

DNA Relatedness among the Pathovar Strains of *Pseudomonas syringae* subsp. *savastanoi* Janse (1982) and Proposal of *Pseudomonas savastanoi* sp. nov.

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We found that *Pseudomonas syringae* subsp. *savastanoi* strains belong to a DNA relatedness group that includes strains of *P. syringae* pv. *glycinea* and *P. syringae* pv. *phaseolicola*. This DNA group was distinct from *P. syringae* pv. *syringae* (including the type strain of *P. syringae*). The results of a numerical analysis were in accord with DNA hybridization data. Thus, *P. syringae* subsp. *savastanoi* (Janse) 1982 is elevated to species level as *Pseudomonas savastanoi* sp. nov., which includes *P. savastanoi* pv. *savastanoi*, *P. savastanoi* pv. *glycinea*, and *P. savastanoi* pv. *phaseolicola*.

In 1908, Smith (22) named "*Bacterium savastanoi*," the bacterium which causes knots on several plants belonging to the family Oleaceae. This species was later transferred to the genus *Pseudomonas* as "*Pseudomonas savastanoi*" by Stevens (24).

In 1978, Young et al. (27) proposed a new nomenclature and classification for plant-pathogenic bacteria and introduced the concept of pathovar, and all fluorescent oxidase-negative *Pseudomonas* species (except *Pseudomonas viridiflava*) were considered to be members of a single species, *Pseudomonas syringae*, which had a number of pathovars. Thus, "*P. savastanoi*" became *P. syringae* pv. *savastanoi* (27). The pathovar is an infrasubspecific subdivision which is not covered by the *International Code of Nomenclature of Bacteria* (14). Thus, the name *P. syringae* pv. *savastanoi* did not appear on the Approved Lists of Bacterial Names (21).

"*Bacterium savastanoi* var. *fraxini*" (2), which was isolated from excrescences on *Fraxinus excelsior* L., and "*Bacterium tonellianum*" (6), which was isolated by Ferraris from galls on *Nerium oleander* L. and which became "*P. savastanoi* var. *nerii*" (21), were included in *P. syringae* pv. *savastanoi* by Young et al. (27).

In 1982, Janse revived the epithet *savastanoi* in designating a new subspecies, *Pseudomonas syringae* subsp. *savastanoi* (17). The following three pathovars were recognized in this subspecies: *P. syringae* subsp. *savastanoi* pv. *oleae*, which causes parenchymatic galls on members of various genera of the Oleaceae; *P. syringae* subsp. *savastanoi* pv. *nerii*, which causes parenchymatic galls or wartlike excrescences on *N. oleander* L. and members of various genera of the Oleaceae; and *P. syringae* subsp. *savastanoi* pv. *fraxini*, which causes wartlike excrescences on *F. excelsior* L. and *Olea europea* L. (12). Although Janse did not specifically describe the taxon, his proposal in fact created *P. syringae* subsp. *syringae* (14).

Several authors have described physiological, nutritional,

and biochemical characteristics of *P. syringae* subsp. *savastanoi* strains isolated from different hosts and compared these organisms with some pathovars of *P. syringae* subsp. *syringae* (10, 11, 16, 25).

In a numerical taxonomy study, 34 strains of *P. syringae* subsp. *savastanoi* constituted a phenon that was closely related to *P. syringae* pv. *glycinea* and *P. syringae* pv. *phaseolicola* (20).

Previously, DNA-DNA hybridization data have shown that *P. syringae* is a heterogeneous species (18).

The purpose of this work was to determine the taxonomic position of *P. syringae* subsp. *savastanoi* and related pathovars by using numerical taxonomy and DNA-DNA hybridization. Our results indicated that *P. syringae* subsp. *savastanoi* should be elevated to species level as *Pseudomonas savastanoi* sp. nov.

MATERIALS AND METHODS

Bacterial strains. Three sets of strains were used. The first set comprised 143 *P. syringae* subsp. *savastanoi* strains that were isolated from various hosts (Table 1). The second set included 50 reference strains of *P. syringae* pathovars and the type strains of *Pseudomonas cichorii*, *P. viridiflava*, and *Pseudomonas amygdali*; the origins of these strains have been published previously (7). The third set contained 35 strains of miscellaneous *Pseudomonas* spp. (Table 2), *Agrobacterium tumefaciens* CFBP 2413^T (T = type strain), and *Xanthomonas campestris* pv. *campestris* CFBP 2350. All of the bacteria were cultured routinely on YBGA (yeast extract, 7 g; Bacto Peptone, 7 g; glucose, 7 g; agar, 15 g; distilled water, 1,000 ml; pH 7.2) incubated at 25°C.

