INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Timilsina *et al.*, *Int J Syst Evol Microbiol*
DOI 10.1099/ijsem.0.003104

MICROBIOLOGY SOCIETY

Reclassification of *Xanthomonas gardneri* (ex Šutič 1957) Jones *et al.* 2006 as a later heterotypic synonym of *Xanthomonas cynarae* Trébaol *et al.* 2000 and description of *X. cynarae* pv. *cynarae* and *X. cynarae* pv. *gardneri* based on whole genome analyses

S. Timilsina,[1] S. Kara,[1,2] M. A. Jacques,[3] N. Potnis,[4] G. V. Minsavage,[1] G. E. Vallad,[5] J. B. Jones[1,*] and M. Fischer-Le Saux[3,*]

Abstract

Multilocus sequence analysis of *Xanthomonas* species revealed a very close relationship between *Xanthomonas cynarae*, an artichoke pathogen and *Xanthomonas gardneri*, a tomato and pepper pathogen. Results of whole genome sequence comparisons using average nucleotide identity between representative strains of *X. gardneri* and *X. cynarae* were well above the threshold of 95–96 %. Inoculations of *X. gardneri* strains in artichoke leaves caused mild disease symptoms, but only weak symptoms were observed in the bracts. Both *X. cynarae* and *X. gardneri* grew equally and caused typical bacterial spot symptoms in pepper after artificial inoculation. However, *X. cynarae* induced a hypersensitive reaction in tomato, while *X. gardneri* strains were virulent. Pathogenicity-associated gene clusters, including the protein secretion systems, type III effector profiles, and lipopolysaccharide cluster were nearly identical between the two species. Based on our results from whole genome sequence comparison, *X. gardneri* and *X. cynarae* belong to the same species. The name *X. cynarae* has priority and *X. gardneri* should be considered as a later heterotypic synonym. An emended description of *X. cynarae* (type strain=CFBP 4188^T, =DSM 16794^T) is given. However, due to the host specificity in artichoke and tomato, two pathovars, *X. cynarae* pv. *cynarae* and *X. cynarae* pv. *gardneri*, are proposed.

In 1954, a bacterial disease was identified on artichoke (*Cynara scolymus* L.) in Brittany (Saint Pol de Léon area) and in the Loire Valley near Angers in France [1]. The symptoms were observed during warm and humid weather conditions and described as water-soaked dark green spots. The disease was called bacterial bract spot of artichoke. The pathogen was described as a new species within the genus *Xanthomonas* and designated as *Xanthomonas cynarae* based on multiphasic phenotypic and genotypic characteristics [2].

Bacterial spot of tomato and pepper is a widespread major disease resulting in significant crop losses in regions with a warm and humid climate that has been well characterized [3]. The disease symptoms consist of necrotic lesions on fruits and foliage that can result in reduced yield and fruit quality. The causal agent of the bacterial spot disease was originally identified as *Bacterium vesicatorium*. The bacterium underwent several taxonomic changes and the nomenclature was changed to *Xanthomonas vesicatoria* and eventually to *X. campestris* pv. *vesicatoria*. Šutič identified bacterial spot on tomato in former Yugoslavia and isolated a yellow bacterium that was originally named *Pseudomonas gardneri* [4]. The *P. gardneri* strain was determined to be synonymous with *X. vesicatoria* as both were pathogenic on tomato and could not be distinguished using physiological assays [5]. Furthermore, the placement of *P. gardneri* in the *Xanthomonas* genus was supported by DNA–DNA hybridization results [6]. Later, De Vos *et al.*, following an

Author affiliations: [1]Plant Pathology Department, University of Florida, Gainesville, FL, 32611, USA; [2]Diyarbakır Plant Protection Research Institute, Silvan Highway 7. Km. 21110, Diyarbakır, Turkey; [3]IRHS, INRA, Agrocampus-Ouest, Université d'Angers, SFR 4207 QUASAV, 49070 Beaucouzé, France; [4]Entomology and Plant Pathology Department, Auburn University, Auburn, AL, 36849, USA; [5]University of Florida, Gulf Coast Research and Education Center, Balm, Florida, 33598, USA.
***Correspondence:** J. B. Jones, jbjones@ufl.edu; M. Fischer-Le Saux, Marion.Le-Saux@inra.fr
Keywords: sequence based taxonomy; emended species; artichoke, tomato and pepper pathogen.
Abbreviations: ANI, Average Nucleotide Identity; BLAST, Basic Local Alignment Search Tool; CFU, Colony Forming Unit; DDH, DNA-DNA hybridization; DNA, Deoxyribonucleic Acid; ECW, Early Calwonder; MLSA, Multilocus Sequence Analysis; NCBI, National Center for Biotechnology Initiative; PAMDB, Plant Associated Microbe Database; rRNA, Ribosomal Ribonucleic Acid.
Two supplementary figures and five supplementary tables are available with the online version of this article.

extensive study, suggested the *P. gardneri* was missnamed and should be placed in the genus *Xanthomonas* [7]. Jones *et al.* elevated the strains to species and designated *X. gardneri* as one of the four *Xanthomonas* species that causes bacterial spot of tomato and pepper along with *X. euvesicatoria*, *X. vesicatoria,* and *X. perforans* [8].

