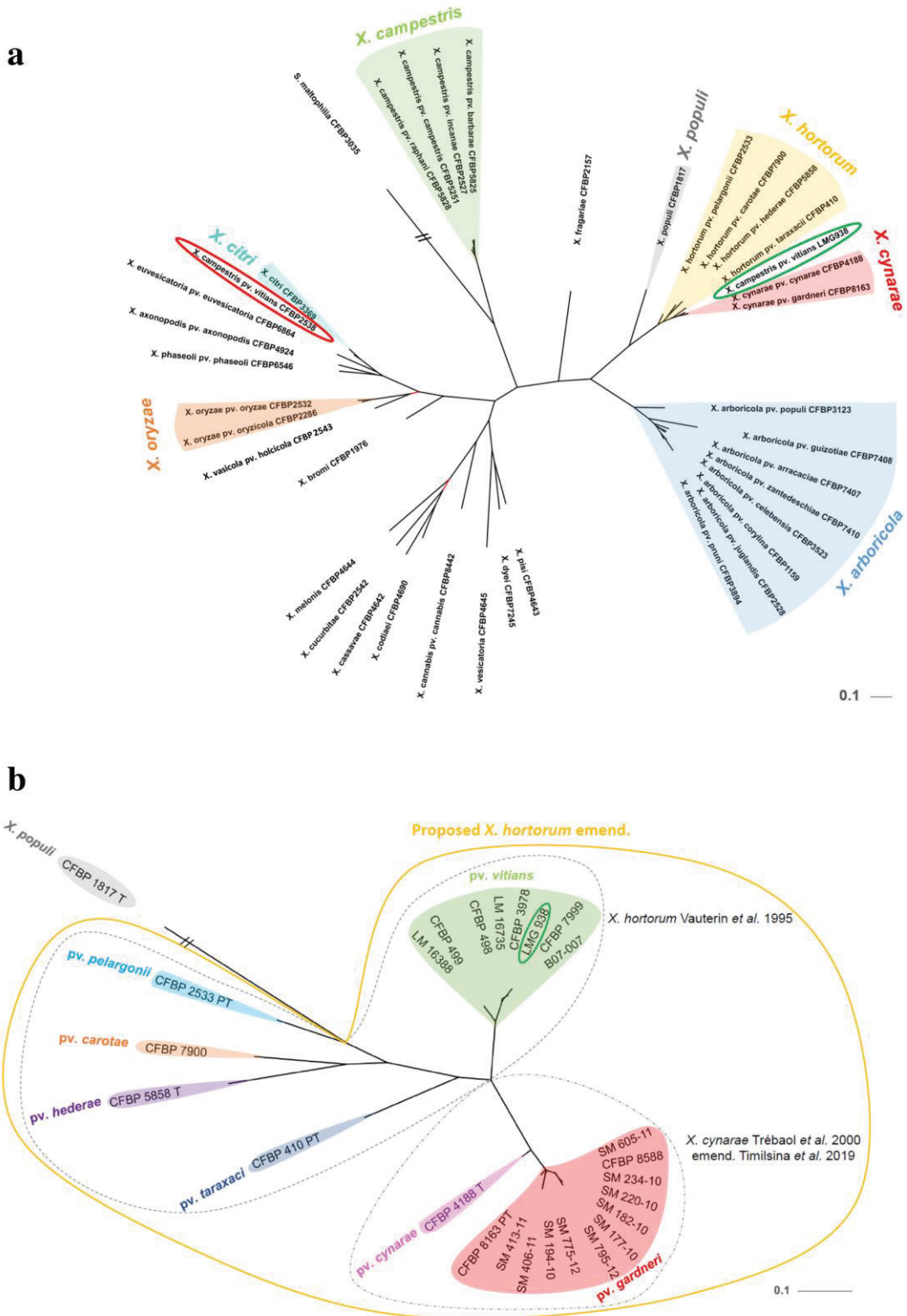
### Pathogenicity assays

All strains were tested for their pathogenicity on two lettuce cultivars (oakleaf cv. Kirinia and leaf lettuce cv. Météore), and 11 strains on tomato cv. Marmande. Strains able to induce a mean disease severity  $\geq 2$  on the 8 plants at the end of the three-week monitoring period were considered pathogenic, whereas strains were determined non-pathogenic when mean disease severity was  $< 2$ . When mean disease severity was  $\geq 2$ , few variations of disease severity were observed between strains or cultivars using

our five-point scale disease index (*data not shown*). All strains labeled as *X. hortorum* pv. *vitians* were able to induce typical BLSL symptoms on the two lettuce cultivars (Table 1), and therefore considered pathogenic on lettuce. Consistent with previous reports [3,53], pathotype strain of *X. campestris* pv. *vitians* CFBP 2538<sup>PT</sup> was non-pathogenic on lettuce. (cvs. Kirinia and Météore). All other pathovars tested were neither pathogenic, except strain *X. cynarae* pv. *gardneri* CFBP 7999 which was surprisingly highly virulent on the two lettuce cultivars and produced unmistakable BLSL symptoms. As expected, the three strains of *X. cynarae* pv. *gardneri* were pathogenic on tomato cv. Marmande, and apart from *X. campestris* pv. *vitians* LM 16735 which was weakly pathogenic, none of the other strains tested were able to induce symptoms on tomato (Table 1).

### Multilocus sequence analysis

Assessment of the genetic diversity of a large panel of 57 BLSL-inducing strains, mostly isolated in France and some in USA and Zimbabwe, was performed using multilocus sequence analysis (MLSA) on housekeeping genes *gapA*, *gyrB* and *rpoD*. Three major phylogenetic groups and six sequence types (STs) were identified based on the concatenated sequences (Fig. 1). Each MLSA group (referred to as A, B and C in this study) were composed of two different STs, respectively A1, A2, B1, B2, C1 and C2. Six different alleles were observed for *gapA* gene, 4 for *gyrB* and only one for *rpoD*. The three MLSA groups were also obtained with *gapA* alone, whereas *gyrB* alone can only discriminate group B (Fig. S1). *rpoD* being identical for all the strains, it was not useful for assessing the intra-pathovar diversity, although it allowed to discriminate most of *X. hortorum* and *X. cynarae* pathovars (Figure S2). The two isolates included into the minor ST B2 were isolated in the same field at the same date, making them potential clones rather than



**Fig. 2.** Whole-genome based phylogenies constructed on a core of ~350 single-copy full-length predicted proteins from the PhyloPhlan database of (a) *Xanthomonas* clade II or (b) proposed *X. hortorum* emend. Strain LMG 938<sup>neOPT</sup> proposed in this article as the neopathotype of *X. hortorum* pv. *vitiens* is circled in green while current pathotype CFBP 2538<sup>PT</sup> is circled in red. In (a), current valid species *X. hortorum* and *X. cynarae* are highlighted in yellow and red respectively while other species useful to set the background (*X. citri*, *X. oryzae*, *X. arboricola*, *X. populi* and *X. campestris*) are highlighted in light blue, orange, blue, gray and green respectively. Branch of *Stenotrophomonas maltophilia* CFBP 3035<sup>T</sup> has been shorten in order to be represented. In (b), current delineation of *X. hortorum* Vauterinet et al. 1995 and *X. cynarae* Trébaol et al. 2000 emend. Timilsina et al. 2019 are displayed in dotted lines whereas proposed *X. hortorum* emend. is circled in yellow. Branch of *X. populi* CFBP 1817<sup>T</sup> has been shorten in order to be represented. Branches with statistical support below 0.85 are (a) displayed in red and (b) collapsed. Tree scales indicate number of amino acid substitution per site. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

real populations. It was the same situation for the 3 isolates of ST C2. For all the other STs described, no pattern of repartition of the strains depending on their geographical origin or year of isolation could be identified. The BLSL strains from USA grouped in STs A1 and B1 and it should be noted that strain LMG 938 from Zimbabwe belonged to major ST C1.

#### Whole-genome based phylogenies

Two phylogenomic trees were built with PHYLOPHLAN: the first one on a set of genomes representing the known diversity of *Xanthomonas* clade II (Fig. 2a) and the second one on a subset containing all available or newly-sequenced genomes of *X. hortorum*, *X. cynarae* and *X. campestris* pv. *vitians* type B (Fig. 2b). Analysis of the clade-scaled phylogeny revealed that the pathotype strain of *X. campestris* pv. *vitians*, currently labeled as *X. axonopodis* pv. *vitians* CFBP 2538<sup>PT</sup> was highly related to the type strain of *X. citri* CFBP 3369<sup>T</sup> (Fig. 2a). This phylogeny also highlighted the genetic relatedness of strains labeled as *X. hortorum*, *X. cynarae* and BLSL-causing *X. campestris* pv. *vitians*. Moreover, a clear cut-off between this group and the closest species *X. populi* was obvious (Fig. 2a). Within this group, *X. hortorum* pvs. *carotae* and *hederae* were more genetically similar to each other than to the other pathovars (Fig. 2b). *X. hortorum* pv. *pelargonii* was the most divergent. The large number of genomes available for *X. cynarae* pv. *gardneri* allowed us to evidence the narrow diversity between the strains in this pathovar. Likewise, all sequenced strains of *X. hortorum* pv. *vitians* had low genetic diversity except for strains LM 16388 and CFBP 499 which clustered together and seemed to have diverged earlier than the others. Surprisingly, the strain CFBP 7999 of *X. cynarae* pv. *gardneri* was more related to BLSL-causing strains of *X. campestris* pv. *vitians* than to strains of *X. cynarae* pv. *gardneri*.

#### Pairwise overall genome relatedness indices comparisons

Four OGRI values (ANIm, ANIb, isDDH and Tetra) were calculated on a subset composed of the genomes of all type, pathotype or representative strains of *X. campestris* pv. *vitians* type A and B, *X. hortorum*, *X. cynarae*, and type strains of *X. populi*, *X. axonopodis* and *X. citri*. ANIm and isDDH similarity matrices are displayed in Table 2, while ANIb and Tetra results are presented in Table S3. As ANIb and Tetra values gave similar results to ANIm and isDDH, they will not be discussed here. Unsurprisingly, *X. campestris* pv. *vitians* type A CFBP 2538<sup>PT</sup> presented only 88.2% ANIm and 34.4% isDDH values compared pairwise with *X. campestris* pv. *vitians* type B LMG 938 but was highly similar to type strain of *X. citri* CFBP 3369<sup>T</sup> (ANIm = 98.7% and isDDH = 89.5%), confirming the observations made on the genus-scaled phylogeny (Fig. 2a). The comparison with the type strain of *X. axonopodis* CFBP 4924<sup>T</sup> (83.4% ANIm and 52.6% isDDH) showed that CFBP 2538 was actually genetically closer to the type strain of *X. citri* than to the one of *X. axonopodis*. *Xanthomonas cynarae* pvs. *cynarae* CFBP 4188<sup>T</sup> and *gardneri* CFBP 8163<sup>PT</sup> differed little on their genomic content, with ANIm and isDDH reaching 99.3% and 94.9% respectively. By contrast, between the several pathovars of *X. hortorum*, values were less substantial but still higher or in the range of ANI > 95–96% and isDDH > 60–70%. Indeed, ANIm varied from 95.4% to 96.5% and isDDH from 64.3% to 71.1%. The lowest values were always obtained when comparing *X. hortorum* pv. *pelargonii* CFBP 2533<sup>PT</sup> and pv. *taraxaci* CFBP 410<sup>PT</sup> and the highest between pv. *hederae* CFBP 5858<sup>T</sup> and pv. *carotae* CFBP 7900. Exploring the relationships among these two species and *X. campestris* pv. *vitians* led to interesting observations. The two *X. cynarae* strains, *X. campestris* pv. *vitians* LMG 938 and *X. hortorum* pv. *taraxaci* CFBP 410<sup>PT</sup> were robustly grouped together by all parameters, with ANIm > 97% and isDDH > 78%, indicating unequivocally that they should belong the same species. However, if *X. campestris* pv. *vitians* LMG 938 shared

**Table 2**  
Pairwise ANIm and isDDH values among draft whole genome sequences of type, pathotype and representative strains of *X. hortorum* and *X. cynarae*. *X. populi* was chosen as an outgroup and type strain of *X. citri* and *X. axonopodis* were added for means of comparison to pathotype strain of *X. campestris* pv. *vitians*. ANIm values (%) are displayed in the lower triangle and isDDH values (%) in the upper triangle. Number in brackets indicates the percentage of aligned sequences used for calculation of ANIm between two genomes, and differences between ANIm reciprocal values were <0.1% in all comparisons. isDDH values are the point estimate plus the 95% model-based confidence intervals obtained with formula 2 as recommended at the GGDC web-server.

Current nomenclature	Proposed nomenclature	1	2	3	4	5	6	7	8	9	10	11
<i>X. cynarae</i> pv. <i>cynarae</i> CFBP 4188 <sup>T</sup>	1 <sup>a</sup> <i>X. hortorum</i> pv. <i>cynarae</i> CFBP 4188 <sup>PT</sup>	–	94.9	87.3	79.5	68.5	68.6	67.3	44.7	34.4	34.4	34.9
<i>X. cynarae</i> pv. <i>gardneri</i> CFBP 8163 <sup>PT</sup>	2 <sup>a</sup> <i>X. hortorum</i> pv. <i>gardneri</i> CFBP 8163 <sup>PT</sup>	99.3 [93.3]	–	85.9	78.6	68	68.4	67.2	44.7	34.3	34.4	34.9
<i>X. campestris</i> pv. <i>vitians</i> LMG 938	3 <i>X. hortorum</i> pv. <i>vitians</i> LMG 938 <sup>neopt</sup>	98.4 [93.2]	98.3 [93.4]	–	80.8	68.2	68	67.1	44.5	34.4	34.3	34.9
<i>X. hortorum</i> pv. <i>taraxaci</i> CFBP 410 <sup>PT</sup>	4 <i>idem</i>	97.5 [92.9]	97.4 [93.2]	97.6 [94.0]	–	64.8	65	64.3	44.4	34.5	34.4	35.0
<i>X. hortorum</i> pv. <i>hederae</i> CFBP 5858 <sup>T</sup>	5 <i>idem</i>	96.0 [80.6]	96.0 [81.3]	96.1 [81.1]	95.6 [80.9]	–	71.1	68.8	44.4	34.8	34.5	35.0
<i>X. hortorum</i> pv. <i>carotae</i> CFBP 7900	6 <i>idem</i>	96.2 [86.8]	96.2 [87.8]	96.2 [87.0]	95.7 [86.3]	96.5 [85.1]	–	68.5	44.7	34.4	34.5	34.9
<i>X. hortorum</i> pv. <i>pelargonii</i> CFBP 2533 <sup>PT</sup>	7 <i>idem</i>	95.8 [86.0]	95.8 [85.8]	95.9 [85.8]	95.4 [89.7]	96.1 [83.4]	96.2 [86.5]	–	44.9	34.3	34.5	35.0
<i>X. populi</i> CFBP 1817 <sup>T</sup>	8 <i>idem</i>	91.2 [80.4]	91.4 [80.4]	91.3 [79.4]	91.3 [79.5]	91.3 [79.4]	91.4 [78.2]	91.4 [81.2]	–	33.5	33.6	33.9
<i>X. axonopodis</i> pv. <i>vitians</i> CFBP 2538 <sup>PT</sup>	9 <i>X. citri</i> CFBP 2538	88.2 [64.3]	88.2 [66.5]	88.2 [66.1]	88.2 [65.5]	88.3 [66.8]	88.2 [66.0]	88.1 [66.7]	87.7 [55.0]	–	52.6	89.5
<i>X. axonopodis</i> pv. <i>axonopodis</i> CFBP 4924 <sup>T</sup>	10 <i>idem</i>	88.1 [67.4]	88.1 [67.5]	88.1 [68.0]	88.1 [67.9]	88.1 [67.9]	88.0 [68.1]	88.1 [68.2]	87.63 [57.9]	93.4 [86.1]	–	53.5
<i>X. citri</i> CFBP 3369 <sup>T</sup>	11 <i>idem</i>	88.3 [64.3]	88.3 [64.6]	88.3 [64.8]	88.4 [64.8]	88.3 [63.4]	88.3 [64.6]	88.3 [64.5]	87.8 [52.6]	98.7 [89.8]	93.5 [85.2]	–

<sup>T</sup> = type strain, <sup>PT</sup> = pathotype strain, <sup>neopt</sup> = neopathotype.

<sup>a</sup> Nomenclature sensu Timilsina et al. 2019.

**Table 3**

Phenotypic profiles of the different studied *Xanthomonas* using standard phenotypic tests and Biolog GEN III microplates with biological triplicates. Discriminative traits of *X. populi* reported in literature allowing to differentiate from *X. hortorum* emend. are highlighted in red. For Biolog GEN III results, characters negative for all strains tested are not displayed, resistance phenotypes are displayed in italic and shared stable traits for all *X. hortorum* emend. strains are highlighted in green.

Species <sup>a</sup>	1	2	3	4	5	6	7	8	9 <sup>b</sup>
<b>Phenotypic tests</b>									
<b>Gram staining</b>									
Optimum growth temperature	na	rod negative 25 - 28°C	rod negative 25 - 28°C	rod negative 25 - 28°C	rod negative 25 - 28°C	rod negative 25 - 28°C	rod negative 25 - 28°C	rod negative 25 - 28°C	rod negative 20 - 23°C
Starch hydrolysis	+	-	-	-	-	-	-	-	v
Gelatin hydrolysis	-	+	+	+	+	+	+	+	-
Motility	+	+	+	+	+	+	+	+	+
Hydrogen sulfide production	+	+	+	+	w	+	+	w	+
Indole production	+	w	w	w	w	w	w	w	-
<b>Litmus milk</b>									
Litmus reduction	-	+	+	+	+	+	+	+	v
Casein hydrolysis	-	+	+	+	+	+	+	+	na
Casein precipitation	+	+	+	+	+	+	+	+	na
<b>Biolog GEN III microplates<sup>b</sup></b>									
Dextrin	+	-	-	w(-)	-	-	-	-	+
D-Maltose	+	-	-	-	-	-	-	-	na
<b>D-Trehalose</b>	+	+	+	+	+	+	+	+	+
<b>D-Cellobiose</b>	+	+	+	+	+	+	+	+	-
β-Gentiobiose	+	+	+	-(+)	+	+	-	-	v
Sucrose	+	+	+	+	+	+	+	+	+
<b>pH 6</b>	+	+	+	+	+	+	+	+	+
<b>pH 5</b>	-	-	-(+)	-	-	-	-(+)	-	na
D-Raffinose	-	-	-	+(+)	-	-	-	-	-
α-D-Lactose	+	-	-	+(+)	-	+(+)	-	+	-
<b>D-Melibiose</b>	+	+	+	+	+	+	+	+	-
<b>N-Acetyl-D-Glucosamine</b>	+	+	+	+	+	+	+	+	v
<b>1% NaCl</b>	+	+	+	+	+	+	+	+	-
<b>4% NaCl</b>	w(+)	+	+	w(-)	-	+	v	+	-
<b>8% NaCl</b>	-	-	-	-	-	-(+)	-	-	-
<b>D-Glucose</b>	+	+	+	+	+	+	+	+	+
<b>D-Mannose</b>	+	+	+	+	+	+	+	+	+
<b>D-Fructose</b>	+	+	+	+	+	+	+	+	+
<b>D-Galactose</b>	+	+	+	+	+	+	+	+	+
D-Fucose	-	-	-	w(-)	-	-	-	-	na
<b>L-Fucose</b>	+	+	+	+	+	+	+	+	-
<b>1% Sodium Lactate</b>	+	+	+	+	+	+	+	+	na
D-Serine #2	-	-	-	-	-	+(+)	-	-	-
Glycerol	+	+	+	+	+	+	+	+	v
D-Fructose-6-Phosphate	w(-)	-	-	-	-	-	-	-	-
Troleandomycin	-	-(+)	v	-	-	-	-	-	na
Rifamycin S1 <sup>c</sup>	+(+)	-	+	-(+)	-(+)	-(+)	+(+)	-	na
<b>Gelatin</b>	+	+	+	+	+	+	+	+	-
Gly-Pro	+	+	+	+	+	+	+	v	na
<b>L-Alanine</b>	+	+	+	+	+	+	w	+	-
L-Aspartic Acid	-	-	-	w(-)	w(-)	v	w(-)	+	-
<b>L-Glutamic Acid</b>	+	+	+	+	+	+	+	+	-
L-Histidine	+	+	-	+	+	-(+)	w(+)	+	-
<b>L-Serine</b>	+	+	+	+	+	+	+	+	-
<b>Lincomycin</b>	+	+	+	+	+	+	+	+	na
<b>Guanidine Hydrochloride</b>	w(-)	+(+)	+	+	+(+)	+(+)	+	+(+)	na
Niaproof	-	+(+)	+	w(-)	v	w(-)	v	-	na
Pectin	+	+	+	+	+	+	-	+	na
D-Glucuronic Acid	-	-	-	w(-)	-	-	-	-	v
<b>Vancomycin</b>	-	v	w(-)	-	-	-	-	-	na
<b>Tetrazolium Violet</b>	+	+	+	-(+)	+(+)	+	+(+)	+	na
<b>Tetrazolium Blue</b>	+	+	+	+	+(+)	+	+	+	na
<b>Methyl Pyruvate</b>	+	+	+	+	+	+	+	+	v
L-Lactic Acid	+	+	w(-)	+	+(+)	+	w	+	na
<b>Citric Acid</b>	+	+	+	+	+	+	+	+	v
<b>α-Keto-Glutaric Acid</b>	+	+	+	+	+	+	+	+	v
<b>L-Malic Acid</b>	+	+	+	+	+	+	+	+	na
<b>Bromo-Succinic Acid</b>	+	w(+)	+	+	+	+	+	+	v
<b>Lithium Chloride</b>	+	+(+)	+	+(+)	-(+)	+(+)	+(+)	w(-)	na
<b>Potassium Tellurite</b>	-	+	+	w(+)	-	+	-	-	na
Tween 40	-	w(+)	+	w	w(+)	+	w(-)	+	-
α-Hydroxy-Butyric Acid	w(-)	-	w(-)	w(-)	v	-	w(-)	-	-
α-Keto-Butyric Acid	+	-	+(+)	+	-(+)	+	v	+	-
Acetoacetic Acid	+	w(+)	-	+	+	w(+)	w	+	na
<b>Propionic Acid</b>	+	+	+	+	+	+	+	+	-
<b>Acetic Acid</b>	+	+	+	+	+	+	+	+	v
Sodium Formate	+	+	+(+)	-(+)	+(+)	w(+)	+(+)	+	na
<b>Aztreonam</b>	+	+	+	+	+	+	+	+(+)	na
<b>Sodium Bromate</b>	-	+	v	-	-	w(+)	v	w(-)	na

<sup>a</sup> Species (names as proposed in this study): **1** = *X. citri* CFBP 2538, **2** = *X. hortorum* pv. *pelargonii* CFBP 2533<sup>PT</sup>, **3** = *X. hortorum* pv. *carotae* CFBP 7900, **4** = *X. hortorum* pv. *hederae* CFBP 5858<sup>T</sup>, **5** = *X. hortorum* pv. *taraxaci* CFBP 410<sup>PT</sup>, **6** = *X. hortorum* pv. *vitians* LMG 938<sup>nePT</sup>, **7** = *X. hortorum* pv. *cynarae* CFBP 4188<sup>PT</sup>, **8** = *X. hortorum* pv. *gardneri* CFBP 8163<sup>PT</sup>, **9** = *X. populi* as reported in Van den Mooter and Swings 1990 [63], Ridé and Ridé 1992 [42] and Vauterin et al. 1995 [62].

<sup>b</sup> Reactions were classified as positive (+), weak (w) or negative (-). Management of triplicates was achieved using the following equivalences: +=(+/+/+) or (++/w), w=(w/w/w), -=(-/-/-) or (-/-/w), +(+)=(+/+/-), -(+)=(-/-/+), w(+)=(w/w/+), w(-)=(w/w/-), v=(-/+/+).

<sup>c</sup> For *X. populi*, (-) and (+) mean that characteristics were either negative or positive in the three studies where data were extracted, (na) that these tests were not conducted and (v) that variable results were described in the different studies.

98.4% ANIm and 87.3% isDDH with type strain of *X. cynarae* CFBP 4188<sup>T</sup>, it also shared 96.6% ANIm and 68.5% isDDH with *X. hortorum* CFBP 5858<sup>T</sup>. As a matter of fact, comparing type strains of *X. cynarae* and *X. hortorum* revealed ANIm and isDDH values of 96.1% and 68.5%, implying that genomic data could support their combination into one single species. Overall, OGRI calculations revealed that all the strains of *X. hortorum*, *X. cynarae* and BSL-causing *X. campestris* pv. *vitians* type B formed a coherent genomic group as no clear cut-off in the distribution of values could be observed. However, such a gap was distinctly observed between the previously described group and the nearest species *X. populi* CFBP 1817<sup>T</sup>, as ANIm and isDDH values drastically fell from above 95% ANIm and 60% isDDH to ~91% and ~45% respectively.

### Phenotype

Resistance to chemical compounds and carbon and nitrogen sources utilization were tested using Biolog GEN III, PM02A and PM03B microplates. The global phenotypic profiles obtained with standardized tests and GEN III microplates are displayed in Table 3, while PM02A and PM03B results are provided in Tables S4 and S5 respectively. Exhaustive phenotypic profiles are depicted in the protologues.

The original description of *X. campestris* pv. *vitians* provided by Brown in 1918 [7] and reworked by Burkholder in Bergey's *Manual of Determinative Bacteriology* of 1957 [6] depicts the pathogen as a Gram-negative motile rod which is feebly amylolytic, liquefies gelatin slowly, produces hydrogen sulfide, feebly produces indole and produces an alkaline reaction in litmus milk with litmus reduction, casein hydrolysis and precipitation. Reproducing these tests (Table 3), we observed that pathotype strain CFBP 2538 differs from the previous descriptions as it was strongly amylolytic, did not liquefy gelatin after 7 days, had a strong production of indole 5 days after inoculation and did not reduce litmus in litmus milk, neither did it hydrolyze casein after 7 days. On the other hand, strain LMG 938 fits completely the previously described characteristics, except for starch hydrolysis as no reaction was observed 4 days after inoculation on starch agar plates. Indeed, none of the strains of *X. hortorum* or *X. cynarae* hydrolyzed starch, all presented a slight production of indole and gave the same reduction reaction in litmus milk.

Moreover, Biolog GEN III microplate assays showed that CFBP 2538 used Dextrin and D-Maltose as carbon sources when none of other strains tested did. Overall, strains of *X. hortorum*, *X. cynarae* and *X. campestris* pv. *vitians* type B exhibited a strong stable core phenotype of 21 highly used carbon sources and 4 chemical resistances, all the other reactions tested being either negative for all strains or highly variable among biological replicates for at least one strain. No stable discriminative trait between *X. cynarae* and *X. hortorum* could be observed in our analyses.

### Discussion

In this research we investigated all of the taxonomic relationships between all the taxonomically relevant members of *X. hortorum*, *X. cynarae* and *X. campestris* pv. *vitians* type A and B, which until this work, have never been explored in a single study. Though, previous studies revealed the high genetic proximity between *X. hortorum* and *X. cynarae* [64,78].

The *X. hortorum* Vauterin et al. 1995 species was proposed mainly on the basis of DNA-DNA reassociation experiments and grouped together *X. campestris* pvs. *hederae*, *taraxaci*, *carotae*, *pelargonii* and *vitians* type B strains [72]. Later, *X. cynarae* Trébaol et al. 2000 was described on artichoke and considered to be a new species because none of the DNA-DNA hybridizations con-

ducted against several other *Xanthomonas* allowed to bind it to a previously described species at a level higher than the species threshold [68]. Unfortunately, the type strain of *X. hortorum* pv. *hederae* CFBP 5858<sup>T</sup> (=CFBP 4925<sup>T</sup> = LMG 733<sup>T</sup>) was not tested as it should have been and only *X. hortorum* pv. *pelargonii* CFBP 2533<sup>PT</sup> (=LMG 7314<sup>PT</sup>) was included and presented a 49% reassociation value against *X. cynarae* CFBP 4188<sup>T</sup> (=ICMP 16775<sup>T</sup>). *Xanthomonas gardneri* Jones et al. 2006 was created based on DNA-DNA hybridization experiments conducted on strains of *Xanthomonas* pathogenic toward tomato and pepper and on representative strains of the *Xanthomonas* diversity [29]. Again, the type strain of *X. hortorum* was not included in that study and the other *X. hortorum* pathovars yielded reassociation values between 53% and 65% toward *X. gardneri* CFBP 8163<sup>T</sup> (=LMG 962<sup>T</sup> = ATCC 19865<sup>T</sup>) except for *X. hortorum* pv. *taraxaci* CFBP 410<sup>PT</sup> (=LMG 870<sup>PT</sup>) for which reassociation values reached 71–75%. However, no reclassification of this latter pathovar was proposed. It is noticeable that in 1990 and 1993, work by Hildebrand et al. [27] and Palleroni et al. [42] classified '*X. gardneri*', whose name was not valid at that time, in the same homology group as *X. hortorum* pathovars *pelargonii*, *carotae* and *taraxaci* due to reassociation values ranging from 64% to 100% (highest values retrieved for pv. *taraxaci*). Finally, using whole genome sequence comparisons, *X. gardneri* (ex Šutič 1957) Jones et al. 2006 has been shown to be a later heterotypic synonym of *X. cynarae* Trébaol et al. 2000 and was transferred in this species as *X. cynarae* pv. *gardneri* (ex Šutič 1957) Timilsina et al. 2019 [64]. Despite their genetic relatedness evidenced by wet-lab and *in silico* DNA-DNA hybridizations, no comprehensive investigation of the taxonomic relationships between *X. hortorum*, *X. cynarae* and their pathovars, using the relevant type and pathotype strains has been conducted to date.

Our comprehensive study yielded many OGRI values around the threshold defined for bacterial species delineation [46], including the comparison between *X. hortorum* and *X. cynarae* type strains. The *in silico* experiments revealed that *X. cynarae* pvs. *cynarae* and *gardneri* and *X. hortorum* pvs. *taraxaci* and *vitians* belong undoubtedly to the same species as ANI and isDDH values are well above 97% and 70% respectively. The relationships between the previous cluster and *X. hortorum* pvs. *hederae*, *carotae* and *pelargonii* may be more ambiguous as ANI and isDDH scores falls in a 'transition zone' [46] with ANI ranging from ~95% to 96.5% and isDDH from ~64% to 68%. The lowest OGRI was isDDH scores of 64–65% obtained between pv. *taraxaci* and the three pathovars *hederae*, *carotae* and *pelargonii*, yet the other OGRIs calculated and its central phylogenetic position demonstrate clearly it belongs to the same species. Tetranucleotide signature frequencies endorsed the hypothesis of one species only as all the pathovars mentioned compared pairwise presented very high values of this correlation coefficient, above 0.999 for most and 0.998 at least, while the determined threshold for species delineation is considered to be 0.990 [46,47]. These results are in accordance with previous wet-lab DDH values; even though some wet-lab DDH values might seem lower than the threshold, they should be considered with caution. Indeed, although DNA-DNA hybridizations had been considered for years as the taxonomic "gold standard" in the scientific community, it has been repeatedly criticized as a cumbersome method, subjected to high standard deviations, sensitive to DNA quality and to the methodology used to measure DNA relatedness, as different laboratories using different methodologies could produce different results for the same comparisons [21,23,46]. Measurement of thermal stability of reassociated DNA ( $\Delta T_m$ ) was recommended to complement DDH and to overcome its drawbacks [22] and it was not unusual to find low  $\Delta T_m$  associated to DDH around 50%. Moreover, the usual threshold of 70% DDH was recommended by the *ad hoc* committee as an approximate cut-off for species delineation and not meant to be a strict boundary [38]. Indeed, its strict application might lead to the

division of taxa into different species without real biological significance. In fact, there is a “transition zone” within 60–70% DDH and 93–96% ANI where the choice to merge or separate species must be led by other criteria such as stable phenotypic diagnostic features and phylogenetic relationships [46,52].

The phenotypic profiles we described endorsed the proposition of synonymy as no stable phenotypes allowed to differentiate strains of *X. cynarae* from strains of *X. hortorum*. It was rather observed that they all shared an invariable core phenotype of 21 highly used carbon sources and 4 chemical resistances. The variable phenotypic traits were strain-specific, unstable between replicates for at least one strain and consisted mostly of weakly used carbon sources and resistances to chemical compounds, which can be considered as accessory phenotypic features irrelevant for taxonomical purposes. Nitrogen source utilization profiles were way more diverse and highly variable, as there was not a single common nitrogen source for all the strains tested. It appeared surprisingly that *X. hortorum* pv. *pelargonii* CFBP 2533<sup>PT</sup> seems to use a large number of different nitrogen sources, maybe related to some particular ecological lifestyle features. On the other hand, the proposed *X. hortorum* emend. can easily be distinguished from its closest species *X. populi* as the latter is able to use dextrin, cannot metabolize D-cellobiose, D-melibiose, L-fucose, gelatin, L-alanine, L-glutamic acid, L-serine and propionic acid, is not able to grow in presence of 1% NaCl, does not liquefies gelatin in tube tests neither does it produces indole, and has an unusual optimum growth temperature between 20 and 23 °C, as systematically and consistently reported in previous descriptions of this species [48,71,72].

To explore the phylogenetic relatedness between *X. hortorum* and *X. cynarae*, we both built a genus-scaled phylogeny using available and reliable type or pathotype strain genomes and another phylogeny focused on all available genomes of *X. hortorum* and *X. cynarae*. Genome-based phylogenetic reconstructions have proven to be robust useful methods to investigate the evolutionary relationships and infer taxonomic assignments for *Xanthomonas* species [37,51]. In this study, we used a recently developed automated pipeline which has been proven to be robust toward horizontal gene transfer (HGT) events [59]. The resulting phylogenetic trees have strong statistical supports (Fig. 2) and, as previously seen by MLSA [78], revealed that *X. hortorum* represent a paraphyletic species that needs taxonomic reclassification. Grouping pathovars of *X. hortorum* and *X. cynarae* in a single species fulfills the monophyly criteria of the species concept and produces a species cluster within which the evolutionary distances are in the range of those observed in other polymorphic *Xanthomonas* species (Fig. 2a).

Nevertheless, in order to achieve these taxonomic proposals formally, we need to address the status of the pathotype strain *X. campestris* pv. *vitians* type A CFBP 2538<sup>PT</sup> (=NCPBB 976<sup>PT</sup> = LMG 937<sup>PT</sup> = ATCC 19320<sup>PT</sup> = ICMP 336<sup>PT</sup>). This particular strain was reported many times in the past to exhibit unusual features compared to other BLSL-causing strains [3,53,62,72]: different colony morphology, protein pattern, rep-PCR or RFLP (restriction fragment length polymorphism) profiles, DNA homology group. However, the most striking aberrant characteristic of this pathotype strain is its absence of pathogenicity toward lettuce [26,53], confirmed by our study on two different lettuce cultivars. In the original description of the South Carolina lettuce disease made by Nellie Brown in 1918, the strain fulfilled the Koch's postulates and was clearly virulent on lettuce though, even a year after its isolation in the field [7]. According to standard 11 of the *International Standards for Naming Pathovars of Phytopathogenic Bacteria* [15], this strain has therefore become unsuitable as the pathotype strain of *X. campestris* pv. *vitians*, as its primary character, i.e. pathogenicity toward lettuce, has drastically changed. In addition to confirming that *X. campestris* pv. *vitians* CFBP 2538<sup>PT</sup> is not pathogenic on lettuce we

demonstrate that it differs from the original description provided by Brown 1918 [7] and complemented by Burkholder in the *Bergey's Manual of Determinative Bacteriology* of 1957 [6] in the following: starch hydrolysis, gelatin liquefaction, indole production and litmus milk reaction. Unfortunately, only one strain was supposedly conserved from this original description and none of the duplicates preserved in culture collections fits the original description. On the other hand, reference strain LMG 938 is undoubtedly pathogenic toward lettuce, and the symptoms it causes are identical to those described by Brown in 1918. Moreover, we demonstrate that this strain matches the description of Brown 1918 and Burkholder 1957 for the following: is a Gram-negative motile rod, liquefies gelatin, produces hydrogen sulfide, slightly produces indole, provokes an alkaline reaction in litmus milk with litmus reduction, casein hydrolysis and precipitation. The only feature differing from Brown's description is that it does not hydrolyzes starch, which was reported to be feebly positive. However, potato starch hydrolysis tests performed in 1918 differ significantly from the starch hydrolysis tests available today and this likely accounts for this difference. In conclusion, to resolve this long-lasting issue, we propose in accordance with standard 11 and 9–4 to officially replace the pathotype strain CFBP 2538<sup>PT</sup> (=NCPBB 976<sup>PT</sup> = LMG 937<sup>PT</sup> = ATCC 19320<sup>PT</sup> = ICMP 336<sup>PT</sup>) by strain LMG 938 as a neopathotype. In our opinion, the most probable hypothesis of what rendered the pathotype of *X. campestris* pv. *vitians* unsuitable is that the original strain deposited was mixed and/or exchanged with a *Xanthomonas* sp. strain at some point during its long history of transfer and before distribution to culture collections.

The data presented here support the transfer of *X. campestris* pv. *vitians* into *X. hortorum* as previously proposed by Vauterin et al. based on DNA-DNA hybridizations experiments [72]. The comparison of strain LMG 938 with type strain of *X. hortorum* confirms they belong to the same species, as they shared 96.1% ANIm, 68.2% isDDH and resulted in a Tetra score of 0.99872. Moreover, the pathogenicity tests we performed demonstrated that none of the existing pathovars of *X. hortorum* nor *X. cynarae* were pathogenic on lettuce, eliminating the possibility of a pathovar synonymy. We therefore propose its transfer in *X. hortorum* as *X. hortorum* pv. *vitians* comb. nov. with strain LMG 938 acting as the neopathotype.

The species-scaled phylogeny revealed that *X. hortorum* pv. *vitians* is most probably divided in two genomic groups which might be further subdivided into close sub-populations. *X. hortorum* pv. *vitians* strains representing MLSA groups A and C formed a tight cluster whereas strains LM 16388 and CFBP 499 (MLSA group B) were slightly divergent (Fig. 2b). The three MLSA clusters identified in this study are identical to those described recently by Fayette et al. [19] in the United States, as representative strains L43, JF196 and L7 of Fayette's study appeared to belong to our major sequence types A1, B1 and C1 based on the comparison of partial *gapA* sequences only (*data not shown*). In both MLSA studies, the clustering is dependent of *gapA* and *gyrB* as other loci show no or little polymorphism. The *gyrB*-based tree resulted in two groups seemingly similar to those obtained using whole-genome alignments, whereas *gapA* is the only locus that discriminates group C strains.

Overall, the evolutionary picture depicted by the species-level phylogeny (Fig. 2b) clearly distinguished the different pathovars, highlighting recent specializations of closely related organisms toward different hosts. *X. hortorum* pv. *pelargonii* branched at the root of the species and pathovars *gardneri* and *cynarae* were the most highly related depicting a recent divergence as demonstrated recently [64]. Apart from the exception discussed below, none of the pathovars have been, to our knowledge, reported to be isolated on another plant than their described hosts in natural conditions [35]. In addition, recent work made on the comparison of host ranges of *X. cynarae* and *X. hortorum* pathovars by cross-

inoculations in artificial conditions revealed that they all differ by at least one host and cannot therefore be considered as synonymous pathovars [26]. It would be of great interest to add to this phylogeny and evaluate the experimental host ranges of some newly identified pathogenic isolates which reportedly belong to *X. hortorum* based on partial *gyrB* sequencing or MLSA. These new variants were isolated on radicchio (*Cichorium intybus*) [80], annual wormwood (*Artemisia annua*) [61], English lavender (*Lavandula angustifolia*) [49], peony (*Paeonia* spp.) [31], poinsettia (*Euphorbia pulcherrima*) [50], pot marigold (*Calendula officinalis*) and avocado (*Persea americana*) [43]. It has also been reported that *X. campestris* pv. *nigromaculans*, pathogenic on greater burdock (*Arctium lappa*) should belong to *X. hortorum* emend. [29,43].

The last interesting point raised in this article is the status of strain CFBP 7999 (=ICMP 7383) from pv. *gardneri* for which the taxonomic singularity has been already depicted [24,63]. According to our phylogenetic analyses and pathogenicity tests, it appears that this strain belongs unequivocally to *X. hortorum* pv. *vitians* rather than pathovar *gardneri*. Indeed, it was demonstrated by its high virulence on the two lettuce cultivars tested resulting in typical BLSL symptoms. Its high virulence on tomato was an unusual feature for a pv. *vitians* strain though. If several strains of *X. hortorum* pv. *vitians* have been already reported to be weakly pathogenic on tomato and pepper [2,53,54], like strain LM 16735 from this study, none was shown to possess such level of aggressiveness on both hosts. While most of the strains we investigated held only one plasmid, preliminary investigations demonstrated that this peculiar strain possess three (*data not shown*). *In silico* comparisons revealed that plasmid pICMP7383.2 resembles highly to the typical plasmid of *X. hortorum* pv. *vitians*, yet plasmids pICMP7383.1 and pICMP7383.3 were found to be strikingly akin to pJS749-3.1 and pJS749-3.2 of *X. hortorum* pv. *gardneri* CFBP 8588 (=JS749-3) [45]. Plasmid pICMP7383.1 was found also to be similar to pLMG911.1 of type strain of *X. vesicatoria* LMG 911<sup>T</sup> (=CFBP 2537<sup>T</sup>), another *Xanthomonas* pathogenic toward tomato and pepper. This particular plasmid feature raises questions about the role of plasmids in the adaptation to different hosts in *Xanthomonas* and should therefore be investigated further. Regardless, it may be involved in the singular phenotype which resulted in the erroneous taxonomic affiliation of this particular strain.

As a conclusion, this polyphasic study has led us to propose to replace the inappropriate pathotype strain CFBP 2538<sup>PT</sup> (=NCPPB 976<sup>PT</sup> = LMG 937<sup>PT</sup> = ATCC 19320<sup>PT</sup> = ICMP 336<sup>PT</sup>) of *X. campestris* pv. *vitians* by the neopathotype strain LMG 938<sup>neoPT</sup> and to transfer this pathovar in *X. hortorum* emend. as *X. hortorum* pv. *vitians* comb. nov. (proposed neopathotype LMG 938<sup>neoPT</sup> = CFBP 8686<sup>neoPT</sup> = NCPPB 2248<sup>neoPT</sup>). This proposition allows to maintain Brown's pathovar epithet priority. If these changes should be rejected by the ISPP-Committee on the Taxonomy of Plant Pathogenic Bacteria, we still propose the creation of a new pathovar named *X. hortorum* pv. *vitians* pv. nov. having the same description and with LMG 938<sup>PT</sup> being the pathotype strain. In addition, the phylogenetic, genomic and phenotypic data presented in this work all support that *X. cynarae* is a later heterotypic synonym of *X. hortorum* and reclassification of *X. cynarae* pv. *cynarae* and *X. cynarae* pv. *gardneri* into *X. hortorum* as *X. hortorum* pv. *cynarae* comb. nov. (pathotype strain CFBP 4188<sup>PT</sup>) and *X. hortorum* pv. *gardneri* comb. nov. (pathotype strain CFBP 8163<sup>PT</sup>). An emended description of *X. hortorum* (type strain CFBP 5858<sup>T</sup>) is provided. Resolving these taxonomic issues will aid in further investigations into the biology and epidemiology of these pathogens. We are currently investigating the race structure of *X. hortorum* pv. *vitians* [8,25]. In future work, we will investigate the molecular determinants underlying the intriguing host-specificity pattern of these pathovars on phylogenetically-distant plants.

## Taxonomy

*Emended description of Xanthomonas hortorum* Vauterin et al. 1995

*Xanthomonas hortorum* (hor.to'rum. L. masc. gen. n. *hortorum*, from gardens)

The general characteristics are as depicted in the first description of the species [72], emended with data from the present study. Based on Biolog GEN III MicroPlates assays, strain CFBP 5858<sup>T</sup> is undoubtedly able to utilize D-trehalose, D-cellobiose, sucrose, D-melibiose, N-acetyl-D-glucosamine, D-glucose, D-mannose, D-fructose, D-galactose, L-fucose, gelatin, L-alanine, L-glutamic acid, L-serine, methyl pyruvate, citric acid,  $\alpha$ -keto-glutaric acid, L-malic acid, bromo-succinic acid, propionic acid and acetic acid. Strain CFBP 5858<sup>T</sup> also undoubtedly grow at pH 6, in presence of 1% NaCl, 1% sodium lactate and lincomycin. Additionally, the Biolog PM03B MicroPlates revealed that pathovars of *X. hortorum* presents highly variable profiles of nitrogen sources utilization.

