Genomic and phenotypic characterization of *Xanthomonas cynarae* sp. nov., a new species that causes bacterial bract spot of artichoke (*Cynara scolymus* L.)

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A bacterial disease of artichoke (*Cynara scolymus* L.) was first observed in 1954 in Brittany and the Loire Valley, France. This disease causes water-soaked spots on bracts and depreciates marketability of the harvest. Ten strains of the pathogen causing bacterial spot of artichoke, previously identified as a member of the genus *Xanthomonas*, were characterized and compared with type and pathotype strains of the 20 *Xanthomonas* species using a polyphasic study including both phenotypic and genomic methods. The ten strains presented general morphological, biochemical and physiological traits and G+C content characteristic of the genus *Xanthomonas*. Sequencing of the 16S rRNA gene confirmed that this bacterium belongs to the genus *Xanthomonas*, and more precisely to the *Xanthomonas campestris* core. DNA–DNA hybridization results showed that the strains that cause bacterial spot of artichoke were 92–100% related to the proposed type strain CFBP 4188^T and constituted a discrete DNA homology group that was distinct from the 20 previously described *Xanthomonas* species. The results of numerical analysis were in accordance with DNA–DNA hybridization data. Strains causing the bacterial bract spot of artichoke exhibited consistent determinative biochemical characteristics, which distinguished them from the 20 other *Xanthomonas* species previously described. Furthermore, pathogenicity tests allowed specific identification of this new phytopathogenic bacterium. Thus, it is concluded that this bacterium is a new species belonging to the genus *Xanthomonas*, for which the name *Xanthomonas cynarae* is proposed. The type strain, CFBP 4188^T, has been deposited in the Collection Française des Bactéries Phytopathogènes (CFBP).

Keywords: *Xanthomonas cynarae*, taxonomy, DNA–DNA hybridization, rRNA gene sequencing, *Cynara scolymus* L.

INTRODUCTION

In France, a bacterial disease of artichoke (*Cynara scolymus* L.) was observed for the first time in 1954, in Brittany (Saint Pol de Léon area) and in the Loire Valley near Angers (Ridé, 1956). The disease has since been reported in south-west France and in North Africa (Ridé, 1965). This disease, called bacterial bract spot of artichoke, results in water-soaked, dark green

spots which occur on the abaxial part of the capitulum bracts during warm and humid periods. As the infection progresses, large brown necrosis spots result. Water-soaked spots on bracts depreciate marketability of the crop. In 1995, for example, this bacterial disease was observed in numerous artichoke crops in Brittany and resulted in substantial economic losses. The causal agent was first isolated from bract spots by Ridé (1956) and was identified as a phytopathogenic bacterium belonging to the genus *Xanthomonas*, but it was never classified at the species level.

Taxonomy of the genus *Xanthomonas* has fluctuated

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The GenBank accession number for the 16S rRNA gene sequence of strain CFBP 4188^T is AF208315.

since the first description of the genus (Dowson, 1939). Previously, taxonomy has been based on host specificity: a bacterium isolated from a new host was described as a new taxon and was considered as a new species (Dye & Lelliott, 1974). These taxa were later grouped within five species: *Xanthomonas albilineans*, *Xanthomonas ampelina* (now *Xylophilus ampelinus*), *Xanthomonas axonopodis*, *Xanthomonas fragariae* and *Xanthomonas campestris* (Young *et al.*, 1978). In the latter species, 129 of the former species described before 1974 were regrouped as pathovars. This classification was not satisfactory because biochemical and phenotypic characteristics could not reliably differentiate each pathovar of *X. campestris* species.

Classification has been partially clarified by Vauterin *et al.* (1995) who described 20 species from amongst the three former species, *X. axonopodis*, *X. fragariae* and *X. albilineans*, and 62 pathovars of *X. campestris*, on the basis of DNA–DNA hybridization data and biochemical and physiological tests (Vauterin *et al.*, 1995). Sixty-six other pathovars of *X. campestris* were not included in their study and remain to be classified among the 20 described species or in other new species.

