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Pseudomonas syringae subsp. *savastanoi* (ex Smith) subsp. nov., nom. rev., the Bacterium Causing Excrescences on *Oleaceae* and *Nerium oleander* L.

J. D. JANSE

Department of Bacteriology, Plant Protection Service, 6700 HC, Wageningen, The Netherlands

From a study of the so-called bacterial canker of ash, caused by a variant of "*Pseudomonas savastanoi*" (Smith) Stevens, it became evident that this variant and the variants of "*P. savastanoi*" which cause olive knot and oleander knot can be distinguished from one another on the basis of their pathogenicity and host range. All isolates of "*P. savastanoi*" were recently classified by Dye et al. (Plant Pathol. **59**:153–168, 1980) as members of a single pathovar of *P. syringae* van Hall. It appears, however, that these isolates differ sufficiently from the other members of *P. syringae* to justify subspecies rank for them. The following classification and nomenclature are therefore proposed: *Pseudomonas syringae* subsp. *savastanoi* (ex Smith) subsp. nov., nom. rev., to include the olive pathogen (pathovar *oleae*), the ash pathogen (pathovar *fraxini*), and the oleander pathogen (pathovar *nerii*). The type strain of *P. syringae* subsp. *savastanoi* is ATCC 13522 (= NCPPB 639).

"*Pseudomonas savastanoi*" (Smith 1908) Stevens 1913 was previously used as the name of the bacterium which causes pernicious excrescences on several species of the *Oleaceae*. (Names in quotation marks are not on the Approved Lists of Bacterial Names [12], have not been validly published since 1 January 1980, and therefore do not have standing in bacterial nomenclature.) This bacterium was first adequately described from *Olea europea* L. by Smith (15), who named it "*Bacterium savastanoi.*" A variant from the common ash (*Fraxinus excelsior* L.) was then described by Brown in 1932 (2) under the name "*B. savastanoi* var. *fraxini*"; it was later named "*P. savastanoi* subsp. *fraxini*" by Dowson in 1943 (5). The variant from *Nerium oleander* L. (*Apocynaceae*), which was first described by Ferraris (7) in 1926 as "*P. tonelliana,*" was described more adequately by Smith (14), who renamed it "*P. savastanoi* subsp. *nerii*" in 1928.

My recent studies of the so-called bacterial canker of common ash (7a, 7b) have yielded biochemical, serological, and pathological data that cast additional light on the nature of the isolates obtained from ash, olive, oleander, privet, and jasmin.

In their proposed nomenclature of the plant-pathogenic fluorescent pseudomonads, Young et al. (23) did not distinguish among the different variants, and they classified all isolates of "*P. savastanoi*" as members of *P. syringae* pathovar *savastanoi*. Their interpretation of the term pathovar, however, is in this case not in agreement with the definition of the term as recommended in the *International Code of Nomenclature of Bacteria* (8).

According to the available data, the present classification and nomenclature of the organisms under discussion are inadequate. The purpose of this paper is to rectify this situation.

MATERIALS AND METHODS

Bacterial strains. The strains studied are listed in Table 1.

Methods. Descriptions of the methods employed are reported elsewhere (7b).

RESULTS

Over 40 biochemical and physiological features were determined for the strains. All "*P. savastanoi*" strains had nearly the same characteristics. Significant variances were found only in the production of levan and in the hydrolysis of pectate (not correlated to host plant or pathogenicity) and in the production of indolyl-acetic acid and cytokinin-like compounds. The latter two substances are not produced (or are produced only in very small amounts) by isolates from ash, whereas they are produced by isolates from other host plants. The tests which appear to be useful in differentiating between "*P. savastanoi*" and *P. syringae* are listed in Table 2. For differential characters, also see Sands et al. (11).

All of the "*P. savastanoi*" strains produced similar titers with an antiserum prepared against strain NCPPB 639 (from *Olea europea*). The antiserum was absorbed with a cross-reacting *P. syringae* strain (NCPPB 191). After cross-ab-