More recently, whole genome sequence-based taxonomy has been suggested for differentiating *Xanthomonas* bacterial species [9]. As a result of advancements in high throughput sequence analysis, whole genome sequences and comparative genomics have been used in lieu of the DNA–DNA hybridization (DDH) method to demarcate bacterial species [10]. Unlike DDH, sequence-based taxonomy can be used to build a central database for future comparisons and is not labour-intensive. Richter and Rosselló-Móra in 2009 proposed average nucleotide identity (ANI) as a statistical approach for taxonomic comparisons of prokaryotic genomes [11]. ANI measures the pairwise identity between two genomes at the nucleotide level. Based on a number of previous studies, the DDH threshold of 70 % for species delineation lies between ~95–96 % ANI [10–12]. Reconstructing phylogeny based on conserved housekeeping genes and comparative genomic tools is often used along with genome indices for subgeneric classification [13].

Young *et al.* recommended various changes in *Xanthomonas* taxonomy based on multilocus sequencing of four housekeeping genes: *dnaK*, *fyuA*, *gyrB,* and *rpoD* [14]. More than 100 *Xanthomonas* strains representing different species were compared to reconstruct a neighbour-joining phylogenetic tree. The phylogenetic analyses suggested that *X. cynarae*, *X. hortorum* and *X. gardneri* form a single heterogeneous group and that *X. gardneri* may be a synonym of *X. cynarae*. Recently, phylogenomic analysis based on the core proteome of the genus *Xanthomonas* confirmed that these three species formed a tight cluster [15]. Trébaol et al. in 2000 used DDH assays between *X. cynarae* and 20 other known *Xanthomonas* species to taxonomically describe *X. cynarae* [2]. DNA hybridization between *X. cynarae* and *X. hortorum* was 49 % [2], significantly lower than the 70 % threshold to be considered as the same species [16]. However, the study did not include DDH comparisons between *X. cynarae* and *X. gardneri* since the latter species was defined only in 2004 [8]. Thus, the objective of this study was to evaluate the taxonomic relationship between *X. cynarae* and *X. gardneri* by comparing the whole genome sequences of respective type strains. Along with the genomic comparisons, pathogenicity assays and bacterial population dynamics were used to determine if the strains could cross-infect their known plant hosts.

The 16S rRNA sequences of type strains from all validly published *Xanthomonas* species were retrieved from National Centre for Biotechnology Information (NCBI) database and EzBioCloud database [17]. The sequences were aligned using MUSCLE tool [18] from MEGA 7.1 [19]. A maximum-likelihood phylogenetic tree was reconstructed using the general time-reversible model with gamma distributed invariable sites. Similar to 16S rRNA sequence analysis, type strains of *X. cynarae* and *X. gardneri* were phylogenetically compared with other closely related *Xanthomonas* species, including *X. hortorum*, using concatenated sequences of six housekeeping genes: *fusA*, *gapA*, *gltA*, *gyrB*, *lacF* and *lepA* [20]. The reference housekeeping genes were downloaded from the Plant Associated and Environmental Microbes Database (PAMDB, www.pamdb.org) [20] or the NCBI database. The single gene sequences were aligned separately and concatenated for phylogenetic analyses. A maximum-likelihood tree was reconstructed using a similar model as described for phylogenetic analysis of 16S rRNA sequences.

The type strains of *X. cynarae* and *X. gardneri* have identical 16S rRNA sequences (Fig. S1, available in the online version of this article) that varied from *X. hortorum* by 2 nt. Additionally, the 16S rRNA sequences of *X. cynarae* and *X. gardneri* were identical to the sequence of *X. campestris* strain ATCC 33913^T. MLSA using six housekeeping genes showed a very close relationship between the type strains of *X. gardneri* and *X. cynarae*. The type strains of *X. cynarae* and *X. gardneri* varied in *gapA* gene by 12 nt, while the other five genes were identical to each other. Phylogenetically, *X. cynarae* and *X. gardneri* also clustered together with *X. hortorum*. However, the housekeeping gene sequences of *X. gardneri* and *X. cynarae* were significantly different from the *X. hortorum* genes with nucleotide identities ranging between 96–99 % (Fig. 1).