The type strain is CFBP 5858<sup>T</sup> = CFBP 4925<sup>T</sup> = LMG 733<sup>T</sup> = NCPPB 939<sup>T</sup> = ICMP 453<sup>T</sup>. The GenBank accession number for its genome assembly is GCA.002940005.1 and its 16 S rRNA gene accession number is NR.026386. The species has a G+C mole % value between 63.3 to 63.9%. The species includes, so far, the following pathovars based on their phytopathogenic specialization: *X. hortorum* pv. *hederae*, *X. hortorum* pv. *pelargonii*, *X. hortorum* pv. *carotae*, *X. hortorum* pv. *taraxaci*, *X. hortorum* pv. *cynarae*, *X. hortorum* pv. *gardneri* and *X. hortorum* pv. *vitians*.

The closest species described is *X. populi* (ex-Ridé 1958) van den Mooter and Swings 1990 [71]. According to the characteristics of *X. populi* described in the valid description, in Ridé and Ridé 1992 [48] and Vauterin et al. 1995 [72], *X. hortorum* strains can be distinguished from *X. populi* by their incapacity to use dextrin, ability to use D-cellobiose, D-melibiose, L-fucose, gelatin, L-alanine, L-glutamic acid, L-serine and propionic acid, to grow in presence of 1% NaCl, to liquefy gelatin and produce indole. Moreover, *X. populi* is a fastidiously cultivable bacteria which has an optimal growth temperature of 20–23 °C and cannot grow at 28 °C, which is the optimal growth temperature of *X. hortorum*. Finally, *X. hortorum* can be phylogenetically discriminated from other *Xanthomonas* species using the 4-genes MLSA scheme proposed by Young et al. [78].

Strains reportedly identified as *X. hortorum* using partial *gyrB* sequencing were isolated from symptomatic radicchio (*Cichorium intybus*) [80], annual wormwood (*Artemisia annua*) [61], English lavender (*Lavandula angustifolia*) [49], peony (*Paeonia* spp.) [31], poinsettia (*Euphorbia pulcherrima*) [50], pot marigold (*Calendula officinalis*) and avocado (*Persea americana*) [43]. It has been also suggested that *X. campestris* pv. *nigromaculans*, pathogenic on greater burdock (*Arctium lappa*), might belong to *X. hortorum* [43].

### *X. hortorum* pv. *vitians* (Brown 1918) comb. nov.

=*X. campestris* pv. *vitians* (Brown 1918) Dye 1978

The description is the same as the species. Additionally, the Biolog GEN III and PM02A MicroPlates revealed the ability of strain LMG 938<sup>neoPT</sup> to utilize  $\beta$ -gentiobiose, glycerol, pectin, L-lactic acid, tween 40,  $\alpha$ -keto-butyric acid, acetoacetic acid, sodium formate, gelatin, laminarin, D-raffinose, N-acetyl-L-glutamic acid, weakly utilize L-tartaric acid, and grow in presence of 4% NaCl, tetrazolium violet, tetrazolium blue, potassium tellurite and sodium bromate. The Biolog PM03B MicroPlates indicated that L-alanine, L-arginine, L-glutamic acid, L-glutamine, L-ornithine, glucuronamide, D-glucosamine, N-acetyl-D-glucosamine, guanine, xanthosine, uric acid, alanyl-glutamine, alanyl-glutamic acid and glycyl-glutamic acid can be used as nitrogen sources.

The primary host is the lettuce (*Lactuca sativa* L.). The pathotype strain of *X. campestris* pv. *vitians* (Brown 1918) Vauterin et al.



1995 CFBP 2538<sup>PT</sup> = LMG 937<sup>PT</sup> = NCPPB 976<sup>PT</sup> = ICMP 336<sup>PT</sup> = ATCC 19320<sup>PT</sup> has been proven to be non-pathogenic on lettuce, phenotypically and genotypically different from all other bacterial leaf spot of lettuce-related strains, as discussed in the present study and in previous ones [3,53,62]. Therefore, in accordance with Standards 9–4 and 11 of the *International Standards for Naming Pathovars of Phytopathogenic Bacteria* [15,75], we propose the replacement of this pathotype by neopathotype strain LMG 938<sup>neoPT</sup> = CFBP 8686<sup>neoPT</sup> = NCPPB 2248<sup>neoPT</sup>. We propose that its subsequent transfer to *X. hortorum* be as *X. hortorum* pv. *vitians* (Brown 1918) Vauterin et al. 1995 comb. nov. in part to ensure the conservation of the priority established by Nellie Brown. However, if the neopathotype is rejected, we propose that this same name be established as *X. hortorum* pv. *vitians* pv. nov. The GenBank accession number for its genome assembly is GCA.012922135.1.

***X. hortorum* pv. *cynarae*** (Trébaol et al. 2000) comb. nov.

=*X. cynarae* Trébaol et al. 2000

=*X. cynarae* pv. *cynarae* (Trébaol et al. 2000) Timilsina et al. 2019

The description is the same as the species. Additionally, the Biolog GEN III and PM02A MicroPlates revealed the ability of strain CFBP 4188<sup>T</sup> to utilize glycerol and L-histidine, weakly utilize L-lactic acid and acetoacetic acid, and grow in presence of guanidine hydrochloride and tetrazolium blue. The Biolog PM03B MicroPlates indicated that N-phthaloyl-L-glutamic acid, guanine and alloxan can be used as nitrogen sources.

The primary host is the common artichoke (*Cynara scolymus* L.). The pathotype strain is CFBP 4188<sup>PT</sup> = ICMP 16775<sup>PT</sup>, the former type strain of *X. cynarae* Trébaol et al. 2000 emend. Timilsina et al. 2019. The GenBank accession number for its genome assembly is GCA.002939985.1.

***X. hortorum* pv. *gardneri*** (ex Sutic 1957) comb. nov.

=*X. gardneri* (ex Šutič 1957) Jones et al. 2006

=*X. cynarae* pv. *gardneri* (ex Šutič 1957) Timilsina et al. 2019

The description is the same as the species. Additionally, the Biolog GEN III and PM02A MicroPlates revealed the ability of strain CFBP 8163<sup>PT</sup> to utilize  $\alpha$ -D-lactose, L-aspartic acid, L-histidine, pectin, L-lactic acid, tween 40,  $\alpha$ -keto-butyric acid, acetoacetic acid, sodium formate, arbutin, D-raffinose, succinamic acid, D-tartaric acid, N-acetyl-L-glutamic acid and L-homoserine, and grow in presence of 4% NaCl, tetrazolium violet and tetrazolium blue. The Biolog PM03B MicroPlates indicated that L-arginine, L-aspartic acid, L-glutamic acid, L-leucine, N-acetyl-L-glutamic acid, D-glucosamine, N-acetyl-D-glucosamine, guanine, xanthosine, alloxan, parabanic acid, alanyl-glycine, alanyl-threonine, glycyL-glutamine and glycyL-glutamic acid can be used as nitrogen sources.

The primary hosts are tomato (*Solanum lycopersicon* L.) and pepper (*Capsicum annuum* L.). The pathotype strain is CFBP 8163<sup>PT</sup> = NCPPB 881<sup>PT</sup> = ATCC 19865<sup>PT</sup>. The GenBank accession numbers for the two versions its genome assembly are GCA.012922265.1 and GCA.000192065.2.

*In order to provide taxonomic data ready to use and easily comparable, and because the original protologues may be difficult to find, we recall hereby the characteristics of the other pathovars of X. hortorum complemented with data from our study:*

***X. hortorum* pv. *hederae*** (Arnaud 1920) Vauterin et al. 1995

=*X. campestris* pv. *hederae* (Arnaud 1920) Dye 1978

The description is the same as the species. Additionally, the Biolog GEN III and PM02A MicroPlates revealed the ability of strain CFBP 5858<sup>T</sup> to utilize pectin, glycerol, L-lactic acid,  $\alpha$ -keto-butyric acid, acetoacetic acid, weakly utilize tween 40, and grow in presence of guanidine hydrochloride and tetrazolium blue. The Biolog PM03B MicroPlates indicated that N-phthaloyl-L-glutamic acid can be used as a nitrogen source. It should be noted that previous studies on multiple strains of *X. hortorum* pv. *hederae* showed their

ability hydrolyze starch and gelatin [40], making these features strain-dependent for CFBP 5858<sup>T</sup>.

The primary host is the common ivy (*Hedera helix* L.), yet strains of *X. hortorum* pv. *hederae* have been reported to be pathogenic on other Araliaceae plants such as umbrella tree (*Schefflera actinophylla*), dwarf umbrella tree (*Schefflera arboricola*), Japanese aralia (*Fatsia japonica*), false aralia (*Pterandra elegantissima*) and ming aralia (*Polyscias fruticosa*) [12,40]. The pathotype strain is also the type strain of *X. hortorum* CFBP 5858<sup>T</sup> (=CFBP 4925<sup>T</sup> = LMG 733<sup>T</sup> = NCPPB 939<sup>T</sup> = ICMP 453<sup>T</sup>).

***X. hortorum* pv. *pelargonii*** (Brown 1923) Vauterin et al. 1995

=*X. campestris* pv. *pelargonii* (Brown 1923) Dye 1978

The description is the same as the species. Additionally, the Biolog GEN III and PM02A MicroPlates revealed the ability of strain CFBP 2533<sup>PT</sup> to utilize  $\beta$ -gentiobiose, glycerol, L-histidine, pectin, L-lactic acid, tween 40, acetoacetic acid, sodium formate, gelatin, laminarin, amygdalin, arbutin, L-alaninamide, N-acetyl-L-glutamic acid, L-homoserine, weakly utilize malonic acid and 3-O- $\beta$ -L-galactopyranosyl-D-arabinose, and grow in presence of 4% NaCl, tetrazolium blue, tetrazolium violet, potassium tellurite and sodium bromate. The Biolog PM03B MicroPlates indicated that ammonia, sodium nitrite, sodium nitrate, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-threonine, L-tryptophan, L-valine, D-alanine, D-asparagine, D-citrulline, L-ornithine, N-acetyl-L-glutamic acid, N-phthaloyl-L-glutamic acid, glucuronamide, D-glucosamine, D-mannosamine, N-acetyl-D-glucosamine, adenosine, cytosine, guanine, xanthosine, uric acid, alloxan, allantoin, parabanic acid,  $\gamma$ -amino-n-butyric acid, alanyl-aspartic acid, alanyl-glutamine, alanyl-glutamic acid, alanyl-glycine, alanyl-histidine, alanyl-leucine, alanyl-threonine, glycyL-asparagine, glycyL-glutamine, glycyL-glutamic acid, glycyL-methionine and methionyl-alanine can be used as nitrogen sources.

The primary host is the ivy-leaved geranium (*Pelargonium peltatum* L'Hér.). The pathotype strain is CFBP 2533<sup>PT</sup> = LMG 7314<sup>PT</sup> = NCPPB 2985<sup>PT</sup> = ICMP 4321<sup>PT</sup>. The GenBank accession number for its genome assembly is GCA.012922215.1.

***X. hortorum* pv. *carotae*** (Kendrick 1934) Vauterin et al. 1995

=*X. campestris* pv. *carotae* (Kendrick 1934) Dye 1978

The description is the same as the species. According to Kendrick (1934), acid is produced from dextrose and glycerin, litmus milk is cleared in 7 days, and strains were pathogenic on leaves, stems and floral parts of *Daucus carota* L. var. *sativa* DC. The pathotype strain CFBP 4997<sup>PT</sup> = LMG 8646<sup>PT</sup> = NCPPB 1422<sup>PT</sup> = ICMP 5723<sup>PT</sup> has been reported many times to be unsuitable as it is not a *Xanthomonas* [75]. However, it was revealed that pathogenic strain CFBP 7900 was able to utilize  $\beta$ -gentiobiose, glycerol, L-aspartic acid, pectin, tween 40, gelatin, D-raffinose, D-tartaric acid, N-acetyl-L-glutamic acid, weakly utilize L-hydroxyproline, and to grow in presence of 4% NaCl, rifamycin SV, guanidine hydrochloride, niaproof, tetrazolium violet, tetrazolium blue, lithium chloride and potassium tellurite according to Biolog GEN III and PM02A MicroPlates. The Biolog PM03B MicroPlates indicated that L-aspartic acid, L-glutamic acid, L-glutamine, N-acetyl-L-glutamic acid, glucuronamide, D-glucosamine, N-acetyl-D-glucosamine, alloxan, alanyl-glutamine, alanyl-glutamic acid and glycyL-glutamic acid can be used as nitrogen sources.

The primary host is the wild carrot (*Daucus carota* L.). Another pathotype strain must be formally described. We used CFBP 7900 = M081 [30] as a pathogenic representative strain because it will be formally proposed as a neopathotype in a near future (MA Jacques personal communication). The GenBank accession number for its genome assembly is GCA.000505565.1.

### *X. hortorum* pv. *taraxaci* (Niederhauser 1943) Vauterin et al. 1995

The description is the same as the species. Additionally, the Biolog GEN III and PMO2A MicroPlates revealed the ability of strain CFBP 410<sup>PT</sup> to utilize β-gentiobiose, glycerol, L-histidine, pectin, acetoacetic acid, gelatin, arbutin, D-tartaric acid and L-homoserine. The Biolog PMO3B MicroPlates indicated that ammonia, sodium nitrate, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamic acid, L-glutamine, glycine, L-leucine, L-lysine, L-proline, D-alanine, L-ornithine, N-acetyl-L-glutamic acid, glucuronamide, N-acetyl-D-glucosamine, adenosine, cytosine, guanine, xanthosine, uric acid, parabanic acid, alanyl-asparagine, alanyl-glutamine, alanyl-glutamic acid, alanyl-glycine, alanyl-leucine, glycyL-asparagine, glycyL-glutamine and glycyL-glutamic acid can be used as nitrogen sources.

The primary host is the Kazakh dandelion (*Taraxacum kok-saghyz* Rodin). The pathotype strain is CFBP 410<sup>PT</sup> = LMG 870<sup>PT</sup> = NCPPB 940<sup>PT</sup> = ICMP 579<sup>PT</sup> = ATCC 19318<sup>PT</sup>. The GenBank accession number for its genome assembly is GCA.012922225.1.

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### Conflict of interest

None declared.

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### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <https://doi.org/10.1016/j.syapm.2020.126087>.

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# CHAPITRE II :

## Caractérisation et conservation du génome essentiel *in vitro* de *X. hortorum* pv. *vitians*

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### Préambule

Une fois effectuée la reclassification taxonomique de *X. hortorum* pv. *vitians* en tant que l'un des sept pathovars de l'espèce étendue *X. hortorum*, nous avons cherché à appliquer la technique du Tn-seq à une souche modèle représentative du principal groupe génétique de *X. hortorum* pv. *vitians* identifié par MLSA et analyses génomiques. Pour ce faire, nous avons engagé une collaboration avec le laboratoire du MAP au cours de laquelle nous avons pu utiliser des outils moléculaires développés initialement dans le cadre d'une analyse de Tn-seq du phytopathogène *Dickeya dadantii* (Royet et al., 2019).

L'objectif premier derrière le choix de cette méthode à haut débit était d'identifier les gènes impliqués dans l'interaction hôte-pathogène, mais il est apparu que cette technique était également un outil puissant permettant l'identification du génome essentiel d'une bactérie. L'étude de ce génome essentiel – outre son intérêt du point fondamental pour étudier l'évolution et la structure des génomes bactériens (Juhas et al., 2011) – présente également un intérêt appliqué en identifiant de nouvelles cibles moléculaires généralement conservées qui pourraient être visées par des stratégies de lutte antibactérienne (Mobegi et al., 2014). Toutefois, l'étude du génome essentiel d'une bactérie tient rarement compte de la conservation de ses éléments au sein de l'espèce, du genre, ou même de la famille. Or, étudier cette conservation présente plusieurs avantages. Tout d'abord, cela permet dans une certaine mesure d'extrapoler les résultats obtenus chez une bactérie à ses apparentées, et de réfléchir aux implications écologiques de la présence ou de l'absence de certains gènes chez des bactéries proches mais présentant des modes de vie différents. Aussi, on peut alors distinguer les gènes essentiels conservés d'autres gènes qui présentent un phénotype d'essentialité, mais qui sont en réalité associés à des éléments génétiques mobiles « égoïstes » neutres ou délétères pour la bactérie (Werren, 2011).

Ces travaux, présentés ici en tant que second chapitre, ont été valorisés par une publication dans le journal *Microbial Genomics* en mars 2021.

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# *In vitro* exploration of the *Xanthomonas hortorum* pv. *vitians* genome using transposon insertion sequencing and comparative genomics to discriminate between core and contextual essential genes

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## Abstract

The essential genome of a bacterium encompasses core genes associated with basic cellular processes and conditionally essential genes dependent upon environmental conditions or the genetic context. Comprehensive knowledge of those gene sets allows for a better understanding of fundamental bacterial biology and offers new perspectives for antimicrobial drug research against detrimental bacteria such as pathogens. We investigated the essential genome of *Xanthomonas hortorum* pv. *vitians*, a gammaproteobacterial plant pathogen of lettuce (*Lactuca sativa* L.) which belongs to the plant-pathogen reservoir genus *Xanthomonas* and is affiliated to the family *Xanthomonadaceae*. No practical means of disease control or prevention against this pathogen is currently available, and its molecular biology is virtually unknown. To reach a comprehensive overview of the essential genome of *X. hortorum* pv. *vitians* LM16734, we developed a mixed approach combining high-quality full genome sequencing, saturated transposon insertion sequencing (Tn-Seq) in optimal growth conditions, and coupled computational analyses such as comparative genomics, synteny assessment and phylogenomics. Among the 370 essential loci identified by Tn-Seq, a majority was bound to critical cell processes conserved across bacteria. The remaining genes were either related to specific ecological features of *Xanthomonas* or *Xanthomonadaceae* species, or acquired through horizontal gene transfer of mobile genetic elements and associated with ancestral parasitic gene behaviour and bacterial defence systems. Our study sheds new light on our usual concepts about gene essentiality and is pioneering in the molecular and genomic study of *X. hortorum* pv. *vitians*.

## DATA SUMMARY

All new genome and sequencing data were deposited in the corresponding databases of the National Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov>), and are associated with BioProject PRJNA530964 and BioSample SAMN11340225. The Illumina and PacBio raw sequencing reads of the complete genome sequencing of strain LM16734 are

accessible at the Sequence Read Archive (SRA) database under accession numbers SRR12066994–SRR12066995. The manually revised annotated assembly carried out at the MicroScope annotation platform (<https://mage.genoscope.cns.fr>) was deposited in the GenBank database under accession number GCA\_014338485.1. The transposon insertion sequencing reads of the two duplicates were also deposited in the SRA database under accession numbers

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**Keywords:** *Xanthomonas hortorum*; Tn-Seq; bacterial leaf spot of lettuce; mobile genetic elements; *Xanthomonadaceae*; essential genome.

**Abbreviations:** BLSL, bacterial leaf spot of lettuce; CDS, coding sequence; COG, Cluster of Orthologous Groups; DEG, database of essential genes; EPS, exopolysaccharide; HMM, hidden markov model; KEGG, kyoto encyclopaedia of genes and genomes; LB, luria-bertani; MGE, mobile genetic element; MTase, methyltransferase; OLD, overcoming lysogenization defect; PCR, polymerase chain reaction; REase, restriction endonuclease; R-M, restriction-modification; Tn-Seq, transposon insertion sequencing; TSA / TSB, tryptic soy agar / tryptic soy broth; TTR, trimmed total reads.

The NCBI GenBank assembly and SRA accession numbers for the complete *X. hortorum* pv. *vitians* LM16734 genome and raw sequencing reads are GCA\_014338485.1 and SRR12066994–SRR12066995, respectively. Transposon insertion sequencing reads are available at SRA under accessions SRR12067051–SRR12067052. All data are associated with BioProject PRJNA530964. Supplementary Material can be found at [10.6084/m9.figshare.13573523](https://doi.org/10.6084/m9.figshare.13573523).

**Data statement:** All supporting data, code and protocols have been provided within the article or through supplementary data files. Seven supplementary tables and three supplementary figures are available with the online version of this article.

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SRR12067051–SRR12067052. The GenBank assembly accession numbers for the other published genome data used in this study are given in Table S1 (available in the online version of this article). Tables S1–S7 and Figs S1–S3 are available at figshare with the following doi: <https://figshare.com/s/aab51b0deba42f67e969>.

## INTRODUCTION

Evolutionary forces shape bacterial genomes through persistence and loss of genes in relation to the fitness cost or benefit they provide to the organism or population. However, for most genes, the conferred fitness depends on occupied environments and ecological niches, or on the lifestyle adopted by the bacterium [1, 2]. A minority of coding sequences are considered indispensable regardless of the environmental context, as they regulate critical cellular processes such as DNA replication, cell wall integrity or energy metabolism [3]. These coding sequences and structural elements as a whole are referred to in the literature as the minimal gene set [4] or essential genome [5] of a given bacterium. However, gene essentiality is usually deduced from indirect observations of lethal mutant phenotypes in a given medium. Thus, observed essentiality could either result from direct alteration of critical functions required for cell survival (e.g. DNA replication, membrane synthesis), or context-dependent effects related to the genome content or environmental conditions (e.g. toxin–antitoxin systems, auxotrophy) [6, 7]. Consequently, the essentiality of some genes varies among strains within the same species [8–10]. Therefore, the essential genome of a bacterium can be seen as a combination of ‘core’ and ‘accessory’ essential genes, and the latter depend on environmental conditions or the genetic context.

Essential genomes have been historically assessed through systematic single-gene knock-out or deletion experiments [11, 12], or identification of conserved genes by comparative genomics [13, 14]. Yet, both methods have substantial limitations. For example, computational approaches based on protein sequence homology yielded artificially small essential genomes because similar functions can be encoded by non-orthologous genes, while experimental approaches were time-consuming and subject to variability due to methodological differences [3, 15]. However, the development and extensive application of genome-wide transposon insertion sequencing – abbreviated Tn-Seq [16] – in the last decade, and of its variants referred to as INSeq [17], TraDIS [18] or HITS [19] has allowed for highly efficient and systematic identification of the essential features of cell survival in a genome. Briefly, Tn-Seq experiments use high-throughput sequencing of a pooled population of mutants covering the entire genome to assess the impact of each mutation site on overall fitness and survival. Experiments can be carried out both in optimal growth conditions to identify genes commonly considered as absolutely essential [5, 20–26], and in diverse environmental conditions (e.g. *in vivo*, in synthetic communities) to reveal conditionally essential genes [27–32]. Essential genomes have been so far characterized *in vitro* for at least 63 bacteria from various phyla (DEG database, accessed September

### Impact Statement

The genus *Xanthomonas* constitutes a huge reservoir of economically and ecologically important plant-pathogenic species that pressure agroecosystems and threaten global food sustainability. Amongst these, *Xanthomonas hortorum* pv. *vitians* causes a bacterial leaf spot of lettuce. This disease attacks all varieties of lettuce around the world, and no sustainable and operational means of control has yet been found. Moreover, little is known about the genetic and molecular functioning of *X. hortorum* species. Previous studies conducted on other organisms have shown that transposon insertion sequencing (Tn-Seq) is a powerful tool for acquiring fundamental knowledge on cell functioning and critical cellular processes because it reliably identifies prospective targets for new antimicrobial compounds. Using the discriminative power of highly saturated genome-wide Tn-Seq and high-quality complete genome sequencing, the present study offers an extensive description and analysis of *X. hortorum* pv. *vitians* LM16734 genes required for cell survival in optimal growth conditions. Portrayal of the essential genome is accompanied by a critical reflection on the concepts underlying gene essentiality based on gene conservation, synteny and phylogenomic analyses. In this way, we discriminated between core essential genes of xanthomonads and horizontally acquired contextual essential genes in this first *Xanthomonas* essential genome.

2020) [33]. However, research has been mostly focused on human-pathogenic bacteria (51 essential genomes out of 63) because systematic essentiality assessment is considered a promising way to identify new targets for antimicrobial drugs [34]. However, apart from the two major  $\alpha$ -proteobacterial plant pathogens *Agrobacterium fabrum* [35] and *Ralstonia solanacearum* [36], no other essential genomes of plant pathogens, especially from the  $\gamma$ -proteobacterial class, have been investigated.

The genus *Xanthomonas* is a large reservoir of important plant-pathogenic  $\gamma$ -proteobacteria, as *Xanthomonas* species are the causative agents of diseases of almost 400 plant species, some of which are major crops worldwide (rice, cabbage, tomato, citrus, etc.) [37]. They belong to the same taxonomic family – *Xanthomonadaceae* – as the environmental and opportunistic pathogenic genus *Stenotrophomonas* and the devastating plant-pathogenic genus *Xylella*. While some *Xanthomonas* species have been extensively studied, e.g. major *X. campestris* or *X. oryzae* species [38], there still remains a lot to discover about most species. Among these, *Xanthomonas hortorum*, which comprises seven distinct pathovars and an increasing number of non-classified pathogenic strains attacking a wide variety of plants, has been scarcely investigated, perhaps partially because of its complex and until recently unresolved



taxonomy [39]. Indeed, apart from a few studies on some type III effectors of the tomato pathogen *X. hortorum* pv. *gardneri* (formerly known as '*X. gardneri*') [40, 41], and a noticeable genomic study of *X. hortorum* pv. *carotae* [42], extensive functional genomic studies and mutagenesis experiments have not yet been attempted on *X. hortorum*.

Our research work focuses primarily on *X. hortorum* pv. *vitians*, the causal agent of the bacterial leaf spot of lettuce disease (BLSL). This foliar disease is characterized by an outburst of small water-soaked lesions that later coalesce to form large necrotic patches [43]. Although sporadic, BLSL seems to have been re-emerging all around the world since the 1990s [39], sometimes dramatically reducing plant yield and quality, and increasing post-harvest losses [44]. Moreover, this pathogen can disseminate across the environment and persist mostly through seed infestation [45], but also in buried plant debris, irrigation water and epiphytically on a wide variety of weeds [46, 47]. It has become a concerning threat for lettuce production in the absence of efficient and practical means of disease control or prevention [48]. Understanding the architecture and evolution of the *X. hortorum* pv. *vitians* genome would certainly support the many attempts made to select resistant lettuce cultivars [49] and prevent predictable resistance by identifying virulence and resistance genes.

The present study aims at giving a comprehensive description of the essential genome of *X. hortorum* pv. *vitians* in optimal growth conditions, and provides an overview of the conservation and horizontal transfer of essential genes within the family *Xanthomonadaceae*. Using high-resolution complete genome sequencing and the design of a highly saturated transposon mutant library, we combined the discriminative power of genome-wide Tn-Seq experiments with critical insights into gene essentiality brought by multiple computational analyses. This innovative approach allowed us to identify core essential features conserved at various taxonomic levels, and genetic-context-dependent essential genes acquired through horizontal transfer events. This primary functional genomic study of *X. hortorum* pv. *vitians* will push forward investigations of this under-investigated species and lead to advances in both fundamental genomic understanding and disease control research.

## METHODS

### Bacterial strains, plasmids and growth conditions

*Xanthomonas hortorum* pv. *vitians* strain LM16734 (=CFBP 8638) was isolated from a diseased lettuce in 2016 in the Rhône-Alpes region, France [39], and routinely cultured at 28 °C on 1/10<sup>th</sup> tryptic soy agar (TSA) plates or in 1/10<sup>th</sup> tryptic soy broth (TSB). *Escherichia coli* MFDpir/pSamEc [27] was grown at 37 °C in LB medium supplemented with diaminiopimelic acid (DAP), for which it is auxotrophic, at 57 µg.ml<sup>-1</sup>. When required, the media were supplemented with 100 µg.ml<sup>-1</sup> ampicillin and 25 µg.ml<sup>-1</sup> kanamycin. The pSamEc plasmid is a mobilizable suicide vector carrying (i) the ampicillin resistance gene *bla*, (ii) a Himar1-C9 transposase gene under the control of the *lac* promoter and (iii) a kanamycin

resistance gene flanked by mariner inverted repeat sequences containing *MmeI* restriction sites. The strains were kept at -80 °C in 30 % (v/v) glycerol vials for long-term storage.

### Complete genome sequencing, assembly and annotation

High-quality complete genome sequencing of *X. hortorum* pv. *vitians* LM16734 was achieved using a combination of long-read and short-read technologies. Genomic DNA was extracted and purified from overnight broth cultures using the standard phenol-chloroform method [50], then sent to GATC Biotech for PacBio long-read sequencing with a PacBio RS instrument (Pacific Biosciences) and to Eurofins Genomics (Eurofins Scientific) for Illumina HiSeq 2×150 bp short-read sequencing on a NovaSeq 6000 instrument (Illumina Inc.). Hybrid assembly of raw reads from the two sequencing strategies was performed using UNICYCLER v0.4.2 [51], and the resulting assembly was deposited and annotated on the MicroScope platform (<https://mage.genoscope.cns.fr>) [52]. Annotation of the genes of interest was cured manually based on homology and synteny analysis following the MicroScope recommendations. The replication origin (*oriC*) was identified based on its homology with the origin of the *X. hortorum* B07-007 genome recorded in the DoriC v10 database (DoriC ID: ORI97015374) [53]. The replication terminus sequence (*dif*) was detected by BLAST-searching the *dif* site of *X. campestris* pv. *campestris* 17 [54]. Both predicted *oriC* and *dif* sequences are presented in Table S7. Finally, leading and lagging strands of replication were determined by analysing the GC-skew bias computed with the GenSkew web tool (<http://genskew.csb.univie.ac.at/>).

### Construction of the transposon library, DNA preparation and sequencing

A transposon mutant library was generated by performing a conjugation of *X. hortorum* pv. *vitians* LM16734 with a donor strain of the mariner transposon Himar1-C9. Briefly, six mixed solutions containing 15 ml of an overnight culture of strain LM16734 and *Escherichia coli* MFDpir/pSamEc were mixed in equal parts, centrifuged at 3344 x g for 20 min, and resuspended each in 200 µl of TSB supplemented with DAP at 57 µg ml<sup>-1</sup>. The resulting suspensions were deposited on over-dried TSA plates and incubated at 28 °C for 2 h, then resuspended in 1 ml of TSB. Conjugation efficiencies were evaluated by plating serially diluted aliquots on TSA plates supplemented with kanamycin. The remaining suspensions of the conjugation experiments were diluted to 1/5<sup>th</sup> and plated on TSA kanamycin plates, and then incubated at 28 °C for 3 days. Finally, the plates were washed with 800 µl of TSB, and colonies were resuspended with a spreader. Bacteria were subsequently mixed with 30 % (v/v) glycerol for long-term storage at -80 °C. The library theoretically consisted of 250,000 individual colonies, i.e. individual transposition events, at a concentration of 10<sup>10</sup> CFU.ml<sup>-1</sup>.

Library DNA preparation and sequencing procedures were derived from Royet *et al.* [27]. The genomic DNA of the

mutant library was extracted in two replicates from 125 µl aliquots using a Promega Wizard Genomic DNA Purification kit (Promega). Then, 50 µg of DNA from each sample was digested with 50 U of *MmeI* in a final volume of 1.2 ml at 37 °C for 1.5 h. Digestions were heat-inactivated for 20 min at 80 °C and purified with a QIAquick PCR Purification kit (Qiagen). The samples were then concentrated with a vacuum concentrator to obtain final volumes of 20 µl, which were deposited on 1% (w/v) agarose gels. Each gel was cut out to extract the digested 1.3–1.8 kb fragments containing the transposon and adjacent genomic DNA using a QIAquick Gel Extraction kit (Qiagen). For each sample, 500 ng of digested and purified DNA was ligated to 0.44 µM of double-stranded barcoded adapters obtained as described previously [27] with 2000 U of T4 DNA ligase in a final volume of 50 µl at 16 °C overnight. Five 18-cycle PCRs were performed per sample to amplify the genomic DNA adjacent to the transposon. Each reaction mix contained 100 ng of DNA, 1 U of Q5 DNA polymerase (New England Biolabs), 1× Q5 buffer, 0.2 mM of dNTPs, and 0.4 µM of the LIB\_PCR\_5 and LIB\_PCR\_3 primers [32]. The five reaction products per sample were pooled and concentrated with a vacuum concentrator to obtain 25 µl samples that were deposited on a 1.8% (w/v) agarose gel. The 125 bp bands were cut out and extracted with a QIAquick Gel Extraction kit (Qiagen). All gel electrophoresis and gel extraction steps were carried out on a blue-light transilluminator to avoid DNA damage, and DNA was stained with GelGreen (Biotium). Finally, DNA samples were dialysed for 4 h on 0.025 µm nitrocellulose membranes (Milipore) deposited on sterile ultra-purified water. Thirty nanograms of each DNA library were sent to MGX (CNRS Sequencing Service) for quality control assessment and high-throughput sequencing on Illumina HiSeq 2500 instruments (Illumina Inc.).

### Assessment of genome essentiality

Raw reads were treated with CUTADAPT v1.15 [55] to retain only those containing the mariner inverted left repeat (ACAGGTTGGATGATAAGTCCCCGGTCTT) and cut the adapter sequence. Then, sequencing yield differences between the two replicates were normalized to the lowest yield by random subsampling with SEQTK v1.2 (<https://github.com/lh3/seqtk>). Trimmed read datasets were mapped to unique TA sites on independent replicons without mismatches on the genome using a modified version of the TPP script [27] from the TRANSIT software program v2.0.2 [56]. Gene essentiality was determined by analysing the datasets with TRANSIT v2.3.4 using the Hidden Markov Model method (HMM) [57]. We performed Trimmed Total Reads (TTR normalization, summed the read counts of the two replicates, applied a LOESS correction for genome positional bias, and discarded the read counts in the 10% N-terminus and C-terminus of the ORF that may have led to erroneous interpretations. Eventually, reciprocal BLASTN of genes classified as essential, growth-defect and growth-advantage were performed with BLAST+ v2.6.0 [58]

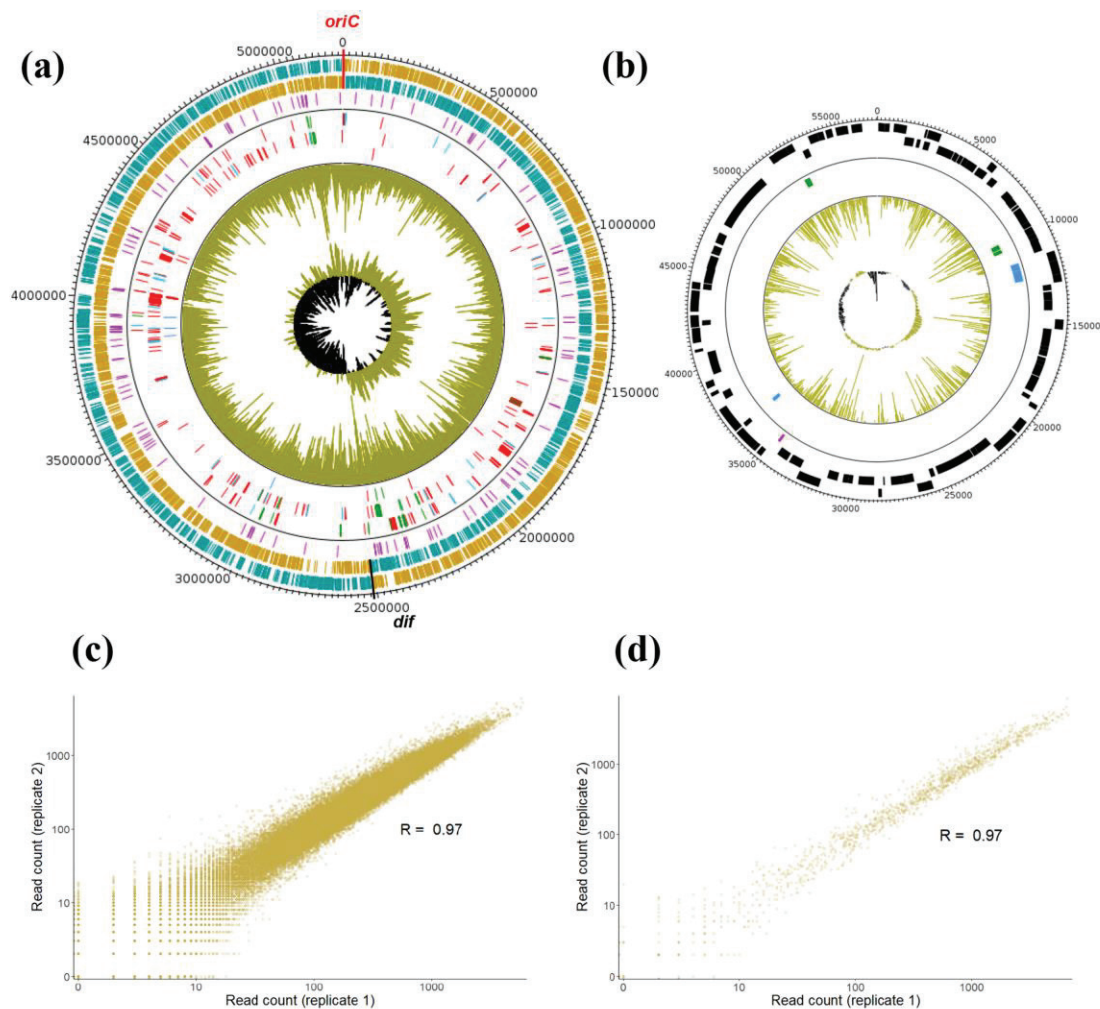
against the genome to exclude the genes present in multiple copies on the same or different replicons, considered as false positives. Visualizations of the circular genome and local genomic regions were plotted with DNAPLOTTER [59].

### Gene set enrichment analysis and cellular pathway mapping

Cluster of Orthologous Groups (COG)- [60] based annotation of genes was retrieved from the MicroScope platform. Gene set enrichment analyses were performed by computing the proportion of each COG category in the different gene states (i.e. non-essential, essential, growth-defect and growth-advantage) reported against their global proportion in the genome. The fold-change values were log-transformed to determine over- and under-represented COG categories depending on gene state. Kyoto Encyclopedia of Genes and Genomes (KEGG) (<https://www.kegg.jp/>) annotations were assigned to genes with BLASTKOALA [61] and mapped to *Xanthomonas hortorum* reference pathways with KEGG MAPPER [62].

### Analysis of essential gene conservation at multiple taxonomic levels

Conservation of the essential genes identified within the genus *Xanthomonas* and within the family *Xanthomonadaceae* was investigated. First, GenBank assemblies of 72 genomes exhaustively representing the taxonomic diversity of *Xanthomonas*, three genomes of *Stenotrophomonas maltophilia* and three genomes of *Xylella fastidiosa* subspecies *fastidiosa*, *multiplex* and *pauca* were retrieved from the NCBI database. Genome accession numbers, taxonomic names and general assembly statistics are given in Table S1. Then, a whole-genome-based phylogeny was constructed using PHYLOPHLAN2 v0.40 (<https://bitbucket.org/nsegata/phylophlan/wiki/phylophlan2>) [63]. Briefly, a core of ~350 single-copy highly conserved proteins from the PhyloPhlan database were retrieved from the genomes using SEARCH v11.0.667 [64], aligned and concatenated with MUSCLE v3.8.31 [65], and a phylogeny was built with FASTTREE v2.1.11 SSE3 [66]. Robustness was assessed locally using 1000 resamples of the Shimodaira–Hasegawa (SH) test [67]. Previously identified homologues of the essential protein-coding genes were then searched for among the 78 genomes using BLASTP with thresholds of 50% coverage, 30% amino-acid identity and an e-value <10<sup>-5</sup>. To check for potential false negatives caused by erroneous automatic annotation, sequences were also searched for using TBLASTN and the same thresholds, and homologous nucleotide sequences were searched for using BLASTN with thresholds of 50% coverage, 70% nucleotide identity and an e-value <10<sup>-5</sup>. Finally, essential and growth-defect genes were searched for in the DEG10 v15.2 database of essential genes (accessed June 2020) [33] with BLASTP using thresholds of 50% coverage, 30% amino-acid identity and an e-value <10<sup>-5</sup>. The phylogenetic tree and BLAST results were plotted together with the *ggtree* R package [68].



**Fig. 1.** Complete genome atlas of *X. hortorum* pv. *vitians* LM16734. The localizations of essential, growth-defect and growth-advantage loci identified by Tn-Seq are indicated on (a) the circular chromosome (5,213,310 bp) and (b) plasmid pLM16734 (57,250 bp). The outermost ring track is a clockwise-arranged base pair (bp) ruler, with ticks every 20,000 bp for (a) and every 200 bp for (b). The next two tracks represent predicted protein-coding gene sequences on the forward and reverse strands, respectively. The chromosome replication origin (*oriC*) and terminus (*dif*) are marked as red and black ticks in (a), respectively, and protein-coding genes on the leading and lagging strands of replication are shown in orange and blue, respectively. The third (purple) track shows predicted ncRNA sequences on both strands. After the separation line, the next two tracks represent essential (red), growth-defect (blue) or growth-advantage (green) protein-coding genes on the forward and reverse strands, while the sixth track indicates important RNAs with the same colour code. Finally, the seventh track (gold) shows the mean distribution of read counts between the two technical replicates throughout the entire genome, and the eighth track depicts the GC-skew (positive in gold and negative in black). (c, d) Correlations between the two technical replicates of the Tn-Seq experiment. The log-transformed numbers of reads per TA site are plotted and compared between replicates, and Pearson correlation coefficients are indicated for (a) the circular chromosome ( $R=0.97$ ) and (b) plasmid pLM16734 ( $R=0.97$ ).

## RESULTS AND DISCUSSION

### Complete genome sequencing of *X. hortorum* pv. *vitians* LM16734 and design of a highly saturated transposon mutant library

The complete genome of *Xanthomonas hortorum* pv. *vitians* LM16734 was sequenced using a combination of PacBio long-read and Illumina HiSeq short-read technologies. PacBio sequencing yielded 94,722 reads with a mean read length of 10,717 bp, while Illumina HiSeq

sequencing yielded more than 7.2 million paired-end reads with a mean read length of 151 bp. Hybrid assembly constructed with UNICYCLER resulted in two replicons: a circular chromosome of 5,213,310 bp (Fig. 1a) and a native plasmid of 57,250 bp, designated pLM16734 (Fig. 1b), with an overall sequencing depth of 587.5 $\times$ . The presence of a pLM16734-like plasmid might be a common feature among *X. hortorum* pv. *vitians* strains. Plasmid detection experiments performed on 18 strains representative of the

**Table 1.** Transposon-insertion sequencing (Tn-Seq) statistics

Replicate	Sequencing yield	No. of reads after normalization <sup>*</sup>	No. of Tn-end containing reads	Replicon	Read count (unique TA sites)	TA hits <sup>†</sup>	Insertion density <sup>‡</sup>	Mean read count over non-zero TA <sup>‡</sup>
1	23,662,661	23,179,825	20,267,199	Chromosome	20,168,343	60,186	70.5	335.1
				pLM16734	938,617	1,197	88.0	784.1
2	23,179,825	23,179,825	20,309,279	Chromosome	20,209,37	60,715	71.2	332.9
				pLM16734	92,4781	1,218	89.5	759.3

<sup>\*</sup>Numbers of reads were normalized based on the lowest sequencing yield (i.e. replicate 2).

<sup>†</sup>The complete *X. hortorum* pv. *vitians* LM16734 genome contains 85 314 TA sites in the chromosome and 1361 in plasmid pLM16734. Insertion density was calculated as the ratio of TA sites with at least one read mapped over the total number of TA sites in the replicon.

<sup>‡</sup>Mean value of mapped reads per TA site with at least one read.

known diversity of this lettuce pathogen indeed revealed that they all contained a single native plasmid ranging from around 40 to 60 kb [39]. The curated annotation carried out at the MicroScope platform detected 4,909 predicted protein-coding sequences (CDSs), 56 tRNAs, six rRNAs, one transfer-messenger RNA (tmRNA), seven regulatory riboswitches and 77 non-coding RNAs (ncRNAs) on the chromosome, and 89 CDSs and one ncRNA on plasmid pLM16734. The protein-coding density was therefore 87.6% on the chromosome and 79.1% on pLM16734, similar to values observed in the complete genome of *X. hortorum* pv. *carotae* M081 and other xanthomonads [42].