In this paper, the causal agent of bacterial spot of artichoke was characterized using a polyphasic study which includes pathogenicity tests, DNA–DNA hybridization data, a numerical analysis of biochemical and physiological tests, G+C content and rRNA gene sequencing data. These data have provided a description of *Xanthomonas cynarae* sp. nov.

METHODS

Bacterial strains and growth conditions. Two sets of strains were used in this study. The first set included ten strains that are pathogenic to artichoke: eight strains isolated from bacterial bract spot of artichoke and two strains isolated from the leaf surface of a common weed found in artichoke fields (*Senecio sp.*) (Table 1). The second set comprised the type and pathotype strains of the 20 *Xanthomonas* species described in the revised classification of Vauterin *et al.* (1995): *X. albilineans* CFBP 2523^T, *Xanthomonas arboricola* pv. *juglandis* CFBP 2528^T, *X. axonopodis* pv. *phaseoli* CFBP 2534^{PT} (PT, PathoType as Dye *et al.*, 1980), *Xanthomonas*

bromi CFBP 3625^{PT}, *X. campestris* pv. *campestris* CFBP 2350^T, *Xanthomonas cassavae* CFBP 4642^T, *Xanthomonas codiae* CFBP 4690^T, *Xanthomonas cucurbitae* CFBP 2542^T, *X. fragariae* CFBP 2057^T, *Xanthomonas hortorum* pv. *pelargonii* CFBP 2533^{PT}, *Xanthomonas hyacinthi* CFBP 1156^T, *Xanthomonas melonis* CFBP 4644^T, *Xanthomonas oryzae* pv. *oryzae* CFBP 2532^T, *Xanthomonas pisi* CFBP 4643^T, *Xanthomonas populi* CFBP 1817^T, *Xanthomonas sacchari* CFBP 4641^T, *Xanthomonas theicola* CFBP 4691^T, *Xanthomonas translucens* pv. *translucens* CFBP 2054^T, *Xanthomonas vasicola* pv. *holcicola* CFBP 2543^T and *Xanthomonas vesicatoria* CFBP 4645^T. Bacteria were grown routinely on a YBGA medium (yeast extract, 7 g l⁻¹; Bacto Peptone, 7 g l⁻¹; glucose, 7 g l⁻¹; agar, 15 g l⁻¹; pH 7.2) at 28 °C for 24 h, except for *X. populi* which was grown at 19 °C.

For BIOLOG tests and DNA extractions for DNA–DNA hybridization experiments, strains were cultured on Tryptic Soy Agar (TSA) medium (Bacto Tryptone, 15 g l⁻¹; Bacto Soytone, 5 g l⁻¹; NaCl, 5 g l⁻¹; agar, 15 g l⁻¹; pH 7.3) at 28 °C.

Morphological, biochemical and physiological characterization

Pathogenicity tests. Pathogenicity tests were performed on detached artichoke bracts placed in sterile Petri dishes. Bacterial suspensions were prepared from 24-h-old cultures grown on YBGA agar slants in sterilized distilled water at a concentration of approximately 1 × 10⁸ c.f.u. ml⁻¹. Surface-sterilized artichoke bracts were scarified with a sterile scalpel and then inoculated with 1 ml bacterial suspension per bract; the control was inoculated with sterile distilled water. Three bracts were inoculated with each strain and incubated at 28 °C. The presence or absence of typical water-soaking symptoms was recorded 4 d after inoculation.

Conventional identification tests. Colony morphology was observed on YBGA plates. Gram tests were performed with 3% (w/v) KOH as described by Suslow *et al.* (1982). Motility was observed from bacterial suspensions grown in nutrient broth (yeast extract, 7 g l⁻¹; Bacto Peptone, 7 g l⁻¹; pH 7) under a compound microscope (magnification × 400). The presence of xanthomonadin pigment was analysed as described by Schaad & Stall (1988). Hugh & Leifson (1953) medium was used for the oxidation-fermentation test. Hypersensitive reaction on tobacco leaves was tested as described by Lelliott *et al.* (1966).

