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DNA relatedness among the pathovars of *Pseudomonas syringae* and description of *Pseudomonas tremae* sp. nov. and *Pseudomonas cannabina* sp. nov. (*ex* Sutic and Dowson 1959)

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A total of 48 pathovars of *Pseudomonas syringae* and eight related species were studied by DNA–DNA hybridization (S1 nuclease method) and ribotyping. The existence of nine discrete genomospecies was indicated. Genomospecies 1 corresponded to *P. syringae sensu stricto* and included *P. syringae* pathovars *syringae, aptata, lapsa, papulans, pisi, atrofaciens, aceris, panici, dysoxyli* and *japonica*. Genomospecies 2 included *P. syringae* pathovars *phaseolicola, ulmi, mori, lachrymans, sesami, tabaci, morsprunorum, glycinea, ciccaronei, eriobotryae, mellea, aesculi, hibisci, myricae, photiniae* and *dendropanacis* and nomenspecies *Pseudomonas savastanoi, Pseudomonas ficuserectae, Pseudomonas meliae* and *Pseudomonas amygdali*, which are thus synonymous. *P. amygdali* is the earliest valid name for this genomospecies. Genomospecies 3 included *P. syringae* pathovars *tomato, persicae, antirrhini, maculicola, viburni, berberidis, apii, delphinii, passiflorae, philadelphi, ribicola* and *primulae*. We recommend strain CFBP 2212 of *P. syringae* pv. *tomato* to serve as the type strain. Genomospecies 4 included '*Pseudomonas coronafaciens*' and *P. syringae* pathovars *porri, garcae, striafaciens, atropurpurea, oryzae* and *zizaniae* and corresponds to '*P. coronafaciens*'. Genomospecies 5 included *P. syringae* pv. *tremae* and corresponds to *Pseudomonas tremae* sp. nov. Genomospecies 6 included *Pseudomonas viridiflava* and the presently misidentified pathotype strains of *P. syringae* pv. *ribicola* and *P. syringae* pv. *primulae* and thus corresponds to *P. viridiflava*. Genomospecies 7 included *P. syringae* pv. *tagetis* and *P. syringae* pv. *helianthi*. We recommend strain CFBP 1694 of *P. syringae* pv. *tagetis* to serve as a reference strain. Genomospecies 8 included *P. syringae* pv. *theae* and *Pseudomonas avellanae* and thus corresponds to *P. avellanae*. Genomospecies 9 included *P. syringae* pv. *cannabina* and corresponds to *Pseudomonas cannabina* sp. nov. Ribotyping (*Sma*I and *Hin*cII endonucleases) could separate seven of the nine genomospecies. The unnamed genomospecies 3 and 7 will be named when phenotypic data are available for identification. Two species are described, *P. tremae* sp. nov. and *P. cannabina* sp. nov. Other species will be named when phenotypic data are available for identification.

Keywords: *Pseudomonas syringae*, taxonomy, ribotyping, DNA–DNA hybridization

INTRODUCTION

The genus *Pseudomonas sensu lato* has been subdivided into several genera following rRNA–DNA hybridization studies and 16S rRNA sequence comparisons (Kersters *et al.*, 1996; Palleroni *et al.*, 1972; Willems *et*

Table 1. Strains used in this study

P. syringae pathovar is abbreviated in the table to *P. s.* pv. Strains were obtained from the Benaki Phytopathological Institute Collection, Athens, Greece (BPIC); the Collection Française des Bactéries Phytopathogènes, Angers, France (CFBP); the International Collection of Microorganisms from Plants, Manaaki Whenua Landcare Research, Auckland, New Zealand (ICMP); the Culture Collection of the Laboratorium woor Microbiologie, Ghent, Belgium (LMG); and the National Collection of Plant-pathogenic Bacteria, Sand Hutton, York, UK (NCPPB). References are as given in Young *et al.* (1992), unless otherwise indicated. NK, Not known.