Biochemical and physiological tests. The presence of oxidase, gelatinase, and arginine dihydrolase, fluorescent pigment production, levan formation, acid production from sucrose, sorbitol, erythritol, and mannitol, reduction of nitrate, hydrolysis of Tween 80 and esculin, and the hypersensitivity reaction on tobacco leaves were tested as described by Lelliott et al. (15). Pectolytic activity was tested by using the method of Prunier and Kaiser (19) and on

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TABLE 1. Origins of the 143 *P. syringae* subsp. *savastanoi* strains tested

No. of strains	Host	Geographic origin (no. of strains)
58	Olive	Algeria (31), Tunisia (1), France (7), Portugal (1), United States (2), Yugoslavia (4), Greece (1), Italy (7), Syria (4)
33	Oleander	France (13), Algeria (4), Greece (5), Yugoslavia (1), Netherlands (1), United States (4), Italy (5)
39	Ash	France (25), Algeria (2), United Kingdom (2), United States (1), Netherlands (9)
6	Privet	Italy (6)
4	<i>Phillyrea</i> sp.	Algeria (4)
3	Jasmine	Greece (3)

pectate gels at pH 5 and 8 (9). Utilization of DL-lactate, L-(+)-tartrate, and D-(-)-tartrate was tested on a basal medium (NH₄H₂PO₄, 1 g; KCl, 0.2 g; MgSO₄ · 2H₂O, 0.2 g; agar, 3 g; bromothymol blue, 0.08 g; distilled water, 1,000 ml; pH 7.2) supplemented with 0.1% (wt/vol) organic acid

(sodium salt). The presence of DNase was tested on DNA agar (Diagnostics Pasteur, Marnes-la-Coquette, France).

Assimilation of 49 carbohydrates, 49 organic acids, and 49 amino acids was studied by using API 50CH, API 50AO, and API 50AA strips (BioMérieux, La Balme-les-Grottes, France) which were incubated at 24°C and examined for growth after 6 days.

Numerical analysis. A total of 167 characters were included in the numerical taxonomy analysis. The distance matrix was calculated by using the Jaccard coefficient (23). Cluster analysis was done by using the unweighted pair group method with averages (23).

DNA extraction. Previously described methods were used to extract and purify DNA (1).

DNA-DNA hybridization. Native DNAs were labeled in vitro by nick translation with tritium-labeled nucleotides (Amersham International, Amersham, England). The procedure used for the hybridization experiments (S1 nuclease-trichloroacetic acid method) has been described previously (3, 8). The reassociation temperature was 60°C. DNA-DNA hybridization tests were carried out by using labeled DNAs from *P. syringae* pv. *syringae* CFBP 1392^T and *P. syringae* subsp. *savastanoi* CFBP 1670^T.

Thermal stability of reassociated DNAs. The temperature at which 50% of reassociated DNA became hydrolyzable by the S1 enzyme (*T_m*) was determined by using the method of

TABLE 2. Origins of 33 fluorescent and nonfluorescent oxidase-positive *Pseudomonas* strains and *A. tumefaciens* and *X. campestris* pv. *campestris* strains