Cytochrome oxidase activity, nitrate reduction, urease activity, indole production, and aesculin, Tween 80, gelatin

Table 1. Strains pathogenic to artichoke used in this study

CFBP strain no.	Source of isolate	Geographical origin	Year of isolation
19	<i>Cynara scolymus</i> L.	Brittany, France	1957
4188 ^T	<i>Cynara scolymus</i> L.	Brittany, France	1996
4189	<i>Cynara scolymus</i> L.	Brittany, France	1996
4190	<i>Cynara scolymus</i> L.	Brittany, France	1996
4199	<i>Cynara scolymus</i> L.	Brittany, France	1996
4209	<i>Senecio sp.</i>	Brittany, France	1996
4827	<i>Cynara scolymus</i> L.	Brittany, France	1997
4828	<i>Cynara scolymus</i> L.	Brittany, France	1997
4829	<i>Cynara scolymus</i> L.	Brittany, France	1997
4830	<i>Senecio sp.</i>	Brittany, France	1997

and starch hydrolysis were tested as described by Lelliott *et al.* (1966). Casein hydrolysis was performed on a medium containing skimmed milk (24 g l⁻¹) and agar (18.8 g l⁻¹), pH 7.5.

Acid production from arabinose, cellobiose, erythritol, galactose, glucose, inositol, mannose and sorbitol was tested on the basal medium of Ayers *et al.* (1919) supplemented with 0.1 % (w/v) carbohydrate source.

DNA base composition. The G+C content (mol%) of the artichoke strain CFBP 4188^T was determined by the thermal denaturation method (Marmur & Doty, 1962) and was calculated by using the equation of Owen & Lapage (1976).

Sequencing of the 16S rRNA gene. Strain CFBP 4188^T was grown overnight on YBGA medium at 27 °C and bacteria were resuspended in 2 ml sterile distilled water and pelleted by centrifugation for 10 min at 7500 r.p.m. (5000 g). The bacterial pellet was resuspended in 180 µl ATL buffer (Qiagen). Bacterial DNA was extracted and purified using the QIAamp Tissue kit according to the protocol proposed by the manufacturer (Qiagen). The 16S rRNA gene was PCR-amplified using primers described by Hauben *et al.* (1997): 16F27 (5'-AGAGTTTGATCMTGGCTCAG-3') and 16R1525 (5'-TTCTGCAGTCTAGAAGGAGGTGWTCCAGCC-3'). Bacterial DNA (100 ng) was added to a solution containing 5 µl 10 × buffer [100 mM Tris/HCl, pH 8.3, 500 mM KCl], 0.01 % (w/v) gelatin, 3 mM MgCl₂, dNTPs (175 mM each), each primer at a concentration of 0.3 µM and 2.5 U AmpliTaq Gold (Perkin Elmer) in a final volume of 50 µl. The samples were heated at 94 °C for 10 min and processed through 35 cycles consisting of 30 s denaturation at 94 °C, 30 s annealing at 55 °C and 1 min primer extension at 72 °C. A final step was performed at 72 °C for 2 min. The amplification products were purified with a QIAquick PCR purification kit according to the manufacturer (Qiagen). DNA was cloned into the pGEM-T easy plasmid according to the manufacturer (Promega). Recombinant plasmids were transferred into *Escherichia coli* strain DH5α (Hanahan, 1983).

The PCR product was sequenced directly from the PCR reactions and from recombinant plasmids. The consensus sequence was established from the data. DNA fragments were sequenced by using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) and a model ABI377 automatic sequencer. A nearly complete 16S rRNA gene sequence was determined by using T7 and SP6 primers on a DNA fragment cloned into the pGEM-T easy plasmid, and by using 16F27 and 16R1525 on PCR amplicons, and 16F530 (5'-TTCGTGCCAGCAGCCGCGG-3') and 16R1087 (5'-CTCGTTGCGGGACTTAACCC-3') (Hauben *et al.*, 1997) on both types of DNA templates.