We designed a transposon mutant library for our Tn-Seq experiment by mating recipient strain LM16734 with an *E. coli* MFDpir donor strain containing a transferable *Himar9* mariner transposon on the suicide vector pSamEc. Approximately 250,000 individual transconjugants were recovered on 1/10<sup>th</sup> TSA rich medium supplemented with kanamycin and pooled. Then, two DNA libraries were prepared from two different aliquots of the mutant pool and massively sequenced by Illumina sequencing. The sequencing yielded more than 23 million reads in each replicate, which were normalized to the lowest value (Table 1). Of these 23 million reads, 87.4% (replicate 1) and 87.6% (replicate 2) were considered as ‘true’ TA-site-disrupted reads because they carried the *Himar9* end sequence. The fact that 99.5% of the *Himar9*-end-harboured reads mapped to unique TA sites on the chromosome in both replicates certified the quasi-certain complete absence of persistence of the vector within the mutant library. This phenomenon often occurs in mariner-based Tn-Seq studies that do not use a suicide vector, and can result in a drastic reduction in the number of informative reads mapping the genome because vector sequences can be amplified during the PCR steps [9, 22]. It is worth noting that the discrepancy between the total number of *Himar9*-end-harboured reads (~20.3 million) and the sum of reads mapped to the two

replicons (~21.1 million) suggested that some sequences of plasmid pLM16734 were identical or highly similar to some chromosomal regions. Mapping these filtered reads to the genome also revealed that out of the 85,314 and 1,361 TA sites potentially disrupted by the transposon on the chromosome and the plasmid, respectively, 60,186 (replicate 1) to 60,715 (replicate 2) of the chromosomal TA sites and 1,197 (replicate 1) to 1,218 (replicate 2) of the plasmid TA sites contained at least one insertion (Table 1). Hence, the insertion density achieved was 70.5–71.2% on the chromosome and 88–89.5% on the plasmid, i.e. one insertion every 85 bp on the chromosome and one every 47 bp on the plasmid on average (Fig. 1a, b). The TA site saturation of the library is a critical parameter for statistical inference of essentiality in Tn-Seq studies [57, 69]. Saturation achieved was higher in the present study than in most mariner-based Tn-Seq studies [10, 22, 24, 57]. Furthermore, correlations between the number of reads at each TA site (i.e. the read count) of the two replicates were optimal on both replicons as asserted by Pearson correlation coefficients of 0.97 in both cases (Fig. 1c, d). In brief, previous analyses of our complete genome sequence and mutant library representativeness showed that we achieved a high quality in all key-point statistics that strongly supported further statistical gene state interpretations.

### ***In vitro* identification of essential and fitness-related genes**

The computational assessment of gene essentiality by Tn-Seq methods generally relies on examination of the number of disrupted sites within a given gene relative to the total number of potential transposon insertion sites (i.e. the insertion rate). This rate theoretically reflects the tolerance of the organism to the loss of the encoded function [57, 70]. Yet, other approaches for Tn-Seq data analysis use the number of reads at insertion sites (i.e. the read count) as a quantitative measure by calculating the mean read count in a sliding window to

identify regions of the genome presenting lower insertion values [71]. Both methods have their drawbacks: the first tend to overestimate the number of essential genes [71] and are sensitive to insertions in the N- and C-terminal regions of genes, incorrectly concluding that they are non-essential [72], while the second have been criticized for their lack of statistical significance [56, 57]. We decided to use the HMM-based approach offered by TRANSIT because it uses both the qualitative insertion rate and quantitative read count parameters. Moreover, it assesses the probability of essentiality for each individual TA site, so that domains of essentiality can be identified in genes. It also performs a four-state classification, as genes and regions can be labelled as essential, non-essential, growth-defect or growth-advantage. The growth-defect state, also referred to as critical in other studies [10, 22], corresponds to observations that a deficit rather than complete lack of insertions within a region might be a sign that it is not directly vital for the cell, but that its disruption would impair the growth of the organism [16]. Likewise, genes or regions presenting a significantly higher read count than the rest of the genome are classified as growth-advantage, meaning that their disruption would lead to a higher growth rate, possibly because they represent an unnecessary metabolic cost in the studied conditions (e.g. secretion of an extracellular enzyme or toxin) [57].

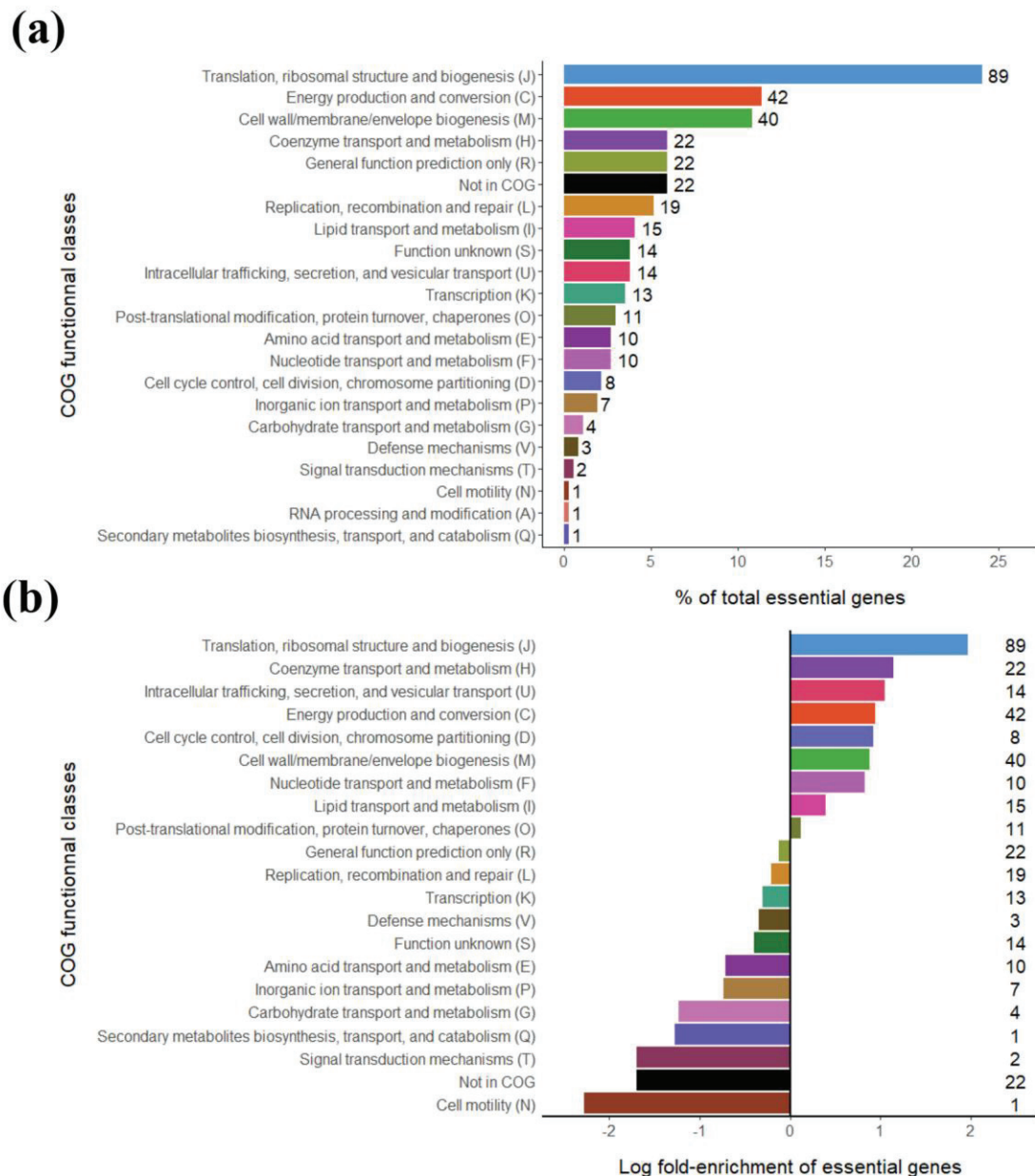
Our HMM-based analysis conducted with TRANSIT primarily identified 571 essential chromosomal loci. Nine were subsequently considered as artefactual CDSs after manual examination on the MicroScope comparative genomics platform, and another 192 were discarded because they were duplicated in the genome, on the same or different replicons, and thus were impossible to classify according to our workflow. Nineteen loci computationally identified as growth-defect were also discarded manually because they were duplicated. Finally, 34 more loci were impossible to categorize because they did not contain TA sites. All these loci were therefore classified as ‘undetermined’ and are detailed in Table S2. Following these refinement steps, 370 chromosomal loci were positively identified as essential (Table S3), consisting of 359 protein-coding genes, eight tRNAs and three ncRNAs. Additionally, 44 protein-coding genes and six tRNAs were categorized as growth-defect (Table S4), and 77 protein-coding genes, one tRNA and one ncRNA were considered as growth-advantage after curation (Table S5). The initial analysis and subsequent manual curation identified three growth-defect and three growth-advantage genes on plasmid pLM16734 (Table S6).

#### **Analysis of essential genes of *X. hortorum* pv. *vitians* associated with core bacterial features**

A striking observation one can make about the number of essential genes in various bacteria is that most of them appear to rely on a restricted set of 300–500 essential genes despite important variability in terms of genome size, architecture or lifestyles [33]. To determine whether the essential genes we identified in *X. hortorum* pv. *vitians* were common across the bacterial kingdom, we searched

for homologues in the Database of Essential Genes (DEG10 v15.2, accessed June 2020), which at the time contained 48 essential genomes from 29 Gram-negative bacteria, 17 from Gram-positive bacteria and two from *Mollicutes*. An overwhelming majority (93.5%) of the essential genes we identified in *X. hortorum* pv. *vitians* had at least one putative homologue in the database (Table S3). Therefore, it seems that essential genes and functions are widely conserved across bacteria. Moreover, the comparison of our growth-defect gene set to the DEG database revealed that 86% of them also had putative homologues in the database, suggesting that they could have been annotated as essential under more stringent experimental conditions.

To further compare the key cellular functions of *X. hortorum* pv. *vitians* and those described in other bacteria, we classified genes into general functional categories or metabolic pathways based on the COG and KEGG databases. The COG classification of essential genes showed that almost half of them were clustered in only three categories: 24% were related to fundamental processes of translation and ribosome biogenesis (J), 11% were associated with energy production and conversion (C), and another 11% were related to cell wall and membrane biogenesis (M) (Fig. 2a). However, it appeared more accurate to consider how enriched the COG categories were in the essential gene set considering their representation in the complete genome. Cellular processes presenting an enrichment in the essential gene set were associated with translation and ribosome structure (J), coenzyme metabolism (H), intracellular trafficking and secretion (U), energy production (C), cell division (D), cell wall and membrane biogenesis (M), and nucleotide metabolism (F) (Fig. 2b). Similar analyses in other bacteria also showed that translation and ribosome structure were the key essential functions of bacterial cells, while the other COG categories enriched in our gene set were usually identified as essential but varied in terms of importance and gene numbers [8, 23, 24, 26, 73]. Other COG-based analyses of non-essential, growth-defect and growth-advantage genes are not discussed here but can be found in Fig. S1. In addition, KEGG pathway clustering reached better precision in the determination of crucial cellular pathways, and underlined that most of the genes required for aminoacyl-tRNA biosynthesis, ribosome structure, protein export, DNA replication and lipopolysaccharide biosynthesis were essential (Table 2). About half of the genes involved in peptidoglycan biosynthesis, the citrate cycle, oxidative phosphorylation and RNA degradation were also required for cell survival. Unlike what was described in other organisms [10, 22, 25, 73], our analysis revealed that genes of the glucose catabolism pathways were apparently not essential for *X. hortorum* pv. *vitians* survival in rich medium (Fig. S2), even though glucose is the main carbon source in TSA medium. The only essential gene in the pentose phosphate pathway encoded the ribose-phosphate pyrophosphokinase (*prsA*, XHV734\_3940), which catalyses the conversion of ribose-5-phosphate into 5-phosphoribosyl-1-pyrophosphate, a key compound



**Fig. 2.** Clustering of essential genes based on Cluster of Orthologous Groups (COG) functional classes. (a) Proportion of each class in the essential gene set, with the number of genes next to the bars; (b) log-fold enrichment of COG classes, calculated as the ratio of the proportion of a given class in the essential gene set to its overall proportion in the genome.

in the biosynthesis pathways of the essential purine and pyrimidine bases [74]. More interestingly, the only essential gene associated with the glycolysis pathway encoded the phosphopyruvate hydratase, also known as enolase (*eno*, XHV734\_2903), which converts 2-phospho-D-glycerate into phosphoenolpyruvate. Considering that this enzyme is multifunctional – it is also involved in the RNA degradosome [75], a pathway containing seven essential genes (Table 2) – this secondary function of the enolase probably accounts more for its essentiality phenotype than does its

role in glycolysis. Overall, these two examples highlight the fact that some genes in our essential gene set act as metabolic hubs, and this makes it difficult to precisely determine which of their many roles is critical and responsible for the observed essentiality phenotype. The absence of essential genes in the usually key pathways of glucose catabolism is probably a reflection of the ability of xanthomonads to rely on the Entner-Doudoroff alternative glucose catabolism pathway (Fig. S2) [76]. A metabolic flux study of the utilization of  $^{13}\text{C}$ -labelled glucose in *X. campestris* revealed

**Table 2.** Essential KEGG pathways in *X. hortorum* pv. *vitians* LM16734 (non-exhaustive)

KEGG pathways	Essential+Critical genes <sup>*</sup>	Total genes <sup>†</sup>
Aminoacyl-tRNA biosynthesis	22+1	24
Ribosome	48	54
Protein export	10+1	16
DNA replication	10	15
Lipopolysaccharide biosynthesis	11	17
Peptidoglycan biosynthesis	12	21
Citrate cycle	13	24
Oxidative phosphorylation	26	51
RNA degradation	7	15
Fatty acid biosynthesis	6+1	19
Pyrimidine metabolism	6+2	25
Amino sugar and nucleotide sugar metabolism	6	34
Purine metabolism	8	55
Biosynthesis of amino acids	9+3	105

<sup>\*</sup>Number of genes identified as essential and growth-defect.

<sup>†</sup>Total number of genes in the model pathway in the *X. campestris* pv. *campestris* ATCC 33913<sup>†</sup> organism-specific KEGG database.

that it was mostly processed through the Entner-Doudoroff pathway to be converted into pyruvate; yet, it could also be marginally handled by the glycolysis and pentose phosphate pathways [77]. Therefore, we can reasonably conclude that no matter which of the three glucose catabolism pathways was disrupted in *X. hortorum* pv. *vitians* in our experiment, the others would be able to take over and prevent the occurrence of deleterious phenotypes. In a nutshell, most of the *X. hortorum* pv. *vitians* essential genome overlapped with key cellular functions described in other organisms, while displaying a few peculiar features.

### Conservation of essential genes among *Xanthomonadaceae* and investigations of specific essential features and mobile genetic elements

As we intended to go beyond the description of common essential bacterial functions, we further looked into the specific essential genes showing no homology in the DEG database and investigated the conservation of our essential gene set among the genus *Xanthomonas*, and also to some extent the family *Xanthomonadaceae*. To that end, we built a genome-based phylogeny of all the known species and pathovars of *Xanthomonas* species, *Stenotrophomonas maltophilia* and *Xylella fastidiosa* using relevant type and pathotype strain genomes (Table S1), and analysed the conservation of *X. hortorum* pv. *vitians* essential genes across the tree (Fig. 3).

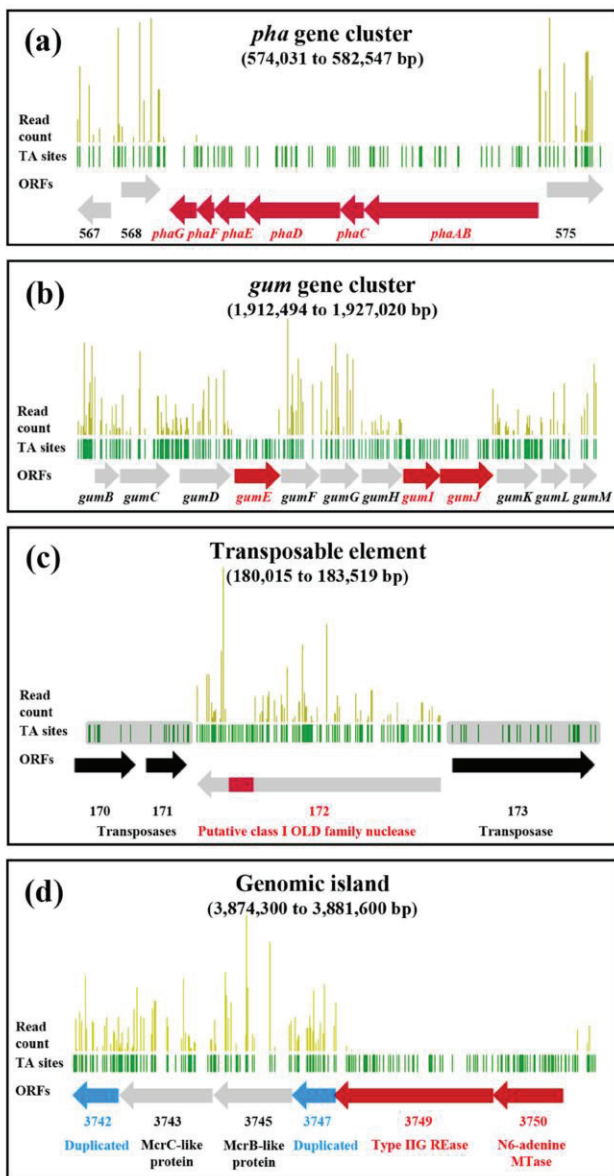
We deliberately chose weakly stringent coverage and identity thresholds to look at the conservation of protein functions and deal with quality heterogeneity in genome assemblies and annotations, but we checked for inconsistencies and potential artefacts manually. Hence, 334 out of 359 protein-coding genes were shared among all investigated *Xanthomonadaceae* (Table S3). The remaining 25 genes could be distinguished into two groups according to their distribution pattern in the phylogeny: (i) those that were mostly conserved among *Xanthomonadaceae* or displayed a phylogeny-related distribution (Fig. 3, filled inverse triangles), and (ii) those that showed a heterogeneous distribution unrelated to phylogeny (Fig. 3, empty inverse triangles).

The genes included in the first category (phylogeny-related distribution) were mainly related to known genes and functions. For example, the six genes of the *pha* operon, which encodes the monovalent cation/proton antiporter system Pha also referred to as Mrp or Mnh, were essential according to our analysis as no in-frame transposon insertions were observed (Fig. 4a). These genes are conserved in all *Xanthomonas* species and *S. maltophilia*, but completely absent in *X. fastidiosa* (Fig. 3). Note that all six genes in the operon are required for the antiporter to be functional [78]. Antiporters of the Pha family are widely distributed across bacteria and usually involved in cytoplasmic pH homeostasis and efflux of toxic cations, but also in a broad range of other resistance and environmental adaptation phenotypes [79]. The *pha* operon is essential for a few bacteria to grow in rich medium [11, 35, 80]. Its absence in *X. fastidiosa* could be a consequence of the extreme genome reduction undergone by this pathogen, which made it fit for only a few restricted homeostatic environments [81], while *Xanthomonas* species and *S. maltophilia* are adapted to living and surviving in more variable and stressful environments. Thus, they might partly rely on Pha antiporters to buffer physicochemical toxicity in challenging environments (e.g. epiphytic life for *Xanthomonas* species).

Similarly, three components of the *gum* gene cluster were also essential (Fig. 4b). This set of 12 genes (*gumB* to *gumM*) encodes the synthesis and export of the well-known *in planta* virulence factor and biotechnologically important exopolysaccharide (EPS) xanthan. The three genes – *gumE*, *gumI* and *gumJ* – turned out to be conserved in most *Xanthomonas* species but absent in *X. sacchari* and *X. albilineans* (Fig. 3), as previously reported [82]. They had no homologues in the DEG database (Table S2), except *gumJ* that showed low but significant homology (85% coverage and 25% amino acid identity, e-value of  $3.27e^{-20}$ ) with the succinoglycan biosynthesis transport protein ExoT of *Agrobacterium tumefaciens*, essential in rich medium [35]. They were also absent in *S. maltophilia*, in line with the inability of *Stenotrophomonas* to synthesize xanthan as one of the major features that led to their differentiation from the genus *Xanthomonas* [83]. Concerning *X. fastidiosa*, it was shown that the absence of *gumG*, *I* and *L* leads to the formation of another type of EPS [84], and independent acquisition of the *gum* cluster by *Xanthomonas* species and *Xylella* species may account







**Fig. 4.** Schematic representations of gene essentiality within the genomic context. Representations are shown for (a) the *pha* gene cluster (574,031...582,547 bp), (b) the *gum* gene cluster (1,912,494...1,927,020 bp), (c) the transposable element containing the putative OLD family endonuclease XHV734\_0172 (180,015...183,519 bp) and (d) the genomic island containing the restriction-modification system composed of restriction endonuclease XHV734\_3749 and methyltransferase XHV734\_3750 (3,868,330...3,884,140 bp). Essential genes or domains are shown in red, non-essential genes or domains in grey, transposases in black in (c), and duplicated loci in blue in (d). Gene names or simplified loci numbers are indicated at the bottom of each box. Green bars indicate the position of TA sites, and golden bars represent the read count at each TA site. In (c), shadowed TA sites indicate unavailability of read counts because of repeated sequences.

that they were all associated with mobile genetic elements (MGEs), i.e. prophages, transposons, genomic islands, etc. For example, locus XHV734\_0172 encodes a putative ATP-dependent endonuclease of the OLD family flanked by transposase genes (Fig. 4c), suggesting transposable-element-mediated horizontal acquisition. The conservation pattern among the *Xanthomonadaceae* implies that it was acquired independently in *X. hortorum* pv. *vitians* and *X. albilineans* (Fig. 3). The absence of this element in strain LM16388 is consistent with the early divergence of this strain from the rest of the pathovar, as we previously reported [39]. According to the classification proposed by Schiltz *et al.* [95], this nuclease belongs to class 1 OLD nucleases, like the canonical *old* gene of phage P2. Exhaustive biological roles of OLD nucleases remain to be unravelled, yet they are known to play a significant role in phage exclusion by protecting lysogenized host genomes against superinfection by other phages, presumably by degrading nucleic acids from newcomers through their ribonuclease and DNA exonuclease activities [95, 96]. Intriguingly, XHV734\_0172 was the only locus in our analysis that displayed only one short domain intolerant to insertions (I422 to Y476) (Fig. 4c). A comparison with the recently described structure of OLD nucleases [95] revealed that this essential domain was located in the C-ter Topoisomerase/Primase (Toprim) catalytic domain that bears the nuclease activity, and more precisely starts with the highly conserved metal A-binding aspartate motif D425xD427 (Fig. S3) [97]. Further knowledge of the *in vivo* biological activity of OLD nucleases would be required to understand why this segment of the Toprim domain is essential in *X. hortorum* pv. *vitians*. One possible explanation would be that transposon insertion near catalytic sites generates truncated proteins displaying uncontrolled exonuclease and ribonuclease activities, leading to cell death. Nevertheless, a genome-wide Tn-Seq screening of *Salmonella enterica* also showed that another OLD nuclease was conditionally essential [30], confirming that some OLD nuclease genes behave as 'essential' genes whose disruption results in a lethal phenotype. Furthermore, knowledge of the phage exclusion-related genes of *X. hortorum* pv. *vitians* could be of importance in the near future, as phage therapy arises as a promising way to control plant diseases caused by *Xanthomonas* species [98].

We identified other MGE-associated essential features related to restriction–modification (R-M) systems. R-M systems are often considered as primitive immune systems as their biological role is to protect bacterial genomes from foreign DNA integration by recognizing self and non-self DNA according to methylation profiles [99]. A typical R-M system involves a restriction endonuclease (REase) that recognizes and cleaves foreign DNA at specific sites, and a methyltransferase (MTase) that methylates the host DNA at the same specific sites to protect it from restriction. We notably identified an essential R-M system composed of a type-II restriction endonuclease (XHV734\_3749) and its cognate N6-adenine methyltransferase (XHV734\_3750) (Fig. 3). This system is located on a small genomic island of 7.3 kb also containing an McrBC-like R-M system and two small duplicated unknown

proteins upstream of each R-M system (Fig. 4d). Interestingly, this genomic island is conserved in the few xanthomonads possessing the essential R-M system (Fig. 3), but also strongly homologous to cluster S13 of *Pseudomonas protegens* Cab57, yet with a completely different genomic environment [100]. From a wider perspective, this genomic island is part of a larger variable genomic region located between *moaE* downstream (XHV734\_3751) and *dnaX* upstream (XHV734\_3720), which seems to have undergone multiple horizontal acquisition events, as indicated by the persistence of several mobility-associated genes (XHV734\_3722, 3725, 3734 and 3735). Searching for homologues of XHV734\_3749 and 3750 in the restriction enzyme database REBASE [101] yielded a perfect match for an identical R-M system in *X. arboricola* pv. *juglandis* CPBF 1521 (M.Xar1521ORF31530P and Xar1521ORF31540P), with *moaE* downstream and an McrB-like protein-coding gene upstream. However, this genomic island was not detected in the type strain of *X. arboricola* pv. *juglandis* CFBP 2528<sup>T</sup> included in our analyses (Fig. 3). This shows some limitations of our taxonomy-based conservation analysis when it comes to MGEs. The presence of this genomic island upstream of the *moa* operon in some of the phylogenetically distant xanthomonads investigated suggests either early acquisition followed by loss events in most strains, or multiple independent site-specific integration events. Nevertheless, it was interesting to observe that both the REase and the cognate MTase were annotated as essential. As R-M systems are often compared to toxin/antitoxin systems because they act as genetic addiction systems [102], we would have expected the sole MTase to be essential. Yet, the similarity with Xar1521ORF31540p and domain analysis using MicroScope revealed that XHV734\_3749 is a type-II REase, meaning that it possesses both the endonuclease and the methyltransferase domains, and that the presence of a cognate separate MTase is accessory [103]. Thus, its disruption would probably lead to post-segregational killing as the cell REase concentration would become too low to successfully protect bacterial DNA from its own endonuclease activity. Nevertheless, the co-occurrence of two distinct restriction modification systems on a small genomic island raises questions about their biological roles. The type-I R-M system McrBC was originally described as part of the so-called 'Immigration Control Region' in *E. coli* K12 [104] because it plays a role in the protection of the host genome against foreign invading DNA. On the other hand, type-II R-M systems frequently behave like 'selfish' genetic elements promoting their own survival to increase their relative frequency [105, 106]. Observations that type-II R-M systems are often associated with MGEs also suggest that they play an important role in the stabilization of genomic islands [99, 107], a perspective that echoes the essentiality of the type-II R-M system of the genomic island we described.

## CONCLUSIONS

In this study, we fully exploited the power of genome-wide Tn-Seq essentiality screening by putting our results in perspective with the insights offered by comparative

genomics, synteny analyses and phylogenomics. Combining these approaches allowed us to separate core essential genes conserved across bacterial phyla from context-dependent essential genes in the essential genome of *X. hortorum* pv. *vitians*. Among these, we identified specific *Xanthomonas* or *Xanthomonadaceae* features that were essential *in vitro*, but also probably play an important role in natural ecological niches. Also, we discovered through systematic synteny and phylogenomic examination that some essential genes were acquired through horizontal gene transfer events, either separately or on genomic islands, and were associated with bacterial defence systems or genetic addiction complexes. Thus, we showed that gene essentiality assessment by Tn-Seq gains significance by systematically examining genome content and gene conservation. Our complete genome sequence and high-density transposon library will reveal useful and reliable tools for the next steps of our research on this important lettuce pathogen, which will aim at discovering *X. hortorum* pv. *vitians* genes associated with virulence and survival *in planta*.

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### Author contributions

F.B. supervised the manuscript, funding acquisition and project administration. E.G. also participated in supervision, methodology development and provided some of the resources. S.L. helped to perform experimental investigations and provided some of the resources used. L.M. conceptualized the study, carried out the investigations, data curation, formal analysis, visualization, and wrote the original draft. All four authors contributed to the reviewing and editing of the manuscript.

### Conflicts of interest

The authors declare that there are no conflicts of interest.

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# CHAPITRE III :

## Bases moléculaires de l'adaptation de *X. hortorum* pv. *vitians* à son hôte végétal

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### Préambule

Dans le chapitre précédent, nous avons fait la démonstration de la qualité de notre librairie de Tn-seq construite chez *X. hortorum* pv. *vitians* en l'utilisant pour caractériser le génome essentiel de cet organisme en milieu riche. Nous avons dès lors émis l'hypothèse que l'application de cette même librairie en interaction avec la laitue permettrait de passer au crible le génome bactérien pour identifier des gènes impliqués dans l'adaptation aux conditions de vie *in planta*. Le principe de l'expérimentation repose dans la comparaison entre l'abondance des différents mutants dans une condition contrôle (ici, l'inoculum cultivé en bouillon liquide) et dans la condition expérimentale (en association avec la plante). Les mutants impactés dans des gènes présentant un avantage pour la valeur sélective des individus *in planta* auront alors une abondance moindre dans la laitue que dans l'inoculum.

Du fait de la redondance fonctionnelle de certains gènes et de l'existence d'un effet de complémentation fonctionnelle dans une population d'individus génotypiquement et phénotypiquement hétérogènes (Cain et al., 2020), certains gènes cruciaux pour le dialogue moléculaire avec l'hôte comme les effecteurs de type III ne peuvent être identifiés par une telle technique (Helmann et al., 2020). Toutefois, ces effecteurs ne sont pas les seuls déterminants moléculaires impliqués dans l'adaptation spécifique à une espèce végétale, et ils interviennent essentiellement au cours de la phase endophyte suivant la pénétration des bactéries dans les tissus (Timilsina et al., 2020). De nombreux autres gènes interviennent au cours de cette phase endophyte ainsi que lors de la phase épiphyte primaire de l'interaction, tels que des gènes de synthèse de polysaccharides, d'expression du système de sécrétion de type II, ou de systèmes d'assimilation des carbohydrates (Mhedbi-Hajri et al., 2011). Certains gènes impliqués dans la résistance et la dissimulation vis-à-vis du système immunitaire végétal sont également des marqueurs importants de cette adaptation fine du pathogène à son hôte (Petrocelli et al., 2012). Nous avons donc cherché en première instance à identifier l'ensemble des gènes nécessaires, au niveau individuel, à la réussite de la colonisation de la laitue par *X. hortorum* pv. *vitians*.

Ce dernier chapitre est présenté sous la forme d'un projet d'article qui sera soumis prochainement dans le journal *mSystems*.



# **A comprehensive overview of the genes and functions required for lettuce infection by the hemibiotrophic phytopathogen *Xanthomonas hortorum* pv. *vitians***

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## **Keywords :**

*Xanthomonas hortorum* pv. *vitians* ; Tn-seq ; lettuce ; nutrient acquisition ; plant immune system

**Data availability** : The NCBI GenBank assembly accession numbers for *X. hortorum* pv. *vitians* LM16734 complete genome is GCA\_014338485.1. Transposon insertion sequencing raw reads are deposited in the NCBI SRA database under accessions SRR14385278 to SRR14385281. All data are associated with BioProjects PRJNA530964 & PRJNA726806. Supplementary tables and figures were made available at figshare ([private temporary link: https://figshare.com/s/eb1f29b098cad3fa887c](https://figshare.com/s/eb1f29b098cad3fa887c)) under DOI: 10.6084/m9.figshare.14527194.

## Abstract

The successful infection of a host plant by a phytopathogenic bacterium depends on a finely tuned molecular dialog between both partners of the interaction. From the pathogen's perspective, it aims at suppressing or evading the plant immune response, transforming the micro-niche, and exploiting the host for nutrition and maintenance of basic metabolic processes. Recently, transposon insertion sequencing techniques (Tn-seq) allowed to assess genome-wide which genes were important for the fitness of several plant-associated bacteria *in planta*. *Xanthomonas hortorum* pv. *vitians* is a re-emerging foliar hemibiotrophic phytopathogen responsible for severe outbreaks of the bacterial leaf spot of lettuce disease all around the world. Despite its agricultural relevance, the dynamic molecular interaction established with its host remains completely unknown. To remedy this situation and decipher the genes and functions mobilized by the pathogen throughout the infection, we conducted a Tn-seq experiment in lettuce leaves which mimics as far as possible the natural infection process. This genome-wide screen identified 170 genes for which disruption causes serious fitness defects in lettuce. A thorough examination of these genes using comparative genomics and gene set enrichment analyses highlighted that several functions and pathways were highly critical for the pathogen's survival. Among them, biosynthesis of amino acids, nucleic acids and exopolysaccharides, secretion of cell wall-degrading enzymes through the type II secretion system, and expression of nutrients scavenging systems were crucial. Also, several genes implicated in the camouflage from the plant immune system and the resistance to oxidative stress were strongly engaged during the host colonization. We hypothesize that these genes may be differentially mobilized during the subsequent epiphytic, endophytic, and necrotic phases of the infection. Overall, our study provides a broad view of the molecular bases sustaining the pathogen's biology within its host plant, which will undoubtedly enlighten our general understanding of *Xanthomonas* host-pathogen interactions.

## Introduction

Most Gram-negative bacterial plant pathogens behave as hemibiotrophs when interacting with their host plant [1]. *Xanthomonas* species are indeed mostly considered as hemibiotrophic pathogens which cause necrosis after a first asymptomatic phase [2–4]. The infection process of a host plant by a non-vascular *Xanthomonas* is thus a series of different sequences in which the pathogen displays different abilities to dialogue with its host and ensure its own survival through nutrient acquisition systems and transformation of its micro-niche [5]. *Xanthomonas* species initially start as epiphytes in the phyllosphere, an environment generally considered as stressful and oligotrophic [6]. There, in addition to deploying strategies to adhere to the leaf surface and survive abiotic stresses [7,8], they rely partly on plant cell wall-degrading enzymes (CWDEs) secreted by the type II secretion system (T2SS) to release carbohydrates that are assimilated through high-affinity TonB-dependent transporters (TBDTs) for nutrition [9–11]. Biofilm formation at this stage participates in creating a safer and stable micro-environment for the population to develop [12].

Then, the pathogen penetrates the leaves through natural openings, i.e. stomata, hydathodes and wounds, and establishes inside the apoplast to initiate its endophytic biotroph phase [5]. It must resist to the early basal responses triggered by the recognition of microbial-associated molecular patterns (MAMPs) by the plant pattern-recognition receptors (PRRs) [13], feed from available nutrients and synthesize compounds that are not directly available within the habitat. Polysaccharides such as the cell-surface lipopolysaccharide (LPS) and the secreted exopolysaccharide (EPS) xanthan help to dampen the host immune response [14,15]. The type III secretion system (T3SS) also plays a major role in this phase, as it allows *Xanthomonas* species to disrupt the immune response signaling cascades and remodel the host metabolism to their own benefit through the intensive secretion of type III effectors (T3Es) and transcription activator-like effectors (TALEs) [16]. Finally, the pathogen transitions to a necrotrophic lifestyle where T2SS, CWDEs and other T3Es induce cell death, allowing the population to feed on leaked intracellular compounds and degraded cell components [1,16].

*Xanthomonas hortorum* pv. *vitians* (Vauterin *et al.* 1995) Morinière *et al.* 2020 is a foliar pathogen of cultivated lettuces (*Lactuca sativa*) which causes a disease called bacterial leaf spot of lettuce (BLSL) [17]. This disease is characterized by the progressive apparition of small necrotic spots a few days after the inoculation of the pathogen which later coalesce to form large necrotic patches, altering the quality and yield of the harvest without killing the

entire plants [18,19]. BLSL has become a significant threat for lettuce producers over the past decades, and *X. hortorum* pv. *vitians* is considered to be a re-emerging pathogen all around the world [17,19–22]. At the present time, very little is known about the biology of this pathogen during the infection, and the molecular bases sustaining the host-pathogen interaction remain completely unelucidated.

To investigate the molecular biology and describe the essential genome *in vitro* of *X. hortorum* pv. *vitians*, a transposon insertion sequencing (Tn-seq) approach has been recently developed in our model strain LM16734 [23]. Tn-seq technics rely on the generation of a saturated mutant library through large-scale transposon mutagenesis coupled with high-throughput next-generation sequencing and statistical analysis to estimate the essentiality and/or fitness contribution of each genetic feature in a given environment [24]. Recently, Tn-seq applications on bacteria of agricultural interest enhanced our understanding of the molecular dialogue between symbiotic or pathogenic bacteria and their host plants [25]. To date, only the soft rot pathogen *Dickeya dadantii* and the foliar pathogen *Pseudomonas syringae* pv. *syringae* have been investigated using *in planta* Tn-seq [26,27]. In both cases, it allowed to provide an overall picture of the cellular functions and processes mobilized by the pathogens during the plant infection, identify novel virulence factors, and in the case of *P. syringae* pv. *syringae* to screen for genes putatively involved in adaptation to different host plants [28].

We used our Tn-seq mutant library to screen for genes implicated in virulence and lettuce colonization by *X. hortorum* pv. *vitians*. First, we carefully set the experimental conditions of our *in planta* Tn-seq experiment to prevent for potential known biases such as bottlenecks. Then, we applied a combination of comparative genomics, gene set enrichment analysis, metabolic and cellular pathway mapping and synteny assessment to draw biological sense out of the list of genes defined as conditionally-essential for lettuce leaf infection. This approach successfully identified critical genetic features involved in amino acids and nucleic acids biosynthesis, secretion of CWDEs through the T2SS, carbohydrates nutrition and iron uptake, cellular polysaccharides synthesis and oxidative stress resistance systems. Overall, we hypothesize that nutrition uptake and catabolism systems are differentially implicated in the subsequent phases of the interaction with the host, and that structural components and stress resistance systems allows *X. hortorum* pv. *vitians* to temper the lettuce immune system. Our pioneering study proposes an insight into the molecular interaction of this pathogen within its host.

## Methods

### **Bacterial strains, growth conditions and Tn-seq library generation.**

*Xanthomonas hortorum* pv. *vitians* LM16734 (= CFBP 8638) was originally isolated from a diseased leaf sample of oakleaf lettuce (*Lactuca sativa*) collected in 2016 in the Rhône-Alpes region, France [17]. The bacterium was routinely cultured on 1/10<sup>th</sup> Tryptic Soy Agar (TSA) plates and in 1/10<sup>th</sup> Tryptic Soy Broth (TSB) at 28°C. The Tn-seq mutant library generation procedure was described previously [23]. Briefly, mating the recipient *X. hortorum* pv. *vitians* LM16734 strain with a donor *Escherichia coli* MFDpir/pSamEc strain containing a Himar1-C9 transposase gene yielded approximately 250,000 individual mutant colonies. They were recovered with 1/10<sup>th</sup> TSB, pooled, and stored in 30 % (v/v) glycerol vials at -80°C. The resulting mutant library population was estimated to 10<sup>10</sup> CFU.mL<sup>-1</sup>.

### **Monitoring of bacterial population *in planta*.**

Bacterial population was estimated over the course of the infection in leaf lettuce cv. Météore for 14 days. Plants were cultivated and inoculated as described previously [17]. Population size was enumerated at 0, 1, 2, 4, 7, 10 and 14 days post-inoculation (DPI). At each time point, three infected leaves from three different plants were randomly collected and treated as independent technical replicates. Except for the initial sampling at 0 DPI which aimed at enumerating the inoculum deposited on the leaves, all other leaf samples were surface-sterilized in a 70 % ethanol bath for 20 s and rinsed in sterile deionized H<sub>2</sub>O (dH<sub>2</sub>O) to count only the bacteria having penetrated the leaf tissue. In any case, 4 disks of 3.14 cm<sup>2</sup> each were die-cut with a sterilized 1-cm diameter punch on both sides of the leaf midrib and pooled in 10 mL of sterile dH<sub>2</sub>O. Leaf disks were crushed using a T25 IKA Ultra-turrax disperser (IKA, Staufen im Breisgau, Germany) at full speed. The resulting leaf homogenates were then serially diluted and plated on 1/10<sup>th</sup> TSA plates supplemented with cycloheximide at 50 µg.mL<sup>-1</sup> with an easySpiral automated plater (Interscience, Saint-Nom-la-Bretèche, France). The plates were incubated for 4 days at 28°C and enumerated with a Scan 1200 automatic colony counter (Interscience). Bacterial population sizes were expressed in CFU.cm<sup>-2</sup> of lettuce leaf.

### **Transposon library inoculation in lettuce.**

Two transposon library aliquots were thawed at 4°C overnight and inoculated in two flasks containing 100 mL of 1/10<sup>th</sup> TSB supplemented with kanamycin at 25 µg.mL<sup>-1</sup>. Cultures were incubated overnight at 28°C with 120 rpm until reaching 1.4 OD<sub>600</sub>. Then,

cultures were centrifuged at 3,344 x g for 20 min at 4°C and cell pellets were washed by resuspension in 25 mL of cold sterile dH<sub>2</sub>O and repeating centrifugation. Each cleaned cell pellet was resuspended in sterile dH<sub>2</sub>O and spectrophotometrically adjusted to obtain 400 mL of inoculum at 0.2 OD<sub>600</sub> supplemented with Tween 80 at 0.08 % (v/v). Half of each inoculum was centrifugated and cell pellets were stored at -20°C for DNA extraction of the control condition. Thirty leaf lettuce cv. Météore were inoculated for each transposon library duplicate. The remaining 200 mL inocula were hand-sprayed until run-off on 30 young plants per duplicate. All plants were immediately incubated in an Ineltec environmental chamber (Ineltec France, Vénissieux, France) at 25 °C, with an 18 h photoperiod and 90 % relative humidity (RH) for 48 h, and then 70 % RH for the rest of the experiment.

### **Bacterial cells recovery from lettuce leaves.**

Diseased lettuce leaves were collected at 10 DPI when small water-soaked leafspot lesions were abundant but not coalescent, and duplicates were treated separately. Leaves were crushed in 500 mL of sterile dH<sub>2</sub>O with a sterilized Moulinex Masterchef 58 electronic blender (SEB Moulinex G S M, Ecully, France) at middle speed for 2–3 min until obtention of < 2 mm fragments. The resulting suspensions were sonicated for 15 min in a sonication water bath 88155 (Fischer Bioblock Scientific, Illkirch, France) to detach bacterial cells from leaf debris. To remove plant debris, suspensions were successively filtered through 1 mm–, 0.5 mm–, 0.25 mm– and 0.05 mm–diameters sieves, twice through 20 µm-diameter coffee filters, and finally vacuum-filtered through 10 µm–ipPORE™ Track-Etched Membrane filters (it4ip, Louvain-la-Neuve, Belgium). In the end, approximately 400 mL of filtered bacterial cell suspensions were recovered per duplicate, centrifugated at 10,647 x g for 20 min at 4°C, and cell pellets were temporary conserved at -20°C.

### **DNA extraction and library preparation.**

Genomic DNA library extraction and preparation was conducted mostly as described previously [23], but with some technical variations. Briefly, cell pellets were thawed, and genomic DNAs were extracted using the Promega Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA) and resuspended in 200 µL of Tris EDTA pH7. Fifty µg of DNA per sample were digested with the MmeI restriction enzyme, and digestion products were purified. Samples were deposited on a 1 % (w/v) agarose gel stained with Atlas ClearSight DNA stain (Bioatlas, Tartu, Estonia) and the 1.3 – 1.8 kb DNA fragments were cut out from the gel and retrieved using a QIAquick Gel Extraction kit (Qiagen, Hilden, Germany). Then, 300 to 500 ng of each samples were ligated to double-stranded barcoded

adapters with the T4 DNA ligase. The 20 bp–genomic DNA regions flanking the transposon were amplified during 22-cycles polymerase chain reactions (PCR) in 50  $\mu$ L final volumes. Reactions contained 2  $\mu$ L of ligated DNA matrix, 1 U of Q5 DNA polymerase (New England Biolabs, Ipswich, USA), 1x of Q5 buffer, 0.2 mM of dNTPs and 0.4  $\mu$ M of the Illumina TruSeq Universal Adapters P5-nextseq and P7-nextseq (Illumina Inc., San Diego, USA). PCR were deposited on a 2 % (w/v) agarose gel and the 125-bp bands were extracted using the QIAquick Gel Extraction kit (Qiagen). Library DNA samples were sent to the I2BC–sequencing platform (I2BC, Gif-sur-Yvette, France) to be sequenced in High Output Single Read 75 bp mode on a NextSeq 500 instrument (Illumina Inc.).

### **Sequencing read data pre-treatment.**

Raw reads were trimmed with CUTADAPT v1.15 [29] to filter those containing the mariner inverted left repeat (ACAGGTTGGATGATAAGTCCCCGGTCTT) and remove the adapter sequences. Trimmed datasets were mapped without mismatches to unique TA sites with a modified version of the TPP script from the TRANSIT software suite v2.0.2 [30] on the complete genome sequence of *X. hortorum* pv. *vitians* LM16734 available at the NCBI GenBank database under accession number GCA\_014338485.1. The script generating the prot\_table file used by TRANSIT was modified to integrate non-coding RNA (ncRNA) features. *In vitro* library sequencing reads available at the NCBI SRA database under accession numbers SRR12067051 and SRR12067052 were retrieved and combined with the newly-generated sequencing reads in a single .wig file, then subjected to the TTR (Trimmed Total Reads) normalization method in TRANSIT v3.2.1.