Taxon	CFBP no.	Host or origin and place and year of isolation
<i>Pseudomonas aeruginosa</i>	2466 ^T	Host, origin, and year unknown
<i>P. alcaligenes</i>	2437 ^T	Water, origin and year unknown
<i>P. caryophylli</i>	2429 ^T	<i>Dianthus caryophyllus</i> , United States, 1951
<i>P. cepacia</i>	2227 ^T	<i>Allium cepa</i> , United States, year unknown
<i>P. cepacia</i>	2234	Hospital, France, year unknown
<i>P. corrugata</i>	2431 ^T	<i>Lycopersicon esculentum</i> , United Kingdom, 1972
<i>P. corrugata</i>	145.41	<i>Lycopersicon esculentum</i> , France, 1982
<i>P. corrugata</i>	30.5	<i>Lycopersicon esculentum</i> , France, 1984
<i>P. corrugata</i>	22.4	<i>Lycopersicon esculentum</i> , France, 1983
<i>P. fluorescens</i>	2102 ^T	Water, United Kingdom, 1951
<i>P. fluorescens</i>	2123	Water, Netherlands, 1966
<i>P. fluorescens</i>	2125	Host, origin, and year unknown
<i>P. fluorescens</i>	2127	Egg, United States, year unknown
<i>P. fluorescens</i>	2129	Water, origin and year unknown
<i>P. fluorescens</i>	2130	Water, origin and year unknown
<i>P. fluorescens</i>	2299	<i>Beta vulgaris</i> (root), France, 1983
<i>P. fuscovaginae</i>	2065 ^T	<i>Oryza sativa</i> , Japan, 1976
<i>P. gladioli</i> pv. <i>gladioli</i>	2427 ^T	<i>Gladiolus</i> sp., origin unknown, 1966
<i>P. gladioli</i> pv. <i>alliicola</i>	2422	<i>Allium cepa</i> , United States, 1939
<i>P. marginalis</i> pv. <i>marginalis</i>	1387 ^T	<i>Cichorium intybus</i> , United States, 1949
<i>P. marginalis</i> pv. <i>pastinacae</i>	2038 ^T	<i>Pastinaca sativa</i> , United States, 1959
<i>P. marginalis</i> pv. <i>alfalfae</i>	2039 ^T	<i>Medicago sativa</i> , United States, 1971
<i>P. putida</i>	2066 ^T	Soil, United States, year unknown
<i>P. putida</i>	2298	<i>Malus sylvestris</i> (root), France, 1983
<i>P. pseudoalcaligenes</i>	2435 ^T	Sinus drainage, origin unknown, 1966
<i>P. rubrilineans</i>	1294	<i>Saccharum officinale</i> , Réunion, 1970
<i>P. rubrisubalbicans</i>	1296	<i>Saccharum officinale</i> , Réunion, 1970
<i>P. solanacearum</i>	2047 ^T	<i>Lycopersicon esculentum</i> , United States, 1953
<i>P. solanacearum</i> (race 3)	1420	<i>Solanum phureja</i> , Colombia, 1965
<i>P. solanacearum</i> (race 2)	1482	<i>Musa</i> sp., Panama, 1958
<i>P. stutzeri</i>	2443 ^T	Spinal fluid, United States, 1970
<i>P. tolaasii</i>	2068 ^T	<i>Agaricus bisporus</i> , United Kingdom, 1965
<i>P. tolaasii</i>	2152	<i>Agaricus bisporus</i> , France, 1975
<i>A. tumefaciens</i>	2413 ^T	Host plant and origin unknown, 1972
<i>X. campestris</i> pv. <i>campestris</i>	2350	<i>Brassica oleracea</i> , United Kingdom, 1957

