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Pseudomonas frederiksbergensis sp. nov., isolated from soil at a coal gasification site

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Phenotypic and genotypic characterization indicated that a group of 29 closely related phenanthrene-degrading bacteria from a coal gasification site in Frederiksberg, Copenhagen, Denmark, belonged to the genus *Pseudomonas*. The strains were isolated at two sampling occasions 2 years apart. The isolates were phenotypically different from any known species of the genus *Pseudomonas* and were therefore subject to further identification. Colonies were smooth and pale yellowish and did not produce pigments fluorescent in UV light when grown on King's B agar. Cells were rod-shaped, approximately 0·5–0·8 × 1·5–3·0 μm, and grew at 4 and 30 °C, but not 37 °C. The bacteria were oxidase- and catalase-positive, accumulated poly-β-hydroxybutyrate and denitrified, but did not utilize D-xylose. The mean G+C content was 59·6 mol%. Phenotypic data and 16S rDNA sequence data information for *Pseudomonas amygdali* and *Pseudomonas corrugata*, and 16S rDNA sequence data for *Pseudomonas chlororaphis* and *Pseudomonas syringae* showed close relationships to these strains. However, DNA–DNA hybridization data showed that the isolates belong to a new species, for which the name *Pseudomonas frederiksbergensis* sp. nov. is proposed. The type strain is JAJ28^T (DSM 13022^T).

Keywords: *Pseudomonas frederiksbergensis* sp. nov., soil, phenanthrene

INTRODUCTION

Pseudomonas species are important decomposers of organic matter in soil, water and food products, but are also pathogens in plants, animals and humans (Palleroni, 1993). Their biotechnological importance is partly due to their potential plant growth-promoting effects and application in biological control of fungal diseases in plants for example (Nielsen *et al*., 1998). Recent interest has also focused on the use of these bacteria in decontamination of soil (bioremediation) because of their capability to degrade xenobiotic compounds (e.g. Campbell *et al*., 1995; Johnsen *et al*., 1996; Bouchez *et al*., 1995; Kiyohara *et al*., 1992).

The genus *Pseudomonas* was previously a heterogeneous group of species, defined by a limited number of phenotypic characters. At present the genus *Pseudomonas* is synonymous with *Pseudomonas* RNA homology group I, true *Pseudomonas*, *Pseudomonas sensu stricto* and fluorescent pseudomonads (Kersters *et al*., 1996; Moore *et al*., 1996). Based on phylogenetic rRNA homology studies, several groups formerly belonging to '*Pseudomonas*' have been reclassified into the genera *Burkholderia*, *Comamonas* and *Methylobacterium*. This classification was extensively reviewed by Kersters *et al*. (1996).

During the past decade several new *Pseudomonas* species have been described, e.g. *Pseudomonas flavescens*, *Pseudomonas rhodesiae* and the plant-pathogenic *Pseudomonas avellanae* (Hildebrand *et al*., 1994; Janse *et al*., 1996; Coroler *et al*., 1996). Two other species, *Pseudomonas monteilii* and *Pseudomonas veronii*, were isolated from mineral waters (Elomari *et al*., 1997, 1996) and genomovar 6 of *Pseudomonas stutzeri* was reclassified as a new species, *Pseudomonas balearica* (Bennasar *et al*., 1996).

In a previous study we described the isolation of 22 closely related phenanthrene-degrading *Pseudomonas* spp. from a coal gasification site in Frederiksberg, Copenhagen, Denmark (Johnsen *et al*., 1996). In the

Abbreviation: PHB, poly-β-hydroxybutyrate.

The EMBL accession number for the 16S rDNA sequence of isolate JAJ28^T is AJ249382.

present study, representatives of these 22 and seven other isolates from a subsequent sampling event (Andersen *et al*., 2001) were characterized by phenotypic and genotypic methods, and confirmed to belong to the genus *Pseudomonas*. However, the isolates were different from any known *Pseudomonas* species. Based on rRNA homology, we demonstrate that the isolates show close phylogenetic relationship to *Pseudomonas chlororaphis*, *Pseudomonas cichorii*, *P. avellanae*, *Pseudomonas amygdali*, *Pseudomonas syringae* and *Pseudomonas corrugata*. Since both phenotypic traits and DNA–DNA hybridization data exclude their affiliation to any of these species, we propose that the phenanthrene-degrading *Pseudomonas* isolates should be assigned to a new species, *Pseudomonas frederiksbergensis* sp. nov., with JAJ28^T (DSM 13022^T) as type strain.

