Pseudomonas lutea sp. nov., a novel phosphate-solubilizing bacterium isolated from the rhizosphere of grasses

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A phosphate-solubilizing bacterial strain designated OK2^T was isolated from rhizospheric soil of grasses growing spontaneously in a soil from Spain. Cells of the strain were Gram-negative, strictly aerobic, rod-shaped and motile. Phylogenetic analysis of the 16S rRNA gene indicated that this bacterium belongs to the γ -subclass of *Proteobacteria* within the genus *Pseudomonas* and that the closest related species is *Pseudomonas graminis*. The strain produced catalase but not oxidase. Cellulose, casein, starch, gelatin and urea were not hydrolysed. Aesculin was hydrolysed. Growth was observed with many carbohydrates as carbon sources. The main non-polar fatty acids detected were hexadecenoic acid (16:1), hexadecanoic acid (16:0) and octadecenoic acid (18:1). The hydroxy fatty acids detected were 3-hydroxydecanoic acid (3-OH 10:0), 3-hydroxydodecanoic acid (3-OH 12:0) and 2-hydroxydodecanoic acid (2-OH 12:0). The G + C DNA content determined was 59.3 mol%. DNA–DNA hybridization showed 48.7% relatedness between strain OK2^T and *P. graminis* DSM 11363^T and 26.2% with respect to *Pseudomonas rhizosphaerae* LMG 21640^T. Therefore, these results indicate that strain OK2^T (=LMG 21974^T = CECT 5822^T) belongs to a novel species of the genus *Pseudomonas*, and the name *Pseudomonas lutea* sp. nov. is proposed.

The genus *Pseudomonas sensu stricto* currently includes several species able to solubilize phosphate *in vitro* (reviewed by Peix *et al.*, 2003), all of them belonging to rRNA group I (Palleroni *et al.*, 1973; Palleroni, 1992). Recently, a novel phosphate-solubilizing species, *Pseudomonas rhizosphaerae*, isolated from the rhizosphere of grasses, was described (Peix *et al.*, 2003). This species forms a group separate from other *Pseudomonas* species together with *Pseudomonas graminis*, which was isolated from the above-ground parts of grasses (Behrendt *et al.*, 1999).

During a study of phosphate-solubilizing rhizospheric bacteria in soils from northern Spain, we isolated a strain that produces a yellow pigment in media containing glucose as carbon source and generates a great transparent 'halo' surrounding its colonies in media containing insoluble bicalcium phosphate as phosphorus source. The ability of this strain to solubilize phosphate is similar to

that of *P. rhizosphaerae* IH5^T, but strain OK2^T solubilizes phosphate more slowly.

Strain OK2^T was isolated as described previously (Peix *et al.*, 2001). Strain OK2^T was grown in nutrient agar medium for 48 h at 22 °C to check for motility by phase-contrast microscopy. Cells were also stained according to the classic Gram procedure described by Doetsch (1981). Strain OK2^T is a Gram-negative, rod-shaped and motile organism (0.7–0.8 × 1.2–1.6 µm). Cells grew as translucent, yellow-coloured colonies on nutrient agar.

For 16S rDNA sequencing, DNA was extracted as described previously (Rivas *et al.*, 2001). Amplification and sequencing of the nearly complete 16S rRNA gene was performed according to methods already described (Rivas *et al.*, 2003). The sequence obtained was compared with those from GenBank using the BLAST program (Altschul *et al.*, 1990). Sequences were analysed using the parameters and methods used previously for description of *P. rhizosphaerae* (Peix *et al.*, 2003). Bootstrap analysis was based on 1000 resamplings. The MEGA 2.1.0 package (Kumar *et al.*, 2001) was used for all analyses. The nearly complete 16S rDNA sequence of strain OK2^T (1531 nucleotides) showed

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99.2 and 98.7% similarity, respectively, to those of *P. graminis* DSM 11363^T and *P. rhizosphaerae* LMG 21640^T. A complete phylogenetic analysis using the parameters and tree building methods used in the description of *P. rhizosphaerae* (Peix *et al.*, 2003) was performed and the same results were obtained from all the methods used (data not shown). In these analyses, all species of genus *Pseudomonas sensu stricto* according to Anzai *et al.* (2000), other species of *Pseudomonas* with validly published names listed by Peix *et al.* (2003), including the novel species *P. rhizosphaerae*, and the three recently described species *Pseudomonas congelans*, *Pseudomonas poae* and *Pseudomonas trivialis* (Behrendt *et al.*, 2003) were included. Fig. 1 shows a reduced phylogenetic tree obtained with Kimura's two-parameter method (Kimura, 1980) and the neighbour-joining method (Saitou & Nei, 1987), showing the phylogenetic placement of strain OK2^T within the genus *Pseudomonas* in a separate group together with *P. rhizosphaerae* LMG 21640^T and *P. graminis* DSM 11363^T.