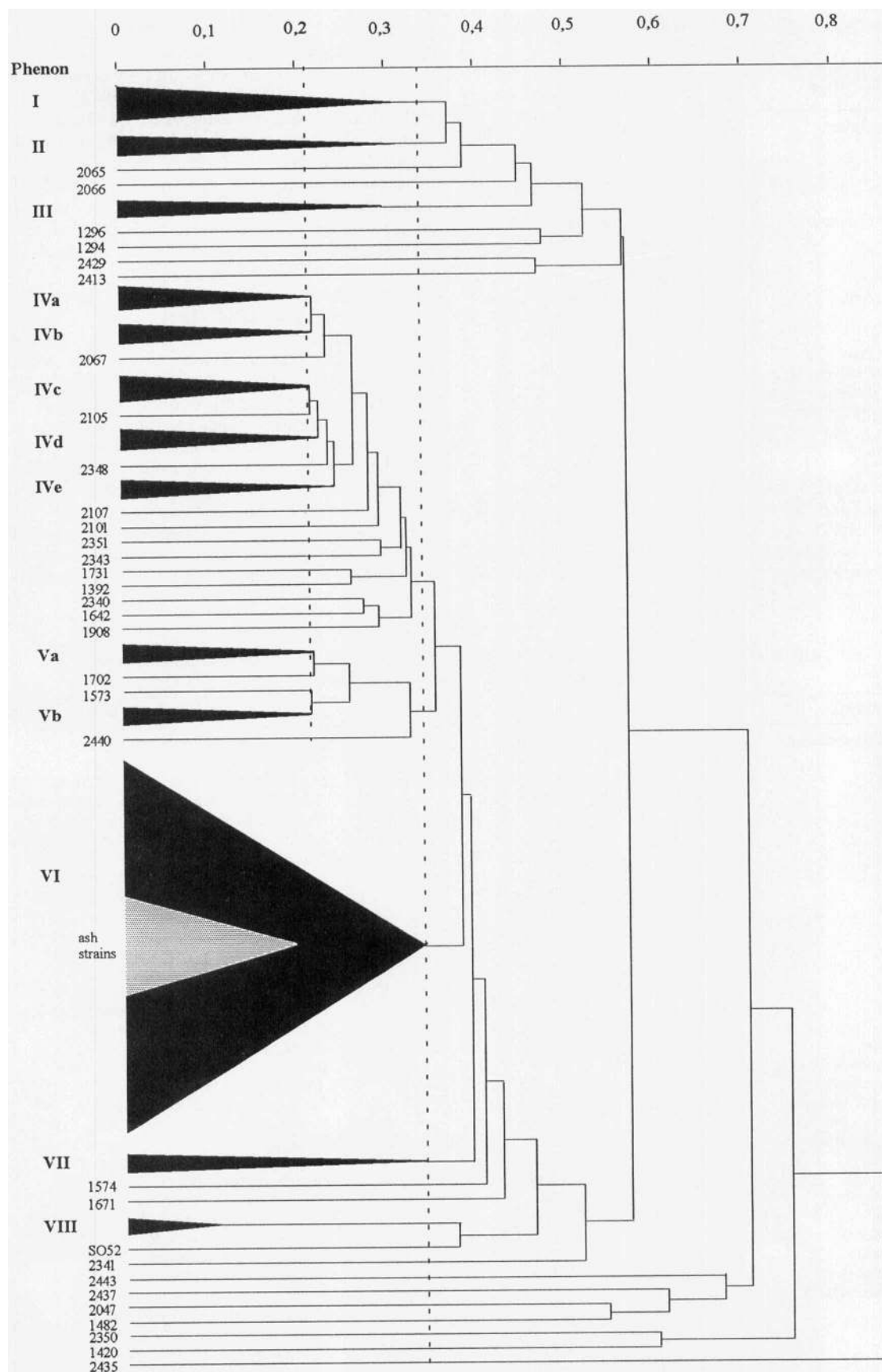


FIG. 1. Dendrogram of the distances among 231 strains of *P. savastanoi*, *P. syringae* sensu lato, and related *Pseudomonas* spp.