Taxon	Strain number as received	Strain	Host	Geographical origin	Year of isolation
P. s. pv. *aceris*	NCPPB 958	CFBP 2339	*Acer* sp.	USA	NK
P. s. pv. *aesculi*	ICMP 8947	CFBP 2894	*Aesculus indica*	India	1980
P. s. pv. *antirrhini*	NCPPB 1817	CFBP 1620	*Antirrhinum majus*	UK	1965
P. s. pv. *apii*	NCPPB 1626	CFBP 2103	*Apium graveolens* var. *dulce*	USA	1942
P. s. pv. *aptata*	NCPPB 871	CFBP 1617	*Beta vulgaris*	USA	1959
P. s. pv. *atrofaciens*	NCPPB 2612	CFBP 2213	*Triticum aestivum*	New Zealand	1968
P. s. pv. *atropurpurea*	NCPPB 2397	CFBP 2340	*Lolium multiflorum*	Japan	1967
P. s. pv. *berberidis*	NCPPB 1356	CFBP 1727	*Berberidis* sp.	New Zealand	1972
P. s. pv. *cannabina*	NCPPB 1437	CFBP 2341	*Cannabis sativa*	Hungary	1957
	NCPPB 1410	CFBP 1619	*Cannabis sativa*	Hungary	1957
	NCPPB 2069	CFBP 1631	*Cannabis sativa*	Yugoslavia	1968
P. s. pv. *ciccaronei*	NCPPB 2355	CFBP 2342	*Ceratonia siliqua*	Italy	1969
P. s. pv. *delphinii*	NCPPB 1879	CFBP 2215	*Delphinium* sp.	New Zealand	1957
P. s. pv. *dendropanacis*	ICMP 9150	CFBP 3226	*Dendropanax trifidus*	Japan	1979
P. s. pv. *dysoxyli*	NCPPB 225	CFBP 2356	*Dysoxylum spectabile*	New Zealand	1949
P. s. pv. *eriobotryae*	NCPPB 2331	CFBP 2343	*Eriobotrya japonica*	USA	1970
P. s. pv. *garcae*	NCPPB 588	CFBP 1634	*Coffea arabica*	Brazil	1956
P. s. pv. *glycinea*	NCPPB 2411	CFBP 2214	*Glycine max*	New Zealand	1968
P. s. pv. *helianthi*	NCPPB 2640	CFBP 2067	*Helianthus annuus*	Mexico	1972
	NCPPB 1055	CFBP 1643	*Helianthus annuus*	Ethiopia	1961
	NCPPB 2639	CFBP 1732	*Helianthus annuus*	Canada	1974
P. s. pv. *hibisci*	ICMP 9623	CFBP 2895	*Hibiscus japonica*	USA	1991
P. s. pv. *japonica*	ICMP 6305	CFBP 2896	*Hordeum vulgare*	Japan	1951
P. s. pv. *lachrymans*	ICMP 3988	CFBP 2440	*Cucumis sativus*	USA	1935
	NCPPB 1096	CFBP 1644	*Cucumis sativus*	Hungary	1957
P. s. pv. *lapsa*	NCPPB 2096	CFBP 1731	*Triticum aestivum*	NK	1968
P. s. pv. *maculicola*	NCPPB 2039	CFBP 1657	*Brassica oleracea*	New Zealand	1965
P. s. pv. *mellea*	NCPPB 2356	CFBP 2344	*Nicotiana tabacum*	USA	1923
P. s. pv. *mori*	NCPPB 1034	CFBP 1642	*Morus alba*	Hungary	1958
P. s. pv. *morsprunorum*	NCPPB 2995	CFBP 2351	*Prunus domestica*	USA	NK
	CFBP 2116		*Prunus cerasus*	France	1974
P. s. pv. *myricae*	ICMP 7118	CFBP 2897	*Myrica rubra*	Japan	1978
P. s. pv. *oryzae*	ICMP 9088	CFBP 3228	*Oryza sativa*	Japan	1983
P. s. pv. *panici*	NCPPB 1498	CFBP 2345	*Panicum* sp.	USA	1963
P. s. pv. *papulans*	NCPPB 2848	CFBP 1754	*Malus sylvestris*	Canada	1973
P. s. pv. *passiflorae*	NCPPB 1387	CFBP 2346	*Passiflora edulis*	New Zealand	1962
P. s. pv. *persicae*	CFBP 1573		*Prunus persicae*	France	1974
P. s. pv. *phaseolicola*	NCPPB 52	CFBP 1390	*Phaseolus vulgaris*	Canada	1949
P. s. pv. *philadelphi*	ICMP 8903	CFBP 2898	*Philadelphus coronarius*	UK	1982
P. s. pv. *photiniae*	ICMP 7840	CFBP 2899	*Photinia glabra*	Japan	1983
P. s. pv. *pisi*	NCPPB 2585	CFBP 2105	*Pisum sativum*	New Zealand	1969
P. s. pv. *porri*	CFBP 1908		*Allium porrum*	France	1978
	CFBP 1687		*Allium porrum*	New Zealand	1949
	CFBP 1770		*Allium porrum*	France	1973
	CFBP 2368		*Allium porrum*	France	1984
P. s. pv. *primulae*	NCPPB 133	CFBP 1660	*Primula* sp.	USA	1939
	LMG 5680	CFBP 11007	*Primula* sp.	UK	1967
P. s. pv. *ribicola*	NCPPB 963	CFBP 2348	*Ribes aureum*	NK	1946

Table 1 (cont.)

Taxon	Strain number as received	Strain	Host	Geographical origin	Year of isolation
	NCPPB 1010	CFBP 10971	*Ribes aureum*	NK	1961
P. s. pv. *sesami*	NCPPB 1016	CFBP 1671	*Sesamum indicum*	Yugoslavia	1961
P. s. pv. *striafaciens*	NCPPB 1898	CFBP 1674	*Avena sativa*	NK	1966
	NCPPB 2480	CFBP 1686	*Avena sativa*	Zimbabwe	1971
P. s. pv. *tabaci*	NCPPB 1427	CFBP 2106	*Nicotiana tabacum*	Hungary	1959
P. s. pv. *tagetis*	NCPPB 2488	CFBP 1694	*Tagetes erecta*	Zimbabwe	1972
	LMG 5684	CFBP 11009	*Tagetes erecta*	Australia	1976
P. s. pv. *theae*	NCPPB 2598	CFBP 2353	*Thea sinensis*	Japan	1970
	LMG 5045	CFBP 11012	*Camellia sinensis*	Japan	1970
	LMG 5687	CFBP 11013	*Camellia sinensis*	Japan	NK
P. s. pv. *tomato*	NCPPB 1106	CFBP 2212	*Lycopersicon esculentum*	UK	1960
	CFBP 1323		*Lycopersicon esculentum*	Switzerland	NK
	CFBP 1427		*Lycopersico nesculentum*	France	1972
	CFBP 10193		*Lycopersicon esculentum*	France	1987
P. s. pv. *tremae*	ICMP 9151	CFBP 3229	*Trema orientalis*	Japan	1979
P. s. pv. *ulmi*	NCPPB 632	CFBP 1407	*Ulmus* sp.	Yugoslavia	1958
P. s. pv. *viburni*	NCPPB 1921	CFBP 1702	*Viburnum* sp.	USA	NK
P. s. pv. *zizaniae*	ICMP 8921	CFBP 11040	*Zizania aquatica*	USA	1983
P. savastanoi	NCPPB 639^T	CFBP 1670	*Olea europaea*	Yugoslavia	1959
	CFBP 1838		*Fraxinus excelsior*	France	1974
	CFBP 2088		*Nerium oleander*	Algeria	1978
	CFBP 4221		*Olea europaea*	Algeria	1977
P. amygdali	NCPPB 2607^T	CFBP 3340^T	*Prunus amygdalus*	Greece	1967
P. avellanae	BPIC 640*	CFBP 11066	*Corylus avellana*	Greece	1976
	BPIC 654	CFBP 11067	*Corylus avellana*	Greece	1976
	NCPPB 3487^T	CFBP 11144^T	*Corylus avellana*	Greece	1976
	NCPPB 3488	CFBP 2637	*Corylus avellana*	Greece	1976
'*P. coronafaciens*'	NCPPB 600^T†	CFBP 2216^T	*Avena sativa*	UK	1958
P. ficuserectae	ICMP 7848^T	CFBP 3224^T	*Ficus erecta*	Japan	1977
P. meliae	ICMP 6289^T	CFBP 3225^T	*Melia azedarach*	Japan	1974
P. syringae	NCPPB 281^T	CFBP 1392^T	*Syringa vulgaris*	UK	1950
	CFBP 600		*Populus* sp.	France	1964
	CFBP 775		*Syringa vulgaris*	France	1965
	CFBP 1685		*Zea mays*	Yugoslavia	1965
P. viridiflava	NCPPB 655^T	CFBP 2107^T	*Phaseolus* sp.	Switzerland	1927

* Janse *et al.* (1996).