Phenotypic analysis was performed as described recently (Peix *et al.*, 2003) using *P. rhizosphaerae* LMG 21640^T and *P. graminis* DSM 11363^T as references. Strain OK2^T showed an ability to solubilize phosphates on YED-P plates comparable to that of *P. rhizosphaerae* LMG 21640^T, although strain OK2^T displayed a lower solubilization rate. Under the same medium and culture conditions, *P. graminis* DSM 11363^T showed limited ability to solubilize phosphates. The optimal growth temperature was 25 °C on nutrient agar. As for *P. rhizosphaerae* LMG 21640^T, the API 20NE and API 50CH systems were used only to characterize strain OK2^T, because, as pointed out by Behrendt *et al.* (1999) and Peix *et al.* (2003), the identification of non-clinical isolates is often wrong with these systems.

Fig. 1. Comparative sequence analysis of 16S rDNA from *P. lutea* sp. nov. OK2^T and representative species from *Pseudomonas sensu stricto*. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 5 substitutions per 100 nt.

Differential phenotypic characteristics among strain OK2^T, the phylogenetically closest species of genus *Pseudomonas sensu stricto*, *P. rhizosphaerae* and *P. graminis* and other phenotypic related species are shown in Table 1. According to the data, strain OK2^T is very similar to *P. rhizosphaerae* and *P. graminis*, forming a phylogenetic group within genus *Pseudomonas* characterized by the absence of oxidase and fluorescent pigment production. Strain OK2^T differs from *P. rhizosphaerae* in assimilation of erythritol, sorbitol, xylitol, melibiose and L-rhamnose and in aesculin hydrolysis and from *P. graminis* in assimilation of sorbitol, xylitol and melibiose.

Analyses of non-polar and hydroxy fatty acids were performed from a culture of strain OK2^T grown for 24 h in TSA medium (Merck) at 28 °C as described by Peix *et al.* (2003). The results of the chemotaxonomic analyses are shown in Table 2. The main non-polar fatty acids detected were hexadecenoic acid (16:1), hexadecanoic acid (16:0) and octadecenoic acid (18:1). The hydroxy fatty acids detected were 3-hydroxydecanoic acid (3-OH 10:0), 3-hydroxydodecanoic acid (3-OH 12:0) and 2-hydroxydodecanoic acid (2-OH 12:0). This fatty acid profile is characteristic of strains from rRNA group I (Oyaizu & Komagata, 1983). According to published data, the cellular fatty acid pattern of strain OK2^T is similar to those of *P. graminis* and *P. rhizosphaerae* (Behrendt *et al.*, 1999;

Table 1. Differential characteristics among *P. lutea* sp. nov. OK2^T and phylogenetically and phenotypically closely related species of *Pseudomonas sensu stricto*

Species: 1, *P. lutea* sp. nov.; 2, *P. rhizosphaerae*; 3, *P. graminis*; 4, *P. tremae*; 5, *P. amygdali*; 6, *P. luteola*; 7, *P. oryzae*. Data for reference species were taken from Behrendt *et al.* (1999), Gardan *et al.* (1999), Kodama *et al.* (1985), Palleroni (1984) and Peix *et al.* (2003), except that data for phosphate utilization were from this study (only the type strain of *P. graminis*, DSM 11363^T, was tested). +, Positive; -, negative; W, weak; S, slow; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Fluorescent pigments	-	-	-	-	-	+	+
Oxidase	-	-	-	ND	ND	-	-
Phosphate solubilization (24 h)	+	(s)	+	W	ND	ND	ND
Utilization of:							
Erythritol	-	+	-	ND	-	ND	ND
Sorbitol	-	+	+	ND	+	-	+
Xylitol	+	-	-	-	ND	ND	ND
Melibiose	+	-	-	-	-	ND	ND
Rhamnose	-	+	-	-	-	+	-
Hydrolysis of:							
Aesculin	+	-	+	-	-	+	-
Casein	-	-	+	*	ND	-	ND
Gelatin	-	-	+	*	-	+	-

*Some strains give a negative result.

