Fluorescent pseudomonads associated with the phyllosphere of grasses; *Pseudomonas trivialis* sp. nov., *Pseudomonas poae* sp. nov. and *Pseudomonas congelans* sp. nov.

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Strains of fluorescent pseudomonads, isolated from the phyllosphere of grasses, were analysed by a polyphasic approach in order to clarify their interspecific position. Classification on the basis of ribotyping revealed six genotypes; four of these, which could be differentiated clearly from each other and from *Pseudomonas* species with validly published names on the basis of phenotypic features, were chosen for detailed phylogenetic analysis. DNA–DNA hybridization studies among representative strains of the four genotypes and closely related *Pseudomonas* species, determined by comparison of 16S rDNA sequences, showed that three of the studied ribotypes represented novel species. Two of them were related to mainly saprophytic fluorescent pseudomonads and could be easily distinguished by a negative arginine dihydrolase reaction. One ribotype, also characterized by a negative arginine dihydrolase reaction, was closely related to potentially plant-pathogenic fluorescent pseudomonads and differed in certain phenotypic features from its phylogenetic neighbours. As a consequence of the phenotypic and phylogenetic analyses, *Pseudomonas trivialis* sp. nov. (type strain: P 513/19^T=DSM 14937^T=LMG 21464^T), *Pseudomonas poae* sp. nov. (type strain: P 527/13^T=DSM 14936^T=LMG 21465^T) and *Pseudomonas congelans* sp. nov. (type strain: P 538/23^T=DSM 14939^T=LMG 21466^T) are proposed.

Fluorescent pseudomonads are typical inhabitants of the phyllosphere and are involved in several interactions with plants (Schroth *et al.*, 1992). Some species have a strictly commensal relationship with their host plants. Furthermore, potentially phytopathogenic pseudomonads can be found on their specific hosts, as well as on plant species that are not susceptible to these pathogens.

Studies of bacterial communities that live in the phyllosphere of grasses showed that predominantly fluorescent *Pseudomonas* species comprised most Gram-negative bacteria present (Behrendt, 2001). Most of them were phenotypically highly similar to the saprophytic species *Pseudomonas fluorescens* or to *Pseudomonas syringae*, a potentially phytopathogenic bacterium. Moreover, strains that showed physiological similarity to the phytopathogenic species *Pseudomonas viridiflava* or *Pseudomonas cichorii* were isolated, but their taxonomic affiliations were ambiguous. Thus, a selection of these strains was characterized by a polyphasic approach to clarify their interspecific position within the genus *Pseudomonas*.

Isolation of strains from the phyllosphere

Samples of grass plots, characterized by different intensities of management, were taken to investigate the community structures of heterotrophic bacteria, as described by Behrendt (2001). Grass material was homogenized in

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The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences analysed in this study are AJ492831 (*Pseudomonas trivialis* P 513/19^T=DSM 14937^T=LMG 21464^T), AJ492829 (*Pseudomonas poae* P 527/13^T=DSM 14936^T=LMG 21465^T), AJ492828 (*Pseudomonas congelans* P 538/23^T=DSM 14939^T=LMG 21466^T), AJ492830 (genotype E1 P 515/12=DSM 14938=LMG 21467), AJ492826 (*Pseudomonas tremae* CFBP 6111^T) and AJ492827 (*Pseudomonas cannabina* CFBP 2341^T).

distilled water by using a Stomacher lab blender and serial dilutions were plated on nutrient agar (SIFIN), supplemented by 0.4 g cycloheximide l⁻¹, to obtain selectivity for bacterial growth. After incubation at 21 °C for 7 days, a representative number of strains was isolated to determine the community structure of culturable heterotrophic bacteria. Isolates were subjected to taxonomic investigations; one set of strains of fluorescent pseudomonads showed a negative arginine dihydrolase reaction and an indefinite taxonomic affiliation after physiological characterization by both conventional tests and application of the commercial Biolog identification system. These strains were used for further studies. Strain numbers of isolates studied and type strains used for comparison are shown in Table 1.