### **Assessment of gene fitness contribution in inoculum and *in planta*.**

Fitness contribution of each genetic feature was determined both in 1/10<sup>th</sup> TSB (inoculum) and in lettuce by comparing pairwise the inoculum condition to the *in vitro* library results, and the lettuce infection condition to the inoculum condition. Reads in the 5 % N–ter and 10 % C–ter of the genetic feature were discarded as they can lead to incorrect interpretations, and a LOESS correction for genome positional bias was applied. The *Resampling* method available in the TRANSIT v3.2.1 suite [31], was used to evaluate the fitness contribution and statistical significance of each genetic feature in the two comparisons. The method calculates the difference in the sums of read counts and  $\log_2$  of the fold-change ( $\log_2$ FC) between the control and experimental conditions, calculates *p*-values using a variation of the non-parametric permutation test, and corrects the *p*-values by performing a Benjamini-Hochberg False Discovery Rate (FDR) test [32] which yields *q*-values expressing

the probabilities of having false positive results. The genetic features presenting a  $q$ -value  $\leq 0.05$  were considered as presenting a significant fitness difference in the control and experimental conditions, and manual checking for potential artefactual features was performed on the MicroScope platform (<https://mage.genoscope.cns.fr>) [33]. Finally, circular genome and data mapping visualization was achieved with the R/Shiny application *shinyCircos* [34].

### **Gene annotation, Gene Ontology term enrichment and KEGG pathway mapping.**

A mixed annotation strategy was applied to assign Gene Ontology (GO) terms [35] to *X. hortorum* pv. *vitians* LM16734 genes. Protein sequences were extracted from the genome using the `cds_extractor` script from the `bac-genomic-scripts` suite [36]. Terms were assigned both using the web-based tool EGGNOG-MAPPER v2 [37] against the eggNOG v5.0 database [38] and INTERPROSCAN v5.0.0 [39] available on the Galaxy for Genome Annotation (GGA) server (<https://annotation.usegalaxy.eu/>) [40]. Finally, a GO term enrichment analysis was conducted with the *Pathway Enrichment Analysis* method available in TRANSIT, based on the *Ontologizer* method [41]. GO terms with a FDR-adjusted  $q$ -value  $\leq 0.05$  were considered as significantly enriched in the gene subset. EC numbers, eggNOG orthologues and Cluster of Orthologous Groups (COG) categories were also assigned with EGGNOG-MAPPER v2. Kyoto Encyclopedia of Genes and Genomes (KEGG) (<https://www.kegg.jp/>) database was used to assign KEGG Orthology (KO) terms to genes with BLASTKOALA v2.2 [42]. KO terms were mapped to the *Xanthomonas hortorum* reference pathways (*xhr*) with KEGG Mapper [43]. Synteny and gene homology assessment were performed on the MicroScope platform following the MicroScope team annotation guidelines [33].

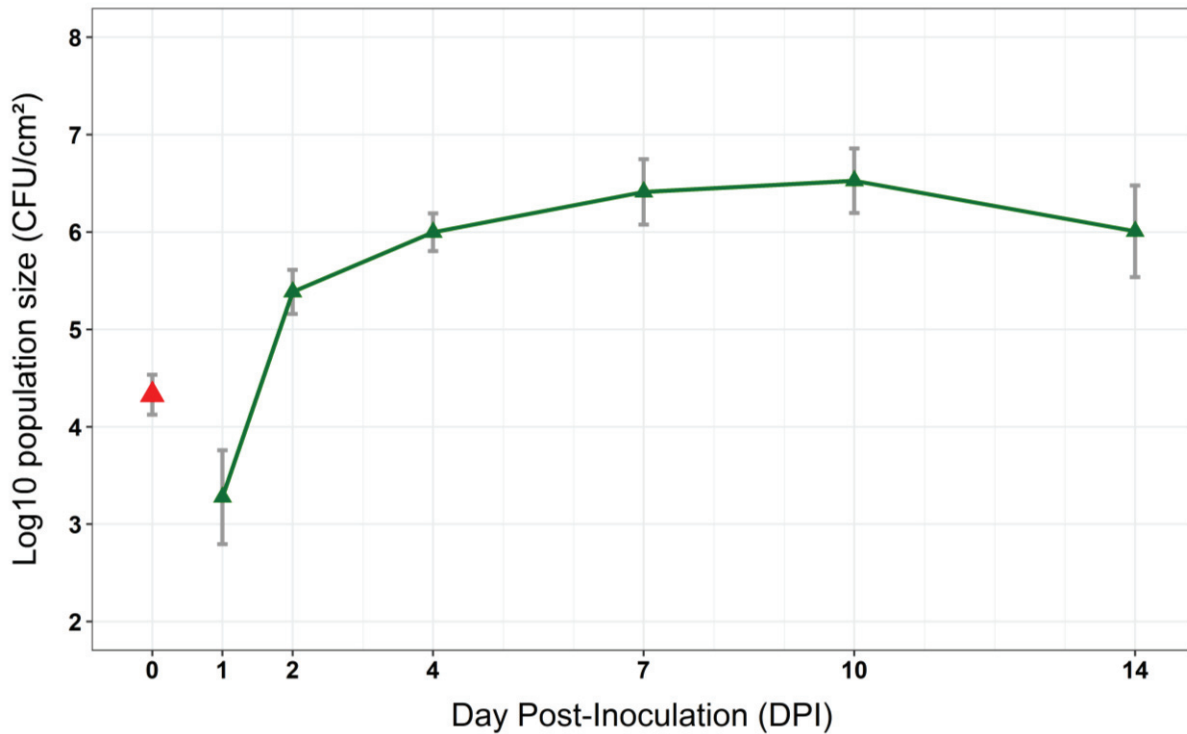


## Results & Discussion

### Tn-seq experimental design validation and bottleneck effect assessment.

To investigate the fitness contribution of each genetic feature of *X. hortorum* pv. *vitians* LM16734 to growth in rich medium broth and during lettuce leaf infection, we used the mariner-based Tn-seq mutant library developed and published previously [23]. This library consists of at least 60,715 chromosomal mutants and 1,218 plasmidic mutants with transposon insertions at individual TA sites, over the total 85,314 chromosomal and 1,361 plasmidic TA sites existing within the *X. hortorum* pv. *vitians* LM16734 complete genome (5.23 Mb circular chromosome and 57 kb native plasmid pLM16734). Insertion density in the library was of ca. 71 % and 89 % on the chromosome and plasmid, respectively.

First, the infection bottleneck effect arising from our inoculation procedure in young lettuce plants was estimated. The central paradigm behind Tn-seq experiments states that the mean read count per genetic feature is correlated with the corresponding mutant fitness in the tested environmental condition [24]. However, sampling bottleneck effects – which are stochastic events of loss of genetic diversity within a population when its size is drastically reduced, e.g. during host penetration and infection by a pathogen – are known to blur data interpretation if not carefully considered in the experimental design, particularly in *in vivo* experiments [44,45]. Moreover, the selective DNA library preparation steps and random nature of mass-sequencing can result in another type of bottleneck, this time by reducing the genetic diversity existing within the extracted transposon mutant genomic DNA [46]. The number of generations experienced by the mutant library is also a critical parameter to consider, as it will directly impact the fitness score calculated for each genetic feature [44,47]. The initial bacterial population size in the experiment must be high enough to reflect the genetic diversity existing within the mutant library, but also sufficiently low to allow enough generation events to occur so as to be able to observe the deleterious effects of mutations in the population. However, the number of generations must also be limited so that mutants presenting an increased growth rate do not overwhelm the others and result in a biased library sampling and sequencing at the end of the experiment.



**Figure 1.** Evolution of the *X. hortorum* pv. *vitians* LM16734 population during the infection in lettuce. Population is expressed as the log<sub>10</sub>-transformed Colony-Forming Unit (CFU) per cm<sup>2</sup> of inoculated leaf. The red triangle indicates the measured epiphytic population following spray-inoculation. Green triangles represent measured endophytic population at each time point. Grey bars show the standard error calculated on the triplicates.

A preliminary experiment was performed to estimate the number of bacterial cells inoculated on lettuce leaves by spraying, the number of cells penetrating inside the leaves, and monitor the population size over the course of the infection. An average of approximately  $2.5 \times 10^4$  CFU.cm<sup>-2</sup> were deposited on the leaves during the inoculation, and  $5 \times 10^3$  CFU.cm<sup>-2</sup> penetrated inside the leaves 24 h after (**Fig. 1**). The successful infection ratio was therefore of 1:5. This experimental bottleneck is weaker than others estimated in animal model infection experiments, in which the successful infection ratio can be as low as 1:1,000 [45]. Contrary to animal models, bottleneck effects *in planta* can be mostly overcome by multiplying the quantity of plant material used for library recovery [27,48,49]. The total foliar surface for 30 plants was estimated to be approximately 6,000 cm<sup>2</sup>, which means that a total of  $1.5 \times 10^8$  CFU were inoculated on the leaves and that  $3 \times 10^7$  CFU have invaded the leaves after 24 h. As stated above, our transposon mutant library consists of at least 60,000 independent mutants. Regarding our estimations, a theoretical total mutant library coverage of 2,500 x was inoculated, and 500 x successfully invaded the plant tissue. Finally, monitoring the bacterial population size over the course of the lettuce infection showed that the highest

population level was achieved at 10 DPI with an average of  $5,6 \times 10^6$  internal CFU.cm<sup>-2</sup> (**Fig. 1**), meaning that virtually 10 bacterial generation events occurred between 1 and 10 DPI, without considering mortality events. In other comparable *in planta* Tn-seq experiments, the output mutant libraries were collected after similar numbers of generations which proved to be adequate for efficient and unbiased fitness score assessment [27,28,49]. Typically, at 10 DPI, a very large number of small water-soaked spots that have not yet coalesced into necrotic patches are visible on lettuce leaves infected by *X. hortorum* pv. *vitians*. This implies that we selected transposon mutants that had the ability to colonize and penetrate the leaves, multiply and survive inside the plant tissue, and to express pathogenesis-related genes.

**Table 1.** Transposon-insertion sequencing (Tn-seq) statistics before TTR normalization

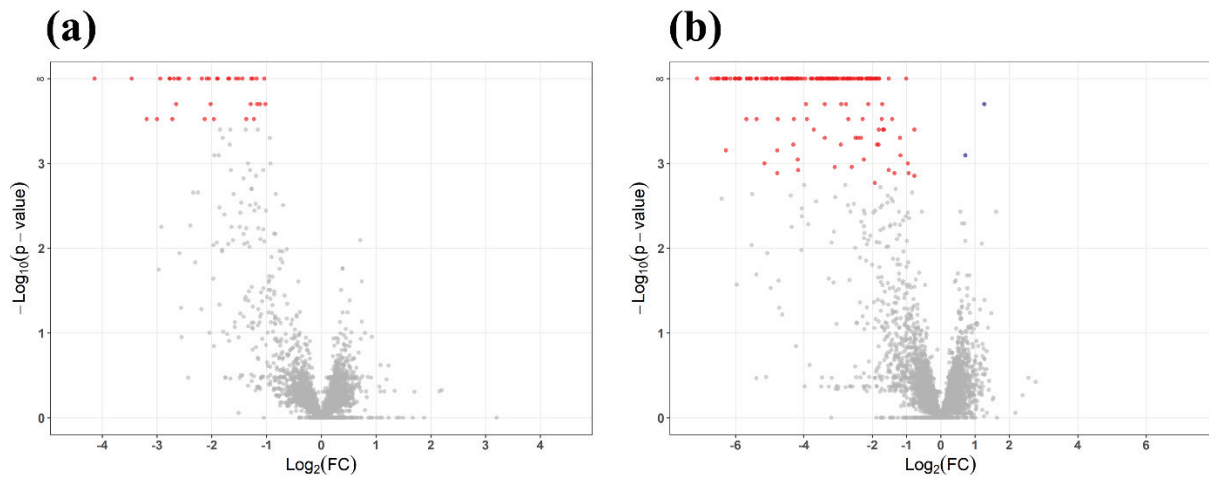
Replicate	Sequencing yield	No. of Tn-end containing reads	Replicon	Read count (unique TA sites)	TA hits <sup>a</sup>	Insertion density <sup>a</sup>	Mean read count over non-zero TA <sup>b</sup>
<b>T1</b>	25,830,465	22,029,814	Chromosome	21,916,438	59,675	69.9	367.3
			pLM16734	1,262,371	1,205	88.5	1047.6
<b>T2</b>	39,102,386	26,462,594	Chromosome	26,330,348	60,579	71.0	434.6
			pLM16734	956,994	1,196	87.9	800.2
<b>P1</b>	30,091,641	26,195,853	Chromosome	26,067,795	52,850	61.9	493.2
			pLM16734	1,227,568	979	71.9	1253.9
<b>P2</b>	34,333,964	30,135,609	Chromosome	29,982,890	55,013	64.5	545.0
			pLM16734	1,236,398	1,052	77.3	1175.3

<sup>a</sup> *X. hortorum* pv. *vitians* LM16734 genome contains 85,314 chromosomal TA sites and 1,361 plasmidic TA sites. Insertion density reflects the percentage of TA sites with at least one read mapped over total number of TA sites on the replicon.

<sup>b</sup> Mean read count per TA site containing at least one read.

The general sequencing and reads mapping statistics showed that 22 to 26 million reads mapped to unique TA sites in the inoculum duplicates (T1 and T2), and 26 to 30 million in the lettuce infection duplicates (P1 and P2) (**Tab. 1**). The insertion density achieved was of 69.9 % and 71.0 % on the chromosome and 88.5 % and 87.9 % on pLM16734 and in the inoculum condition, and of 61.9 and 64.5 % on the chromosome and 71.9 and 77.3 % on pLM16734 in the *in planta* condition. Almost all the mutants from the library were still present in the inoculum, and ca. 90 % chromosomal and 80 % plasmidic mutants were recovered after 10 days in lettuce. Moreover, Pearson correlation coefficients of read counts at unique TA sites between duplicates were of 0.98 and 0.97 for the chromosome and plasmid pLM16734 in the inoculum condition, and of 0.77 and 0.84 in the lettuce condition (**Fig. S1**).

These sequencing statistics confirmed that we successfully bypassed the experimental bottleneck in our experimental design, and that the technical bottleneck resulting from the DNA library preparation steps and sequencing was neglectable.



**Figure 2.** Volcano plots of the Tn-seq results for **(a)** the 1/10<sup>th</sup> TSB control condition and **(b)** the experimental lettuce leaf condition. Plots represent the  $\log_2$  fold-change of each genetic feature as a function of the negative  $\log_{10}$  of the resampling test  $p$ -value. Genetic features which passed the FDR adjustment test ( $q$ -value  $\leq 0.05$ ) are indicated in red if they are conditionally-essential, and in blue if growth-advantage. Non-significant genetic features are in grey.

### Identification of 170 conditionally-essential genes *in planta* mostly involved in anabolism.

The resampling analyses conducted with TRANSIT identified 170 genes critical ( $\log_2\text{FC} < 0$  and  $q$ -value  $\leq 0.05$ ) and two growth-advantage ( $\log_2\text{FC} > 0$  and  $q$ -value  $\leq 0.05$ ) in lettuce leaf (**Fig. 2a**), and 36 critical in 1/10<sup>th</sup> TSB (**Fig. 2b**). The complete lists of these genes and associated resampling metrics are available in supplementary tables **S1-S4**. There was a partial overlap between the two critical gene sets, as 29 genes in lettuce leaf were already considered as critical in 1/10<sup>th</sup> TSB. Additionally, we applied an extra filter by considering that mutants presenting a gene mean read count less than 5 in the 1/10<sup>th</sup> TSB condition showed a decreased fitness *in vitro*, and 8 more genes were classified as critical in 1/10<sup>th</sup> TSB. Examining the distribution of the genes critical in lettuce along the *X. hortorum* pv. *vitians* LM16734 chromosome revealed that a significant part seemed to be co-localized and organized in operons or gene clusters (**Fig. 3**).



**Figure 3.** Genome atlas of the essential genetic features required for lettuce infection by *X. hortorum* pv. *vitians* LM16734. Inner to outer track: mean read count over 1 kb in 1/10<sup>th</sup> TSB and in lettuce; gene state in *in vitro* library as described in [23]; log<sub>2</sub>FC score in lettuce leaves compared to inoculum; clustering of essential genes, pathways, functions and structural components in lettuce; gene names or locus tag identifiers; chromosomal track.

To determine which biological processes, pathways and functions were over-represented in our lettuce critical gene set, Gene Ontology (GO) terms [35] and KEGG orthologs (KO) [42] identifiers were assigned to all *X. hortorum* pv. *vitians* LM16734 genes. The GO term enrichment analysis indicated that 43 GO terms were significantly enriched in our gene set (**Tab. 2**). Among the 35 biological processes-related enriched GO terms, nucleotide, amino acid, polysaccharide, carbohydrate, nitrogen, and phosphorus metabolisms associated-terms were the most abundant. Overall, biosynthetic processes seemed to more represented than degradation processes. For example, 67.7 % of genes related to “Nucleobase biosynthetic process” (GO:0046112) vs. 54.5 % for “Nucleobase metabolic process” (GO:0009112), 42.5 % of genes associated with “Cellular amino acid biosynthetic process” (GO:0008652) vs. 34.9 % for “Cellular amino acid metabolic process” (GO:0006520) and 34.8 % of genes linked to “Polysaccharide biosynthetic process” (GO:0000271) vs. “Polysaccharide metabolic process” (GO:0005976) were identified. Interestingly, all 4 genes involved in the “Urea cycle” (GO:0000050) were essential, as well as 5 out 7 genes for “Phosphate ion transport” (GO:0006817).

The KEGG pathway analysis yielded similar results, although achieving more precision in the identification of the most critical metabolic and cellular pathways *in planta* (**Table 3**). For instance, it highlighted that even though a total of 49.5 % of genes involved in the *de novo* biosynthesis of amino acids were critical in lettuce, relative proportions of genes varied between the particular amino acid pathways. The most impacted biosynthetic pathways were those enabling the synthesis of arginine (92.3 % of genes) valine, leucine and isoleucine (76.9 %), and alanine, aspartate and glutamate (64 %). Genes involved in these pathways were often co-localized, like the *leu* and *ilv* genes encoding part of the leucine and isoleucine biosynthesis, or the *arg* and *his* operons for arginine and histidine, respectively (**Fig. 3**). Similarly, 19.6 % of carbon metabolism genes were critical, but the prevalent pathways were those of 2-oxocarboxylic acid metabolism (57.1 %), pentose phosphate pathway (36 %), starch and sucrose metabolism (31.6 %), and glycolysis / gluconeogenesis (29 %). Also, this analysis allowed to pinpoint that 22.9 % of genes associated with bacterial secretion system were critical, which was not detected by the GO term enrichment analysis. Finally, genes related to polysaccharide metabolism were mostly implicated in O-antigen nucleotide sugar biosynthesis (56.3 %).

**Table 2.** Significantly enriched Gene Ontology (GO) terms in the critical genes in lettuce leaf subset.

GO identifier	GO term	Total genes <sup>a</sup>	Subset genes <sup>b</sup>	Enrichment score <sup>c</sup>	q-value <sup>d</sup>
<b>BIOLOGICAL PROCESSES</b>					
<b>General processes</b>					
GO:0006082	Organic acid metabolic process	298	64	2.686	0.0
GO:0016053	Organic acid biosynthetic process	167	49	2.188	0.0
GO:0044283	Small molecule biosynthetic process	226	56	1.965	0.0
GO:1901362	Organic cyclic compound biosynthetic process	233	39	1.696	0.006043
GO:0018130	Heterocycle biosynthetic process	223	37	1.7	0.008476
GO:0019438	Aromatic compound biosynthetic process	199	38	1.984	0.000196
GO:0046176	Aldonic acid catabolic process	4	3	5.8	0.033252
GO:0040007	Growth	90	14	2.543	0.035375
<b>Nucleotide metabolism</b>					
GO:0052803	Imidazole-containing compound metabolic process	12	6	5.887	0.011874
GO:0034654	Nucleobase-containing compound biosynthetic process	138	22	2.025	0.013182
GO:0055086	Nucleobase-containing small molecule metabolic process	124	26	1.901	0.015897
GO:0042435	Indole-containing compound biosynthetic process	6	5	5.292	0.017839
GO:0046112	Nucleobase biosynthetic process	9	6	4.451	0.022403
GO:1901293	Nucleoside phosphate biosynthetic process	73	20	2.205	0.000229
GO:0042430	Indole-containing compound metabolic process	11	7	7.289	0.000771
GO:0009112	Nucleobase metabolic process	11	6	4.42	0.03223
<b>Amino acid metabolism</b>					
GO:0006520	Cellular amino acid metabolic process	166	53	3.702	0.0
GO:0008652	Cellular amino acid biosynthetic process	94	40	2.453	0.0
GO:0009072	Aromatic amino acid family metabolic process	24	9	3.338	0.027736
GO:0000105	Histidine biosynthetic process	8	6	3.642	0.035375
<b>Polysaccharide metabolism</b>					
GO:0000271	Polysaccharide biosynthetic process	23	8	7.75	3.8e-05
GO:0033692	Cellular polysaccharide biosynthetic process	19	7	8.4	7.7e-05
GO:0044264	Cellular polysaccharide metabolic process	26	8	7.754	0.000214
GO:0005976	Polysaccharide metabolic process	35	9	6.524	0.00034
GO:0008653	Lipopolysaccharide metabolic process	12	6	5.546	0.003351
<b>Carbohydrate metabolism</b>					
GO:1901135	Carbohydrate derivative metabolic process	178	29	1.95	0.008547
GO:0046394	Carboxylic acid biosynthetic process	167	49	1.346	0.009947
GO:0006757	ATP generation from ADP	15	4	4.938	0.028161
<b>Nitrogen and phosphate metabolism</b>					
GO:0019637	Organophosphate metabolic process	147	25	2.12	0.008476
GO:0044106	Cellular amine metabolic process	17	7	5.333	0.008547
GO:0019627	Urea metabolic process	4	4	8.25	0.009169
GO:0071941	Nitrogen cycle metabolic process	6	4	7.754	0.027141
GO:0000050	Urea cycle	4	4	5.515	0.028161
GO:0006793	Phosphorus metabolic process	244	34	1.602	0.042509
GO:0006817	Phosphate ion transport	7	5	2.5	0.04326

**Table 2.** Continued

MOLECULAR FUNCTIONS						
GO:0016829	Lyase activity	93	21	3.246	0.000101	
GO:0016614	Oxidoreductase activity, acting on CH-OH group of donors	49	11	3.6	0.002604	
GO:0016879	Ligase activity, forming carbon-nitrogen bonds	42	12	1.907	0.008476	
GO:0048037	Cofactor binding	251	28	1.86	0.015897	
GO:0005315	Inorganic phosphate transmembrane transporter activity	4	3	10.533	0.018301	
GO:0016638	Oxidoreductase activity, acting on the CH-NH2 group of donors	8	4	8.333	0.024953	
GO:0042802	Identical protein binding	144	19	1.661	0.033252	
GO:0070279	Vitamin B6 binding	32	7	4.238	0.034385	

<sup>a</sup> Total number of genes associated with the GO term in *X. hortorum* pv. *vitans* LM16734 genome.

<sup>b</sup> Number of genes associated with the GO term in the subset of genes conditionally essential *in planta*

<sup>c</sup> The simplified formula for the enrichment score (without pseudocounts) is  $\log_2 \left[ \frac{b/q}{m/p} \right]$ , where  $b$  is the number of genes with the GO term in the subset,  $q$  is the number of genes in the subset with a parent of the GO term,  $m$  is the total number of genes with the GO term in the genome, and  $p$  is the number of genes in the genome with a parent of the GO term. Thus, the enrichment score is the log-transformed ratio of the two relative abundances of genes with the GO term compared to those with a parent GO term (i) in the subset and (ii) in the complete genome. Calculation is derived from the *Ontologizer* method [41].

<sup>d</sup> Adjusted  $p$ -value using the Benjamini-Hochberg False Discovery Rate (FDR) test. GO terms with a  $q$ -value  $< 0.05$  were considered significantly enriched.

Surprisingly, our Tn-seq screen did not identify the T3SS genes (XHV734\_0611 to 633) as significantly critical in lettuce, although almost all displayed moderate to strong fitness defect (**Fig. 3**). The only critical gene related to the T3SS was *hrpF* (XHV734\_0597), a translocon that is required for T3Es translocation into the host cell [50]. The inconclusive determination of T3SS and CWDEs genes as important for survival in lettuce was predictable in our Tn-seq screen, albeit they are considered as major virulence factors in *Xanthomonas* species. One of the principal limitations of Tn-seq studies is their low efficiency for screening secreted products that can be shared with others in the phenotypically heterogeneous mutant population [26,27,51]. This phenomenon is related to the intrinsic duality between cooperation and competition in plant pathogen populations which can cause the apparition of social cheaters that do not contribute to the common efforts – i.e. secretion of CWDEs, EPS, T3Es, siderophores etc. – while still profiting from these “public goods” [52].

Overall, our results matched those of the few transposon insertion sequencing studies conducted so far on plant-pathogenic bacteria *in planta*. Random Tn mutant screening in various *Xanthomonas* species had already exposed that auxotrophic mutants unable to synthesize amino acids and various mutants impacted in carbohydrates metabolism or polysaccharide biosynthesis were impaired for growth and virulence *in planta* [53–56]. Helmann *et al.* [27,28] discovered that the most important genes for both apoplastic and epiphytic colonization by *P. syringae* pv. *syringae* in tomato, pepper and bean leaves were related to amino acid metabolism and transport, polysaccharide synthesis and regulation, and



nucleotide metabolism and transport. Similarly, Royet *et al.* [26] found that many genes involved in purine and pyrimidine biosynthesis, cysteine, methionine, lysine and leucine biosynthesis were critical in chicory leaves inoculated with a Tn-seq library of *D. dadantii*. Even the human enteric pathogen *Salmonella enterica* serovar Typhimurium showed very strong fitness defects in mutants impacted in amino acids and nucleic acids biosynthesis, lipopolysaccharide biosynthesis and glycolysis when it colonizes tomato fruits [57]. It suggests that the central metabolic pathways required for plant aerial parts colonization are equivalent across biological models and pathosystems, probably because bacteria face similar challenges in these harsh environments, e.g. scarce nutrient availability and the necessity to hide from the plant immune system. Interestingly, metabolic genes implicated in nucleotide, amino acid and cofactors metabolism were overexpressed during the biotrophic phase of the hemibiotrophic fungal plant pathogen *Phytophthora infestans*, while the necrotrophic fungus *Pythium ultimum* overexpressed genes associated with carbohydrates, lipids and inorganic nitrogen and sulfur metabolism [58]. Thus, amino acid and nucleic acid biosynthesis could be a marker of the biotrophic phase of *X. hortorum* pv. *vitians*.

**Table 3.** Critical KEGG pathways *in planta* (non-exhaustive)

Pathway code	KEGG pathways	Critical <sup>a</sup>	Total genes <sup>b</sup>	% of genes in pathway
<b>General and diverse pathways</b>				
xhr01110	Biosynthesis of secondary metabolites	84	301	27.9
xhr01240	Biosynthesis of cofactors	19	128	14.8
xhr00910	Nitrogen metabolism	9	14	64.3
xhr03070	Bacterial secretion system	16	70	22.9
<b>Amino acid metabolism</b>				
xhr01230	Biosynthesis of amino acids	54	109	49.5
xhr00220	Arginine biosynthesis	12	13	92.3
xhr00290	Valine, leucine and isoleucine biosynthesis	10	13	76.9
xhr00250	Alanine, aspartate and glutamate metabolism	16	25	64
xhr00400	Phenylalanine, tyrosine and tryptophan biosynthesis	11	26	42.3
xhr00260	Glycine, serine and threonine metabolism	14	35	40
xhr00340	Histidine metabolism	6	18	33.3
xhr00270	Cysteine and methionine metabolism	11	37	29.7
<b>Carbon compounds metabolism</b>				
xhr01200	Carbon metabolism	18	92	19.6
xhr01210	2-Oxocarboxylic acid metabolism	12	21	57.1
xhr00030	Pentose phosphate pathway	9	25	36
xhr00500	Starch and sucrose metabolism	12	38	31.6
xhr00010	Glycolysis / Gluconeogenesis	9	31	29
xhr00052	Galactose metabolism	5	20	25
xhr00051	Fructose and mannose metabolism	5	21	23.8

**Table 3.** Continued

<b>Nucleobase metabolism</b>				
xhr00240	Pyrimidine metabolism	7	25	28
xhr00230	Purine metabolism	11	54	20.4
<b>Polysaccharide metabolism</b>				
xhr00541	O-antigen nucleotide sugar biosynthesis	9	16	56.3

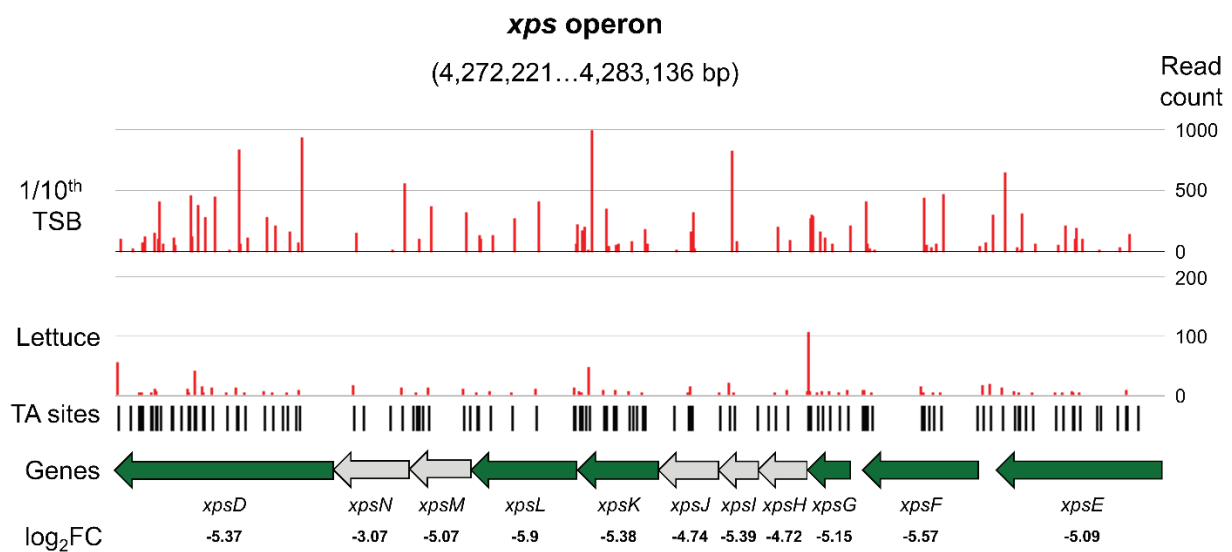
<sup>a</sup> Number of genes identified as conditionally essential *in planta* ( $q$ -value  $\leq 0,05$ )  
<sup>b</sup> Total number of genes in model pathway in *X. hortorum* organism-specific KEGG pathway database

### The type II secretion system and carbohydrates and iron-siderophore scavenging systems ensure nutrition during lettuce infection.

Beside the amino acid and nucleic acid metabolism pathways, numerous mutants in the carbohydrates metabolism were strongly impaired in their fitness *in planta* (**Tab. 2 & 3**). Interestingly, many genes in the central carbohydrates catabolism pathways, i.e. glycolysis, the pentose phosphate pathway and the Entner-Doudoroff pathway were critical in lettuce, while we demonstrated earlier that none were indispensable in a glucose-rich medium [23]. This time, most genes in these three pathways were conditionally-essential either in lettuce or in 1/10<sup>th</sup> TSB (**Fig. S2**), as well as some crucial genes in the starch and sucrose degradation and fructose and mannose degradation pathways (**Tab. 3**). Thus, *X. hortorum* pv. *vitians* carbon nutrition *in planta* seems more diversified, and lacking the ability to catabolize some sugars is most probably detrimental to the individual fitness. Unfortunately, there is no information regarding the chemical composition of the lettuce leaves apoplast or surface. Overall, the most abundant amino acids in the few analyzed eudicot apoplasts seem to be the non-proteogenic  $\gamma$ -aminobutyric acid (GABA), aspartate and glutamate, and sucrose and malate are generally some of the most abundant carbohydrates [59–64].

Most of the *xps* T2SS mutants (XHV734\_4180 to 4190) were positively screened as fitness-deficient in our experiment (**Fig. 4**). This is consistent with numerous reports showing that *xps*-deficient *Xanthomonas* spp. mutants presented reduced virulence *in planta* [53,55,56,65]. Similar to *X. hortorum* pv. *gardneri* [66], we found that *X. hortorum* pv. *vitians* LM16734 genome contained a large repertoire of genes encoding redundant CWDEs, including various pectinolytic, cellulolytic and hemicellulolytic enzymes (**Tab. S1**). Once the cell wall polymers have been degraded by the type II-secreted CWDEs, breakdown products and simple oligosaccharides must be internalized by the pathogen to be catabolized. This role has been mainly attributed to TonB-dependent transporters (TBDTs) in *Xanthomonas* species,

since they were found to be overrepresented in their genomes compared to other Gram-negative bacteria [9]. These TBDTs, primarily known for their role in iron-siderophore uptake, are often associated with carbohydrates sensor or degrading genes in what has been called carbohydrates scavenging systems or carbohydrate utilization with TBDT (CUT) loci [9,11]. They allow the scavenging of carbohydrates with high affinity and are thought to play an important role in the adaptation of bacteria to oligotrophic environments such as plants for phytopathogens [10]. Consequently, we searched for TBDTs genes in our critical gene set and found three loci, i.e. XHV734\_0066, 1016 and 1310, annotated as TonB-dependent receptors.



**Figure 4.** Schematic representation of the critical genes in lettuce within the *xps* T2SS operon of *X. hortorum* pv. *vitians* LM16734. Numbers in brackets indicate the range of the viewing window on the LM16734 genome. Critical genes in lettuce are indicated by green arrows, non-significant genes by grey arrows, and genes names and log<sub>2</sub>FC values are displayed below. Localizations of TA sites are indicated by black bars, and bar plots represent read counts at each TA sites in the control and experimental conditions (values > 1000 not displayed).

First, XHV734\_1016 was located in the middle of a cluster of 3 critical genes (XHV734\_1015 to 1017) (**Fig. 3**) which displays a very strong homology and synteny with the *sux* CUT of *X. campestris* pv. *campestris*, responsible for sucrose-specific uptake and degradation [9]. The *sux* gene cluster consists of *suxA* (= XHV734\_1016), a sucrose TBDT which conveys sucrose into the periplasm, *suxB* (= XHV734\_1015), an intracellular amylosucrase with a hydrolytic function cleaving sucrose into glucose-1-P and fructose [67], *suxC* (= XHV734\_1017), an inner membrane sucrose transporter, and *suxR* (= XHV734\_1018), a LacI/GalR-family transcriptional repressor of sucrose import, which

was not critical in our screen. Afterward, the phosphomannomutase encoded by *cpsG* (= XHV734\_4228) which allows the glucose-1-P to enter the glycolysis pathway was also screened as critical. The essentiality of this sucrose utilization locus in lettuce correlates with a previous study which demonstrated that sucrose was the primary carbon source of *X. campestris* pv. *campestris* in low molecular weight cabbage leaf extract, and that it was primarily imported with malate to be used by the bacterium during the first hours of incubation [68]. Moreover, some *Xanthomonas* species are known to use TALEs to hijack the SWEET sucrose-efflux plant transporters to their benefice [69]. Sucrose can also stimulate the production of diffusible signal factor (DSF) [70], the *Xanthomonas*-quorum sensing signaling molecule which plays a major role in virulence regulation and survival *in planta* [71]. Thus, we hypothesize that sucrose could well be the preferential carbon source of *X. hortorum* pv. *vitians* during the early biotrophic phase of lettuce leaves invasion. It is directly available and assimilable, and its consumption enhances the accumulation of DSF, leading to the coordinated production of virulence factors and exopolysaccharides which are required for the success of the infection process.

Secondly, XHV734\_0066 was nearly identical to the *cirA* gene of *X. citri* subsp. *citri* 306, which is thought to be an oligoxyloside-specific TBDT involved into the uptake and utilization of xylan breakdown products [72]. This *cirA* ortholog was adjacent to XHV734\_0067, a xylose repressor-like transcriptional regulator strongly homologous to *xylR* controlling the expression of genes involved in xylan degradation [10]. Xylan from the cell wall hemicelluloses is primarily degraded in xylooligosaccharides by  $\beta$ -1,4-endoxylanases [73]. Then, *cirA* has been hypothesized to transport methylglucuronoxylotriose (MeGX<sub>3</sub>), a tetrasaccharide intermediate of xylan degradation, into the periplasm where it can then be converted into xylotriase by a membrane-bound  $\alpha$ -glucuronidase [72]. There, xylotriase may be either separated into D-xylose or directly transported to the cytoplasm. Once in the cytoplasm, D-xylose is converted into D-xylulose-5-P by XylA1, XylA2 and XylB (= XHV734\_2842, 4908 and 2843) and subsequently processed by the pentose phosphate pathway (PPP) to be assimilated [10]. Intriguingly, the *tktA* gene encoding the transketolase of the PPP which catalyzes the conversion of D-xylulose-5-P into glyceraldehyde-3-P was determinant for fitness, but not the *xylA* and *xylB* genes, leading to hypothesize that their function could be redundant or ensured by other enzymes. Xylan exploitation is important for *Xanthomonas* nutrition during the early phyllosphere colonization stage [10] but could also be an important nutrient during the late necrotrophic phase when host cells are actively degraded.

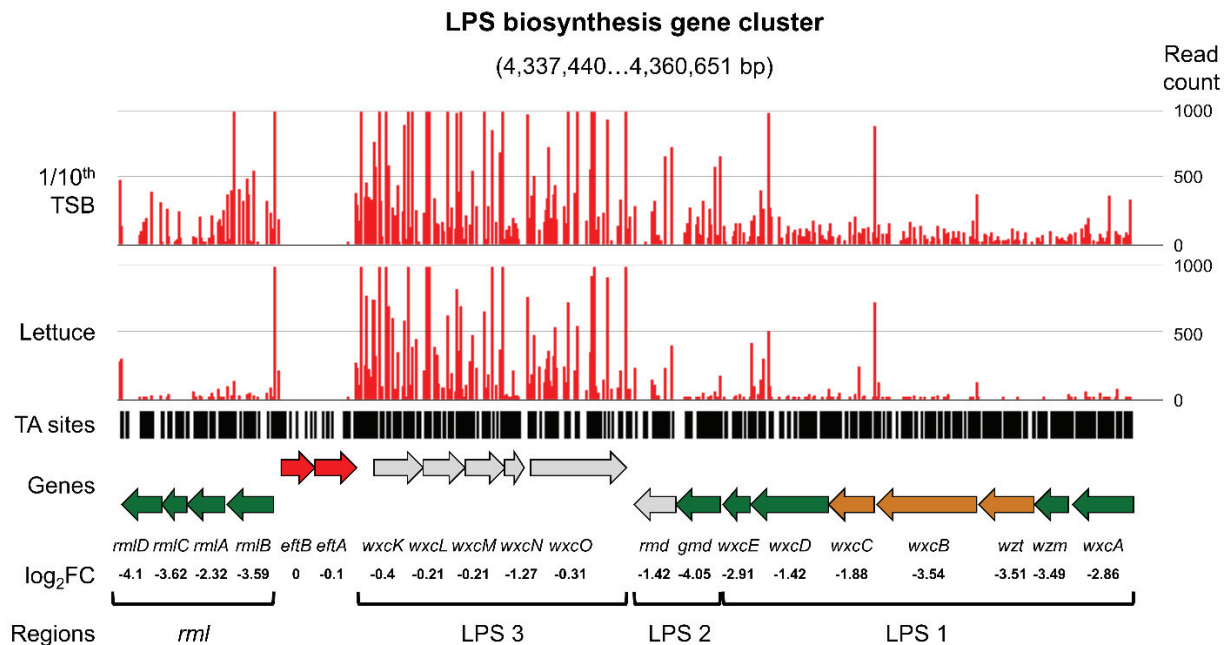
Finally, XHV734\_1310 encodes a homolog of the ferric enterobactin receptor *bfeA* of various *Xanthomonas* spp., which is flanked by two other similar copies (XHV734\_1309 and 1312) and located upstream of the xanthoferrin siderophore biosynthetic and uptake gene cluster *xss* (XHV734\_1298 to 1305) [74]. The organization of these three *bfeA*-like genes upstream of *xss* is strikingly similar to what can be observed in *X. citri* subsp. *citri* [75] and *X. oryzae* pv *oryzae* [54]. Wang *et al.* [54] named the genes *bfeA*, *bfeA2* and *bfeA3* and reported that mutating *bfeA* alone ended in a reduced virulence phenotype *in planta*. A recent proteomic analysis suggested that BfeA protein abundance could be directly correlated with virulence [76], which could partially explain why the gene is present in three co-localized copies. Iron uptake using siderophores is a well-known crucial feature of most bacterial phytopathogens necessary for successful host invasion [77].

### **The LPS O-antigen is required to evade the plant immune response.**

The Tn-seq screen in lettuce also highlighted some critical genetic factors allowing *X. hortorum* pv. *vitians* to evade and counteract the lettuce immune response triggered by the recognition of the pathogen during leaf invasion. Following the identification of cellular polysaccharides metabolism as significantly enriched in the GO term enrichment analysis (**Tab. 2**), we looked for genes associated with these processes in our critical gene subset. As a result, 2 genes of the *gum* gene cluster encoding the synthesis of the xanthan EPS and 19 genes involved in the biosynthesis and assembly of the cell-surface LPS, clustered in 3 distinct genomic regions, were identified (**Fig. 3**). We already discussed the lethal phenotypes observed in our Tn-seq library for the *gumE*, *gumI* and *gumJ* mutants [23], but this time only *gumB* (XHV734\_1853) and *gumC* (XHV734\_1854) were essential in lettuce leaf. These two genes, along with *gumE*, are responsible for the polymerization of the lipid-linked pentasaccharide units and subsequent export of the final xanthan EPS out of the periplasm [78]. Moreover, production of xanthan in *gumB* and *gumC* mutants of *X. campestris* is completely abolished *in vivo* [79]. Xanthan gum is required for full epiphytic survival and virulence in *Xanthomonas* species [12,54,80].

The remaining 19 genes associated with LPS biosynthesis and assembly emphasized the crucial importance of these surface components in the interaction with the host plant. Three main gene clusters were identified (**Fig. 3**), each encoding specific functions. The first region contained 4 genes: the conserved LPS assembly gene *lapB* (XHV734\_2249) [81], a homolog of the *rfb303* gene of *X. citri* subsp. *citri* (XHV734\_2250) putatively associated with the LPS core biosynthesis [82], a putative sugar epimerase gene (XHV734\_2251) and the

*galU* gene (XHV734\_2252) involved in O-antigen nucleotide sugar biosynthesis [78,83]. Another small gene cluster consisting of XHV734\_4495 and 4497 was related to LPS biosynthesis. XHV734\_4497 is an O-antigen ligase-like enzyme putatively responsible for the ligation between the O-antigen and the Lipid A core.



**Figure 5.** Schematic representation of the critical genes in lettuce or in 1/10<sup>th</sup> TSB within the LPS biosynthetic gene cluster of *X. hortorum* pv. *vitians* LM16734. Numbers in brackets indicate the range of the viewing window on the LM16734 genome. Critical genes in lettuce are indicated by green arrows, critical genes in 1/10<sup>th</sup> TSB by gold arrows, essential genes in *in vitro* library by red arrows, and non-significant genes by grey arrows. Genes names, log<sub>2</sub>FC values and grouping in functional regions as described in [84] are displayed below. Localizations of TA sites are indicated by black bars, and bar plots represent read counts at each TA sites in the control and experimental conditions (values > 1000 not displayed).

