European Journal of Plant Pathology **104:** 603–609, 1998.

Phenotypic variability in different strains of *Pseudomonas syringae* subsp. *savastanoi* isolated from different hosts

F. Alvarez, J.E. García de los Ríos, P. Jimenez, A. Rojas, P. Reche and M.T. Troya
Area de Fitopatología. Fac. CC. Experimentales y Técnicas, Univ. San Pablo CEU, Crtra. Boadilla del Monte Km. 5,3. Boadilla del Monte, 28660 Madrid, Spain

Accepted 25 May 1998

Key words: heterogeneity, *Pseudomonas syringae*, *Retama sphaerocarpa*

Abstract

One hundred and sixty strains of *Pseudomonas syringae* subsp. *savastanoi* from *Olea europaea*, *Olea europaea* var. *sylvestris*, *Nerium oleander*, *Fraxinus angustifolia* and *Retama sphaerocarpa*, and four type strains of other pathovars were studied, investigating 102 phenotypic traits, among which we include biochemical characteristics, assimilation of different carbon sources, sensitivity or resistance to antibiotics and indoleacetic acid (IAA) production. Results were analysed with an affinity dendrogram via the Jaccard coefficient. They indicate an influence of environmental factors on the formation of the 15 phenons obtained, since isolated (knot) strains from the same species but different geographical areas are segregated. Segregation, also detected in strains from different hosts within the same area, added to the pathogenicity test helps to characterise these strains as different pathovars.

Introduction

Pseudomonas syringae subsp. *savastanoi* Janse 1982 (Janse, 1982) is a pathogenic bacteria of *Olea europaea* L. (Smith, 1908; Young et al., 1978), *Retama sphaerocarpa* (Boiss.) L. (García de los Ríos, 1989), *Nerium oleander* L. (Wilson, 1965) and *Fraxinus angustifolia* (García de los Ríos, 1989), among others. These bacteria cause hyperplasia of the affected tissues, mainly the branches, where the knots are clearly visible. The fact that these bacteria produce indoleacetic acid (IAA), which is necessary for the induction of these knots (Smith and Kosuge, 1978; Comai and Kosuge, 1982; Surico et al., 1985; Iacobellis et al., 1994), has been known for some time (Beltrá, 1959; Magie et al., 1963).

All the bacterial strains isolated from knots in these plants have traditionally been grouped in the *Pseudomonas syringae* pv. *savastanoi* (Dye et al., 1980), although controversies have arisen over important variations found in the pathological, biochemical and genetic traits, as well as incompatibilities in the cross inoculations among different plants (Sutic & Dowson, 1963; Janse, 1981; Surico et al., 1985; Caponero et al., 1995). For this reason, various authors propose a new classification of the bacteria, officially known as *Pseudomonas syringae* pv. *savastanoi* (Dye et al., 1980), as *Pseudomonas syringae* subsp. *savastanoi*, and within that subspecies, different pathovars (Janse, 1982). In any event, the ISPP Subcommittee on the Taxonomy of Plant Pathogenic Bacteria (Young et al., 1991) grouped the proposed pathovars into *Pseudomonas syringae* subsp. *savastanoi*, and maintained the nomenclature.

Recent discoveries support Janse's proposal (1982): strains from the olive and oleander are separated into homogeneous groups on the basis of their fatty acids profile (Janse, 1991; Wells et al., 1991); these two groups of strains show differences in the location of genes (chromosomic or extrachromosomic) involved in the production of IAA (Comai and Kosuge, 1980; Comai et al., 1982; Caponero et al., 1995); there are differences in the production of bacteriocins (Iacobellis et al., 1995) and differences in DNA restriction fingerprints (Mugnai et al., 1994), although these groups share the plasmidic location of the genes involved in the production of cytokinins (Mac Donald et al., 1986; Powell and Morris, 1986). However, other authors,