Considering the high percentage sequence identity of the 16S rRNA and housekeeping genes between *X. gardneri* and *X. cynarae*, the strains were compared using whole genome sequencing. The genome sequence and sequencing information of *X. gardneri* ATCC 19865^T were published previously and are publicly available in the NCBI database with the GenBank assembly accession no. GCA_000192065.2 [21]. Similarly, the sequences of type strains of *X. cynarae* CFBP 4188^T and *X. hortorum* CFBP 4925^T (LMG 733^T) are also available in the NCBI database with the accession numbers GCA_002939985 and GCF_002940005, respectively (Table S1) [15]. The assembled genomes were annotated through the Integrated Microbial Genome/Joint Genome Institute platform (img.jgi.doe.gov) [22].

Pairwise ANI, based on BLAST, between the whole genome sequences of the type strains of *X. cynarae*, *X. gardneri* and *X. hortorum* were compared using jSpecies version 1.2.1 [11]. Likewise, the Genome-to-Genome Distance Calculator (GGDC) was used for calculating *in silico* DDH values [23]. Similar results were observed from both calculations, as the ANI results identified 99.31 % sequence identity between the *X. cynarae* and *X. gardneri* genomes, a value well above the species cut-off threshold. Likewise, the *in silico* DDH value between the two genomes (GGDC algorithm, recommended formula 2) was estimated to be between 89.9 and 93.7 %, a value well above the 70 % threshold for species demarcation. However, *X. hortorum* varied by approximately 4 % from both *X. gardneri* and *X. cynarae* genomes with ~96 % ANI (Table 1) and GGDC estimated

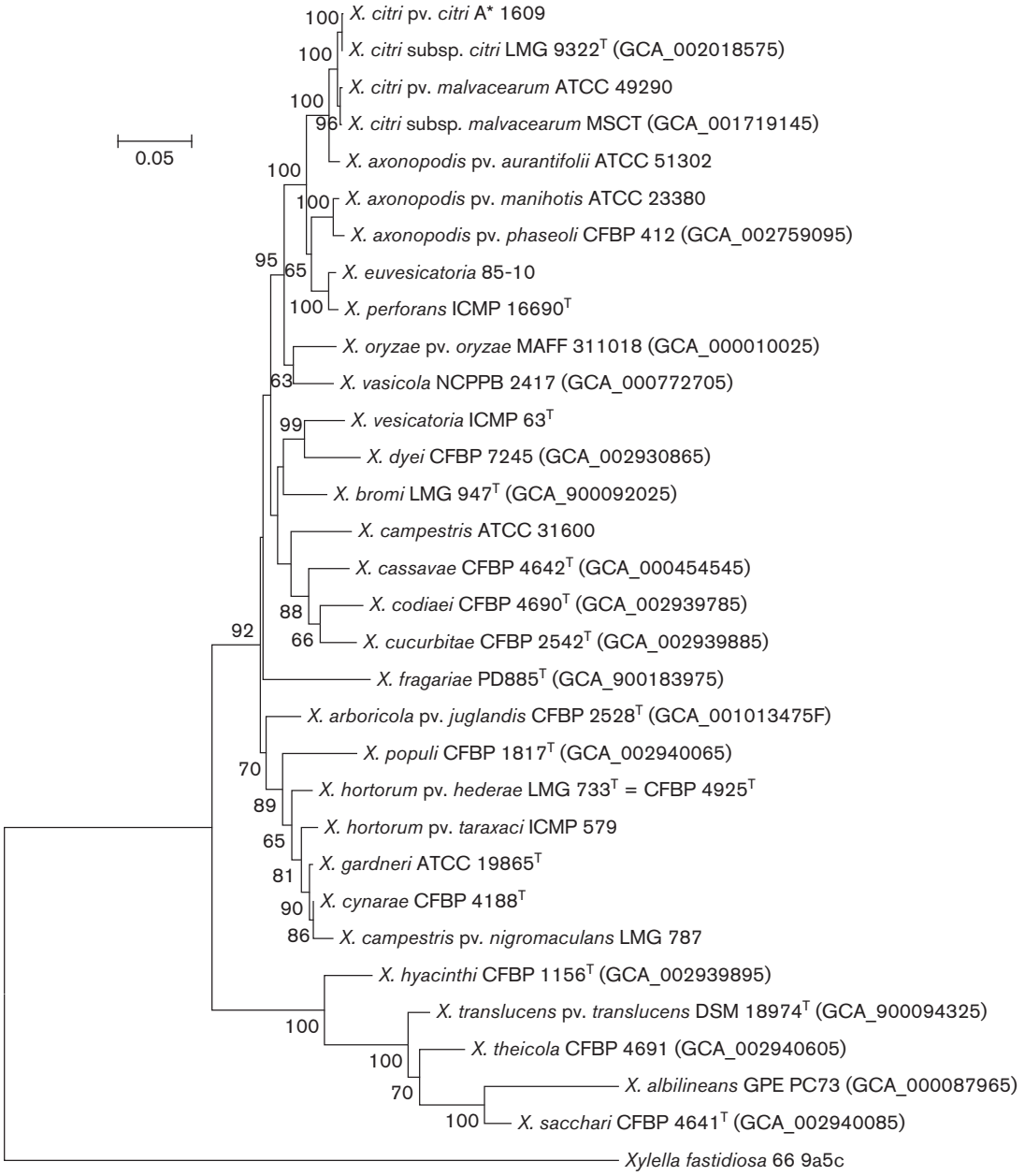

Fig. 1. Maximum-likelihood phylogenetic tree reconstructed using six conserved housekeeping genes (*fusA*, *gapA*, *gltA*, *gyrB*, *lacF* and *lepA*). NCBI accession numbers are provided in parentheses and other reference sequences are publicly available in the PAMDB (www.pamdb.org).