† Pecknold & Grogan (1973).

al., 1992). Fluorescent phytopathogenic pseudomonads cluster with species of the genus *Pseudomonas sensu stricto*, within rRNA-similarity group I (Palleroni, 1984), corresponding to the γ branch of the *Proteobacteria* (Kersters *et al.*, 1996).

The taxonomy of *Pseudomonas syringae* and related phytopathogenic bacteria has evolved and been debated over the last four decades (Young *et al.*, 1992). In the 8th edition of *Bergey's Manual of Determinative Bacteriology*, *P. syringae* was considered to be a species which represented most of the fluorescent phytopathogenic *Pseudomonas* nomenspecies (Doudoroff & Palleroni, 1974). A complete revision of the taxonomy of *P. syringae* was presented in the 1st edition of *Bergey's Manual of Systematic Bacteriology* (Palleroni, 1984), which placed 41 nomenspecies as pathovars of *P. syringae*. This classification of *P. syringae* pathovars was supported by the International Society for Plant Pathology, Subcommittee on Taxonomy of Plant-pathogenic Bacteria (Dye *et al.*, 1980). The reference strain of each pathovar is referred to as the pathotype strain (Dye *et al.*, 1980). Consequently, *P. syringae*, *Pseudomonas amygdali*, *Pseudomonas meliae*, *Pseudomonas coronafaciens*, *Pseudomonas ficuserectae* and *Pseudomonas viridiflava* were the only fluorescent,

oxidase-negative, phytopathogenic *Pseudomonas* species included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1989). Most of the pathovars of *P. syringae* were described on the basis of plant source and limited cross-pathogenicity tests. Furthermore, several of the pathovars were poorly described. Although the phenotypic relationships between *P. syringae* and related bacteria have been studied previously (Gardan *et al.*, 1991; Hildebrand & Schroth, 1972; Hildebrand *et al.*, 1988; Lelliott *et al.*, 1966; Misaghi & Grogan, 1969; Sands *et al.*, 1970; Young & Triggs, 1994), it is impossible to identify correctly each of the pathovars by means of routine biochemical tests (Bradbury, 1986). Consequently, some pathovars may be synonymous (Young, 1992).

The first genomic studies based on DNA–DNA hybridization involved six nomenspecies, later considered to be *P. syringae* pathovars, and showed striking genomic heterogeneity among strains (Palleroni *et al.*, 1972). Pecknold & Grogan (1973) found three DNA-relatedness groups, designated as '*syringae*', '*morsprunorum*' and '*tomato*', among 15 nomenspecies (later considered as pathovars of *P. syringae*). Denny *et al.* (1988) showed that *P. syringae* pv. *tomato* formed a DNA-relatedness group distinct from the type strain of *P. syringae*. Strains of *P. syringae* pv. *savastanoi*, isolated from different host plants, formed a DNA-relatedness group distinct from *P. syringae sensu stricto*, thus constituting a discrete species, *Pseudomonas savastanoi* (Gardan *et al.*, 1992). A recent polyphasic study including DNA–DNA hybridization has reclassified *P. syringae* pv. *avellanae* as *Pseudomonas avellanae* (Janse *et al.*, 1996).

The confusing state of *P. syringae* taxonomy necessitates a comprehensive DNA-relatedness study. The purposes of the present work were: (i) to analyse all available *P. syringae* pathovars; (ii) to delineate genomospecies by DNA–DNA hybridization; and (iii) to characterize DNA–DNA groups by rRNA gene restriction patterns (ribotyping). The outcome of the study is the delineation of nine DNA-hybridization groups, which generally can also be distinguished by ribotyping.

METHODS

Bacterial strains. The type strains of *P. syringae*, *P. amygdali*, *P. coronafaciens*, *P. ficuserectae*, *P. meliae*, *P. savastanoi*, *P. avellanae* and *P. viridiflava* and at least one strain (pathotype) of each pathovar of *P. syringae* were studied (Table 1).

DNA extraction. DNA was extracted and purified following a published procedure (Brenner *et al.*, 1982).

DNA–DNA hybridization. Native DNAs from *P. syringae* (CFBP 1392^T), *P. syringae* pv. *tomato* (CFBP 2212), *P. syringae* pv. *porri* (CFBP 1908), *P. syringae* pv. *tagetis* (CFBP 1694), *P. syringae* pv. *theae* (CFBP 2353), *P. avellanae* (CFBP 11144^T), *P. syringae* pv. *helianthi* (CFBP 2067), *P. syringae* pv. *cannabina* (CFBP 2341), *P. syringae* pv. *tremae* (3229), *P. savastanoi* (CFBP 1670^T) and *P. viridiflava* (CFBP 2107^T) were labelled *in vitro* by nick translation with ^{3}H-labelled nucleotides (Amersham). The S1 nuclease/trichloroacetic acid procedure used has been described previously (Crosa *et al.*, 1973). The reassociation temperature was 70 °C. DNA experiments were repeated twice, or three times for results giving reassociations above 65%. Percentage relative reassociation will be referred to as DNA relatedness. To determine the thermal stability of reassociated DNAs, the temperature at which 50% of reassociated DNA became hydrolysable by nuclease S1 (T_m) was determined by using the method of Crosa *et al.* (1973). The ΔT_m was the difference between the T_m of the homoduplex and the T_m of the heteroduplex.

DNA base composition. The G+C contents of *P. syringae* pv. *tremae* CFBP 3229 and *P. syringae* pv. *cannabina* CFBP 2341 were determined by the thermal denaturation temperature method (Marmur & Doty, 1962) and were calculated by using the equation of Owen & Lapage (1976).

Ribotyping. DNA samples were cleaved by restriction endonucleases *Hin*cII and *Sma*I following the supplier's instructions (Amersham). Electrophoresis of restriction fragments, vacuum transfer and hybridization with *N*-acetylaminofluorene-labelled 16S+23S rRNA (Eurogentec) were done as described previously (Grimont & Grimont, 1995). Computer interpretations of ribotyping data were obtained by using the TAXOTRON package (Institut Pasteur, Paris, France) with a colour Macintosh computer as described previously (Grimont & Grimont, 1995). DNA–DNA hybridization and ribotyping were done in two laboratories in a blind fashion.

Phenotypic analysis with the Biotype 100 system. Carbon source utilization tests were done with Biotype 100 strips (bioMérieux), which contain 99 carbon sources. Biotype medium 1 was used to inoculate the cupules of the strips, which were incubated at 28 °C. Growth was recorded after 2 and 4 d for each cupule.