Phylogenetic analysis

To classify the isolates on the basis of phylogenetic heterogeneity, restriction analysis of 16S rDNA was performed according to Behrendt *et al.* (1999). 16S rRNA genes were amplified with primers described by Weisburg *et al.* (1991), resulting in amplification of a single fragment of approximately 1500 bp. Digestion of these PCR products by a set

of endonucleases (*TaqI*, *HinfI*, *AluI*, *MspI*, *CfoI*, *HaeIII* and *ScrFI*) showed identical patterns for almost all isolates; only two, P 516/20 and P 538/23, revealed differences in patterns for two of the enzymes (*AluI* and *ScrFI*). These results indicate high 16S rDNA sequence similarity. Thus, genetic classification of strains according to differences in physiological features could not be carried out by using this method. In order to classify the isolates by a method of higher taxonomic resolution, strains were studied by ribotyping with the restriction enzyme *EcoRI*, which was species-specific within the genus *Pseudomonas* (Sikorski *et al.*, 2001). These analyses were performed with the automated RiboPrinter Microbial Characterization system (Qualicon DuPont). Band patterns were compared by using the BioNumerics software (Applied Maths). Clustering was carried out by UPGMA based on Pearson's correlation coefficient (optimization coefficient, 1.2 %). This procedure revealed grouping of isolates into six genotypes (Fig. 1). Four of these genotypes (A, B, D and E1), which had similarities between ribopatterns of <60 %, were chosen for further phylogenetic studies. Strains of genotype C were not considered, as no effective physiological characteristics were found to differentiate them clearly from isolates of genotype A (Table 2). Although a few

Table 1. Reference and isolated grass-associated strains used in this study

Abbreviations: ATCC, American Type Culture Collection, Manassas, VA, USA; CCEB, Institute of Entomology, CSAV Czechoslovak Academy of Sciences, Department of Insect Pathology, Prague, Czech Republic; CFBP, Collection Française des Bactéries Phytopathogènes, Angers, France; CFML, Collection de la Faculté de Médecine de Lille, Lille, France; CIP, Collection de l'Institut Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; IAM, Institute of Applied Microbiology, University of Tokyo, Institute of Molecular and Cellular Bioscience, Tokyo, Japan; ICMP, International Collection of Microorganisms from Plants, Plant Disease Division, DSIR, Auckland, New Zealand; LMG, Laboratorium voor Mikrobiologie, Universiteit Gent, Gent, Belgium; NCIB (=NCIMB=NCMB), National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NCPPB, National Collection of Plant-Pathogenic Bacteria, Central Science Laboratory, York, UK; P, culture at the collection in the Institute of Primary Production and Microbial Ecology, Centre for Agricultural Landscape and Land Use Research Müncheberg (ZALF), Paulinenaue, Germany; PDDCC, Plant Division Culture Collection, New Zealand Department of Scientific and Industrial Research, Auckland, New Zealand.

Species/ribotype	Strains
<i>Pseudomonas cannabina</i>	CFBP 2341 ^T (=ICMP 2823 ^T =NCPPB 1437 ^T)
<i>Pseudomonas cedrina</i>	CIP 105541 ^T (=CFML 96-198 ^T)
<i>Pseudomonas rhodesiae</i>	DSM 14020 ^T (=CFML 92-111 ^T =CIP 104664 ^T =NCIMB 13509 ^T)
<i>Pseudomonas savastanoi</i> pv. <i>glycinea</i>	LMG 5066 (=ICMP 2189=NCPPB 2411=PDDCC 2189)
<i>Pseudomonas synxantha</i>	LMG 2190 ^T (=ATCC 9890 ^T =CCEB 607 ^T =CIP 59.22 ^T =IAM 12356 ^T =NCIB 8178 ^T)
<i>Pseudomonas tremae</i>	CFBP 6111 ^T (=ICMP 9151 ^T =NCPPB 3465 ^T)
<i>Pseudomonas veronii</i>	DSM 11331 ^T (=CIP 104663 ^T)
Ribotype:	
A (<i>Pseudomonas trivialis</i>)	P 513/19 ^T (=DSM 14937 ^T =LMG 21464 ^T), P 514/01, P 517/12, P 517/22, P 527/30, P 528/25, P 530/13
B (<i>Pseudomonas poae</i>)	P 527/13 ^T (=DSM 14936 ^T =LMG 21465 ^T), P 527/10, P 529/17
C	P 501/02, P 501/16, P 515/18, P 527/17, P 527/22
D (<i>Pseudomonas congelans</i>)	P 538/23 ^T (=DSM 14939 ^T =LMG 21466 ^T), P 516/20
E1	P 515/12 (=DSM 14938=LMG 21467), P 515/10, P 530/23, P 517/04
E2	P 515/15, P 515/17

Fig. 1. Riboprint patterns of *EcoRI*-restricted DNA of the grass isolates studied. Analysis was done with the RiboPrinter Microbial Characterization system (Qualicon DuPont). The riboprint pattern was analysed with the BioNumerics software package GeneExplore version 1.0 (Applied Maths). VCA, RiboPrinter internal designation for an *EcoRI* batch.

phenotypic features were found that differentiated ribotype E2 from E1, the former was also excluded from further studies due to high similarity between ribopatterns.