~65 % DDH between the strains. Thus, based on previous DDH assay results [2] along with inconclusive ANI- and GGDC-based DDH calculations, *X. hortorum* was not considered as a synonymous species of *X. cynarae* and *X. gardneri*. However, based on MLSA, *X. hortorum* is likely a paraphyletic group including both *X. gardneri* and *X. cynarae* (Fig. 1).

Phenotypic and genomic results strongly suggest that *X. gardneri* and *X. cynarae* are synonymous species. As the bacteria were previously described from different hosts [2, 8], representative strains of *X. gardneri* and *X. cynarae* were used to evaluate pathogenicity on differential hosts. Bacterial strains of *X. gardneri* pathogenic on tomato (ATCC 19865^T), tomato and pepper (Xg444 and Xg51), and representative *X. cynarae* strains, which were isolated from artichoke, were used for inoculations in plant hosts (Table 2). The bacterial suspensions, adjusted to ~10^8 Colony Forming Unit (CFU) ml^{-1} in sterile tap water, were spray-inoculated on 3 and 5 week old greenhouse grown seedlings of Bonny Best tomato and Early Calwonder

Table 1. Pairwise average nucleotide identity values, based on BLASTn, between the type strains of *X. cynarae*, *X. gardneri,* and *X. hortorum*

	***X. cynarae* CFBP 4188^T**	***X. gardneri* ATCC 19865^T**	***X. hortorum* CFBP 4925^T (=LMG 733)**
X. cynarae CFBP 4188^T	–	99.31	96.22
X. gardneri ATCC 19865^T	99.31	–	96.17
X. hortorum CFBP 4925^T	96.21	96.16	–

(ECW) pepper plants, respectively. Inoculated plants were bagged using clear polyethylene bags and placed in a growth chamber at 28 °C. Plants were unbagged 48 h post inoculation and placed in the greenhouse for further observation. Typical disease symptoms were observed in pepper from both *X. gardneri* and *X. cynarae* strains, but only *X. gardneri* resulted in pathogenicity in tomato.

Along with spray inoculation, representative *X. cynarae* and *X. gardneri* strains were infiltrated using a syringe in tomato and pepper leaves at 10^3, 10^5, or 10^8 CFU ml^{-1} for pathogenicity testing and population assays. Infiltration of *X. gardneri* strains at 10^8 CFU ml^{-1} resulted in a susceptible reactions in both tomato and pepper, whereas *X. cynarae* strains elicited a hypersensitive reaction (HR) in tomato (Fig. 2b). To further confirm pathogenicity of *X. cynarae* on pepper, the leaves were infiltrated with bacterial suspensions of *X. cynarae* and *X. gardneri* adjusted to ~10^3 CFU ml^{-1}. Inoculated plants were kept at 25–30 °C during 12 h light and 15–20 °C during 12 h dark periods and the plants were assessed for 2–3 weeks post inoculation for lesion development. Typical bacterial spot lesions were observed in pepper after infiltration with both *X. cynarae* and *X. gardneri,* confirming pathogenicity (Fig. 2a). Additionally, bacterial populations post-infiltration were measured in ECW pepper leaves. Bacterial suspensions of *X. gardneri* Xg51 and *X. cynarae* CFBP 4188^T were infiltrated at 10^5 CFU ml^{-1} into pepper leaves. Infiltrated areas of leaves were sampled every 2 days for 10 days to calculate bacterial population. Representative strains of *X. cynarae* and *X. gardneri* grew to statistically equivalent population levels of approximately 7.25×10^7 CFU cm^{-2} (Fig. 3a).