RESULTS AND DISCUSSION

DNA relatedness

The results of DNA-relatedness experiments are shown in Tables 2 and 3. In all, nine genomospecies were delineated. Relatedness within genomospecies was between 66 and 100% with ΔT_m values of 0·0 to 4·5 °C. Relatedness between genomospecies was 0–59%. Relatedness among all genomospecies except genomospecies 5 was 10–59%. Some genomospecies included type strains of species. Of the 48 pathovars of *P. syringae* and eight related species tested, 51 belonged to six discrete DNA-hybridization groups (Table 2).

DNA-hybridization group 1 included 10 strains of different pathovars of *P. syringae* that demonstrated 71–100% relatedness to the type strain of *P. syringae*, CFBP 1392^T, with ΔT_m values ranging from 0·0 to 3·0 °C. Genomic DNA from three strains of *P. syringae* used as controls was 70–77% related to ^{3}H-labelled DNA from CFBP 1392^T (data not shown). Genomospecies 1 corresponded to *P. syringae sensu stricto*. Thus *P. syringae* and pathovars *aptata*, *lapsa*, *papulans*, *pisi*, *atrofaciens*, *aceris*, *panici*, *dysoxyli* and *japonica* belong to *P. syringae sensu stricto*. This genomospecies corresponds to the DNA–DNA group '*syringae*' of Pecknold & Grogan (1973).

Table 2. DNA relatedness among pathovars of *P. syringae* and related species

The values in the table represent DNA relatedness, expressed as percentage relative reassociation of the particular combination of ^{3}H-labelled and unlabelled DNA. Numbers in parentheses represent ΔT_m (°C). *P. syringae* pathovar is abbreviated in the table to *P. s.* pv. Strain designations are according to CFBP. NT, Not tested.

Source of unlabelled DNA		Source of ^{3}H-labelled DNA					
Taxon	**Strain**	***P. syringae* CFBP 1392^T**	***P. savastanoi* CFBP 1670^T**	***P. s.* pv. *tomato* CFBP 2212**	***P. s.* pv. *porri* CFBP 1908**	***P. s.* pv. *tremae* CFBP 3229**	***P. viridiflava* CFBP 2107^T**
Genomospecies 1							
P. syringae	1392^T	100 (0·0)	59	46	45	6	38
P. s. pv. *aptata*	1617	78 (0·0)	48	38	39	19	30
P. s. pv. *lapsa*	1731	82 (0·0)	46	38	38	7	31
P. s. pv. *papulans*	1754	71 (3·0)	58	44	46	5	33
P. s. pv. *pisi*	2105	80 (1·0)	48	48	52	8	30
P. s. pv. *atrofaciens*	2213	87 (0·0)	53	42	47	8	35
P. s. pv. *aceris*	2339	67 (2·0)	38	42	47	1	40
P. s. pv. *panici*	2345	84 (1·0)	51	49	54	5	47
P. s. pv. *dysoxyli*	2356	71 (3·0)	51	40	36	6	33
P. s. pv. *japonica*	2896	90	38	45	35	2	40
Genomospecies 2							
P. savastanoi	1670^T	44	100 (0·0)	43	48	2	42
P. ficuserectae	3224^T	37	74 (2·5)	53	40	0	29
P. meliae	3225^T	52	77	47	43	2	30
P. amygdali	3340^T	54	83	52	45	3	38
P. s. pv. *phaseolicola*	1390	50	73 (1·0)	43	42	1	29
P. s. pv. *ulmi*	1407	50	73 (3·0)	45	44	3	30
P. s. pv. *mori*	1642	51	85	37	42	2	26
P. s. pv. *lachrymans*	1644	47	72 (1·5)	50	52	NT	31
P. s. pv. *sesami*	1671	57	83	54	48	12	43
P. s. pv. *tabaci*	2106	52	75	42	45	6	30
P. s. pv. *morsprunorum*	2116	53	82	56	50	1	33
P. s. pv. *glycinea*	2214	46	83	46	54	4	34
P. s. pv. *ciccaronei*	2342	52	88	52	54	1	48
P. s. pv. *eriobotryae*	2343	51	84	47	47	1	47
P. s. pv. *mellea*	2344	54	83	35	44	1	32
P. s. pv. *aesculi*	2894	51	81	50	43	2	30
P. s. pv. *hibisci*	2895	57	73 (0·0)	44	43	1	30
P. s. pv. *myricae*	2897	50	88	53	53	0	39
P. s. pv. *photiniae*	2899	52	84	47	45	0	34
P. s. pv. *dendropanacis*	3226	51	82	46	41	3	30
Genomospecies 3							
P. s. pv. *tomato*	2212	41	53	100	53	4	30
P. s. pv. *persicae*	1573	47	50	79	48	4	27
P. s. pv. *antirrhini*	1620	47	50	82	46	20	37
P. s. pv. *maculicola*	1657	47	50	90	47	5	33
P. s. pv. *viburni*	1702	46	50	71 (1·0)	43	1	33
P. s. pv. *berberidis*	1727	46	53	91	57	7	35
P. s. pv. *apii*	2103	47	44	85	49	9	10
P. s. pv. *delphinii*	2215	46	40	79	50	0	34
P. s. pv. *passiflorae*	2346	43	50	74 (2·5)	49	0	33
P. s. pv. *morsprunorum*	2351	46	46	80	51	0	37
P. s. pv. *lachrymans*	2440	47	45	83	44	0	13
P. s. pv. *philadelphi*	2898	41	45	76	42	0	32
P. s. pv. *ribicola*	10971	56	47	79	53	NT	36
P. s. pv. *primulae*	11007	53	43	71	49	NT	32
Genomospecies 4							
P. s. pv. *porri*	1908	42	43	51	100	4	34
P. s. pv. *garcae*	1634	44	46	43	80	7	32
P. s. pv. *striafaciens*	1674	41	31	47	83	4	27
P. s. pv. *striafaciens*	1686	37	32	42	93	NT	31
'*P. coronafaciens*'	2216^T	43	40	50	89	3	29
P. s. pv. *atropurpurea*	2340	42	43	38	78	2	37
P. s. pv. *oryza*	3228	45	46	47	87	3	35
P. s. pv. *zizaniae*	11040	35	48	47	95	NT	30
Genomospecies 5							
P. s. pv. *tremae*	3229	3	4	20	2	100	3
Genomospecies 6							
P. viridiflava	2107^T	33	36	37	38	7	100
P. s. pv. *primulae*	1660	34	31	28	27	1	71
P. s. pv. *ribicola*	2348	33	37	13	29	0	80
Other pathovars							
P. s. pv. *tagetis*	1694	50	53	43	45	13	30
P. s. pv. *helianthi*	2067	62	54	48	40	0	37
P. s. pv. *theae*	2353	44	42	59	41	0	31
P. s. pv. *avellanae*	11144	45	47	49	45	3	31
P. s. pv. *cannabina*	2341	41	42	52	51	0	38

Table 3. DNA relatedness among four pathovars of *P. syringae, tagetis, helianthi, theae* and *cannabina*, and *P. avellanae*

See legend to Table 2.

