# 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. *vitians*, 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 nomenspecies 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. *vitians.*

Then, whole-genome based phylogenies and overall genome relatedness indices calculated on taxonomically relevant strains exhibited the intermediate position of *X. campestris* pv. *vitians* 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. *vitians* as *X. hortorum* pv. *vitians* 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.

# 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 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$^{PT}$ (= NCPPB 976$^{PT}$ = LMG 937$^{PT}$) was grouped with *X. axonopodis* Vauterin *et al.* 1995 as *X. axonopodis pv. vitians*. It is important to note that CFBP 2538$^{PT}$ 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 = NCPPB 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$^{PT}$ [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$^{PT}$ 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$^{PT}$ 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$^{neoPT}$ = CFBP 8686$^{neoPT}$ = NCPPB 2248$^{neoPT}$ 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$^{th}$ tryptic soy broth (TSB) containing 30 % glycerol and stored at -80°C. Bacterial cultures were made in 1/10$^{th}$ tryptic soy broth or on 1/10$^{th}$ tryptic soy agar (TSA) plates and cultivated 24 to 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 to 4 weeks. Overnight bacterial cultures

(0.8 to 1.0 $OD_{600nm}$) were spectrophotometrically adjusted to 0.2 $OD_{600nm}$ in sterile deionized water, corresponding to approximately $10^8$ 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 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 β subunit) and *rpoD* (RNA polymerase σ70 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 µL 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

182 min of initial denaturation at 94°C, 30 cycles of 30 s at 94°C, 30 s at appropriate annealing
183 temperature, 1 min at 72°C, and a final extension for 5 min at 72°C. PCR products were then
184 purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany).
185 DNA quality and concentrations were assessed with a Nanophotometer NP80 (Implen,
186 Munich, Germany) before Sanger sequencing of one strand at Genoscreen (Lille, France) or
187 GATC Biotech (Konstanz, Germany) with primers XgyrB1F (*gyrB*), rpoDXSoR6 (*rpoD*) or
188 gap1F (*gapA*). Sequences of *gyrB* and *rpoD* loci of the CFBP strains were provided by the
189 CIRM-CFBP. Sequences were aligned, trimmed and concatenated following the alphabetical
190 order using CLUSTALΩ [60], resulting in a 2,331 bp sequence. The best-fitted nucleotide
191 substitution model was evaluated in MEGA 7.0.26 based both on the corrected Akaike
192 information criterion (AICc) and the maximized log likelihood (lnL).Maximum likelihood
193 phylogeny was constructed in MEGA 7.0.26 [33] using the Generalized Time Reversible
194 substitution model (GTR) with a gamma distribution of rate variation among sites (shape
195 parameter = 4) and acceptance of invariant sites (I). Branch support was assessed with 500
196 bootstrap replicates. *X. populi* CFBP 1817$^{T}$ was chosen as an outgroup. All the sequences
197 generated were deposited in the National Center for Biotechnology Information GenBank
198 under accession numbers MK610462 to MK610526 for *gapA*, MK610527 to MK610591 for
199 *gyrB* and MK610592 to MK610656 for *rpoD*. The full concatenated alignment is provided
200 along with the supplementary material.

201 **Genome sequencing, assembly, annotation and acquisition**

202 Genomic DNAs were extracted and purified from 25 ml 1/10$^{th}$ TSB overnight cultures using
203 the standard phenol-chloroform method [55]. The genomes of five strains of *X. campestris*
204 pv. *vitians* type B (LM 16388, LM 16735, CFBP 498, CFBP 499 and CFBP 3978), pathotype
205 strain *X. campestris* pv. *vitians* type A CFBP 2538$^{PT}$, pathotype strains of *X. hortorum*
206 pv. *taraxaci* CFBP 410$^{PT}$, *X. hortorum* pv. *pelargonii* CFBP 2533$^{PT}$ and *X. cynarae*

pv. *gardneri* CFBP 8163$^{PT}$ were sequenced with Illumina technology in HiSeq paired-end 2*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*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$^{T}$ 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 (*is*DDH) [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$^{th}$ 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 1x was prepared by addition of manufacturer's Dye Mix A at 1.2 %. Finally, a 50x stock solution composed of sodium succinate 1M and ferric citrate 100 µM was prepared and sterilized by filtration on 0.2 µm 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 µL 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 pathotype 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 safranine 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 labelled 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$^{PT}$ 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 (Figure 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 (Figure 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 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 (Figure 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 (Figure 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$^{PT}$ was highly related to the type strain of *X. citri* CFBP 3369$^{T}$ (Figure 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 (Figure 2a). Within this group, *X. hortorum* pvs. *carotae* and *hederae* were more genetically similar to each other than to the other pathovars (Figure 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, *is*DDH 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 *is*DDH 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 *is*DDH, they will not be discussed here. Unsurprisingly, *X. campestris* pv. *vitians* type A CFBP 2538$^{PT}$ presented only 88.2 % ANIm and 34.4 % *is*DDH values compared pairwise

with *X. campestris* pv. *vitians* type B LMG 938 but was highly similar to type strain of *X. citri* CFBP 3369$^{T}$ (ANIm = 98.7 % and *is*DDH = 89.5 %), confirming the observations made on the genus-scaled phylogeny (Figure 2a). The comparison with the type strain of *X. axonopodis* CFBP 4924$^{T}$ (83.4 % ANIm and 52.6 % *is*DDH) 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$^{T}$ and *gardneri* CFBP 8163$^{PT}$ differed little on their genomic content, with ANIm and *is*DDH 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 *is*DDH > 60~70 %. Indeed, ANIm varied from 95.4 to 96.5 % and *is*DDH from 64.3 to 71.1 %. The lowest values were always obtained when comparing *X. hortorum* pv. *pelargonii* CFBP 2533$^{PT}$ and pv. *taraxaci* CFBP 410$^{PT}$ and the highest between pv. *hederae* CFBP 5858$^{T}$ 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$^{PT}$ were robustly grouped together by all parameters, with ANIm > 97 % and *is*DDH > 78 %, indicating unequivocally that they should belong the same species. However, if *X. campestris* pv. *vitians* LMG 938 shared 98.4 % ANIm and 87.3 % *is*DDH with type strain of *X. cynarae* CFBP 4188$^{T}$, it also shared 96.6 % ANIm and 68.5 % *is*DDH with *X. hortorum* CFBP 5858$^{T}$. As a matter of fact, comparing type strains of *X. cynarae* and *X. hortorum* revealed ANIm and *is*DDH 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 BLSL-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$^{T}$, as