The principal LPS gene cluster corresponded to the *rml* / *wxc* major cluster of LPS biosynthesis [84]. The Tn-seq screen allowed to distinguish which parts of this cluster were essential, and consequently which particular functions in the LPS biosynthetic process were required for survival in lettuce (**Fig. 5**). First, the *rmlBACD* essential genes (XHV734\_4231 to 4233) encode a set of enzymes converting glucose-1-phosphate into the nucleotide sugar dTDP-L-rhamnose [85], which is the direct precursor of the rhamnose moieties of the *Xanthomonas hortorum* pv. *vitians* LPS core and O-antigen [86]. Then, the three functional regions of the LPS biosynthesis gene cluster [84] were differentially essential (**Fig. 5**). The LPS 1 region located from *wxcA* (XHV734\_4249) to *wxcE* (XHV734\_4243) was entirely

critical in lettuce and is responsible for the biosynthesis of the water-soluble O-antigen [84]. Genes *wzt*, *wxcB* and *wxcC* (XHV734\_4247, 4246 and 4245) were also critical the inoculum, suggesting that formation of a functional O-antigen is necessary even in rich medium. The *gmd* gene of LPS region 2 (XHV734\_4242) was also critical in lettuce. This region is implicated in the biosynthesis of the LPS core oligosaccharide [84]. Finally, none of the LPS 3 region genes, ranging from *wxcK* (XHV734\_4236) to *wxcO* (XHV734\_4240), were screened as critical. Genes within this region probably encode the accessory synthesis and transfer of N-acetyl-3-amino hexose [84] and contribute to the side branching of the O-antigen [87]. Mutating these genes resulted in the production of modified O-antigens in *X. campestris* pv. *campestris* [87].

Altogether, our findings indicate that genes directly implicated in the biosynthesis of structurally intact LPS core oligosaccharide and O-antigen are indispensable for successful invasion and survival inside lettuce leaves. This is supported by the previous demonstration that multiple *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae* mutants defective in the O-antigen biosynthesis genes showed reduced or abolished virulence *in planta* [53,54]. Gram-negative bacterial LPS have a general protection role against stresses in diverse environments [88], including oxidative stress [82], and paradoxically camouflage pathogens from the host immune system while being simultaneously one of the major microbial-associated molecular pattern (MAMP) recognized by hosts [89,90]. Recently, the long O-antigen of *Xylella fastidiosa* has been demonstrated to delay the pathogen recognition and consequent plant basal immune response in grapes [91]. Some glycosyltransferases putatively involved in the biosynthesis of the O-antigen of *P. syringae* pv. *syringae* were also screened as conditionally-essential *in planta* by RB-TnSeq [27].

### **Protection against the oxidative burst is a key feature for surviving the immune response.**

The rapid and localized accumulation of reactive oxygen species (ROS) inside the plant tissues, following MAMPs recognition by the plant receptors, is referred to as the oxidative burst [13]. In general, protection against the damages caused by ROS, which includes lipid peroxidation, enzyme inactivation and nucleic acid degradation, can be achieved in three different ways: (i) prevention of ROS generation, (ii) detoxification of radicals and (iii) repair of damaged elements [92]. We found a set of 10 essential genes in lettuce which we hypothesized to actively participate in protecting the pathogen against the oxidative burst by detoxifying ROS and repairing damaged structures (**Fig. 3**).

First, the Mn-superoxide dismutase encoding gene *sodA* (XHV734\_2154) was critical *in planta*. SodA has a pivotal role for the virulence of several plant pathogenic bacteria [93]. The expression of this gene is induced within the first 3 hours of the plant infection in *X. campestris* pv. *campestris* and *D. dadantii* [94,95], proving that it acts as a very early protection against the oxidative burst. Interestingly, *sodA* is not directly induced by ROS in *X. campestris* pv. *campestris*, but by redox cycling agents like plumbagin, a plant quinone with antimicrobial properties [96]. Another gene, XHV734\_2318, encodes an YgfZ-family folate-dependent protein which might be also involved in plumbagin resistance [97]. On the other hand, *phaC* encodes a polymerase producing poly (3-hydroxyalkanoate) granules (PHA), a polyester which can both serve as a form of carbon storage and enhance the resistance to cold and oxidative stresses in pseudomonads [93].

Then, a variety of genes were implicated in the recycling of misfolded proteins and damaged nucleic acids. The *clpX* (XHV734\_3771), *clpP* (XHV734\_3772) and *ftsH* (XHV734\_2889) genes encode the ClpX-ClpP serine protease and an ATP-dependent Zn-metalloprotease, respectively. The ClpX-ClpP protease recycle misfolded proteins in the cytoplasm [98], and its inactivation in *X. campestris* pv. *campestris* through *clpX* or *clpP* mutations lead to pleiotropic effects, including an increased stress sensitivity [99,100]. Moreover, oxidative stress induces the expression of *ftsH* in *Lactobacillus plantarum* and  $\Delta$ *ftsH* mutants are highly sensitive to various stresses [101]. Thus, it seems that these two proteases are required to buffer the damaging effects of the oxidative burst on the bacterial proteome.

Finally, the RNase R-encoding *rnr* gene (XHV734\_3096) and homologous recombination system *recBCD* genes (XHV734\_5034, 5035 and 5033) are all critical in lettuce and probably involved in repairing the damages caused to nucleic acids. Indeed, the RNase R is required for the *trans*-translation pathway which direct deficient proteins and transcripts for degradation while rescuing stalled ribosomes and is important for pathogenesis in many animal pathogens [102]. This protein is overexpressed in *X. citri* subsp. *citri* during orange tree infection, supporting the hypothesis of its importance for *Xanthomonas* virulence [103]. At last, the RecBCD homologous recombination system is required for virulence in *S. enterica*, where it is hypothesized to be needed for repairing double-strand breaks caused by the host immune response [104]. However, the *recC* mutants were also impaired for growth in 1/10<sup>th</sup> TSB, suggesting that they may already present growth-defects *in vitro*. Nevertheless, our results present an interesting and comprehensive view of the genes and



functions that are required to hide from the plant immune response and to resist to the early oxidative burst response during lettuce invasion.

## **Conclusion**

The present study presented the first overview of some of the molecular processes employed by *X. hortorum* pv. *vitians* to colonize and infect lettuce leaves, using the discriminative power of a genome-wide Tn-seq approach and the critical considerations brought by multiple comparative genomic investigations. We demonstrated that amino acid and nucleic acid biosynthesis, expression of the T2SS, carbohydrates acquisition and metabolism, xanthan gum biosynthesis and secretion, LPS core and O-antigen biosynthesis and oxidative burst resistance mechanisms were critical for successful infection of lettuce by the pathogen. We hypothesize that these processes are sequentially mobilized during the consecutive epiphytic, biotrophic and necrotrophic phases of the infection by this hemibiotrophic pathogen. Further investigations on the distinctive contribution of each of these genes and pathways along the time course of the infection in lettuce would be required to test our hypotheses. In any event, the *in planta* Tn-seq screen proved to be a powerful method to identify metabolic pathways mobilized along the plant-pathogen interaction and structures and processes required for dialoguing with the plant immune system.

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# DISCUSSION GÉNÉRALE & PERSPECTIVES

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## 1. Rappel du contexte et des problématiques

Les bactéries du genre *Xanthomonas* sont au cœur des préoccupations agroéconomiques et de santé publique compte tenu des actuelles émergences (ou réémergences) des pathologies végétales auxquelles elles sont associées, et de l'aggravation anticipée des dommages qu'elles provoquent sur les cultures à cause des changements climatiques (Catara et al., 2021). Parmi ces espèces phytopathogènes, *Xanthomonas hortorum* était jusqu'alors restée relativement méconnue, vraisemblablement en raison de l'absence de cadre taxonomique clair circonscrivant les limites de l'espèce et la diversité des pathogènes de plantes qu'elle recouvre. Qui plus est, le manque criant d'informations génomiques, moléculaires et écologiques concernant la plupart de ces pathogènes constitue un frein important à leur étude et à la recherche de solutions viables permettant de contrôler leurs ravages dans les cultures. Débuter l'étude de *Xanthomonas hortorum* à travers le modèle de l'agent causal de la bactériose foliaire de la laitue, *X. hortorum* pv. *vitians*, constituait une bonne première approche pour aborder cette espèce phytopathogène. Ce ravageur de laitues fait paradoxalement partie des pathovars de *X. hortorum* dont le cycle de vie et l'épidémiologie ont été les plus étudiés, mais également de ceux dont les aspects génomiques et moléculaires d'interaction avec l'hôte végétal ont été jusque-là presque complètement laissés de côté. De plus, les problèmes liés à sa classification ont grandement contribué à alimenter les incertitudes sur la taxonomie de l'espèce.

Les objectifs affichés des présents travaux de thèse visaient donc à (i) étudier la diversité des souches associées à la bactériose foliaire de la laitue et mettre en place une approche polyphasique pour résoudre enfin un problème taxonomique persistant depuis plus de 20 ans, (ii) adapter la technique du Tn-seq au modèle de *X. hortorum* pv. *vitians*, en l'associant à un séquençage de génome complet pour (iii) offrir pour la première fois une perspective d'ensemble des déterminants moléculaires, traits écologiques et composants cellulaires structurels mobilisés par le pathogène lors de son interaction avec la laitue. Chaque chapitre de cette thèse a été pensé comme autonome et est donc accompagné d'une discussion extensive des résultats présentés. Nous allons désormais tenter de prendre de la hauteur et de nous interroger sur les nouvelles hypothèses qu'il serait possible de formuler suite à ces

travaux sur l'espèce *X. hortorum* et sur *X. hortorum* pv. *vitians* en particulier. Dans un premier temps, nous allons entamer une réflexion sur l'histoire évolutive et l'émergence de *X. hortorum*, les parallèles qu'il est possible de dresser avec d'autres espèces de *Xanthomonas*, et les études qu'il serait judicieux d'entreprendre pour approfondir notre compréhension de la diversité chez cette espèce. Ensuite, nous reviendrons sur le détail de l'interaction hôte-pathogène entre *X. hortorum* pv. *vitians* et la laitue, en opérant une réflexion critique sur la notion de « pathogène », sur les forces et les limitations de notre analyse de Tn-seq, et sur les perspectives qu'offre l'utilisation de cette librairie.

## **2. Histoire évolutive et émergence de *Xanthomonas hortorum***

La domestication des végétaux est un processus de coévolution entre les humains et les plantes sauvages (Purugganan, 2019). De la même manière, l'évolution et l'émergence des populations de microorganismes phytopathogènes sont intrinsèquement liées à la domestication de leurs hôtes (Stukenbrock & McDonald, 2008). Celle-ci a en effet conduit à une réduction drastique de la diversité génétique au sein des populations végétales, à une forte sélection positive d'un petit nombre de gènes d'intérêt pour la domestication et à l'accumulation de mutations légèrement délétères (Glémin & Bataillon, 2009; Purugganan, 2019; Stukenbrock & Bataillon, 2012). En conséquence, les défenses naturelles que les espèces végétales avaient développées pour résister aux agressions des phytopathogènes ont été compromises (Stukenbrock & Bataillon, 2012). La domestication des plantes et leur mise en culture amènent également à concentrer dans un espace restreint une population importante d'hôtes génétiquement homogènes (Stukenbrock & McDonald, 2008). De plus, les activités anthropogéniques peuvent favoriser l'émergence de populations phytopathogènes en sélectionnant des variants résistants aux méthodes de lutte ou en favorisant la propagation sur de longues distances via le commerce des graines et des plantules (Straub et al., 2020). Dans l'ensemble, ces processus ont favorisé l'émergence dans les agroécosystèmes de nouveaux agents phytopathogènes spécialisés, finement adaptés à ces nouvelles niches écologiques (Vinatzer et al., 2014). Toutefois, ces évolutions ont aussi occasionné une réduction de la diversité existante au sein des populations de microorganismes phytopathogènes, en favorisant la dispersion rapide de lignées clonales spécialisées et/ou la spéciation vis-à-vis d'une seule plante hôte (Stukenbrock & McDonald, 2008).

Chez les bactéries phytopathogènes, il n'existe pas de mécanismes de brassage génétique par reproduction sexuée qui permettrait d'accélérer l'adaptation à de nouvelles plantes hôtes. Il existe toutefois trois mécanismes permettant d'introduire du polymorphisme dans le fond génétique d'une population : (i) les mutations ponctuelles, (ii) les réarrangements de segments d'ADN et les remplacements alléliques par recombinaison homologue, et (iii) l'insertion sans remplacement de nouveaux éléments génétiques exogènes par recombinaison site-spécifique (Bartoli et al., 2016; Merda, 2016; Vinatzer et al., 2014). Ces deux derniers modes d'évolution peuvent être regroupés sous le terme général de transfert horizontal de gène (THG) qui englobe également les phénomènes d'acquisition de plasmides (Vinatzer et al., 2014). Depuis les travaux fondamentaux de John Maynard Smith (1920-2004) sur les dynamiques évolutives des bactéries pathogènes chez l'humain (Maynard-Smith et al., 1993, 2000), le rôle majeur des THG dans les phénomènes de saut d'un hôte végétal à un autre chez les phytopathogènes a été largement reconnu et documenté (McCann, 2020; Morris & Moury, 2019; Vinatzer et al., 2014). De nouvelles espèces de bactéries phytopathogènes peuvent ainsi émerger par recombinaison au sein du microbiote végétal des plantes cultivées ou sauvages dans les environnements agricoles, ou bien au sein de réservoirs environnementaux autres que les hôtes végétaux (dits réservoirs non hôtes) (McCann, 2020). La population globale à partir de laquelle émerge les pathogènes, génétiquement diverse et occupant de multiples environnements est alors appelée *population recombinante*, ou « réseau recombinant » (Merda et al., 2016).

En fonction de la contribution relative de chacun de ces mécanismes dans le polymorphisme génétique existant au sein d'une espèce bactérienne pathogène, on peut distinguer deux types de structure de populations : des populations clonales dites *épidémiques* dont la diversité génétique est principalement influencée par des mutations ponctuelles et des populations panmictiques aussi appelées *endémiques* au sein desquelles les phénomènes de recombinaison sont fréquents (Dykhuizen & Kalia, 2007; Maynard-Smith et al., 1993). D'une part, une population *épidémique* résulte de l'expansion rapide de lignées clonales ayant émergé de la population recombinante totale. L'ensemble des hôtes infectés au cours d'un épisode épidémique le sera donc par des pathogènes issus d'un petit nombre de populations clonales présentant peu de diversité allélique (Merda et al., 2016; Vinatzer et al., 2014). D'autre part, on parle de population *endémique* lorsque des hôtes différents sont infectés par un large ensemble de populations clonales distinctes ayant toutes pour origine commune une émergence ancienne de la population recombinante (Vinatzer et al., 2014). Dans ce cas, la

coexistence de multiples populations apparentées sur une même gamme d'hôtes favorise la recombinaison entre elles (Dykhuizen & Kalia, 2007). La distinction de ces deux types de structure de populations chez les bactéries phytopathogènes permet d'orienter les choix en matière de stratégie de sélection variétale et d'utilisation de moyens de lutte (Vinatzer et al., 2014).

En ce qui concerne les *Xanthomonas*, il a été suggéré que les mutations ponctuelles et les phénomènes de recombinaison ont contribué de manière équivalente à l'évolution de *X. campestris* pv. *campestris* (Fargier et al., 2011) et de l'espèce *X. axonopodis* (Mhedbi-Hajri et al., 2013). En règle générale, les évènements de recombinaison semblent fréquents chez les populations de *Xanthomonas* (Hamza et al., 2012; Fischer-Le Saux et al., 2015), et environ 10 % du génome commun au genre (*core genome*) aurait été impacté par des phénomènes de recombinaison homologue allélique (Huang et al., 2015). La grande majorité (88 %) des évènements observés auraient eu lieu au niveau intraspécifique (Huang et al., 2015). Toutefois, les transferts de grands fragments d'ADN par recombinaison site-spécifique auraient également joué un rôle important dans l'évolution du système de sécrétion de type III au sein du genre *Xanthomonas*, et donc dans l'évolution de la pathogénicité (Merda et al., 2017). D'ailleurs, l'espèce *X. hortorum* serait un des plus importants donneurs dans ces évènements de transfert, tandis que l'espèce voisine *X. arboricola* serait à l'inverse un des receveurs principaux (Merda et al., 2017). Ces deux espèces ont en commun de pouvoir occuper une gamme large et variée d'hôtes végétaux (Fischer-Le Saux et al., 2015 ; cf. **Synthèse bibliographique**), lesquels pourraient dès lors constituer des niches écologiques favorables aux évènements de recombinaisons interspécifiques. Enfin, dans une étude de MLST basée sur 6 gènes de ménage et conduite sur une collection de souches américaines de *X. hortorum* pv. *vitians*, Fayette *et al.* (Fayette, Raid, et al., 2016) ont montré que si les mutations ponctuelles étaient plus fréquentes que les évènements de recombinaison, ces derniers contribuaient plus au polymorphisme génétique des populations.

Dans le cadre des travaux présentés dans le premier chapitre, nous avons mis en évidence par une approche de MLSA l'existence de trois groupes phylogénétiques (A, B et C) au sein des populations de *X. hortorum* pv. *vitians*, chacun étant divisé en deux *sequence types* (STs). Sur la base des combinaisons d'allèles observées pour les trois gènes de ménage utilisés (*gyrB*, *rpoD* et *gapA*), cette structure pourrait donc correspondre à l'existence de six populations clonales distinctes, mais très proches comme l'indique le faible polymorphisme des séquences. L'origine géographique des souches ou leur période d'isolement ne semble pas

avoir d'influence sur la répartition au sein de chacun de ces groupes. De manière intéressante, les analyses phylogénétiques basées sur les génomes de 8 souches représentatives des trois groupes de MLSA n'ont en revanche montré qu'une séparation en deux groupes. Un premier cluster correspond aux groupes A et C et un second contient les deux génomes des souches du groupe B, qui auraient divergé récemment du cluster principal. Bien que ces génomes soient clairement distingués dans les phylogénies, les valeurs d'ANI calculées entre tous les génomes de *X. hortorum* pv. *vitians* sont toutes clairement supérieures à 99.9 %, ce qui signifie bien que la diversité génétique reste faible au sein des populations de ce pathovar.

Dans une étude de génomique populationnelle des souches responsables de la gale bactérienne de la tomate, Timilsina *et al.* (Timilsina *et al.*, 2015) ont montré que les 28 souches de *X. hortorum* pv. *gardneri* testées présentaient exactement les mêmes allèles pour chacun des 6 gènes séquencés et ce, quel que soit le pays d'origine. Seule la souche ICMP 7383 présentait un profil allélique différent, ce que la reclassification en *X. hortorum* pv. *vitians* démontrée dans le premier chapitre permet d'expliquer. La soudaine expansion géographique de *X. hortorum* pv. *gardneri* au cours des dernières décennies et l'absence de variabilité génétique par rapport à la première souche isolée en 1953 suggèrent donc que *X. hortorum* pv. *gardneri* est un clone épidémique dont l'émergence est vraisemblablement récente (Hamza *et al.*, 2012; Timilsina *et al.*, 2015). Nos données suggèrent que les populations de *X. hortorum* pv. *vitians* pourraient également présenter une structure épidémique, mais dont l'émergence serait plus ancienne que pour *X. hortorum* pv. *gardneri*. Les six STs observés par MLSA pourraient donc correspondre à des clones épidémiques issus d'une même population clonale ancestrale.

Une étude de MLVA initiée sur une large collection de souches de *X. hortorum* pv. *carotae* aurait également révélé que les souches de ce pathogène se séparaient en deux lignées génétiques très proches partageant une ascendance commune (Jacques *et al.*, 2013), ce qui pourrait aussi être le signe d'une structure épidémique. S'il n'existe pas d'étude génétique des populations de *X. hortorum* pv. *pelargonii*, le typage moléculaire d'une collection de 124 souches par SDS-PAGE (*Sodium Dodecyl Sulphate – PolyAcrylamide Gel Electrophoresis*) du contenu protéique cellulaire total a révélé que tous les profils des souches étaient extrêmement similaires (Vauterin *et al.*, 1990). Le typage moléculaire de 19 autres souches par ERIC-PCR (*Enterobacterial Repetitive Intergenic Consensus – PCR*) et REP-PCR (*Repetitive Element Palindromic – PCR*) a aussi abouti à observer des profils très conservés entre les souches de ce pathovar (Sulzinski *et al.*, 1995).

On peut donc émettre l'hypothèse que *X. hortorum* pv. *pelargonii* soit également structuré en populations clonales selon un mode épidémique. De manière similaire, le profilage moléculaire d'une large collection de souches de *X. hortorum* pv. *cynarae* par RAPD (*Randomly Amplified Polymorphic DNA*) a permis de montrer qu'elles formaient un groupe génétiquement homogène subdivisé en deux sous-groupes légèrement polymorphiques (Trébaol et al., 2001). À l'inverse, une étude de la structure de *X. hortorum* pv. *hederae* a montré que les souches associées à ce pathovar se structuraient en populations génétiquement et phénotypiquement hétérogènes (Norman et al., 1999). Il n'existe malheureusement aucune donnée concernant d'autres souches de *X. hortorum* pv. *taraxaci* que la souche pathotype. Toutefois, nous avons isolé au cours d'une de nos campagnes d'échantillonnage une souche de *X. hortorum* (= LM16389) sur un pissenlit commun symptomatique (*T. officinale*) en bordure d'un champ de laitue (cf. **Chapitre I**). Une fois sa pathogénicité sur pissenlit vérifiée, l'inclusion de cette souche dans la MLSA de *X. hortorum* a révélé qu'elle se regroupait bien avec la souche pathotype de *X. hortorum* pv. *taraxaci*. Des populations de ce pathogène semblent donc être bien présentes dans l'environnement.

Chez l'espèce proche *X. arboricola*, une approche de taxonomie polyphasique incluant notamment une analyse de MLST sur une grande collection de souches représentatives de la diversité pathogénique de l'espèce et d'autres souches non-pathogènes a mis en évidence une structure de population épidémique (Fischer-Le Saux et al., 2015). Les trois pathovars les plus agressifs (pvs. *pruni*, *juglandis* et *corylina*) formaient des groupes monophylétiques clonaux, compatibles avec une émergence récente de clones spécialisés sur une gamme d'hôtes restreinte. Les autres pathovars, généralement peu virulents, étaient principalement regroupés en groupes polyphylétiques comme relevé précédemment (Vandroemme, Cottyn, Pothier, et al., 2013), à l'exception du pv. *populi* mais dont la population ne correspondait pas à un groupe clonal (Fischer-Le Saux et al., 2015). De plus, l'existence d'une population recombinante de *X. arboricola* dans l'environnement est attestée par plusieurs études (Fernandes et al., 2021; Garita-Cambronero et al., 2017; Merda et al., 2016).

Dans l'ensemble, les éléments présentés semblent supporter l'hypothèse d'un mode d'évolution épidémique de l'espèce *Xanthomonas hortorum* qui impliquerait l'existence d'une population recombinante dans l'environnement à partir de laquelle auraient émergé à plusieurs reprises des clones virulents spécialisés. La quantité de rapports faisant état de l'isolement de souches associées à l'espèce *X. hortorum* sur une grande variété de plantes sauvages et domestiquées, et également dans le microbiote de graines de plantes

asymptomatiques (cf. **Synthèse bibliographique**), tend à accréditer cette hypothèse. Aussi, la présence de populations épiphytes de *X. hortorum* pv. *vitians* sur des plantes asymptomatiques dans les champs de laitue semble bien plus fréquente que ce que l'on pourrait imaginer pour une bactérie principalement considérée comme pathogène. Une étude métagénomique du microbiote phyllosphérique de 88 laitues collectées à travers la Californie et ne présentant aucun symptôme apparent a montré que 33 échantillons contenaient des séquences associées au genre *Xanthomonas* et au moins 13 une forte quantité de séquences spécifiques de *X. hortorum* pv. *vitians* (Rastogi et al., 2012).

Pour vérifier nos hypothèses, il serait tout d'abord nécessaire de réaliser de nouvelles campagnes d'échantillonnage pour chacun des pathovars décrits de *X. hortorum*, et également de recueillir des souches de *X. hortorum* commensales sur des plantes non-hôtes et potentiellement dans d'autres types de réservoirs environnementaux, notamment les graines (cf. **Synthèse bibliographique**). À l'échelle locale, une approche de génomique populationnelle à haute résolution comme la MLVA (*MultiLocus Variable Number of Tandem Repeats Analysis*) sur *X. hortorum* pv. *vitians* permettrait de déterminer la structure des populations présentes sur le territoire, leur degré de clonalité, et de fournir des informations sur leurs modes de dissémination. À l'échelle mondiale, une nouvelle approche de MLSA plus ambitieuse sur un grand panel de souches représentatives de la diversité pathologique et des réservoirs occupés par *X. hortorum* serait capable de nous renseigner sur l'histoire évolutive de l'espèce et les relations entre les différents pathovars (Merda et al., 2016). À une époque où les séquençages de génome sont de plus en plus accessibles, on pourrait tout à fait imaginer de séquencer un grand nombre de génomes représentatifs de la diversité observée par MLSA. Cela permettrait d'étudier l'impact des mutations spontanées et des transferts de gènes sur la diversification de l'espèce.

On pourrait également vérifier la présence ou l'absence du cluster *hrp* et de certains effecteurs de type III « marqueurs » comme cela a été fait récemment pour *X. arboricola* pv. *juglandis* (Fernandes et al., 2021) afin de rechercher des signes de la présence de la fraction non-pathogène de la population recombinante environnementale. On pourrait même aller encore plus loin et entreprendre une grande analyse de la conservation d'un grand nombre de gènes associés à la virulence et à la vie *in planta* entre tous ces génomes (cluster *hrp* et répertoire de T3Es, système de sécrétion de type VI et effecteurs associés, système de sécrétion de type IV, gènes flagellaires, gènes de synthèse de polysaccharides, gènes codant des enzymes de dégradation de la paroi cellulaire végétale...)



(Gétaz et al., 2020; Vandroemme, Cottyn, Baeyen, et al., 2013). En étudiant également leur environnement génétique et en s'intéressant à leur synténie, des flux de ces gènes médiés par THG au sein de *X. hortorum* pourraient être identifiés. Cela permettrait de comprendre l'histoire évolutive de *X. hortorum* et les mécanismes génétiques sous-tendant l'émergence de ces différents pathovars.

### **3. Bases moléculaires de l'adaptation aux hôtes végétaux et à l'environnement**

En parallèle de ces études populationnelles et évolutives, il serait également intéressant d'étudier de manière fonctionnelle les mécanismes permettant l'adaptation spécifique à une espèce végétale et le passage d'un hôte à un autre. Actuellement, de nombreux éléments tendent à indiquer que les phénomènes de sauts d'hôtes (*host jumps*) ont plus contribué à façonner le spectre d'hôte actuel des bactéries phytopathogènes que les mécanismes de coévolution (Morris & Moury, 2019). En revanche, on observe tout de même une tendance des pathogènes à infecter des plantes proches les uns des autres, ce qui fait de l'étude des distances phylogénétiques entre espèces végétales un bon moyen de prédire des potentiels sauts d'hôtes (Morris & Moury, 2019). Ces passages d'un hôte à un autre peuvent être liés à l'acquisition de matériel génétique par transfert horizontal, par recombinaison, par délétion totale ou partielle d'un simple gène, ou par fixation de mutations spontanées (Morris & Moury, 2019).

En ce qui concerne les *Xanthomonas*, les modifications du spectre d'hôte peuvent advenir par adaptation sympatrique ou allopatrique, mais on observe également des sauts d'hôtes rapides par transfert horizontal de matériel génétique d'origine chromosomique ou plasmidique (Jacques et al., 2016). Par exemple, l'omniprésence du plasmide pXap41 dans les génomes de *X. arboricola* pv. *pruni*, et son contenu en gènes de virulence, suggère un rôle primordial dans la pathogénicité et la spécificité d'hôte de ce pathovar (Pothier et al., 2011; Fischer-Le Saux et al., 2015). La présence de deux plasmides supplémentaires dans une souche atypique de *X. hortorum* pv. *vitians* pathogène à la fois de laitue et de tomate, et notamment du plasmide pICMP7383.1, similaire à ceux présents chez d'autres espèces pathogènes de tomate, pourrait dès lors constituer la raison de cette adaptation soudaine à un nouvel hôte végétal distant phylogénétiquement de l'hôte d'origine. Une manière de le vérifier serait de tenter de transférer ce plasmide à d'autres souches de *X. hortorum* pv. *vitians*

incapables d'infecter la tomate, puis de vérifier à nouveau ce phénotype. En effet, ce plasmide contient un système de transfert conjugatif de type IV complet, et confère en plus une résistance au sulfate de cuivre, ce qui permettrait d'opérer une sélection des clones transconjugants.

On peut également s'interroger sur le rôle des plasmides natifs chez *X. hortorum* pv. *vitians*, dans la mesure où toutes les souches semblent posséder au moins un plasmide de taille similaire. D'ailleurs, l'annotation du plasmide pLM16734 de notre souche modèle *X. hortorum* pv. *vitians* LM16734 révèle que celui-ci contient une pectate lyase (XHV734\_p0058), deux effecteurs de type III (XHV734\_p0064 et p0070), deux gènes d'avirulence des familles *avrBs1* (XHV734\_p0066) et *avrBs3* (XHV734\_p0083) et un autre gène d'avirulence non-caractérisé (XHV734\_p0093). La présence d'un effecteur TAL (*Transcription Activator-Like*) de la famille AvrBs3/PthA sur ce plasmide est particulièrement intéressante, dans la mesure où l'acquisition de ces effecteurs d'un type particulier peuvent être associés à l'adaptation à de nouvelles plantes hôtes (Ruh et al., 2017). On pourrait d'ailleurs rechercher la présence d'autres effecteurs TAL dans les génomes de *X. hortorum* et, s'il en existe, les comparer, en utilisant des outils de prédiction informatique (Grau et al., 2016; Pérez-Quintero et al., 2015). Ce rapide examen suggère donc un rôle important de ces plasmides dans la pathogénicité de *X. hortorum* pv. *vitians* dont il serait intéressant d'approfondir l'étude, par exemple en réalisant des mutants de délétion pour ces gènes plasmidiques d'intérêt et en approfondissant l'annotation des autres gènes plasmidiques par génomique comparative. Le séquençage complet de plusieurs génomes de *X. hortorum* pourrait aussi permettre de réaliser des analyses phylogénétiques sur ces plasmides afin de comprendre leur transmission et leur maintien au sein de l'espèce.

Les principaux déterminants moléculaires de l'interaction hôte-pathogène et de la spécificité d'hôte chez les *Xanthomonas* sont donc généralement considérés comme étant les effecteurs de type III (Timilsina et al., 2020). L'adaptation spécifique à un hôte végétal n'est pas liée à l'acquisition d'un ou de quelques nouveaux effecteurs particuliers qui apporteraient une nouvelle fonction indispensable, mais plutôt à la combinaison des effecteurs entre eux, selon l'hypothèse du « répertoire de répertoire » d'effecteurs de type III (Hajri et al., 2009). On parle d'ailleurs désormais d'« *effectomes* » pour désigner le fait que l'action coordonnée de nombreux effecteurs aboutissant à la virulence sur une plante constitue ce que l'on pourrait appeler une propriété émergente (Arroyo-Velez et al., 2020). L'absence d'un large cœur commun d'effecteurs de type III (*core type III effectome*) entre les pathovars de *X. campestris*,

limité à trois gènes seulement, laisse envisager une redondance fonctionnelle d'effecteurs non-apparentés qui agiraient alors sur des protéines végétales cibles conservées (Roux et al., 2015).

Toutefois, ces effecteurs ne sont pas les seuls déterminants génétiques de l'adaptation à l'hôte végétal. En effet, ceux-ci ne sont mobilisés que lors de la phase endophyte du cycle de vie infectieux (An et al., 2020; Timilsina et al., 2020). Le succès de la première phase de vie épiphyte des *Xanthomonas* dépend aussi d'autres facteurs tels que des adhésines, des systèmes senseurs et régulateurs associés au chimiotactisme, et des systèmes d'acquisition de nutriments (An et al., 2020). À titre d'exemple, les répertoires de *Methyl-accepting Chemotaxis Proteins* (MCPs), de *Sensor of Two-Component Regulatory Systems* (STCRS) et de *TonB-Dependent Transporters* (TBDTs) sont corrélés à la structuration en pathovars des espèces de *Xanthomonas* et sont donc bien directement impliqués dans l'adaptation à une gamme d'hôtes particulière (Mhedbi-Hajri et al., 2011). Bien qu'assez souvent négligé chez des phytopathogènes aussi fortement dépendants du SST3 que les *Xanthomonas* (Kraepiel & Barny, 2016), l'expression d'un système de sécrétion de type II et d'un répertoire d'enzymes dégradant la paroi cellulaire végétale (*Cell Wall-Degrading Enzymes*, CWDEs) est aussi un facteur crucial pour la réussite de l'interaction avec l'hôte végétal au cours des deux phases épiphytes et endophytes (Jacques et al., 2016; Timilsina et al., 2020). Il est d'ailleurs très intéressant de constater que l'acquisition ou la perte d'une seule cellobiohydrolase chez *X. translucens* agit comme un « interrupteur phénotypique » (*phenotypic switch*) permettant de passer d'un mode de vie endophyte vasculaire à un mode de vie non-vasculaire (Gluck-Thaler et al., 2020).

La mise en place d'une stratégie de Tn-seq chez notre modèle *X. hortorum* pv. *vitians* a tout d'abord fait l'objet d'une validation technique, qui a permis dans un même temps de décrire le génome essentiel de ce pathogène et de s'interroger sur sa conservation au sein du genre *Xanthomonas* dans le second chapitre. Grâce à ce travail de génomique fondamentale, nous avons pu notamment nous questionner sur le rôle du transporteur de cation/proton Pha/Mrp/Mnh dans l'adaptation à des environnements variés et générateurs de stress auxquels seraient exposés les *Xanthomonas* (Ito et al., 2017), ainsi que sur la structure fonctionnelle et transcriptionnelle de l'opéron *gum* encodant la synthèse de xanthane, un important facteur de virulence et d'adaptation (Aslam et al., 2008; Darsonval et al., 2009). Ces travaux ont aussi identifié plusieurs éléments génétiques mobiles qui pourraient être impliqués dans la résistance aux bactériophages, comme des systèmes de restriction-modification et une

endonucléase de la famille OLD. D'ailleurs, le rôle de certains systèmes de restriction-modification dans la résistance naturelle aux bactériophages dans les écosystèmes vient d'être confirmé par une large étude chez le pathogène d'huîtres *Vibrio crassostreae* (Piel et al., 2021). Les bactériophages ont une influence importante dans la composition et l'abondance des différents taxa du microbiote phyllosphérique, et leur présence affecte même la capacité des microorganismes à coloniser de nouveaux hôtes végétaux (Morella et al., 2018). On observe également un intérêt grandissant de la communauté des phytobactériologues pour l'étude des bactériophages dans la mesure où ceux-ci constituent des alternatives prometteuses aux produits phytosanitaires traditionnels pour lutter contre les pathogènes de plantes (Ansaldi et al., 2020; Balogh et al., 2010). Ces dernières années, plusieurs études ont fait état de l'efficacité de stratégies de phagothérapie dans le biocontrôle de certains *Xanthomonas* (Gašić et al., 2018; Nga et al., 2021; Ranjani et al., 2018). Dans la continuité de cette thèse, une étude est en cours au laboratoire pour identifier les cibles moléculaires impliquées dans l'infectivité de trois bactériophages environnementaux sur *X. hortorum* pv. *vitians* grâce à l'utilisation de la librairie de Tn-seq en conditions *in vitro*. Cette utilisation du Tn-seq pour identifier les cibles des bactériophages a en effet fait la démonstration de son efficacité chez *E. coli* récemment (Cowley et al., 2018; Kortright et al., 2020).

Par la suite, l'application de la librairie de Tn-seq dans des conditions mimant au mieux l'infection naturelle de la laitue par *X. hortorum* pv. *vitians* dans le dernier chapitre a permis d'identifier pour la première fois 170 gènes critiques pour l'interaction hôte-pathogène. Dans l'ensemble, les résultats montrent que le Tn-seq possède un très fort pouvoir discriminant pour identifier ce qui relève de fonctions métaboliques et de composants cellulaires structuraux indispensables aux cellules bactériennes à titre individuel. En revanche, la limitation principale de cette méthode réside dans son incapacité à identifier des éléments indispensables au niveau de la population, qui peuvent être regroupés sous l'appellation de « biens communs » (*common goods*) (Friesen, 2020). Ainsi, nous n'avons identifié ni effecteurs de type III, ni CWDEs parmi les 170 gènes dont la mutation confère un défaut de valeur sélective chez *X. hortorum* pv. *vitians* dans la laitue. Une autre étude de RB-TnSeq chez *P. syringae* pv. *syringae* a abouti aux mêmes conclusions récemment (Helmann et al., 2019). Le Tn-seq ne semble donc pas approprié pour étudier l'importance des effecteurs et des enzymes sécrétées dans l'adaptation pathogène. Pour cela, il faudrait mettre au point une technique fiable de mutagenèse ciblée permettant de réaliser des mutants individuels pour des gènes d'intérêt préalablement identifiés par voie computationnelle.

Toutefois, il est possible de reconsidérer cette limitation technique de manière positive : en ne permettant pas d'étudier les effecteurs de type III, nous avons été amenés à réfléchir en dehors du cadre théorique principal qui voudrait parfois, de manière réductrice, que ceux-ci constituent l'alpha et l'oméga de l'adaptation moléculaire à la plante. Ainsi, la capacité à assimiler et métaboliser les nutriments disponibles dans l'environnement à un moment donné particulier du cycle infectieux, via des TBDTs par exemple, peut être considérée comme tout autant importante pour la valeur sélective d'un individu ou d'une population (Fatima & Senthil-Kumar, 2015). Les plantes peuvent d'ailleurs limiter la disponibilité de certains nutriments essentiels aux microorganismes afin de freiner leur développement, constituant ce qu'on appelle parfois une « immunité nutritionnelle » (Friesen, 2020). De la même manière, la capacité de dissimulation et de résistance de la bactérie vis-à-vis du système immunitaire végétal est une condition *sine qua non* pour la vie en association avec l'hôte végétal, et ce également pour des microorganismes commensaux ou mutualistes (Schlechter et al., 2019).

En guise de poursuite de ces travaux, on peut envisager de réaliser de nouvelles expériences de Tn-seq *in planta* avec plusieurs points de mesure au cours du temps, afin de vérifier nos hypothèses concernant l'implication différentielle de certains gènes au cours des phases de vie épiphyte, endophyte biotrophe et endophyte nécrotrophe. Il serait par ailleurs très intéressant de construire des bibliothèques de Tn-seq chez d'autres pathovars de *X. hortorum*, génétiquement proches du pv. *vitians* comme les pathovars *gardneri* et *cynarae*, ou bien plus distants comme les pv. *carotae* et *pelargonii*. La comparaison des gènes impliqués dans l'adaptation de chacun de ces pathovars à son hôte spécifique pourrait mettre en évidence à la fois un génome essentiel *in planta* commun, mais aussi potentiellement des déterminants spécifiques à chaque couple hôte-pathogène. Dans la mesure où seulement une partie du cycle de vie de *X. hortorum* pv. *vitians* se déroule en association avec la laitue, on pourrait également envisager d'utiliser la bibliothèque de Tn-seq pour mettre en évidence des déterminants moléculaires permettant la survie en conditions non-hôtes, par exemple en association épiphyte avec des adventices, dans le cycle de l'eau ou en association avec des débris végétaux enfouis.

Nous avons généralement tendance à ne considérer les microorganismes « pathogènes » que dans le cadre de leur cycle infectieux, qui ne représente souvent qu'une fraction des conditions environnementales et des modes trophiques qu'ils expérimentent au cours du cycle de vie. Cette vision fixiste du caractère pathogène des microorganismes

constitue un héritage historique du développement initial de la microbiologie dans le domaine de l'infectiologie humaine ou animale (Méthot & Alizon, 2014), mais ne recouvre pas la complexité des formes de vie que peuvent adopter les bactéries dites « phytopathogènes ». Par exemple, l'infection par *X. hortorum* pv. *vitians* ne présente pas un risque léthal pour les laitues, la transmission se fait de manière verticale par les graines, l'expression des symptômes ne se fait que dans des conditions pédoclimatiques bien particulières, les populations peuvent survivre de manière épiphyte et asymptomatique pendant de longues périodes... On voit bien ici que la pathogénicité n'est que le résultat d'une interaction complexe et multifactorielle, et non une propriété intrinsèque (Van Baalen & Jansen, 2001).



## CONCLUSION GÉNÉRALE

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Au début de ces travaux de thèse, il semblait que tout (ou presque) restait encore à étudier chez *Xanthomonas hortorum*. L'espèce était réduite à quelques pathovars peu étudiés, on utilisait encore l'appellation confuse de '*X. campestris* pv. *vitians*', et il n'existait pratiquement aucune donnée moléculaire ou génétique concernant ce pathogène de laitue et ses apparentés. Au fil des quelques années qu'ont duré ces travaux, nous nous sommes attachés à instaurer des fondations solides à l'étude de cette espèce phytopathogène atypique en la faisant entrer enfin dans l'ère de la génomique. Pour commencer, les contours taxonomiques de *X. hortorum* pv. *vitians* et de l'espèce en général ont été redéfinis à travers une approche innovante de taxonomie polyphasique mêlant des techniques anciennes et modernes. Ces premiers travaux ont permis de révéler que l'espèce *X. hortorum* était bien plus étendue et complexe qu'anticipé. Ensuite, la première étude moléculaire approfondie d'un génome de *X. hortorum* pv. *vitians* a été réalisée en caractérisant le génome essentiel par une approche de Tn-seq. L'étude de la conservation de génome essentiel au sein de la famille des *Xanthomonadaceae* a permis de créer du sens à partir de la simple liste de gènes essentiels en éclairant certains à travers une perspective écologique et évolutive. Enfin, la transposition de cette même technique dans le cadre de l'interaction hôte-pathogène entre *X. hortorum* pv. *vitians* et la laitue a révélé un panorama d'ensemble d'une partie des gènes et fonctions impliqués dans l'adaptation à l'hôte végétal. La contextualisation de ces résultats dans une perspective trophique amène à prendre de la hauteur et à considérer de manière dynamique l'interaction hôte-pathogène. En somme, sans prétendre avoir réglé tous les problèmes ni avoir répondu à toutes les questions, nous pouvons affirmer en revanche avoir ouvert grand la voie pour permettre à d'autres de se lancer dans l'étude de *X. hortorum* et de *X. hortorum* pv. *vitians* en particulier. En effet, l'ensemble des informations recueillies et des découvertes effectuées au cours de cette thèse a permis de révéler que la place de *X. hortorum* dans les écosystèmes est probablement bien plus importante que ce qui était attendu.





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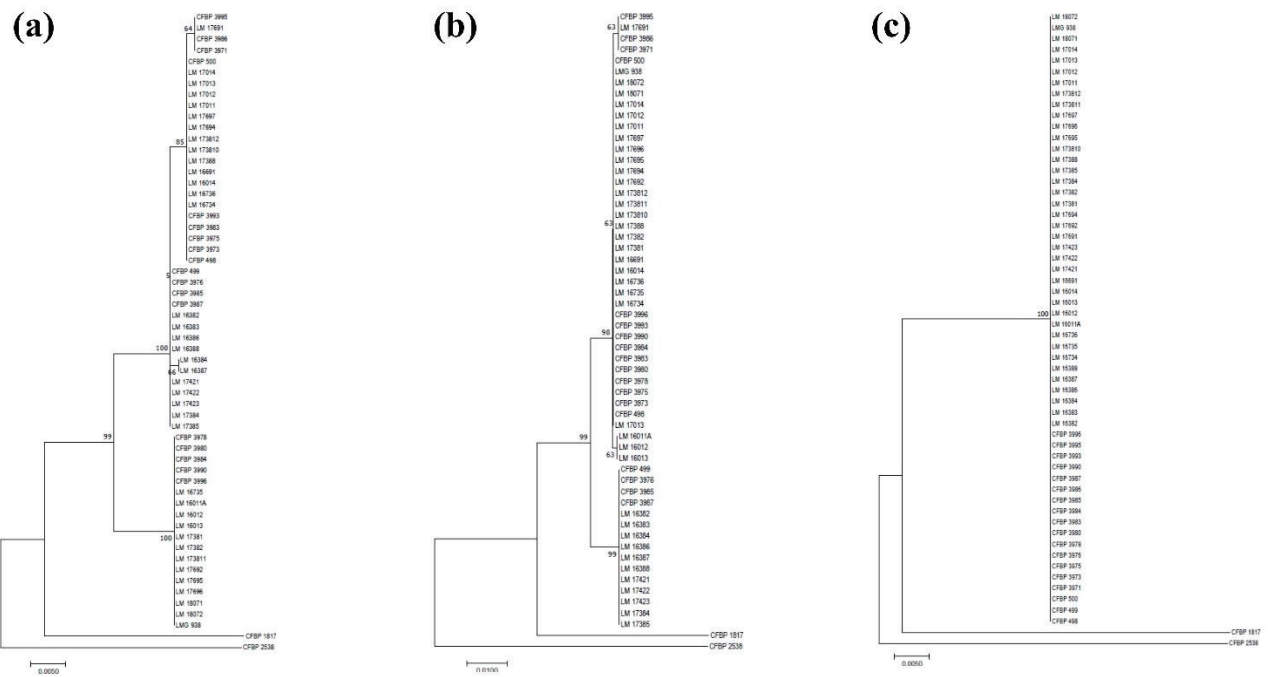
# ANNEXES

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Pour des raisons de place et afin de faciliter la lisibilité de ce manuscrit, les tableaux supplémentaires ne sont pas tous reproduits ici, mais sont accessibles en ligne.