Source of unlabelled DNA		Source of ^{3}H-labelled DNA				
Taxon	Strain	*P. s.* pv. *tagetis* CFBP 1694	*P. s.* pv. *helianthi* CFBP 2067	*P. s.* pv. *theae* CFBP 2353	*P. avellanae* CFBP 11144^T	*P. s.* pv. *cannabina* CFBP 2341
Genomospecies 7						
P. s. pv. *tagetis*	1694	100	85	39	49	39
	11009	90	93			
P. s. pv. *helianthi*	2067	84	100	41	53	43
	1732	91	77			
	1643	78	82			
Genomospecies 8						
P. s. pv. *theae*	2353	50	51	100	81	49
	11012			86	73	
	11013			84	74	
P. avellanae	11144^T	48	42	67 (1·5)	100	42
	11067			66 (3·0)	90	
	11066			76 (1·5)	88	
	2637			69 (4·5)	83	
Genomospecies 9						
P. s. pv. *cannabina*	2341	37	38	44	45	100
	1619				56	85
	1631				52	84

DNA-hybridization group 2 included 20 strains: 16 different pathovars of *P. syringae* and type strains of four related species. *P. savastanoi* (CFBP 1670^T), *P. ficuserectae* (CFBP 3224^T), *P. meliae* (CFBP 3225^T) and *P. amygdali* (CFBP 3340^T) showed 72–100% binding to the type strain of *P. savastanoi*, CFBP 1670^T, with ΔT_m values ranging from 0·0 to 3·0 °C. Three strains of *P. savastanoi* used as controls were 79–93% related to CFBP 1670^T (data not shown). Genomospecies 2 included the type strains of *P. savastanoi* (Janse 1982) Gardan *et al.* 1992, *P. ficuserectae* Goto 1983, *P. meliae* Ogimi 1981 and *P. amygdali* Psallidas and Panagopoulos 1975. Thus, these species should be considered synonymous and the correct name for this species should be *P. amygdali*. This genomospecies corresponds to DNA group '*morsprunorum*' of Pecknold & Grogan (1973). Unfortunately *P. amygdali*, as delineated by DNA–DNA hybridization, could not be differentiated from other genomospecies by phenotypic tests (Table 4).

DNA-hybridization group 3 included 14 strains of different pathovars of *P. syringae* that demonstrated 71–100% relatedness to the pathotype strain of *P. syringae* pv. *tomato*, CFBP 2212. Strains of *P. syringae* pv. *viburni* and *P. syringae* pv. *passiflorae* showed the lowest DNA-relatedness values (71 and 74%, respectively), with ΔT_m values below 2·5 °C. Three strains of *P. syringae* pv. *tomato* used as controls showed 92–98 % relatedness to CFBP 2212 (data not shown). Genomospecies 3 did not include the type strain of any validly described species. By using DNA-relatedness data, Denny *et al.* (1988) showed that *P. syringae* pv. *tomato* was distinct from *P. syringae*, but no formal proposal was made. This genomospecies corresponds to the DNA–DNA group '*tomato*' of Pecknold & Grogan (1973). We recommend that strain CFBP 2212 (*P. syringae* pv. *tomato*) should serve as the type strain.

DNA-hybridization group 4 included '*P. coronafaciens*' and seven strains of different pathovars that were 78–100% related to the pathotype strain of *P. syringae* pv. *porri*, CFBP 1908. Three strains of *P. syringae* pv. *porri* used as controls were 90–98% related to CFBP 1908 (data not shown). Genomospecies 4 included the type strain of '*P. coronafaciens*' Schaad and Cunfer 1979. Thus, this genomospecies represents '*P. coronafaciens*'.

DNA-hybridization group 5 was represented by a single strain of *P. syringae* pv. *tremae* CFBP 3229, distantly related (less than 20% DNA–DNA binding) to the other strains tested. The DNA G+C content was 60·5 mol%. Genomospecies 5 included only the pathotype strain of *P. syringae* pv. *tremae* and thus comprises the species *Pseudomonas tremae* sp. nov.

DNA-hybridization group 6 included three strains, *P. viridiflava*, *P. syringae* pv. *ribicola* and *P. syringae* pv. *primulae*, that demonstrated 71–100% relatedness to the type strain of *P. viridiflava*, CFBP 2107^T. Genomospecies 6 included the type strain of *P. viridiflava*. Thus, this genomospecies represents *P. viridiflava*.

P. syringae pathovars *morsprunorum, lachrymans, ribicola* and *primulae* were represented by their pathotype strains (CFBP 2351, CFBP 2440, CFBP 2348 and CFBP 1660, respectively), which are non-pathogenic, and a typical phytopathogenic strain (CFBP 2116, CFBP 1644, CFBP 10971 and CFBP 11007, respectively). Unfortunately, for each of these pathovars, the pathotype strains and phytopathogenic strains did not belong to the same genomospecies. Thus, the pathotype strains of these four pathovars should be changed.

Further studies were carried out on further strains of five pathovars that did not belong to any of the six

above-mentioned DNA-hybridization groups (Table 3).

DNA-hybridization group 7 included two strains of *P. syringae* pv. *tagetis* and three strains of *P. syringae* pv. *helianthi*. Genomospecies 7 did not include the type strain of any validly described species. We recommend strain CFBP 1694 of *P. syringae* pv. *tagetis* to serve as the reference strain.

DNA-hybridization group 8 included three strains of *P. syringae* pv. *theae* and four strains of *P. avellanae*. When ^{3}H-labelled DNA from CFBP 11144^T (*P. avellanae*) was used, the seven strains showed 73–100% relatedness. However, when ^{3}H-labelled DNA from CFBP 2353 (*P. syringae* pv. *theae*) was used, strains of *P. syringae* pv. *theae* showed 84–100% relatedness, whereas strains of *P. avellanae* showed 66–76% relatedness, with ΔT_m values below 4·5 °C. Genomospecies 8 included the type strain of *P. avellanae* (Janse *et al.*, 1996), and thus represents *P. avellanae*.