ANIm and *is*DDH values drastically fell from above 95 % ANIm and 60 % *is*DDH 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 conducted 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$^{T}$ (= CFBP 4925$^{T}$ = LMG 733$^{T}$) was not tested as it should have been and only *X. hortorum* pv. *pelargonii* CFBP 2533$^{PT}$ (= LMG 7314$^{PT}$) was included and presented a 49 % reassociation value against *X. cynarae* CFBP 4188$^{T}$ (= ICMP 16775$^{T}$). *Xanthomonas gardneri* Jones *et al*. 2006 was created based on DNA-DNA hybridization experiments conducted on strains of *Xanthomonas* pathogenic towards 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 to 65 % towards *X. gardneri* CFBP 8163$^{T}$ (= LMG 962$^{T}$ = ATCC 19865$^{T}$) except for *X. hortorum* pv. *taraxaci* CFBP 410$^{PT}$ (= LMG 870$^{PT}$) 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* 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* 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 *is*DDH 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 *is*DDH scores falls in a 'transition zone' [46] with ANI ranging from ~95 to 96.5 % and *is*DDH from ~64 to 68 %. The lowest OGRI was *is*DDH 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 (ΔTm) was recommended to complement DDH and to overcome its drawbacks [22] and it was not unusual to find low ΔTm 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 to 70 % DDH and 93 to 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$^{PT}$ seems to use a large number of different nitrogen sources, maybe related to

457 some particular ecological lifestyle features. On the other hand, the proposed *X. hortorum*
458 emend. can easily be distinguished from its closest species *X. populi* as the latter is able to use
459 dextrin, cannot metabolize D-cellobiose, D-melibiose, L-fucose, gelatin, L-alanine, L-
460 glutamic acid, L-serine and propionic acid, is not able to grow in presence of 1 % NaCl, does
461 not liquefies gelatin in tube tests neither does it produces indole, and has an unusual optimum
462 growth temperature between 20 to 23°C, as systematically and consistently reported in
463 previous descriptions of this species [48,71,72].

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

477 Nevertheless, in order to achieve these taxonomic proposals formally, we need to
478 address the status of the pathotype strain *X. campestris* pv. *vitians* type A CFBP 2538$^{PT}$
479 (= NCPPB 976$^{PT}$ = LMG 937$^{PT}$ = ATCC 19320$^{PT}$ = ICMP 336$^{PT}$). This particular strain was
480 reported many times in the past to exhibit unusual features compared to other BLSL-causing
481 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 towards 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 towards lettuce, has drastically changed. In addition to confirming that *X. campestris* pv. *vitians* CFBP 2538$^{PT}$ 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 towards 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$^{PT}$ (= NCPPB 976$^{PT}$ = LMG 937$^{PT}$ = ATCC 19320$^{PT}$ = ICMP 336$^{PT}$) 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 % *is*DDH 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 (Figure 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 (Figure 2b) clearly distinguished the different pathovars, highlighting recent specializations of closely related organisms towards 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

556 in typical BLSL symptoms. Its high virulence on tomato was an unusual feature for a pv.
557 *vitians* strain though. If several strains of *X. hortorum* pv. *vitians* have been already reported
558 to be weakly pathogenic on tomato and pepper [2,53,54], like strain LM16735 from this study,
559 none was shown to possess such level of aggressiveness on both hosts. While most of the
560 strains we investigated held only one plasmid, preliminary investigations demonstrated that
561 this peculiar strain possess three (*data not shown*). *In silico* comparisons revealed that plasmid
562 pICMP7383.2 resembles highly to the typical plasmid of *X. hortorum* pv. *vitians*, yet
563 plasmids pICMP7383.1 and pICMP7383.3 were found to be strikingly akin to pJS749-3.1 and
564 pJS749-3.2 of *X. hortorum* pv. *gardneri* CFBP 8588 (= JS749-3) [45]. Plasmid pICMP7383.1
565 was found also to be similar to pLMG911.1 of type strain of *X. vesicatoria* LMG 911$^{T}$
566 (= CFBP 2537$^{T}$), another *Xanthomonas* pathogenic towards tomato and pepper. This
567 particular plasmid feature raises questions about the role of plasmids in the adaptation to
568 different hosts in *Xanthomonas* and should therefore be investigated further. Regardless, it
569 may be involved in the singular phenotype which resulted in the erroneous taxonomic
570 affiliation of this particular strain.

571 As a conclusion, this polyphasic study has led us to propose to replace the
572 inappropriate pathotype strain CFBP 2538$^{PT}$ (= NCPPB 976$^{PT}$ = LMG 937$^{PT}$ = ATCC
573 19320$^{PT}$ = ICMP 336$^{PT}$) of *X. campestris* pv. *vitians* by the neopathotype strain LMG 938$^{neoPT}$
574 and to transfer this pathovar in *X. hortorum* emend. as *X. hortorum* pv. *vitians* comb. nov.
575 (proposed neopathotype LMG 938$^{neoPT}$ = CFBP 8686$^{neoPT}$ = NCPPB 2248$^{neoPT}$). This
576 proposition allows to maintain Brown's pathovar epithet priority. If these changes should be
577 rejected by the ISPP-Committee on the Taxonomy of Plant Pathogenic Bacteria, we still
578 propose the creation of a new pathovar named *X. hortorum* pv. *vitians* pv. nov. having the
579 same description and with LMG 938$^{PT}$ being the pathotype strain. In addition, the
580 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$^{PT}$) and *X. hortorum* pv. *gardneri* comb. nov. (pathotype strain CFBP 8163$^{PT}$). An emended description of *X. hortorum* (type strain CFBP 5858$^{T}$) 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$^{T}$ 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, α-keto-glutaric acid, L-malic acid, bromo-succinic acid, propionic acid and acetic acid. Strain CFBP 5858$^{T}$ 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$^{T}$ = CFBP 4925$^{T}$ = LMG 733$^{T}$ = NCPPB 939$^{T}$ = ICMP 453$^{T}$. The GenBank accession number for its genome assembly is GCA_002940005.1 and its 16S 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 to 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$^{neoPT}$ to utilize β-gentiobiose, glycerol, pectin, L-lactic acid, tween 40, α-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$^{PT}$ = LMG 937$^{PT}$ = NCPPB 976$^{PT}$ = ICMP 336$^{PT}$ = ATCC 19320$^{PT}$ 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*

