SHORT COMMUNICATION

CHARACTERIZATION OF BACTERIAL STRAINS OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* ISOLATED FROM PEPPER LEAF SPOT IN MACEDONIA

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SUMMARY

A new bacterial leaf spot disease on pepper seedlings (*Capsicum annuum* cv. 'Kurtovska kapija') was observed in 1995 in Macedonia. *Pseudomonas* bacteria were isolated, belonging to LOPAT group Ia. Symptoms similar to natural symptoms were reproduced following inoculation on pepper seedlings. Some isolates produced syringomycin and none of them were pathogenic to lilac. In a numerical taxonomic study of five pepper isolates in comparison with 58 pathovars of *P. syringae* and 10 related species, the five pepper isolates clustered in one phenon. Considering phenotypic characteristics, serology, DNA relatedness and pathogenicity tests, it was concluded that the pepper strains belong to *P. syringae* pv. *syringae*.

Key words: Pseudomonas syringae, phenotypy, serology, DNA-DNA hybridization, pathogenicity, numerical taxonomy.

Pseudomonas syringae van Hall 1902, originally isolated from lilac (*Syringa vulgaris* L.), has subsequently been isolated from many plant species belonging to many genera and families (Young, 1991). *P. syringae* is a group comprising more than 50 plant pathogens classified as pathovars on the basis of host specificity (Young *et al.,* 1996). From pepper plants (*Capsicum annuum* L. and *Capsicum* spp.), bacteria have been isolated which belong to the following pathovars of *P. syringae*: pv. *aptata*, pv. *syringae* and pv. *tomato*, (Morton and Ratcliffe, 1964; Bradbury, 1986). Person (1964) first reported isolation of *P. syringae* from leaf spots on naturally infected pepper in 1962.

In Macedonia, symptoms of a new bacterial disease on pepper were observed in 1995, around Strumica city. During spring, especially at the beginning of May, leaf spots were observed on pepper seedlings (cv. 'Kurtovska kapija'). The disease was seen under high relative humidity in plastic tunnels, as a result of intensive irrigation, poor aeration and average temperatures of 22°C. Initial spots were small, water-soaked, and dark green, later becoming larger and black. Characteristically, this disease begins with the above-mentioned symptoms and the plants become necrotic. Similar symptoms were found in different areas of the Strumica district, and in some years, the disease had great economic impact.

The bacteria were isolated from leaf spots on pepper seedlings on King's medium B. Fourteen representative isolates were chosen from the Strumica area during 1995 and 1996: 1050 (CFBP 11835); 1051 (CFBP 11836); 1052 (CFBP 11837); 1053 (CFBP 11844); 1054 (CFBP 11838); 1152 (CFBP 11839); 1153 (CFBP 11840); 1154 (CFBP 11841); 1155 (CFBP 11842); 1156 (CFBP 11843); P-221 (CFBP 11923); P-222 (CFBP 11924); P-223 (CFBP 11925) and P-224 (CFBP 11926). The other pathotype strains of *P. syringae* used for numerical taxonomy were listed in a previous paper (Gardan *et al.*, 1999). Twenty classical biochemical and physiological tests and assimilation of carbon sources using Biotype 100 strips (BioMerieux, La Balme-les-Grottes, France) were performed according to Gardan *et al.* (1999). The 14 isolates from pepper produced a fluorescent pigment on King's medium B and belonged to LOPAT group Ia (+ - - - +) of Lelliott *et al.* (1966). The isolates utilized sucrose, erythritol, mannitol and sorbitol, DL-lactate, D(-)-tartrate, and hydrolysed esculin. The isolates gave negative reactions for utilization of L(+)-tartrate, liquefaction of gelatin, hydrolysis of Tween 80, hydrolysis of polypectate at pH 5.0 and pH and 8.3, reduction of nitrate and presence of DNase.

A numerical taxonomy analysis, using the Jaccard coefficient and cluster analysis by the unweighted pair group method of average with arithmetic mean (UPGMA), was performed according to Gardan *et al.* (1999). The dendrogram displaying the relationships amongst the 74 strains is shown in Fig. 1. Cutting at a distance of 0.13 gathered the five pepper strains studied and the reference strain of *P. syringae* pv. *syringae* CFBP 1543 in the same phenon 1. Eight other phena clustering two

