International Journal of Systematic and Evolutionary Microbiology (2002), **52**, 2065–2074 DOI: 10.1099/ijs.0.02225-0

NOTE

Pseudomonas salomonii sp. nov., pathogenic on garlic, and *Pseudomonas palleroniana* sp. nov., isolated from rice

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A total of 26 strains, including 15 strains isolated from garlic plants with the typical symptoms of 'Café au lait' disease and 11 strains isolated from diseased or healthy rice seeds and sheaths infested by *Pseudomonas fuscovaginae*, were compared with 70 type or reference strains of oxidase-positive pathogenic or non-pathogenic fluorescent pseudomonads. The strains were characterized by using a polyphasic taxonomic approach. Numerical taxonomy of phenotypic characteristics showed that the garlic and rice strains were related to each other. However, they clustered into separate phenons, distinct from those of the other strains tested, and were different in several nutritional tests. On the basis of DNA–DNA hybridization, the garlic and rice strains constituted two distinct DNA hybridization groups, indicating that they belonged to separate species. The two groups of strains were also well differentiated by siderotyping. Garlic strains were pathogenic to garlic plants and either weakly pathogenic or non-pathogenic on rice; rice strains were either weakly pathogenic or non-pathogenic on rice and non-pathogenic on garlic. A phylogenetic analysis of 16S rRNA gene sequences confirmed that the two groups of strains belonged to the *γ-Proteobacteria* and to the genus *Pseudomonas*. The names *Pseudomonas salomonii* sp. nov. and *Pseudomonas palleroniana* sp. nov. are respectively proposed for the garlic strains and the rice strains. The type strains are *P. salomonii* CFBP 2022^T (= ICMP 14252^T = NCPPB 4277^T) and *P. palleroniana* CFBP 4389^T (= ICMP 14253^T = NCPPB 4278^T).

Keywords: *Pseudomonas salomonii*, *Pseudomonas palleroniana*, polyphasic taxonomy, DNA–DNA hybridization, phenotypic characteristics

Phytopathogenic, fluorescent pseudomonads are clustered into the *γ*-subclass of the *Proteobacteria* (Woese, 1987). They are divided into two main groups, the oxidase-negative pseudomonads, which include *Pseudomonas syringae* pathovars and related bacteria, and the oxidase-positive pseudomonads, including *Pseudomonas agarici*, *Pseudomonas cichorii*, *Pseudomonas corrugata*, *Pseudomonas fuscovaginae*, *Pseudomonas tolaasii*, *Pseudomonas asplenii* and *Pseudomonas marginalis*, which are respectively pathogenic to mushroom, lettuce, tomato, rice, mushroom, asplenium and miscellaneous host plants (Palleroni, 1984; Young *et al.*, 1992).

The causal agent of a bacterial disease of garlic (*Allium sativum*), in France called 'Café au lait', was initially assigned to a particular strain of biovar I of *Pseudomonas fluorescens* by Samson (1982). This disease was further reported to occur in Italy (Calzolari & Bazzi, 1985). Girard *et al.* (1994) described *P. fluorescens* biovar V strains isolated from garlic in La Réunion as being closely related to *P. fuscovaginae* and pathogenic to both garlic and rice. In the traditional garlic-culture areas of southern France, the disease symptoms appear in April and May. Garlic plants develop yellowing and wilting of one or two leaves in connection with brown rot of the pseudostem, but the first lesions generally

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA sequences of strains CFBP 2022^T and CFBP 4389^T are AF091528 and AF091527.

Table 1. Origins of the garlic and rice strains used in this study

All strain accession numbers are from the CFBP.

Strain	Year of isolation	Host plant	Geographical origin
P. salomonii sp. nov.			
2022^T, 4273, 4274, 4281, 4378, 4381	1976	*Allium sativum**	France†
4283	1977	*Allium sativum*	France
4276, 4277, 4278, 4279, 4280, 4380	1978	*Allium sativum*	France
4275, 4282	1978	Soil	France
P. palleroniana sp. nov.			
4386, 4397	1988	*Oryza sativa*	La Réunion, France
4389^T, 4398, 4399	1988	*Oryza sativa*	Cameroon
4390, 4391, 4392, 4393, 4394, 4395	1988	*Oryza sativa*	Madagascar

* Various cultivars.

† *P. salomonii* strains were isolated from various locations in southern France.

appear at the base of the leaf sheath on internal leaves. The occurrence of the most severe cases is related to very rainy periods, and the disease leads to the death of the plant. Most frequently, bulb formation is not affected and the disease is restricted to the tunics, which become torn and dark brown (Samson, 1982).

From a collection of 85 garlic-pathogenic strains, isolated from garlic-cultivation plots from various locations, 15 (referred to as garlic strains) were selected for inclusion in the present study. Other strains isolated from rice in La Réunion, Cameroon and Madagascar in 1988 and initially referred to as *P. fluorescens* were later shown to be phenotypically closely related to the garlic strains. Since the original description (Samson, 1982), no comprehensive study has been done on the strains pathogenic to garlic, and the strains isolated from rice have never been well characterized. Therefore, garlic and rice strains were included in the present study for extended comparison purposes. Thus, we undertook a polyphasic study to characterize these two groups of bacteria, using numerical analysis of phenotypic characteristics, siderophore-typing, DNA–DNA hybridization, 16S rRNA gene sequencing and G+C content.