Table 1. List of *Pseudomonas syringae* subsp. *savastanoi* strains, host and location

Strains	Host	Geographic origin
Ps1, Ps2, Ps3, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20.	Olive (*Olea europaea)*	Raña de la Mascura (Toledo) **T1**
IF 3026	Olive	Benidorm (Alicante) **B1**
Ps4, 21, 22, 23, 24.	Wild olive (*Olea europaea* var. *sylvestris*)	Navahermosa (Toledo) **T2**
25, 26, 27, 28, 29, 30.	Olive	Villaviciosa de Odón (Madrid) **M1**
31, 32, 33, 34, 35.	Olive	Mentrida (Toledo) **T3**
PsONH1, PsO2, PsO3, PsO4, PsO5, 36, 37, 38, 39, 40.	Olive	Montepríncipe (Madrid) **M2**
PsA, PsAPEP, PsA2, PsA3, PsA4, PsA5, 41, 42, 43, 44, 45, 46, 47, 48, 49.	Oleander (*Neium oleander*)	Madrid-North **M3**
50, 51, 52, 53, 54.	Oleander	Madrid-South **M4**
55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65.	Oleander	**M2**
PsR, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, RC1, RC2, RC3, RC4, RC5.	Spanish Broom (*Retama sphaerocarpa*)	**T2**
76, 77, 78, 79, 80, 81.	Spanish Broom	**M2**
82, 83, B2, B3, B4, B5, B6, B10, B11.	Spanish Broom	Boadilla del Monte (Madrid) **M5**
1APs, 4APs, 5Ps, 6Ps, 6APs, 7Ps, 9Ps, 11Ps, 12APs, 13APs, 16APs, 17APs, 18APs, 19APs, 20APs, 21APs, 22APs.	Spanish Broom	Pozuelo de Alarcón (Madrid) **M6**
Tr9, Tr10, Tr14, Tr15, Tr18, Tr25, Tr30, Tr32, Tr33, Tr34, Tr36, Tr38, Tr39, Tr40.	Spanish Broom	Torrelaguna (Madrid) **M7**
PsF, 84, 85, 86, 87, 88, 89, 90, 91, 92.	Ash (*Fraxinus angustifolia*)	**T2**
T1, T2, T10, T11, T16, T17.	Spanish Broom	Torrelodones (Madrid) **M8**

such as Ercolani (1983), believe in the potential influence of a wide range of environmental conditions which may have an impact on the nutritional and physiological requirements of these bacteria.

The purpose of this study is to obtain additional information concerning the populations of pathogenic *Pseudomonas syringae* subsp. *savastanoi* of the olive tree, oleander, Spanish broom (*Retama sphaerocarpa*) and ash tree (*Fraxinus angustifolia*), from various locations in the center of Spain, so as to define whether the variations in phenotypic traits are due to abiotic or other factors, or whether these strains differ sufficiently even to be considered pathovars.

Materials and methods

Sampling and isolation

Sampling was performed in different areas in the center of Spain (Raña de la Mascura, Navahermosa, and Mentrida (Toledo); Villaviciosa de Odón, Montepríncipe, Madrid, Boadilla del Monte, Pozuelo de Alarcón, Torrelaguna and Torrelodones (Madrid)). The strains and their sources are listed in Table 1.

To isolate the strains, young knots about 10 mm in diameter, greenish-grey, were cut with a sterile blade in aseptic conditions. Pieces of tissue with a hydropic appearance were taken from the cut surface, plated onto King's B agar medium (KB) and incubated at 25 °C for 48 h. Single colonies of a yellowish, fluorescent bacterium consistently isolated from knot tissues were transferred onto fresh KB agar to assure purity. Specific antibodies were used to identify them.