DNA-DNA hybridization. A previously described method was used to extract and purify DNA (Brenner *et al.*, 1982). Native DNA of the artichoke strain CFBP 4188^T was radio-labelled *in vitro* with tritium-labelled nucleotides (Amersham International) by random priming using Megaprime DNA labelling system RPM 1604. The S1 nuclease-trichloroacetic acid method used for DNA-DNA hybridization experiments was described by Crosa *et al.* (1973) and modified by Grimont *et al.* (1980). The reassociation temperature used was 70 °C. DNA-DNA hybridization experiments were repeated twice.

Assimilation tests with the BIOLOG GN microplate system. Ninety-five carbon source utilization tests were performed by using the BIOLOG GN microplate system. GN microplates were used as recommended by the manufacturer. Microplates

were incubated at 28 °C for 48 h. Formazan reduction was recorded with an ELISA microplate reader (MR 5000; Dynatech) and the accuracy of the results was checked by visual observations of the colour reaction.

Numerical analysis. A total of 112 physiological characteristics were included in a numerical taxonomy analysis (17 conventional tests and 95 carbohydrate reactions on BIOLOG microplates). The distance matrix was calculated using the Jaccard coefficient. Cluster analysis was performed by using the unweighted pair group method of averages with arithmetic mean (UPGMA; Sneath & Sokal, 1973). At a chosen distance level and for the different phena, the amount of information for each test was measured by calculating the diagnostic ability coefficient to determine the discriminating biochemical characteristics of the strains (Descamps & Véron, 1981).

RESULTS AND DISCUSSION

Pathogenicity tests

The eight strains isolated from artichoke bract spot and the two strains isolated from the leaf surface of *Senebiera* sp. produced typical water-soaked lesions on inoculated, detached artichoke bracts. The same bacteria were re-isolated in pure culture from these lesions. None of the reference strains belonging to the 20 described *Xanthomonas* species produced symptoms. It was confirmed that this kind of test can be a good indicator of pathogenicity (Endert & Ritchie, 1984; Randhawa & Civerolo, 1985). These results also showed that strains pathogenic to artichoke could be isolated from leaf surface of a weed (*Senebiera* sp.) grown in naturally infected artichoke crops. Moreover, this suggests that bacteria responsible for bract spot disease of artichoke might survive on the leaf surface of weeds present in artichoke fields. In addition, the ten strains used in this study induced typical hypersensitive reactions on tobacco leaves, indicating that they were pathogenic xanthomonads. In conclusion, the specificity of pathogenicity tests on detached bracts can be used to discriminate the strains pathogenic to artichoke from the 20 other species of *Xanthomonas*.

Xanthomonas characteristics of the bacterium responsible for bacterial bract spot disease

The ten strains pathogenic to artichoke grown on YBGA medium gave yellow, mucoid, round, domed, smooth and shining colonies. They were Gram-negative. Cells were rod-shaped, alone or in pairs, and motile by means of one polar flagellum. Glucose was utilized oxidatively and the respiratory metabolism was strictly aerobic. Acid was produced from galactose and mannose, but not from erythritol and inositol. Aesculin was hydrolysed. Catalase activity was observed, but cytochrome *c* oxidase and urease activities were not detected. Indole was not produced. Nitrate was not reduced into nitrite. Acetoin was not formed and litmus milk was not acidified. The yellow pigment analysed by TLC as described by Schaad & Stall (1988) gave a mean *R_f* value of 0.445, which is

Table 2. Levels of DNA reassociation between *Xanthomonas cynarae* CFBP 4188^T, other strains of this species and the type and pathotype strains of 20 *Xanthomonas* species