TABLE 1. Strains used in this study

Strain[a]	Origin	
	Host	Country
"P. savastanoi"		
PD 109	*F. excelsior* L.	Netherlands
PD 116	*F. excelsior* L.	Netherlands
PD 119	*F. excelsior* L.	Netherlands
PD 120	*F. excelsior* L.	Netherlands
PD 159	*F. excelsior* L.	Netherlands
PD 160	*F. excelsior* L.	Netherlands
PD 161	*F. excelsior* L.	Netherlands
PD 179	*F. excelsior* L.	Netherlands
PD 206	*F. excelsior* L.	Netherlands
NCPPB 1464	*F. excelsior* L.	U.K.
NCPPB 1006	*F. excelsior* L.	U.K.
CNBP 1838	*F. excelsior* L.	France
NCPPB 639	*O. europea* L.	Yugoslavia
NCPPB 2327	*O. europea* L.	Italy
NCPPB 640	*N. oleander* L.	Yugoslavia
PD 181	*N. oleander* L.	Spain
NCPPB 2328	*Ligustrum japonicum* Thbg.	Italy
CNBP 1751	*Jasminum* sp.	Greece
P. syringae		
NCPPB 281	*Syringa vulgaris* L.	U.K.
NCPPB 191	*Persea americana* Mill.	Israel
NCPPB 981	*Populus canadensis* Mnch. 'Eugenei'	U.K.
"P. mors-prunorum"		
NCPPB 560	Host unknown	U.K.
"P. maculicola"		
IPO 154	*Brassica oleracea* L.	Netherlands
Saprophytic fluorescent pseudomonads		
PD 117	*F. excelsior* L.	Netherlands
NCPPB 1465[b]	*F. excelsior* L.	U.K.

[a] The strains are maintained under these names in the collections referred to as follows: PD, Culture Collection of the Plant Protection Service, Wageningen, The Netherlands; NCPPB, National Collection of Plant-Pathogenic Bacteria, Harpenden, U.K.; CNBP, Collection Nationale de Bactéries Phytopathogènes, Angers, France; IPO, Culture Collection of the Research Institute for Plant Protection, Wageningen, The Netherlands.

[b] NCPPB 1465 was described as a deviating pathogenic strain of *"P. savastanoi* subsp. *fraxini"* by Šutić and Dowson (19). In my hands it deviated morphologically, biochemically, and serologically from *P. syringae* subsp. *savastanoi* and was found to be nonpathogenic on four different hosts. It should therefore be regarded as a saprophytic pseudomonad.

sorption, only the *"P. maculicola"* strain showed close antigenic relationship to *"P. savastanoi"* strains.

No significant morphological differences were found between the strains of *"P. savastanoi,"* and only small differences were observed between these strains and the other phytopathogenic pseudomonads tested.

The results of the pathogenicity tests are presented in Table 3. The host plants used were *Fraxinus excelsior* L., *Olea europea* L., *Nerium oleander* L., and *Forsythia intermedia* Zab.

DISCUSSION

From my previous studies (7b) and those of others (2, 11, 18, 19, 21, 22), it has become apparent that *"P. savastanoi"* isolates from different hosts are almost indistinguishable morphologically, biochemically, and serologically. Only the production of indolyl-acetic acid and cytokinin-like compounds differentiated between the isolates from ash and those from other host plants. These substances are most likely related to pathogenicity, as will be discussed below.

However, the isolates from these different host plants show different pathogenicities and host ranges (4, 7b, 14, 18, 20). The isolates from ash can be clearly distinguished from those of other hosts by a deviating pathogenicity (18; Janse, in press). They evoke wartlike necrotic bark swellings with abundant periderm instead of large, parenchymatous galls. This can possibly be explained by the restricted production of growth substances by isolates from ash. The other isolates of *"P. savastanoi"* were found to produce these substances in rather large amounts in vitro, and elevated levels were also

TABLE 2. Biochemical tests useful in differentiating *P. syringae* subsp. *savastanoi* from *P. syringae* subsp. *syringae*[a]

Tests	*P. syringae* subsp. *savastanoi*	*P. syringae* subsp. *syringae*
Hydrolysis of:		
Gelatin	−	+
Esculin	−	+
Arbutin	− or weak	+
Casein	− or weak	+
Acid from:		
D-(+)-Raffinose	−	+
Erythritol	−	+
Alkali from:		
L-(+)-Tartrate	+	−

[a] Also see Sands et al. (11).

found in their galls (1, 17, 22). The host range of the isolates from ash is limited to the *Oleaceae*.

The isolates from olive differ from the isolates from ash by producing large galls instead of necrotic bark swellings; they differ from isolates from oleander in host range (Table 3). Isolates from olive usually do not infect *N. oleander*, and it was for this reason that the oleander organism was originally described as a separate species, *"P. tonelliana"* (7). Although on two occasions (10, 22) strains from olive have been reported to infect *N. oleander*, this is generally not the case.

