Pseudomonas reinekei sp. nov., *Pseudomonas moorei* sp. nov. and *Pseudomonas mohnii* sp. nov., novel species capable of degrading chlorosalicylates or isopimaric acid

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Three bacterial strains, designated MT1^T, RW10^T and IpA-2^T, had been isolated previously for their ability to degrade chlorosalicylates or isopimaric acid. 16S rRNA gene sequence analysis demonstrated that these bacteria are related to species of the genus *Pseudomonas*. Analysis of the results of DNA–DNA hybridization with several close phylogenetic neighbours revealed a low level of hybridization (less than 57 %). On the basis of phenotypic characteristics, phylogenetic analysis, DNA–DNA relatedness data and chemotaxonomic analysis, it is concluded that these isolates represent separate novel species, for which the names *Pseudomonas reinekei* sp. nov. (type strain MT1^T = DSM 18361^T = CCUG 53116^T), *Pseudomonas moorei* sp. nov. (type strain RW10^T = DSM 12647^T = CCUG 53114^T) and *Pseudomonas mohnii* sp. nov. (type strain IpA-2^T = DSM 18327^T = CCUG 53115^T) are proposed.

The genus *Pseudomonas sensu lato* has been subdivided into several genera following rRNA–DNA hybridization studies (Palleroni *et al.*, 1972, 1973) and 16S rRNA gene sequence comparisons (Anzai *et al.*, 2000; Moore *et al.*, 1996). The species belonging to the genus *Pseudomonas sensu stricto* are now restricted to rRNA similarity group I of Palleroni (1984), which belongs to the class *Gammaproteobacteria* (Palleroni, 1984). Polyphasic taxonomic studies and 16S rRNA gene sequence analysis have largely contributed to the stabilization of the taxonomy of *Pseudomonas*; however, the value of chemotaxonomy (Wilkinson, 1968) in helping to delineate novel taxa is still largely overlooked.

Strains belonging to the genus *Pseudomonas* are widely distributed in soils and sediments and are well known for having a broad metabolic versatility. Since the first observation of *Pseudomonas* strains capable of degrading chloroaromatics (Dorn *et al.*, 1974), various other *Pseudomonas* isolates able to degrade chloroaromatics have been described

(Pieper & Reineke, 2004). As in the case for chloroaromatics, the release of resin acids into the environment has led to considerable environmental concern and, among bacterial isolates able to degrade resin acids, *Pseudomonas* strains were frequently observed (Martin *et al.*, 1999).

Strain MT1^T is the most abundant organism in a four-member 4-chlorosalicylate-degrading community isolated from a polluted aerobic sediment of the River Elbe near Bitterfeld, Germany (Pelz *et al.*, 1999). Degradation of 4- and 5-chlorosalicylate by MT1^T occurs via 4-chlorocatechol as a central intermediate (Nikodem *et al.*, 2003); however, degradation of 4-chlorocatechol by this strain proceeds by a novel metabolic route distinct from the previously described chlorocatechol pathway and involves a *trans*-dienelactone hydrolase as a key enzyme. Strain RW10^T was isolated from an enrichment culture inoculated with aerobic sediments from the River Elbe, Hamburg, Germany (Wittich *et al.*, 1999). Like MT1^T, RW10^T is capable of utilizing 4- or 5-chlorosalicylate without involvement of the chlorocatechol pathway, possibly by a metabolic route similar to the one elucidated in MT1^T (Wittich *et al.*, 1999). Strain IpA-2^T was enriched from a laboratory sequencing batch reactor treating pulp mill wastewater (Wilson *et al.*, 1996) on the basis of its ability to degrade the resin acid isopimaric acid, and was characterized previously as a member of *Pseudomonas* (Mohn *et al.*, 1999). So far, no attempt has been made to

Abbreviations: LPS, lipopolysaccharide; ME, minimum evolution.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MT1^T, RW10^T and IpA-2^T are respectively AM293565–AM293567, those for the partial *atpD* gene sequences of strains MT1^T, RW10^T and IpA-2^T are respectively AM293552–AM293554 and those for the partial *gyrB* gene sequences of strains MT1^T, RW10^T and IpA-2^T are respectively AM293559–AM293561.

identify these three organisms to the species level. In the present study, we have undertaken an investigation of the phylogenetic, physiological/biochemical and chemotaxonomic properties of these strains, together with appropriate type strains. Based on the phenotypic and molecular data obtained, three novel species are proposed within the genus *Pseudomonas*.