Source of unlabelled DNA	CFBP no.	Relative binding with labelled DNA from <i>X. cynarae</i> CFBP 4188 ^T (%)
<i>X. cynarae</i>	4188 ^T	100
	19	96
	4199	99
	4209	92
<i>X. fragariae</i>	2057 ^T	23
<i>X. hortorum</i> pv. <i>pelargonii</i>	2533 ^T	49
<i>X. populi</i>	1817 ^T	49
<i>X. arboricola</i> pv. <i>juglandis</i>	2528 ^T	48
<i>X. cassavae</i>	4642 ^T	35
<i>X. codiae</i>	4690 ^T	36
<i>X. bromi</i>	3625 ^{PT}	36
<i>X. cucurbitae</i>	2542 ^T	15
<i>X. axonopodis</i> pv. <i>phaseoli</i>	2534 ^{PT}	44
<i>X. oryzae</i> pv. <i>oryzae</i>	2532 ^T	37
<i>X. vasicola</i> pv. <i>holcicola</i>	2543 ^T	38
<i>X. pisi</i>	4643 ^T	40
<i>X. melonis</i>	4644 ^T	31
<i>X. campestris</i> pv. <i>campestris</i>	2350 ^T	40
<i>X. vesicatoria</i>	4645 ^T	38
<i>X. translucens</i> pv. <i>translucens</i>	2054 ^T	17
<i>X. hyacinthi</i>	1156 ^T	21
<i>X. theicola</i>	4691 ^T	17
<i>X. sacchari</i>	4641 ^T	14
<i>X. albilineans</i>	2523 ^T	15

characteristic of the xanthomonadin pigment. Control R_t values from *X. campestris* pv. *campestris* and *X. axonopodis* pv. *phaseoli* were 0.45 and 0.5, respectively. The DNA G+C content of artichoke strain CFBP 4188^T, determined by the thermal denaturation method, was 63 mol%, which is consistent with the range for *Xanthomonas* strains of 63.3–69.7 mol% (Vauterin *et al.*, 1995). Thus, the ten strains isolated from artichoke bract spots and from the leaf surface of the weed *Senebiera* sp. showed physiological and biochemical characteristics, and a DNA G+C content that are identical to bacteria belonging to the genus *Xanthomonas*.

Sequencing of 16S rRNA gene

Sequencing reactions performed with both types of templates (PCR-amplified fragments and cloned DNA fragments) showed the same nucleotide sequences, allowing a continuous stretch of 1552 bases to be determined. This stretch was identical to the partial 16S rRNA gene sequence of *X. campestris* pv. *campestris* LMG 568^T (EMBL accession number X95917; Hauben *et al.*, 1997). Hauben *et al.* (1997) showed that the sequence similarity between species of the *Xanthomonas* core they described was 98.9–100.0%. Results indicated that the strains pathogenic

to artichoke belonged to the genus *Xanthomonas* and more precisely to the *X. campestris* core (according to the 16S rRNA gene sequence analysis of 46 xanthomonad strains). It does not mean that strains pathogenic to artichoke are more related to *X. campestris* species than to the other species because the level of interspecific sequence similarity is remarkably high within the *X. campestris* core. Hauben *et al.* (1997) reported that no nucleotide differences were observed among the sequences of the type strains of *X. campestris* and *X. arboricola*, whereas strains belonging to *X. axonopodis* showed up to eight nucleotide differences. Our results illustrate again the limitation of 16S rRNA gene sequence analysis in the determination of relationships at the species level (Stackebrandt & Goebel, 1994).