Sequencing of amplified 16S rDNA of strains representative for the selected genotypes was performed by using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and the primer described by Chun & Goodfellow (1995). For phylogenetic analysis, 16S rDNA sequences of 77 strains,

that represented type strains of all authentic species of the genus *Pseudomonas* were chosen (except for *Pseudomonas gelidicola*, for which no 16S rDNA sequence was available). Furthermore, some strains that represented species without validly published names were involved. Sequences were aligned by using the CLUSTALX algorithm (Thompson *et al.*, 1997) and trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms (PHYLIP version 3.57; Felsenstein, 1993). Both trees showed that the investigated isolates clustered clearly within the genus *Pseudomonas* (data not shown). Species that formed a closely related cluster with the grass isolates were selected for more detailed phylogenetic analyses. A phylogenetic tree based on 1282 nt (*Escherichia coli* positions 99–1376) was constructed for this subset of species, as shown in Fig. 2.

Representative strains of genotypes A, B and E1 clustered at a branch that corresponds to the '*P. fluorescens* group' of Anzai *et al.* (2000), whereas the strain with genotype D was in the '*P. syringae* group'. As indicated by restriction analysis of 16S rDNA, sequences were highly similar among grass isolates. In particular, a comparison of genotypes A and B revealed 99.93 % similarity. The closest phylogenetic neighbours of both isolates were *Pseudomonas veronii* and *Pseudomonas rhodesiae*, with a similarity between 99.3 and 99.4 % (based on a binary comparison of the available length of sequences). The representative strain of genotype E1 displayed highest similarity to *Pseudomonas cedrina* (99.9 %) and *Pseudomonas synxantha* (99.0 %), whereas the closest phylogenetic neighbours of genotype D were pathovars of *Pseudomonas savastanoi*, which displayed similarities of 99.6–99.8 %. Such high conformity in 16S rDNA sequence between species was, to a

Table 2. Physiological characteristics that differ between the genotypes of grass isolates, as determined by ribotyping

+, Positive; –, negative; D, different reaction between tested strains (reaction of type strain in parentheses); W, weak.

Characteristic	Ribotype					
	A*	B†	C	D‡	E1	E2
No. tested strains (<i>n</i>)	7	3	5	2	4	2
Oxidase	W	W	W	–	+	+
Casein hydrolysis	D (–)	D (–)	+	+	–	–
Ice nucleation activity	–	–	D	+	–	–
Utilization of:						
D-Sorbitol	D (–)	–	+	+	–	–
Sucrose	–	+	D	+	–	–
D-Trehalose	+	+	+	+	–	+
α-Ketovaleic acid	+	–	+	–	+	D
i-Erythritol	–	–	–	+	–	–
p-Hydroxyphenylacetic acid	D (–)	–	–	–	–	+

**Pseudomonas trivialis*.

†*Pseudomonas poae*.

‡*Pseudomonas congelans*.

Fig. 2. Phylogenetic tree showing the relationships of the strains studied within a subset of closely related *Pseudomonas* species. The tree is based on a 1282 bp alignment of 16S rDNA 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, *E. coli* (GenBank accession no. J01695) was used as an outgroup. Values are the number of times that a branch appeared in 100 bootstrap replications. Strains characterized in this study are in bold. *Pathovar reference strain. Bar, 0.01 relative sequence divergence.

large extent, found in the studied subset of pseudomonads shown in Fig. 2. Accordingly, most bootstrap values were relatively low and only certain branches were confirmed by the topology of the maximum-likelihood tree.