644 *of Phytopathogenic Bacteria* [15,75], we propose the replacement of this pathotype by
645 neopathotype strain LMG 938$^{neoPT}$ = CFBP 8686$^{neoPT}$ = NCPPB 2248$^{neoPT}$. We propose that its
646 subsequent transfer to *X. hortorum* be as ***X. hortorum*** pv. *vitians* (Brown 1918) Vauterin *et al.*
647 1995 comb. nov. in part to ensure the conservation of the priority established by Nellie Brown.
648 However, if the neopathotype is rejected, we propose that this same name be established as ***X.***
649 *hortorum* pv. *vitians* pv. nov. The GenBank accession number for its genome assembly is
650 SMED00000000.
651 ***X. hortorum* pv. *cynarae* (Trébaol *et al.* 2000) comb. nov.**
652 **=** *X. cynarae* Trébaol *et al.* 2000
653 = *X. cynarae* pv. *cynarae* (Trébaol *et al*. 2000) Timilsina *et al.* 2019
654 The description is the same as the species. Additionally, the Biolog GEN III and PM02A
655 MicroPlates revealed the ability of strain CFBP 4188$^{T}$ to utilize glycerol and L-histidine,
656 weakly utilize L-lactic acid and acetoacetic acid, and grow in presence of guanidine
657 hydrochloride and tetrazolium blue. The Biolog PM03B MicroPlates indicated that
658 N-phthaloyl-L-glutamic acid, guanine and alloxan can be used as nitrogen sources.
659 The primary host is the common artichoke (*Cynara scolymus* L.). The pathotype strain is
660 CFBP 4188$^{PT}$ = ICMP 16775$^{PT}$, the former type strain of *X. cynarae* Trébaol 2000 emend.
661 Timilsina *et al.* 2019. The GenBank accession number for its genome assembly is
662 GCA_002939985.1.
663 ***X. hortorum* pv. *gardneri* (Jones *et al.* 2006) comb. nov.**
664 **=** *X. gardneri* (ex-Sutić 1957) Jones *et al.* 2006
665 = *X. cynarae* pv. *gardneri* (Jones *et al.* 2006) Timilsina *et al.* 2019
666 The description is the same as the species. Additionally, the Biolog GEN III and PM02A
667 MicroPlates revealed the ability of strain CFBP 8163$^{PT}$ to utilize α-D-lactose, L-aspartic acid,
668 L-histidine, pectin, L-lactic acid, tween 40, α-keto-butyric acid, acetoacetic acid, sodium
669 formate, arbutin, D-raffinose, succinamic acid, D-tartaric acid, N-acetyl-L-glutamic acid and
670 L-homoserine, and grow in presence of 4 % NaCl, tetrazolium violet and tetrazolium blue.
671 The Biolog PM03B MicroPlates indicated that L-arginine, L-aspartic acid, L-glutamic acid,
672 L-leucine, N-acetyl-L-glutamic acid, D-glucosamine, N-acetyl-D-glucosamine, guanine,
673 xanthosine, alloxan, parabanic acid, alanyl-glycine, alanyl-threonine, glycyl-glutamine and
674 glycyl-glutamic acid can be used as nitrogen sources.
675 The primary hosts are tomato (*Solanum lycopersicon* L.) and pepper (*Capsicum annuum* L.).
676 The pathotype strain is CFBP 8163$^{PT}$ = NCPPB 881$^{PT}$ = ATCC 19865$^{PT}$. The GenBank

accession numbers for the two versions its genome assembly are SMDW00000000 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 $5858^T$ to utilize pectin, glycerol, L-lactic acid, α-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 $5858^T$.

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 Araliaceaous plants such as umbrella tree (*Schefflera actinophylla*), dwarf umbrella tree (*Schefflera arboricola*), Japanese aralia (*Fatsia japonica*), false aralia (*Plerandra elegantissima*) and ming aralia (*Polyscias fruticola*) [12,40].

The pathotype strain is also the type strain of *X. hortorum* CFBP $5858^T$ (= CFBP $4925^T$ = LMG $733^T$ = NCPPB $939^T$ = ICMP $453^T$).

***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 $2533^{PT}$ to utilize β-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-β-D-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, L-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, γ-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$^{PT}$ = LMG 7314$^{PT}$ = NCPPB 2985$^{PT}$ = ICMP 4321$^{PT}$. The GenBank accession number for its genome assembly is SMDX00000000.

***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$^{PT}$ = LMG 8646$^{PT}$ = NCPPB 1422$^{PT}$ = ICMP 5723$^{PT}$ 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 β-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 the 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 PM02A MicroPlates revealed the ability of strain CFBP 410$^{PT}$ to utilize β-gentiobiose, glycerol, L-histidine, pectin, acetoacetic acid, gelatin, arbutin, D-tartaric acid and L-homoserine. The Biolog PM03B 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 $410^{PT}$ = LMG $870^{PT}$ = NCPPB $940^{PT}$ = ICMP $579^{PT}$ = ATCC $19318^{PT}$. The GenBank accession number for its genome assembly is SMDY00000000.