TABLE 3. Distribution of species and pathovars among eight phenons

Phenon or group	Subphenon	No. of strains	Strain(s) or no. of strains
I		15	<i>P. corrugata</i> CFBP 2431 ^T , CFBP 145.41, CFBP 30.5, and CFBP 22.4, <i>P. tolaasii</i> CFBP 2068 ^T and CFBP 2152, <i>P. fluorescens</i> CFBP 2299, CFBP 2129, CFBP 2102 ^T , CFBP 2123, and CFBP 2125, <i>P. marginalis</i> pv. <i>pastinacea</i> CFBP 2038 ^T , <i>P. marginalis</i> pv. <i>marginalis</i> CFBP 1387 ^T , <i>P. marginalis</i> pv. <i>alfalfae</i> CFBP 2039 ^T , <i>P. putida</i> CFBP 2298
II		3	<i>P. fluorescens</i> CFBP 2127 and CFBP 2130, <i>P. aeruginosa</i> CFBP 2466 ^T
III		4	<i>P. gladioli</i> pv. <i>gladioli</i> CFBP 2427 ^T , <i>P. gladioli</i> pv. <i>alliicola</i> CFBP 2422, <i>P. cepacia</i> CFBP 2227 ^T , and CFBP 2234
IV	IVa	3	<i>P. syringae</i> pv. <i>photiniae</i> CFBP 2899, <i>P. syringae</i> pv. <i>myricae</i> CFBP 2897, <i>P. syringae</i> pv. <i>aesculi</i> CFBP 2894
	IVb	2	<i>P. syringae</i> pv. <i>thea</i> CFBP 2353, <i>P. syringae</i> pv. <i>tagetis</i> CFBP 1694,
	IVc	11	<i>P. syringae</i> pv. <i>japonica</i> CFBP 2896, <i>P. syringae</i> pv. <i>hibisci</i> CFBP 2895, <i>P. syringae</i> pv. <i>mellea</i> CFBP 2344, <i>P. syringae</i> pv. <i>tabaci</i> CFBP 2106, <i>P. syringae</i> pv. <i>dysoxylis</i> CFBP 2356, <i>P. syringae</i> pv. <i>aptata</i> CFBP 1617, <i>P. syringae</i> pv. <i>panici</i> CFBP 2345, <i>P. syringae</i> pv. <i>papulans</i> CFBP 1754, <i>P. syringae</i> pv. <i>atrofaciens</i> CFBP 2213, <i>P. syringae</i> pv. <i>primulae</i> CFBP 1660, <i>P. syringae</i> pv. <i>aceris</i> CFBP 2339
	IVd	3	<i>P. syringae</i> pv. <i>tomato</i> CFBP 2212, <i>P. syringae</i> pv. <i>maculicola</i> CFBP 1657, <i>P. syringae</i> pv. <i>apii</i> CFBP 2103
	IVe	4	<i>P. syringae</i> pv. <i>passiflorae</i> CFBP 2346, <i>P. syringae</i> pv. <i>delphinii</i> CFBP 2215, <i>P. syringae</i> pv. <i>coronafaciens</i> CFBP 2216, <i>P. syringae</i> pv. <i>striaefaciens</i> CFBP 1674
		12	Isolated phenotypes of <i>P. syringae</i> pv. <i>helianthi</i> CFBP 2067, <i>P. syringae</i> pv. <i>psidi</i> CFBP 2105, <i>P. syringae</i> pv. <i>ribicola</i> CFBP 2348; <i>P. syringae</i> pv. <i>viridiflava</i> CFBP 2107, <i>P. syringae</i> pv. <i>cichorii</i> CFBP 2101, <i>P. syringae</i> pv. <i>morsprunorum</i> CFBP 2351, <i>P. syringae</i> pv. <i>erobotryae</i> CFBP 2343, <i>P. syringae</i> pv. <i>lapsa</i> CFBP 1731, <i>P. syringae</i> pv. <i>syringae</i> CFBP 1392, <i>P. syringae</i> pv. <i>atropurpurea</i> CFBP 2340, <i>P. syringae</i> pv. <i>mori</i> CFBP 1642, <i>P. syringae</i> pv. <i>porri</i> CFBP 1908
V	Va	4	<i>P. syringae</i> pv. <i>philadelphia</i> CFBP 2898, <i>P. syringae</i> pv. <i>berberidis</i> CFBP 1727, <i>P. syringae</i> pv. <i>anthrithini</i> CFBP 1620, <i>P. syringae</i> pv. <i>viburni</i> CFBP 1702
	Vb	4	<i>P. syringae</i> pv. <i>persicae</i> CFBP 1573, W24-1, W24-2, and W24-3
VI		1	Isolated phenotype of <i>P. syringae</i> pv. <i>lacrymans</i> CFBP 2440
VII		142	<i>P. syringae</i> subsp. <i>savastanoi</i> (57 strains), strains from oleander (33 strains), ash (39 strains), privet (6 strains), <i>Phillyrea</i> sp. (4 strains), and jasmine (3 strains)
VIII		3	<i>P. syringae</i> pv. <i>glycinea</i> CFBP 2214, <i>P. syringae</i> pv. <i>phaseolicola</i> CFBP 1390, <i>P. syringae</i> pv. <i>ulmi</i> CFBP 1407
VIII		2	<i>P. amygdali</i> CFBP 2354 and CFBP W28-1
Isolated phenotypes		17	<i>P. fuscovaginae</i> CFBP 2065 ^T , <i>P. putida</i> CFBP 2066 ^T , <i>P. rubisubalbicans</i> CFBP 1296, <i>P. rubrilineans</i> CFBP 1294, <i>P. caryophylli</i> CFBP 2429 ^T , <i>A. tumefaciens</i> CFBP 2413 ^T , <i>P. syringae</i> pv. <i>syringae</i> CFBP 1574, <i>P. syringae</i> pv. <i>sesami</i> CFBP 1671, <i>P. syringae</i> subsp. <i>savastanoi</i> SO52, <i>P. syringae</i> pv. <i>cannabina</i> CFBP 2341, <i>P. stutzeri</i> CFBP 2443 ^T , <i>P. alcaligenes</i> CFBP 2437 ^T , <i>P. solanacearum</i> CFBP 2047 ^T , CFBP 1482, and CFBP 1420, <i>X. campestris</i> pv. <i>campestris</i> CFBP 2350, <i>P. pseudoalcaligenes</i> CFBP 2435 ^T

Crosa et al. (3). The ΔT_m was the difference between the T_m of the heteroduplex and the T_m of the homoduplex.