DNA-hybridization group 9 included three strains of *P. syringae* pv. *cannabina* (Table 3). The DNA G+C content was 60·2 mol%. Genomospecies 9 included only the pathotype strain of *P. syringae* pv. *cannabina* and thus comprises *Pseudomonas cannabina* sp. nov.

The genomic heterogeneity (three groups) of the *P. syringae* complex (different pathovars and related species), as described in 1973 from a study of 15 nomenspecies (Pecknold & Grogan, 1973), was confirmed and extended by distributing 48 pathovars and eight related species into nine genomospecies.

Ribotyping

Digestion of genomic DNA (from 63 strains) with *Sma*I and hybridization with 16S+23S rRNA generated 55 unique ribotypes (Fig. 1). With *Hin*cII, 42 unique ribotypes were observed (Fig. 1). Mean linkage clustering of combined ribotype data yielded 56 combined ribotypes distributed within 10 ribogroups designated A to J and two isolated ribotypes (K and L) (Fig. 1).

Studies have shown that ribotypes carry taxonomic information in addition to typing information (Brosch *et al.*, 1996; Grimont & Grimont, 1986). In a previous report, all the species studied that are genomically valid were separated by ribotyping with endonucleases *Sma*I and *Hin*cII (Brosch *et al.*, 1996). An attempt was therefore made to use ribotyping to differentiate the genomospecies.

All but one strain of genomospecies 1 belonged to ribogroup A. The 20 strains of genomospecies 2 studied were distributed within four ribogroups, B to E. The strains of genomospecies 4 constituted ribogroup F. The single strain in genomospecies 5 represented an isolated ribotype (ribogroup K). The three strains of genomospecies 6 constituted ribogroup J. The two strains of genomospecies 7 formed ribogroup G. The single strain of genomospecies 9 represented an isolated ribotype (ribogroup L).

Genomospecies 3 and 8 were not clearly distinguished by ribotyping. Nine strains of genomospecies 3 formed ribogroup H and three strains of genomospecies 3 and six strains of genomospecies 8 formed ribogroup I. However, several restriction fragments were common to all strains of both genomospecies. More endonucleases would need to be tested in an attempt to separate these genomospecies. Thus, ribotyping was able to discriminate seven of the nine genomospecies. It should be noted that the type strains of both new species described in this work (*P. tremae* sp. nov. and *P. cannabina* sp. nov.) yielded discrete ribotypes. This partial correlation between ribotyping and DNA relatedness should allow the screening of large collections of strains, since ribotyping is faster than DNA–DNA hybridization.

Phenotypic analysis

In an attempt to characterize the genomospecies phenotypically, the percentages of strains giving positive reactions with the substrates of Biotype 100 strips were calculated for each genomospecies. Of the strains studied, 90–100% utilized (+)-D-glucose, (+)-D-fructose, (+)-D-galactose, (+)-D-mannose, sucrose, (−)-D-ribose, (+)-L-arabinose, glycerol, D-saccharate, mucate, (−)-L-malate, citrate, D-gluconate, 5-aminovalerate, succinate, fumarate, L-aspartate, L-glutamate, L-proline, L-alanine and L-serine. Fewer than 10% of the strains utilized (+)-D-trehalose, (+)-L-sorbose, (+)-D-melibiose, (+)-D-raffinose, maltotriose, maltose, lactose, lactulose, methyl β-galactopyranoside, methyl α-galactopyranoside, (+)-D-cellobiose, β-gentiobiose, methyl β-D-glucopyranoside, palatinose, L-rhamnose, (−)-L-fructose, (+)-D-melezitose, (−)-L-arabitol, xylitol, dulcitol, D-tagatose, maltitol, (+)-D-turanose, adonitol, hydroxyquinoline-β-glucuronide and methyl α-D-glucopyranoside. The carbon sources allowing the most discrimination are given in Table 4. They permitted the discrimination of genomospecies 5 and 9 from the seven other genomospecies. The utilization of other carbon sources was variable, without discriminating between the genomospecies.

Previous studies, limited to a phenotypic comparison of only a few pathovars (Gardan *et al.*, 1992; Janse *et al.*, 1996), led to the elevation of *P. syringae* pv. *avellanae* and *P. syringae* pv. *savastanoi* to species status. Unfortunately, our more comprehensive study yielded nine genomospecies that cannot be differentiated systematically by phenotypic tests. Thus, *P. avellanae* and *P. savastanoi*, as delineated by DNA–DNA hybridization, can no longer be identified by biochemical tests.

Genomospecies should be named formally only when phenotypic characters are available to differentiate them (Wayne *et al.*, 1987). Unfortunately, at this

