Pseudomonas lurida sp. nov., a fluorescent species associated with the phyllosphere of grasses

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The taxonomic position of a group of fluorescent pseudomonad strains isolated from the phyllosphere of grasses was investigated through a polyphasic approach. Ribotyping analysis revealed highly similar patterns for the investigated strains which supported, together with the agreement of many phenotypic characteristics, their affiliation to the same species. A comparison of 16S rRNA gene sequences of strain P 513/18^T, a representative strain from the grass isolates, revealed that it was affiliated to the cluster of the '*Pseudomonas fluorescens* group', with *Pseudomonas costantinii* as the closest phylogenetic neighbour. However, DNA–DNA hybridization showed a clear demarcation at the species level between strain P 513/18^T and *P. costantinii*. Furthermore, a comparison of riboprint patterns with *Pseudomonas* species clustering next to the novel grass isolates on the basis of 16S rRNA gene sequences supported their separate species status at the phylogenetic level. Based on phenotypic features, the novel isolates could also be differentiated from the other fluorescent *Pseudomonas* species that share positive arginine dihydrolase and oxidase reactions. As a consequence of these phenotypic and phylogenetic analyses, the isolates from the grass phyllosphere represent a novel species for which the name *Pseudomonas lurida* sp. nov. is proposed. The type strain is P 513/18^T (=DSM 15835^T =LMG 21995^T).

INTRODUCTION

The ecological significance of fluorescent pseudomonads has been demonstrated by some extensive studies of several habitats (Moore *et al.*, 2006). Analysis of microbial community structures using a polyphasic approach has shown high diversity within this bacterial group and has already led to the description of numerous novel species in recent years (Baida *et al.*, 2002; Behrendt *et al.*, 2003; Dabboussi *et al.*, 2002; Delorme *et al.*, 2002; Gardan *et al.*, 2002; Ivanova *et al.*, 2002; Kwon *et al.*, 2003; Munsch *et al.*, 2002; Park *et al.*, 2005; Reddy *et al.*, 2004).

Fluorescent pseudomonads form a considerable part of the microbial community in the phyllosphere of plants. Through their interactions, they influence plant growth in

different ways (Bailey, 2004; Walsh *et al.*, 2001) and thus are the object of many studies. In the phyllosphere of grasses on fenland, the community of pseudomonads was shown to be influenced by the intensity of management (Behrendt, 2001). In this context, a group of grass isolates was found to display the phenotypic features of fluorescent pseudomonads while phylogenetic analyses of selected strains suggested that they represented a novel species. As a consequence, an extensive study was performed to clarify the taxonomic affiliation of these novel isolates from grass.

Phylogenetic analysis

A representative strain, P 513/18^T, was chosen from a subset of three phenotypically similar pseudomonads (P 513/18^T, P 239/01 and P 240/09) for the 16S rRNA gene sequence comparison. Sequence analysis was performed as described by Behrendt *et al.* (2003). The closest phylogenetic neighbour as determined by a binary sequence comparison was *Pseudomonas costantinii* with a similarity of 99.9 %, followed

Abbreviation: PVD-IEF, pyoverdine-iso-electrofocusing.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Pseudomonas lurida* sp. nov. P 513/18^T is AJ581999.

by *Pseudomonas trivialis* and *Pseudomonas poae*, displaying 99.7 and 99.8 % similarity, respectively.

All recognized species of the genus *Pseudomonas* were included in the sequence comparison (except for *Pseudomonas gelidicola* for which no 16S rRNA gene sequence was available). Strain P 513/18^T clustered at a branch that corresponds to the '*Pseudomonas fluorescens* group' of Anzai *et al.* (2000). Species that were found to be highly related to strain P 513/18^T were selected for more detailed phylogenetic analysis (Fig. 1). Sequences were aligned using the CLUSTAL_X algorithm (Thompson *et al.*, 1997). Phylogenetic trees, based on 1379 nt (*Escherichia coli* position 93–1467), were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms (PHYLP version 3.6; Felsenstein, 1993). As shown in Fig. 1, *Pseudomonas* species found clustering next to strain P 513/18^T in the neighbour-joining method also had the same arrangement in the maximum-likelihood algorithm. The separate clustering of novel isolate P 513/18^T and the closest phylogenetic neighbour *P. costantinii* was supported by a relatively high bootstrap value (75).