Characterization of isolated strains

Each one of the strains isolated was examined for 102 phenotypic traits: basic biochemical test (API20 NE), carbon source assimilation (API50 CH), sensitivity and resistance to antibiotics (Bauer et al., 1966), nitrogen fixation, via the indirect technique of the reducing activity of acetylene (ARA) (Hardy et al., 1971). Ice nucleation activity, in which cells were suspended in buffer phosphate pH 7.2 and 1 ml drops deposited in sterile tubes and submitted to cooling

at the rate of 1 °C/2 min in a cryostat (Frigiterm 6000382, Selecta©), were also examined. The production of IAA was estimated with a colorimetric method (Gordon and Weber, 1951).

Pathogenetic test

The bacteria were preincubated on a solid medium for 48 h at 25 °C, followed by suspension in sterile saline solution, adjusting turbidness to 0.5 Mc Farland. Prior to inoculation, the area of the plant to be infected was washed with Benzalconium Chloride at 1/1.000, in order to eliminate the normal epiphytic flora, and the tested zone was pierced slightly, without applying pressure, with a Becton Dickinson 0.33 × 13 mm Micro-Fine insulin needle.

Different pathovars were inoculated into *Olea europaea*, *Nerium oleander*, *Retama sphaerocarpa* and *Fraxinus angustifolia*. All the plants were kept in a greenhouse at 22–24 °C, 75–80% humidity. The development of symptoms was observed during three months. In all the cases, controls inoculated with saline solution were used.

Statistical analysis

The results from all tests to observe qualitative traits were registered as '1' for a positive trait, antibiotic-sensitive test, and '0' for a negative trait, antibiotic-resistant test. To observe the degree of association among strains, an affinity dendrogram using the Jaccard coefficient (Sneath and Sokal, 1973) was performed. Cluster analysis was done using the unweighted pair group method with averages (UPGMA) (Sneath and Sokal, 1973).

Results

Of the 102 phenotypic traits tested, 23.33% showed variability for the strains studied.

The following are the tests where all the strains studied showed identical results:

Basic biochemical test

oxidation of glucose (+), fermentation of glucose (-), catalase (+), cytochrome oxidase (-), reduction of nitrates to nitrites (-), nitrogen fixation (-), arginine dihydrolase (-), indole production (-), β-galactosidase (-), H_2S production (-) and urease (-).

Carbon source assimilation

mannose (+), gluconate (+), fructose (+), glycerol (+), maltose (-), adipate (-), malate (-), phenyl-acetate (-), N-acetyl-glucosamine (-), rhamnose (-), salicin (-), starch (-), dulcitol (-), L-xylose (-), D-arabinose (-), β-methyl-D-xyloside (-), sorbose (-), β-gentiobiose (-), α-methyl-D-mannoside (-), amygdalin (-), cellobiose (-), α-methyl-D-glucoside (-), lactose (-), melezitose (-), glycogen (-), xylitol (-), D-arabitol (-), L-arabitol (-), D-fucose (-), inulin (-), D-tagatose (-), D-turanose (-) and D-lyxose (-).

Production of levan (+)
Hypersensitive reaction on tobacco leaves (+)
Ice nucleation activity (+)
IAA production (+)

Sensitivity to antibiotics

colistin, polymyxin B, chloramphenicol, oxytetracycline, neomycin, tetracycline, kanamycin, streptomycin, gentamycin, doxicyclin and tobramycin.

Resistance to antibiotics

cephalotin, vancomycin, penicillin G and cloxacillin.

The strains studied showed variability in the hydrolysis of esculin and gelatine, in the assimilation of L-arabinose, ribose, D-xylose, galactose, inositol, sorbitol, saccharose, trehalose, rafinose, caprate, citrate, erythritol, adonitol, arbutin, melibiose, mannitol, 2 keto-gluconate and 5 keto-gluconate, and erythromycin, nalidixic acid, sulphamethoxazol, bacitracin, amoxicilin, novobiocin, sulfadiazine and nitrofurantoin sensibility.