Our results demonstrate that the above-mentioned garlic strains and rice strains represent two novel species, for which the names *Pseudomonas salomonii* sp. nov. and *Pseudomonas palleroniana* sp. nov. are respectively proposed.

Bacterial strains

A total of 96 strains were included in this study. A first group of strains contained 15 strains isolated from garlic plants grown in southern France and showing symptoms typical of 'Café au lait' disease (Table 1). The 11 strains of a second group were isolated in 1988 from rice grown in La Réunion, Cameroon and Madagascar from healthy or necrotic rice seeds and from diseased tissue from leaf sheaths (Table 1). The 70 other type and reference strains are listed in Table 2. The bacteria were routinely cultured on YBGA [0·7% yeast extract; 0·7% Bacto peptone (Difco); 0·7% glucose and 1·5% agar; pH 7·2] at 25 °C (Gardan *et al*., 1992).

Numerical taxonomy of biochemical and physiological tests

A total of 167 tests, including assimilation of 147 carbohydrate sources (API 50 CH, AA and AO strips; bioMérieux) and 20 biochemical and physiological tests, were performed and included in a numerical taxonomy as indicated by Gardan *et al*. (1992).

A dendrogram displaying distance relationships between the strains is shown in Fig. 1. At a distance of 0·15, we observed eight phenons, among which were five phenons that contained four or more strains, and 19 unclustered strains.

Phenon 1 contained 26 strains that were divided at a distance of 0·13 into two subphenons, which clustered the 15 strains isolated from garlic (phenon 1a) and the 11 strains isolated from rice (phenon 1b). Thus, the garlic strains and the rice strains constituted discrete, homogeneous phenotypic groups. Phenon 2 contained eight *P. tolaasii* strains, including the type strain, CFBP 2068^T. Phenon 3 contained two strains isolated from mushroom, '*Pseudomonas gingeri*' and *P. tolaasii*. Phenons 4 and 5 respectively included four and two strains of *P. marginalis*. Phenon 6 contained 14 strains of *P. fuscovaginae*, including the type strain, CFBP 2065^T. Phenon 7 contained 14 *P. corrugata* strains, including the type strain, CFBP 2431^T. Phenon 8 contained seven of nine *P. cichorii* strains tested; the

other two strains were clustered at a greater distance. The 19 unclustered strains corresponded to type or reference strains of fluorescent, oxidase-positive *Pseudomonas* species included in this study (Table 2; Fig. 1).

The phenotypic characteristics that differentiate the eight phenons are shown in Table 3. At least two tests were able to discriminate phenons and unclustered strains from each other. The strains belonging to phenons 1a (garlic strains) and 1b (rice strains) were discriminated by three biochemical tests: phenon 1a gave positive results in adonitol, xylitol and L-arabitol tests, whereas phenon 1b gave negative results. In addition, 5-ketogluconate utilization was positive for 86% of phenon 1a strains but negative for phenon 1b strains; conversely, assimilation of L-methionine was negative for phenon 1a strains and positive for 81% of phenon 1b strains (data not shown).

Siderotyping behaviour

Cultures for pyoverdine production and the electrophoretic characterization of the pyoverdine isoforms that accumulated in growth media were done according to Meyer *et al.* (1998), with the exception that isoelectric pH (pI) values were determined by using an internal standard made of a mixture of pyoverdines with defined pI values, as described by Fuchs *et al.* (2001). Purification of pyoverdines by the XAD chromatographic procedure and their use in pyoverdine-mediated ^{59}Fe uptake were performed as described previously (Meyer *et al.*, 1998).

As illustrated in Table 4, the garlic strains had an identical pyoverdine IEF pattern (pI 8·5, 7·3, 7·2), which was different from the pattern characterizing the rice strains (pI 8·8, 7·3, 7·2). None of the pyoverdine IEF patterns of strains belonging to other fluorescent *Pseudomonas* species was identical to those of the garlic or rice strains (Table 4). Pyoverdine-mediated iron-uptake studies confirmed the uniqueness of the pyoverdine system within each of the new groups. Each strain within a new group was able to incorporate, at the same efficiency as its own pyoverdine, the pyoverdine of the respective type strain (data not shown). Furthermore, pyoverdine-mediated iron cross-incorporation tests, involving the type strain of one new group with the pyoverdine of the other new group, also allowed easy discrimination between the two groups. No incorporation was observed when the type strain isolated from rice was tested with the pyoverdine of garlic strains as iron transporter, whereas weak incorporation, representing 17% or less of the homologous incorporation, was detectable when garlic strains were tested with the pyoverdine of rice strains. Thus, the siderotyping data allowed the easy and rapid discrimination of garlic and rice strains, the two groups, as shown by IEF and by uptake, forming well-differentiated novel siderovars. These conclusions reinforce the view that precise taxonomic allocation can be achieved through siderotyping, as already demonstrated for other recently described species, i.e. *Pseudomonas brassicacearum* and *Pseudomonas thivervalensis* (Achouak *et al.*, 2000), *Pseudomonas jessenii*, *Pseudomonas mandelii*, *Pseudomonas monteilii*, *Pseudomonas rhodesiae* and *Pseudomonas veronii* (Meyer, 2000) or *P. tolaasii* (Munsch *et al.*, 2000).