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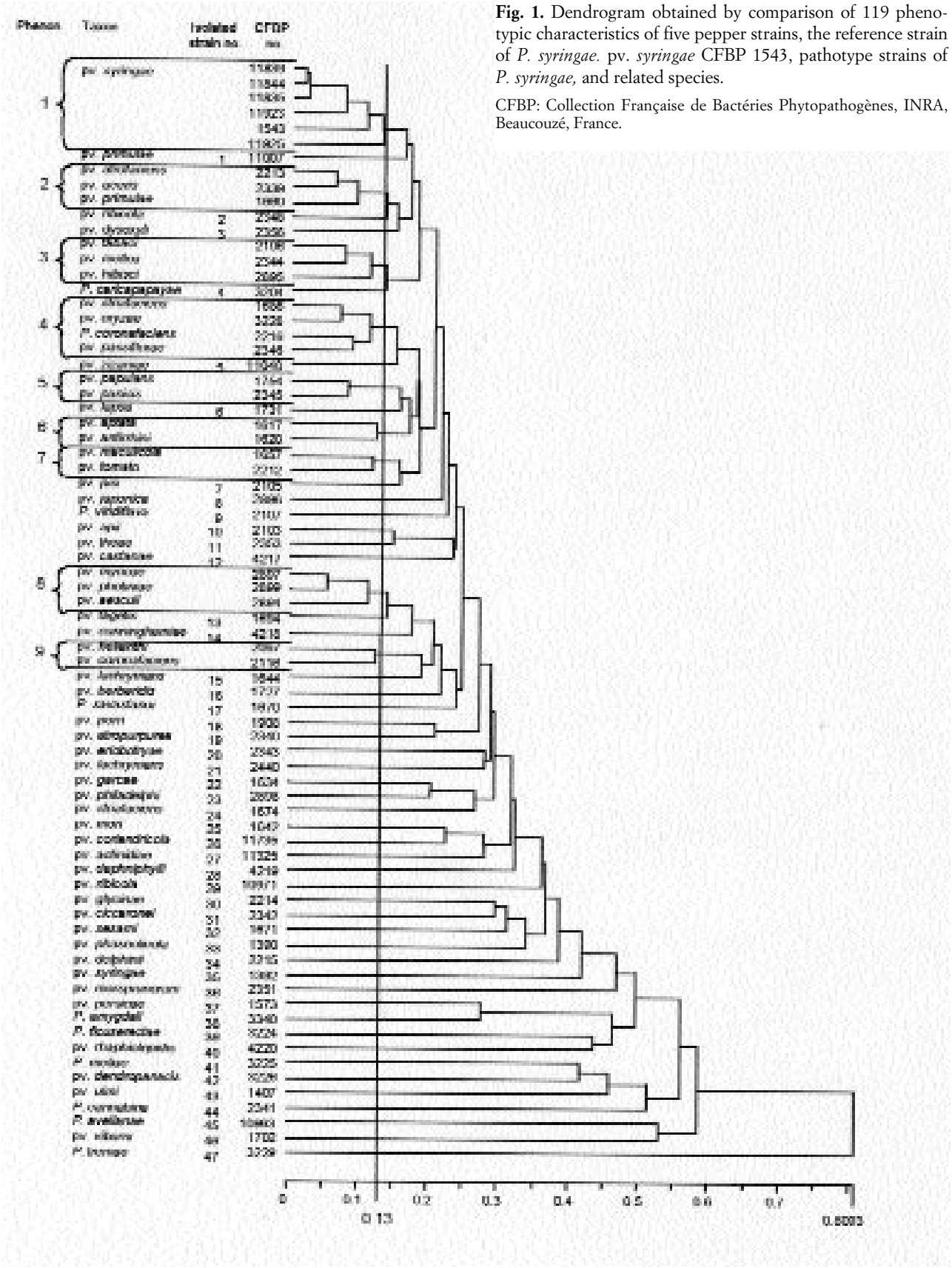

Fig. 1. Dendrogram obtained by comparison of 119 phenotypic characteristics of five pepper strains, the reference strain of *P. syringae.* pv. *syringae* CFBP 1543, pathotype strains of *P. syringae,* and related species.

CFBP: Collection Française de Bactéries Phytopathogènes, INRA, Beaucouzé, France.

to four strains, and 47 isolated strains were observed. By calculating the diagnostic ability coefficient (DAC), the biochemical and physiological characteristics that differentiate phenon 1 strains from the others were deduced (Table 1). The type strain of *P. syringae* CFBP 1392^T was distantly clustered from pepper strains and CFBP 1543; CFBP 1392^T was already considered as atypical and not truly representative of *P. syringae* pv. *syringae* (Gardan *et al.,* 1991).

Syringomycin production was tested using a bioassay with strain CFBP 3389 of *Rhodotorula pilimanae* (Gross and De Vay, 1977). Two pepper isolates, CFBP 11923, 11925 and the type strain of *P. syringae* CFBP 1392^T produced syringomycin, while three other pepper isolates CFBP 11835, 11839, 11844 and *P. syringae* pv. *syringae* CFBP 1543 did not.