METHODS

Bacterial isolates and growth conditions. A total of 29 bacterial isolates were isolated in two different sampling events from a former coal gasification site in Frederiksberg, Copenhagen, Denmark, using *Pseudomonas* selective Gould's S1 agar (Gould *et al*., 1985; Kragelund *et al*., 1996; Johnsen & Nielsen, 1999). All isolates produced clearing zones on phenanthrene-coated minimal agar medium (Kiyohara *et al*., 1982) and did not produce fluorescent pigment on King's B medium. Twenty-two of the strains (JAJ strains) were isolated in 1994 and were tentatively identified as *Pseudomonas fluorescens* biovar III, and designated subgroup IIIc (Johnsen *et al*., 1996). In 1996, the additional seven strains (SMA strains) were isolated from the same soil and also found to belong to subgroup IIIc (Andersen *et al*., 2001) based on a number of phenotypic tests (Johnsen *et al*., 1996). Five of the 29 isolates, JAJ28^T, JAJ137, JAJ149, SMA1 and SMA5 (the JAJ28 group) were selected for further study. The five strains showed a close relationship, but with some heterogeneity based on REP-PCR (De Bruijn, 1992) and Biolog GN, and so represent the whole group. Strain JAJ28^T was selected as type strain and underwent 16S rDNA sequencing and electron microscopy. Additional tests were performed by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany. Table 1 shows the tests performed on different strains.

Unless otherwise stated, isolates were grown in Luria–Bertani (LB) medium (Maniatis *et al*., 1982) supplemented with 1 g glucose l^{-1} or streaked on LB agar plates with 1·5% (w/v) BiTek Agar (Difco). Strains from frozen cultures (−80 °C) were grown for 2–3 d on LB agar plates before further studies. The growth temperature was 30 °C, unless otherwise stated.

Biochemical and nutritional tests. Table 1 shows the tests performed on each of the 29 isolates. The five isolates in the JAJ28 group (JAJ28^T, JAJ137, JAJ149, SMA1 and SMA5) were all further tested using a number of standard assays. These were API20NE, (classical/phenotypic tests) for characterization and tentative identification (Sørensen *et al*., 1992; Johnsen *et al*., 1996) of *Pseudomonas* spp. The Biolog GN test kit was chosen to examine respiration of 95 carbon compounds. The JAJ28 group underwent further growth tests on approximately 140 different carbon sources by using API50CH, and Biolog SF-N and Biolog SF-P test kits. The medium used for API50CH inoculation was AUX medium, also used in API20NE, as suggested by the supplier (MEDA); examination was done after 48 h. Examination of Biolog SF-N and Biolog SF-P tests was done after 8 d. Poly-*β*-hydroxybutyrate (PHB) accumulation was tested by studying the FT-IR pattern and accumulation of PHB in phase-contrast microscopy with slides stained with Sudan black after growth in complex medium and minimal medium with DL-*β*-hydroxybutyrate. Tolerance to temperature was tested by growth at 4, 37 and 41 °C. Tolerance to salinity was tested by growth on tryptic soy broth (Difco) solidified with 1·5% (w/v) BiTek agar, supplemented with 2, 4 and 6% (w/v) NaCl. Tests were also performed for the presence of arginine dihydrolase (Thornley, 1960), lipase (Tween 80; Sierra, 1957), amylase (Stanier *et al*., 1966), lecithinase and gelatinase. Finally, strain JAJ28^T was selected for further phenotypic and phylogenetic characterization, as shown in

Table 1. Tests performed on different isolates from the coal gasification site

Test	Isolate
Classical tests*, API20NE, Biolog GN	JAJ16, 20, 21, 23, 25, 28^T, 29, 31, 33, 37, 39, 83, 85, 86, 97, 98, 137, 138, 139, 146, 148, 149 SMA1, 2, 4, 5, 7, 8, 9
API50CH, Biolog SF-N and Biolog SF-P, temperature and salt tolerance, PHB-accumulation†, gelatinase†, lecithinase†, presence of arginine dihydrolase, lipase, amylase, DNA–DNA filter hybridization	JAJ28^T, 137, 149 SMA1, 5
DSMZ tests†, 16S rDNA and electron microscopy	JAJ28^T

*Classical tests included: Gram stain, motility, denitrification, oxidase and catalase. Growth on *meso*-inositol, sorbitol and tryptophan, production of levan on sucrose-rich agar, blue pigment on potato dextrose agar and UV fluorescence, as described by Johnsen *et al*. (1996).