DNA base composition

The G+C contents of the DNA of strains CFBP 2022^T and CFBP 4389^T were determined by the thermal denaturation temperature method of Marmur & Doty (1962) and were calculated by using the equation of Owen & Lapage (1976). The DNA G+C contents of the two garlic strains CFBP 2022^T and CFBP 4282 were respectively 60·2 and 58·7 mol%. For rice strains CFBP 4389^T and CFBP 4392, the values were respectively 60·2 and 61·2 mol%. Thus, the G+C contents of the garlic strains and rice strains were 58·7–61·2 mol%, within the range of the genus *Pseudomonas* (Palleroni, 1984).

DNA–DNA hybridization

DNA–DNA hybridization experiments were done with labelled DNA (^{3}H nucleotides; Amersham) from strains CFBP 2022^T and CFBP 4389^T as described by Gardan *et al.* (1992). The reassociation temperature was 70 °C.

The results of DNA relatedness studies are shown in Table 4. The strains isolated from garlic gave hybridization values of 79–100% with strain CFBP 2022^T and gave lower values (10–39%) with rice strains and with type strains or other reference strains tested. For rice strain CFBP 4389^T, which exhibited the highest percentage of reassociation (47%), the ΔT_m value was 6·4 °C. *P. fluorescens* CFBP 2102^T and '*Pseudomonas orientalis*' CFBP 4863, the two closest strains according to 16S rDNA sequencing, were respectively 40 and 39% related to garlic strain CFBP 2022^T. Thus, the garlic strains can be considered as members of a single DNA hybridization group.

The rice strains were 68–100% related to strain CFBP 4389^T; for strains CFBP 4386 and 4398, showing the two lowest DNA relatedness values (68%), the ΔT_m values were respectively 2·2 and 1·9 °C. The rice strains gave very low hybridization values with the garlic strains (45–48%) and type or reference strains of the species tested (10–42%). The type strain of *P. tolaasii*, CFBP 2068^T, the closest strain according to rDNA sequencing, was only 41% related to rice strain CFBP 4389^T. For garlic strain CFBP 2022^T (48% reassociation), the ΔT_m value was 7·6 °C. Thus, the rice strains constitute a unique DNA hybridization group. The results of our DNA studies clearly indicate that the garlic and rice strains constitute two homogeneous genomospecies as defined by Wayne *et al.* (1987).

Table 2. Origin of *Pseudomonas* reference strains used

Strain accession numbers as received are given, together with the corresponding CFBP accession number if different. bv., Biovar; NK, host plant or origin unknown; pv., pathovar.

Strain	Host plant or origin and place
P. marginalis pv. *alfalfae* NCPPB 2644 (= CFBP 2039)	*Medicago sativa*, USA
P. aeruginosa ATCC 10145^T (= CFBP 2466^T)	NK
P. agarici ICMP 2656^T (= CFBP 2063^T)	*Agaricus bisporus*, New Zealand
P. asplenii ICMP 3944^T (= CFBP 3279^T)	*Asplenium nidus*
P. aureofaciens ATCC 13985^T (= CFBP 2133^T)	
P. chlororaphis ATCC 9446^T (= CFBP 2132^T)	
P. cichorii	
CFBP 4400	*Allium sativum*, La Réunion, France
CFBP 4401	*Allium sativum*, La Réunion, France
CFBP 4402	*Lactuca sativa*, France
CFBP 4403	*Cichorium endivia*, France
CFBP 4404	*Lycopersicon esculentum*, France
CFBP 4406	*Cichorium endivia*, France
CFBP 4405	*Cichorium endivia*, France
CFBP 4407	*Lactuca sativa*, France
NCPPB 943^T (= CFBP 2101^T)	*Cichorium endivia*
P. corrugata	
CFBP 4562	*Lycopersicon esculentum*, Italy
CFBP 4563	*Lycopersicon esculentum*, Italy
ICMP 4898 (= CFBP 4043)	*Lycopersicon esculentum*, New Zealand
ICMP 8270 (= CFBP 4038)	*Medicago sativa*, USA
ICMP 8271 (= CFBP 4039)	*Medicago sativa*, USA
ICMP 8890 (= CFBP 4040)	*Lycopersicon esculentum*, New Zealand
ICMP 8892 (= CFBP 4041)	*Lycopersicon esculentum*, New Zealand
ICMP 8894 (= CFBP 4042)	*Lycopersicon esculentum*, New Zealand
NCPPB 2445^T (= CFBP 2431^T)	*Lycopersicon esculentum*, UK
NCPPB 2447 (= CFBP 4025)	*Lycopersicon esculentum*, UK
NCPPB 2451 (= CFBP 4026)	*Lycopersicon esculentum*, UK
NCPPB 2457 (= CFBP 4561)	*Lycopersicon esculentum*, UK
NCPPB 2457 (= CFBP 4027)	*Lycopersicon esculentum*, UK
NCPPB 247 (= CFBP 4031)	*Lactuca sativa*, USA
'*P. flectens*' ICMP 745 (= CFBP 3281)	*Phaseolus vulgaris*, Australia
P. fluorescens bv. II ATCC 17482 (= CFBP 2125)	
P. fluorescens bv. III ATCC 17400 (= CFBP 2127)	Egg, USA
P. fluorescens bv. V ATCC 17386 (= CFBP 2130)	Water
P. fluorescens bv. V CFBP 2298	*Malus sylvestris*, France
P. fluorescens bv. V CFBP 4558	*Allium sativum*, La Réunion, France
P. fuscovaginae	
CFBP 2065^T	*Oryza sativa*, Japan
CFBP 2801	*Oryza sativa*, Japan
CFBP 2803	*Oryza sativa*, Japan
CFBP 2804	*Oryza sativa*, Japan
CFBP 2805	*Oryza sativa*, Burundi
CFBP 2808	*Oryza sativa*, Madagascar
CFBP 3078	*Triticum* sp., Mexico
CFBP 3080	*Secalotriticum* sp., Mexico
CFBP 3081	*Triticum durum*, Mexico
CFBP 3082	Grass, Mexico
CFBP 3083	Grass, Mexico
CFBP 3084	*Triticum* sp., Mexico
CFBP 4558	*Allium sativum*, La Réunion, France