Antisera were produced in rabbits using whole bacterial cells as antigens, and O-serogroups were determined by Ouchterlony double-diffusion (Saunier *et al.,* 1996). The pepper isolates belonged to two distinct O-serogroups: PHA for the strains CFBP 11835, 11836, 11837, 11838, 11839, 11840, 11841, 11842, 11843, 11844, and APTPIS for the strains CFBP 11923, 11924, 11925 and 11926. The isolates were not distributed at random within the known O-serogroups as could be expected in the case of *P. syringae* pv. *syringae* strains forming part of the normal plant epiphytic flora (Grondeau *et al.,* 1992). Pepper isolates reacted either as PHA or APTPIS. The O-serogroup PHA occurs mainly in *P. syringae* pv. *phaseolicola* but it has already been noticed amongst some *P. syringae* pv. *syringae* isolates (R. Samson, personal communication). The LPS corresponding to the O-serogroup APTPIS is present in several pathovars of the genomospecies 1 (pv. *aptata*, pv. *pisi*, pv. *atrofaciens*) and in *P. savastanoi* pv. *glycinea* (genomospecies 2). Such double serotyping, already reported for *P. syringae* pv. *atrofaciens* and *P. savastanoi* pv. *phaseolicola,* would remain compatible with the hypothesis of a single new pathovar (Saunier *et al.,* 1996). Serotyping was therefore considered a useful complement for identification of Macedonian pepper isolates.

Bacterial suspensions in sterile de-ionized water (1 x 10^7 cfu ml^{-1}) obtained from 24-48 h NA cultures, were used to inoculate leaves of young pepper plants (cv. 'Kurtovska kapija') by injection with a needle and by gentle spraying. The young pepper plants reacted intensely by giving, after 2-4 days, characteristic spots which appeared around the needle wound and turned darker. Necrosis progressively spread and whole leaves were destroyed. Spraying of bacterial suspension on the leaf surface of young plants led to similar symptoms. Pepper fruits, as well as the green fruits of cherry, sour cherry, lemon, pear, plum, and tomato, reacted very quickly with the appearance of a dark brown discoloration of 1 to 2 cm after two days, gradually became necrotic and, after a few days, fell off.

Young seedlings of lilac (*Syringa vulgaris*, Vilmorin no. 47 82 800) were inoculated by placing a bacterial suspension at *ca.* 1 x 10^8 cfu ml^{-1} on a main vein cut with a razor-blade. Plants were maintained in an illuminated incubator at 22°C. The five pepper strains inoculated (CFBP 11835, 11839, 11844, 11923 and 11925) produced no symptoms on lilac, whereas the strains of *P. syringae* pv. *syringae* CFBP 1392^T and 1543 caused development of black necrotic lesions.

DNA-DNA hybridization tests were carried out by using labeled DNA from pepper strain CFBP 11835 and unlabeled DNA of *P. syringae* pv. *syringae* CFBP 1392^T, *P. savastanoi* pv. *savastanoi* CFBP 1670^T, *P. syringae* pv. *tomato* CFBP 2212, *P. syringae* pv. *porri* CFBP 1908, *P. syringae* pv. *tagetis* CFBP 1694, *P. caricapapayae* CFBP 3204^T and *P. viridiflava* CFBP 2107^T, according to Gardan *et al.* (1999). The pepper isolate CFBP 11844 and the type strain of *P. syringae* pv. *syringae* CFBP 1392^T were 99 and 75% respectively related to the strain CFBP 11835, thus corresponding to the same genomospecies 1, *P. syringae* (Table 2). The seven other reference strains *(Pseudomonas* spp. and pathovars of *P. syringae)* were only 29 to 61% related to the strain CFBP 11835.

In conclusion, although the pepper strains isolated in Macedonia were not pathogenic to lilac, there was insufficient evidence to recognize these pepper strains as delineating a new pathovar. From the overall results of phenotypic characteristics, serology, DNA relatedness and pathogenicity tests, it was concluded that pepper strains belong to *P. syringae* pv. *syringae*.

P. syringae pv. *syringae* was reported for the first time in the former Yugoslavia (Vojvodine, Serbia, and Croatia) on pepper fruits in the field and later on seedlings (Arsenijevic and Balaz, 1978). More recently, this pathogen has been observed in important pepper production areas in the Balkan region where it has caused significant losses (Obradovic *et al.,* 1995). This is the first report of bacterial leaf-spot of pepper in Macedonia. According to previous investigations, applications of copper-based preparations and combination of copper and zineb could help to control the disease.

Table 1. Biochemical characteristics of 47 isolated taxa and the nine phena delineated in Fig. 1.