Figure 1 shows the affinity dendrogram obtained as a result of applying the Jaccard coefficent to the qualitative test where there is variability among the 164 strains studied.This analysis shows the appearance, at distance level of 0.23, of 15 phenons and five isolated strains where there is clear-cut segregation in terms of the host plant and its place of origin. The number, the name, and the origin of the strains clustered in the different phenons are given in Table 2.

Phenon 15 in the affinity dendrogram displays a group of 10 strains with very specific traits. All these strains were isolated from *Fraxinus angustifolia* knots from area T2. Strains isolated from *Retama sphaerocarpa* are placed in different phenons depending on place of origin; thus, strains from the T2 area are found in phenon 3, M2 strains in phenon 4, M5 strains in

Table 2. Strains in each phenon, host and location

Phenon	N° of strains	Strains	Host and location
1	20	Ps1, 4, 5, 7,8, 9, 10, 11, 13, 15, 17, 19, 20, 12, 14, 6, 18, 16.	Olive (T1)
2	8	Ps2, Ps3.	Olive (T1)
		IF 3026	Olive (B1)
		Ps4, 21, 22, 23, 24.	Wild olive (T2)
3	16	PsR, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, RC1, RC2, RC3, RC4, RC5.	Spanish Broom (T2)
4	17	3.	Olive (T1)
		PsA, 41, 42, 43, 44, 45, 46, 47 ,48, 49.	Oleander (M3)
		76, 77, 78, 79, 80, 81.	Spanish Broom (M2)
5	24	25.	Olive (M1)
		55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65.	Oleander (M2)
		PsAPEP, PsA2, PsA3, PsA4, PsA5.	Oleander (M3)
		50, 51, 52, 53, 54.	Oleander (M4)
		T2, T11.	Spanish Broom (M8)
6	8	26, 27, 28, 29, 30.	Olive (M1)
		31, 33, 35.	Olive (T3)
7	13	32, 34.	40.Olive (T3)
		PsONH1, PsO2, PsO3, PsO4, PsO5, 36, 37, 38, 39,	Olive (M2)
8	2	82, 83.	Spanish Broom (M5)
9	7	B2, B3,B4, B5, B6, B10, B11	Spanish Broom (M5)
10	21	P.s. subsp. savastanoi. NCPPB 1464* 1APs, 6Ps, 11Ps.	Spanish Broom (M6)
		Tr9, Tr10, Tr14, Tr18, Tr25, Tr30, Tr32, Tr33, Tr34, Tr36, Tr38, Tr39, Tr40.	Spanish Broom (M7)
		T1, T10, T16, T17.	Spanish Broom (M8)
11	4	5Ps, 7Ps, 12APs, 13APs.	Spanish Broom (M6)
12	2	16APs, 20APs.	Spanish Broom (M6)
13	2	9Ps, 17APs.	Spanish Broom (M6)
14	6	4APs, 6APs, 18APs, 19APs, 21APs, 22APs.	Spanish Broom (M6)
15	10	PsF, 84, 85, 86, 87, 88, 89,90, 91, 92.	Ash (T2)
Strains	1	Tr15.	Spanish Broom (M6)
	1	P.s. pv. phaseicola. NCPPB 52*	
	1	P.s. pv. tabaci. NCPPB 1408*	
	1	P.s. pv. tomato. NCPPB 996*	
	1	P.s. pv. syringae. NCPPB 93*	

(*) NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, UK.

phenons 6 and 9, M6, M7 and M8 strains in phenon 10, along with NCPPB 1464 type strain, and lastly, strains from M6 in phenons 11, 12, 13 and 14. Strains from olive knots are likewise grouped in different phenons; T1 area strains in phenon 1, M1 and T3 area strains in phenon 6, and T3 and M2 strains in phenon 7. In phenon 2 there are wild olive strains with two olive strains from T1 and one from B1. Lastly, the strains from the oleanders from various locations are in phenons 4 and 5. It is important to highlight that, of the five strains that appear by themselves in the dendrogram, four are type strains belonging to pathovars different to those that were studied.