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

| Proposed nomenclature[a] | Strain no. | Other collection no. | Former nomenclature[b] | Host of isolation | Geographic origin | Year of isolation | Pathogenicity [¥] | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lettuce cv. Kirinia | Lettuce cv. Météore | Tomato cv. Marmande | |
| *X. citri* | CFBP 2538 [*] | ATCC 19320<br>ICMP 336<br>LMG 937<br>NCPPB 976 | *X. campestris* pv. *vitians* (Brown 1918) Dye 1978<br>*X. axonopodis* pv. *vitians* (Brown 1918) Vauterin *et al.* 1995 | *Lactuca sp.* | United States | 1917 | - | - | NA | [5] |
| *X. hortorum* pv. *hederae* | **CFBP 5858$^{T}$** | CFBP 4925<br>LMG 733<br>NCPPB 939<br>ICMP 453 | | *Hedera helix* | United States | 1944 | - | - | NA | [63] |
| *X. hortorum* pv. *carotae* | CFBP 7900 [+] | M081 | | *Daucus carota* | United States | 2011 | - | - | - | [24] |
| *X. hortorum* pv. *cynarae* | **CFBP 4188$^{PT}$** | ICMP 16775 | *X. cynarae* Trébaol *et al.* 2000<br>*X. cynarae* pv. *cynarae* (Trébaol *et al.* 2000) Timilsina *et al.* 2019 | *Cynara scolymus* | Bretagne, France | 1996 | - | - | NA | [60] |
| *X. hortorum* pv. *gardneri* | **CFBP 8163$^{PT}$** | ATCC 19865<br>NCPPB 881 | *X. gardneri* (ex Šutič 1957) Jones *et al.* 2006<br>*X. cynarae* pv. *gardneri* (Jones *et al.* 2006) Timilsina *et al*. 2019 | *Solanum lycopersicum* | Yugoslavia | 1953 | - | - | + | [23] |
| | CFBP 8588 | JS749-3 | | *Solanum lycopersicum* | La Réunion, France | 1997 | - | - | + | [56] |
| *X. hortorum* pv. *pelargonii* | **CFBP 2533$^{PT}$** | LMG 7314<br>NCPPB 2985<br>ICMP 4321 | | *Pelargonium peltatum* | New Zealand | 1974 | - | - | NA | [63] |
| *X. hortorum* pv. *taraxaci* | **CFBP 410$^{PT}$** | ATCC 19318<br>LMG 870<br>NCPPB 940 | | *Taraxacum kok-sahgyz* | United States | 1942 | - | - | - | [63] |
| | LM 16389 | CFBP 8644 | | *Taraxacum sp.* | Isère, France | 2016 | - | - | NA | This study |
| *X. hortorum* pv. *vitians* | **LMG 938$^{neoPT}$** | CFBP 8686<br>NCPPB 2248 | *X. campestris* pv. *vitians* (Brown 1918) Dye 1978<br>*"X. hortorum* pv. *vitians (*Brown 1918) Vauterin *et al.* 1995" | *Lactuca sativa* | Zimbabwe | 1966 | + | + | NA | [63] |
| | CFBP 498 | NCPPB 232 | | *Lactuca sp.* | United States | 1949 | + | + | NA | This study |
| | CFBP 499 | NCPPB 969 | | *Lactuca scariola* | United States | 1961 | + | + | NA | This study |
| | CFBP 500 | NCPPB 992 | | *Lactuca sp.* | United States | 1949 | + | + | NA | This study |
| | CFBP 3971 | | | *Lactuca sativa* | France | 1994 | + | + | NA | This study |
| | CFBP 3973 | | | *Lactuca sativa* | France | 1994 | + | + | NA | This study |
| | CFBP 3975 | | | *Lactuca sativa* | France | 1994 | + | + | NA | This study |
| | CFBP 3976 | | | *Lactuca sativa* | France | 1994 | + | + | NA | This study |
| | CFBP 3978 | | | *Lactuca sativa* | France | 1994 | + | + | NA | This study |
| | CFBP 3980 | | | *Lactuca sativa* | Isère, France | 1995 | + | + | NA | This study |
| | CFBP 3983 | | | *Lactuca sativa* | Jura, France | 1995 | + | + | NA | This study |
| | CFBP 3984 | | | *Lactuca sativa* | Vaucluse, France | 1994 | + | + | NA | This study |
| | CFBP 3985 | | | *Lactuca sativa* | Rhône, France | 1995 | + | + | NA | This study |
| | CFBP 3986 | | | *Lactuca sativa* | France | 1994 | + | + | NA | This study |
| | CFBP 3987 | | | *Lactuca sativa* | France | 1994 | + | + | NA | This study |
| | CFBP 3990 | | | *Lactuca sativa* | France | 1995 | + | + | NA | This study |
| | CFBP 3993 | | | *Lactuca sativa* | Loiret, France | 1995 | + | + | NA | This study |
| | CFBP 3995 | | | *Lactuca sativa* | Isère, France | 1996 | + | + | NA | This study |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| CFBP 3996 | | | *Lactuca sativa* | Isère, France | 1996 | + | + | NA | This study |
| CFBP 7999 | ICMP 7383 | *X. gardneri* (ex Šutič 1957) Jones et al. 2006 | *Solanum lycopersicum* | New Zealand | 1980 | + | + | + | [56] |
| LM 16382 | | | *Lactuca sativa* cv. Escale | Isère, France | 2016 | + | + | NA | This study |
| LM 16383 | | | *Lactuca sativa* cv. Escale | Isère, France | 2016 | + | + | NA | This study |
| LM 16384 | | | *Lactuca sativa* cv. Escale | Isère, France | 2016 | + | + | NA | This study |
| LM 16386 | | | *Lactuca sativa* cv. Escale | Isère, France | 2016 | + | + | NA | This study |
| LM 16387 | | | *Lactuca sativa* cv. Minestrone | Isère, France | 2016 | + | + | NA | This study |
| LM 16388 | CFBP 8640 | | *Lactuca sativa* cv. Minestrone | Isère, France | 2016 | + | + | - | This study |
| LM 16734 | CFBP 8638 | | *Lactuca sativa* cv. Parrinice | Savoie, France | 2016 | + | + | - | This study |
| LM 16735 | CFBP 8639 | | *Lactuca sativa* cv. Almagro | Savoie, France | 2016 | + | + | w | This study |
| LM 16736 | | | *Lactuca sativa* cv. Almagro | Savoie, France | 2016 | + | + | NA | This study |
| LM 16011A | CFBP 8641 | | *Lactuca sativa* | Ain, France | 2016 | + | + | - | This study |
| LM 16012 | | | *Lactuca sativa* | Ain, France | 2016 | + | + | NA | This study |
| LM 16013 | | | *Lactuca sativa* | Ain, France | 2016 | + | + | NA | This study |
| LM 16014 | | | *Lactuca sativa* | Ain, France | 2016 | + | + | NA | This study |
| LM 16691 | CFBP 8642 | | *Lactuca sativa* cv. Funride | Rhône, France | 2016 | + | + | NA | This study |
| LM 17421 | | | *Lactuca sativa* cv. Almagro | Loire, France | 2017 | + | + | NA | This study |
| LM 17422 | | | *Lactuca sativa* cv. Celesti | Loire, France | 2017 | + | + | NA | This study |
| LM 17423 | | | *Lactuca sativa* cv. Almagro | Loire, France | 2017 | + | + | NA | This study |
| LM 17691 | | | *Lactuca sativa* cv. Olana | Rhône, France | 2017 | + | + | NA | This study |
| LM 17692 | | | *Lactuca sativa* cv. Olana | Rhône, France | 2017 | + | + | NA | This study |
| LM 17694 | | | *Lactuca sativa* | Rhône, France | 2017 | + | + | NA | This study |
| LM 17695 | | | *Lactuca sativa* cv. Kisheri | Rhône, France | 2017 | + | + | - | This study |
| LM 17696 | | | *Lactuca sativa* cv. Oseka | Rhône, France | 2017 | + | + | NA | This study |
| LM 17697 | | | *Lactuca sativa* cv. Analota | Rhône, France | 2017 | + | + | NA | This study |
| LM 17381 | | | *Taraxacum sp.* | Isère, France | 2017 | + | + | - | This study |
| LM 17382 | | | *Lactuca sativa* cv. Impression | Isère, France | 2017 | + | + | NA | This study |
| LM 17384 | | | *Lactuca sativa* cv. Lilybel | Isère, France | 2017 | + | + | NA | This study |
| LM 17385 | | | *Lactuca sativa* cv. Tourbillon | Isère, France | 2017 | + | + | NA | This study |
| LM 17388 | | | *Lactuca sativa* cv. Tourbillon | Isère, France | 2017 | + | + | NA | This study |
| LM 173810 | | | *Lactuca sativa* cv. Julena | Isère, France | 2017 | + | + | NA | This study |
| LM 173811 | | | *Lactuca sativa* cv. Kisheri | Isère, France | 2017 | + | + | NA | This study |
| LM 173812 | | | *Lactuca sativa* cv. Kisheri | Isère, France | 2017 | + | + | - | This study |
| LM 17011 | | | *Lactuca sativa* | Ain, France | 2017 | + | + | NA | This study |
| LM 17012 | | | *Lactuca sativa* | Ain, France | 2017 | + | + | NA | This study |
| LM 17013 | | | *Lactuca sativa* | Ain, France | 2017 | + | + | NA | This study |
| LM 17014 | | | *Lactuca sativa* | Ain, France | 2017 | + | + | NA | This study |
| LM 18071 | | | *Lactuca sativa* | Ardèche, France | 2018 | + | + | NA | This study |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| LM 18072 | | *Lactuca sativa* | Ardèche, France | 2018 | + | + | NA | This study |