Table 2 (*cont.*)

Strain	Host plant or origin and place
CFBP 4559	*Allium sativum*, La Réunion, France
'*P. gingeri*' NCPPB 3146 (= CFBP 2810)	*Agaricus bisporus*, UK
P. marginalis	
ICMP 6005 (= CFBP 4051)	Russia
ICMP 6993 (= CFBP 4044)	*Allium porrum*, New Zealand
P. marginalis pv. *marginalis*	
NCPPB 1558 (= CFBP 4034)	*Phaseolus coccineus*, UK
NCPPB 1679 (= CFBP 4036)	*Chrysanthemum moriflorum*, UK
NCPPB 2630 (= CFBP 4037)	*Phragmipedium vittatum*, UK
NCPPB 667^T (= CFBP 3300^T)	*Cichorium intybus*, USA
P. marginalis pv. *pastinacea*	
NCPPB 806 (= CFBP 2038)	*Pastinaca sativa*, USA
P. putida ATCC 12633^T (= CFBP 2066^T)	Soil, USA
P. tolaasii	
CFBP 2152	*Agaricus bisporus*, France
ICMP 4227 (= CFBP 4054)	*Agaricus bisporus*, New Zealand
ICMP 6553 (= CFBP 4055)	*Agaricus bisporus*, Canada
ICMP 6643 (= CFBP 4056)	*Agaricus bisporus*, Australia
ICMP 6955 (= CFBP 4058)	*Agaricus bisporus*, New Zealand
ICMP 7065 (= CFBP 4059)	*Agaricus bisporus*, New Zealand
NCPPB 2192^T (= CFBP 2068^T)	*Agaricus bisporus*, UK
NCPPB 2325 (= CFBP 4030)	*Agaricus bisporus*, Italy
NCPPB 741 (= CFBP 4029)	*Agaricus bisporus*, The Netherlands

Sequencing of 16S rDNA and phylogenetic analysis

The 16S rRNA gene was amplified by PCR using universal primers 16F27 and 16R1525 (Hauben *et al.*, 1998). Amplified 16S rDNAs from strains CFBP 2022^T and CFBP 4389^T were purified using the QIAquick PCR purification kit (Qiagen) and then subjected to direct sequencing using the ABI PRISM Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems) according to the instructions of the supplier. DNA sequencing was performed using primers described previously (Achouak *et al.*, 1999). The 16S rDNA sequences were aligned automatically and then manually by reference to a database of 35000 previously aligned bacterial 16S rDNA sequences. Phylogenetic analysis combining neighbour-joining, maximum likelihood, parsimony and bootstrap analysis were performed as detailed by Ivanova *et al.* (2002). For Fig. 2, retaining only sequences of the genus *Pseudomonas*, mostly for reference strains (66 sequences), allowed the inclusion in the analysis of almost the entire available 16S rDNA sequences, corresponding to positions 1–1176 of the garlic strain CFBP 2022^T sequence (some shorter sequences did not allow the 164 nt at the 3′ end of the sequences to be taken into account). The topology shown is that of the bootstrap analysis using the BIONJ algorithm; as a result, there is no distance bar in this tree. The analysis was done with the 66 sequences. Fig. 2 is a subset (41 sequences) of the total analysis.

16S rDNA sequence analysis revealed that the bacteria studied are members of the *γ-Proteobacteria* and, more precisely, that they are included in the clade formed by the genus *Pseudomonas*.