As reference strains, *Pseudomonas koreensis* DSM 16610^T, *Pseudomonas jessenii* DSM 17150^T, *Pseudomonas vancouverensis* DSM 17555^T and *Pseudomonas umsongensis* DSM 16611^T were obtained from the DSMZ. *Pseudomonas* sp. IpA-2^T was obtained from William Mohn. All strains were cultured in Luria–Bertani (LB) media at 30 °C, unless otherwise indicated. *Pseudomonas fluorescens* DSM 50090^T and *Pseudomonas aeruginosa* DSM 50071^T were used for comparative purposes in the chemotaxonomic studies.

16S rRNA, *atpD* and *gyrB* gene sequence analysis and DNA–DNA hybridization

Genomic DNA was prepared from single colonies which were suspended in 100 µl TE buffer, heated to 95 °C for 5 min and centrifuged briefly; the supernatant was used. 16S rRNA gene amplification and sequencing was carried out as described previously (Moore *et al.*, 1996).

For *atpD* amplification, previously designed primers (Hilario *et al.*, 2004) were modified based on an upgraded alignment of recently published *Pseudomonas atpD* gene sequences to give primers atpD-F (5'-CTGGGCCGATC-ATGGACG-3') and atp-R (5'-GTCCATRCCCAGGATSGC-3'). PCR amplifications [50 µl final volume: 20 pmol of each primer, 5 nmol dNTPs (Pqlab), 2.5 U *Taq* polymerase (Qiagen) and 1 µl DNA template] were performed on an AB9700 thermal cycler (Applied Biosystems) with an initial denaturation at 94 °C for 2 min, 35 cycles of denaturation (94 °C, 30 s), annealing (58 °C, 45 s) and elongation (72 °C, 60 s) and a final elongation at 72 °C for 5 min. *gyrB* gene amplifications (approx. 900 bp fragment) were performed as indicated above but with 10 pmol of each primer (Yamamoto *et al.*, 2000). PCR conditions included an initial denaturation at 96 °C for 2 min followed by 5 cycles of denaturation (95 °C, 30 s), annealing (62 °C, 60 s) and elongation (72 °C, 60 s), 30 further cycles as before but with annealing at 57 °C for 45 s and a final elongation at 72 °C for 5 min. Purified amplicons were sequenced using the PCR primers described above. Reactions contained 10 pmol primer, 2 µl BigDye 1.1 premix (Applied Biosystems), 1 µl buffer and approx. 10 ng DNA in a 10 µl reaction. Cycle sequencing was carried out at the appropriate annealing temperatures for 25 cycles. DNA sequences were resolved on a 3130xl DNA sequencer (Applied Biosystems). Sequences were edited and assembled with Sequencher software (Gene Codes Co.) and consensus sequences were aligned using CLUSTAL X (Thompson *et al.*, 1997). Alignments were imported and edited manually in MEGA3 software (Kumar *et al.*, 2004), bootstrap analysis was performed by 1000

resamplings and graphical representations of the data were calculated using Jukes–Cantor distances.

PCR amplification and cycle sequencing of *atpD* and *gyrB* genes yielded PCR products of the expected length and without any ambiguous nucleotide positions. In contrast, the 16S rRNA gene sequences of strains RW10^T and IpA-2^T included ambiguous nucleotides at several positions situated in helix 18 (RW10^T and IpA-2^T) and helix 37 (RW10^T), indicating sequence heterogeneities. It is unlikely that these heterogeneities were due to contamination, but were rather the effect of the presence of multiple heterogeneous 16S rRNA operons, an anomaly also reported for a variety of bacterial taxa including other *Pseudomonas* species (Nelson *et al.*, 2002).

16S rRNA gene sequence dissimilarities were calculated from 1426 nucleotide positions. In the sequences of strains IpA-2^T and RW-10^T, there were 8 and 18 ambiguous positions, which might be due to heterogeneities between multiple operons (Coenye & Vandamme, 2003). These positions overlapped and were found in regions of the 16S rRNA gene sequence which showed the greatest degree of variability. These differences might reflect real differences between the different operons within a single strain, but their resolution would not necessarily contribute to solving the taxonomic question addressed in this paper. Given the high degree of similarity between the strains used in this study, the resolution of the 16S rRNA gene sequences is not sufficient to allow delineation of the various species, and the circumscription of the novel species depends primarily on data other than the 16S rRNA gene sequence. Removing the stretch of 18 nucleotides from the analysis did not affect the position of this group of strains relative to other *Pseudomonas* species. The group of strains studied here formed a distinct cluster within the genus *Pseudomonas* (Kwon *et al.*, 2003), sharing >98 % sequence similarity within the cluster, but <97 % sequence similarity to other species within the genus *Pseudomonas*. Because of the removal of the 18 polymorphic positions, these values are fairly conservative. In addition to the 16S rRNA gene sequence data, there are indications of heterogeneity within the genus *Pseudomonas*, such as differences in chemotaxonomic properties (Stead, 1992; Vancanneyt *et al.*, 1996), which we have used to reduce the number of species included in this taxonomic study. As a consequence, sequence distances between strains MT1^T, IpA-2^T and RW10^T and the type strains of *P. umsongensis*, *P. vancouverensis*, *P. koreensis* and *P. jessenii* were <0.01 (i.e. 1 % sequence divergence; Fig. 1a) and thus could not be used to identify interspecific relationships between related taxa.