To clarify the interspecific position of the investigated genotypes, DNA–DNA hybridization studies were performed according to a method described previously (Martin *et al.*, 1997). Representative strains of genotypes were tested against each other and against species with validly published names that were the closest phylogenetic neighbours, as estimated by binary comparison of 16S rDNA sequences. As shown in Table 3, all tested pairings

of genotypes A, B and D resulted in reassociation values <70 %, indicating separate species status. However, hybridization studies between genotype E1 and *P. cedrina* revealed similarities of 76.4 and 78.3 %, suggesting an affiliation to the species *P. cedrina*.

Morphological and physiological characterization

Morphological and physiological characterization was performed as described by Behrendt *et al.* (1999). All grass isolates studied were Gram-negative, non-spore-forming, rod-shaped organisms that occurred as single

Table 3. DNA reassociation values (%) among *Pseudomonas* strains tested

Strain	Genotype			
	A	B	D	E1
<i>Pseudomonas trivialis</i> P 513/19 ^T (genotype A)	–	60.0	32.9	53.7
<i>Pseudomonas poae</i> P 527/13 ^T (genotype B)	60.0	–	29.5	50.7
<i>Pseudomonas congelans</i> P 538/23 ^T (genotype D)	32.9	29.5	–	29.0
Genotype E1 (P 515/12)	53.7	50.7	29.0	–
<i>Pseudomonas rhodesiae</i> DSM 14020 ^T	43.8	41.7	–	–
<i>Pseudomonas veronii</i> DSM 11331 ^T	41.5	31.7	–	–
<i>Pseudomonas savastanoi</i> pv. <i>glycinea</i> LMG 5066*	–	–	44.8	–
<i>Pseudomonas synxantha</i> LMG 2190 ^T	–	–	–	48.9
<i>Pseudomonas cedrina</i> CIP 105541 ^T	–	–	–	76.4†

*Pathovar reference strain.

†Repetition: 78.3.

cells. The cells were motile by means of one or two polar flagella. Colonies were colourless, translucent and formed a green fluorescent pigment on media B and A of King *et al.* (1954).

Each strain gave positive results for catalase, aesculin hydrolysis and aerobic acid formation from glucose (Hugh & Leifson, 1953). The following characteristics were negative for all strains: hydrolysis of starch, formation of levan from sucrose, reduction of nitrate to nitrite, denitrification, arginine dihydrolase, DNase, indole production, formation of H₂S from sodium thiosulphate, utilization of D-tartrate on Dye's medium (Sands, 1990) and β -haemolysis of sheep blood. Production of oxidase was assayed on an early growth-phase culture with the aid of Bactident Oxidase test strips (Merck). Strains that developed a purple colour after 20–60 s, the time-span given by the manufacturers for a positive reaction, were considered oxidase-weak. Results of oxidase tests are shown in Table 2.

Assimilation of carbon substrates was determined by using Biolog GN MicroPlates as recommended by the manufacturers (MicroLog System, release 4.0). Results were read visually after 48 h incubation at 30 °C. A survey of the assimilation of substances is given in the species descriptions. Physiological tests that were different among the genotypes determined by ribotyping are shown in Table 2.

Taxonomic conclusions

The fluorescent pseudomonads studied, isolated from the phyllosphere of grasses, are composed of six genotypes as determined by ribotyping. The four that showed highest dissimilarity in ribopatterns and physiological features were chosen for further phylogenetic studies. Comparison of 16S rDNA sequences between representative strains and almost all authentic *Pseudomonas* species revealed a clear affiliation to the genus *Pseudomonas sensu stricto*. DNA–DNA hybridization studies of these strains and their

nearest phylogenetic neighbours (as determined by 16S rDNA sequence comparisons) indicated separate species status for three of the genotypes studied.

This result is supported by phenotypic features. The yellow-pigmented, halophilic, marine bacterium *P. gelidicola*, which was not used in 16S rDNA analysis, differed clearly from the grass isolates in its ability to hydrolyse agar and requirement of NaCl for growth (Holt *et al.*, 1994). By 16S rDNA analysis, strains of genotypes A and B (*Pseudomonas trivialis* sp. nov. and *Pseudomonas poae* sp. nov., respectively) were grouped with species characterized by a positive arginine dihydrolase reaction (Rudolph *et al.*, 1990; Coroler *et al.*, 1996; Elomari *et al.*, 1996; Dabboussi *et al.*, 1999; Baïda *et al.*, 2002; Gardan *et al.*, 2002; Ivanova *et al.*, 2002; Munsch *et al.*, 2002). They can thus be easily distinguished from their nearest phylogenetic neighbours. Phenotypic features that are effective for differentiation from physiologically similar species, characterized in particular by a negative arginine dihydrolase reaction, are shown in Table 4.