Table 2. List of strains used for pathogenicity assay

Strain	Host	Source
X. gardneri		
ATCC 19865^T	Tomato	[21]
Xg 444	Pepper	This study
Xg 51	Pepper	This study
X. cynarae		
CFBP 19	Artichoke	www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria
CFBP 2044	Artichoke	www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria
CFBP 4188^T	Artichoke	www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria
CFBP 4189	Artichoke	www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria
CFBP 4190	Artichoke	www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria
CFBP 4719	Artichoke	www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria
CFBP 4927	Artichoke	www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria
CFBP 4942	Artichoke	www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria
CFBP 4943	Artichoke	www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria

In artichoke leaves infiltrated with bacterial suspensions at ~10^5 CFU ml^{-1}, the *in planta* population of *X. gardneri* strain Xg444 reached 5.0×10^7 CFU cm^{-2} similar to *X. cynarae* CFBP 4188^T, which was 6.0×10^7 CFU cm^{-2}. However, population of another *X. gardneri* strain, Xg51, was significantly lower and reached approximately 4.0×10^6 CFUcm^{-2} 9 days post inoculation (Fig. 3b). Pathogenicity tests on detached artichoke bracts were conducted as previously described [2] with *X. cynarae* strains CFBP 4188^T and CFBP 4927 and *X. gardneri* strains ATCC 19865^T and Xg51. The bracts were scarified and inoculated with bacterial suspensions of ~10^8 CFU ml^{-1}. A total of 13 bracts per strain were inoculated and incubated at 28 °C. Symptoms were recorded 7 days post inoculation. Sterile tap water was used as a negative control. Inoculation with *X. cynarae* strains produced typical water-soaked lesions with exudates, while inoculation with *X. gardneri* strains produced a surface-limited reddish-brown necrosis along the scarified tissue. No exudate was observed after inoculation with *X. gardneri* strains. (Fig. 2c). The results suggest that only *X. cynarae* strains produced typical water-soaked lesions on artichoke.

Given the high sequence similarity between *X. cynarae* and *X. gardneri* and pathogenicity results on the hosts described above, we compared pathogenicity-related genes, as the variation in the genomes could reflect the bacterial host specificity [21]. The *hrp* (hypersensitive response and pathogenicity) genes encode proteins associated with the type III secretion system (T3SS) that is used by the Gram-negative plant pathogenic *Xanthomonas* species to deliver effectors and infect their hosts [24–26]. The type three secretion clusters were similar among *X. cynarae, X. gardneri,* and *X. hortorum* genomes (Fig. 4). Considering the nucleotide identity among 20 genes in the *hrp* operons [27], *hrcJ*, *hrcV*, *hrcQ*, *hrcR*, *hpaA*, *hrcD*, *hrpE*, *hpaB,* and *hrcW* varied between *X. gardneri* and *X. cynarae*, while the other 11 genes were identical. For the nine variable *hrp* genes annotated, the genes were 99 % identical between the two strains except for *hrcV* and *hrpE* that shared 97 % sequence

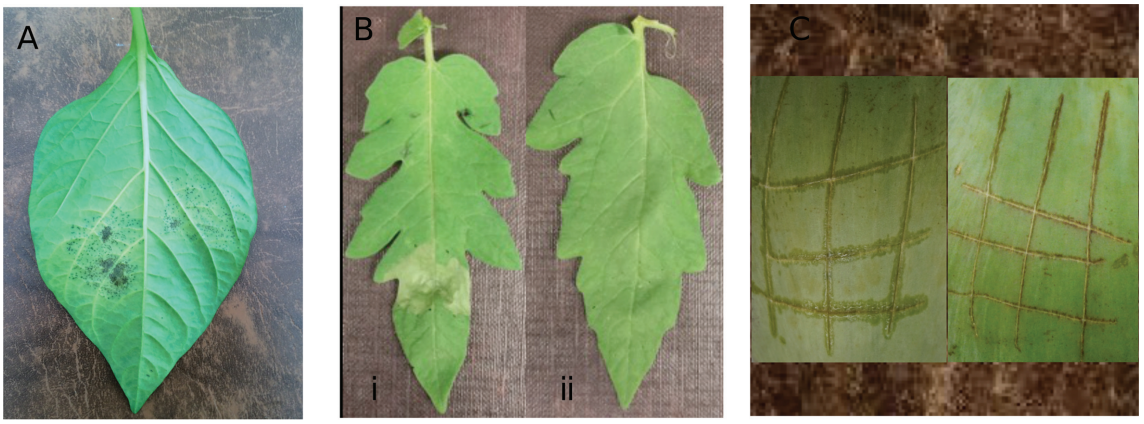

Fig. 2. Disease development in (A) Early Calwonder pepper leaves following infiltration with 10^3 CFU ml^{-1} of *X. garnderi* (left side of leaf) and *X. cynarae* (right side of leaf). Both strains produced typical lesions, (B) hypersensitive reaction on Bonny Best tomato leaflets following infiltration with i; *X. cynarae* at 10^8 CFU ml^{-1} but not with ii; *X. gardneri* at the same concentration, and (C) typical water-soaked lesions with bacterial exudate on Calico artichoke bract after inoculation with ~10^8 CFU ml^{-1} *X. cynarae* (left), whereas reddish-brown necrosis were observed after *X. gardneri* inoculation in same conditions.

identity. However, *X. hortorum hrp* genes differed from both *X. gardneri* and *X. cynarae hrp* genes that ranged between 93–99 % and *hrpE* was not detected in *X. hortorum* (Table S2). The distribution of genes coding for type III secreted effectors (T3Es) was also compared in this study. The presence or absence of T3Es is associated with host specificity of *Xanthomonas* species and other plant-pathogenic Gram-negative bacteria [27, 28]. XopD, which is a core effector among xanthomonads causing bacterial spot in tomato and pepper [3, 28], was observed in *X. cynarae* to contain an insertion sequence thus rendering the effector non-functional (Table S3). However, comparison of transcription activation-like (TAL) effectors was limited due to the short sequence reads of the Illumina sequencing platform that prevented successful assembly of the repeat sequences of these genes and their subsequent comparison.