RESULTS

Numerical analysis. A dendrogram displaying the distance relationships among the 231 strains which we studied is shown in Fig. 1.

At a distance of 0.346, eight phenons and 17 isolated strains were observed. The distribution of species, subspecies, and pathovars in the eight major phenons is shown in Table 3.

Phenon I and II contained fluorescent oxidase-positive *Pseudomonas* strains. Phenon III contained only nonfluorescent phytopathogenic *Pseudomonas* strains.

Phenon IV to VIII included reference strains of fluorescent and nonfluorescent (*P. amygdali*) phytopathogenic *Pseudomonas* spp. that were either oxidase negative or oxidase positive (*P. cichorii*), corresponding to groups I through III of Lelliott et al. (15).

Phenon IV was subdivided into five subphenons (subphenons

IVa to IVe), and phenon V was subdivided into subphenons Va and Vb.

Phenon VI contained *P. syringae* subsp. *savastanoi* strains. All but one strain of *P. syringae* subsp. *savastanoi* fell into this phenon. All 39 strains isolated from ash were clustered in one subphenon. The strains isolated from olive and oleander did not constitute a subphenon within phenon VI.

Only 4 of the 17 unclustered strains were identified as *P. syringae* strains (*P. syringae* subsp. *savastanoi* SO52, *P. syringae* pv. *cannabina* CFBP 2341, *P. syringae* pv. *syringae* CFBP 1574, and *P. syringae* pv. *sesami* CFBP 1671) (Table 3).

Phenon I to III were clearly differentiated by biochemical tests from phenons IV to VIII. Strains in phenons IV to VIII could not utilize *N*-acetylglucosamine, acetate, *N*-valerate, arginine, spermine, tyrosine, 2-ketogluconate, ethanolamine, L-tryptophan, L-ornithine, DL-kynurenine, isobutyrate, citraconate, itaconate, mesaconate, levulinate, and

TABLE 4. Characteristics that differentiate phenon IV to VIII

Phenon or subphenon	Utilization of:										Levan production	Fluorescence
	meso-Tartrate	DL-Hydroxybutyrate	Erythritol	DNase	Esculin	L-(+)-Tartrate	DL-Lactate	Glucosamine	β -Alanine	D-(-)-Tartrate		
IVa	+	-	-	+	-	+	-	-	-	-	+	+
IVb	+	d	-	-	-	d	-	+	+	-	+	+
IVc	+	+	+	-	+	d	d	d	-	d	+	+
IVd	+	+	-	d	-	-	+	-	-	+	+	+
IVe	+	-	+	-	+	-	-	-	-	-	+	+
Va	+	-	-	d	d	-	-	-	-	+	+	+
Vb	-	d	-	+	-	-	-	-	-	+	+	+
VI	-	-	-	+	-	+	-	-	-	-	-	+
VII	d	-	d	d	-	+	-	-	-	d	+	+
VIII	-	-	-	-	-	-	-	-	-	-	+	-

^a +, 90 to 100% of the strains are positive; -, 0 to 10% of the strains are positive; d, 11 to 89% of the strains are positive.

p-aminobenzoate and could not reduce nitrate. All of the strains in phenon I to III gave the opposite reactions.

Characteristics that differentiate phenon IV to VIII are shown in Table 4. Identification of a strain as a phenon IV or V strain requires identification at the subphenon level (Table 4). The reactions exhibited by the phenon VI strains differed somewhat when the source of isolation was considered (Table 5).