In contrast to the other genotypes, the representative strain of genotype D (*Pseudomonas congelans* sp. nov.) was a close neighbour to mostly oxidase- and arginine dihydrolase-negative species of *Pseudomonas*, which are potentially plant-pathogenic. Phenotypic features that are effective for differentiation from phylogenetically related species are shown in Table 4. *Pseudomonas savastanoi*, *Pseudomonas ficuserectae*, *Pseudomonas meliae* and *Pseudomonas amygdali* were included as separate species, although extensive DNA–DNA hybridization studies by Gardan *et al.* (1999) revealed that, together with certain pathovars of *P. syringae*, they formed a single genomospecies and should therefore be considered as synonymous. Unfortunately, the authors did not formally propose an emended species description, as no phenotypic features were found to distinguish them from six other genomospecies delineated

Table 4. Differentiating characteristics of arginine dihydrolase-negative *Pseudomonas* species that are physiologically similar to the proposed novel *Pseudomonas* species

Taxa: 1, genotype A (*P. trivialis*); 2, genotype B (*P. poae*); 3, genotype D (*P. congelans*); 4, *P. agarici* [data from Young (1970), Hildebrand *et al.* (1994) and Delorme *et al.* (2002) (Biotype 100)]; 5, *P. amygdali* [Psallidas & Panagopoulos (1975)]; 6, *P. avellanae* [Janse *et al.* (1996)]; 7, *P. cannabina* [Gardan *et al.* (1999) (Biotype 100)]; 8, *P. cichorii* [Billing (1970), Delorme *et al.* (2002) (Biotype 100) and Biolog database (release 4.0)]; 9, *P. ficuserectae* [Hu *et al.* (1991)]; 10, *P. meliae* [Hu *et al.* (1991)]; 11, *P. kilonensis* [Sikorski *et al.* (2001) (Biolog GN, API 20NE and 50CH)]; 12, *P. savastanoi* pv. *savastanoi* [Psallidas & Panagopoulos (1975) and Gardan *et al.* (1992) (API 50AA, AO and CH)]; 13, *P. savastanoi* pv. *glycinae* [Billing (1970), Gardan *et al.* (1992) (API 50AA, AO and CH), Hildebrand *et al.* (1994) and Biolog database (release 4.0)]; 14, *P. tremiae* [Gardan *et al.* (1999)]; 15, *P. viridiflava* [Billing (1970), Hildebrand *et al.* (1994) and Biolog database (release 4.0)]. Abbreviations: +, positive reaction; –, negative reaction; D, reaction differs among strains; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fluorescent pigments	+	+	+	+	–	+	+	+	–	–	+	+	+	–	+
Oxidase	W	W	–	+	–	–	–	+	–	–	+	–	–	–	–
Levan sucrase	–	–	–	–	ND	+	+	–	+	–	ND	–	+	–	–
Aesculin hydrolysis	+	+	+	–	–	–	–	–	ND	–	–	–	–	–	+
Nitrate reduction	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
Gelatin hydrolysis	D	D	+	–	–	–	–	–	–	–	+	–	–	–	+
Utilization of:															
L-Arabinose	+	+	+	–	ND	+	–	+	–	–	+	+	D	–	+
D-Arabitol	+	+	+	ND	ND	ND	–	D	ND	ND	+	ND	–	–	+
<i>m</i> -Inositol	+	+	+	ND	–	+	–	D	–	–	+	V	+	–	+
D-Mannitol	+	+	+	+	+	+	–	+	ND	ND	+	+	+	–	+
Sucrose	–	+	+	–	+	+	ND	–	+	–	+	+	+	ND	–
D-Galacturonic acid	+	+	+	–	ND	ND	ND	+	ND	ND	–	ND	–	ND	+
D-Glucosaminic acid	+	+	+	–	ND	ND	ND	–	ND	ND	+	ND	–	ND	–
D-Glucuronic acid	+	+	+	–	ND	ND	ND	+	ND	ND	–	ND	–	ND	+
L-Asparagine	+	+	+	ND	+	–	ND	+	ND	ND	+	ND	+	ND	+
L-Pyrogutamic acid	+	+	+	ND	ND	ND	ND	D	ND	ND	–	ND	–	ND	+
L-Threonine	+	+	+	ND	ND	–	ND	D	ND	ND	+	ND	–	ND	+
D-Trehalose	+	+	+	–	–	–	ND	–	–	–	+	ND	–	ND	–
Erythritol	–	–	+	ND	–	ND	ND	–	–	–	–	–	–	ND	+
D-Tartrate	–	–	–	–	–	–	ND	–	ND	ND	ND	–	–	ND	+
Glycerol	+	+	+	ND	–	+	+	D	ND	ND	+	ND	D	–	–