## CHAPITRE I SUPPLEMENTARY DATA

Accessibilité : <https://doi.org/10.1016/j.syapm.2020.126087>



**Figure S1.** Neighbor-joining trees of strains associated with bacterial leaf spot of lettuce based on partial sequences of **(a)** *gapA*, **(b)** *gyrB* and **(c)** *rpoD*. Bootstrap values are indicated in percentages and were calculated on 500 replicates. Tree scales reflect nucleotide substitution per site. *X. populi* type strain CFBP 1817<sup>T</sup> and erroneous pathotype strain *X. axonopodis* pv. *vitians* CFBP 2538<sup>PT</sup> were chosen as outgroups.



**TABLE S3.** Pairwise ANIb (OrthoANI) and tetra nucleotide frequencies (Tetra) values among draft whole genome sequences of type, pathotype and representative strains of *X. hortorum* and *X. cynarae*. *X. populi* was chosen as an outgroup and type strains of *X. citri* and *X. axonopodis* were added for means of comparison to pathotype strain of *X. campestris* pv. *vitians*. ANIb values (%) are displayed in the lower triangle and Tetra values (%) in the higher triangle.

Proposed nomenclature	1	2	3	4	5	6	7	8	9	10	11	
<i>X. hortorum</i> pv. <i>cynarae</i> CFBP 4188 <sup>PT</sup>	1	-	0.99977	0.99973	0.99969	0.99855	0.9992	0.99952	0.99482	0.99263	0.99224	0.99202
<i>X. hortorum</i> pv. <i>gardneri</i> CFBP 8163 <sup>PT</sup>	2	99.2	-	0.99975	0.99969	0.99888	0.99925	0.99959	0.99473	0.9928	0.99238	0.99231
<i>X. hortorum</i> pv. <i>vitians</i> LMG 938 <sup>neopPT</sup>	3	98.3	98.1	-	0.99971	0.99872	0.99923	0.99969	0.99478	0.99297	0.99258	0.99251
<i>X. hortorum</i> pv. <i>taraxaci</i> CFBP 410 <sup>PT</sup>	4	97.4	97.3	97.5	-	0.99852	0.99923	0.99964	0.99461	0.99271	0.99242	0.99213
<i>X. hortorum</i> pv. <i>hederæ</i> CFBP 5858 <sup>T</sup>	5	95.8	95.6	95.8	95.4	-	0.99842	0.99865	0.99385	0.99321	0.99272	0.99243
<i>X. hortorum</i> pv. <i>carotæ</i> CFBP 7900	6	96.0	96.0	95.8	95.4	96.3	-	0.99911	0.99529	0.99289	0.99244	0.99274
<i>X. hortorum</i> pv. <i>pelargonii</i> CFBP 2533 <sup>PT</sup>	7	95.7	95.6	95.7	96.0	95.8	96.0	-	0.99465	0.99253	0.99224	0.99231
<i>X. populi</i> CFBP 1817 <sup>T</sup>	8	90.9	91.0	90.9	90.8	90.8	91.0	91.1	-	0.9897	0.98885	0.98951
<i>X. citri</i> CFBP 2538	9	86.6	86.7	86.6	86.7	86.7	86.6	86.6	86.0	-	0.99981	0.99842
<i>X. citri</i> CFBP 3369 <sup>T</sup>	10	86.7	86.7	86.8	86.9	86.7	86.6	86.6	86.1	98.4	-	0.99826
<i>X. axonopodis</i> pv. <i>axonopodis</i> CFBP 4924 <sup>T</sup>	11	86.4	86.5	86.4	86.4	86.5	86.4	86.4	85.7	93.0	93.0	-

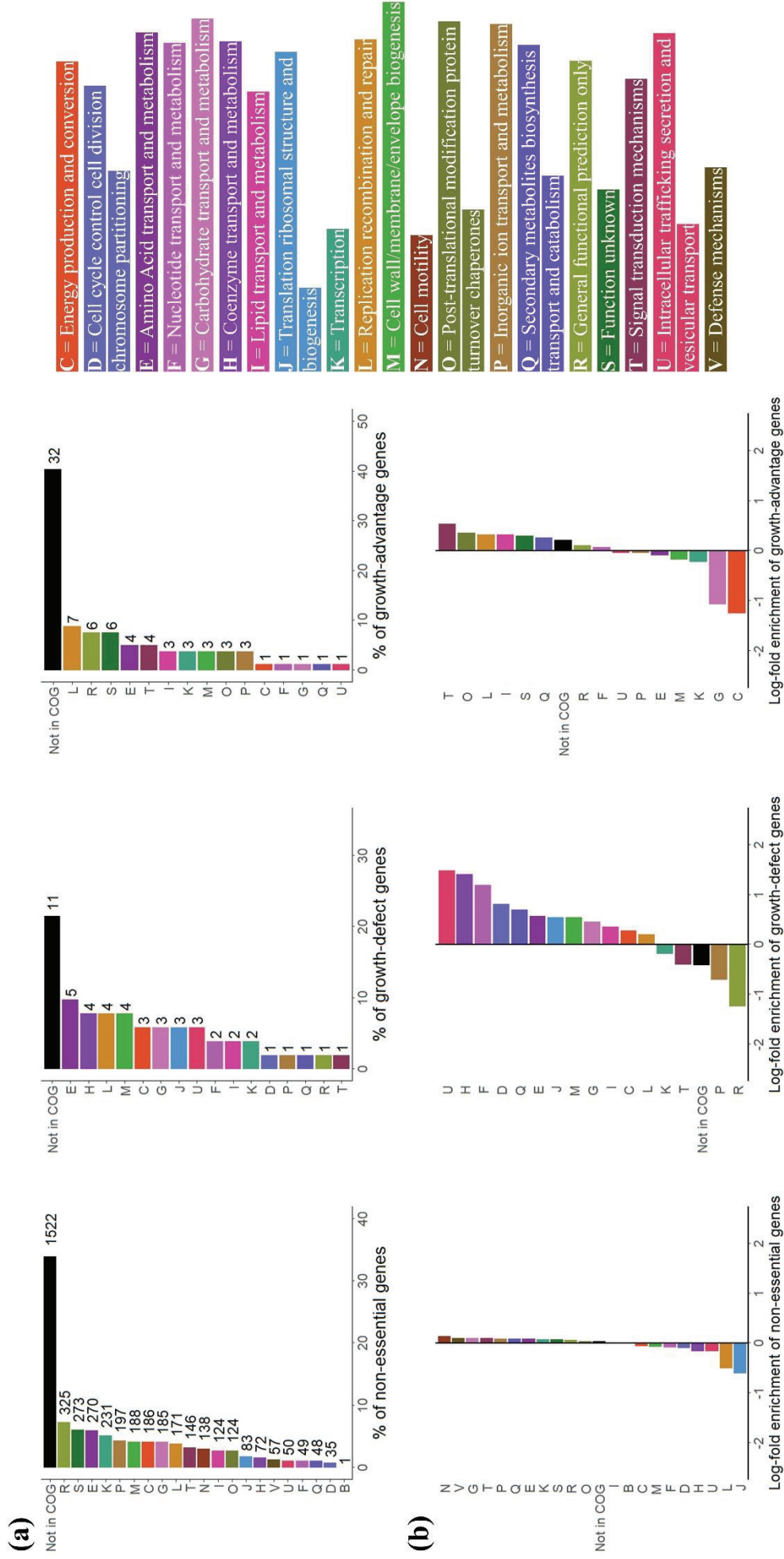
<sup>T</sup> = type strain, <sup>PT</sup> = pathotype strain, <sup>neopPT</sup> = neopathotype

# **CHAPITRE II**

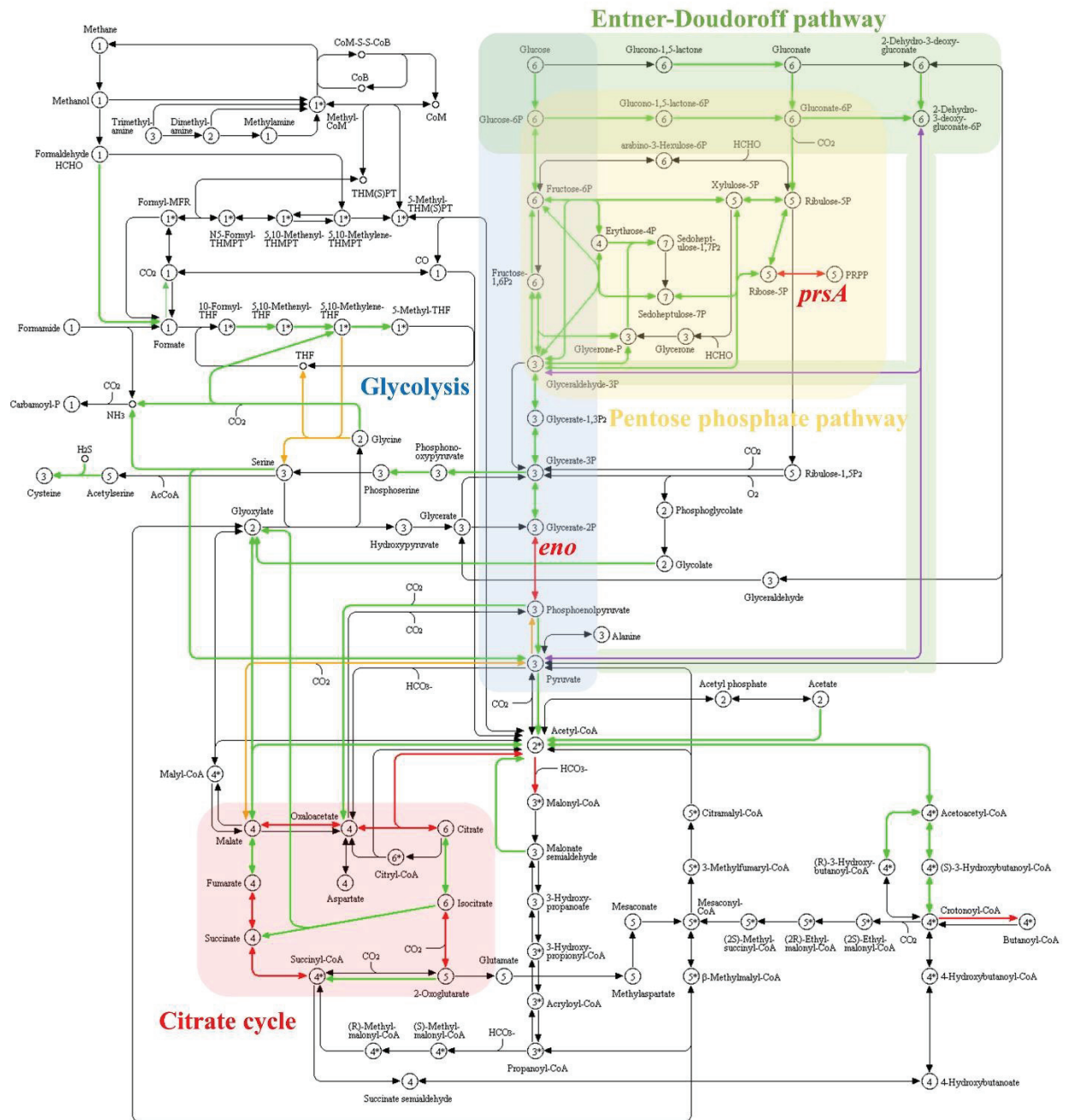
## **SUPPLEMENTARY DATA**

Accessibilité : [https:// figshare. com/ s/ aab51b0deba42f67e969](https://figshare.com/s/aab51b0deba42f67e969)

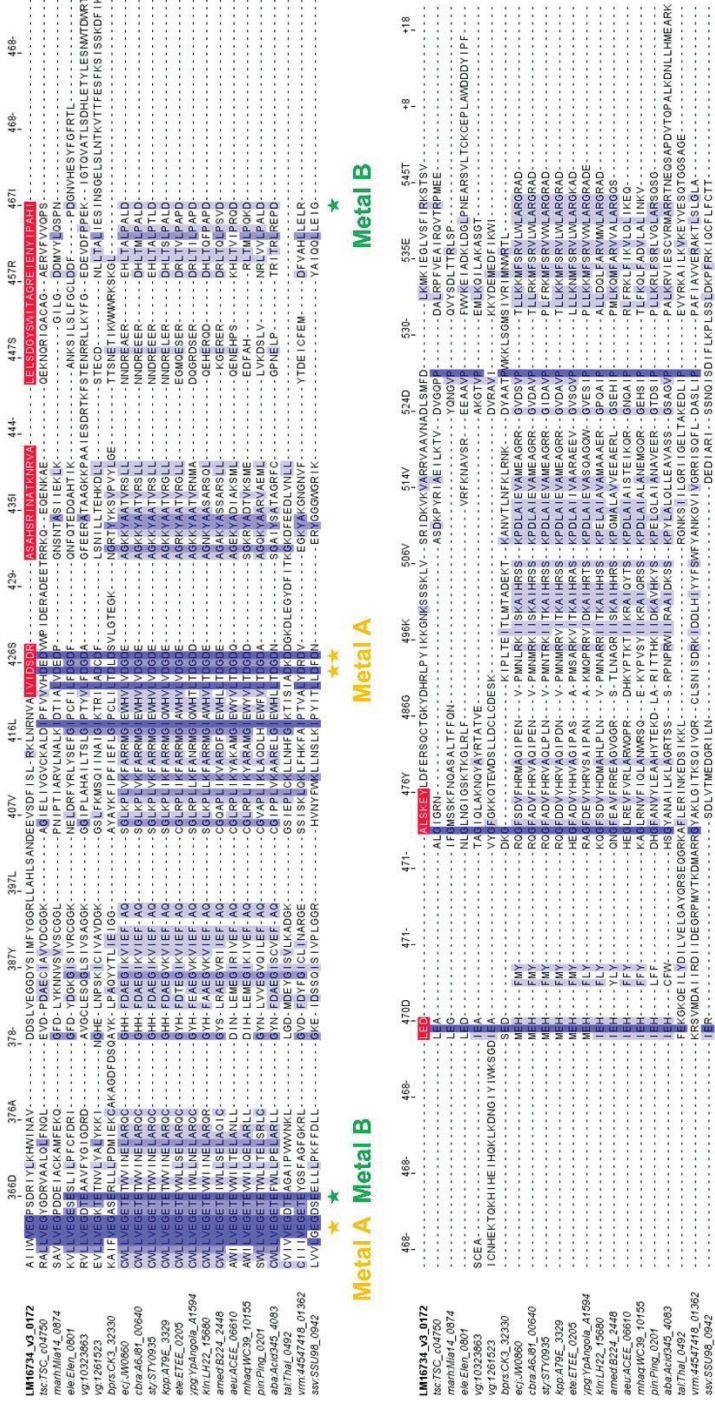




**Figure S1.** Cluster of Orthologous Groups (COG) analyses of genes identified as non-essential, growth-defect and growth-advantage. Number of genes and proportions of COG classes are displayed in **(a)** and log-fold enrichment of COG categories are shown in **(b)**.



**Figure S2.** KEGG Mapper-generated carbon metabolism pathway map of *X. hortorum* pv. *vitians* LM16734. Number of carbons for each compound is noted in circle except for cofactors that are represented by asterisks. Green lines correspond to non-essential reactions, red lines to essential reactions, orange lines to growth-defect reactions, purple lines to undetermined reactions and black lines to reactions not detected in *Xanthomonas*. Shaded areas correspond to glycolysis pathway (blue), pentose phosphate pathway (yellow), Entner-Doudoroff pathway (green), and citrate cycle (red). Essential genes *eno* (enolase) and *prsA* (ribose-phosphate pyrophosphokinase) discussed in the article are indicated in red next to the associated reactions.



**Figure S3.** TOPRIM domains alignment of representative class 1 OLD family endonucleases, according to Schiltz *et al.* 2020 (96). Alignment was produced with MAFFT using the E-INS-I method, and visualization was achieved with Jalview. Essential residues of XHV734\_0172 protein are shown in red and metal B binding residues as described in Schiltz *et al.* 2020 are indicated by orange and green stars. Blue gradient represents sequence conservation. XHV734\_0172 sequence was used as a reference for the scale above alignment. Sequence identifiers corresponds to KEGG IDs for OLD nucleases from *Thermus scotoductus* (tsc:TSC\_c04750), *Escherichia coli* phage P2 (vg:1261523), *Escherichia coli* (ecj:JW0860), *Actinobacillus equuli* (aeu:ACEE\_06610), *Aeromonas media* (amed:B224\_2448), *Citrobacter braakii* (cbra:A6J81\_00640), *Edwardstiella anguillarum* (ete:ETEE\_0205), *Klebsiella pneumoniae* (kpp:A79E\_3329), *Mannheimia haemolytica* (mhaq:WC39\_10155), *Pantoea rwandensis* (kln:LH22\_15680), *Psychromonas ingrahamii* (pin:Ping\_0201), *Salmonella enterica* (sty:STY0935), *Yersinia pestis* Angola (ypg:YpAngola\_A1594), *Candidatus Micrarchaeota* (math:Mia14\_0874), *Koribacter versatilis* (aba:Acid345\_4083), *Eggerthella lenta* (ele:Eleen\_0801), *Thermocrinis albus* (tai:Thal\_0492), Butyrate-producing bacterium SS3/4.

**TABLE S1.** Genomes used in this study

Taxonomic name	Strain	GenBank assembly accession number	Sequencing technology	Genome size (Mb)	G+C content (%)	No. of contigs	Contig N50 (bp)	Coverage	Reference
<b><i>Xanthomonas</i></b>									
<i>X. albilineans</i>	CFBP 2523 <sup>T</sup>	GCA_002939705.1	Illumina HiSeq	3.68	63.1	151	168,122	302.0x	Merda <i>et al.</i> 2017
<i>X. arboricola</i> pv. <i>juglandis</i>	CFBP 2528 <sup>T</sup>	GCA_001013475.1	Illumina HiSeq	5.09	65.5	19	431,859	607.0x	Cesbron <i>et al.</i> 2015
<i>X. arboricola</i> pv. <i>arracaciae</i>	CFBP 7407 <sup>PT</sup>	GCA_002940565.1	Illumina HiSeq	5.16	65.2	232	62,305	118.0x	Merda <i>et al.</i> 2017
<i>X. arboricola</i> pv. <i>celebensis</i>	CFBP 3523 <sup>PT</sup>	GCA_000724925.1	Illumina HiSeq	4.91	65.6	75	172,772	140.0x	Harrison <i>et al.</i> 2016
<i>X. arboricola</i> pv. <i>corylina</i>	CFBP 1159 <sup>PT</sup>	GCA_002939845.1	Illumina HiSeq	5.11	65.5	125	134,548	154.0x	Merda <i>et al.</i> 2017
<i>X. arboricola</i> pv. <i>guizotiae</i>	CFBP 7408 <sup>PT</sup>	GCA_002940205.1	Illumina MiSeq	4.94	65.6	161	105,503	60.0x	Merda <i>et al.</i> 2017
<i>X. arboricola</i> pv. <i>populi</i>	CFBP 3123 <sup>PT</sup>	GCA_002939945.1	Illumina HiSeq	4.65	65.5	28	597,835	128.0x	Merda <i>et al.</i> 2017
<i>X. arboricola</i> pv. <i>pruni</i>	CFBP 3894 <sup>PT</sup>	GCA_001741965.1	Illumina HiSeq	5.06	65.4	78	162,135	~50.0x	López-Soriano <i>et al.</i> 2016
<i>X. arboricola</i> pv. <i>zantedeschiae</i>	CFBP 7410 <sup>PT</sup>	GCA_002940625.1	Illumina HiSeq	4.98	65.5	37	645,945	293.0x	Merda <i>et al.</i> 2017
<i>X. axonopodis</i> pv. <i>axonopodis</i>	CFBP 4924 <sup>T</sup>	GCA_001304695.1	Illumina MiSeq	4.48	64.5	325	25,955	158.0x	Midha and Patil 2014
<i>X. bromi</i>	CFBP 1976 <sup>T</sup>	GCA_002939755.1	Illumina HiSeq	4.90	64.0	223	130,879	379.0x	Merda <i>et al.</i> 2017
<i>X. campestris</i> pv. <i>campestris</i>	CFBP 5251 <sup>T</sup>	GCA_000007145.1	NA	5.08	65.1	1	-	NA	da Silva <i>et al.</i> 2002
<i>X. campestris</i> pv. <i>incanae</i>	CFBP 2527 <sup>PT</sup>	GCA_00059035.1	Illumina HiSeq	4.93	65.1	6	2,485,103	1127.0x	Roux <i>et al.</i> 2015
<i>X. campestris</i> pv. <i>raphani</i>	CFBP 5828 <sup>PT</sup>	GCA_000590355.1	Illumina HiSeq	4.91	65.4	11	1,210,840	1017.0x	Roux <i>et al.</i> 2015
<i>X. campestris</i> pv. <i>barbarae</i>	CFBP 5825 <sup>PT</sup>	GCA_000590375.1	Illumina HiSeq	5.06	65.1	8	2,773,827	687.0x	Roux <i>et al.</i> 2015
<i>X. cannabidis</i> pv. <i>cannabidis</i>	CFBP 8442 <sup>T</sup>	GCA_000802365.1	Illumina HiSeq	4.76	65.8	257	38,306	69.0x	Jacobs <i>et al.</i> 2015
<i>X. cassavae</i>	CFBP 4642 <sup>T</sup>	GCA_000454545.1	Illumina HiSeq	5.26	65.2	83	158,383	3045.0x	Bolot <i>et al.</i> 2013
<i>X. citri</i> subsp. <i>citri</i>	CFBP 3369 <sup>T</sup>	GCA_002018575.1	Illumina MiSeq	5.14	64.6	206	84,312	142.0x	Bansal <i>et al.</i> 2017
<i>X. citri</i> pv. <i>fuscans</i>	CFBP 6165 <sup>PT</sup>	GCA_000788075.2	Illumina MiSeq	4.96	64.9	136	74,01	40.0x	Aritua <i>et al.</i> 2015
<i>X. citri</i> pv. <i>glycines</i>	CFBP 2526 <sup>PT</sup>	GCA_000495275.1	Illumina HiSeq	5.25	64.6	25	423,865	2941.0x	Darrasse <i>et al.</i> 2013
<i>X. citri</i> pv. <i>mahveaarum</i>	CFBP 2530 <sup>PT</sup>	GCA_003064125.1	Illumina MiSeq	5.23	64.4	179	81,416	107.0x	Michalopoulou <i>et al.</i> 2018
<i>X. citri</i> pv. <i>punicae</i>	CFBP 8455 <sup>PT</sup>	GCA_000285775.1	Roche 454	4.95	64.9	217	45,854	100.0x	Sharma <i>et al.</i> 2012
<i>X. citri</i> pv. <i>vignicola</i>	CFBP 7112 <sup>PT</sup>	GCA_002218265.1	PacBio	5.16	64.7	3	-	100.0x	Ruh <i>et al.</i> 2017
<i>X. citri</i> pv. <i>viticola</i>	CFBP 7660 <sup>PT</sup>	GCA_000723725.1	Roche 454	5.10	64.5	50	306,265	58.0x	Midha and Patil 2014
<i>X. codiae</i>	CFBP 4690 <sup>T</sup>	GCA_002939785.1	Illumina HiSeq	5.04	66.0	110	134,676	289.0x	Merda <i>et al.</i> 2017
<i>X. cucurbitae</i>	CFBP 2542 <sup>T</sup>	GCA_002939885.1	Illumina HiSeq	4.42	65.4	181	105,102	229.0x	Merda <i>et al.</i> 2017
<i>X. dyei</i>	CFBP 7245 <sup>T</sup>	GCA_002939865.1	Illumina HiSeq	5.31	64.3	152	112,759	269.0x	Merda <i>et al.</i> 2017
<i>X. euvesicatoria</i> pv. <i>euvesicatoria</i>	CFBP 6864	GCA_001854165.1	PacBio	5.42	64.6	4	-	192.0x	Seong <i>et al.</i> 2016
<i>X. euvesicatoria</i> pv. <i>alfalfae</i>	CFBP 3836 <sup>PT</sup>	GCA_000488955.1	Illumina HiSeq	5.08	64.7	22	763,181	3086.0x	Jacques <i>et al.</i> 2013
<i>X. euvesicatoria</i> pv. <i>allii</i>	CFBP 6369 <sup>PT</sup>	GCA_000730305.1	Illumina HiSeq	5.43	64.3	13	1,415,587	700.0x	Gagnevin <i>et al.</i> 2014

**TABLE S1. Continued**

<i>X. euvesicatoria</i> pv. <i>citrumelonis</i>	<b>CFBP 3371<sup>PT</sup></b>	GCA_002939715.1	Illumina HiSeq	4.93	65.0	49	357,474	125.0x	Merdah <i>et al.</i> 2017
<i>X. euvesicatoria</i> pv. <i>perforans</i>	<b>CFBP 7293<sup>PT</sup></b>	GCA_001976075.1	Illumina MiSeq	5.01	64.9	32	508,791	80.0x	Richard <i>et al.</i> 2017
<i>X. floridensis</i>	<b>CFBP 8601<sup>T</sup></b>	GCA_001642575.1	Illumina HiSeq	5.25	64.5	58	150,157	378.0x	Vicente <i>et al.</i> 2017
<i>X. fragariae</i>	<b>CFBP 2157<sup>T</sup></b>	GCA_900380235.1	Illumina HiSeq	3.97	62.1	329	25,701	87.0x	Gétaz <i>et al.</i> 2018
<i>X. hortorum</i> pv. <i>hederae</i>	<b>CFBP 5858<sup>T</sup></b>	GCA_002940005.1	Illumina HiSeq	5.32	63.8	313	41,465	214.0x	Merda <i>et al.</i> 2017
<i>X. hortorum</i> pv. <i>carotae</i>	CFBP 7900	GCA_000505565.1	Illumina GAIIX	5.03	63.8	287	29,662	525.0x	Kimbrel <i>et al.</i> 2011
<i>X. hortorum</i> pv. <i>cynarae</i>	<b>CFBP 4188<sup>PT</sup></b>	GCA_002939985.1	Illumina HiSeq	5.06	63.7	103	145,505	221.0x	Merda <i>et al.</i> 2017
<i>X. hortorum</i> pv. <i>gardneri</i>	<b>CFBP 8163<sup>PT</sup></b>	GCA_012922265.1	Illumina HiSeq	5.15	63.7	121	125,969	311.8x	Morinière <i>et al.</i> 2020
	CFBP 8588	GCA_001908755.1	PacBio	5.42	63.5	3	-	144.0x	Richard <i>et al.</i> 2017
<i>X. hortorum</i> pv. <i>pelargonii</i>	<b>CFBP 2533<sup>PT</sup></b>	GCA_012922215.1	Illumina HiSeq	5.21	63.8	94	134,256	360.1x	Morinière <i>et al.</i> 2020
<i>X. hortorum</i> pv. <i>taraxaci</i>	<b>CFBP 410<sup>PT</sup></b>	GCA_012922225.1	Illumina HiSeq	4.92	63.9	124	76,729	456.4x	Morinière <i>et al.</i> 2020
<i>X. hortorum</i> pv. <i>vitians</i>	<b>LMG938<sup>noPT</sup></b>	GCA_012922135.1	Illumina MiSeq	5.03	63.8	119	141,718	77.4x	Morinière <i>et al.</i> 2020
	LM16734	GCA_014338485.1	PacBio, Illumina NovaSeq	5.27	63.6	2	-	587.5x	This study
	LM 16388	GCA_012922175.1	Illumina HiSeq	5.08	63.7	121	115,523	523.1x	Morinière <i>et al.</i> 2020
	LM 16735	GCA_012922125.1	Illumina HiSeq	5.19	63.7	138	118,265	420.8x	Morinière <i>et al.</i> 2020
	CFBP 498	GCA_012922255.1	Illumina HiSeq	5.57	63.3	147	129,226	527.6x	Morinière <i>et al.</i> 2020
	CFBP 499	GCA_012922335.1	Illumina NovaSeq	5.17	63.7	132	118,955	353.8x	Morinière <i>et al.</i> 2020
	CFBP 3978	GCA_012922195.1	Illumina HiSeq	5.14	63.7	131	141,718	612.5x	Morinière <i>et al.</i> 2020
	B07-007	GCA_002285515.1	PacBio, Illumina HiSeq	5.25	63.6	2	-	2681.9x	Xu <i>et al.</i> 2017 (unpublished)
	CFBP 7999	GCA_001908775.1	PacBio	5.63	63.3	4	-	167.0x	Richard <i>et al.</i> 2017
<i>X. hyacinthi</i>	<b>CFBP 1156<sup>T</sup></b>	GCA_002939895.1	Illumina HiSeq	4.91	68.1	104	208,336	114.0x	Merda <i>et al.</i> 2017
<i>X. 'naliensis'</i>	<b>CFBP 7942<sup>T</sup></b>	GCA_009192945.1	Illumina MiSeq	5.20	66.2	141	77,591	155.0x	Triplett <i>et al.</i> 2015
<i>X. melonis</i>	<b>CFBP 4644<sup>T</sup></b>	GCA_002940015.1	Illumina HiSeq	4.68	66.1	37	418,571	105.0x	Merda <i>et al.</i> 2017
<i>X. nasturtii</i>	<b>CFBP 8602<sup>T</sup></b>	GCA_001660815.1	Illumina HiSeq	4.87	64.5	89	101,995	387.0x	Vicente <i>et al.</i> 2017
<i>X. phaseoli</i> pv. <i>phaseoli</i>	<b>CFBP 6546<sup>T</sup></b>	GCA_000588855.1	Illumina HiSeq	4.99	64.9	28	399,888	2100.0x	Chen <i>et al.</i> 2018
<i>X. phaseoli</i> pv. <i>dieffenbachiae</i>	<b>CFBP 3133<sup>PT</sup></b>	GCA_001564415.1	Illumina HiSeq	5.04	64.9	10	1,343,781	768.0x	Robene <i>et al.</i> 2016
<i>X. phaseoli</i> pv. <i>manihotis</i>	<b>CFBP 7153<sup>PT</sup></b>	GCA_001482665.1	Illumina MiSeq	5.02	64.8	205	68,71	169.0x	Midha and Patil 2015 (unpublished)
<i>X. pisi</i>	<b>CFBP 4643<sup>T</sup></b>	GCA_002940045.1	Illumina HiSeq	5.33	65.1	81	208,648	178.0x	Merda <i>et al.</i> 2017
<i>X. populi</i>	<b>CFBP 1817<sup>T</sup></b>	GCA_002940065.1	Illumina HiSeq	4.33	63.3	410	25,166	148.0x	Merda <i>et al.</i> 2017
<i>X. prunicola</i>	<b>CFBP 8353<sup>T</sup></b>	GCA_002846205.1	Illumina MiSeq	5.32	64.0	95	767,534	300.0x	López <i>et al.</i> 2018
<i>X. sacchari</i>	<b>CFBP 4641<sup>T</sup></b>	GCA_002940085.1	Illumina HiSeq	4.92	69.1	57	222,197	213.0x	Merda <i>et al.</i> 2017
<i>X. theicola</i>	<b>CFBP 4691<sup>T</sup></b>	GCA_002940605.1	Illumina HiSeq	4.54	68.4	435	32,023	305.0x	Merda <i>et al.</i> 2017
<i>X. translucens</i> pv. <i>translucens</i>	<b>CFBP 2054<sup>T</sup></b>	GCA_900094325.1	PacBio, Illumina MiSeq	4.72	67.7	1	-	223.0x	Jaenicke <i>et al.</i> 2016
<i>X. translucens</i> pv. <i>arrhenatheri</i>	<b>CFBP 2056<sup>PT</sup></b>	GCA_001282805.1	Illumina MiSeq	4.75	68.4	89	89,164	109.0x	Hersemann <i>et al.</i> 2016

**TABLE S1.** Continued

<i>X. translucens</i> pv. <i>cerealis</i>	<b>CFBP 2041<sup>PT</sup></b>	GCA_000807145.1	Illumina HiSeq	4.52	67.3	31	1,339,657	1926.0x	Pesce et al. 2015
<i>X. translucens</i> pv. <i>graminis</i>	<b>CFBP 2053<sup>PT</sup></b>	GCA_001269865.1	Illumina HiSeq	4.34	68.3	48	126,262	691.0x	Pesce et al. 2015
<i>X. translucens</i> pv. <i>phlei</i>	<b>CFBP 2062<sup>PT</sup></b>	GCA_001282885.1	Illumina MiSeq	4.40	68.4	142	59,681	315.0x	Hersemann et al. 2016
<i>X. translucens</i> pv. <i>poae</i>	<b>CFBP 2057<sup>PT</sup></b>	GCA_001282765.1	Illumina MiSeq	4.61	68.3	190	37,264	249.0x	Hersemann et al. 2016
<i>X. translucens</i> pv. <i>undulosa</i>	Xtu4699	GCA_001021935.1	PacBio, Illumina MiSeq	4.56	68.1	1	-	> 60.0x	Peng et al. 2016
<i>X. oryzae</i> pv. <i>oryzae</i>	<b>CFBP 2532<sup>T</sup></b>	GCA_000482445.1	Illumina HiSeq	4.29	63.9	299	28,674	NA	Kyprides et al. 2013 (unpublished)
<i>X. oryzae</i> pv. <i>oryzicola</i>	<b>CFBP 2543<sup>PT</sup></b>	GCA_001042735.1	PacBio	5.01	64.0	2	-	296.0x	Wilkins et al. 2015
<i>X. vasicola</i> pv. <i>holcicola</i>	<b>CFBP 2543<sup>T</sup></b>	GCA_000772705	Illumina HiSeq, Illumina MiSeq	4.93	63.3	96	125,807	215.0x	Studholm et al. 2014 (unpublished)
<i>X. vesicatoria</i>	<b>CFBP 4645<sup>T</sup></b>	GCA_000192025.2	Roche 454, Illumina GA2	5.53	64.1	296	41,380	7.78x, 115x	Potnis et al. 2011
<b><i>Stenotrophomonas</i></b>									
<i>Stenotrophomonas maltophilia</i>	<b>CFBP 3035<sup>T</sup></b>	GCA_900186865.1	PacBio	5.00	66.1	1	-	156.0x	<a href="https://www.sanger.ac.uk/resources/downloads/bacteria/hctc/">https://www.sanger.ac.uk/resources/downloads/bacteria/hctc/</a>
	K279a	GCA_000072485.1	Sanger	4.85	66.3	1	-	11.05x	Crossman et al. 2008
	AB550	GCA_002189545.2	PacBio	4.94	66.5	1	-	306.0x	Glady-Croue et al. 2018
<b><i>Xylella</i></b>									
<i>Xylella fastidiosa</i> subsp. <i>fastidiosa</i>	<b>CFBP 7970<sup>T</sup></b>	GCA_004016315.1	Illumina MiSeq	2.49	51.5	92	104,928	900.0x	Denancé et al. 2019
<i>Xylella fastidiosa</i> subsp. <i>multiplex</i>	<b>CFBP 8173<sup>T</sup></b>	GCA_000428665.1	Illumina HiSeq	2.42	51.6	66	102,517	NA	Kyprides et al. 2013 (unpublished)
<i>Xylella fastidiosa</i> subsp. <i>paucica</i>	De Donno	GCA_002117875.1	PacBio, Illumina HiSeq	2.54	52.0	2	-	636.0x	Giampetruzzi et al. 2017

**Table S3.** List of the 370 essential loci of *X. hortorum* pv. *vitians* LM16734 in rich medium identified through Tn-Seq

Locus	Gene	Function	Number of TA sites	Number of essential sites	Mean read count over non-zero TA sites	Insertion rate	COG categories <sup>a</sup>	COG identifier	KO identifier	Number of homologs in DEGI10 <sup>b</sup>	Conservation <sup>c</sup>	
											Family	Genus
<b>Protein-coding genes</b>												
XHV734_0001	<i>dnaA</i>	Chromosomal replication initiator protein DnaA, DNA-binding transcriptional dual regulator	11	11	0	0	L	COG0593	K02313	34		
XHV734_0002	<i>dnaN</i>	DNA polymerase III, beta subunit	13	13	0	0	L	COG0592	K02338	17		
XHV734_0005	<i>gyrB</i>	DNA gyrase, subunit B	29	28	21	0.0345	L	COG0187	K02470	73		
XHV734_0153	<i>fabY</i>	Enoyl-[acyl-carrier-protein] reductase [NADH]	15	14	3	0.0667	S	COG3007	K00209	3		
XHV734_0172	-	Putative ATP-dependent endonuclease, OLD family	117	15	53.21	0.4872	R	COG1106	-	none		
XHV734_0508	<i>ubiA</i>	p-hydroxybenzoate octaprenyltransferase	17	16	47	0.0588	H	COG0382	K03179	16		
XHV734_0570	<i>phaF</i>	Putative K(+)/H(+) antiporter subunit F	6	5	20	0.1667	P	COG2212	K05563	2		
XHV734_0571	<i>phaE</i>	Na(+)/H(+) antiporter subunit E	6	6	0	0	P	COG1863	K05562	2		
XHV734_0572	<i>phaD</i>	Na(+)/H(+) antiporter subunit D	20	20	2	0.05	C	COG0651	K05561	5		
XHV734_0573	<i>phaC</i>	Putative K(+)/H(+) antiporter subunit C	4	4	0	0	P	COG1006	K05560	4		
XHV734_0574	<i>phaAB</i>	Putative K(+)/H(+) antiporter subunit A/B	28	28	1	0.0357	C	COG1009	K05559	7		
XHV734_0646	<i>speD</i>	S-adenosylmethionine decarboxylase	24	23	62.5	0.0833	E	COG1586	K01611	none		
XHV734_0649	<i>rpmM</i>	50S ribosomal subunit protein L13	15	15	1	0.0667	J	COG0102	K02871	37		
XHV734_0650	<i>rpsL</i>	30S ribosomal subunit protein S9	6	4	18	0.1667	J	COG0103	K02996	29		
XHV734_0686	<i>accC</i>	Acetyl-CoA carboxylase, biotin carboxylase subunit	15	15	1	0.0667	I	COG0439	K01961	34		
XHV734_0688	<i>accB</i>	Acetyl CoA carboxylase, BCCP subunit	3	3	0	0	I	COG0511	K02160	23		
XHV734_0696	<i>groS</i>	Cpn10 chaperonin GroES, small subunit of GroESL	5	5	0	0	O	COG0234	K04078	31		
XHV734_0697	<i>groL</i>	Cpn60 chaperonin GroEL, large subunit of GroESL	11	11	0	0	O	COG0459	K04077	32		
XHV734_0869	<i>ubiE</i>	Bifunctional 2-octaprenyl-6-methoxy-1,4-benzoquinone methyltransferase	12	11	37	0.0833	H	COG2226	K03183	22		
XHV734_0884	<i>mreB</i>	Cell wall structural complex MreBCD, actin-like component MreB	11	11	0	0	D	COG1077	K03569	24		
XHV734_0885	<i>mreC</i>	Rod shape-determining protein MreC	7	7	0	0	M	COG1792	K03570	7		
XHV734_0886	<i>mreD</i>	Rod shape-determining protein MreD	7	7	0	0	M	COG2891	K03571	7		
XHV734_0887	<i>mrdA</i>	Peptidoglycan D,D-transpeptidase MrdA	28	28	0	0	M	COG0768	K05515	15		
XHV734_0888	<i>mrdB</i>	Cell wall shape-determining protein	12	11	6	0.0833	D	COG0772	K05837	39		
XHV734_0890	<i>mltB</i>	Membrane-bound lytic murein transglycosylase B precursor	21	21	0	0	M	COG2951	K08305	none		
XHV734_0891	<i>rlpA</i>	Rare lipoprotein A precursor	20	19	294	0.05	M	COG0797	K03642	1*		
XHV734_0895	-	Proposed lipote regulatory protein YbeD	2	2	0	0	S	COG2921	K09158	1*		
XHV734_0896	<i>lipB</i>	Octanoyltransferase	11	11	0	0	H	COG0321	K03801	15		
XHV734_0897	<i>lipA</i>	Lipoate synthase	11	11	0	0	H	COG0320	K03644	15		
XHV734_0917	-	Conserved protein of unknown function	21	18	20.25	0.1905	S	COG1565	-	4		

**Table S3.** Continued

XHV734_0947	<i>cca</i>	Fused tRNA nucleotidyl transferase ; 2'3'-cyclic phosphodiesterase and 2'nucleotidase and phosphatase	21	21	0	0	J	COG0617	K00974	19
XHV734_0961	<i>gshA</i>	Glutamate--cysteine ligase	19	18	50	0.1053	H	COG3572	K01919	5
XHV734_1010	<i>rsgA</i>	Small ribosomal subunit biogenesis GTPase RsgA	13	13	1	0.0769	R	COG1162	K06949	6
XHV734_1040	<i>waaA</i>	3-deoxy-D-manno-octulosonic acid transferase	23	23	0	0	M	COG1519	K02527	20
XHV734_1074	<i>adk</i>	Adenylate kinase	6	6	0	0	F	COG0563	K00939	29
XHV734_1123	<i>spoT</i>	Bifunctional (p)ppGpp synthetase II and guanosine-3',5'-bis pyrophosphate 3'-pyrophosphohydrolyase	23	22	50	0.087	K	COG0317	K01139	18
XHV734_1127	<i>rpmE</i>	50S ribosomal protein L31 type B	9	9	1	0.2222	J	COG0254	K02909	11
XHV734_1128	<i>glbA</i>	Citrate synthase	26	23	65.25	0.1538	C	COG0372	K01647	11
XHV734_1248	<i>coaE</i>	Dephospho-CoA kinase	11	10	164	0.0909	H	COG0237	K00859	30
XHV734_1249	<i>xpsO</i>	Leader peptidase / N-methyltransferase	13	13	0	0	N	COG1989	K02654	1
XHV734_1255	<i>sucC</i>	Succinyl-CoA synthetase, beta subunit	12	12	1	0.1667	C	COG0045	K01903	8
XHV734_1256	<i>sueD</i>	Succinyl-CoA synthetase, NAD(P)-binding, alpha subunit	8	7	183.5	0.25	C	COG0074	K01902	8
XHV734_1264	<i>bamD</i>	Outer membrane protein assembly factor BamD	25	25	1	0.04	R	COG4105	K05807	9
XHV734_1327	<i>aspS</i>	Aspartyl-tRNA synthetase	20	20	0	0	J	COG0173	K01876	35
XHV734_1335	<i>ybgC</i>	Acyl-CoA thioesterase	9	9	0	0	R	COG0824	K07107	4
XHV734_1336	<i>tolQ</i>	Membrane spanning protein in TolA-TolQ-TolR complex	12	12	7	0.0833	U	COG0811	K03562	8
XHV734_1337	<i>tolR</i>	Tol-Pal system protein TolR	4	4	0	0	U	COG0848	K03560	11
XHV734_1338	<i>tolA</i>	TolA protein	9	8	5	0.1111	M	COG3064	K03646	none
XHV734_1339	<i>tolB</i>	Periplasmic protein	32	31	126	0.0312	U	COG0823	K03641	9
XHV734_1340	<i>pal</i>	Peptidoglycan-associated lipoprotein	9	8	61.5	0.2222	M	COG2885	K03640	8
XHV734_1376	<i>flh</i>	Signal Recognition Particle (SRP) component with 4.5S RNA (ffs)	8	8	0	0	U	COG0541	K03106	56
XHV734_1380	<i>rpsP</i>	30S ribosomal subunit protein S16	6	6	0	0	J	COG0228	K02959	28
XHV734_1381	<i>rimM</i>	Ribosome maturation factor RimM	7	7	0	0	J	COG0806	K02860	18
XHV734_1382	<i>trmD</i>	tRNA (guanine-1-)-methyltransferase	10	10	186.5	0.2	J	COG0336	K00554	30
XHV734_1438	<i>lepB</i>	Signal peptidase I	12	12	1	0.0833	U	COG0681	K03100	19
XHV734_1439	-	Conserved protein of unknown function	10	6	49.75	0.4	Not in COG	-	-	none
XHV734_1440	<i>rnk</i>	RNase III	5	5	1	0.2	K	COG0571	K03685	15
XHV734_1441	<i>era</i>	Membrane-associated, 16S rRNA-binding GTPase	9	6	241.5	0.2222	R	COG1159	K03595	48
XHV734_1520	<i>metG</i>	Methionyl-tRNA synthetase	27	26	54	0.037	J	COG0143	K01874	16
XHV734_1594	<i>accA</i>	Acetyl-CoA carboxylase, carboxytransferase, alpha subunit	13	11	48.5	0.1538	I	COG0825	K01962	27
XHV734_1595	<i>dnaE</i>	DNA polymerase III alpha subunit	35	35	0	0	L	COG0587	K02337	36
XHV734_1597	<i>lpxB</i>	Tetraacyldisaccharide-1-P synthase	17	17	0	0	M	COG0763	K00748	23
XHV734_1598	<i>lpxA</i>	UDP-N-acetylglucosamine acetyltransferase	7	7	0	0	M	COG1043	K00677	28
XHV734_1599	<i>fabZ</i>	(3R)-hydroxymyristol acyl carrier protein dehydratase	8	8	1	0.125	I	COG0764	K02372	29
XHV734_1600	<i>lpxD</i>	UDP-3-O-(3-hydroxymyristoyl)-glucosamine N-acyltransferase	8	8	0	0	M	COG1044	K02536	24
XHV734_1602	<i>bamA</i>	Outer membrane protein assembly factor BamA	45	45	0	0	M	COG4775	K07277	15