Of the 28 biochemical traits showing variability, we selected the 20 which differentiated the 15 phenons from each other (Table 3), since the remaining 8 biochemical traits, assimilation of erythritol, adonitol, arbutin, melibiose, mannitol, 2 keto-gluconate y 5 keto-gluconate, and erythromycin sensibility, only

Table 3. Biochemical characteristics of the 15 phenons delineated in the analysis. + = 90% or more strains are positive; – = 90% or more strains are negative; d = 10 to 89% of strains are positive

Test phenons	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Hydrolysis of:															
Esculin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Gelatin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Assimilation of:															
L–Arabinose	d	+	–	d	–	+	–	d	d	+	+	+	+	+	+
Ribose	–	–	–	–	–	–	–	+	+	–	–	–	–	–	+
D–Xylose	–	–	–	–	–	–	d	+	d	+	–	–	–	–	–
Galactose	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–
Inositol	+	+	d	+	+	+	+	+	+	d	+	+	–	+	+
Sorbitol	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
Saccharose	+	d	+	+	d	d	+	+	+	+	+	+	+	+	+
Trehalose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Rafinose	–	–	–	–	–	–	–	–	d	–	–	–	–	–	–
Caprate	d	+	–	+	+	+	+	d	+	+	+	+	+	+	+
Citrate	–	d	+	+	+	–	–	+	+	+	+	–	+	d	+
Sensibility to:															
Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Sulphamethoxazol	+	–	d	+	–	–	–	+	–	–	–	+	–	+	–
Bacitracin	–	–	–	–	–	–	–	–	–	–	d	+	–	+	–
Amoxicillin	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–
Novobiocin	–	–	–	–	–	–	–	–	–	d	+	+	+	–	–
Sulfadiazine	+	+	+	+	d	+	+	+	–	d	+	–	–	–	+
Nitrofurantoin	–	–	+	–	–	–	–	+	+	–	–	–	+	–	–
N. of strains	20	8	16	17	24	8	12	2	7	21	4	2	2	6	10

Table 4. Results of the inoculations of the different strains in the plants tested: ++ all strains induce knots; + all strains produce a slight swelling; – all strains test negative

	Olive	S. broom	Oleander	Ash	Control*
N° of strains	15	15	10	10	
O. europaea	++	–	–	–	–
N. oleander	+	–	++	–	–
R. sphaerocarpha	–	++	–	–	–
F. angustifolia	–	–	–	+	–

* All plants were inoculated with sterile saline solution.

show variability with the strains which are grouped together in the analysis.

In the pathogenetic test (Table 4), we observed that each strain induced knots only in the specific plants in which they were found. Only one cross pathogenesis was detected when strains from *Olea europaea* were inoculated in *Nerium oleander*, where they produced a slight swelling, but not a knot. Strains from *Retama sphaerocarpa*, inoculated in *Retama sphaerocarpa* plants, produced much more rapid knot formation than when the other strains were inoculated in their respective host plants. Also, when strains from *Fraxinus angustifolia* were inoculated in plants other than ash trees, these strains developed necrosis in the inoculation area (hypersensitivity reaction). Lastly, all the control inoculations with saline solution tested negative.

Discussion

As several authors point out, there are clear variations among *Pseudomonas syringae* subsp. *savastanoi* (Ercolani, 1983; Wells et al., 1991; Surico and Iacobellis, 1992; Mugnai et al., 1994; Caponero et al., 1995; Iacobellis et al., 1994 and 1995). According to Ercolani (1983), variations are due to environmental factors, whereas other authors (Janse, 1981; Young et al., 1991) believe that they are different pathovarieties,