In order to achieve greater interspecific resolution at the genus level, two housekeeping genes, *atpD* (Hilario *et al.*, 2004) and *gyrB* (Yamamoto *et al.*, 2000), were sequenced. Analysis of approximately 775 nucleotides of the *atpD* genes revealed that strain MT1^T had the highest degree of similarity to the type strains of *P. jessenii* (99.1 %) and *P. koreensis* (98.7 %) (Fig. 1b). The translated AtpD amino

Fig. 1. Similarity dendrograms based on comparison of nearly complete 16S rRNA gene sequences (a), partial *atpD* gene sequences (b) and partial *gyrB* gene sequences (c). The dendrograms were calculated with the ME method (Nei & Kumar, 2000) after distance correction as described by Jukes & Cantor (1969) and as implemented in the MEGA3 software package (Kumar *et al.*, 2004). Reliability of the inferred trees was tested by bootstrapping using 1000 resamplings. Only columns in which all nucleotides were determined unambiguously were included in the calculation. The *atpD* sequence of the type strain of *Pseudomonas putida* was not available from public databases. Bars, 1 estimated substitution per 100 bases.

acid sequences of these three strains were identical, indicating that the changes in the nucleotide sequences were the result of synonymous substitutions. Strains *IpA-2*^T and *RW10*^T differed from each other (2.8 % sequence dissimilarity) and from strain *MT1*^T (5.8 and 5.1 % sequence dissimilarity, respectively) and, according to their *atpD* sequences, were most closely related to *P. vancoverensis* DSM 17555^T (3.8 and 4.2 % sequence dissimilarity, respectively), a strain reported previously to be able to degrade the resin acid dehydroabietic acid (Mohn *et al.*, 1999).

Dissimilarities of partial *gyrB* gene sequences deduced from comparison of 890 nucleotide sites revealed greater sequence dissimilarities than observed for the 16S rRNA and *atpD* gene sequences (Fig. 1c). The sequence dissimilarity between strains *IpA-2*^T and *RW10*^T was found to be 3.3 %, and the distances to strain *MT1*^T were 7.4 and 6.7 %, respectively.

The overall tree topology derived from the minimum-evolution (ME; Kumar *et al.*, 2004) dendrogram of the 16S rRNA gene sequences was consistent with maximum-parsimony and neighbour-joining trees and did not change when different distance models were chosen for calculation in the latter method of analysis. As shown in Fig. 1(b), the clustering of the *atpD* gene sequences of the three strains was similar to that observed from the 16S rRNA gene sequence dendrogram. In all cases, strains *IpA-2*^T and *RW10*^T clustered with *P. vancoverensis* DSM 17555^T and strain *MT1*^T clustered with *P. jessenii* DSM 17150^T and *P.*

koreensis DSM 16610^T. Whilst the *AtpD* dendrograms derived from translated amino acid sequences exhibited very short internal branches, which resulted in changes in the internal branching order (data not shown), the overall clustering remained constant.

The tree topology of the *gyrB* nucleotide sequence-derived dendrograms differed slightly depending on the method of calculation used. It was, however, consistent for the trees constructed from translated amino acid sequences, despite weak statistical support as expressed by low bootstrap values. Comparison of the aligned nucleic acid and translated amino acid sequences revealed that, similar to *atpD*, most nucleotide exchanges were synonymous, resulting in highly similar amino acid sequences (data not shown).