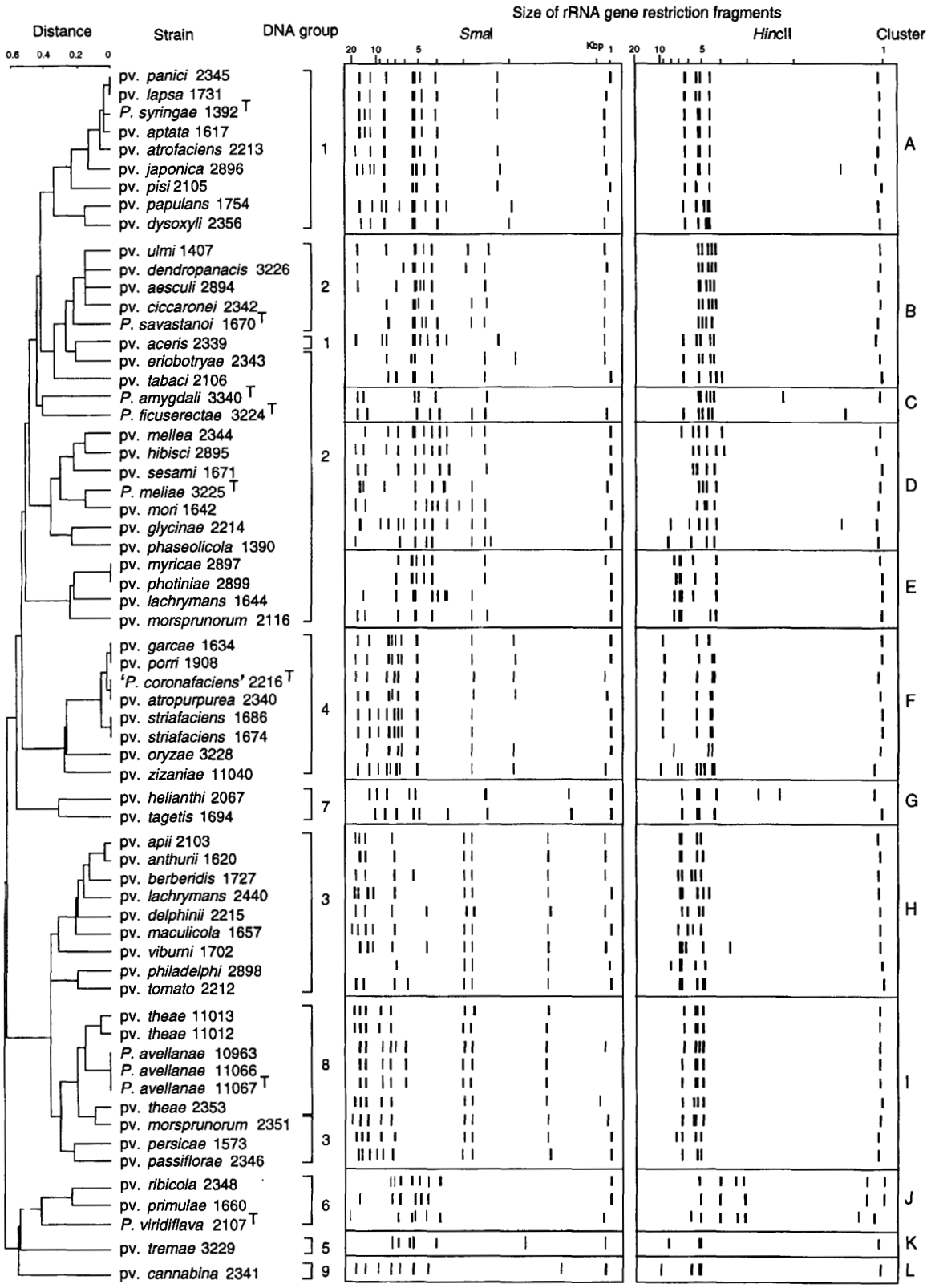

Fig. 1. Dendrogram obtained by comparison of *Sma*I and *Hin*cII ribotyping patterns from 63 strains of *P. syringae sensu lato* pathovars and related species. Strains compared are pathovars of *P. syringae* unless otherwise indicated; strain numbers given are those allocated by CFBP. The dendrogram was established by using DENDROGRAPH, a program from the TAXOTRON software package (Institut Pasteur, Paris), with the complement of Dice similarity coefficients. A fragment length tolerance of 4% and mean linkage were used.

Table 4. Assimilation by nine *Pseudomonas* DNA–DNA-hybridization groups on eight carbon sources in Biotype 100 strips

The table shows the percentage of strains tested from a given genomospecies that were able to assimilate the carbon source shown.

Genomospecies	No. of strains tested	Carbon source							
		L-Alanine	D-Fructose	Glycerol	D-Gluconate	Fumarate	D-Malate	Succinate	DL-Lactate
1 (*P. syringae*)	10	90	100	100	100	100	90	100	90
2 (*P. amygdali*)	20	85	100	85	95	95	85	100	0
3 ('*P. tomato*')	14	100	93	79	92	92	86	93	0
4 ('*P. coronafaciens*')	8	100	100	100	100	89	88	100	0
5 (*P. tremae*)	1	0	0	0	0	100	100	100	0
6 (*P. viridiflava*)	3	100	100	100	100	100	100	100	100
7 ('*P. theae*')	2	100	100	100	100	100	100	100	0
8 (*P. avellanae*)	2	50	100	100	50	100	100	100	0
9 (*P. cannabina*)	1	100	100	100	100	0	0	0	0

point, we do not have reliable means to differentiate all nine genomospecies phenotypically, and thus only two new species are described below.

Genomic, phenotypic and pathogenicity data supporting a proposal to change the pathotype strains of *P. syringae* pv. *morsprunorum* (CFBP 2351), *P. syringae* pv. *lachrymans* (CFBP 2440), *P. syringae* pv. *ribicola* (CFBP 2348) and *P. syringae* pv. *primulae* (CFBP 1660) will be presented to the ISPB Subcommittee on Taxonomy of Plant-pathogenic Bacteria.

The delineation of nine genomospecies in an otherwise confused taxonomic group should stimulate more work, such as cross-pathogenicity studies within genomospecies, the design of genomospecies-specific DNA probes or correlations with other genomic or phenotypic methods.

Description of *Pseudomonas tremae* sp. nov.

Pseudomonas tremae (tre'ma.e. M. L. gen. fem. n. *tremae* of *Trema*, generic name of the host plant, *Trema orientalis* BL).

Rather slow growing with white or cream, smooth colonies on YPGA. Gram-negative, aerobic rods, motile by means of one to four polar flagella. Metabolism is respiratory. Does not produce fluorescent pigment on King B medium. Results of LOPAT tests are −, −, −, − and + (Lelliott *et al.*, 1966). Nitrate is not reduced. No hydrolysis of aesculin or starch. Arginine test (Thornley), indole production and gelatin liquefaction are negative. Hydrolysis of Tween 80 and production of reducing substances from sucrose are negative. Assimilates D-saccharate, D-malate, L-malate, citrate, succinate, fumarate, L-aspartate and L-glutamate and does not assimilate the 91 other carbon sources of the Biotype 100 strips (bioMérieux). The DNA G+C content is 60·5 mol%. Pathogenic only for *Trema orientalis*. The type strain is CFBP 3229^T = ICMP 9151^T = NCPPB 3465^T.

Description of *Pseudomonas cannabina* (*ex* Sutic and Dowson 1959) sp. nov.

Pseudomonas cannabina (can.na'bi.na. L. fem. adj. *cannabina* pertaining to *Cannabis*, the generic name of the host plant, *Cannabis sativa* L.).

Gram-negative rods that are 1·1–3·0 μm wide × 3·0–4·0 μm long and motile by means of one to four polar flagella. Colonies have a grey colour and are slightly convex on YPGA. Metabolism is respiratory. Produces a fluorescent pigment on King B medium. Results of LOPAT tests are +, −, −, − and + (Lelliott *et al.*, 1966). Nitrate is not reduced. No hydrolysis of aesculin or starch. Arginine test (Thornley), indole production and gelatin liquefaction are negative. Assimilates D-glucose, D-fructose, D-galactose, D-mannose, D-ribose, glycerol, D-saccharate, mucate, citrate, D-gluconate, L-histidine, L-aspartate, L-glutamate, L-proline, L-alanine and L-serine and does not assimilate the other 83 carbon sources of the Biotype 100 strips (bioMérieux). The DNA G+C content is 60·2 mol%. In nature, pathogenic on *Cannabis sativa* L. and by inoculation on *Phaseolus vulgaris* L. The type strain is CFBP 2341^T = ICMP 2823^T = NCPPB 1437^T.