DNA relatedness. Results of DNA relatedness experiments are shown in Table 6. *P. syringae* subsp. *savastanoi* strains isolated from six hosts were 75 to 100% related to type strain CFBP 1670. For the two lowest relatedness values (75 and 79%), the ΔT_m values were 1.5 and 3.0°C, respectively. The reference strains of *P. syringae* pv. *glycinea* and *P. syringae* pv. *phaseolicola* were 72 and 83% related to strain CFBP 1670^T, respectively. The ΔT_m value calculated for *P. syringae* pv. *glycinea* and *P. syringae* subsp. *savastanoi* hybridized DNAs was 2.5°C. Thus, *P. syringae* subsp. *savastanoi*, *P. syringae* pv. *glycinea*, and *P. syringae* pv. *phaseolicola* are members of a single DNA hybridization group.

The type strain of *P. syringae* pv. *syringae*, strain CFBP 1392, was 43% related to strain CFBP 1670^T when DNA from strain CFBP 1670^T was labeled; the level of relatedness was 54% when DNA from strain CFBP 1392^T was labeled. *P. syringae* pv. *persicae*, *P. syringae* pv. *tomato*, and the other *Pseudomonas* species which we studied were less than 51% related to either *P. syringae* subsp. *savastanoi* CFBP 1670^T or *P. syringae* pv. *syringae* CFBP 1392^T; thus, these strains are members of DNA groups other than the *P. syringae* pv. *syringae* or *P. syringae* subsp. *savastanoi* DNA group. In this study the *P. syringae* pv. *syringae* DNA group was represented only by the type strain.

DISCUSSION

Before 1978, a *Pseudomonas* strain that was isolated for the first time from a new diseased host was considered a species. Young et al. (27) and Dye et al. (5) considered the named, phytopathogenic, oxidase-negative fluorescent, *Pseudomonas* spp. to be pathovars of *P. syringae*. At the present time, 45 pathovars are recognized (5, 26).

Janse (11) found that strains of *P. syringae* pv. *savastanoi* that were isolated from different hosts had nearly identical biochemical and physiological characteristics. Variations were observed only in the production of levan, in the hydrolysis of pectate, and in the production of indoleacetic acid and cytokininlike compounds. Janse also observed a marked variation in the results of pathogenicity tests among the strains that were isolated from different hosts. For these reasons he named this group of strains *P. syringae* subsp. *savastanoi* and proposed the following three pathovars: *P. syringae* subsp. *savastanoi* pv. *oleae*, *P. syringae* subsp. *savastanoi* pv. *nerii*, and *P. syringae* subsp. *savastanoi* pv. *fraxini* (12, 17).

Cross-pathogenicity is currently being tested by one of us (L.G.) to determine whether pathogenicity characteristics match taxonomic grouping. Pathogenicity of olive strains on oleander has not been demonstrated (6a, 11). All strains of *P. syringae* subsp. *savastanoi* are pathogenic on ash. Ash strains are pathogenic only on ash, and 80% of oleander strains are pathogenic on olive. However, cross-pathogenicity data for different hosts are not complete.

On the basis of DNA-DNA hybridization, physiological and biochemical characteristics, we propose that *P. syringae* subsp. *savastanoi* should be elevated to species level as

TABLE 5. Reactions of phenon VI strains (*P. syringae* subsp. *savastanoi*) isolated from different hosts

Host	No. of isolates	% of strains positive										Hydrolysis of polypectate (pH 5)	Levan production
		Utilization of:											
		L-Serine	<i>n</i> -Caproate	DL-5-Amino-valerate	L-Leucine	Raffinose	2-Amino-benzoate	L-Tyrosine	D-Xylose	Trigonelline	L-Arabinose		
Olive	58	100	12	0	50	0	0	0	45	50	37	13	0
Ash	39	21	82	53	92	23	13	18	92	0	95	100	21
Oleander	33	100	18	0	76	0	0	0	3	0	79	97	0
Jasmine	3	100	66	0	100	0	0	0	0	0	100	100	0
<i>Phillyrea</i> sp.	4	100	0	0	100	0	0	0	0	0	100	100	0
Privet	6	100	0	0	16	0	0	0	0	0	100	16	16