[a] Proposed nomenclature in accordance with the *International Code of Nomenclature of Prokaryotes* and the *International Standards for Naming Pathovars of Phytopathogenic Bacteria*
[b] 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
[*] 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
[+] Representative strain CFBP 7900 of *X. hortorum* pv. *carotae* was chosen as the actual pathotype CFBP 4997 is known to be inconsistent
[¥] Pathogenicity assays conducted in this study: + = pathogenic, - = non-pathogenic, w = weakly pathogenic, NA = non-tested

**TABLE 2.** Pairwise ANIm and *is*DDH 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 *is*DDH 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. *is*DDH 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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *X. cynarae* pv. *cynarae* CFBP 4188$^{T}$ | 1[a] | *X. hortorum* pv. *cynarae* CFBP 4188$^{T}$ | - | 94.9 | 87.3 | 79.5 | 68.5 | 68.6 | 67.3 | 44.7 | 34.4 | 34.4 | 34.9 |
| *X. cynarae* pv. *gardneri* CFBP 8163$^{PT}$ | 2[a] | *X. hortorum* pv. *gardneri* CFBP 8163$^{PT}$ | 99.3 [93.3] | - | 85.9 | 78.6 | 68 | 68.4 | 67.2 | 44.7 | 34.3 | 34.4 | 34.9 |
| *X. campestris* pv. *vitians* LMG 938 | 3 | *X. hortorum* pv. *vitians* LMG 938$^{neoPT}$ | 98.4 [93.2] | 98.3 [93.4] | - | 80.8 | 68.2 | 68 | 67.1 | 44.5 | 34.4 | 34.3 | 34.9 |
| *X. hortorum* pv. *taraxaci* CFBP 410$^{PT}$ | 4 | *idem* | 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 |
| *X. hortorum* pv. *hederae* CFBP 5858$^{T}$ | 5 | *idem* | 96.1 [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 |
| *X. hortorum* pv. *carotae* CFBP 7900 | 6 | *idem* | 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 |
| *X. hortorum* pv. *pelargonii* CFBP 2533$^{PT}$ | 7 | *idem* | 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 |
| *X. populi* CFBP 1817$^{T}$ | 8 | *idem* | 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 |
| *X. axonopodis* pv. *vitians* CFBP 2538$^{PT}$ | 9 | *X. citri* 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 |
| *X. axonopodis* pv. *axonopodis* CFBP 4924$^{T}$ | 10 | *idem* | 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 |
| *X. citri* CFBP 3369$^{T}$ | 11 | *idem* | 88.3 [64.3] | 88.3 [64.6] | 88.3 [64.8] | 88.4 [64.4] | 88.3 [63.4] | 88.3 [64.6] | 88.3 [64.5] | 87.8 [52.6] | 98.7 [89.8] | 93.5 [85.2] | - |