**Table S3. Continued**

XHV734_1603	<i>rseP</i>	Regulatory intramembrane protein RIP zinc protease	17	6	41	0.3529	M	COG0750	K11749	7
XHV734_1604	<i>dvr</i>	1-deoxy-D-xylose 5-phosphate reductoisomerase	5	5	0	0	I	COG0743	K00099	30
XHV734_1605	<i>cdsA</i>	CDP-diglyceride synthase	3	3	0	0	I	COG0575	K00981	21
XHV734_1606	<i>ispU</i>	Undecaprenyl pyrophosphate synthase	4	4	0	0	I	COG0020	K00806	35
XHV734_1607	<i>fir</i>	Ribosome recycling factor	2	2	0	0	J	COG0233	K02838	39
XHV734_1608	<i>pyrH</i>	Uridylate kinase	6	5	233	0.1667	F	COG0528	K09903	37
XHV734_1610	<i>tsf</i>	Protein chain elongation factor EF-Ts	5	4	116	0.2	J	COG0264	K02357	32
XHV734_1611	<i>rpsB</i>	30S ribosomal subunit protein S2	9	9	0	0	J	COG0052	K02967	32
XHV734_1618	<i>map</i>	Methionine aminopeptidase	14	14	0	0	J	COG0024	K01265	31
XHV734_1619	<i>glnD</i>	[Protein-PiI] uridylyltransferase / [Protein-PiI]-UMP uridylyl-removing enzyme	15	14	696	0.1333	O	COG2844	K00990	1
XHV734_1620	<i>dapD</i>	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	14	14	0	0	E	COG2171	K00674	16
XHV734_1623	<i>dapE</i>	N-succinyl-diaminopimelate deacylase	12	12	0	0	E	COG0624	K01439	19
XHV734_1634	<i>parC</i>	DNA topoisomerase IV, subunit A	28	28	0	0	L	COG0188	K02621	56
XHV734_1653	<i>fpr</i>	Ferredoxin--NADP reductase	10	10	1	0.1	C	COG1018	K00528	11
XHV734_1677	<i>dnaB</i>	Replicative DNA helicase	18	17	12	0.0556	L	COG0305	K02314	39
XHV734_1685	<i>leuS</i>	Leucyl-tRNA synthetase	29	29	0	0	J	COG0495	K01869	35
XHV734_1686	<i>lptE</i>	LPS-assembly lipoprotein RlpB precursor (Rare lipoprotein B)	9	9	1	0.1111	M	COG2980	K03643	1
XHV734_1687	<i>holA</i>	DNA polymerase III delta subunit	7	7	0	0	L	COG1466	K02340	11
XHV734_1708	<i>tilS</i>	tRNA(Ile)-lysidine synthase	15	15	0	0	D	COG0037	K04075	18
XHV734_1709	-	Alkaline phosphatase	19	18	47	0.0526	P	COG1785	K01077	1*
XHV734_1751	<i>psd</i>	Phosphatidylserine decarboxylase proenzyme	19	19	0	0	I	COG0688	K01613	10
XHV734_1757	<i>usg</i>	Putative semialdehyde dehydrogenase	11	10	300	0.0909	E	COG0136	K00133	14
XHV734_1767	<i>accD</i>	Acetyl-CoA carboxylase, beta (carboxyltransferase) subunit	6	6	0	0	I	COG0777	K01963	28
XHV734_1768	<i>glmM</i>	Phosphoglucosamine mutase	13	13	0	0	G	COG1109	K03431	29
XHV734_1781	<i>nuoA</i>	NADH-quinone oxidoreductase subunit A	8	8	1	0.125	C	COG0838	K00330	7
XHV734_1782	<i>nuoB</i>	NADH-quinone oxidoreductase subunit B	12	12	1	0.25	C	COG0377	K00331	12
XHV734_1783	<i>nuoC</i>	NADH-quinone oxidoreductase subunit C	10	10	0	0	C	COG0852	K00332	6
XHV734_1784	<i>nuoD</i>	NADH-quinone oxidoreductase subunit D	29	29	1	0.1379	C	COG0649	K00333	13
XHV734_1785	<i>nuoE</i>	NADH-quinone oxidoreductase subunit E	9	9	1	0.1111	C	COG1905	K00334	8
XHV734_1786	<i>nuoF</i>	NADH:ubiquinone oxidoreductase, chain F	22	22	1	0.0909	C	COG1894	K00335	9
XHV734_1787	<i>nuoG</i>	NADH-ubiquinone oxidoreductase chain G	21	21	0	0	C	COG1034	K00336	9
XHV734_1788	<i>nuoH</i>	NADH-ubiquinone oxidoreductase subunit H	18	18	1	0.0556	C	COG1005	K00337	11
XHV734_1789	<i>nuoI</i>	NADH-ubiquinone oxidoreductase subunit I	10	10	0	0	C	COG1143	K00338	13
XHV734_1790	<i>nuoJ</i>	NADH-ubiquinone oxidoreductase subunit J	7	7	0	0	C	COG0839	K00339	9
XHV734_1791	<i>nuoK</i>	NADH-ubiquinone oxidoreductase subunit K	2	2	0	0	C	COG0713	K00340	14
XHV734_1792	<i>nuoL</i>	NADH-ubiquinone oxidoreductase subunit L	37	37	1	0.0541	C	COG1009	K00341	14
XHV734_1793	<i>nuoM</i>	NADH-ubiquinone oxidoreductase subunit M	29	29	1	0.1379	C	COG1008	K00342	12

**Table S3. Continued**

	<i>nuoN</i>		29	26	19.5	C	COG1007	K00343	9
XHV734_1794	<i>nuoN</i>	NADH-quinone oxidoreductase subunit N							
XHV734_1796	<i>rmpP</i>	Ribosome maturation factor RmpP	6	6	0	S	COG0779	K09748	14
XHV734_1797	<i>nusA</i>	Transcription termination/antitermination L factor	15	15	0	K	COG0195	K02600	28
XHV734_1799	<i>infB</i>	Translation initiation factor IF-2	29	26	35.5	J	COG0532	K02519	37
XHV734_1802	<i>rpsO</i>	30S ribosomal subunit protein S15	4	4	0	J	COG0184	K02956	22
XHV734_1803	<i>pnp</i>	Polynucleotide phosphorylase/polyadenylase	27	26	41.5	J	COG1185	K00962	14
XHV734_1844	<i>thrS</i>	Threonyl-tRNA synthetase	37	37	0	J	COG0441	K01868	38
XHV734_1845	<i>infC</i>	Protein chain initiation factor IF-3	1	1	0	J	COG0290	K02520	36
XHV734_1847	<i>rplM</i>	50S ribosomal subunit protein L35	5	5	0	J	COG0291	K02916	27
XHV734_1848	<i>rplT</i>	50S ribosomal subunit protein L20	14	14	0	J	COG0292	K02887	31
XHV734_1849	<i>pheS</i>	Phenylalanine tRNA synthetase, alpha subunit	9	9	0	J	COG0016	K01889	38
XHV734_1850	<i>pheT</i>	Phenylalanine tRNA synthetase, beta subunit	20	20	0.05	J	COG0072	K01890	35
XHV734_1856	<i>gumE</i>	Xanthan biosynthesis exopolysaccharide polymerase GumE	23	22	133	Not in COG	-	KI3662	none
XHV734_1860	<i>gumI</i>	GDP-mannose:glycolipid 4-beta-D-mannosyltransferase	18	18	0	M	COG0438	K13658	none
XHV734_1861	<i>gumJ</i>	Xanthan biosynthesis oligosaccharidyl-lipid flippase GumJ	22	21	239	R	COG2244	-	2*
XHV734_1876	<i>dxs</i>	1-deoxyxylulose-5-phosphate synthase, thiamine-requiring, FAD-requiring	22	22	0	H	COG1154	K01662	31
XHV734_1894	<i>ftsY</i>	Signal recognition particle receptor FtsY	15	15	0	U	COG0552	K03110	30
XHV734_1946	<i>yajC</i>	Preprotein translocase subunit YajC (TC 3.A.5.1.1)	2	2	0	U	COG1862	K03210	14
XHV734_1947	<i>secD</i>	SecYEG protein translocase auxiliary subunit	23	23	0	U	COG0342	K03072	22
XHV734_1948	<i>secE</i>	SecYEG protein translocase auxiliary subunit	5	5	0	U	COG0341	K03074	20
XHV734_2033	-	Conserved membrane protein of unknown function	18	17	19	Not in COG	-	-	none
XHV734_2034	<i>ybeX</i>	Putative protein involved in divalent ion export	7	7	1	P	COG4535	K06189	4
XHV734_2068	-	N-acetylneuramyl-L-alanine amidase	16	15	10.5	M	COG0860	K01448	10*
XHV734_2089	<i>hemE</i>	Uroporphyrinogen decarboxylase	13	13	1	H	COG0407	K01599	22
XHV734_2137	<i>lptB</i>	Putative lipopolysaccharide transport protein B: ATP-binding component of ABC superfamily	9	9	0	R	COG1137	K06861	81
XHV734_2138	<i>lptA</i>	Lipopolysaccharide export system protein LptA	8	8	0	S	COG1934	K09774	7
XHV734_2139	<i>lptC</i>	Lipopolysaccharide export system protein LptC	8	8	0	S	COG3117	K11719	2*
XHV734_2140	<i>kdsC</i>	3-deoxy-D-manno-oculosonate 8-phosphate phosphatase	6	6	0	R	COG1778	K03270	3
XHV734_2141	<i>kdsD</i>	D-arabinose 5-phosphate isomerase	6	6	5	M	COG0794	K06041	12
XHV734_2143	<i>murA</i>	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	12	12	0	M	COG0766	K00790	31
XHV734_2149	-	Conserved exported protein of unknown function	19	18	217	S	COG3249	K09938	none
XHV734_2150	-	Putative permease often clustered with de novo purine synthesis	19	19	0	R	COG0628	-	3*
XHV734_2151	<i>hda</i>	DnaA regulatory inactivator Hda	13	11	177	L	COG0593	K10763	8
XHV734_2156	<i>subB</i>	Inositol monophosphatase	12	12	1	G	COG0483	K01092	15
XHV734_2164	<i>ubiG</i>	Bifunctional 3-demethylubiquinone-9 3-methyltransferase and 2-octaprenyl-6-hydroxy phenol methylase	11	11	0	H	COG2227	K00568	21
XHV734_2184	<i>suflE</i>	Cysteine desulfuration protein SufE	8	8	0	R	COG2166	K02426	8

**Table S3. Continued**

XHV734_2186	<i>cysS</i>	Cysteine--tRNA ligase	20	20	0	0	J	COG0215	K01883	39
XHV734_2241	-	N-carbamoylputrescine amidase (3.5.1.53)	13	13	1	0.0769	R	COG0388	K12251	6
XHV734_2245	<i>cmk</i>	Cytidylate kinase	10	6	60	0.4	F	COG0283	K00945	30
XHV734_2246	<i>rpsA</i>	30S ribosomal subunit protein S1	15	10	69	0.2667	J	COG0539	K02945	24
XHV734_2256	<i>guaB</i>	IMP dehydrogenase	13	13	1	0.0769	F	COG0516	K00088	21
XHV734_2274	<i>orn</i>	Oligoribonuclease	8	7	20	0.125	A	COG1949	K13288	12
XHV734_2319	-	Conserved protein of unknown function	2	2	0	0	S	COG5508	-	none
XHV734_2320	<i>sdhC</i>	Succinate dehydrogenase cytochrome b-556 subunit	9	9	0	0	C	COG2009	K00241	5
XHV734_2321	<i>sdhD</i>	Succinate dehydrogenase hydrophobic membrane anchor protein	3	3	0	0	C	COG2142	K00242	3
XHV734_2322	<i>sdhA</i>	Succinate dehydrogenase, flavoprotein subunit	31	31	0	0	C	COG1053	K00239	26
XHV734_2323	<i>sdhB</i>	Succinate dehydrogenase, FeS subunit	14	14	1	0.0714	C	COG0479	K00240	10
XHV734_2324	-	Conserved protein of unknown function	6	6	0	0	S	COG2938	K09159	4
XHV734_2326	<i>lolC</i>	Lipoprotein-releasing system transmembrane protein LolC	22	22	0	0	M	COG4591	K09808	31
XHV734_2327	<i>lolD</i>	Outer membrane-specific lipoprotein transporter subunit ; ATP-binding component of ABC superfamily	5	4	1111	0.2	V	COG1136	K09810	158
XHV734_2331	<i>exbD4</i>	Biopolymer transport protein ExbD/ToIR	7	7	0	0	U	COG0848	K03559	7
XHV734_2332	<i>msbA</i>	Fused lipid transporter subunits of ABC superfamily; membrane component ; ATP-binding component	21	21	1	0.0476	V	COG1132	K11085	33
XHV734_2333	<i>lpxK</i>	Lipid A 4'kinase	18	18	0	0	M	COG1663	K00912	20
XHV734_2334	<i>kdsB</i>	3-deoxy-manno-octulosonate cytidylyltransferase	12	12	0	0	M	COG1212	K00979	22
XHV734_2338	<i>pgsA</i>	Phosphatidylglycerophosphate synthetase	9	9	1	0.1111	I	COG0558	K00995	32
XHV734_2439	<i>rne</i>	Fused ribonucleaseE; endoribonuclease ; RNA-binding protein ; RNA degradosome binding protein	39	23	172.06	0.4103	J	COG1530	K08300	7*
XHV734_2475	-	Putative phage-related protein	12	9	172	0.25	Not in COG	-	-	none
XHV734_2549	<i>der</i>	GTPase involved in ribosome synthesis and maintenance	16	15	405.5	0.125	R	COG1160	K03977	36
XHV734_2550	<i>bamB</i>	Outer membrane protein assembly factor BamB	21	21	0	0	S	COG1520	K17713	3
XHV734_2551	-	Putative membrane protein	5	5	0	0	S	COG2976	-	1*
XHV734_2565	<i>trxB</i>	Thioredoxin reductase, FAD/NAD(P)-binding	10	10	0	0	O	COG0492	K00384	17
XHV734_2569	<i>infA</i>	Translation initiation factor IF-1	8	7	212	0.125	J	COG0361	K02518	33
XHV734_2698	<i>prfB</i>	Peptide chain release factor 2	13	13	0	0	J	COG1186	K02836	65
XHV734_2714	<i>feoB</i>	Ferrous iron transport protein B	21	21	1	0.0476	P	COG0370	K04759	2
XHV734_2715	<i>feoA</i>	Ferrous iron transport protein	3	2	11	0.3333	P	COG1918	K04758	2
XHV734_2751	<i>hisS</i>	Histidine--tRNA ligase	20	19	411	0.05	J	COG0124	K01892	9
XHV734_2811	-	Conserved protein of unknown function	1	1	0	0	Not in COG	-	-	none
XHV734_2812	-	Methyltransferase	14	14	0	0	Q	COG0500	-	2*
XHV734_2813	<i>pyrD</i>	Dihydro-orotate oxidase, FMN-linked	16	16	0	0	F	COG0167	K00254	9
XHV734_2814	<i>murB</i>	UDP-N-acetylenolpyruvoylglucosamine reductase	13	13	0	0	M	COG0812	K00075	17
XHV734_2818	<i>ispG</i>	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin)	16	15	847	0.0625	I	COG0821	K03526	28

**Table S3.** Continued

XHV734_2833	<i>folK</i>	2-amino-4-hydroxy-6-hydroxymethylidihydropteridine pyrophosphokinase	4	3	254	0.25	H	COG0801	K00950	22
XHV734_2834	<i>pcnB</i>	Poly(A) polymerase I	15	15	1	0.0667	J	COG0617	K00970	2
XHV734_2860	<i>dapA</i>	Dihydrodipicolinate synthase	13	13	0	0	E	COG0329	K01714	28
XHV734_2877	<i>alaS</i>	Alanyl-tRNA synthetase	29	29	0	0	J	COG0013	K01872	36
XHV734_2901	<i>ispD</i>	4-diphosphocytidylyl-2-C-methyl-D-erythritol synthase	8	7	77	0.125	I	COG1211	K00991	22
XHV734_2902	<i>fisB</i>	Cell division protein FisB	3	3	0	0	D	COG2919	K05589	8
XHV734_2903	<i>eno</i>	Enolase	16	16	2	0.0625	G	COG0148	K01689	26
XHV734_2905	<i>kdsA</i>	2-dehydro-3-deoxyphosphooctonate aldolase	3	3	0	0	M	COG2877	K01627	19
XHV734_2979	<i>ssb</i>	Single-stranded DNA-binding protein	10	10	1	0.1	L	COG0629	K03111	19
XHV734_2980	<i>ispB</i>	Octaprenyl diphosphate synthase	9	9	0	0	H	COG0142	K02523	42
XHV734_2981	<i>murD</i>	UDP-N-acetylmuramoyl-L-alanine--L-glutamate ligase	23	22	376	0.0435	M	COG0771	K23169	10
XHV734_2982	<i>murL</i>	UDP-N-acetyl-alpha-D-muramoyl-L-alanyl-L-glutamate epimerase	19	19	0	0	Not in COG	-	K23170	none
XHV734_2983	-	Conserved membrane protein of unknown function	8	4	87	0.25	Not in COG	-	-	none
XHV734_3008	<i>surR</i>	Iron-sulfur cluster regulator IscR	3	3	0	0	K	COG1959	-	2
XHV734_3009	<i>surB</i>	Component of SufBCD complex	28	28	0	0	O	COG0719	K09014	14
XHV734_3010	<i>surC</i>	Component of SufBCD complex, ATP-binding component of ABC superfamily	8	8	1	0.125	O	COG0396	K09013	30
XHV734_3011	<i>surD</i>	Iron-sulfur cluster assembly protein SufD	13	13	0	0	O	COG0719	K09015	4
XHV734_3012	<i>surS</i>	Selenocysteine lyase, PLP-dependent	11	11	0	0	E	COG0520	K11717	22
XHV734_3036	<i>gyrA</i>	DNA gyrase (type II topoisomerase), subunit A	38	38	0	0	L	COG0188	K02469	56
XHV734_3040	<i>ligA</i>	DNA ligase, NAD(+)-dependent	29	28	5	0.0345	L	COG0272	K01972	38
XHV734_3045	<i>rpsR</i>	30S ribosomal subunit protein S18	7	5	200	0.2857	J	COG0238	K02963	24
XHV734_3046	<i>rpsF</i>	30S ribosomal subunit protein S6	8	8	1	0.125	J	COG0360	K02990	22
XHV734_3048	<i>asns</i>	Asparaginyl tRNA synthetase	20	20	0	0	J	COG0017	K01893	20
XHV734_3062	<i>nadK</i>	NAD kinase	10	10	0	0	G	COG0061	K00858	22
XHV734_3126	<i>purB</i>	Adenylosuccinate lyase	13	13	0	0	F	COG0015	K01756	16
XHV734_3131	<i>sucA</i>	2-oxoglutarate decarboxylase, thiamin-requiring	40	40	1	0.025	C	COG0567	K00164	19
XHV734_3132	<i>sucB</i>	Dihydrolipoyltranssuccinase	8	8	2	0.125	C	COG0508	K00658	28
XHV734_3133	<i>lpd</i>	Dihydrolipoyl dehydrogenase	11	8	42.5	0.3636	C	COG1249	K00382	23
XHV734_3146	<i>dnaK</i>	Chaperone Hsp70, co-chaperone with DnaJ	13	11	108	0.2308	O	COG0443	K04043	35
XHV734_3253	<i>glxX</i>	Glutamyl-tRNA synthetase	24	24	0	0	J	COG0008	K01885	41
XHV734_3362	<i>serS</i>	Seryl-tRNA synthetase, also charges selenocysteinylyl-tRNA with serine	18	17	66	0.0556	J	COG0172	K01875	38
XHV734_3400	<i>prfC</i>	Peptide chain release factor RF-3	12	12	0	0	J	COG4108	K02837	3
XHV734_3409	-	Conserved exported protein of unknown function	9	9	0	0	Not in COG	-	-	none
XHV734_3548	<i>ileS</i>	Isoleucyl-tRNA synthetase	40	40	0	0	J	COG0060	K01870	32
XHV734_3549	<i>ribF</i>	Bifunctional riboflavin kinase and FAD synthetase	14	14	1	0.0714	H	COG0196	K11753	31
XHV734_3550	<i>murJ</i>	Putative lipid II flippase MurJ	19	18	76	0.0526	R	COG0728	K03980	17



**Table S3.** Continued

XHV734_3915	<i>rpsC</i>	30S ribosomal subunit protein S3	16	16	0	0	J	COG0092	K02982	38
XHV734_3916	<i>rplV</i>	50S ribosomal subunit protein L22	4	4	0	0	J	COG0091	K02890	32
XHV734_3917	<i>rpsS</i>	30S ribosomal subunit protein S19	7	7	0	0	J	COG0185	K02965	29
XHV734_3918	<i>rplB</i>	50S ribosomal subunit protein L2	18	18	1	0.0556	J	COG0090	K02886	37
XHV734_3919	<i>rplW</i>	50S ribosomal subunit protein L23	7	7	0	0	J	COG0089	K02892	32
XHV734_3920	<i>rplD</i>	50S ribosomal subunit protein L4	10	10	0	0	J	COG0088	K02926	40
XHV734_3921	<i>rplC</i>	50S ribosomal subunit protein L3	14	14	0	0	J	COG0087	K02906	39
XHV734_3922	<i>rpsJ</i>	30S ribosomal subunit protein S10	5	5	0	0	J	COG0051	K02946	31
XHV734_3924	<i>ftsA</i>	Protein chain elongation factor EF-G, GTP-binding	29	29	0	0	J	COG0480	K02355	37
XHV734_3925	<i>rpsG</i>	30S ribosomal subunit protein S7	9	9	0	0	J	COG0049	K02992	37
XHV734_3926	<i>rpsL</i>	30S ribosomal subunit protein S12	8	8	0	0	J	COG0048	K02950	35
XHV734_3927	<i>rpoC</i>	RNA polymerase, beta prime subunit	54	52	150	0.037	K	COG0086	K03046	34
XHV734_3928	<i>rpoB</i>	RNA polymerase, beta subunit	57	57	0	0	K	COG0085	K03043	28
XHV734_3930	<i>rplL</i>	50S ribosomal subunit protein L7/L12	2	2	0	0	J	COG0222	K02935	34
XHV734_3931	<i>rplJ</i>	50S ribosomal subunit protein L10	4	4	1	0.25	J	COG0244	K02864	25
XHV734_3932	<i>rplA</i>	50S ribosomal subunit protein L1	8	8	0	0	J	COG0081	K02863	28
XHV734_3933	<i>rplK</i>	50S ribosomal subunit protein L11	9	9	0	0	J	COG0080	K02867	33
XHV734_3934	<i>nusG</i>	Transcription termination factor	7	7	0	0	K	COG0250	K02601	29
XHV734_3935	<i>secE</i>	Preprotein translocase subunit SecE (TC 3.A.5.1.1)	7	7	0	0	U	COG0690	K03073	6
XHV734_3939	<i>rplY</i>	50S ribosomal protein L25	6	6	0	0	J	COG1825	K02897	1
XHV734_3940	<i>prsA</i>	Phosphoribosylpyrophosphate synthase	10	10	0	0	E	COG0462	K00948	36
XHV734_3941	<i>ispE</i>	4-diphosphoethyl-2-C-methylerythritol kinase	5	5	0	0	I	COG1947	K00919	28
XHV734_3942	<i>lolB</i>	Outer-membrane lipoprotein LolB	6	6	0	0	M	COG3017	K02494	2
XHV734_3943	-	FOG: TPR repeat	19	19	0	0	R	COG0457	-	none
XHV734_3944	<i>hemA</i>	Glutaryl tRNA reductase	18	17	14.5	0.1111	H	COG0373	K02492	16
XHV734_3945	<i>ptfA</i>	Peptide chain release factor RF-1	16	16	0	0	J	COG0216	K02835	65
XHV734_3962	-	Putative FeS assembly SUF system protein SufT	10	10	1	0.2	R	COG2151	-	7
XHV734_3992	<i>glmS</i>	Glutaryl-tRNA synthetase	25	25	0	0	J	COG0008	K01886	21
XHV734_4024	<i>lptD</i>	LPS-assembly protein LptD	69	63	30.5	0.029	M	COG1452	K04744	2
XHV734_4025	<i>surA</i>	Chaperone SurA	20	20	0	0	O	COG0760	K03771	6
XHV734_4037	<i>thyA</i>	Thymidylate synthetase	11	11	0	0	F	COG0207	K00560	28
XHV734_4038	<i>lgt</i>	Phosphatidylglycerol-prolipoprotein diacylglyceryl transferase	13	13	0	0	M	COG0682	K13292	19
XHV734_4080	<i>metK</i>	Methionine adenosyltransferase 1	12	11	253	0.0833	H	COG0192	K00789	19
XHV734_4109	<i>secA</i>	Preprotein translocase subunit, ATPase	42	41	128	0.0476	U	COG0653	K03070	35
XHV734_4112	<i>lpxC</i>	UDP-3-O-acetyl N-acetylglucosamine deacetylase	14	12	106.5	0.1429	M	COG0774	K02535	23
XHV734_4113	<i>ftsZ</i>	GTP-binding tubulin-like cell division protein	5	5	0	0	D	COG0206	K03531	39
XHV734_4114	<i>ftsA</i>	ATP-binding cell division protein involved in recruitment of FtsK to Z ring	10	10	0	0	D	COG0849	K03590	31
XHV734_4115	<i>ftsQ</i>	Cell division protein FtsQ	9	9	0	0	M	COG1589	K03589	11

**Table S3.** Continued

XHV734_4116	<i>ddlB</i>	D-alanine:D-alanine ligase	14	14	0	0	0	0	0	M	COG1181	K01921	26
XHV734_4117	<i>murC</i>	UDP-N-acetylmuramate:L-alanine ligase	13	13	0	0	0	0	0	M	COG0773	K01924	34
XHV734_4118	<i>murG</i>	N-acetylglucosaminyl transferase	14	14	0	0	0	0	0	M	COG0707	K02563	30
XHV734_4119	<i>ftsW</i>	Putative peptidoglycan glycosyltransferase FtsW	16	16	0	0	0	0	0	D	COG0772	K03588	35
XHV734_4120	<i>mraY</i>	Phospho-N-acetylmuramoyl-pentapeptide transferase	15	15	2	0.0667	0	0	0	M	COG0472	K01000	33
XHV734_4121	<i>murF</i>	UDP-N-acetylmuramoyl-tripeptide:D-alanyl-D-alanine ligase	16	16	0	0	0	0	0	M	COG0770	K01929	30
XHV734_4122	<i>murE</i>	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate:meso-diaminopimelate ligase	9	9	0	0	0	0	0	M	COG0769	K01928	30
XHV734_4123	<i>ftsI</i>	Transpeptidase involved in septal peptidoglycan synthesis (penicillin-binding protein 3)	27	27	0	0	0	0	0	M	COG0768	K03587	23
XHV734_4124	<i>ftsL</i>	Cell division protein FtsL	2	2	0	0	0	0	0	D	COG3116	K03586	9
XHV734_4125	<i>mraW</i>	S-adenosyl-dependent methyltransferase activity on membrane-located substrates	7	7	0	0	0	0	0	M	COG0275	K03438	20
XHV734_4126	<i>mraZ</i>	Transcriptional regulator MraZ	6	6	0	0	0	0	0	S	COG2001	K03925	2
XHV734_4145	<i>nusB</i>	Transcription antitermination protein	7	7	0	0	0	0	0	K	COG0781	K03625	19
XHV734_4146	<i>ribE</i>	Riboflavin synthase beta chain	2	2	0	0	0	0	0	H	COG0054	K00794	19
XHV734_4148	<i>ribB</i>	3,4-dihydroxy-2-butanone 4-phosphate synthase	7	4	153	0.2857	0	0	0	H	COG0108, COG0807	K14652	15
XHV734_4149	<i>ribE</i>	Riboflavin synthase	4	4	1	0.5	0	0	0	H	COG0307	K00793	17
XHV734_4157	<i>yjJK</i>	Fused putative transporter subunits of ABC superfamily: ATP-binding components	18	17	395	0.0556	0	0	0	R	COG0488	K06020	10
XHV734_4201	-	Putative permease	24	23	386	0.0417	0	0	0	R	COG0795	K11720	9
XHV734_4202	-	Putative permease	16	16	0	0	0	0	0	R	COG0795	K07091	9
XHV734_4203	<i>pepA</i>	Aminopeptidase A, a cyteinyglycinase	16	16	0	0	0	0	0	E	COG0260	K01255	3
XHV734_4204	<i>holC</i>	DNA polymerase III chi subunit	7	7	0	0	0	0	0	L	COG2927	K02339	2
XHV734_4205	<i>vaiS</i>	Valyl-tRNA synthetase	45	44	133.5	0.0444	0	0	0	J	COG0525	K01873	35
XHV734_4210	-	Alanine acetyltransferase	5	5	0	0	0	0	0	J	COG0532	-	none
XHV734_4211	<i>pssA</i>	CDP-diacetylgllycerol--serine O-phosphatidyltransferase	11	11	0	0	0	0	0	I	COG1183	K17103	13
XHV734_4213	<i>pros</i>	Prolyl-tRNA synthetase	20	20	0	0	0	0	0	J	COG0442	K01881	28
XHV734_4221	-	Electron transfer flavoprotein-ubiquinone oxidoreductase	24	24	1	0.0417	0	0	0	C	COG0644	K00311	7
XHV734_4234	<i>efrB</i>	Electron transfer flavoprotein subunit beta	7	7	0	0	0	0	0	C	COG2086	K03521	15
XHV734_4235	<i>efrA</i>	Electron transfer flavoprotein subunit alpha	10	10	1	0.1	0	0	0	C	COG2025	K03522	12
XHV734_4285	<i>glmS</i>	L-glutamine:D-fructose-6-phosphate aminotransferase	28	27	29	0.0357	0	0	0	M	COG0449	K00820	28
XHV734_4296	<i>glmU</i>	Fused N-acetyl glucosamine-1-phosphate uridylyltransferase ; glucosamine-1-phosphate acetyl transferase	13	12	36	0.1538	0	0	0	M	COG1207	K04042	31
XHV734_4299	<i>atpC</i>	F1 sector of membrane-bound ATP synthase, epsilon subunit	5	3	71	0.4	0	0	0	C	COG0355	K02114	11
XHV734_4300	<i>atpD</i>	F1 sector of membrane-bound ATP synthase, beta subunit	20	20	0	0	0	0	0	C	COG0055	K02112	29
XHV734_4301	<i>atpG</i>	F1 sector of membrane-bound ATP synthase, gamma subunit	14	14	0	0	0	0	0	C	COG0224	K02115	24

**Table S3. Continued**

XHV734_4302	<i>atpA</i>	F1 sector of membrane-bound ATP synthase, alpha subunit	21	21	0	0	C	COG0056	K02111	28
XHV734_4303	<i>atpH</i>	ATP synthase subunit delta	4	4	0	0	C	COG0712	K02113	10
XHV734_4304	<i>atpF</i>	F0 sector of membrane-bound ATP synthase, subunit b	4	4	0	0	C	COG0711	K02109	9
XHV734_4306	<i>atpE</i>	ATP synthase subunit c	1	1	0	0	C	COG0636	K02110	9
XHV734_4307	<i>atpB</i>	F0 sector of membrane-bound ATP synthase, subunit a	8	8	0	0	C	COG0356	K02108	10
XHV734_4308	-	Conserved membrane protein of unknown function	11	11	0	0	Not in COG	-	-	none
XHV734_4336	<i>alr</i>	Alanine racemase	13	10	485.33	0.2308	M	COG0787	K01775	15
XHV734_4460	-	DUF1629-domain containing protein	14	10	8	0.2143	Not in COG	-	-	none
XHV734_4462	-	Cytosine-specific methyltransferase	32	30	106.5	0.0625	L	COG0270	K00558	2*
XHV734_4488	<i>rpoD</i>	RNA polymerase, sigma 70 (sigma D) factor	20	19	32	0.05	K	COG0568	K03086	20
XHV734_4500	<i>fnt</i>	10-formyltetrahydrofolate:L-methionyl-tRNA(fMet) N-formyltransferase	13	13	3	0.0769	J	COG0223	K00604	34
XHV734_4507	<i>topA</i>	DNA topoisomerase I	29	29	0	0	L	COG0550	K03168	25
XHV734_4508	<i>yrdC</i>	Putative ribosome maturation factor	3	3	0	0	J	COG0009	K07566	24
XHV734_4519	<i>prfA</i>	Primosomal protein N'	23	6	1.92	0.5217	L	COG1198	K04066	19
XHV734_4528	<i>rpoH</i>	RNA polymerase, sigma 32 (sigma H) factor	12	11	76	0.0833	K	COG0568	K03089	30
XHV734_4535	<i>rho</i>	Transcription termination factor Rho	24	24	0	0	K	COG0513, COG1158	K03628	21
XHV734_4576	<i>gcp</i>	O-sialoglycoprotein endopeptidase	14	11	121	0.1429	O	COG0533	K01409	29
XHV734_4580	<i>dnaG</i>	DNA primase	21	20	190	0.0476	L	COG0358	K02316	25
XHV734_4601	<i>tyrS</i>	Tyrosine--tRNA ligase	19	19	0	0	J	COG0162	K01866	16
XHV734_4623	<i>argS</i>	Arginine--tRNA ligase	30	29	151	0.0333	J	COG0018	K01887	14
XHV734_4629	<i>speA</i>	Biosynthetic arginine decarboxylase	32	32	1	0.0625	E	COG1166	K01585	2
XHV734_4630	<i>speE</i>	Polyamine aminopropyltransferase	14	13	211	0.0714	E	COG0421	K00797	none
XHV734_4691	<i>trpS</i>	Tryptophan--tRNA ligase	18	17	11.33	0.1667	J	COG0180	K01867	32
XHV734_4704	<i>birA</i>	Bifunctional ligase/repressor BirA	9	9	0	0	H	COG0340	K03524	12
XHV734_4709	<i>phoQ</i>	Sensor protein PhoQ	19	19	0	0	T	COG0642	K07637	2
XHV734_4710	<i>phoP</i>	DNA-binding response regulator in two-component regulatory system with PhoQ	11	11	0	0	K	COG0745	K07660	62
XHV734_4726	<i>hemB</i>	Delta-aminolevulinic acid dehydratase	11	11	0	0	H	COG0113	K01698	18
XHV734_4756	<i>trxA</i>	Thioredoxin	4	4	0	0	C	COG0526	K03671	1
XHV734_4757	-	Flavodoxins	5	5	0	0	C	COG0716	-	4*
XHV734_4758	<i>nrdB</i>	Ribonucleoside-diphosphate reductase subunit beta	21	21	0	0	F	COG0208	K00526	28*
XHV734_4759	<i>nrdA</i>	Ribonucleoside-diphosphate reductase subunit alpha	48	48	1	0.0417	F	COG0209	K00525	8
XHV734_4772	-	Ankyrin-like protein	20	19	62	0.05	R	COG0477, COG0666	K06867	none
XHV734_4773	-	YcgL domain-containing protein XGA_2463	6	6	1	0.1667	S	COG3100	K09902	none
XHV734_4797	<i>hemF</i>	Coproporphyrinogen III oxidase	17	15	57.75	0.2353	H	COG0408	K00228	13
XHV734_4823	<i>rpmB</i>	50S ribosomal subunit protein L28	2	2	0	0	J	COG0227	K02902	23
XHV734_4891	<i>glyS</i>	Glycine tRNA synthetase, beta subunit	27	27	0	0	J	COG0751	K01879	27



**Table S3.** Continued

XHV734_4892	<i>glyQ</i>	Glycine tRNA synthetase, alpha subunit	22	22	0	0	J	COG0752	K01878	26
XHV734_4901	<i>hemH</i>	Ferrochelatase	17	16	34.75	0.2353	H	COG0276	K01772	21
XHV734_4943	<i>plsB</i>	Glycerol-3-phosphate acyltransferase	35	34	158	0.0571	I	COG2937	K00631	9
XHV734_5093	<i>yidC</i>	Membrane protein insertase YidC	24	24	0	0	U	COG0706	K03217	15
XHV734_5094	<i>rnpA</i>	Ribonuclease P protein component	4	4	0	0	J	COG0594	K03536	9
XHV734_5095	<i>rpmH</i>	50S ribosomal subunit protein L34	2	2	0	0	J	COG0230	K02914	26

**RNA**

XHV734_ncRNA3	-	sX9	3	2	416	0.3333	Not in COG	-	-	-
XHV734_ncRNA65	-	RNaseP_bact_a	12	12	0	0	Not in COG	-	-	-
XHV734_ncRNA67	-	FMN riboswitch	4	4	0	0	Not in COG	-	-	-
XHV734_tRNA14	-	Pro tRNA	4	4	0	0	Not in COG	-	-	-
XHV734_tRNA23	-	Cys tRNA	3	3	0	0	Not in COG	-	-	-
XHV734_tRNA46	-	Trp tRNA	2	2	0	0	Not in COG	-	-	-
XHV734_tRNA47	-	Thr tRNA	3	3	0	0	Not in COG	-	-	-
XHV734_tRNA48	-	Gly tRNA	2	2	0	0	Not in COG	-	-	-
XHV734_tRNA49	-	Tyr tRNA	3	3	0	0	Not in COG	-	-	-
XHV734_tRNA50	-	Gln tRNA	2	2	0	0	Not in COG	-	-	-
XHV734_tRNA8	-	Met tRNA	2	2	0	0	Not in COG	-	-	-

<sup>a</sup> COG categories identifiers stands for :

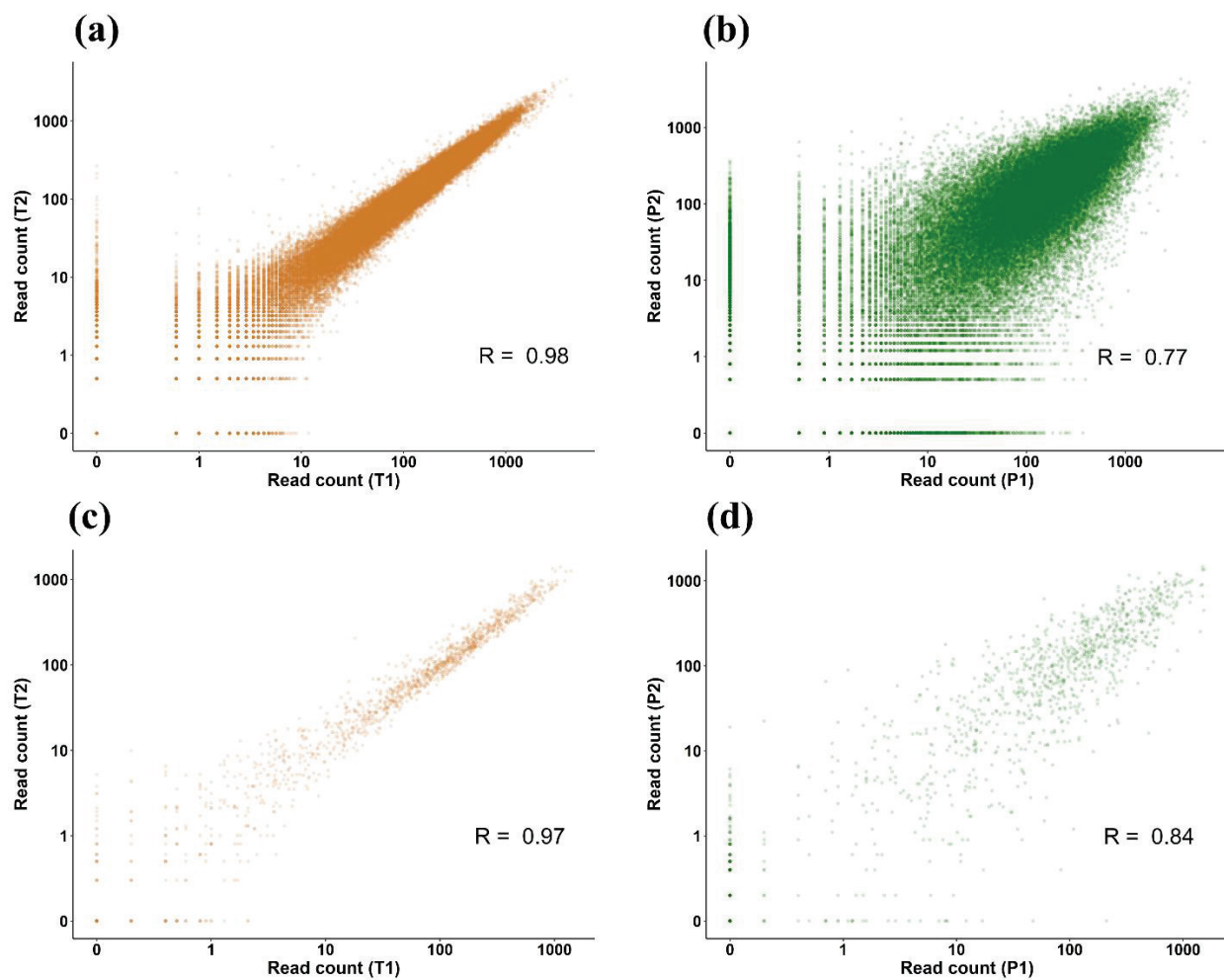
C = Energy production and conversion, D = Cell cycle control and mitosis, E = Amino Acid metabolism and transport, F = Nucleotide metabolism and transport, G = Carbohydrate metabolism and transport, H = Coenzyme metabolism, I = Lipid metabolism, J = Translation, K = Transcription, L = Replication and repair, M = Cell wall/membrane/envelope biogenesis, N = Cell motility, O = Post-translational modification, protein turnover, chaperone functions, P = Inorganic ion transport and metabolism, Q = Secondary structure, R = General functional prediction only, S = Function unknown, T = Signal transduction, U = Intracellular trafficking and secretion, V = Defense mechanisms

<sup>b</sup> Homologs were retrieved using BLASTp in DEG10 v15.2 essential gene database applying thresholds of 50 % coverage, 30 % identity and e-value > 10<sup>-5</sup>. Numbers with a \* indicate lower confidence homologs with identity values > 20 % and/or coverage > 40 %.