by DNA–DNA hybridization studies of 48 *P. syringae* pathovars.

Genomic heterogeneity of *P. syringae* pathovars, as revealed by DNA–DNA hybridization studies (Gardan *et al.*, 1999), is linked with phenotypic differences as shown by Gardan *et al.* (1991) and makes species demarcation more difficult. Thus, several physiological features in Table 4 would be listed as variable, because of differences between pathovars. Therefore, *P. syringae* was not included in Table 4. However, phenotypic characterization of 48 *P. syringae* pathovars and eight related species by Gardan *et al.* (1999) showed that <10 % of strains studied were able to utilize D-trehalose; they can thus be differentiated from the grass isolates of genotypes A, B and D (*P. trivialis*, *P. poae* and *P. congelans*, respectively), which are positive for this feature. Furthermore, characterization of phenotypic relationships by Billing (1970), Gardan *et al.* (1991), Hildebrand *et al.* (1994) and Young & Triggs (1994) showed that pathovars of *P. syringae* can be differentiated from the grass isolates by a combination of the following features: levan sucrase,

aesculin and gelatin hydrolysis, oxidase, ice nucleation activity and utilization of erythritol.

DNA–DNA hybridization studies of the representative strain of genotype E1 revealed an affiliation to the species *P. cedrina*. However, in contrast to the strains of *P. cedrina* that were isolated from Lebanese spring water (Dabboussi *et al.*, 1999), grass-associated isolates were characterized by negative arginine dihydrolase and levan sucrase reactions. Furthermore, strains of genotype E1 were not able to assimilate D-trehalose, erythritol, D-sorbitol or adonitol. Thus, the grass isolates showed differences in physiological features that are normally used to distinguish species.

In summary, three of the four characterized grass-associated genotypes can be distinguished from all *Pseudomonas* species with validly published names on the basis of phenotypic and phylogenetic features. Consequently, the names *Pseudomonas trivialis* sp. nov., *Pseudomonas poae* sp. nov. and *Pseudomonas congelans* sp. nov. are proposed.

General description of novel *Pseudomonas* species and strains of genotype E1

Cells are Gram-negative, non-spore-forming rods that occur as single cells. Colonies on King A and B media are white-yellowish, smooth with regular margins and produce a pigment with light yellow-green fluorescence on irradiation with UV light. Metabolism is respiratory. Optimal growth temperature is 21 °C. Growth can be observed at 4 °C, but none of the isolates is able to grow at 41 °C.

Each species is positive for catalase, aesculin hydrolysis and aerobic acid formation from glucose. The following characteristics are negative: hydrolysis of starch, formation of levan from sucrose, reduction of nitrate to nitrite, denitrification, arginine dihydrolase, DNase, indole production, formation of H₂S from sodium thiosulphate, utilization of D-tartrate and β -haemolysis of sheep blood.

Results obtained with Biolog GN MicroPlates show that the species oxidize the following substrates: L-arabinose, D-arabitol, bromosuccinic acid, D-fructose, D-galactose, *m*-inositol, D-mannitol, D-mannose, methyl pyruvate, acetic acid, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -ketoglutaric acid, malonic acid, propionic acid, D-saccharic acid, succinic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, itaconic acid, L-leucine, L-pyrogutamic acid, L-serine, L-threonine, Tween 80, γ -aminobutyric acid, urocanic acid, inosine, uridine and glycerol. None of the strains assimilated *N*-acetyl-D-galactosamine, adonitol, D-arabitol, α -cyclodextrin, dextrin, L-fucose, gentiobiose, glucose 1-phosphate, methyl β -D-glucoside, α -D-lactose, lactulose, maltose, D-melibiose, D-raffinose, turanose, xylitol, sebacic acid, L-phenylalanine, thymidine, phenylethylamine or 2,3-butanediol.