Rice strain CFBP 4389^T clustered robustly with *P. tolaasii*, for which two slightly different sequences were available from two different sources. Comparisons of aligned sequences showed that sequence Z76670 for *P. tolaasii* had a few undetermined nucleotides and errors at positions 5 (T instead of A), 30 (C for G), 650–681 (errors and incorrect insertions of nucleotides), 709 (C for G), 714 (T for A) and 111 (G missing). These errors were deleted because they corresponded to conserved nucleotides in all other sequences. Thus, according to the 16S rDNA sequences, rice strain CFBP 4389^T can be clustered with *P. tolaasii*, and DNA–DNA hybridization studies are the only means of concluding that they are distinct species.

For garlic strain CFBP 2022^T, phylogenetic analyses of 16S rDNA sequences showed no robust clustering with any recognized species, thus suggesting that this strain represents a novel species within the genus *Pseudomonas*. Both results are consistent with phenotypic characteristics and DNA–DNA hybridization experiments.

Pathogenicity tests on rice and garlic

In order to test pathogenicity, 10–18 rice seedlings were inoculated with the following 17 strains: a reference strain of *P. fuscovaginae* (CFBP 3078), five

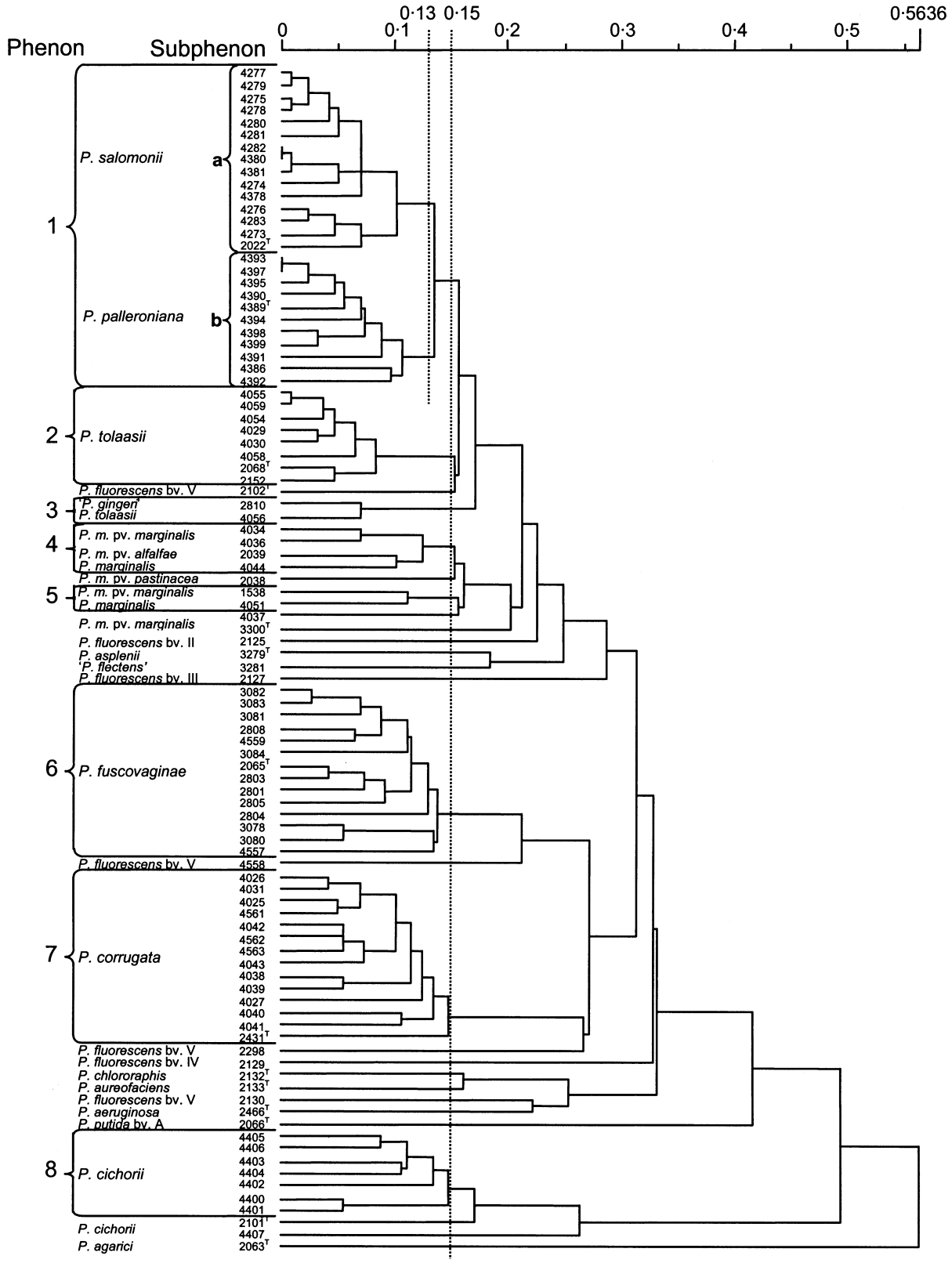

Fig. 1. Dendrogram of phenotypic distances of the 96 strains under study. Strain accession numbers are from the CFBP.