Genes coding for the cell-wall degrading enzymes, the lipopolysaccharide cluster and diffusible signal factor (DSF) cell–cell signalling system that co-ordinates virulence and biofilm gene expression were compared between *X. cynarae* and *X. gardneri* [29]. The genetic composition and organization of both cell-wall degrading enzymes and genes within the lipopolysaccharide cluster were identical between the *X. gardneri* and *X. cynarae* genomes (Table S4, Fig. S2). However, in both cases, the organization of the genes in *X. hortorum* CFBP 4925^T and their sequence identity varied significantly from *X. gardneri* ATCC 19865^T and *X. cynarae* CFBP 4188^T. The lipopolysaccharide cluster is flanked by *metB* and *etfA* genes in xanthomonads [30] that were highly similar between *X. cynarae* and *X. gardneri* type strains. The *metB* gene varied by only one nucleotide between *X. cynarae* and *X. gardneri*, but *metB* from *X. hortorum* CFBP 4925^T

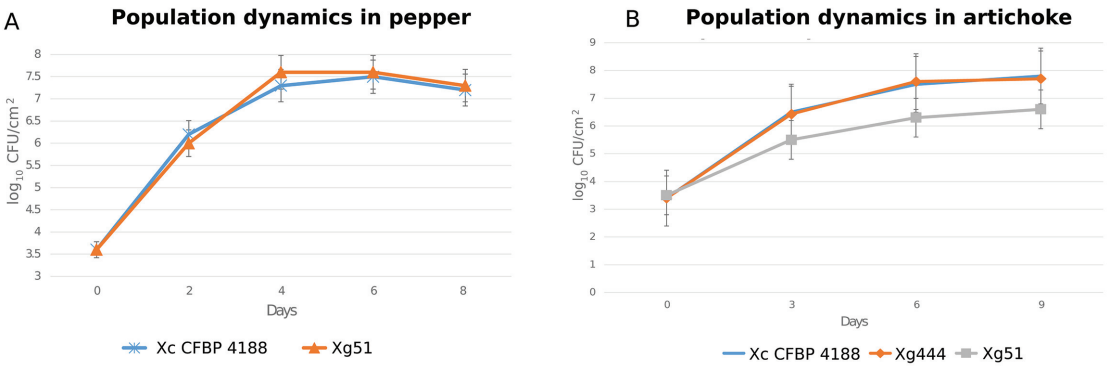

Fig. 3. Population dynamics of *Xanthomonas* strains on (A) pepper and (B) artichoke. Bacterial suspensions were adjusted to ~10^5 CFU ml^{-1} for infiltration.

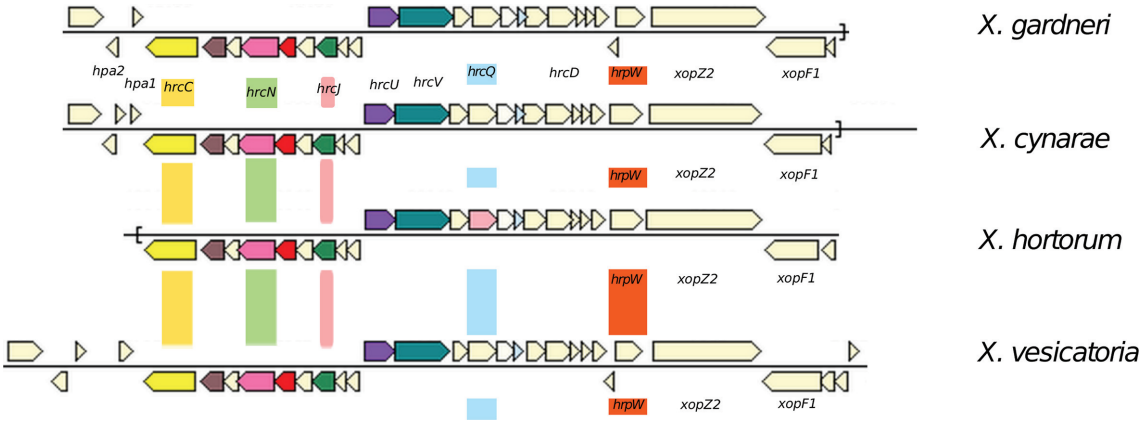

Fig. 4. Type III secretion gene clusters in four strains are shown. Boxes of same colour indicate orthologous genes. *X. gardneri*, *X. cynarae*, and *X. hortorum* have nearly identical cluster orientation. However, *X. gardneri* and *X. cynarae* shared higher nucleotide sequence identity compared to the *X. hortorum* sequences. The gene cluster of *X. vesicatoria* was used as a reference.