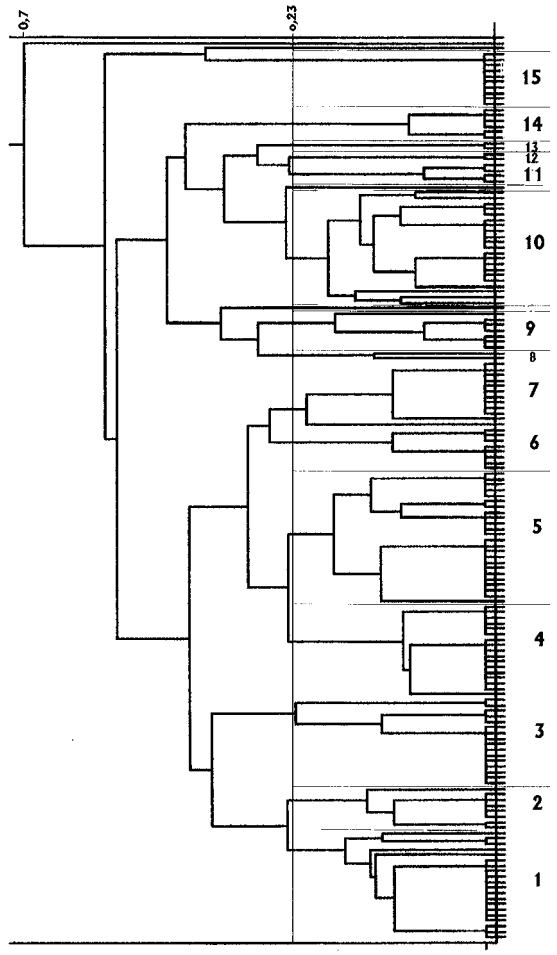

Figure 1. Affinity dendrogram based on the distance of 160 strains of *Pseudomonas syringae* subsp. *savastanoi* and 4 type strains of other pathovars. The numbers correspond to the phenons (1-15).

even subspecies (Janse, 1981), when pathogenetic tests are added to this variability.

In the classification analysis, according to the phenons obtained, possible influence from environmental factors is established, as proven by the fact that strains isolated from knots from the same plant species but different geographic origin are segregated (Figure 1 and Table 2). This fact tallies with Ercolani's hypothesis (1983) that abiotic factors are the cause of the heterogeneity registered in the populations of *Pseudomonas syringae*. When the human factor is added to the abiotic factors, these differences among strains from plants belonging to the same species and location are heightened, as is the case for strains from Spanish broom from the M6 area (very industrialized, high density traffic), which are grouped in different phenons (10, 11, 12, 13 y 14). They show resistances and sensitivities to antibiotics different from the other strains infecting broom. Likewise, in this analysis, strains isolated from *Fraxinus angustifolia* from the T2 area are clearly segregated in phenon 15. This fact, added to the clear-cut difference vis-a-vis with the rest of the strains studied, considering hydrolysis of esculin, and gelatine and trehalose assimilation, as well as incompatibilities in cross inoculations, may lead to the belief that these strains are a different pathovariety. They might be considered different from the pathovariety proposed by Janse (1982) from *Fraxinus excelsior*, which in our analysis is in phenon 10, along with various strains from *Retama sphaerocarpa.*

Moreover, as is pointed out in Young et al.'s revision (1991), we think there are homogeneous groups based on pathological and biochemical aspects, a fact also observed by Surico and Iacobellis (1992) and Iacobellis et al. (1995). This is because we have noted that strains from different hosts, but the same location (hence subject to the same environmental factors), also appear segregated in the foregoing analysis. They display clear differences, as observed in the strains from different host plants from the M2 area and the T2 area. This segregation, added to the incompatibilities observed in the pathogenetic test, as well as the tests carried out by Sutic and Dowson (1963); Janse (1981); Surico et al. (1985) and Iacobellis et al. (1995), might indicate that these strains are different pathovarieties, as Janse (1981) and Young et al. (1991) point out. Ultimately, the existing variation could be the result of the interaction of two factors acting simultaneously, i.e., environmental conditions and host plant specificity.