DNA–DNA hybridization

Results of DNA–DNA hybridization are shown in Table 2. The three strains CFBP 19, 4199 and 4209, pathogenic to artichoke, were 92–100% related to strain CFBP 4188^T. All the other 20 type or pathotype strains tested were only 14–49% related to strain CFBP 4188^T. Thus, the four strains tested that were pathogenic to artichoke constituted a discrete DNA hybridization group. The approved molecular

Table 3. Conventional tests and BIOLOG carbohydrate tests which differentiate the artichoke strains from 19 *Xanthomonas* species

+, 90–100 % of the strains are positive; –, 0–10 % of the strains are positive. Species: 1, *X. cynarae*; 2, *X. fragariae* CFBP 2057^T; 3, *X. hortorum* pv. *pelargonii* CFBP 2533^T; 4, *X. arboricola* pv. *juglandis* CFBP 2528^T; 5, *X. cassavae* CFBP 4642^T; 6, *X. codiae* CFBP 4690^T; 7, *X. bromi* CFBP 3625^{PT}; 8, *X. cucurbitae* CFBP 2542^T; 9, *X. axonopodis* pv. *phaseoli* CFBP 2534^{PT}; 10, *X. oryzae* pv. *oryzae* CFBP 2532^T; 11, *X. vasicola* pv. *holcicola* CFBP 2543^T; 12, *X. pisi* CFBP 4643^T; 13, *X. melonis* CFBP 4644^T; 14, *X. vesicatoria* CFBP 4645^T; 15, *X. campestris* pv. *campestris* CFBP 2350^T; 16, *X. translucens* pv. *translucens* CFBP 2054^T; 17, *X. hyacinthi* CFBP 1156^T; 18, *X. theicola* CFBP 4691^T; 19, *X. sacchari* CFBP 4641^T; 20, *X. albilineans* CFBP 2523^T.

Test	<i>Xanthomonas</i> species																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Conventional tests																				
Gelatin hydrolysis	+	+	+	+	+	+	+	–	–	–	+	+	+	+	–	–	+	–	+	–
Starch hydrolysis	–	–	+	+	+	+	–	–	+	–	–	+	+	+	+	–	–	–	+	–
Milk proteolysis	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	–
Tween esterase	+	–	+	+	–	+	–	–	+	–	+	+	+	–	–	+	–	–	+	+
Acidification of:																				
Arabinose	–	–	–	–	–	–	–	+	–	–	+	+	+	+	–	+	–	–	–	–
Cellobiose	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Glucose	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Mannose	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
BIOLOG tests																				
D-Raffinose	+	–	+	–	–	+	–	–	+	–	–	+	+	–	+	–	–	–	+	–
Formic acid	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–
α-Ketobutyric acid	+	–	–	+	+	+	–	–	–	–	–	+	+	+	+	–	–	–	+	–
Propionic acid	–	–	–	+	–	+	–	–	+	–	–	+	+	–	+	–	–	–	+	–
D-Saccharic acid	–	–	–	–	–	+	–	+	–	–	–	+	+	–	+	–	–	–	+	–
L-Asparagine	+	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–
L-Aspartic acid	+	+	–	+	–	+	+	+	–	–	–	+	–	–	–	+	+	–	+	–
L-Ornithine	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
L-Threonine	+	–	+	+	+	–	–	–	–	–	–	+	+	–	+	–	–	–	+	–
Urocanic acid	+	–	–	+	–	–	+	+	–	+	–	+	–	+	+	–	–	–	+	–
Uridine	+	–	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	+	–
D,L-α-Glycerophosphate	+	–	+	+	–	–	–	–	+	–	–	+	–	–	+	+	+	–	+	–

definition of a bacterial genomic species (genomospecies) is applied to strains whose level of DNA relatedness is around 70 % (Wayne *et al.*, 1987). Thus, our results of DNA–DNA hybridization indicated clearly that the strains pathogenic to artichoke belong to a distinct genomic species and corresponded to a new genomospecies.

Biochemical and physiological tests

The results of nine biochemical tests allowed strains that were pathogenic to artichoke to be distinguished from most of the 19 other species of *Xanthomonas* (Table 3).