varied by 69 and 70 nt from the *X. cynarae* and *X. gardneri* genes, respectively. The gene *etfA* varied by 18 nt between *X. cynarae* and *X. gardneri* genomes, whereas this gene from *X. hortorum* varied by 23 and 25 nt from the *X. gardneri* and *X. cynarae* genomes, respectively. Among the genes involved in DSF cell–cell signalling, *rpfB*, *rpfF* and *rpfG* were identical and *rpfC* and *rpfH* genes varied by <0.4 % between the type strains of *X. gardneri* and *X. cynarae* and the nucleotide identity with *X. hortorum* genes ranged from 97 to 98.88 % (Table S5).

In conclusion, our study provides significant information to conclude that *X. cynarae* and *X. gardneri* should be considered as synonymous. The species name *X. cynarae* was proposed before *X. gardneri* and has priority, thus an emendation of the species *X. cynarae* is proposed to include the tomato and pepper pathogens. Considering the host specificity associated with artichoke, two pathovars, *X. cynarae* pv. *cynarae* and *X. cynarae* pv. *gardneri*, encompassing strains previously described in respective species group should be considered within *X. cynarae* emend. Although *X. hortorum* has an ANI value that is slightly greater than 95 % with *X. gardneri* and *X. cynarae*, previous DDH assays [2] along with phylogenetic and comparative genomics observations suggest *X. hortorum* should be considered as a separate species from *X. cynarae*.

EMENDED DESCRIPTION OF *XANTHOMONAS CYNARAE* TRÉBAOL *ET AL.* 2000

Xanthomonas cynarae (cy.na′rae. N.L. fem. gen. n. *cynarae* from the genus of artichoke, *Cynara scolymus* L.).

Xanthomonas cynarae is a Gram-negative, rod-shaped bacterium with a single polar flagellum [2]. Strains are aerobic and form slightly convex smooth colonies on yeast–peptone–glucose agar at 28 °C. Based on Biolog GENIII MicroPlates, *X. cynarae* strains grow at pH 5 and utilize N-acetyl-D-glucosamine, D-glucose, D-mannose, D-frucotse, D-galactose, trehalose, cellobiose, L-fucose, gelatin, glycyl-L-proline, L-glutamic acid, L-serine, sucrose, methyl pyruvate, citric acid, α-keto-glutaric acid, L-malic acid, propionic acid and acetic acid. The strains are insensitive to 1 % sodium lactate, 1 % sodium chloride, lincomycin and tetrazolium blue. The type strain is CFBP 4188^T (=CIP 106774^T=ICMP 16775^T=DSM 16794^T).

DESCRIPTION OF *XANTHOMONAS CYNARAE* PV. *CYNARAE*

Xanthomonas cynarae pv. *cynarae* (cy.na′rae. N.L. fem. gen. n. cynarae from the genus of artichoke, *Cynara scolymus* L.).

The pathovar description is the same as *X. cynarae* described by Trébaol *et al.* [2]. The pathovar *X. cynarae* pv. *cynarae* may be distinguished based on host specificity to artichoke. Phenotypically, *X. cynarae* pv. *cynarae* can be differentiated by its glycerol utilization and insensitivity to guanidine hydrochloride and aztreonam. The *X. cynarae* type strain, CFBP 4188^T, also represents the *X. cynarae* pv. *cynarae* pathotype strain.

DESCRIPTION OF *XANTHOMONAS CYNARAE* PV. *GARDNERI*

Xanthomonas cynarae pv. *gardneri* (gard′ne.ri. N.L. gen. n. gardneri, named after M. W. Gardner, an American plant pathologist).

The pathovar description is the same as the description of *X. gardneri* by Jones *et al.* [8]. Host specificity on tomato can distinguish *X. cynarae* pv. *gardneri* from other

pathovars. *X. cynarae* pv. *gardneri* strains are insensitive to tetrazolium violet. The pathotype strain is ATCC 19865^{PT}.