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REFERENCES

Bradbury, J. F. (1986). *Pseudomonas* Migula 1894. In *Guide to Plant Pathogenic Bacteria*, pp. 110–184. Egham, UK: International Mycobiological Institute.

Brenner, D. J., McWorter, A. C., Leete Knutson, J. K. & Steigerwalt, A. G. (1982). *Escherichia vulneris*: a species of *Enterobacteriaceae* associated with human wounds. *J Clin Microbiol* **15**, 1133–1140.

Brosch, R., Lefèvre, M., Grimont, F. & Grimont, P. A. D. (1996). Taxonomic diversity of pseudomonads revealed by computer interpretation of ribotyping data. *Syst Appl Microbiol* **19**, 541–555.

Crosa, J. M. D., Brenner, D. J. & Falkow, S. (1973). Use of a single-strand-specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *J Bacteriol* **115**, 904–911.

Denny, J. P., Gilmour, M. N. & Selander, R. K. (1988). Genetic diversity and relationships of two pathovars of *Pseudomonas syringae*. *J Gen Microbiol* **134**, 1949–1960.

De Vos, P., Goor, M., Gillis, M. & De Ley, J. (1985). Ribosomal ribonucleic acid cistron similarities of phytopathogenic *Pseudomonas* species. *Int J Syst Bacteriol* **35**, 169–184.

Doudoroff, M. & Palleroni, N. J. (1974). Genus I. *Pseudomonas* Migula 1894. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 217–243. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

Dye, D. W., Bradbury, J. F., Goto, M., Hayward, A. C., Lelliott, R. A. & Schroth, M. N. (1980). International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. *Rev Plant Pathol* **59**, 153–158.

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

Gardan, L., Bollet, C., Abu Ghorrah, M., Grimont, F. & Grimont, P. A. D. (1992). DNA relatedness among the pathovar strains of *Pseudomonas syringae* subsp. *savastanoi* Janse (1982) and proposal of *Pseudomonas savastanoi* sp. nov. *Int J Syst Bacteriol* **42**, 606–612.

Grimont, F. & Grimont, P. A. D. (1986). Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. *Ann Inst Pasteur Microbiol* **137B**, 165–175.

Grimont, F. & Grimont, P. A. D. (1995). Determination of rRNA restriction patterns. In *Diagnostic Bacteriology Protocols*, pp. 181–200. Edited by J. Howard & D. M. Whitcombe. Totowa: Humana Press.

Hildebrand, D. C. & Schroth, M. N. (1972). Identification of the fluorescent pseudomonads. In *Proceedings of the Third International Conference on Plant Pathogenic Bacteria*, pp. 281–287. Edited by H. P. Maas Geesteranus. Wageningen: Pudoc.

Hildebrand, D. C., Schroth, M. N. & Sands, D. C. (1988). Pseudomonads. In *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 2nd edn, pp. 60–80. Edited by N. W. Schaad. St Paul, MN: American Phytopathological Society Press.

Janse, J. D., Rossi, P., Angelucci, L., Scortichini, M., Derks, J. H. J., Akermans, A. D. L., De Vrijer, R. & Psallidas, P. G. (1996). Reclassification of *Pseudomonas syringae* pv. *avellanae* as *Pseudomonas avellanae* (spec. nov.) the bacterium causing canker of hazelnut (*Corylus avellanae* L.). *Syst Appl Microbiol* **19**, 589–595.

Kersters, K., Wolfgang, L., Vancanneyt, M., De Vos, P., Gillis, M. & Schleifer, K. H. (1996). Recent changes in the classification of the pseudomonads: an overview. *Syst Appl Microbiol* **19**, 465–477.

Lelliott, R. A., Billing, E. & Hayward, A. C. (1966). A determinative scheme for the fluorescent plant pathogenic pseudomonads. *J Appl Bacteriol* **29**, 470–489.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Misaghi, I. & Grogan, R. G. (1969). Nutritional and biochemical comparisons of plant pathogenic and saprophytic fluorescent pseudomonads. *Phytopathology* **59**, 1436–1451.

Owen, R. J. & Lapage, S. P. (1976). The thermal denaturation of partly purified bacterial deoxyribonucleic acid and its taxonomic implications. *J Appl Bacteriol* **41**, 335–340.

Palleroni, N. J. (1984). Genus I *Pseudomonas* Migula 1894. In *Bergey's Manual of Systematic Bacteriology*, pp. 141–199. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Palleroni, N. J., Ballard, R. W., Ralston, E. & Doudoroff, M. (1972). Deoxyribonucleic acid homologies among some *Pseudomonas* species. *J Bacteriol* **110**, 1–11.

Pecknold, P. C. & Grogan, R. G. (1973). Deoxyribonucleic acid homology groups among phytopathogenic *Pseudomonas* species. *Int J Syst Bacteriol* **23**, 111–121.

Sands, D. C., Schroth, M. N. & Hildebrand, D. C. (1970). Taxonomy of phytopathogenic pseudomonads. *J Bacteriol* **101**, 9–23.

Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (editors) (1989). *Approved Lists of Bacterial Names*. Washington, DC: American Society for Microbiology.

Wayne, L. G., Brenner, D. J., Colwell, R. R. and 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Willems, A., De Vos, P., Gillis, M. & Kersters, K. (1992). Toward improved classification of Pseudomonas. In *Identification Methods in Applied and Environmental Microbiology*, vol. 29, pp. 21–43. Edited by R. G. Board, D. Jones & F. A. Skinner. Oxford: Blackwell Scientific Publications.

Young, J. M. (1992). *Pseudomonas syringae* pv. *japonica* (Mukoo 1955) Dye *et al.* 1980 is a junior synonym of *P. syringae* pv. *syringae* van Hall 1902. *Lett Appl Microbiol* **15**, 129–130.

Young, J. M. & Triggs, C. M. (1994). Evaluation of determinative tests for pathovars of *Pseudomonas syringae* van Hall 1902. *J Appl Bacteriol* **77**, 195–207.

Young, J. M., Takikawa, Y., Gardan, L. & Stead, D. E. (1992). Changing concepts in the taxonomy of plant pathogenic bacteria. *Annu Rev Phytopathol* **30**, 67–105.