Metabolic activities on BIOLOG tests of the ten strains pathogenic to artichoke

The percentage of positive responses to BIOLOG tests was calculated (Table 4) to compare the results obtained using strains pathogenic to artichoke (Table

4) to those presented by Vauterin *et al.* (1995). Four to 18 discriminating tests between strains pathogenic to artichoke and 17 species of *Xanthomonas* were found (a discriminating test is a test that yielded at least 90 % positive reactions in one species and less than 10 % positive reactions in the other species; as defined by Vauterin *et al.*, 1995). For *X. vesicatoria*, *X. arboricola* and *X. axonopodis*, only two, one and zero discriminating tests were observed. *X. axonopodis* comprises 37 different pathovars and the results of mean metabolic activities were calculated with 85 strains that represented a very large potential source of variability (Vauterin *et al.*, 1995). If a positive test was considered to be one in which 80 % or more of the strains tested produced positive responses, and a negative test was considered to be one in which less than 19 % of all strains tested give positive responses, then *X. vesicatoria*, *X. arboricola* and *X. axonopodis* were differentiated from artichoke strains by tests BIOLOG 11, five and four, respectively. In addition, the results of BIOLOG GN microplate tests confirm that strains

Table 4. Percentage of metabolic activities of *X. cynarae* on 95 carbon compounds in the BIOLOG GN microplate assay

The names of the carbon sources used, as indicated by letters (rows) and numbers (columns) are those given by BIOLOG. (–), 0–10% of the strains are positive; (+), 90–100% of the strains are positive; (d), 11–89% of the strains are positive.

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	0	100	100	100	100	0	100	0	0	0	100
		(–)	(+)	(+)	(+)	(+)	(–)	(+)	(–)	(–)	(–)	(+)
B	20	100	100	90	100	100	0	60	100	100	0	100
		(d)	(+)	(+)	(+)	(+)	(–)	(d)	(+)	(+)	(–)	(+)
C	100	60	100	100	0	20	100	100	100	0	100	100
		(+)	(d)	(+)	(+)	(–)	(d)	(+)	(+)	(–)	(+)	(+)
D	100	80	90	0	0	0	10	0	0	100	80	30
		(+)	(d)	(+)	(–)	(–)	(–)	(–)	(–)	(+)	(d)	(d)
E	30	70	100	100	40	100	30	10	0	10	20	100
		(d)	(d)	(+)	(+)	(d)	(+)	(d)	(–)	(–)	(d)	(+)
F	100	100	0	100	90	100	80	90	100	100	50	90
		(+)	(+)	(–)	(+)	(+)	(d)	(+)	(+)	(+)	(d)	(+)
G	90	40	10	10	0	100	10	0	100	100	0	0
		(+)	(d)	(–)	(–)	(–)	(+)	(–)	(+)	(+)	(–)	(–)
H	100	80	100	0	0	0	0	20	90	90	90	60
		(+)	(d)	(+)	(–)	(–)	(–)	(d)	(+)	(+)	(+)	(d)

pathogenic to artichoke belong to the genus *Xanthomonas*. Indeed, Vauterin *et al.* (1995) identified 24 carbon sources which are oxidized by at least 94% of strains. Our results obtained with the ten artichoke strains are in accordance with the results of Vauterin *et al.* (1995) for these 24 carbon substrates.

Numerical analysis of biochemical and physiological tests

One-hundred-and-twelve tests (17 conventional biochemical and physiological tests and the 95 BIOLOG tests) were used in numerical taxonomy analysis. A dendrogram displaying the distance relationships among the ten strains pathogenic to artichoke and the type or pathotype strains of 19 species described by Vauterin *et al.* (1995) is shown in Fig. 1. The type strain of *X. populi* was not included in this analysis because of its poor growth on different media and in tests. At a distance of 0.185, three phenon and 15 isolated strains were delimited. Phenon 1 included the ten strains pathogenic to artichoke. Phenon 2 contained *X. arboricola* pv. *juglandis* and *X. pisi*, and phenon 3 contained *X. cucurbitae* and *X. translucens* pv. *translucens*. Thus, the ten strains pathogenic to artichoke formed a homogeneous phenotypic group that was distinct from all type and pathotype strains of the 19 species tested. Using the diagnostic ability coefficient of Descamps & Véron (1981), 12 additional tests from the 95 BIOLOG tests were selected that differentiate the ten strains pathogenic to artichoke from the other species tested (Table 3). However, for five type strains some different results from those of Vauterin *et al.*