Description of *Pseudomonas trivialis* sp. nov.

Pseudomonas trivialis (tri.vi.a'lis. L. fem. adj. *trivialis* trivial).

Cells are motile by one polar flagellum and are 1.6–3.3 μ m long and about 0.8 μ m wide. Oxidase reaction is weak and ice nucleation activity is negative. In addition to the general description, the following substances are assimilated: formic acid, α -D-glucose, α -ketovaleric acid, glycyl L-glutamic acid, L-histidine, β -hydroxybutyric acid, α -ketobutyric acid, D-alanine, L-proline, quinic acid, monomethyl succinate, succinamic acid, D-trehalose and Tween 40. None of the strains assimilates *i*-erythritol, glucose 6-phosphate, glycogen, glycolyl L-aspartic acid or D-psicose. Gelatinase, casein hydrolysis and utilization of *N*-acetyl-D-glucosamine, D-cellobiose, L-rhamnose, α -hydroxybutyric acid, γ -hydroxybutyric acid, DL-lactic acid, hydroxy-L-proline, L-ornithine, D-serine, DL-carnitine, putrescine, 2-aminoethanol, DL- α -glycerolphosphate, *p*-hydroxyphenylacetic acid and D-sorbitol are strain-dependent.

The type strain, P 513/19^T (=DSM 14937^T=LMG 21464^T), was isolated from the phyllosphere of grasses in Paulinenau (Germany).

Description of *Pseudomonas poae* sp. nov.

Pseudomonas poae (po'ae. Gr. n. *poa* grass; N.L. gen. n. *poae* of/from grass).

Cells are motile by one polar flagellum and are 2.5–3.3 μ m long and about 0.8 μ m wide. Strains show a weak oxidase reaction. Ice nucleation activity is negative. In addition to the general description, the following substances are assimilated: 2-aminoethanol, β -hydroxybutyric acid, L-proline, L-histidine, DL-lactic acid, putrescine, quinic acid, succinamic acid, sucrose, D-trehalose and Tween 40. None of the strains assimilates γ -hydroxybutyric acid, D-cellobiose, *i*-erythritol, glucose 6-phosphate, DL- α -glycerol phosphate, glycyl L-aspartic acid, *p*-hydroxyphenylacetic acid, α -ketovaleric acid, D-sorbitol or L-rhamnose. Gelatinase, casein hydrolysis and utilization of glycogen, *N*-acetyl-D-glucosamine, α -D-glucose, D-psicose, monomethyl succinate, formic acid, α -hydroxybutyric acid, α -ketobutyric acid, glycyl L-glutamic acid, hydroxy-L-proline, L-ornithine, D-serine and DL-carnitine are strain-dependent.

The type strain, P 527/13^T (=DSM 14936^T=LMG 21465^T), was isolated from the phyllosphere of grasses in Paulinenau (Germany).

Description of *Pseudomonas congelans* sp. nov.

Pseudomonas congelans (con.ge'lans. L. part. adj. *congelans* freezing/forming ice).

Cells are motile by one polar flagellum and are 2.5–2.9 μ m wide and about 0.8 μ m long. Strains are positive for gelatinase, casein hydrolysis and ice nucleation activity. Oxidase reaction is negative. In addition to the general description, the following substances are assimilated: DL-lactic acid, 2-aminoethanol, *i*-erythritol, α -D-glucose, glycyl L-glutamic acid, DL- α -glycerol phosphate, α -hydroxybutyric acid, β -hydroxybutyric acid, formic acid, L-histidine, monomethyl succinate, L-proline, D-psicose, quinic acid, D-sorbitol, D-serine, succinamic acid, sucrose, D-trehalose and Tween 40. None of the strains assimilates *N*-acetyl-D-glucosamine, DL-carnitine, D-cellobiose, glucose 6-phosphate, glycyl L-aspartic acid, γ -hydroxybutyric acid, hydroxy-L-proline, glycogen, *p*-hydroxyphenylacetic acid, α -ketovaleric acid, L-ornithine, putrescine or L-rhamnose.

The type strain, P 538/23^T (=DSM 14939^T=LMG 21466^T), was isolated from the phyllosphere of grasses in Paulinenau (Germany).

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