† DSMZ tests: PHB accumulation, aminopeptidase, gelatinase, lecithinase, urease, G+C content, spectroscopic DNA–DNA hybridization.

Table 2. DNA homology between JAJ28^T and other *Pseudomonas* strains, including type strains from culture collections

Source of unlabelled DNA	DNA homology*	
	DNA–DNA filter hybridization	Spectroscopic DNA–DNA hybridization†
JAJ28^T (DSM 13022^T)	100	100
JAJ137	100	ND
JAJ149	100	ND
SMA1	73	ND
SMA5	72	ND
Pseudomonas alcaligenes DSM 50342^T	25	ND
'*Pseudomonas aureofaciens*' ATCC 13985	33	ND
Pseudomonas mendocina DSM 50017^T	15	ND
Pseudomonas pseudoalcaligenes DSM 50188^T	11	ND
Pseudomonas putida DSM 291^T	28	ND
Pseudomonas stutzeri DSM 5190^T	14	ND
Escherichia coli DSM 498	9	ND
Pseudomonas aeruginosa DSM 50071^T	18	ND
Pseudomonas avellanae DSM 11809^T	19	ND
Pseudomonas cichorii DSM 50259^T	20	ND
Pseudomonas chlororaphis DSM 50083^T	18	ND
Pseudomonas fluorescens DSM 50090^T	19	45
Pseudomonas corrugata DSM 7228^T	19	52
Pseudomonas syringae DSM 6693^T	20	43
Pseudomonas amygdali DSM 7298^T	ND	54

* ND, Not determined.

† DSMZ test.

Table 1, including examination for extracellular production of aminopeptidase and urease.

DNA base composition. The G+C content (mol%) of DNA from JAJ28^T was determined. Cells were disrupted with a French pressure cell and DNA was purified on a hydroxyapatite column according to Cashion *et al.* (1977) before subsequent hydrolysis with P1 nuclease. Nucleotides were dephosphorylated with bovine alkaline phosphatase (Mesbah *et al.*, 1989) and analysed by HPLC.

16S rDNA sequencing. PCR amplification of 16S rDNA from JAJ28^T was performed as described by Rainey *et al.* (1992). The PCR product was purified using a PCR purification kit (Qiagen) and served as template for sequencing with the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Perkin Elmer). Sequencing was performed on a 373 PRISM DNA sequencer (ABI). The nucleotide sequence was aligned against a ribosomal database using the ARB program package for alignment (arbu@mikro.biologie.tu-muenchen.de; Lehrstuhl für Mikrobiologie, Technical University of Munich, Munich, Germany). Final adjustments of the alignment were made manually. A phylogenetic tree was constructed by maximum-likelihood using the FastDNAmL program (Olsen *et al.*, 1994) and the phylogenetic analysis was performed using facilities in the ARB program package.

Spectroscopic DNA–DNA hybridization. Spectroscopic DNA–DNA hybridization studies were performed between the isolate JAJ28^T and four other *Pseudomonas* type strains from the DSMZ culture collection (Table 2). DNA was isolated by chromatography on hydroxyapatite (Cashion *et al.*, 1977) and DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) with the modifications described by Huss *et al.* (1983) and Escara & Hutton (1980). A Gilford System model 2600 spectrometer equipped with a Gilford model 2527-R thermoprogrammer and plotter was used. Renaturation rates were computed with the TRANSFER.BAS program reported by Jahnke (1992).