These results confirm that the populations of the different pathotypes of *Pseudomonas syringae* subsp. *savastanoi* which infect olive trees, oleander, ash and broom are different from one another and distinguishible: through qualifiable phenotype characters; through traits such as fatty acid profiles (Janse, 1991; Wells et al., 1991); the production of bacteriocines (Iacobellis et al., 1995); as well as through different DNA restriction fingerprints (Mugnai et al., 1994).

Moreover, we believe that pathogenesis is correlated to phenotypic properties, as opposed to Gardan et al's results (1991) in their study of phenotypic heterogeneity in *Pseudomonas syringae* pv. *syringae,* since our results (Table 4) indicate high host specificity, and strains obtained from different hosts are generally grouped in different phenons.

In the light of these results, the conclusion reached is as follows: there is clear phenotypic heterogeneity in the *Pseudomonas syringae* subsp. *savastanoi* strains

and this heterogeneity in biochemical traits may be due to environmental factors, to the availability of resources in the host plant, or to the fact that they are different pathovars.

Acknowledgements

We wish to thank Ana Goslyn and Linda Hamalainen for linguistic assistance and Raquel Matellanes for help with the references. This work was supported by the grant 17/97, from the Facultad de Ciencias Experimentales y Técnicas, Universidad San Pablo CEU, Spain.

References

Bauer AW, Kirby WMM, Sherris JC and Turck M (1966) Antibiotic susceptibility testing by a standardized simple disk method. Amer J Clin Pathol 45: 493–496

Beltrá R (1959) El ácido beta-indol acético y los tumores vegetales de origen bacteriano. Revista Latinoam Microbiol 2: 23–32

Caponero A, Contesini AM and Iacobellis NS (1995) Population diversity of *Pseudomonas syringae* subsp. *savastanoi* on olive and oleander. Plant Pathology 44: 848–855

Comai L and Kosuge T (1980) Involvement of plasmid Deoxyribonucleic Acid in Indolacetic Acid synthesis in *Pseudomonas savastanoi*. J Bacteriol 143: 950–957

Comai L and Kosuge T (1982) Cloning and characterization of 'iaaM', a virulence determinant of *Pseudomonas savastanoi*. J Bacteriol 149: 40–46

Comai L, Surico G and Kosuge T (1982) Relation of plasmid DNA to Indolacetic Acid production in different strains of *Pseudomonas syringae pv. savastanoi*. J Gen Microbiol 128: 2157–2163

Dye DW, Bradbury JF, Gato M, Hayward AC, Lelliot RA and Schroth MN (1980) International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. Annu Rev Plant Pathol 59: 153–168

Ercolani GL (1983) Variability among Isolates of *Pseudomonas syringae* pv. *savastanoi* from the Philloplane of the Olive. J Gen Microbiol 129: 901–916

García de los Ríos JE (1989) Estudios acerca de la tuberculosis del olivo. Tesis Doctoral. Universidad Complutense de Madrid

Gardan L, Cottin S, Bollet C and Hunault G (1991) Phenotypic heterogeneity of *Pseudomonas syringae* van Hall. Res Microbiol 142: 995–1003

Gordon SA and Weber RP (1951) Colorimetric estimation of indolacetic acid. Plant Phisiol 26: 192–195

Hardy RWF, Burns RC and Holsten RD (1971) Applications of acetylene-ethylene assay for measurement of nitrogen fixation. 12th. Symp. Nitrogen Econ. Plant Comm. Pac. Sc. Cong. Camberra

Iacobellis NS, Sisto A, Surico G, Evidente A and Di Maio E (1994) Pathogenicity of *Pseudomonas syringae* subsp. *savastanoi* mutants defective in phytohormone production. J Phytopathol 140: 238–248

Iacobellis NS, Contesini AM and Surico G (1995) Bacteriocin production by *Pseudomonas syringae* subsp. *savastanoi*. Phytopathol Med 34: 15–22