Several attempts have been made to resolve the intrageneric structure of the genus *Pseudomonas* by analyses of the sequences of the 16S rRNA gene (Anzai *et al.*, 2000; Moore *et al.*, 1996) and protein-coding genes (Ait Tayeb *et al.*, 2005; Hilario *et al.*, 2004; Yamamoto & Harayama, 1995; Yamamoto *et al.*, 2000). These studies revealed that defined clusters exist within the genus. However, as observed in this study (Fig. 1), tree topologies were incongruent and poorly supported at a statistical level. Nevertheless, nucleotide sequence distances of *gyrB* and *atpD* allowed differentiation of the three strains from closely related species.

A good correlation between *gyrB* sequence dendrograms and DNA–DNA association data for the genus *Acinetobacter* was

reported (Yamamoto *et al.*, 1999). The authors reported an estimated species limit of 0.041 sequence distance for *gyrB*, corresponding to a DNA–DNA hybridization value of >70% for species within this genus. In the present study, DNA for DNA–DNA hybridization studies was isolated by using a French pressure cell and purified by chromatography on hydroxyapatite (Cashion *et al.*, 1977). DNA–DNA reassociation was performed under optimal conditions (2 × SSC plus 5% formamide at 70 °C) and recorded using a Gilford 2600 spectrophotometer. With the exception of the pair IpA-2^T and RW10^T (distance 0.033), all distances found for the sequence set used in the dendrograms (Fig. 1c) were >0.05; however, the DNA–DNA hybridization value for IpA-2^T and RW10^T was only 50.2% (53.4% reciprocal value; Table 1).

DNA–DNA hybridization was carried out with closely related type strains, which were selected on the basis of their 16S rRNA gene sequence similarities. The three novel strains shared the highest DNA relatedness (45–56%) among the species compared, none of which gave a value for DNA–DNA relatedness higher than the 70% guideline value (Wayne *et al.*, 1991).

Chemotaxonomy

Respiratory lipoquinones and polar lipids were extracted from freeze-dried cell material (100 mg) using a two-stage method (Tindall, 1990a, b). Lipoquinones were separated by TLC and UV-absorbing bands were removed from the plate and analysed further by reverse-phase HPLC. Polar lipids were separated by two-dimensional, silica-gel TLC; total lipid material and specific functional groups were detected using dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate–Schiff (α-glycols), Dragendorff (quaternary nitrogen) and anisaldehyde–sulfuric acid (glycolipids) as described previously (Tindall, 1990a, b). Fatty acids were analysed as the methyl ester derivatives prepared from 10 mg dry cell material. Cells were subjected to differential hydrolysis to detect ester-linked and non-ester-linked

(amide-bound) fatty acids (Labrenz *et al.*, 1998). Fatty acid methyl esters were analysed by GC using a 0.2 μm × 25 m non-polar capillary column and flame-ionization detection under conditions as follows: injection and detector port temperature, 300 °C; inlet pressure, 60 kPa; split ratio, 50:1; injection volume, 1 μl; temperature program, 130–310 °C at a rate of 4 °C min⁻¹.

All strains produced ubiquinones, with ubiquinone 9 (Q9) as the predominant respiratory lipoquinone. The fatty acid compositions (Table 2) are consistent with those published previously for members of the genus *Pseudomonas* (*Pseudomonas* RNA group 1) (Stead, 1992; Vancanneyt *et al.*, 1996). However, additional observations could be made which are relevant to any future evaluation of the taxonomy of this genus. All strains contained saturated and unsaturated C₁₆ and C₁₈ fatty acids as the main components, although this is not particularly unusual for members of the *Gamma-proteobacteria*. Discrimination lies at the level of the 'less-important' fatty acids, some of which are probably components of the lipopolysaccharides (LPS), rather than being present in the polar lipids. This is particularly true of the 2-OH and 3-OH fatty acids, where the exact structure of the LPS from *P. aeruginosa* strains has been described (Ernst *et al.*, 1999). The differential hydrolysis methods used here indicated that the 3-OH 10:0 and 2-OH 12:0 fatty acids are ester-linked, while the 3-OH 12:0 fatty acid appears to be amide- and/or ester-linked, depending on the strain examined. With the exception of strain RW10^T, the (genetically) closely related group of strains examined contained only amide-linked 3-OH 12:0. In contrast, both *P. aeruginosa* DSM 50071^T and *P. fluorescens* DSM 50090^T contained both amide- and ester-linked 3-OH 12:0. These results are the first indication that such methods might help to differentiate groups within '*Pseudomonas* RNA group 1'. It is interesting to note that all closely related strains examined here produced 12:0 fatty acids (up to 6% of the total fatty acid composition) and that 14:0 fatty acids were present in relatively small amounts (<1.0% of total fatty acid composition). The presence of 2-OH 12:0 fatty acids in all strains examined is characteristic for the majority of species within the genus *Pseudomonas* (Vancanneyt *et al.*, 1996). Strains that produce more than trace amounts (>1%) of 14:0 fatty acids are clearly definable (Vancanneyt *et al.*, 1996) and also group together based on the combined analysis of the nucleotide sequences of the *gyrB* and *rpoD* genes (Yamamoto *et al.*, 2000) as a distinct subgroup that is included in intrageneric cluster I within the genus *Pseudomonas*, as well as on the basis of 16S rRNA gene sequence data (Moore *et al.*, 1996). In the strains studied here, only trace amounts of 14:0 fatty acids were detected, confirming that the strains belong in the intrageneric cluster II defined previously (Yamamoto *et al.*, 2000). It is interesting to notice that the different levels of 12:0 in the small number of taxa examined here indicate that this fatty acid might also be useful to help detect infrastructure within the genus *Pseudomonas*. Clearly there is more scope for the evaluation of intrageneric groupings within the genus *Pseudomonas* (RNA