Table 3. Phenotypic characteristics that differentiate phenon 1 from other phenons

Phenons represent *P. salomonii* sp. nov. and *P. palleroniana* sp. nov. (phenon 1), *P. tolaasii* (2), '*P. gingeri*' (3), *P. marginalis* (4 and 5), *P. fuscovaginae* (6), *P. corrugata* (7) and *P. cichorii* (8). +, 90–100 % of strains positive; −, 90–100 % of strains negative; d, 11–89 % of strains positive (numbers in parentheses are percentages of strains that tested positive).

Characteristic	Phenon							
	1*	2	3	4	5	6	7	8
Number of strains in phenon	26	9	2	5	2	14	14	7
Production of pyoverdin	+	+	+	+	+	+	−	d (85)
Arginine dihydrolase	+	+	+	+	+	+	+	−
Gelatin hydrolysis	+	+	+	+	d (50)	d (64)	+	−
Nitrate reduction	−	−	−	+	+	−	+	d (14)
Aesculin	−	−	−	d (75)	+	−	−	+
Pectin	−	d (12)	−	+	+	−	d (35)	−
Hypersensitivity reaction	−	−	−	−	−	d (28)	d (35)	+
Utilization of carbohydrates and polyalcohols:								
Sucrose	+	−	−	+	+	−	+	−
L-Arabinose	+	−	d (50)	+	+	+	+	+
Sorbitol	+	+	+	+	+	−	−	−
Xylitol	+/−, d (57)	+	+	d (75)	d (50)	−	−	−
L-Arabitol	+/−, d (61)	+	−	+	−	−	−	−
Adonitol	+/−, d (57)	+	d (50)	+	−	−	−	−
Inositol	+	+	+	+	+	−	+	+
Erythritol	+	+	+	+	−	−	−	d (14)
Utilization of organic salts:								
D(−)-Tartrate	−	−	−	d (75)	+	−	d (28)	+
L(+)-Tartrate	−	−	−	−	−	−	d (14)	+
Itaconate	+	+	+	+	+	−	−	−
meso-Tartrate, mesaconate, 2-ketogluconate	+	+	+	d (75)	+	+	−	+
Acetate	+	+	+	+	+	+	+	−
p-Aminobenzoate	+	+	−	+	+	+	−	−
Citraconate	+	+	+	+	+	+	−	−
Utilization of amino acids								
L-Tryptophan	+	+	−	+	+	−	−	−
Trigonelline	−	−	−	+	+	d (78)	+	+
L-Citrulline	+	d (12)	d (50)	d (50)	+	d (78)	−	−
Glucosamine, L-leucine, L-phenylalanine	+	+	+	+	+	+	+	−
Histamine	−	−	+	−	−	−	−	−
DL-Kynurenine	+	d (87)	−	+	+	−	−	−
Spermine	+	+	+	+	+	−	+	−

* Where phenons 1a and 1b gave different reactions, the results for 1a/1b are given, followed by the percentage of phenon 1 strains that tested positive.

garlic strains and the 11 rice strains. Bacterial cultures grown for 24 h on King's medium B (King *et al.*, 1954) were harvested in sterile distilled water. Aqueous cell suspensions (10^8 c.f.u. ml^{-1}) were injected with a syringe into 3-week-old rice seedlings (variety IRAT 13) between leaf sheaths about 5 cm above the soil until the inoculum filled up the intersheath spaces. Plants were grown in a greenhouse according to Rott *et al.* (1991). The pathogenicity tests were repeated twice.

Bacterial suspensions (5×10^8 c.f.u. ml^{-1}) of 15 garlic strains were injected by syringe into the parenchyma of garlic leaves through the plant pseudostem. Inoculations were performed in the glasshouse in winter (three plants of cultivar 'Blanc de Beaumont') and in the field at the beginning of April (10 plants with five to six leaves of two cultivars, 'Meristem Violet' and 'Blanc de Beaumont'). A disease scale from 1 (limited chlorotic stripe at the inoculation point) to 5 (total soft rot and plant death) was used to record disease. The symptoms appeared 8–14 days after inoculation. The inoculation tests were repeated three or four times for each strain.

After inoculation on garlic plants, all garlic strains induced collapse of the infiltrated areas followed by a

Table 4. Levels of DNA relatedness and pyoverdine isoforms

Values in parentheses are ΔT_m values (°C). NT, Not tested; Pvd (−), pyoverdine not produced.