Filter DNA–DNA hybridization. To extend the DNA–DNA hybridization, filter hybridization studies were performed according to Seldin & Dubnau (1985) using [^{35}S]dATP (Amersham Pharmacia Biotech) and the Random Primed DNA labelling kit (Roche Molecular Biochemicals) to label JAJ28^T DNA for use as probe. Filter hybridizations were done against the strains listed in Table 2. Genomic DNA was prepared according to the CTAB DNA isolation method described by Ausubel *et al.* (1987), including an RNase A treatment (Sigma cat. no. R-6513; 50 μg ml^{-1}, 30 min, 37 °C) after proteinase K digestion (Qiagen cat. no. 19131). Concentration and purity of genomic DNA was measured spectrophotometrically according to Maniatis *et al.* (1982) using a Lambda 12 UV/VIS Spectrometer (Perkin-Elmer). Genomic DNA was loaded on nylon membranes (Roche Molecular Biochemicals) using a PR600 slot blot system (Hoefer Scientific Instruments). Hybridizations were carried out in a Mini 10 hybridization oven (Hybaid) at 65 °C overnight. Filters were first washed twice for 15 min at room temperature (stringency 2% SSC, 0·1% SDS) and then subsequently twice for 15 min at 37 °C (stringency 0·1%

SSC, 0·1 % SDS), as opposed to the method of Seldin & Dubnai (1985) who only used washings at room temperature. The percentage hybridization was measured with a Cyclone Storage Phosphor System (Packard).

Electron microscopy. Electron microscopy of JAJ28^T was performed using the method described in Glauert (1975). The strain was grown in LB medium overnight and subsequently resuspended in fresh medium. The cells were allowed to grow for 3 h before they were harvested by centrifugation (10 min, 2000 ***g***). Primary fixation was done in 0·1 M sodium phosphate buffer with 2 % glutaraldehyde, followed by resuspension in the buffer. Osmium fixation was done with 1 % OsO_4 (in buffer) for 1 h before resuspension in buffer and subsequently in distilled water. The cells were dehydrated by serial transfer in 15, 30, 50, 70, 96 and 99 % ethanol and 100 % propylene oxide solutions (10 min each). Transmission electron microscopy was done with a JEOL 100SX microscope.

RESULTS

Biochemical and nutritional characteristics

Table 3 shows a number of phenotypic traits that separates the JAJ28 group (JAJ28^T, JAJ137, JAJ149, SMA1 and SMA5) from *Pseudomonas aeruginosa*, *P. fluorescens*, *P. chlororaphis*, *P. corrugata*, *P. amygdali* and *P. syringae*, the latter two as the only oxidase-negative representatives. These include well established *Pseudomonas* species (*P. aeruginosa*, *P. fluorescens*, *P. chlororaphis* and *P. syringae*).

In Table 3 the JAJ28 group is placed in the non-fluorescent group between *P. corrugata* and *P. amygdali* because it shares several major phenotypic traits with either one or both of these strains. Notably, these traits are the absence of fluorescence on King's B agar (King *et al.*, 1954) and absence of an arginine dihydrolase reaction. Several characters relate the JAJ28 group to *P. corrugata* rather than to *P. amygdali*, e.g. the combination of positive oxidase and denitrification reactions, growth on *meso*-inositol and the accumulation of PHB, which is not found in the other *Pseudomonas* spp. shown in Table 3. Differences do exist, as members of the JAJ28 group do not grow at 37 °C, show no growth on D-xylose, but do grow on benzylamine.

DNA base composition

Table 3 shows that the G+C content of DNA from strain JAJ28^T is 59·6 mol%, which is typical for several *Pseudomonas* spp. (Palleroni, 1984), including *P. fluorescens*, *P. corrugata* and *P. syringae*, flanking the JAJ28^T isolate in Table 3.

Phylogenetic characterization

The majority of the 16S rDNA gene in the JAJ28^T isolate was recovered by PCR amplification of rDNA and the full sequence of the PCR product was obtained. After exclusion of the primer regions, the sequence included 1464 nt, which is more than 95 % of the full sequence. The recorded sequence was manually

Table 3. Characteristics differentiating *Pseudomonas frederiksbergensis* JAJ28^T from other *Pseudomonas* species

+, Positive; −, negative; d, 11–89 % positive; ?, no information available.