Table 1. Results of DNA–DNA hybridization experiments

Values are percentage results from hybridization at 70 °C. Reciprocal values are given in parentheses. ND, Not done.

Strain	MT1 ^T	RW10 ^T	IpA-2 ^T
<i>P. koreensis</i> DSM 16610 ^T	35.1 (41.5)	39.5 (33.6)	ND
<i>P. vancouverensis</i> DSM 17555 ^T	28.9 (29.3)	15.1 (8.2)	30.7 (37.1)
<i>P. umsongensis</i> DSM 16611 ^T	22.4 (34.5)	40.5 (42.6)	23.3 (29.9)
<i>P. jessenii</i> DSM 17150 ^T	44.8 (49.2)	56.4 (55.7)	ND
Strain RW10 ^T	33.4 (41.2)	–	50.2 (53.4)
Strain MT1 ^T	–	33.4 (41.2)	ND

Table 2. Fatty acid composition (%) of the strains used in this study

Methods 1 (M1) and 2 (M2) refers to the method of differential hydrolysis (see text), which distinguishes between ester- and amide-linked fatty acids. M1 releases ester-linked and M2 releases ester-linked plus amide-linked fatty acids.

Fatty acid	MT1 ^T		RW10 ^T		IpA-2 ^T		P. koreensis DSM 16610 ^T		P. jessenii DSM 17150 ^T		P. vancoverensis DSM 17555 ^T		P. umsongensis DSM 16611 ^T		P. fluorescens DSM 50090 ^T		P. aeruginosa DSM 50071 ^T	
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
3-OH 10:0	2.49	3.34	2.30	2.92	4.48	3.93	1.90	2.24	3.12	3.12	2.64	3.37	2.76	3.51	2.27	2.53	1.56	2.38
12:0	4.22	3.55	3.24	2.80	4.99	3.53	2.06	1.63	5.39	3.61	4.13	3.39	3.22	2.92	0.97	1.03	5.51	5.00
Unidentified		0.48								0.59		0.51		0.37				0.72
2-OH 12:0	5.01	4.28	4.57	3.51	5.08	3.70	5.97	4.98	5.55	3.41	4.20	3.37	3.96	3.37	6.40	6.29	3.23	2.82
3-OH 12:0		4.78	0.84	3.74		4.19		3.98		3.85		3.88		3.49	2.37	4.51	1.07	4.23
14:0	0.83	0.72	0.87	0.66		0.77	0.96	0.73		0.49	0.74	0.66	0.81	0.61	0.49	0.49	1.06	1.26
16:1 ω 7c	10.57	9.72	15.28	13.37	26.52	23.86	39.27	36.72	36.61	30.05	24.44	22.10	38.53	35.61	13.45	13.17	9.32	8.69
16:1 ω 7t	2.93	2.50	3.54	3.14		1.20		0.58	6.43	5.49	2.99	2.52	0.89	0.67	1.40	1.18	0.68	0.49
16:0	36.59	36.48	35.05	36.21	32.95	33.24	34.09	33.33	30.04	34.89	37.17	35.70	34.00	33.26	35.86	35.59	37.48	36.72
cyclo 17:0	27.22	22.25	22.80	21.25	15.03	11.70	2.42	2.07	1.56	1.32	14.90	13.91	2.19	1.95	18.41	17.29	27.53	24.71
18:1 ω 9c		0.79				0.75											0.34	
18:1 ω 7c	8.63	9.56	10.15	10.30	10.96	12.47	13.34	13.02	11.31	12.47	8.80	9.81	13.65	13.51	16.66	15.80	10.76	10.87
18:0	0.71	0.81		0.88		0.66		0.73		0.72		0.78		0.75	1.59	1.57	0.89	0.87
cyclo 19:0	0.82	0.73	1.37	1.22										0.62	0.56	0.92	0.92	0.89

group I of Palleroni) (Palleroni *et al.*, 1972, 1973) based on both genetic and epigenetic datasets.