Source of unlabelled DNA	Relative binding (%) at 70 °C with labelled DNA from:		pI values of pyoverdine isoforms
	P. salomonii CFBP 2022^T	*P. palleroniana* CFBP 4389^T	
P. salomonii sp. nov.			
CFBP 2022^T	100	48 (7·6)	8·5, 7·3, 7·2
CFBP 4273	85	47	8·5, 7·3, 7·2
CFBP 4379	85	NT	8·5, 7·3, 7·2
CFBP 4274	95	NT	8·5, 7·3, 7·2
CFBP 4275	89	NT	8·5, 7·3, 7·2
CFBP 4280	79	NT	8·5, 7·3, 7·2
CFBP 4282	85	45	8·5, 7·3, 7·2
CFBP 4277	83	NT	8·5, 7·3, 7·2
P. palleroniana sp. nov.			
CFBP 4389^T	47 (6·4)	100	8·8, 7·3, 7·2
CFBP 4390	46	88	8·8, 7·3, 7·2
CFBP 4394	34	88	8·8, 7·3, 7·2
CFBP 4397	33	81	8·8, 7·3, 7·2
CFBP 4386	NT	68 (2·2)	8·8, 7·3, 7·2
CFBP 4391	NT	76	8·8, 7·3, 7·2
CFBP 4392	NT	80	8·8, 7·3, 7·2
CFBP 4393	NT	79	8·8, 7·3, 7·2
CFBP 4395	NT	80	8·8, 7·3, 7·2
CFBP 4398	NT	68 (1·9)	8·8, 7·3, 7·2
CFBP 4399	NT	84	8·8, 7·3, 7·2
P. fluorescens bv. V CFBP 4557	16	27	Pvd (−)
P. fluorescens bv. I CFBP 2102^T	40	42	8·7, 7·3, 7·1
P. tolaasii CFBP 2068^T	28	41	9·1, 7·5, 7·3
P. marginalis pv. *marginalis* CFBP 3300^T	32	40	8·8, 7·7
P. cichorii CFBP 2101^T	10	13	7·2, 5·7, 4·5
P. fuscovaginae CFBP 2065^T	16	20	9·0, 7·4, 5·1, 4·3
P. corrugata CFBP 2431^T	15	21	Pvd (−)
P. aeruginosa CFBP 2466^T	13	15	8·8, 8·4, 7·4, 5·1
P. putida bv. A CFBP 2066^T	15	10	4·6, 4·2, 4·0
P. agarici CFBP 2063^T	14	17	NT
P. asplenii CFBP 3279^T	26	20	7·8, 5·7, 4·7
'*P. orientalis*' CFBP 4863	39	NT	8·8, 8·7, 7·3

soft rot of several leaves, often leading to the death of the plant (Table 5). Similar results were obtained with the two garlic cultivars that were used. The reference strains (including *P. fluorescens* CFBP 2102^T) inoculated as controls did not induce any reaction, with the exception of *Pseudomonas chlororaphis* CFBP 2132^T and *P. tolaasii* CFBP 2068^T. These two strains rapidly caused a white collapsed stripe to appear on a few leaves, but without further progress of the symptoms. They were therefore not considered to be pathogenic to garlic.

The five garlic strains were non-pathogenic, or were very weakly pathogenic, on rice. A few plants showed a restricted necrosis that did not reach 50% of the sheath length in experiment 1, and none of the inoculated plants showed symptoms in experiment 2. The 11 rice strains were either non-pathogenic or weakly pathogenic, and never caused death of the rice plants within the 1-month observation period after inoculation. Some strains never caused symptoms, while others induced limited necrosis that reached 50% or more of the sheath length. The rice strains are mostly secondary invaders of rice lesions caused by *P. fuscovaginae*. Typical symptoms of bacterial sheath brown rot of rice were obtained with *P. fuscovaginae* CFBP 3078 but not with the garlic strains or with the rice strains. The bacterial strains clearly differed in virulence on rice.

On the basis of phenotypic properties, DNA–DNA reassociation values, 16S rDNA sequencing, G+C

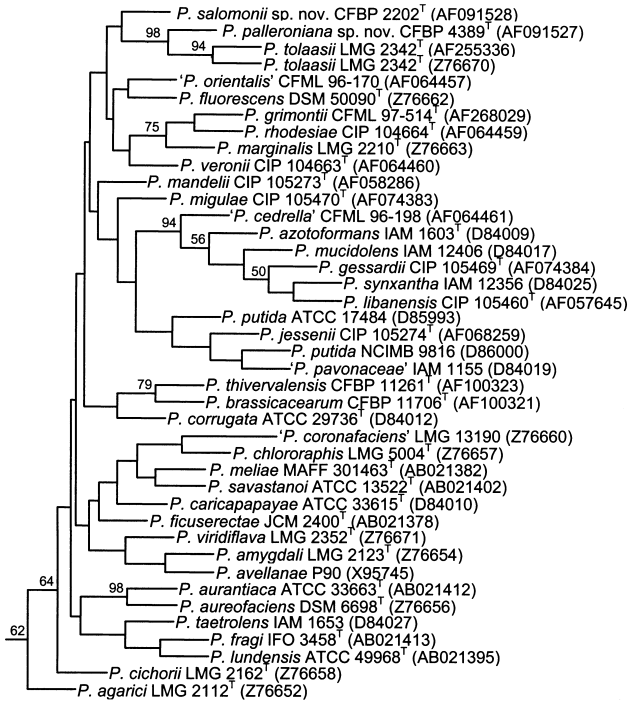

Fig. 2. Phylogenetic tree obtained by 16S rDNA sequence analysis. The topology shown is a restricted subset of a larger analysis including every type species of the genus *Pseudomonas*. This tree was obtained using the bioNJ algorithm and 500 bootstrap replications. Percentages of bootstrap support are indicated only for branches that were also retrieved by maximum parsimony and maximum likelihood ($P < 0{\cdot}01$); these branches should be considered as the only robust clusters identified by this analysis.