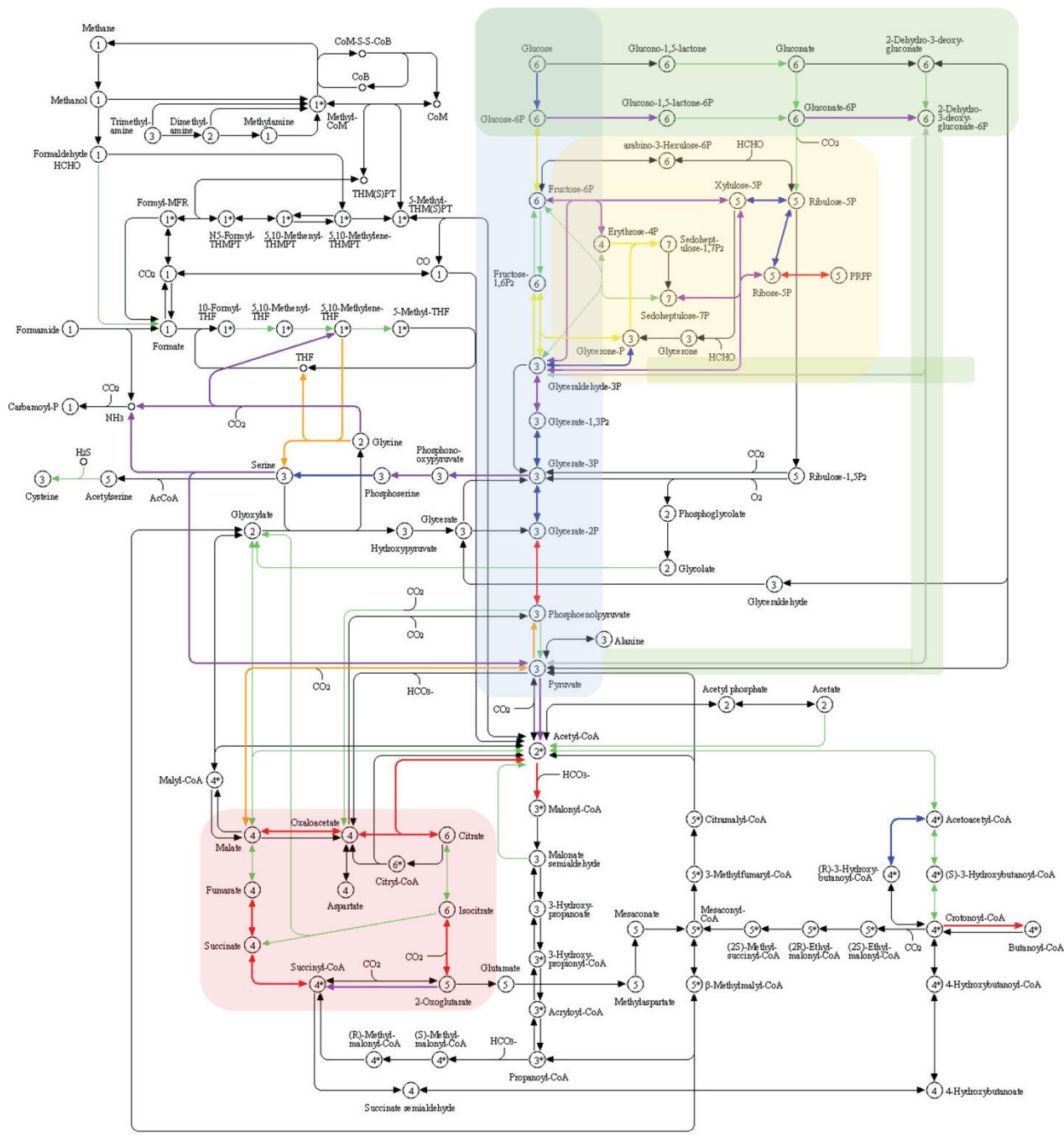
<sup>c</sup> Gene conservation among the genomes used in this study, at the family (*Xanthomonadaceae*) and genus (*Xanthomonas*) levels. Black indicates presence and white absence.

**CHAPITRE III**  
**SUPPLEMENTARY DATA**

Accessibilité : <https://figshare.com/s/eb1f29b098cad3fa887c>



**Figure S1.** Read count correlations among Tn-seq duplicates for *X. hortorum* pv. *vitians* LM16734 chromosome in (a) 1/10<sup>th</sup> TSB and (b) lettuce leaf, and for plasmid pLM16734 in (c) 1/10<sup>th</sup> TSB and (d) lettuce leaf.



**Colours legend :**

- Essential in library
- Growth-defect in library
- Critical in 1/10<sup>th</sup> TSB
- Critical in lettuce
- Non-significant in lettuce but log2FC < -1
- Not assessed (duplicated)
- Neutral (non-significant)
- Absent in KEGG *xhr* organism-specific pathways map
- Citrate cycle (TCA)
- Glycolysis
- Pentose Phosphate Pathway
- Entner-Doudoroff pathway

**Figure S2.** Critical genes in the carbon metabolism pathways of *X. hortorum* pv. *vitians* LM16734 in the different Tn-seq experimental conditions, created with *KEGG Mapper*. Carbon number in compounds are indicated in circles or by asterisks for cofactors.

**Table S2.** List of 170 critical genes in lettuce leaf (see online version for full annotation data). RC stands for read count, log2FC for log2 fold-change.

Locus	Gene	Product	TA sites count	Mean RC in control	Mean RC in experiment	Log2FC	Sum RC in control	Sum RC in experiment	Δ Mean RC	Permutation test p-value	FDR test q-value	Gene state in library	Fitness in control
XHV734_0038	<i>gltD</i>	glutamate synthase, 4Fe-4S protein, small subunit	15	133.5	12.4	-3.33	4006.4	372.28	-121.1	0	0	NE	-
XHV734_0039	<i>gltB</i>	glutamate synthase, large subunit	49	404.5	37.6	-3.39	39643.6	3680.71	-367	0	0	NE	-
XHV734_0066	<i>cirA</i>	TonB-dependent receptor	26	268.7	41.9	-2.65	13973	2176.38	-226.9	0	0	NE	-
XHV734_0130	<i>tyrB</i>	tyrosine aminotransferase, tyrosine-repressible, PLP-dependent	12	85.6	0.1	-6.35	2054.1	1.42	-85.5	0	0	NE	-
XHV734_0250	-	cardiolipin synthase	24	109.4	55.7	-0.96	5250.3	2671.23	-53.7	0.001	0.03016	NE	down
XHV734_0259	<i>glnA</i>	glutamine synthetase	18	116.8	10.8	-3.33	4205.6	387.16	-106.1	0	0	NE	-
XHV734_0262	-	Two-component system sensor protein	6	86.1	10.5	-2.92	1033	126.04	-75.6	6,00E-04	0.019	NE	-
XHV734_0263	<i>glnG</i>	fused DNA-binding response regulator, sigma54 interaction protein	5	63	3.1	-3.96	630	31.13	-59.9	1,00E-04	0.0039	NE	-
XHV734_0278	<i>secB</i>	protein export chaperone	5	17.4	0	-4.2	174.4	0	-17.4	1,00E-04	0.0039	NE	-
XHV734_0296	<i>oleC</i>	Olefin beta-lactone synthetase	16	4.4	0.3	-2.04	139.7	9.75	-4.1	0	0	NE	down
XHV734_0384	-	DNA (Cytosine-5)-methyltransferase	40	7.2	1.2	-1.88	578.3	98.23	-6	0	0	GD	-
XHV734_0458	<i>ilvD</i>	dihydroxyacid dehydratase	24	76.1	0.8	-5.4	3653.7	39.8	-75.3	0	0	NE	-
XHV734_0506	<i>bioB</i>	biotin synthase	10	77.5	2.1	-4.64	1549.3	42.78	-75.3	0	0	NE	-
XHV734_0589	<i>purC</i>	Phosphoribosylaminoimidazole-succinocarboxamide synthase	13	12.8	0.3	-3.45	332.8	6.72	-12.5	0	0	NE	-
XHV734_0595	<i>trpE</i>	Anthraniolate synthase component 1	22	233.8	2	-6.28	10287.7	88.82	-231.8	0	0	NE	-
XHV734_0597	<i>hrpF</i>	HrpF protein	23	119.3	41.1	-1.52	5488.6	1888.65	-78.3	0.0012	0.03534	NE	-
XHV734_0639	-	Nitroacetate monooxygenase component B	7	221	43	-2.33	3093.4	602.3	-177.9	5,00E-04	0.01625	NE	-
XHV734_0640	<i>trpD</i>	Anthraniolate phosphoribosyltransferase	5	197.5	1.6	-6.28	1975.4	15.51	-196	7,00E-04	0.0219	NE	-
XHV734_0642	<i>trpC</i>	Indole-3-glycerol phosphate synthase	8	79.4	0.6	-5.68	1270.6	9.08	-78.8	3,00E-04	0.01042	NE	-
XHV734_0670	<i>purD</i>	phosphoribosylglycinamide synthetase phosphoribosylamine-glycine ligase fused IMP cyclohydrolase;	10	29.3	0.2	-4.62	585.2	4.65	-29	0	0	NE	-
XHV734_0671	<i>purH</i>	phosphoribosylaminoimidazolecarboxamide formyltransferase	17	10	0.3	-3.07	339.7	10.52	-9.7	0	0	NE	-
XHV734_0691	-	Cytochrome c-type biogenesis protein DsbD, protein-disulfide reductase	17	41.7	11.1	-1.82	1416.8	377.22	-30.6	6,00E-04	0.019	NE	-
XHV734_0747	<i>aceE</i>	pyruvate dehydrogenase, decarboxylase component E1, thiamin-binding	37	6.9	0.2	-2.7	510.3	15.78	-6.7	0	0	NE	down
XHV734_0831	<i>opgH</i>	Glucans biosynthesis glucosyltransferase H	21	233.4	57.4	-2.01	9802.3	2410.51	-176	0	0	NE	-

**Table S2.** Continued

XHV734_0943	-	Transcriptional regulator of N-acetylglucosamine utilization, LacI family	15	45.3	0.7	-4.78	1358.6	20.64	-44.6	0	0	NE	-
XHV734_0998	<i>aspC</i>	putative aspartate aminotransferase	20	66.7	1	-5.1	2666.1	39.09	-65.7	0	0	NE	-
XHV734_1015	<i>suxB</i>	amylsucrase or alpha amylase	33	58.3	12.1	-2.18	3849.2	800.95	-46.2	0	0	NE	-
XHV734_1016	<i>suxA</i>	putative sucrose-specific TonB-dependent receptor	40	82.6	35.7	-1.19	6606.7	2858.6	-46.9	5,00E-04	0.01625	NE	-
XHV734_1017	<i>suxC</i>	Sugar transporter	21	82.6	28.2	-1.52	3469.8	1184.45	-54.4	0	0	NE	-
XHV734_1041	-	Type I secretion outer membrane protein, TolC precursor	28	91	28.1	-1.66	5097.4	1571.12	-63	4,00E-04	0.01343	NE	-
XHV734_1046	<i>leuC</i>	3-isopropylmalate isomerase subunit, dehydratase component	8	232	0.7	-7.12	3711.4	10.72	-231.3	0	0	NE	-
XHV734_1047	<i>leuD</i>	3-isopropylmalate isomerase subunit	9	70.4	0.2	-5.89	1266.7	3.61	-70.2	0	0	NE	-
XHV734_1049	<i>leuB</i>	3-isopropylmalate dehydrogenase	15	68.7	0.1	-5.98	2062	3.18	-68.6	0	0	NE	-
XHV734_1050	<i>leuA</i>	2-isopropylmalate synthase	12	47.6	2.7	-3.71	1142	65.04	-44.9	4,00E-04	0.01343	NE	-
XHV734_1051	<i>tdcB</i>	L-threonine dehydratase catabolic TdcB	13	95.5	3.7	-4.37	2482.3	95.16	-91.8	0	0	NE	-
XHV734_1053	<i>ilvG</i>	Acetolactate synthase isozyme 2 large subunit	11	170.7	1.4	-6.15	3755.5	31.12	-169.3	0	0	NE	-
XHV734_1054	<i>ilvC</i>	Ketol-acid reductoisomerase (NADP(+))	15	180.9	1.8	-6.02	5427.3	54.34	-179.1	0	0	NE	-
XHV734_1096	<i>hemL</i>	glutamate-1-semialdehyde aminotransferase (aminomutase)	13	4.7	0	-2.45	122.8	1.22	-4.7	0	0	NE	down
XHV734_1124	<i>yjgF</i>	ketoacid-binding protein	4	117.8	6.9	-3.91	942.1	55.15	-110.9	3,00E-04	0.01042	NE	-
XHV734_1145	<i>tkfA</i>	transketolase 1, thiamin-binding	21	18.2	0	-4.2	762.4	1.88	-18.1	0	0	NE	-
XHV734_1167	<i>gapA</i>	glyceraldehyde-3-phosphate dehydrogenase A	8	21.6	4.2	-2.12	345.9	67.48	-17.4	2,00E-04	0.00741	NE	-
XHV734_1174	-	putative fructose-bisphosphate aldolase class 1	9	3.5	0	-2.17	62.7	0	-3.5	0	0	NE	down
XHV734_1210	-	sulfur deprivation response regulator	9	3	0.1	-1.9	54	1.3	-2.9	1,00E-04	0.0039	NE	down
XHV734_1213	<i>thiG</i>	thiamine biosynthesis ThiGH complex subunit	11	167.8	31.2	-2.39	3691.3	687.49	-136.5	0	0	NE	-
XHV734_1242	-	two-component system sensor protein	14	27.1	0	-4.81	757.6	0	-27.1	0	0	NE	-
XHV734_1251	<i>ecpC</i>	Fimbrial protein EcpC	17	58.8	17.3	-1.71	1998.7	588.64	-41.5	2,00E-04	0.00741	NE	-
XHV734_1262	<i>nadE</i>	Glutamine-dependent NAD(+) synthetase	23	7.2	0	-3.03	330.9	0	-7.2	0	0	NE	down
XHV734_1310	-	Ferric enterobactin receptor	38	163.2	84.7	-0.94	12405.3	6437.05	-78.5	0.0013	0.03763	NE	-
XHV734_1330	-	hypothetical protein	4	22.2	3.3	-2.42	177.8	26.77	-18.9	5,00E-04	0.01625	NE	-
XHV734_1332	<i>ruvA</i>	component of RuvABC resolvase, regulatory subunit	6	75.2	15.1	-2.25	902.6	180.79	-60.2	9,00E-04	0.02747	NE	-
XHV734_1333	<i>kup</i>	putative potassium transport system protein kup	20	15.9	1.7	-2.65	636.5	67.85	-14.2	0	0	NE	down

**Table S2.** Continued

XHV734_1625	<i>asnB</i>	asparagine synthetase B	24	110.1	16.7	-2.65	5283	799.86	-93.4	0	0	NE	-
XHV734_1688	-	Nicotinate-nucleotide adenyltransferase	7	40.4	0.3	-4.97	564.9	4.45	-40	0	0	NE	-
XHV734_1731	<i>dcd</i>	dCTP deaminase	7	6.1	0.1	-2.72	85.6	1.14	-6	0	0	NE	-
XHV734_1753	<i>prmb</i>	N5-glutamine methyltransferase	12	3.9	0	-2.28	92.5	0	-3.9	3,00E-04	0.01042	NE	down
XHV734_1754	-	conserved membrane hypothetical protein	5	64.8	1.2	-4.9	647.8	12.05	-63.6	0	0	NE	-
XHV734_1756	<i>gyaR</i>	Glyoxylate reductase	12	27.4	0.6	-4.17	658.5	13.81	-26.9	0.0012	0.03534	NE	-
XHV734_1761	<i>trpF</i>	N-(5'-phosphoribosyl)anthranilate isomerase	6	83	0.1	-6.26	996.1	1.15	-82.9	0	0	NE	-
XHV734_1763	<i>trpB</i>	tryptophan synthase, beta subunit	14	315.2	2.4	-6.53	8826	67.63	-312.8	0	0	NE	-
XHV734_1765	<i>trpA</i>	Tryptophan synthase alpha chain	8	491.9	3.7	-6.7	7870.4	59.88	-488.2	0	0	NE	-
XHV734_1808	<i>purK</i>	N5-carboxyaminoimidazole ribonucleotide synthase	8	114.9	1.8	-5.38	1837.6	28.42	-113.1	3,00E-04	0.01042	NE	-
XHV734_1853	<i>gumB</i>	Polysaccharide biosynthesis protein GumB	9	84.5	19	-2.09	1521.3	342.83	-65.5	0	0	NE	-
XHV734_1854	<i>gumC</i>	Xanthan biosynthesis chain length determinant protein GumC	18	69.6	13.2	-2.32	2506.8	474.41	-56.5	1,00E-04	0.0039	NE	-
XHV734_1945	<i>tgt</i>	tRNA-guanine transglycosylase	17	8.5	0.5	-2.65	289.9	17.65	-8	0	0	NE	-
XHV734_1977	<i>gdh</i>	NAD-specific glutamate dehydrogenase	58	2.1	0.5	-1.01	243.1	62.18	-1.6	0	0	GD	down
XHV734_2093	<i>pdxH</i>	Pyridoxine/pyridoxamine 5'-phosphate oxidase	4	38.8	0.4	-4.78	310.6	3.57	-38.4	7,00E-04	0.0219	NE	-
XHV734_2132	<i>hprK</i>	HPr kinase/phosphorylase	9	25.9	2.3	-3.03	466.2	41.25	-23.6	1,00E-04	0.0039	NE	-
XHV734_2148	<i>purM</i>	phosphoribosylaminoimidazole synthetase	9	84.7	1.3	-5.23	1524.7	23.21	-83.4	0	0	NE	-
XHV734_2154	<i>sodA</i>	superoxide dismutase, Mn	8	6.2	0	-2.85	99.6	0	-6.2	0	0	NE	-
XHV734_2163	<i>mtaD</i>	5-methylthioadenosine/S-adenosylhomocysteine deaminase	13	32.8	7.6	-1.97	853.4	198.18	-25.2	1,00E-04	0.0039	NE	-
XHV734_2180	-	Dihydrorotase	12	16.9	0	-4.16	405.4	0	-16.9	0	0	NE	down
XHV734_2181	-	C4-type zinc finger protein, DksA/TraR family	14	2.5	0	-1.81	69.9	0	-2.5	4,00E-04	0.01343	NE	down
XHV734_2188	<i>argF</i>	N-acetylmethionine carbamoyltransferase	12	171.6	14.5	-3.47	4117.7	348.98	-157	0	0	NE	-
XHV734_2189	<i>argG</i>	Argininosuccinate synthase	18	176.7	18.7	-3.17	6363	674.88	-158	0	0	NE	-
XHV734_2190	-	Acetylmethionine deacetylase	8	133.3	11.8	-3.39	2132.6	188.62	-121.5	2,00E-04	0.00741	NE	-
XHV734_2191	<i>argB</i>	Acetylglutamate kinase	9	311.1	25.6	-3.55	5600.2	461.57	-285.5	0	0	NE	-
XHV734_2193	<i>argC</i>	N-acetyl-gamma-glutamyl-phosphate reductase	13	97.4	12.9	-2.82	2531.4	336.11	-84.4	0	0	NE	-
XHV734_2194	<i>argH</i>	Argininosuccinate lyase	15	185.5	7.5	-4.45	5564	225.09	-178	0	0	NE	-
XHV734_2195	-	Cupin	3	97.3	10.5	-3.1	583.6	62.75	-86.8	0.0011	0.03278	NE	-
XHV734_2249	<i>lapB</i>	Lipopolysaccharide assembly protein B	17	103.9	4	-4.38	3531.5	137.33	-99.8	0	0	NE	-

**Table S2.** Continued

XHV734_2250	-	UDP-N-acetylmuramyl pentapeptide phosphotransferase/UDP-N-acetylglucosamine-1-phosphate transferase	11	61.2	5.5	-3.25	1345.4	121.29	-55.6	0	0	NE	-
XHV734_2251	-	Epimerase/dehydratase protein	25	50.8	10.6	-2.15	2539.3	531.92	-40.1	0	0	NE	down
XHV734_2252	<i>galU</i>	UTP--glucose-1-phosphate uridylyltransferase	9	95	0.1	-6.48	1709.4	1.31	-94.9	0	0	NE	-
XHV734_2257	<i>guaA</i>	GMP synthetase (glutamine aminotransferase)	16	5.1	0.1	-2.53	162.6	1.67	-5	0	0	NE	down
XHV734_2285	<i>phaC</i>	Poly(3-hydroxyalkanoate) polymerase subunit PhaC	18	277.3	64.7	-2.08	9984.3	2327.91	-212.7	0	0	GA	-
XHV734_2313	<i>edd</i>	6-phosphogluconate dehydratase	15	121.1	31.6	-1.91	3633.8	947	-89.6	0	0	NE	-
XHV734_2316	<i>zwf</i>	glucose-6-phosphate dehydrogenase	18	190.9	83.8	-1.18	6870.6	3017.33	-107	8,00E-04	0.02472	NE	-
XHV734_2318	-	Folate-dependent protein for Fe/S cluster synthesis/repair in oxidative stress	11	12.8	0.2	-3.5	282.3	4.84	-12.6	0	0	NE	-
XHV734_2707	<i>carB</i>	carbamoyl-phosphate synthase large subunit	38	7.9	0	-3.15	600.2	0	-7.9	0	0	NE	down
XHV734_2708	-	conserved membrane hypothetical protein	4	57.8	0	-5.88	462.1	0	-57.8	1,00E-04	0.0039	NE	-
XHV734_2709	<i>carA</i>	carbamoyl phosphate synthetase small subunit, glutamine amidotransferase	12	9.9	0	-3.44	236.6	0	-9.9	0	0	NE	down
XHV734_2726	<i>serA</i>	D-3-phosphoglycerate dehydrogenase	11	42.8	1.2	-4.29	942.5	27.34	-41.6	3,00E-04	0.01042	NE	-
XHV734_2735	<i>mtnB</i>	Methylthioribulose-1-phosphate dehydratase	6	228.3	133.2	-0.77	2740.1	1598.22	-95.2	4,00E-04	0.01343	NE	-
XHV734_2737	<i>mtnC</i>	Enolase-phosphatase E1	12	6.7	0	-2.95	160.9	0	-6.7	0	0	NE	-
XHV734_2742	<i>hisF</i>	imidazole glycerol phosphate synthase, catalytic subunit with HisH	9	104.6	4.3	-4.31	1882	77.5	-100.3	6,00E-04	0.019	NE	-
XHV734_2743	<i>hisA</i>	N-(5'-phospho-L-ribosyl-formimino)-5-amino-1-(5'-phosphoribosyl)-4-imidazolecarboxamide isomerase	8	164.7	9.8	-3.94	2635.7	157.08	-154.9	2,00E-04	0.00741	NE	-
XHV734_2744	<i>hisH</i>	imidazole glycerol phosphate synthase, glutamine amidotransferase subunit with HisF	8	141.5	3	-5.14	2264.4	48.61	-138.5	0	0	NE	-
XHV734_2745	<i>hisB</i>	fused histidinol-phosphatase; imidazoleglycerol-phosphate dehydratase	13	42.5	0.9	-4.55	1105.2	22.25	-41.7	0	0	NE	-
XHV734_2747	<i>hisD</i>	bifunctional histidinol dehydrogenase and histidinol dehydrogenase	13	194	9.5	-4.21	5043.8	247.36	-184.5	0	0	NE	-
XHV734_2748	<i>hisG</i>	ATP phosphoribosyltransferase	7	136.4	5.4	-4.41	1909	76.21	-130.9	0	0	NE	-
XHV734_2755	<i>thrC</i>	Threonine synthase	14	132.4	0.4	-6.6	3706.8	10.39	-132	0	0	NE	-
XHV734_2757	-	EthD family reductase	7	256.3	2.3	-6.28	3588.1	32.47	-254	0	0	NE	-
XHV734_2758	<i>thrB</i>	Homoserine kinase	10	78.9	1.3	-5.1	1577.7	26.71	-77.6	0	0	NE	-
XHV734_2759	<i>thrA</i>	fused aspartokinase I; homoserine dehydrogenase I	26	116.4	1.4	-5.59	6051.4	74.85	-114.9	0	0	NE	-



Table S2. Continued

XHV734_2829	<i>pgi</i>	Glucose-6-phosphate isomerase	14	25.6	0.4	-4.24	715.6	11.28	-25.2	0	0	NE	down
XHV734_2832	<i>panB</i>	3-methyl-2-oxobutanoate hydroxymethyltransferase	8	2.8	0	-1.92	44.7	0	-2.8	0	0	NE	down
XHV734_2852	<i>dgoA</i>	2-oxo-3-deoxygalactonate 6-phosphate aldolase	6	273.6	25.2	-3.39	3282.6	302.1	-248.4	5,00E-04	0.01625	NE	-
XHV734_2853	<i>dgoD</i>	galactonate dehydratase	15	194.1	13.3	-3.77	5823.4	398.85	-180.8	0	0	NE	-
XHV734_2887	<i>miaA</i>	delta(2)-isopentenylpyrophosphate tRNA-adenosine transferase	9	34.6	2.8	-3.22	623.2	50.98	-31.8	0	0	NE	-
XHV734_2889	<i>ftsH</i>	protease, ATP-dependent zinc-metallo	15	6.2	0	-2.86	187.4	0	-6.2	0	0	NE	-
XHV734_2989	<i>pyrB</i>	Aspartate carbamoyltransferase	6	26.2	0	-4.76	314	0	-26.2	3,00E-04	0.01042	NE	-
XHV734_3043	<i>smc</i>	Chromosome partition protein Smc	22	36	9.7	-1.8	1585.5	425.28	-26.4	0	0	NE	-
XHV734_3052	<i>can</i>	Carbonic anhydrase 2	8	8.6	0	-3.26	137.5	0	-8.6	0	0	NE	down
XHV734_3084	<i>pstS</i>	phosphate transporter subunit	11	193.2	2.8	-5.67	4250.3	61.82	-190.4	0	0	NE	-
XHV734_3085	<i>pstC</i>	phosphate transporter subunit	10	120.7	4.4	-4.48	2414.8	88.9	-116.3	0	0	NE	-
XHV734_3086	<i>pstA</i>	phosphate transporter subunit	9	50.8	2.9	-3.74	914.3	51.59	-47.9	1,00E-04	0.0039	NE	-
XHV734_3087	<i>pstB</i>	phosphate transporter subunit	10	88	3.5	-4.32	1759.5	69.31	-84.5	0	0	NE	-
XHV734_3096	<i>rnr</i>	exoribonuclease R, RNase R	30	66.3	25.4	-1.35	3979.5	1523.77	-40.9	0.0013	0.03763	NE	-
XHV734_3113	<i>udg</i>	UDP-glucose 6-dehydrogenase	19	78.3	2.7	-4.41	2976.8	104.03	-75.6	0	0	NE	-
XHV734_3118	-	Transcriptional regulator, GntR family	3	17.2	0	-4.18	103	0	-17.2	9,00E-04	0.02747	NE	-
XHV734_3365	<i>aroA</i>	3-phosphohikimate 1-carboxyvinyltransferase	10	12.9	0	-3.8	257.6	0	-12.9	0	0	NE	-
XHV734_3366	<i>pheA</i>	Chorismate mutase / Prephenate dehydratase	11	83.4	0.5	-5.86	1835.5	9.99	-83	0	0	NE	-
XHV734_3367	<i>serC</i>	3-phosphoserine/phosphohydroxythreonine aminotransferase	14	124.4	1.5	-5.65	3483.6	41.82	-122.9	0	0	NE	-
XHV734_3398	<i>metXS</i>	Homoserine O-succinyltransferase	13	122.3	37.3	-1.69	3178.9	970.08	-85	4,00E-04	0.01343	NE	-
XHV734_3482	-	Glycosyltransferase	27	82.8	19	-2.06	4470.7	1027.88	-63.8	0	0	NE	down
XHV734_3578	-	Polyketide cyclase	17	38	4.7	-2.77	1291.1	160.79	-33.2	2,00E-04	0.00741	NE	-
XHV734_3771	<i>cipX</i>	ATPase and specificity subunit of CipX-CipP ATP-dependent serine protease	11	5.6	0.3	-2.32	122.5	6.96	-5.3	1,00E-04	0.0039	NE	-
XHV734_3772	<i>cipP</i>	proteolytic subunit of CipA-CipP and CipX-CipP ATP-dependent serine protease	11	7.8	0	-3.14	172	0	-7.8	0	0	NE	-
XHV734_3840	<i>phoB</i>	DNA-binding response regulator in two-component regulatory system with PhoR (or CreC)	6	312.7	2.4	-6.52	3751.9	29.11	-310.2	1,00E-04	0.0039	NE	-
XHV734_3851	<i>purF</i>	amidophosphoribosyltransferase	17	80.7	3.1	-4.32	2742.6	105.27	-77.6	0	0	NE	-

Table S2. Continued

XHV734_3889	<i>bipA</i>	GTP-binding protein	12	4.3	0.3	-2.04	104.3	7.11	-4.1	0	0	NE	down
XHV734_4155	<i>gljA</i>	serine hydroxymethyltransferase	17	0.7	0	-0.77	23.9	0	-0.7	0.0014	0.04029	GD	down
XHV734_4180	<i>xpsD</i>	Secretin XpsD	31	148.4	2.6	-5.37	9200.2	161.99	-145.8	0	0	NE	-
XHV734_4183	<i>xpsL</i>	Type II secretion system protein L	5	171.5	1.9	-5.9	1715	18.87	-169.6	0	0	NE	-
XHV734_4184	<i>xpsK</i>	Type II secretion system protein K	14	217.2	4.3	-5.38	6082.5	119.17	-213	0	0	NE	-
XHV734_4188	<i>xpsG</i>	Type II secretion system protein G	5	101.8	1.9	-5.15	1018.2	18.87	-99.9	0.001	0.03016	NE	-
XHV734_4189	<i>xpsF</i>	Type II secretion system protein F	6	143.3	2	-5.57	1719.8	24.36	-141.3	0	0	NE	-
XHV734_4190	<i>xpsE</i>	Type II secretion system protein E	16	63.1	0.9	-5.09	2019	28.22	-62.2	0	0	NE	-
XHV734_4196	<i>purL</i>	phosphoribosylformyl-glycineamide synthetase	24	36.3	0.1	-5.08	1741.5	4.91	-36.2	0	0	NE	-
XHV734_4222	-	hypothetical protein	29	84.2	11.6	-2.75	4881.3	673.93	-72.5	0	0	NE	down
XHV734_4228	<i>cpsG</i>	phosphomannomutase	17	174.6	32.2	-2.4	5935.5	1093.75	-142.4	0	0	NE	-
XHV734_4229	<i>cpsB</i>	mannose-1-phosphate guanylyltransferase	16	11.4	0	-3.63	363.7	0	-11.4	0	0	NE	down
XHV734_4230	<i>rmlD</i>	dTDP-4-dehydrohamnose reductase subunit, NAD(P)-binding, of dTDP-L-rhamnose synthase	14	34	1	-4.1	952.4	29.34	-33	0	0	NE	-
XHV734_4231	<i>rmlC</i>	dTDP-4-deoxyhamnose-3,5-epimerase	9	29.1	1.4	-3.62	523.3	25.95	-27.6	1,00E-04	0.0039	NE	-
XHV734_4232	<i>rmlA</i>	glucose-1-phosphate thymidyltransferase	18	26.1	4.4	-2.32	938	159.71	-21.6	0	0	NE	-
XHV734_4233	<i>rmlB</i>	dTDP-glucose 4,6-dehydratase	17	105.3	7.8	-3.59	3580.2	265.82	-97.5	0	0	NE	-
XHV734_4242	<i>gmd</i>	GDP-D-mannose dehydratase, NAD(P)-binding	17	44.6	1.8	-4.05	1516.9	59.69	-42.9	0	0	NE	-
XHV734_4243	<i>wxcE</i>	Glycosyltransferase	10	26	2.6	-2.91	520.8	52.03	-23.4	2,00E-04	0.00741	NE	-
XHV734_4244	<i>wxcD</i>	Glycosyltransferase	33	59.1	21.5	-1.42	3899.8	1421.05	-37.6	3,00E-04	0.01042	NE	-
XHV734_4245	<i>wxcC</i>	Glycosyl transferase	26	22.7	5.4	-1.88	1178.6	282.22	-17.2	0	0	NE	down
XHV734_4246	<i>wxcB</i>	Kinase	41	20.8	0.9	-3.54	1705.3	71.51	-19.9	0	0	NE	down
XHV734_4247	<i>wzt</i>	ATP binding component of ABC-transporter	34	10.4	0	-3.51	706.3	0	-10.4	0	0	NE	down
XHV734_4248	<i>wzm</i>	Transport permease protein	15	10.2	0	-3.49	306.8	0	-10.2	0	0	NE	-
XHV734_4249	<i>wxcA</i>	Glycosyl transferase	36	19.5	1.8	-2.86	1400.9	130.31	-17.6	0	0	NE	-
XHV734_4311	-	Dihydropyrimidine dehydrogenase of pyruvate dehydrogenase complex	9	20	2.7	-2.49	359.7	49.12	-17.3	5,00E-04	0.01625	NE	-
XHV734_4313	<i>aceF</i>	pyruvate dehydrogenase, dihydropyrimidinyltransferase component E2	12	3.4	0	-2.12	80.4	0	-3.4	1,00E-04	0.0039	NE	down
XHV734_4495	-	Putative CDP-glycerol glycerophosphotransferase	12	70.4	10.8	-2.6	1690.3	258.76	-59.6	0.0011	0.03278	NE	-
XHV734_4497	-	Lipid A core-O-antigen ligase-like enzyme	15	107.9	28.9	-1.86	3238.1	867.74	-79	6,00E-04	0.019	NE	-

**Table S2.** Continued

XHV734_4542	<i>icd</i>	Isocitrate dehydrogenase [NADP]	22	14.6	3.7	-1.72	642.1	164.84	-10.8	3.00E-04	0.01042	NE	-
XHV734_4574	<i>bgIX</i>	Periplasmic beta-glucosidase	23	75.4	14.5	-2.3	3467.2	666.13	-60.9	0	0	NE	-
XHV734_4610	<i>pyrE</i>	orotate phosphoribosyltransferase	4	32.3	0.2	-4.78	258.2	1.68	-32.1	0.0013	0.03763	NE	-
XHV734_4644	<i>desA</i>	NADPH-dependent stearyl-CoA 9-desaturase	16	155.7	23	-2.71	4982.9	736.22	-132.7	0	0	NE	-
XHV734_4646	-	Flavodoxin reductases (ferredoxin-NADPH reductases) family 1	7	156.6	17.7	-3.08	2192.4	247.38	-138.9	0	0	NE	-
XHV734_4721	<i>aroE</i>	Shikimate dehydrogenase (NADP(+))	7	189.7	2	-6.01	2655.5	27.49	-187.7	0	0	NE	-
XHV734_4897	<i>tatC</i>	TatABC protein translocation system subunit	10	188.2	52.8	-1.81	3763.2	1055.67	-135.4	0	0	NE	-
XHV734_4949	<i>pyrF</i>	Orotidine 5'-phosphate decarboxylase	4	80.7	0	-6.35	645.2	0	-80.7	1.00E-04	0.0039	NE	-
XHV734_4950	-	Acid phosphatase	9	45.5	0	-5.54	818.7	0	-45.5	0	0	NE	-
XHV734_5033	<i>recD</i>	RecBCD enzyme subunit RecD	20	10.2	0.9	-2.58	408.2	34.97	-9.3	0	0	NE	-
XHV734_5034	<i>recB</i>	RecBCD enzyme subunit RecB	39	10.9	0.2	-3.28	846.5	17.37	-10.6	0	0	NE	-
XHV734_5035	<i>recC</i>	RecBCD enzyme subunit RecC	37	5.6	0.2	-2.4	411	17.65	-5.3	0	0	NE	down

**Table S3.** List of 36 critical genes in 1/10<sup>th</sup> TSB

Locus	Gene	Product	TA sites count	Mean RC in library	Mean RC in 1/10 <sup>th</sup> TSB	Log2FC	Sum RC in library	Sum RC in 1/10 <sup>th</sup> TSB	Δ Mean RC	Permutation test p-value	FDR test q-value	Gene state in library	Fitness in lettuce
XHV734_0250	-	cardiolipin synthase	24	223.2	109.4	-1.02	10712	5250.26	-113.8	2,00E-04	0.03473	NE	down
XHV734_0306	<i>ubiB</i>	2-octaprenylphenol hydroxylase	18	6.4	0.7	-2.09	229	26.16	-5.6	0	0	NE	-
XHV734_0747	<i>aceE</i>	pyruvate dehydrogenase, decarboxylase component E1, thiamin-binding	37	16.9	6.9	-1.18	1248.6	510.27	-10	1,00E-04	0.0219	NE	down
XHV734_1096	<i>hemL</i>	glutamate-1-semialdehyde aminotransferase (aminomutase)	13	22.7	4.7	-2.05	589.4	122.8	-17.9	0	0	NE	down
XHV734_1262	<i>nadE</i>	Glutamine-dependent NAD(+) synthetase	23	21.3	7.2	-1.44	978.8	330.91	-14.1	0	0	NE	down
XHV734_1333	<i>kup</i>	putative potassium transport system protein <i>kup</i>	20	38.8	15.9	-1.23	1551	636.54	-22.9	3,00E-04	0.04197	NE	down
XHV734_1592	-	conserved membrane hypothetical protein	7	23.2	2.8	-2.65	324.5	39.8	-20.3	2,00E-04	0.03473	NE	-
XHV734_1721	-	Metallopeptidase	24	134.7	59.4	-1.17	6466.5	2851.88	-75.3	2,00E-04	0.03473	NE	-
XHV734_1977	<i>gdh</i>	NAD-specific glutamate dehydrogenase	58	7.8	2.1	-1.51	907.6	243.07	-5.7	0	0	GD	down
XHV734_2180	-	Dihydroorotase	12	95	16.9	-2.42	2280.8	405.4	-78.1	1,00E-04	0.0219	NE	down
XHV734_2209	-	Transport ATP-binding protein <i>CydD</i>	12	16.7	0	-4.14	400.6	0	-16.7	0	0	NE	-
XHV734_2210	-	Transport ATP-binding protein <i>CydC</i>	12	8.2	0	-3.19	195.7	0	-8.2	3,00E-04	0.04197	NE	-
XHV734_2216	<i>ccmF</i>	heme lyase, <i>CcmF</i> subunit	21	95.4	36.3	-1.37	4005.3	1523.37	-59.1	3,00E-04	0.04197	NE	-
XHV734_2251	-	Epimerase/dehydratase protein	25	152	50.8	-1.56	7599.5	2539.25	-101.2	0	0	NE	down
XHV734_2257	<i>guaA</i>	GMP synthetase (glutamine aminotransferase)	16	26.6	5.1	-2.18	851.8	162.64	-21.5	0	0	NE	down
XHV734_2271	<i>mntH</i>	Divalent metal cation transporter <i>MntH</i>	14	57.7	12.4	-2.13	1615.2	347.49	-45.3	3,00E-04	0.04197	NE	-
XHV734_2430	-	AAA ATPase central domain protein	21	53.4	13.7	-1.89	2241.1	575.13	-39.7	0	0	NE	-
XHV734_2555	<i>ndk</i>	multifunctional nucleoside diphosphate kinase and apyrimidinic endonuclease and 3'-phosphodiesterase	4	17.3	3.7	-1.96	138.7	29.84	-13.6	3,00E-04	0.04197	NE	-
XHV734_2707	<i>carB</i>	carbamoyl-phosphate synthase large subunit	38	53.7	7.9	-2.62	4078.3	600.19	-45.8	0	0	NE	down
XHV734_2709	<i>carA</i>	carbamoyl phosphate synthetase small subunit, glutamine amidotransferase	12	70.4	9.9	-2.72	1689.8	236.58	-60.6	3,00E-04	0.04197	NE	down
XHV734_2829	<i>pgi</i>	Glucose-6-phosphate isomerase	14	202.8	25.6	-2.94	5678.4	715.56	-177.2	0	0	NE	down
XHV734_2831	<i>panC</i>	pantothenate synthetase	8	18.5	2.2	-2.59	295.9	35.92	-16.2	1,00E-04	0.0219	NE	-
XHV734_2832	<i>panB</i>	3-methyl-2-oxobutanoate hydroxymethyltransferase	8	24.9	2.8	-2.77	398.8	44.68	-22.1	0	0	NE	down
XHV734_2876	<i>csrA</i>	Translational regulator <i>CsrA</i>	4	13.9	0.9	-3	110.9	6.86	-13	3,00E-04	0.04197	GD	-

**Table S3.** Continued

XHV734_3039	<i>epmA</i>	Elongation factor P--(R)-beta-lysine ligase	12	2.4	0.1	-1.7	57.9	1.21	-2.4	0	0	GD	-
XHV734_3052	<i>can</i>	Carbonic anhydrase 2	8	37.8	8.6	-2.02	605.1	137.51	-29.2	2,00E-04	0.03473	NE	down
XHV734_3072	-	Peptidyl-prolyl cis-trans isomerase	10	56.7	4.2	-3.46	1134	84.7	-52.5	0	0	NE	-
XHV734_3482	-	Glycosyltransferase	27	204.3	82.8	-1.29	11034.8	4470.68	-121.6	2,00E-04	0.03473	NE	down
XHV734_3702	<i>fabF</i>	3-oxoacyl-[acyl-carrier-protein] synthase II	11	2.4	0.1	-1.68	53.3	1.52	-2.4	0	0	GD	-
XHV734_3779	-	hypothetical protein	20	5.7	0	-2.69	227.6	1.51	-5.7	0	0	GD	-
XHV734_4222	-	hypothetical protein	29	173.8	84.2	-1.04	10078.1	4881.32	-89.6	1,00E-04	0.0219	NE	down
XHV734_4229	<i>cpsB</i>	mannose-1-phosphate guanylyltransferase	16	83.5	11.4	-2.77	2672.4	363.68	-72.1	0	0	NE	down
XHV734_4245	-	Glycosyl transferase	26	50.5	22.7	-1.12	2625.5	1178.57	-27.8	2,00E-04	0.03473	NE	down
XHV734_4246	-	Kinase	41	51.8	20.8	-1.28	4251.1	1705.32	-31	0	0	NE	down
XHV734_4247	-	ATP binding component of ABC-transporter	34	41.5	10.4	-1.9	2822.8	706.31	-31.1	0	0	NE	down
XHV734_5035	<i>recC</i>	RecBCD enzyme subunit RecC	37	14.6	5.6	-1.25	1079.3	410.96	-9	0	0	NE	down



## **Étude polyphasique de *Xanthomonas hortorum* à l'ère de la génomique : focus sur *X. hortorum* pv. *vitians*, agent causal de la bactériose foliaire de la laitue**

Le genre *Xanthomonas* représente un réservoir majeur d'espèces bactériennes phytopathogènes. Parmi celles-ci, les différents pathovars de *X. hortorum* s'attaquent à une grande variété d'espèces végétales phylogénétiquement distantes. La bactériose foliaire de la laitue, causée par *X. hortorum* pv. *vitians*, est l'une des de ces pathologies dont les conséquences pour les cultures sont les plus importantes à l'échelle mondiale. Toutefois, les questions liées à l'étiologie de cette maladie infectieuse végétale nécessitent une étude approfondie pour être élucidées. De plus, les aspects moléculaires et génétiques de l'interaction hôte-pathogène et de l'adaptation environnementale n'avaient jamais été investigués. À travers une approche mixte alliant les principes de la taxonomie polyphasique et le criblage par Tn-seq des gènes contribuant à la valeur sélective de *X. hortorum* pv. *vitians* en conditions *in vitro* et *in planta*, cette étude entend répondre à ces problématiques en se reposant sur les dernières évolutions dans le champ de la génomique. La taxonomie de *X. hortorum* a tout d'abord été remaniée en proposant sa combinaison avec *X. cynarae*. Ensuite, le génome essentiel de *X. hortorum* pv. *vitians* a été caractérisé et distingué entre gènes conservés et gènes accessoires « égoïstes » acquis par transferts horizontaux. Enfin, les gènes et fonctions importants pour la valeur sélective du pathogène lors des phases successives épiphytes et endophytes de l'interaction avec la laitue ont été identifiés. Ces travaux révèlent la complexité insoupçonnée de l'espèce *X. hortorum* et éclairent les bases génétiques de la plasticité écologique de *X. hortorum* pv. *vitians*.

**Mots-clés** : *Xanthomonas hortorum* ; bactériose de la laitue ; taxonomie ; Tn-seq ; génomique

## **A polyphasic study of *Xanthomonas hortorum* in the genomic era: focus on *X. hortorum* pv. *vitians*, the causal agent of the bacterial leaf spot of lettuce**

The genus *Xanthomonas* constitutes a major reservoir of phytopathogenic bacterial species. Among them, the pathovars of *X. hortorum* attacks a wide variety of phylogenetically distant botanical species. The bacterial leaf spot of lettuce caused by *X. hortorum* pv. *vitians* is one of the most destructive of these diseases at a worldwide scale. However, substantial interrogations remain regarding the etiology of this disease. Moreover, the molecular and genetic determinants sustaining the host-pathogen interaction and the environmental adaptation have never been investigated. Through a mixed approach combining the principles of polyphasic taxonomy and the genome-wide Tn-seq screening of genes contributing to the fitness of *X. hortorum* pv. *vitians* in *in vitro* and *in planta* conditions, this study aims at solving these issues using the latest developments in the field of genomics. First, investigations on the taxonomy of this phytopathogenic bacteria led to reconsider the genomic diversity existing within the species and to propose the combination of *X. hortorum* and *X. cynarae*. Then, the essential genome of *X. hortorum* pv. *vitians* was characterized and essential genes were discriminated between core and “selfish” accessory ones, the latter being acquired through horizontal gene transfer events. At last, a comprehensive overview of the genes and functions required for the fitness of *X. hortorum* pv. *vitians* during the successive epiphytic and endophytic phases of the lettuce infection was provided. This work reveals the unsuspected complexity of the *X. hortorum* species and enlighten the genetic bases of the ecological plasticity of *X. hortorum* pv. *vitians*.

**Keywords**: *Xanthomonas hortorum* ; bacterial leaf spot of lettuce ; taxonomy ; Tn-seq ; genomics