The next step in the phylogenetic analysis was to investigate the similarity of the subset of isolates from the grass phyllosphere. Ribotyping has been proven to be a powerful method for classifying pseudomonads both at the species and at the strain level (Behrendt *et al.*, 2003; Sikorski *et al.*, 2001). The three novel grass isolates and the type strains of the phylogenetically related species that form an internal cluster,

as revealed by 16S rRNA comparisons (Fig. 1), were studied by ribotyping with the *EcoRI* restriction enzyme (Fig. 2). The analysis was performed with the automated RiboPrinter microbial characterization system (Qualicon Du Pont). Band patterns were compared using BioNumerics software (Applied Maths) and clustering was carried out by UPGMA based on Pearson's correlation coefficient (optimization coefficient, 1.2 %). As shown in Fig. 2, the novel isolates from grass clustered together at a high similarity level (>90 %), suggesting that the three strains belong to the same species. The most similar ribopatterns found were those of *Pseudomonas extremorientalis* and *P. costantinii* which showed small shifts in the single bands or differences in band intensities. In contrast, the remaining type strains of the related species showed salient differences in their respective patterns.

To clarify the taxonomic position of the novel strains at the species level, the closest phylogenetic neighbour, *P. costantinii* as determined by 16S rRNA gene sequence comparisons, and the highly related species, *P. extremorientalis*, which showed the next most similar ribopattern, were chosen for DNA–DNA hybridization. The DNA–DNA hybridization study, performed with 2 × SSC and 10 % dimethylsulfoxide at 67 °C according to the method of Martin *et al.* (1997), revealed reassociation values of 33.6 and 51.5 %, respectively. According to the recommendation by Wayne *et al.* (1987) for species delineation, this result clearly indicates a separate species position for strain P 513/18^T, as had already been revealed by 16S rRNA gene sequence analysis and by comparisons of ribopatterns.

Fig. 1. Phylogenetic tree showing the relationship of strain P 513/18^T within a subset of closely related *Pseudomonas* species. The tree is based on a 1379 bp alignment of 16S rRNA gene sequences and was constructed by using the neighbour-joining method (Saitou & Nei, 1987). Dots indicate branches of the tree that were also formed by using the maximum-likelihood method (Felsenstein, 1981). To estimate the root position of the tree, *Escherichia coli* (GenBank accession no. J01695) was used as an outgroup (not shown). Values are the relative proportions that a branch appeared in 1000 bootstrap replications. Bar, 0.01 relative sequence divergences.

Pearson correlation (Opt:1.20 %) [0.0–100.0 %]

Fig. 2. Riboprint patterns of *Eco*RI-restricted DNA of the novel isolates and type strains of highly related *Pseudomonas* species as determined by 16S rRNA gene sequence comparisons. Analysis was performed with the RiboPrinter microbial characterization system (Qualicon Du Pont). The riboprint pattern was analysed with the BioNumerics GeneExplore version 1.0 software (Applied Maths).

Phenotypic analysis

Morphological and physiological characterization of the novel isolates was performed as described in Behrendt *et al.* (1999). Type strains of phenotypically related species that share positive oxidase and arginine dihydrolase reactions (Table 1) were also included in the study. An extensive investigation of the carbon substrate assimilation was conducted using Biotype 100 strips (bioMérieux) and Biolog GN MicroPlates (MicroLog System) as recommended by the manufacturers. Results were read after 48 h incubation at 30 °C.

The novel isolates were able to utilize a wide range of carbohydrates. Morphological and physiological features of the investigated strains are given in the species description below. Characteristics that enable the novel grass isolates to be differentiated from the related fluorescent pseudomonads displayed in the phylogenetic tree (Fig. 1) and characterized by a positive arginine dihydrolase and oxidase reaction are presented in Table 1. Each recognized species could be distinguished from the novel grass isolates on the basis of the selected features. The physiological features of the novel grass isolates and those of the closest phylogenetic neighbour, *P. costantinii*, were, however, very similar. Out of 99 substrates tested by the Biotype 100 strips, only the assimilation of four substances was different. Thus, on the basis of physiological characteristics, the utilization of α -L-rhamnose, i-erythritol, 5-keto-D-gluconate and histamine are the only effective features for distinguishing these species. In contrast, many differences were revealed between the novel grass isolates and *P. extremorientalis*, the species that showed the most similar ribopattern. As shown in Table 1, the novel isolates and *P. extremorientalis* differed in the reduction of nitrate, hydrolysis of gelatin and in the assimilation of α -L-rhamnose. Moreover, according to the characteristics of *P. extremorientalis* as described by Ivanova *et al.* (2002), the assimilation of sebacic acid, hydroxy-L-proline, L-ornithine, dextrin, D-cellobiose, α -D-glucose, L-histidine, lactulose, maltose, D-psicose, L-rhamnose, turanose, thymidine, putrescine, DL- α -glycerol phosphate and glucose 1-phosphate are additional features that can be used to distinguish this species from the novel isolates.