$^{T}$ = type strain, $^{PT}$ = pathotype strain, $^{neoPT}$ = neopathotype
[a] = 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[a] | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9**[#] |
|---|---|---|---|---|---|---|---|---|---|
| Phenotypic tests | | | | | | | | | |
| Gram staining | rod negative | rod negative | rod negative | rod negative | rod negative | rod negative | rod negative | rod negative | rod negative |
| Optimum growth temperature | na | 25 - 28°C | 25 - 28°C | 25 - 28°C | 25 - 28°C | 25 - 28°C | 25 - 28°C | 25 - 28°C | 20 - 23°C |
| Starch hydrolysis | + | - | - | - | - | - | - | - | v |
| Gelatin hydrolysis | - | + | + | - | + | + | + | + | - |
| Motility | + | + | + | + | + | + | + | + | + |
| Hydrogen sulfide production | + | + | + | + | w | + | + | w | + |
| Indole production | + | w | w | w | w | w | w | w | - |
| Litmus milk | | | | | | | | | |
| Litmus reduction | - | + | + | + | + | + | + | + | v |
| Casein hydrolysation | - | + | + | + | + | + | + | + | na |
| Casein precipitation | + | + | + | + | + | + | + | + | na |
| Biolog GEN III microplates[b] | | | | | | | | | |
| Dextrin | + | - | - | w(-) | - | - | - | - | + |
| D-Maltose | + | - | - | - | - | - | - | - | na |
| **D-Trehalose** | + | + | + | + | + | + | + | + | + |
| **D-Cellobiose** | + | + | + | + | + | + | + | + | - |
| β-Gentiobiose | + | + | + | -(+) | + | + | - | - | v |
| **Sucrose** | + | + | + | + | + | + | + | + | + |
| ***pH 6*** | + | + | + | + | + | + | + | + | + |
| *pH 5* | - | - | -(+) | - | - | - | -(+) | - | na |
| D-Raffinose | - | - | - | +(-) | - | - | - | - | - |
| α-D-Lactose | + | - | - | +(-) | - | +(-) | - | + | - |
| **D-Melibiose** | + | + | + | + | + | + | + | + | - |
| **N-Acetyl-D-Glucosamine** | + | + | + | + | + | + | + | + | v |
| ***1% NaCl*** | + | + | + | + | + | + | + | + | - |
| *4% NaCl* | w(+) | + | + | w(-) | - | + | v | + | - |
| *8% NaCl* | - | - | - | - | - | -(+) | - | - | - |
| **D-Glucose** | + | + | + | + | + | + | + | + | + |
| **D-Mannose** | + | + | + | + | + | + | + | + | + |
| **D-Fructose** | + | + | + | + | + | + | + | + | + |
| **D-Galactose** | + | + | + | + | + | + | + | + | + |
| D-Fucose | - | - | - | w(-) | - | - | - | - | na |
| **L-Fucose** | + | + | + | + | + | + | + | + | - |
| ***1% Sodium Lactate*** | + | + | + | + | + | + | + | + | na |
| D-Serine #2 | - | - | - | - | - | +(-) | - | - | - |
| Glycerol | + | + | + | + | + | + | + | - | v |
| D-Fructose-6-Phosphate | w(-) | - | - | - | - | - | - | - | - |
| *Troleandomycin* | - | -(+) | v | - | - | - | - | - | na |
| *Rifamycin SV* | +(-) | - | + | -(+) | -(+) | -(+) | +(-) | - | na |
| **Gelatin** | + | + | + | + | + | + | + | + | - |
| Gly-Pro | + | + | + | + | + | + | + | v | na |
| **L-Alanine** | + | + | + | + | + | + | w | + | - |
| L-Aspartic Acid | - | - | + | w(-) | w(-) | v | w(-) | + | - |
| **L-Glutamic Acid** | + | + | + | + | + | + | + | + | - |
| L-Histidine | + | + | - | + | + | -(+) | w(+) | + | - |
| **L-Serine** | + | + | + | + | + | + | + | + | - |
| ***Lincomycin*** | + | + | + | + | + | + | + | + | na |
| *Guanidine Hydrochloride* | w(-) | +(-) | + | + | +(-) | +(-) | + | +(-) | na |
| *Niaproof* | - | +(-) | + | w(-) | v | w(-) | v | - | na |
| Pectin | + | + | + | + | + | + | - | + | na |
| D-Glucuronic Acid | - | - | - | w(-) | - | - | - | - | v |
| *Vancomycin* | - | v | w(-) | - | - | - | - | - | na |
| *Tetrazolium Violet* | + | + | + | -(+) | +(-) | + | +(-) | + | na |
| *Tetrazolium Blue* | + | + | + | + | +(-) | + | + | + | na |
| **Methyl Pyruvate** | + | + | + | + | + | + | + | + | v |
| L-Lactic Acid | + | + | w(-) | + | +(-) | + | w | + | na |
| **Citric Acid** | + | + | + | + | + | + | + | + | v |
| **α-Keto-Glutaric Acid** | + | + | + | + | + | + | + | + | v |
| **L-Malic Acid** | + | + | + | + | + | + | + | + | na |
| **Bromo-Succinic Acid** | + | w(+) | + | + | + | + | + | + | v |
| *Lithium Chloride* | + | +(-) | + | +(-) | -(+) | +(-) | +(-) | w(-) | na |
| *Potassium Tellurite* | - | + | + | w(+) | - | + | - | - | na |
| Tween 40 | - | w(+) | + | w | w(+) | + | w(-) | + | - |