The polar lipids were characterized by the presence of three major phospholipids, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine (Fig. 2). In the case of *P. aeruginosa* DSM 50071^T, we were unable to detect any significant amounts of phosphatidylcholine unambiguously, although genes encoding its biosynthesis have been reported in a strain of this species (Sohlenkamp *et al.*, 2003). Biosynthesis might be dependent on an external source of choline.

Based on the data collected here, it seems that, even though the different species are not characterized by individual fatty acid or polar lipid patterns, typical patterns might be observed at the level of 'groups of species'. Given the relatively large 16S rRNA gene sequence divergences between members of the genus *Pseudomonas* (Moore *et al.*, 1996), it is possible that the infrastructure indicated in this and previous work (Stead, 1992; Vancanney *et al.*, 1996) might correlate with groups of species within this genus. Clearly, more detailed studies on the role of fatty acid distribution in the LPS would appear to be a line of investigation that might be profitable. These groups might also correlate, in turn, with other features, such as the distribution of certain biochemical pathways.

Based on the results presented here, we conclude that strains MT1^T, IpA-2^T and RW10^T represent novel species of the genus *Pseudomonas* within the *Gammaproteobacteria*.

Physiological and biochemical characteristics

Lecithinase and hydrolysis of Tween 80 (Gerhardt *et al.*, 1994) and levan formation (Cowan & Steel, 1974) were determined as previously described. Fluorescent pigment production was tested on *Pseudomonas* B agar (King *et al.*, 1954). Salinity tolerance was analysed by growth on trypticase soy broth supplemented with 5 and 7% NaCl. Temperature tolerance was tested by determining growth at 41 °C.

Physiological and biochemical tests were performed on all seven strains at 28 °C using API 20NE strips and Biolog GN2 microplates, according to the manufacturers' instructions. All tests were inoculated with cells pre-grown on trypticase soy yeast extract agar (per litre distilled water: 30.0 g trypticase soy broth, 3.0 g yeast extract, 15 g agar). API 20NE strips were examined after 48 h and Biolog GN2 plates were observed after 25 and 48 h of incubation.

Differential physiological and biochemical characteristics of the novel strains and related *Pseudomonas* species are summarized in Table 3. MT1^T is the only strain that has the ability to form levan, and it differs from RW10^T and IpA-2^T in the absence of arginine dihydrolase. Strains RW10^T and IpA-2^T can not be differentiated by the characteristics specified in Table 3, which is reflected in the elevated value in DNA–DNA relatedness (Table 1), as well as by the

Fig. 2. Polar lipid profiles of strains MT1^T, RW10^T and IpA-2^T and six reference strains after separation by two-dimensional TLC. DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

similarity depicted in the phylogenetic trees (Fig. 1). However, a detailed analysis of carbon source dissimilation capabilities by the Biolog system (Table 4) revealed that strain RW10^T differed from IpA-2^T in its ability to dissimilate L-histidine and L-pyrroglutamic acid.

Description of *Pseudomonas reinekei* sp. nov.

Pseudomonas reinekei (rei'ne.ke.i. N.L. gen. n. *reinekei* of/ from Reineke, named in honour of Walter Reineke, for his pioneering contributions to the study of the biodegradation of chlorinated aromatics).

Cells are Gram-negative and non-spore-forming. Colonies are circular, white-yellow and mucoid on LB agar and become mucoid after 2 days of incubation. No fluorescent pigments are produced on King's B medium. Cells are oxidase-positive and are able to form levan, but show no hydrolysis of arginine, hydrolysis of Tween 80 or reduction of nitrate. Lecithinase, urease and β -galactosidase reactions are negative and there is no hydrolysis of aesculin or gelatin. Indole is not produced from tryptophan and there is no glucose fermentation. Growth does not occur at 41 °C or in media supplemented with 5 or 7 % NaCl. Results obtained with Biolog GN2 microplates indicate that the following

Table 3. Characteristics that differentiate strains MT1^T, RW10^T and IpA-2^T from other closely related *Pseudomonas* species

Strains: 1, MT1^T; 2, RW10^T; 3, IpA-2^T; 4, *P. koreensis* DSM 16610^T; 5, *P. jessenii* DSM 17150^T; 6, *P. Vancouverensis* DSM 17555^T; 7, *P. umsongensis* DSM 16611^T.