Another approach for phenotypic characterization that has been shown to be a powerful method for taxonomic studies of the pseudomonads is siderotyping (Meyer *et al.*, 2002; Meyer & Geoffroy, 2004). The pyoverdine production of the novel isolates was compared with that of related fluorescent species. Cultures for pyoverdine production and the electrophoretic characterization of the pyoverdine isoforms that accumulated in the growth media were performed according to Meyer *et al.* (1998), with the exception of the isoelectric pH (pI) values which were determined using an internal standard made from a mixture of pyoverdines with defined pI values, as described in Fuchs *et al.* (2001). The purification of pyoverdines with the XAD chromatographic procedure and their use in pyoverdine-mediated ⁵⁹Fe uptake were performed as described previously (Meyer *et al.*, 1998). The novel isolates revealed two different pyoverdine-isoelectrofocusing (PVD-IEF) patterns. Strains P 513/18^T and P 240/09 displayed an identical PVD-IEF pattern with two isoform bands at pI 8.3 and 7.2, respectively, while the pyoverdine of strain P 239/01 was characterized by three isoforms at pI 8.2, 8.1 and 7.2. This heterogeneity was confirmed by studying the pyoverdine-mediated iron-uptake capacity of the isolates. Strain P 513/18^T was able to incorporate the pyoverdine of strain P 240/09 at the same efficiency as its own pyoverdine. The same was found for the reciprocal test. In contrast, a much weaker incorporation (10 to 30 % efficiency compared with the homologous system) was observed when the pyoverdine of strain P 239/01 was tested on the two other strains or when strain P 239/01 was tested with the pyoverdine of strains P 513/18^T or P 240/09. In addition, the testing of a collection of structurally known pyoverdines as iron transporters revealed that strain P 513/18^T was able to use the pyoverdine of strain *Pseudomonas* sp. CFML 95-275 at 100 % efficiency, while strain P 239/01 recognized the pyoverdine of strain *Pseudomonas* sp. CFML 96-318 as efficiently as its own (data not shown). The comparisons were also valid for the respective PVD-IEF patterns of these strains, strongly suggesting that strains P 513/18^T and P 240/09 produce a pyoverdine identical to PVD (95-275) and that strain P 239/01 produces a pyoverdine identical to PVD (96-318). Interestingly, the structures of these two pyoverdines, Ser-Orn-FOHOrn-Ser-Ser-(Lys-Ser-FOHOrn) for PVD

Table 1. Characteristics that differentiate *P. lurida* from phylogenetically related species characterized by positive oxidase and arginine dihydrolase reactions