Characteristic	*P. aeruginosa**	*P. chlororaphis**†	*P. fluorescens**	*P. corrugata*‡§	*P. frederiksbergensis*‖¶	*P. amygdali**#	*P. syringae**
G+C content of DNA (mol%)	67·2	63·5	59·4–61·3	58·4–60·8	59·6	?	59–61
Growth at 4 °C	−	+	d	+‖	+	+	d
Growth at 37 °C	+	−‖	−‖	+	−	−	+
Fluorescence on King's B agar	d	d	+	−	−	−	+
Arginine dihydrolase	+	+	+	d	−	−	−
Oxidase	+	+	+	+	+	−	−
Denitrification	+	d	d	+	+	−	−
Levan production	−	+	d	−	−	?	d
Accumulation of PHB	−	−	−	+	+	−	−
Gelatinase	+	+	+	d	+	−	d
Lecithinase	−	d	d	+	d	−	d
Growth on:							
Benzylamine	−	−	d	−	+	?	−
D-Xylose	−	−	d	+	−	?	d
Sorbitol	−	−	d	−	−	+	d
meso-Inositol	−	+	d	+	+	−	d

* Palleroni (1984).

† Johnson & Palleroni (1989).

‡ Scarlett *et al.* (1978).

§ Sutra *et al.* (1997).

‖ This study.

¶ Johnsen *et al.* (1996).

Psallidas & Panagopoulos (1975).

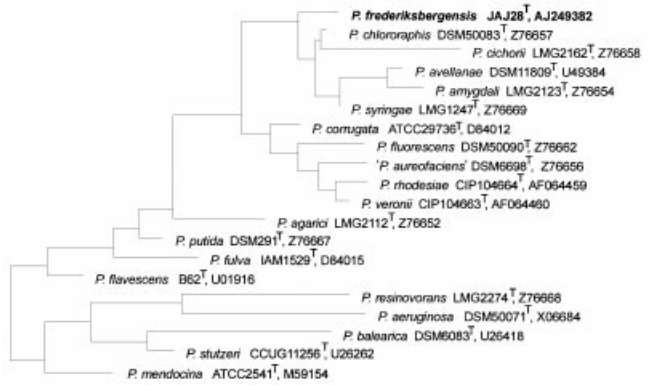

Fig. 1. Maximum-likelihood tree showing the phylogenetic position of *Pseudomonas frederiksbergensis* strain JAJ28^T compared to species of the genus *Pseudomonas*. The strain number and the EMBL accession number of the 16S rDNA sequence are shown after the species name. The sequences in the tree were selected from a comparison with 16S rDNA sequences of all described *Pseudomonas* spp.

aligned against all known *Pseudomonas* spp. in the EMBL database and was found to be most homologous to published sequences representing several species of the genus *Pseudomonas*. Fig. 1 shows the phylogenetic tree, which assigns the JAJ28^T isolate to a phylogenetic branch consisting of *P. chlororaphis* (99·2 % homology), *P. syringae* (99·0 %) and *P. amygdali* (98·2 %). Two other *Pseudomonas* species, *P. avellanae* (98·3 %) and *P. cichorii* (97·6 %), were also in the phylogenetic branch, both belonging to the group of fluorescent phytopathogens including *P. syringae*. With a homology of 98·4 %, *P. corrugata* also showed a high phylogenetic relationship being placed in a separate branch together with *P. fluorescens* (97·5 %), '*Pseudomonas aureofaciens*' (98·2 %), *P. rhodesiae* (97·1 %) and *P. veronii* (98·2 %).

DNA–DNA hybridization

Spectroscopic DNA–DNA hybridization (Table 2) was performed to test the JAJ28^T isolate against some closely related *Pseudomonas* species, as described above. All showed low DNA homology values (43–54 %). To extend the hybridization studies, we performed DNA–DNA filter hybridization studies with JAJ28^T against JAJ137, JAJ149, SMA1, SMA5 and the culture collection strains (Table 2). The data showed high homology values between JAJ28^T and JAJ137, JAJ149, SMA1 and SMA5 (all above 70 %), showing that JAJ28^T is synonymous with the JAJ28 group. Hybridization against the culture collection strains all gave values below 35 %.

All test results presented were based on independent duplicate determinations. Lipase activity (Tween 80) was negative after 5 d, but weak after 6 d and subsequently. Stanier *et al.* (1966) recommend that reading should not be done after 6 d, as haloes may be false after this time. Lecithinase activity was variable for the JAJ28 group and negative/weak for JAJ28^T. Phenotypic discrepancies within the JAJ28 group (one or more strains) were observed for the following compounds (result of JAJ28^T in parentheses): malonate (−), D-turanose (−), 2-aminoethanol (+), succinamate (+) and uridine 5′-monophosphate (+); 2-deoxyadenosine gave variable results for JAJ28^T. The following compounds yielded contradictory results for the JAJ28 group in different tests, e.g. between respiration tests (Biolog GN) and growth tests (API20NE, API50CH, Biolog SF-N or Biolog SF-P), or among two or more different growth tests: acetate, 2,3-butanediol, dextrin, formate, L-fucose, DL-α-glycerol phosphate, glycyl-L-glutamate, glycogen, α-hydroxybutyrate, α-ketobutyrate, maltose, methylsuccinate, L-phenylalanine, propionate, D-psicose, D-saccharate, D-serine and L-threonine.