(1995) were observed: L-aspartic acid for *X. fragariae* (+ in Table 3); formic acid for *X. arboricola* (+ in Table 3); urocanic acid for *X. bromi* and *X. cucurbitae* (+ in Table 3); D-raffinose and urocanic acid for *X. sacchari* (+ in Table 3). Our experiments were repeated two or three times, but no objective explanations for these differences were determined. These discrepancies could be due to the weak growth of strains, to the different ways of reading the tests, or to differences in strains.

All the results presented here show that the ten strains pathogenic to artichoke belong to the genus *Xanthomonas*. The strains are phenotypically and genetically very closely related to one another and form a homogeneous group distinct from the 20 other species of *Xanthomonas* described by Vauterin *et al.* (1995). These data are consistent with the definition of a bacterial species (Wayne *et al.*, 1987) and it is proposed that the strains causing bacterial bract spot of artichoke be designated a new species. The name *Xanthomonas cynarae* is proposed for this new species and strain CFBP 4188^T is the type strain.

Description of *Xanthomonas cynarae* sp. nov.

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

Typical symptoms caused by *Xanthomonas cynarae* consist of water-soaked then dark green spots which occur on the abaxial part of the capitulum bracts. As the infection progresses, large brown necrosis spots

Fig. 1. Dendrogram of phenotypic distances between ten *Xanthomonas cynarae* strains and 19 type and pathotype strains of *Xanthomonas*.

result. Bacterial cells are Gram-negative, non-spore-forming, straight rods, motile by one polar flagellum. Colonies grown on YBGA for 2 d at 28 °C are circular (2 mm in diameter), smooth, glistening, slightly convex and produce the yellow xanthomonadin pigment. They are strictly aerobic. They do not produce cytochrome *c* oxidase, nitrate reductase, urease or indole. They hydrolyse casein, gelatin and aesculin, but not starch. Acid is produced from galactose, glucose and mannose, but not from arabinose, erythritol, inositol or sorbitol. Using BIOLOG GN microplates, *Xanthomonas cynarae* gives metabolic activity on the following carbon substrates: dextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α -D-glucose, lactulose, maltose, D-mannose, D-melibiose, D-psicose, D-raffinose, sucrose, D-trehalose, turanose, methylpyruvate, monomethylsuccinate, acetic acid, citric acid, α -hydroxybutyric acid, α -ketobutyric acid, α -ketoglutaric acid, DL-lactic acid, succinic acid, bromo-

succinic acid, succinamic acid, alaninamide, D-alanine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid, L-histidine, L-proline, L-serine, L-threonine, urocanic acid, uridine, glycerol, DL- α -glycerophosphate and glucose 1-phosphate. It lacks metabolic activity on: α -cyclodextrin, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, *meso*-inositol, D-mannitol, L-rhamnose, xylitol, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, propionic acid, quinic acid, D-saccharic acid, glucuronamide, L-leucine, L-ornithine, L-phenylalanine, L-pyrroglutamic acid, D-serine, DL-carnitine, γ -aminobutyric acid, thymidine, phenyl ethylamine, putrescine and 2-aminoethanol. *Xanthomonas cynarae* induces a hypersensitive reaction on tobacco leaves. Strains pathogenic to artichoke (*Cynara scolymus* L.) give typical symptoms after inoculation on detached scarified bracts. The DNA G + C content is 63 mol %. The type strain is CFBP

4188^T and has been deposited in the Collection Française de Bactéries Phytopathogènes (CFBP), Angers, France.

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