Phena [a]																								
1	+	+	+	-	+	+	+	+	-	83	83	+	-	+	83	+	+	+	+	+	+	+	+	83
2	+	+	+	+	+	+	+	+	+	+	66	+	-	+	+	+	+	+	+	+	+	+	+	33
3	+	+	+	+	+	+	+	66	33	+	66	+	+	+	+	+	-	+	+	+	+	+	+	+
4	+	+	-	+	+	+	75	75	75	50	-	+	-	75	25	+	-	+	+	+	+	+	+	-
5	+	+	50	+	50	+	+	+	-	-	-	+	-	+	50	+	+	+	+	+	+	+	+	50
6	+	50	+	+	+	+	-	-	-	-	+	+	-	-	-	+	50	+	+	+	+	+	+	-
7	+	-	+	+	+	+	+	+	50	50	+	+	-	+	-	+	-	+	+	+	+	+	+	50
8	-	-	+	+	66	+	+	+	-	66	-	33	+	+	66	+	-	+	+	66	+	+	+	-
9	-	+	+	+	+	+	50	-	+	-	-	+	+	50	-	50	-	+	+	+	+	+	+	50
Isolated taxa																								
1	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-
3	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-
4	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
5	+	-	-	+	+	+	-	+	-	-	-	+	-	-	+	+	-	+	+	+	+	+	+	-
6	+	+	+	+	+	+	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+
7	+	-	+	-	+	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	+	+	-
8	+	+	+	+	-	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-
10	-	-	+	+	+	-	+	+	-	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+
11	-	-	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	+	+	+	+	+	+	-
12	-	+	-	-	+	+	+	+	-	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+
13	-	-	+	+	+	+	+	+	-	-	-	+	+	+	-	+	-	+	+	+	+	+	+	-
14	-	-	+	-	+	+	+	-	-	+	-	+	+	+	-	+	-	+	+	+	+	+	+	-
15	+	+	-	+	-	+	+	+	+	-	-	+	+	+	-	+	-	+	+	+	-	+	+	-
16	-	-	+	+	+	+	+	-	-	-	-	-	+	+	-	+	-	+	+	+	-	-	+	+
17	-	+	-	+	+	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	+	+	-
18	-	-	-	+	-	-	+	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	-
19	-	+	-	+	+	-	+	+	-	-	-	+	-	+	-	+	-	+	+	+	-	+	+	-
20	+	+	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-	+	+	+	+	+	+	+
21	+	-	+	+	-	+	-	+	+	-	+	-	-	-	-	-	-	+	+	+	+	+	+	+
22	+	-	-	+	-	-	-	-	-	+	+	+	-	-	-	+	-	-	+	-	+	+	+	-
23	-	-	+	+	-	-	-	-	-	+	+	+	-	-	-	+	-	-	+	-	+	+	+	-
24	+	-	-	+	-	+	-	-	-	+	-	+	-	-	+	+	-	+	+	+	+	+	+	+
25	-	-	-	+	-	+	+	+	+	+	-	+	-	+	-	+	-	+	-	+	-	-	-	-
26	-	-	-	-	-	+	+	+	-	-	+	-	-	+	-	+	-	+	-	-	+	+	-	-
27	-	-	-	-	+	-	+	+	+	+	-	+	-	+	+	+	-	+	-	-	+	+	+	-
28	-	-	+	-	-	+	+	-	+	-	-	+	+	+	-	-	-	+	+	-	+	+	+	-
29	-	-	+	-	-	-	+	+	+	-	-	-	-	+	-	+	-	-	+	+	+	+	+	-
30	-	-	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	+	-	-	+	-
31	-	-	+	+	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	+	-	+	-
32	-	-	-	+	-	+	+	-	-	-	-	+	-	+	-	+	-	+	-	+	-	-	+	-
33	-	-	+	-	+	-	+	+	-	-	-	+	-	+	-	-	-	-	+	+	-	-	-	-
34	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+
35	+	+	+	-	+	-	+	-	+	-	-	-	-	+	+	+	+	-	-	+	-	+	-	-
36	+	+	+	+	-	-	-	+	-	+	+	-	-	+	+	-	-	+	-	+	+	+	-	-
37	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	+	-	-
38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
39	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-
40	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-
41	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-
43	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-
44	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-
46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] Phena and isolated taxa are listed as ordered in the Fig. 1 ; pepper isolates are in phenon 1.

Table 2. DNA relatedness among pepper strains and *Pseudomonas* spp.

Unlabelled DNA from	CFBP number	Average % of relative binding at 70°C with labeled DNA from strain CFBP 11835
pepper strain	11835 (P-150)	100
pepper strain	11844 (P-153)	99
P. syringae pv. *syringae*	1392^T	75
P. savastanoi pv *savastanoi*	1670^T	55
P. syringae pv. *tomato*	2212	45
P. syringae pv. *porri*	1908	39
P. syringae pv. *tagetis*	1694	47
P. caricapapaye	3204^T	61
P. viridiflava	2107^T	29

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Received 19 May 1999
Accepted 9 February 2000