DISCUSSION

On the basis of phenotypic and genotypic characters, the type strain JAJ28^T represents a total of 29 phenanthrene-degrading bacteria from a coal gasification site and is clearly a member of the genus *Pseudomonas*, as seen from the numerous biochemical properties studied and 16S rDNA sequence analysis. Isolate JAJ28^T shares many characteristics with both fluorescent and non-fluorescent species of the genus *Pseudomonas*, but also differs in several important traits. Strain JAJ28^T is thus distinct from other closely related *Pseudomonas* spp. as shown by the properties listed in Table 3. In particular, the absence of fluorescence in UV light on King's B agar, arginine dihydrolase activity and growth at 37 °C, and the presence of denitrification and PHB accumulation are important characteristics of the new group. It is interesting that the phylogenetic values show a very close relationship between JAJ28^T and the fluorescent *P. chlororaphis* (99·2 %), but apart from this, phenotypic differences and DNA–DNA filter hybridization do not suggest any relationship. It is also remarkable that the level of 16S rDNA sequence homology with the fluorescent '*P. aureofaciens*' (98·2 %) is lower than the value for *P. chlororaphis*, since the two species have been combined into one taxon, *P. chlororaphis* (Johnson & Palleroni, 1989). However, close 16S rDNA homology values do not always imply a close relationship at the species level, as demonstrated by Stackebrandt & Goebel (1994).

In contrast, the emended description of *P. corrugata* by Sutra *et al.* (1997) revealed a very similar biochemical profile and a high 16S rDNA homology value of 98·4 % between strain JAJ28^T and this species. However, the absence of growth at 37 °C and D-xylose utilization, and the presence of benzylamine utilization separates isolate JAJ28^T from *P. corrugata*. Likewise, 16S rDNA sequence analysis indicated strong homology between JAJ28^T and *P. syringae* and *P. amygdali*

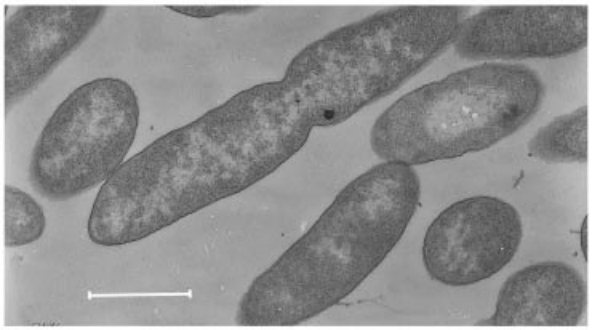

Fig. 2. Transmission electron microscopy showing cells of JAJ28^T in both transverse and longitudinal section. Bar, 1 μm.

(homology values of 99·0 and 98·2 %, respectively). However, *P. syringae* shows fluorescence in UV light on King's B agar, does not denitrify or accumulate PHB and is oxidase-negative as opposed to strain JAJ28^T. Similarly, *P. amygdali* does not denitrify or accumulate PHB, utilizes sorbitol and is oxidase-negative in contrast to JAJ28^T. Finally, DNA–DNA hybridization data comparing JAJ28^T with the relevant type strains from culture collections (Table 2) showed values too low to warrant assignment of JAJ28^T into any of these species. Filter hybridization against *P. amygdali* was not included, but the spectroscopic data from DSMZ support the phenotypic data that exclude a relationship with the strain JAJ28 group at the species level. We used medium stringency in the filter hybridization as used by others (Nielsen *et al.*, 1995; Johansen *et al.*, 1999; Bredholt *et al.*, 1999). Other studies have done the final washings at room temperature (Meyer & Schleifer, 1978; Ezaki *et al.*, 1988) and so obtained higher percentage hybridization. Comparison of our data obtained by filter hybridization and the spectroscopic data obtained by DSMZ for *P. fluorescens*, *P. syringae* and *P. corrugata* (Table 2) does not make it probable that filter hybridization against *P. amygdali* should exceed 20–30 %. The DNA–DNA hybridization values were thus far below the 70 % limit established by Wayne *et al.* (1987) to separate species. We therefore propose a new species, *Pseudomonas frederiksbergensis* sp. nov., to be assigned to the JAJ28 group.