content and siderotyping, the strains included in phenons 1a (garlic strains) and 1b (rice strains) should be considered as two novel species. The name *Pseudomonas salomonii* sp. nov. is proposed for the garlic strains, with strain CFBP 2022^T as the type strain, and the name *Pseudomonas palleroniana* sp. nov. is proposed for the strains isolated from rice, with CFBP 4389^T as the type strain.

Description of *Pseudomonas salomonii* sp. nov.

Pseudomonas salomonii (sa.lo.mo′ni.i. N.L. gen. n. *salomonii* of Salomon, referring to Max Salomon, the agricultural technical advisor who first observed the symptoms of the new garlic disease 'Café au lait').

Gram-negative, motile, non-spore-forming rods. Forms typical white-cream, circular, smooth colonies, 3–4 mm in diameter after 48 h growth on YBGA at 28 °C. A fluorescent pigment is produced on King's medium B. Strictly aerobic. Pectolytic on Hildebrand's medium (pH 8·3). Oxidase, arginine dihydrolase, levan production and gelatinase reactions are positive. Nitrate reduction and aesculin hydrolysis are negative. Growth is observed on D-xylose, D-glucose, trehalose, lyxose, malonate, DL-glycerate, D-malate, L-isoleucine, L-valine, L-tyrosine, L-ornithine, L-arginine, *β*-alanine, *p*-aminobenzoate, sarcosine, ethanolamine, diaminobutyrate and on the carbon sources listed as positive in Table 3. There was no assimilation of L-rhamnose, arbutin, aesculin, melibiose, raffinose, butyrate, isovalerate, DL-2-aminobutyrate, L-methionine or the carbon sources mentioned as negative in Table 3. The G+C content of the DNA of the type strain, CFBP 2022^T, is 60·2 mol%. The hypersensitive reaction on tobacco leaves is positive just after isolation and negative after subculturing several times. Strains are pathogenic to *Allium sativum*, producing the typical symptoms of 'Café au lait' disease. The type strain has been deposited in the Collection Française des Bactéries Phytopathogènes (CFBP), Angers, France, as CFBP 2022^T, in the International Collection of Microorganisms from Plants (ICMP), Auckland, New Zealand, as ICMP 14252^T and in the National Collection of Plant-Pathogenic Bacteria (NCPPB), Sand Hutton, York, UK, as NCPPB 4277^T.

Table 5. Results of pathogenicity tests on garlic

All strain accession numbers are from the CFBP. Disease index: 0, no reaction; 1, limited chlorotic stripe at the inoculation point; 2, white collapsed stripe on one leaf; 3, two or three collapsed leaves; 4, deformation and wilting of the plant; 5, plant death.

Strains	Disease index
Garlic isolates	
4273, 4280, 4281, 4282, 4378	5
4276, 4380, 4275, 4279, 4274, 4283, 4379, 4381	4–4·7
2022^T, 4278, 4277	3·2–3·6
P. chlororaphis 2132^T, *P. tolaasii* 2068^T	1·3
P. aureofaciens 2133^T, *P. marginalis* pv. *pastinacea* 2038	0·8
P. fluorescens bv. V 2130, *P. marginalis* pv. *alfalfae* 2039, *P. agarici* 2063^T, *P. fuscovaginae* 2065^T, *P. putida* 2066^T, *P. cichorii* 2101^T, *P. fluorescens* 2102^T, *P. viridiflava* 2107^T, *P. marginalis* pv. *marginalis* 3300^T	0

Description of *Pseudomonas palleroniana* sp. nov.

Pseudomonas palleroniana (pal.le.ron.i.a′na. N.L. fem. adj. *palleroniana* pertaining to N. J. Palleroni, the famous microbiologist).

Gram-negative, motile, non-spore-forming rods. Forms typical white-cream colonies, 3–4 mm in diameter after incubation on YBGA at 28 °C. Produces a fluorescent pigment on King's medium B. Shares the same characteristics as those described above for *P. salomonii* except for the results of assimilation of adonitol, xylitol, L-arabitol and 5-ketogluconate, which are negative. Assimilation is variable for isobutyrate, D-hydroxybenzoate, L-methionine, L-lysine and tryptamine. The G+C content of the DNA of the type strain, CFBP 4389^T, is 60·2 mol%. The hypersensitive reaction on tobacco leaves is negative. The strains are either weakly pathogenic or non-pathogenic on rice. The type strain is strain CFBP 4389^T (= ICMP 14253^T = NCPPB 4278^T).

Acknowledgements

We thank D. Tharreau for his help with the rice pathogenicity tests. We are grateful to Solange Belouin and Alain Huard for technical assistance.

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