Species: 1. *P. lurida* sp. nov.; 2. *P. constantinii*; 3. *P. extremorientalis*; 4. *P. fluorescens* biovar I; 5. *P. fluorescens* biovar II; 6. *P. fluorescens* biovar III; 7. *P. fluorescens* biovar IV; 8. *P. fluorescens* biovar V; 9. *P. grimontii*; 10. *P. lini*; 11. *P. marginalis*; 12. *P. migulae*; 13. *P. orientalis*; 14. *P. palleroniana*; 15. *P. rhodesiae*; 16. *P. salomonii*; 17. *P. tolaasii*; 18. *P. veronii*. +, Positive; –, negative; D, different reaction; W, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Denitrification	–	–§	ND	– ⁿ	+ ⁿ	+ ⁿ	+ ⁿ	– ⁿ	+ ^a	ND	–§	+ ^o	– ^e	–§	– ^c	ND	–§	+ ^{eg}
Nitrate reduction	–	– ^m	+ ^j	+ ^d	+ ^{df}	– ^d	D ^d	D ^d	+ ^a	+ ^f	+ ^{hmj} – ^b	+ ^o	+ ^o – ^f	– ^h	+ ^c	– ^{hm}	– ^h	+ ^{dm}
Production of:																		
Gelatinase	+	+ ^m	– ^j	+ ^{cn}	D ^f /+ ^{cn}	+ ^{cn}	+ ^{cn}	+ ^{cn}	–§	+ ^f	D ^h	– ^o	+ ^f –	+ ^h	– ^c	+ ^h	+ ^{hi}	D ^g
Lecithinase	+	+§	+ ^j	+ ⁿ	W ⁿ	+ ⁿ	+ ⁿ	D ⁿ	– ^a	ND	W§	+ ^o	D ^e	+§	+ ^c	ND	+§	– ^{eg}
Amylase	–	–§	– ^j	– ⁿ	– ⁿ	– ⁿ	– ⁿ	– ⁿ	+ ^a	ND	–§	– ^o	ND	–§	– ^c	ND	– ⁱ	– ^a
Levan sucrase	–	– ^m	ND	+ ⁿ	+ ⁿ – ^f	– ⁿ	+ ⁿ	– ⁿ	+ ^a	D ^f	+ ^m	+ ^o	+ ^{ef}	+ ^h	+ ^c	+ ^h	– ⁱ	+ ^a
Assimilation of:																		
α-L-Rhamnose	+	– ^m	– ^j	– ^{cn}	D ^{cn}	D ^{cn}	– ^{cn}	D ^{cn}	+ ^a	– ^f	ND	– ^o	D ^e	– ^h	– ^c	– ^h	– ⁱ	– ^{eg}
D-Xylose	+	+ ^m	+ ^j	+ ^{cn}	– ^f /D ^{cn}	D ^{cn}	D ^{cn}	D ^{cn}	+ ^a	– ^f	+ ^{bm}	+ ^o	+ ^{ef}	+ ^h	+ ^c	+ ^h	– ⁱ	+ ^{egm}
D-Lyxose	+	+ ^m	ND	ND	– ^f	ND	ND	ND	ND	– ^f	ND	– ^o	– ^{ef}	+ ^h	– ^c	+ ^h	+ ⁱ	ND
Sucrose	–	D	+ ^j	+ ^{cn}	+ ^{efn}	– ^{cn}	+ ^{cn}	D ^{nc}	+ ^a	+ ^f	+ ^h	– ^o	+ ^f	+ ^h	+ ^c	+ ^h	– ^{hi}	+ ^{gm}
5-Keto D-gluconate	–	+ ^m	ND	ND	ND	ND	ND	ND	ND	– ^f	+ ^m	– ^m	– ^{em}	ND	– ^c	ND	+ ⁱ	+ ^m
i-Erythritol	–	+ ^m	– ^j	+ ⁿ	D ⁿ	D ⁿ	– ⁿ	D ⁿ	+ ^a	– ^f	D ^h /+ ^m	– ^o	– ^e	+ ^h	– ^c	+ ^h	+ ^{hi}	+ ^{eg}
Adonitol	+	+ ^m	– ^j	+ ^{cn}	– ^{efn}	D ^{cn}	– ^{cn}	D ^{cn}	– ^a	– ^f	D ^h	– ^o	+ ^{ef}	– ^h	– ^c	+ ^h	+ ^{hi}	– ^{egm}
Benzoate	+	+ ^m	ND	D ^c	D ^c	D ^c	+ ^c	D ^c	D ^a	D ^f	– ^{bm}	+ ^o	– ^{em}	– ^h	– ^c	– ^m	– ⁱ	– ^{gm} /+ ^e
DL-α-Amino -N-butyrate	+	+ ^m	+ ^j	– ^c	– ^c	– ^c	– ^c	– ^c	+ ^a	+ ^f	ND	+ ^o	+ ^e	ND	+ ^c	ND	D ⁱ	+ ^e
L-Threonine	+	ND	+ ^j	– ⁿ	– ⁿ	– ⁿ	– ⁿ	– ⁿ	ND	ND	ND	ND	ND	ND	+ ^c	ND	+ ⁱ	ND
L-Tryptophan	–	– ^m	ND	+ ^c	D ^c	D ^c	– ^c	D ^c	– ^a	D ^f	+ ^h	– ^o	– ^e	+ ^h	– ^c	+ ^h	+ ⁱ	+ ^{eg}
Histamine	–	+ ^m	ND	D ^c	– ^{ef}	D ^c	– ^c	D ^c	– ^a	– ^f	– ^{bh}	+ ^o	– ^{ef}	– ^h	– ^c	– ^h	– ^{hi}	– ^{egm}
Trigonelline	–	– ^m	ND	D ^c	D ^c /– ^f	D ^c	– ^c	D ^c	+ ^a	– ^f	+ ^h	– ^o	+ ^{ef}	– ^h	– ^c	– ^h	– ^{hi}	+ ^{egm}
DL-Lactate	+	+ ^m	+ ^j	+ ⁿ	+ ⁿ	+ ⁿ	+ ⁿ	+ ⁿ	+ ^a	+ ^f	– ^b	+ ^o	ND	ND	+ ^c	ND	+ ⁱ	ND
D-Tartrate	–	ND	ND	– ^{cn}	D ^{cn}	– ^{cn}	– ^{cn}	D ^{nc}	D ^a	D ^f	+ ^{bh}	– ^o	– ^e	– ^h	– ^c	– ^h	– ^{hi}	– ^{eg}
meso-Tartrate	+	+ ^m	ND	– ^{cn}	– ^{efn}	D ^{cn}	– ^{cn}	D ^{cn}	– ^a	– ^f	– ^b /D ^h	+ ^o	– ^{ef}	+ ^h	– ^c	+ ^h	+ ^{hi}	– ^{eg}
PVD isoforms	8.3, 7.2;	9.1,	8.1, 7.3,	8.7, 7.3,	7.1 ^m	9.2,	7.3,	8.4,	8.7,	7.55, 5.30,	8.8,	9.6,	8.8,	8.8,	8.4,	8.4, 7.3 ^m ,	9.1,	7.5,
(pI values)‡	8.2, 8.1, 7.2	7.5, 7.3 ^m	5.2, 4.3§	7.1 ^{hm}		7.6‡	5.3 ^m	7.4, 5.5 ^m	8.6, 7.3§	5.20 ^f , 5.25, 4.45 ^f	7.7 ^h	9.3, 7.7§	8.7, 7.3 ^h	7.3, 7.2 ^h	7.5, 7.4 ^k	8.5, 7.3, 7.2 ^h	7.5, 7.3 ^l	7.3, 5.2 ^k