Description of *Pseudomonas frederiksbergensis* sp. nov.

Pseudomonas frederiksbergensis (fre.de.riks.ber.gen′-sis. M.L. adj. *frederiksbergensis* pertaining to Frederiksberg near Copenhagen, Denmark, from where the organism was isolated).

Cells are Gram-negative, motile, non-spore-forming rods of approximately 0·5–0·8 × 1·5–3·0 μm. A transmission electron micrograph is shown in Fig. 2. Colonies are smooth and pale yellowish on LB agar. No fluorescent pigments are produced on King's B or Gould's S1 agar. The species is oxidase- and catalase-positive, denitrifying and shows hydrolysis of gelatin and accumulation of PHB, but no levan production on sucrose-rich agar or blue pigment formation on potato dextrose agar. Indole is not produced during growth on tryptophan. The species shows no acidification of glucose or hydrolysis of aesculin or starch. No arginine dihydrolase, urease or β-galactosidase reactions are seen. Growth takes place from 4 to 30 °C, but not at 37 °C, and no growth occurs in the presence of 4 % NaCl. Growth and respiration occurs on *N*-acetyl-D-glucosamine, *cis*-aconitate, alaninamide, D-alanine, L-alanine, L-alanylglycine, γ-aminobutyrate, L-arabinose, D-arabitol, L-asparagine, L-aspartate, bromosuccinate, carnitine, citrate, D-fructose, D-galactonate lactone, D-galactose, D-galacturonate, D-gluconate, D-glucosaminate, α-D-glucose, glucuronamide, D-glucuronate, L-glutamate, α-ketoglutarate, glycerol, L-histidine, β-hydroxybutyrate, hydroxy-L-proline, inosine, *meso*-inositol, DL-lactate, L-leucine, D-mannitol, D-mannose, methylpyruvate, L-ornithine, L-proline, putrescine, L-pyroglutamate, quinate, L-serine, succinate, sucrose, D-trehalose, Tween 40, Tween 80, uridine and urocanate. Growth (respiration not tested) also occurs on *N*-acetyl-L-glutamate, adenosine, benzylamine, caprate, citraconate, 2-ketogluconate, lactamide, L-lactate, D-malate, L-malate, pyruvate, D-ribose and L-valine. No growth or respiration occurs on *N*-acetyl-β-D-galactosamine, adonitol, D-cellobiose, α-cyclodextrin, *meso*-erythritol, β-gentiobiose, glucose 1-phosphate, glucose 6-phosphate, glycyl-L-aspartate, γ-hydroxybutyrate, *p*-hydroxyphenylacetate, itaconate, α-D-lactose, lactulose, L-rhamnose, D-melibiose, β-methyl-D-glucoside, phenylethylamine, D-raffinose, sebacate, D-sorbitol, thymidine, α-ketovalerate or xylitol. No growth (respiration not tested) occurs on *N*-acetyl-β-D-mannosamine, adenosine-5′-monophosphate, adipate, starch, amygdalin, D-arabinose, L-arabitol, arbutin, β-cyclodextrin, dulcitol, aesculin, fructose 6-phosphate, D-fucose, 5-ketogluconate, inulin, D-lactate methyl ester, β-D-lactose, D-lyxose, maltotriose, D-mandelate, L-mandelate, mannan, D-melezitose, α-methyl-D-galactoside, β-methyl-D-galactoside, α-methyl-D-glucoside, α-methyl-D-mannoside, 3-methylglucose, β-methylxyloside, palatinose, phenylacetate, salicin, sedoheptulosan, L-sorbose, stachyose, D-tagatose, thymidine-5′-monophosphate, L-tryptophan, D-xylose or L-xylose. Mean G+C content is 59·6 mol%. Isolated from tar-polluted soil on Gould's S1 agar and subsequently screened on phenanthrene-coated minimal agar. The type strain, JAJ28^T, has been deposited at DSMZ under DSM 13022^T.

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