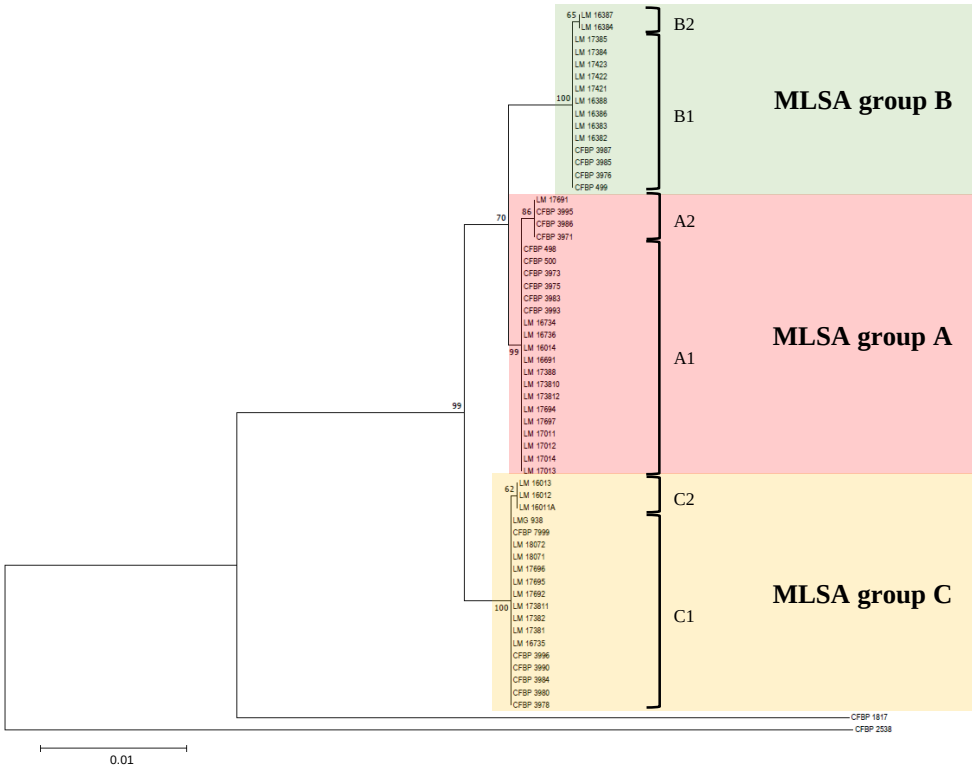
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| α-Hydroxy-Butyric Acid | w(-) | - | w(-) | w(-) | v | - | - | w(-) | - |
| α-Keto-Butyric Acid | + | - | +(-) | + | -(+) | + | v | + | - |
| Acetoacetic Acid | + | w(+) | - | + | + | w(+) | w | + | na |
| **Propionic Acid** | + | + | + | + | + | + | + | + | - |
| **Acetic Acid** | + | + | + | + | + | + | + | + | v |
| Sodium Formate | + | + | +(-) | -(+) | +(-) | w(+) | +(-) | + | na |
| *Aztreonam* | + | + | + | + | + | + | + | +(-) | na |
| *Sodium Bromate* | - | + | v | - | - | w(+) | v | w(-) | na |

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

[b] 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 = (-/w/+)

[#] 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.

1020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CFBP 3985
CFBP 3976
CFBP 499
B2
B1
MLSA group B
LM 17691
86
CFBP 3995
CFBP 3986
CFBP 3971
70
CFBP 498
CFBP 500
CFBP 3973
CFBP 3975
CFBP 3983
CFBP 3993
LM 16734
LM 16736
99
LM 16014
LM 16691
LM 17388
LM 173810
LM 173812
LM 17694
LM 17697
LM 17011
LM 17012
LM 17014
LM 17013
A2
A1
MLSA group A
99
62
LM 16013
LM 16012
LM 16011A
LMG 938
CFBP 7999
LM 18072
LM 18071
LM 17696
LM 17695
LM 17692
100
LM 173811
LM 17382
LM 17381
LM 16735
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CFBP 2538
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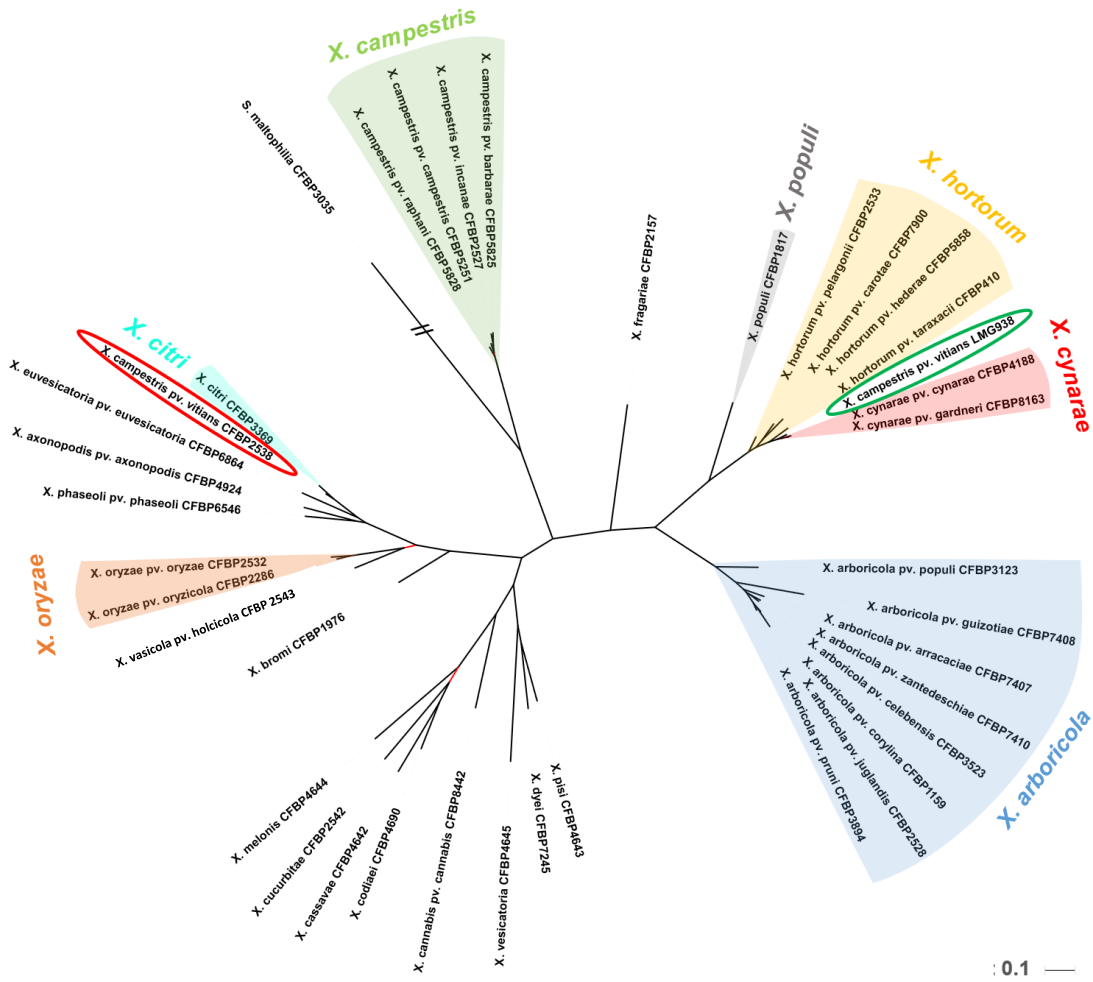
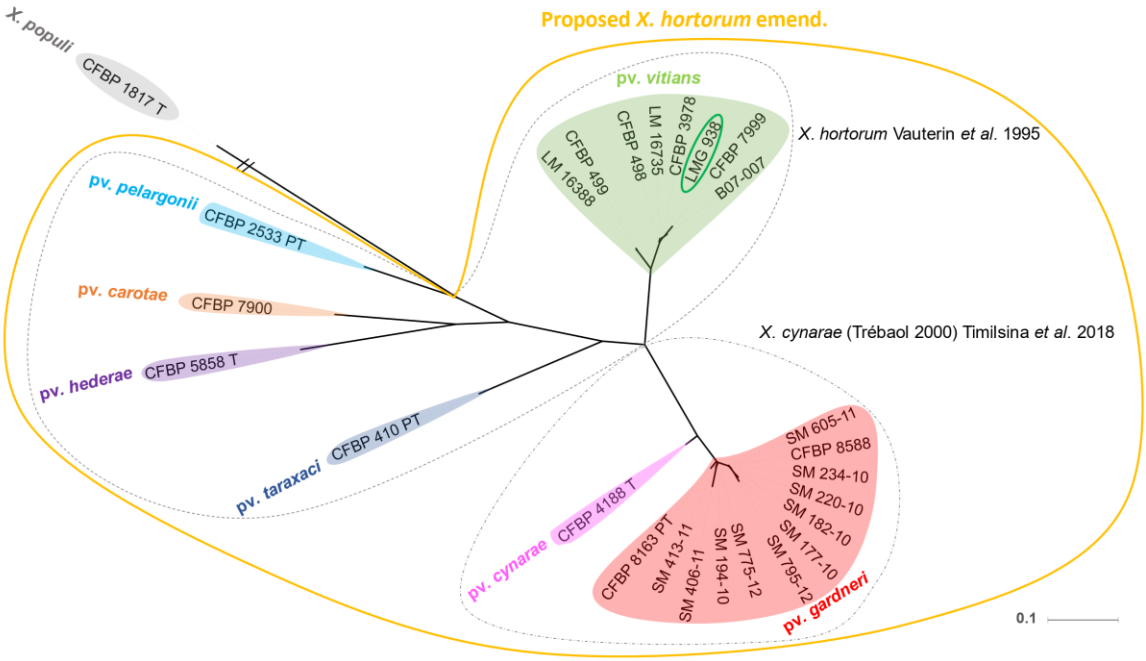