*Data are from this study and studies by ^aBaida *et al.* (2002); ^bCatara *et al.* (2002); ^cCoroler *et al.* (1996); ^dDabboussi *et al.* (1999a, b); ^fDelorme *et al.* (2002); ^gElomari *et al.* (1996); ^hGardan *et al.* (2002); ⁱGoor *et al.* (1986); ^jIvanova *et al.* (2002); ^kMeyer (2000); ^{lm}Munsch *et al.* (2000, 2002); ⁿPalleroni (1984) and ^oVerhille *et al.* (1999).

‡Determined by the same method.

§Following strains were used: *P. extremorientalis* CFBP 6729^T, *P. fluorescens* biovar III ATCC 17400, *P. grimontii* CFML 97-514^T, *P. marginalis* LMG 2210^T, *P. migulae* CFML 95-321^T, *P. palleroniana* CFBP 4389^T, *P. tolaasii* CFBP 2068^T.

(96-318) (Schlegel *et al.*, 2001) and Ser-Ser-FOHOrn-Ser-Ser-(Lys-Ser-Lys-FOHOrn) for PVD (95-275) (Sultana *et al.*, 2000), reveal strong similarities with the common motif FOHOrn-Ser-Ser in the linear part of the peptides and three common amino acids (Lys, Ser and FOHOrn) in the cyclic peptidic parts of the pyoverdines. Thus, the pyoverdines that characterize the two siderotypes of the novel grass isolates appear to be structurally related, a feature that could explain the partial cross-reactivity shown during the iron uptake studies and also reflects the close phylogenetic relationship established for these strains through ribotyping. The PVD-IEF patterns found for the novel isolates were indeed different from those obtained for the related fluorescent species described in Fig. 2 and the phenotypically similar species shown in Table 1, thus confirming, together with the uptake studies, that the novel grass isolates display siderotypes not associated with the recognized fluorescent *Pseudomonas* species. This result was in agreement with the separate species position of the novel isolates that was demonstrated by phylogenetic analysis and biochemical and physiological characterizations. According to this combined evidence, the isolates from the grass phyllosphere represent a novel species, for which the name *Pseudomonas lurida* sp. nov. is proposed.