Funding information
The authors received no specific grant from any funding agency.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Ridé M. Sur une maladie nouvelle de l'artichaut (*Cynara scolymus*). *C R SeUances Acad Sci* 1956;243:174–177.
2. Trébaol G, Gardan L, Manceau C, Tanguy JL, Tirilly Y *et al.* Genomic and phenotypic characterization of *Xanthomonas cynarae* sp. nov., a new species that causes bacterial bract spot of artichoke (*Cynara scolymus* L.). *Int J Syst Evol Microbiol* 2000;50:1471–1478.
3. Potnis N, Timilsina S, Strayer A, Shantharaj D, Barak JD *et al.* Bacterial spot of tomato and pepper: diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Mol Plant Pathol* 2015;16:907–920.
4. Šutič D. Bakterioze crvenog patlidzana (Tomato bacteriosis). In: *Posebna Izd Inst Zasht Bilja Beograd (Special Edition)*, vol. 6. Beograd: Institute of Plant Protein; 1957. pp. 1–65 (English summary: Rev App Mycl 36:734-735).
5. Dye DW. Cultural and biochemical reaction of additional *Xanthomonas* species. *New Zeal J Sci* 1966;9:913–919.
6. de Ley J. Modern molecular methods in bacterial taxonomy: evaluation, application, prospects. In: *Proceedings of the 4th International Conference of Plant Pathogenic Bacteria*, vol. 1; 1978. pp. 347–357.
7. de Vos P, Goor M, Gillis M, de Ley J. Ribosomal ribonucleic acid cistron similarities of phytopathogenic *Pseudomonas* species. *Int J Syst Bacteriol* 1985;35:169–184.
8. Jones JB, Lacy GH, Bouzar H, Stall RE, Schaad NW. Reclassification of the xanthomonads associated with bacterial spot disease of tomato and pepper. *Syst Appl Microbiol* 2004;27:755–762.
9. Constantin EC, Cleenwerck I, Maes M, Baeyen S, van Malderghem C *et al.* Genetic characterization of strains named as *Xanthomonas axonopodis* pv. *dieffenbachiae* leads to a taxonomic revision of the *X. axonopodis* species complex. *Plant Pathol* 2016;65:792–806.
10. Kim M, Oh HS, Park SC, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:346–351.
11. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
12. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P *et al.* DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
13. Chun J, Rainey FA. Integrating genomics into the taxonomy and systematics of the Bacteria and Archaea. *Int J Syst Evol Microbiol* 2014;64:316–324.
14. Young JM, Park DC, Shearman HM, Fargier E. A multilocus sequence analysis of the genus *Xanthomonas*. *Syst Appl Microbiol* 2008;31:366–377.
15. Merda D, Briand M, Bosis E, Rousseau C, Portier P *et al.* Ancestral acquisitions, gene flow and multiple evolutionary trajectories of the type three secretion system and effectors in *Xanthomonas* plant pathogens. *Mol Ecol* 2017;26:5939–5952.
16. Wayne LG, Moore WEC, Stackebrandt E, Kandler O, Colwell RR *et al.* Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.
17. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
18. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797.
19. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
20. Almeida NF, Yan S, Cai R, Clarke CR, Morris CE *et al.* PAMDB, a multilocus sequence typing and analysis database and website for plant-associated microbes. *Phytopathology* 2010;100:208–215.
21. Potnis N, Krasileva K, Chow V, Almeida NF, Patil PB *et al.* Comparative genomics reveals diversity among xanthomonads infecting tomato and pepper. *BMC Genomics* 2011;12:146.
22. Markowitz VM, Chen IM, Chu K, Szeto E, Palaniappan K *et al.* IMG/M-HMP: a metagenome comparative analysis system for the Human Microbiome Project. *PLoS One* 2012;7:e40151.
23. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
24. Galán JE, Collmer A. Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* 1999;284:1322–1328.
25. Büttner D, He SY. Type III protein secretion in plant pathogenic bacteria. *Plant Physiol* 2009;150:1656–1664.
26. Noël L, Thieme F, Nennstiel D, Bonas U. Two novel type III-secreted proteins of *Xanthomonas campestris* pv. *vesicatoria* are encoded within the *hrp* pathogenicity island. *J Bacteriol* 2002;184:1340–1348.
27. Block A, Li G, Fu ZQ, Alfano JR. Phytopathogen type III effector weaponry and their plant targets. *Curr Opin Plant Biol* 2008;11:396–403.
28. Schwartz AR, Potnis N, Timilsina S, Wilson M, Patané J *et al.* Phylogenomics of *Xanthomonas* field strains infecting pepper and tomato reveals diversity in effector repertoires and identifies determinants of host specificity. *Front Microbiol* 2015;6:535.
29. Slater H, Alvarez-Morales A, Barber CE, Daniels MJ, Dow JM. A two-component system involving an HD-GYP domain protein links cell-cell signalling to pathogenicity gene expression in *Xanthomonas campestris*. *Mol Microbiol* 2000;38:986–1003.
30. Patil PB, Sonti RV. Variation suggestive of horizontal gene transfer at a lipopolysaccharide (lps) biosynthetic locus in *Xanthomonas oryzae* pv. *oryzae*, the bacterial leaf blight pathogen of rice. *BMC Microbiol* 2004;4:40.