Characteristic	1	2	3	4	5	6	7
Fluorescence	–	–	–	+	+	+	+
Formation of levan	+	–	–	–	–	–	–
Hydrolysis of Tween 80	–	–	–	+	–	–	–
Growth in the presence of 5% NaCl	–	–	–	+	–	–	–
Reduction of nitrate	–	–	–	–	+	+	+
Presence of arginine dihydrolase	–	+	+	+	–	–	+
Assimilation of:							
Arabinose	+	+	+	+	+	–	+
Mannitol	+	+	+	+	+	+	–
<i>N</i> -Acetylglucosamine	–	–	–	+	+	+	–
Phenylacetic acid	+	+	+	–	+	+	+

substrates are utilized: L-arabinose, D-arabitol, D-galactose, α -D-glucose, methyl pyruvate, monomethyl succinate, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, β -hydroxybutyric acid, α -ketoglutaric acid, DL-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline, L-pyrroglutamic acid, urocanic acid, phenylethylamine and glycerol. Weak utilization is observed of D-glucosaminic acid, α -hydroxybutyric acid, malonic acid, propionic acid, alaninamide, L-alanine, L-serine, DL-carnitine and 2-aminoethanol. The other organic substrates included in the Biolog GN2 microplates are not utilized. Consistent with the chemical properties of the genus, the major respiratory lipoquinone present is ubiquinone 9 (Q9) and the major phospholipids are phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. The fatty acids present include 3-OH 10:0, 12:0, 2-OH 12:0, 3-OH 12:0, 14:0, 16:1 ω 7c, 16:1 ω 7t, 16:0, cyclo 17:0, 18:1 ω 9c, 18:1 ω 7c, 18:0 and cyclo 19:0. The level of 14:0 does not exceed 1.5%. The 3-OH 10:0 and 2-OH 12:0 are ester-linked, while the 3-OH 12:0 is amide-linked.

The type strain is MT1^T (=DSM 18361^T=CCUG 53116^T).

Description of *Pseudomonas moorei* sp. nov.

Pseudomonas moorei (moo're.i. N.L. gen. n. *moorei* of/from Moore, named in honour of Edward R. B. Moore, for his important contributions to the study of the genus *Pseudomonas*).

Cells are Gram-negative and non-spore-forming. Colonies are circular and white–yellow on LB agar. Cells do not produce a fluorescent pigment on King's B medium. Cells are oxidase-positive and show hydrolysis of arginine, but do not reduce nitrates or hydrolyse Tween 80. Does not show

Table 4. Differences in carbon source dissimilation according to Biolog GN2 for *Pseudomonas* type strains

Strains: 1, MT1^T; 2, RW10^T; 3, IpA-2^T; 4, *P. koreensis* DSM 16610^T; 5, *P. jessenii* DSM 17150^T; 6, *P. Vancouverensis* DSM 17555^T; 7, *P. umsongensis* DSM 16611^T. b, Borderline (values between 20 and 25% of the highest reaction value after 25 h of incubation).