TABLE 6. Levels of DNA relatedness among *Pseudomonas* strains

Taxon	Strain (host)	% of relative binding at 60°C with labeled DNA from:	
		<i>P. syringae</i> CFBP 1392 ^T	<i>P. savastanoi</i> CFBP 1670 ^T
<i>P. savastanoi</i> pv. <i>savastanoi</i> (phenon VI)	CFBP 1670 ^T (olive)	54	100
	K124-4 (olive)	53	82
	T35-1 (olive)	49	87
	T12-6 (olive)	64	83
	K23-15 (olive)	49	93
	CFBP 2088 (oleander)	58	79 (3) ^a
	L145-2 (oleander)	52	88
	T37-6 (oleander)	49	90
	L36-7 (oleander)	50	84
	L86-1 (oleander)	53	81
	CFBP 1838 (ash)	58	86
	CFBP 2093 (ash)	58	97
	T36-3 (ash)	55	92
	T5-1 (ash)	47	75 (1.5)
	T12-7 (jasmine)	60	92
CFBP 1751 (jasmine)	53	88	
T12-10 (jasmine)	54	83	
Phi (<i>Phillyrea</i> sp.)	54	100	
T51-3 (<i>Phillyrea</i> sp.)	62	93	
T51-1 (<i>Phillyrea</i> sp.)	50	81	
T37-11 (privet)	59	86	
T35-10 (privet)	57	86	
T37-15 (privet)	53	90	
<i>P. savastanoi</i> pv. <i>phaseolicola</i> (phenon VII)	CFBP 1390 ^T	56	83
<i>P. savastanoi</i> pv. <i>glycinea</i> (phenon VII)	CFBP 2214 ^T	50	72 (2.5)
<i>P. syringae</i> pv. <i>syringae</i> (isolated phenotype)	CFBP 1392 ^T	100	43
<i>P. syringae</i> pv. <i>persicae</i> (phenon IVb)	CFBP 1573 ^T	46	41
<i>P. syringae</i> pv. <i>tomato</i> (phenon IVd)	CFBP 2212 ^T	51	47
<i>P. viridiflava</i> (isolated phenotype)	CFBP 2107 ^T	49	44
<i>P. cichorii</i> (isolated phenotype)	CFBP 2101 ^T	25	24
<i>P. marginalis</i> (phenon I)	CFBP 1387 ^T	21	22
<i>P. fluorescens</i> (phenon II)	CFBP 2102 ^T	20	21
<i>P. putida</i> (phenon I)	CFBP 2066 ^T	9	10

^a The values in parentheses are ΔT_m values (in degrees Celsius).

Pseudomonas savastanoi and that this species should include three pathovars, *P. savastanoi* pv. *savastanoi*, *P. savastanoi* pv. *glycinea*, and *P. savastanoi* pv. *phaseolicola*.

Description of *Pseudomonas savastanoi* (Smith). *Pseudomonas savastanoi* (sa.vas.ta'no.i. L.gen.n. *savastanoi* of Savastano, the first worker who studied olive knot disease). Gram-negative rods that are 0.4 to 0.8 by 1.0 to 3.0 μ m and motile by means of one to four polar flagella. Rather slow growing. Colonies are white or cream, smooth, flat, and glistening with entire or erose margins on YBGA. Produces a hypersensitive reaction on tobacco leaves. Metabolism is respiratory. Oxidase negative. Nitrates are not reduced. Blue fluorescent pigment is produced under UV light on King B medium. Arginine test (Thornley) negative. Esculin, gelatin, and starch are not hydrolyzed. Assimilates sucrose, L-arabinose, gluconate, caprylate, fumarate, DL-glycerate, L-malate, pyruvate, citrate, D- α -alanine, and L-proline. Does not assimilate lactose, L-xylose, adonitol, 2-aminobutyrate, DL-lactate, DL-3-hydroxybutyrate, D(-)-tartrate, L-cysteine, L-methionine, and L-valine. Strains isolated from members of various genera of the Oleaceae and *N. oleander* do not produce levan, whereas strains isolated from *F. excelsior*

L. (21%), *Phaseolus vulgaris*, and *Glycine max* L. do produce levan. The G+C content of the DNA is 60 mol% (4).

P. savastanoi pv. *savastanoi* causes knots, galls, and cankers on members of the various genera of the Oleaceae and *N. oleander* L.; *P. savastanoi* pv. *glycinea* causes bacterial blight of soybean; and *P. savastanoi* pv. *phaseolicola* causes halo blight of bean.

The type strain is strain NCPPB 639 (= ATCC 13522 = ICMP 4352 = CFBP 1670).

Description of the type strain. Strain NCPPB 639^T was isolated from *O. europea* L. in Yugoslavia. This strain has physiological and biochemical characteristics that are typical of the species. In addition, it does not assimilate sorbitol and *trans*-aconitate.

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