X. campestris
X. campestris pv. barbarae CFBP5825
X. campestris pv. incanae CFBP2527
X. campestris pv. campestris CFBP5251
X. campestris pv. raphani CFBP5828
S. maltophilia CFBP3035
X. fragariae CFBP2157
X. populi
X. populi CFBP1817
X. hortorum
X. hortorum pv. pelargonii CFBP2533
X. hortorum pv. carotae CFBP7900
X. hortorum pv. hederae CFBP5858
X. hortorum pv. taraxacii CFBP410
X. hortorum pv. vitians LMG938
X. cynarae
X. campestris pv. vitians LMG938
X. cynarae pv. cynarae CFBP4188
X. cynarae pv. gardneri CFBP8163
X. citri
X. citri CFBP3389
X. campestris pv. vitians CFBP2538
X. euvesicatoria pv. euvesicatoria CFBP6864
X. axonopodis pv. axonopodis CFBP4924
X. phaseoli pv. phaseoli CFBP6546
X. oryzae
X. oryzae pv. oryzae CFBP2532
X. oryzae pv. oryzicola CFBP2286
X. vasicola pv. holcicola CFBP 2543
X. bromi CFBP1976
X. arboricola
X. arboricola pv. populi CFBP3123
X. arboricola pv. guizotiae CFBP7408
X. arboricola pv. arracaciae CFBP7407
X. arboricola pv. zantedeschiae CFBP7410
X. arboricola pv. celebensis CFBP3523
X. arboricola pv. corylina CFBP1159
X. arboricola pv. juglandis CFBP2528
X. arboricola pv. pruni CFBP3894
X. melonis CFBP4644
X. cucurbitae CFBP2542
X. cassavae CFBP4642
X. codiaei CFBP4690
X. cannabis pv. cannabis CFBP8442
X. vesicatoria CFBP4645
X. dyei CFBP7245
X. pisi CFBP4643
0.1


Proposed X. hortorum emend.
X. populi
CFBP 1817 T
pv. vitians
CFBP 499
LM 16388
CFBP 498
LM 16735
CFBP 3978
LMG 938
CFBP 7999
B07-007
X. hortorum Vauterin et al. 1995
pv. pelargonii
CFBP 2533 PT
pv. carotae
CFBP 7900
X. cynarae (Trébaol 2000) Timilsina et al. 2018
pv. hederae
CFBP 5858 T
pv. taraxaci
CFBP 410 PT
pv. cynarae
CFBP 4188 T
CFBP 8163 PT
SM 413-11
SM 406-11
SM 194-10
SM 775-12
SM 795-12
SM 177-10
SM 182-10
SM 220-10
SM 234-10
CFBP 8588
SM 605-11
pv. gardneri
0.1