Table 2. Cellular fatty acid composition of *P. lutea* sp. nov., *P. rhizosphaerae* and *P. graminis*

Species/strain: 1, *P. lutea* sp. nov. OK2^T; 2, *P. rhizosphaerae* IH5^T (data from Peix *et al.*, 2003); 3, *P. graminis* (Behrendt *et al.*, 1999). Values are percentages of total fatty acids. Results were obtained using comparable methods. ND, Not detected.

Fatty acid	1	2	3
3-OH 10:0	2.4	2.9	ND
2-OH 12:0	2.7	2.7	0.1–2.3
3-OH 12:0	3.3	4.1	0.5–2.6
12:0	4.4	4.3	0–3.4
14:0	ND	0.3	0.2–0.3
16:1	39.0	38.6	50.1–61.4
16:0	29.0	23.3	27.4–35.5
17:0	ND	0.2	ND
18:1	18.6	23.2	2.4–17.6
18:0	0.5	0.5	ND

Peix *et al.*, 2003). The main differences between the strain OK2^T and *P. graminis* were the presence of hydroxydecanoic acid (3-OH 10:0), which was not detected in the latter species, and the presence of tetradecanoic acid (14:0) in *P. graminis*, which was not detected in strain OK2^T. With respect to *P. rhizosphaerae*, the main differences were the presence in this species of tetradecanoic acid (14:0) and heptadecanoic acid (17:0), which were not detected in strain OK2^T.

For base composition analysis, DNA was prepared according to Chun & Goodfellow (1995). The DNA G + C content was determined as 59.3 mol% using the thermal denaturation method (Mandel & Marmur, 1968). This value is similar to those obtained for *P. graminis* and *P. rhizosphaerae* (Behrendt *et al.*, 1999; Peix *et al.*, 2003).

For DNA–DNA hybridization analyses, DNA was isolated by hydroxyapatite chromatography by the procedure of Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) with the modification described by Huß *et al.* (1983) and Escara & Hutton (1980). Renaturation rates were computed with the program TRANSFER.BAS (Jahnke, 1992). DNA–DNA relatedness was tested [in 2 × SSC plus 10% (v/v) DMSO at 68 °C] among strain OK2^T, *P. rhizosphaerae* LMG 21640^T and *P. graminis* DSM 11363^T. The results of DNA–DNA hybridization showed relatedness of 26.2% between strain OK2^T and *P. rhizosphaerae* LMG 21640^T and 48.7% between strain OK2^T and *P. graminis* DSM 11363^T. These results indicate that strain OK2^T does not belong to either of these species when the recommendation of a threshold value of 70% DNA–DNA relatedness for definition of species is considered (Wayne *et al.*, 1987).

Therefore, on the basis of phylogenetic, chemotaxonomic and phenotypic data, isolate OK2^T should be classified as representing a novel species, for which we propose the name *Pseudomonas lutea* sp. nov.

Description of *Pseudomonas lutea* sp. nov.

Pseudomonas lutea (lu'te.a. L. fem. adj. *lutea* yellow, referring to the yellowish pigment produced by this bacterium).

Gram-negative, strictly aerobic, non-spore-forming, motile, rod-shaped cells, 1.2–1.6 µm long and 0.7–0.8 µm in diameter. Colonies on YED are circular convex, yellow, translucent and usually 1–2 mm in diameter within 2 days growth at 25 °C. Able to oxidize glucose in medium containing ammonium nitrate as nitrogen source, but unable to ferment glucose in the same medium. Produces catalase and does not produce oxidase, gelatinase, caseinase, urease, arginine dehydrolase, tryptophan deaminase, β-galactosidase, indole or H₂S. Aesculin is hydrolysed. Utilizes L-arabinose, D-arabinose, D-xylose, ribose, mannose, galactose, D-fructose, L-lyxose, D-fucose, L-fucose, melibiose, inositol, mannitol, adonitol, glycerol, D-arabitol, L-arabitol, xylitol, caprate, malate, gluconate, 2-ketogluconate and citrate as sole carbon sources. Does not utilize L-xylose, L-sorbose, methyl β-xyloside, methyl α-D-mannoside, methyl α-D-glucoside, L-rhamnose, amygdalin, arbutin, salicin, cellobiose, lactose, sucrose, trehalose, inulin, melezitose, D-raffinose, starch, glycogen, erythritol, sorbitol, dulcitol, N-acetylglucosamine, maltose, β-gentiobiose, D-turanose, D-tagatose, adipate, 5-ketogluconate or phenylacetate.

The type strain, OK2^T (=LMG 21974^T=CECT 5822^T), has a DNA G + C content of 59.3 mol%.

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