Description of *Pseudomonas lurida* sp. nov.

Pseudomonas lurida (lu'ri.da. L. fem. adj. *lurida* yellowish, to indicate the yellow-greenish fluorescent pigment of the organism).

Cells are Gram-negative, non-spore-forming rods that occur as single cells. The white-yellowish colonies that form on King's A and B medium are smooth with regular margins and produce a pigment showing a light yellow-green fluorescence by irradiation with UV-light at 350 nm. The optimal growth temperature is 21 °C. At 4 °C growth can be observed, but none of the isolates are able to grow at 41 °C. Cells are motile by means of one polar flagellum. Each strain is positive in tests for catalase, oxidase, arginine dihydrolase, gelatinase and lecithinase activities and for the hydrolysis of casein. Produces Tween esterase on Tween 60; hydrolysis of Tween 80 is strain dependent. Negative result in tests for the following: hydrolysis of aesculin and starch, formation of levan from sucrose, DNase, production of indole, formation of H₂S from sodium thiosulphate, ice nucleation activity, reduction of nitrate to nitrite and denitrification. Results from tests using Biolog GN microplates show that the following substrates are utilized: *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-fructose, D-galactose, α -D-glucose, *myo*-inositol, D-mannitol, D-mannose, L-rhamnose, D-sorbitol, D-trehalose, methyl pyruvate, monomethyl succinate, acetic acid, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, β -hydroxybutyric acid, itaconic acid, α -ketoglutaric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, formic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine,

L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-proline, L-pyrogutamic acid, L-serine, α -hydroxybutyric acid, γ -aminobutyric acid, α -ketobutyric acid, α -ketovaleric acid, urocanic acid, inosine, putrescine, 2-aminoethanol, L-threonine and glycerol. None of the strains assimilate α -cyclodextrin, dextrin, *N*-acetyl-D-galactosamine, i-erythritol, D-cellobiose, L-fucose, gentiobiose, α -D-lactose, lactulose, maltose, D-melibiose, methyl β -D-glucoside, *p*-hydroxyphenylacetic acid, D-psicose, D-raffinose, turanose, sebacic acid, L-phenylalanine, thymidine, phenylethylamine, 2,3-butanediol, DL- α -glycerol phosphate, glucose 1-phosphate or glucose 6-phosphate. Utilization of the following substrates is strain-dependent: sucrose, glycogen, xylitol, γ -hydroxybutyric acid, glycyl L-aspartic acid, D-serine, DL-carnitine and uridine. Using the Biotype 100-system, tests positive for the utilization of the following additional substrates: D-ribose, L-arabitol, D-xylose, D-lyxose, D-saccharate, mucate, *meso*-tartrate, D-malate, L-malate, *cis*-aconitate, *trans*-aconitate, citrate, D-galacturonate, 2-keto-D-gluconate, D-glucuronate, D-gluconate, protocatechuate, *p*-hydroxybenzoate, quinate, benzoate, betaine, DL- α -amino-*N*-butyrate, DL-lactate, caprate, caprylate, succinate, fumarate, glutarate, DL- α -amino-*N*-valerate, ethanolamine, D-glucosamine, itaconate, DL- β -hydroxybutyrate, L-aspartate, L-glutamate, malonate, propionate, L-tyrosine and α -ketoglutarate. None of the strains assimilate L-sorbose, maltotriose, 1-0-methyl- β -galactopyranoside, 1-0-methyl- α -galactopyranoside, D-cellobiose, 1-0-methyl- β -D-glucopyranoside, palatinose, D-melezitose, dulcitol, D-tagatose, maltitol, hydroxyquinoline- β -glucuronide, 1-0-methyl- α -D-glucopyranoside, 3-0-methyl-D-glucopyranose, L-tartrate, D-tartrate, tricarballoylate, 5-keto-D-gluconate, L-tryptophan, phenylacetate, gentisate, *m*-hydroxybenzoate, 3-phenylpropionate, *m*-coumarate, trigonelline, histamine or tryptamine. In contrast to the tests using the Biolog GN microplates, L-histidine was not used. The assimilation of DL-glycerate is strain-dependent.

The type strain, P 513/18^T (= DSM 15835^T = LMG 21995^T), was isolated from the phyllosphere of grasses in Paulinenaue, Germany.

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