Janse JD (1981) The bacterial disease of ash (*Fraxinus excelsior*), caused by *Pseudomonas syringae* subsp. *savastanoi* pv. *fraxini*, II. Etiology and taxonomic considerations. Eur J Forest Pathol 11: 425–438

Janse JD (1982) *Pseudomonas syringae* subsp. *savastanoi* (ex Smith) subsp. nov. rev., the bacterium causing excrescences on Oleaceae and *Nerium oleander* L. Inter J Systematic Bacteriol 32: 166–169

Janse JD (1991) Pathovars discrimination within *Pseudomonas syringae* subsp. *savastanoi* using whole-cell fatty acids and pathogenicity as criteria. Systematic and Applied Microbiol 13: 79–84

Mac Donald EMS, Powell GK, Regier DA, Glass NL, Roberto F, Kosuge T and Morris RO (1986) Secretion of zeatin, ribosylzeatin, and ribosyl-1 -methylzeatin by *Pseudomonas savastanoi*. Plant Physiology 82: 742–747

Magie AR, Wilson EE and Kosuge T (1963) Indolacetamide as an intermediate in the synthesis of indolacetic acid in *Pseudomonas savastanoi*. Science 141: 1281–1282

Mugnai L, Giovanetti L, Ventura S and Surico G (1994) The grouping of strains of *Pseudomonas syringae* subsp. *savastanoi* by DNA restriction fingerprinting. J Phytopathol 142: 209–218

Powell GK and Morris RO (1986) Nucleotide sequence and expression of a *Pseudomonas savastanoi* cytokinin biosynthetic gene, homology with *Agrobacterium tumefaciens* tmr and tzs loci. Nucleic Acids Research 6: 225–235

Smidt M and Kosuge T (1978) The role of indole-3-acetic acid accumulation by alpha methyl tryptophan resistant mutants of *Pseudomonas savastanoi* in gall formation on oleanders. Physiol Plant Pathol 13: 203–214

Smith EF (1908) Recent studies of the olive-tubercle organism. Bull US Dept Agr Bur Plant Ind 131: 25–43

Sneath PHA and Sokal RR (1973) Numerical taxonomy. The principles and practice of numerical classification. Freeman & Co., San Francisco

Surico G, Iacobellis NS and Sisto S (1985) Studies on the role of indole-3-acetic acid and cytokinins in the formation of knots on olive and oleander plants by *Pseudomonas syringae* pv. *savastanoi*. Physiol Plant Pathol 26: 309–320

Surico G and Iacobellis NS (1992) Phytohormones and olive knot disease. In: Verma DPS, (ed.) Molecular Signals in Plant-Microbe Communications. Boca Raton, FL, USA: CRC Press, 209–227

Sutic D and Dowson DJ (1963) The reaction of olive, oleander and ash, cross inoculated with some strains and forms of *Pseudomonas savastanoi* (Smith) Stevens. Phytopathologische Zeitschrift 46: 305–314

Wells JM, Casano FJ and Surico G (1991) Fatty acid composition of *Pseudomonas syringae* pv. *savastanoi*. J Phytopathol 133: 152–162

Wilson EE (1965) Pathological histogenesis in oleander tumors induced by *Pseudomonas savastanoi*. Phytopathology 55: 1244–1249

Young JM, Dye DW, Bradbury JF, Panagopoulos CG and Robbs CF (1978) A proposed nomenclature and classification for plant pathogenic bacteria. N.Z. J Agric Res 21: 153–177

Young JM, Bradbury JF, Davis RE, Dickey RS, Ercolani GL, Hayward AC and Vidader AK (1991) Nomenclatural revisions of plant pathogenic bacteria and list of names 1980–1988. ISPP Subcommitee on Taxonomy of Phytopathogenic Bacteria. Rev Plant Pathology 70: 211–221