The isolates from oleander form galls and can therefore be distinguished from isolates from ash; they differ from isolates from olive in host range (Table 3).

On the basis of these facts, it is concluded that the bacterial isolates causing excrescences on ash, olive, and oleander must be ranked separately at the level of pathovar as defined in the *Bacteriological Code* (8) and not as interpreted by Dye et al. (6). Determination of the status of the jasmin and privet isolates requires a more comprehensive host-range study.

Isolates of *"P. savastanoi"* were recently named *P. syringae* pathovar *savastanoi* (6, 23) as a result of investigations (e.g., 3, 9, 11) which have shown that *"P. savastanoi"* is closely related to *P. syringae* van Hall. However, isolates belonging to *"P. savastanoi"* can be readily distinguished biochemically, serologically, and pathologically from *P. syringae* (Table 2) and its subgroups (11); they should therefore be considered at the subspecies level. As the epithet *"savastanoi"* has had no standing in bacterial nomenclature since 1 January 1980 (12), it is here revived for bacterial pathogens causing excrescences on *Oleaceae* and *N. oleander* L.

Description of *Pseudomonas syringae* subsp. *savastanoi* (ex Smith 1908) subsp. nov., nom. rev. (sa·vas·ta'no·i· M.L. gen. noun *savastanoi* of Savastano, named for L. Savastano, the first to study olive knot).

Gram-negative, nonsporeforming rods with rounded ends, 0.3 to 0.7 by 1.0 to 1.8 μm, occurring singly or in pairs; motile by means of one to five polar flagella. Rather slow-growing, gray-white, smooth, glistening, raised and circular or slightly irregular to undulate colonies are produced on nutrient agar; levan negative or levan positive on nutrient-sucrose (5%) agar; produces a weak, blue-green fluorescent, diffusible pigment on King's B medium; some strains produce a brown diffusible pigment. Metabolism is respiratory. Oxidase negative, catalase positive. Acid is produced from D-(+)-galactose, glucose, D-(+)-ribose, sucrose (slow), D-(+)-xylose, and mannitol; no acid is produced from maltose, D-(+)-raffinose, erythritol, or salicin; alkali is produced from L-(+)-tartrate; esculin, arginine, gelatin, and starch are not hydrolyzed; generally, arbutin and casein are not hydrolyzed. Nitrates are not reduced. H_2S is not produced from cysteine. No growth occurs in nutrient broth at 37°C or with 5% NaCl. Hypersensitivity is produced in tobacco leaves. The deoxyribonucleic acid contains 60 mol% guanine plus cytosine (3). Causes galls and wartlike excrescences on various species of *Oleaceae* and *N. oleander* L. Gall-forming isolates produce indolyl-acetic acid and cytokinin-like substances in vitro.

The type strain of this subspecies is ATCC 13522 (= NCPPB 639). This strain was isolated by D. Šutić from *Olea europea* in Yugoslavia. Its description is identical to that of the subspecies, but with the following modifications: levan negative on nutrient-sucrose (5%) agar; casein hydrolysis is weak; produces galls on *O. europea* L. and *F. excelsior* L.

The following pathovars of *P. syringae* subsp. *savastanoi* are recognized: pathovar *oleae*, causing parenchymatic galls on various species of the *Oleaceae* (15, 16); pathovar *nerii*, causing parenchymatic galls or wartlike excrescences on *N. oleander* L. and various species of the *Olea-*

TABLE 3. Results of pathogenicity tests with isolates of *P. syringae* subsp. *savastanoi* on different host plants

Isolate(s) from:	Pathogenicity[a] on:			
	Ash	Olive	Oleander	Forsythia
Ash	+	+	−	−
Olive	⊕	⊕	−	−
Oleander	⊕	⊕	⊕	−
Jasmin	−	⊕	−	⊕
Privet	⊕	⊕	−	−

[a] Symbols: +, Necrotic swellings; ⊕, parenchymatous galls; −, no pathogenic reaction.

ceae (14); and pathovar *fraxini*, causing wartlike excrescences on *F. excelsior* L. and *O. europea* L. (2, 5, 13).

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REPRINT REQUESTS

Address reprint requests to: J. D. Janse, Department of Bacteriology, Plant Protection Service, Geertjesweg 15, Postbus 9102, 6700 HC, Wageningen, The Netherlands.

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