Carbon source	1	2	3	4	5	6	7
Dextrin	–	–	–	–	–	–	b
Tween 40	–	–	–	–	+	–	b
Tween 80	–	–	–	–	+	b	+
<i>N</i> -Acetyl-D-glucosamine	–	–	–	+	+	+	–
L-Arabinose	+	+	+	+	+	–	+
D-Arabitol	+	–	–	+	–	–	–
D-Fructose	–	–	–	+	+	b	+
D-Galactose	+	+	+	+	+	–	–
D-Mannitol	–	+	+	+	+	+	–
D-Mannose	–	–	b	+	+	–	–
Methyl pyruvate	+	+	+	b	+	+	b
Monomethyl succinate	+	+	b	+	+	+	b
Acetic acid	+	–	–	–	+	+	+
Formic acid	+	–	b	–	+	–	–
D-Galactonic acid lactone	+	+	+	+	+	–	+
D-Galacturonic acid	+	+	+	–	–	–	+
D-Glucosaminic acid	b	+	b	+	–	+	–
D-Glucuronic acid	–	+	+	–	–	–	+
α -Hydroxybutyric acid	b	–	–	–	–	–	–
<i>p</i> -Hydroxyphenylacetic acid	–	–	–	–	+	–	–
α -Ketoglutaric acid	+	+	+	+	+	+	–
Malonic acid	b	–	b	+	+	+	b
Propionic acid	b	–	b	+	+	+	+
Quinic acid	+	+	+	+	+	–	+
D-Saccharic acid	+	+	+	+	+	–	+
Succinamic acid	–	–	–	–	–	+	–
Glucuronamide	–	–	–	–	–	–	+
Alaninamide	b	–	–	–	+	b	–
D-Alanine	–	b	+	+	+	+	+
L-Alanine	b	b	+	+	+	+	b
L-Alanyl glycine	–	–	–	b	+	–	–
L-Histidine	–	+	–	–	+	–	+
Hydroxy-L-proline	–	+	+	+	+	+	+
L-Leucine	–	–	–	–	+	–	b
L-Ornithine	–	–	–	–	+	+	–
L-Phenylalanine	–	–	–	–	–	b	–
L-Pyrroglutamic acid	+	+	–	+	+	–	+
D-Serine	–	–	–	–	+	–	–
L-Serine	b	+	+	+	+	+	+
L-Threonine	–	–	–	–	+	–	–
DL-Carnitine	b	b	+	+	b	+	b
γ -Aminobutyric acid	–	+	+	+	+	+	–
Inosine	–	–	–	+	–	b	–
Uridine	–	–	–	+	–	–	–
Phenylethylamine	+	–	b	–	+	b	+
Putrescine	–	b	b	+	+	+	+
2-Aminoethanol	b	b	–	+	+	–	–
DL- α -Glycerol phosphate	–	–	–	b	+	–	–

lecithinase, urease or β -galactosidase reactions or hydrolysis of aesculin or gelatin. Indole is not produced on tryptophan and there is no glucose fermentation. Does not grow at 41 °C or in media supplemented with 5 or 7 % NaCl. Results obtained with Biolog GN2 microplates indicate that the following substrates are utilized: L-arabinose, D-galactose, α -D-glucose, D-mannitol, methyl pyruvate, monomethyl succinate, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, β -hydroxybutyric acid, α -ketoglutaric acid, DL-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-proline, L-pyroglyutamic acid, L-serine, γ -aminobutyric acid, urocanic acid and glycerol. Weak utilization is observed of D- and L-alanine, DL-carnitine, putrescine and 2-aminoethanol. The other organic substrates included in the Biolog GN2 microplates are not utilized. Consistent with the chemical properties of the genus, the major respiratory lipoquinone present is ubiquinone 9 (Q9) and the major phospholipids are phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. The fatty acids present include 3-OH 10:0, 12:0, 2-OH 12:0, 3-OH 12:0, 14:0, 16:1 ω 7c, 16:1 ω 7t, 16:0, cyclo 17:0, 18:1, 18:1 ω 7c and 18:0. The level of 14:0 does not exceed 1.5 %. The 3-OH 10:0 and 2-OH 12:0 are ester-linked, while the 3-OH 12:0 is amide-linked.

The type strain is RW10^T (= DSM 12647^T = CCUG 53114^T).

Description of *Pseudomonas mohnii* sp. nov.

Pseudomonas mohnii (moh'ni.i. N.L. gen. n. *mohnii* of/from Mohn, named in honour of William Mohn, for his pioneering contributions to the study of the biodegradation of resin acids).

Cells are Gram-negative and do not form spores. Colonies are circular and white–yellow on LB agar. Cells do not produce a fluorescent pigment on King's B medium. Cells are oxidase-positive and show hydrolysis of arginine, but do not reduce nitrates or hydrolyse Tween 80. Does not show lecithinase, urease or β -galactosidase reactions or hydrolysis of aesculin or gelatin. Indole is not produced on tryptophan and there is no glucose fermentation. Does not grow at 41 °C or in media supplemented with 5 or 7 % NaCl. Results obtained with Biolog GN2 microplates indicate that the following substrates are utilized: L-arabinose, D-galactose, α -D-glucose, D-mannitol, methyl pyruvate, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, β -hydroxybutyric acid, α -ketoglutaric acid, DL-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, D-alanine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, hydroxy-L-proline, L-proline, L-serine, DL-carnitine, γ -aminobutyric acid, urocanic acid and glycerol. Weak utilization is observed of D-mannose, monomethyl succinate, formic acid, D-glucosaminic acid, malonic acid, propionic acid, phenylethylamine and putrescine. The other organic

substrates included in the Biolog GN2 microplates are not utilized. Consistent with the chemical properties of the genus, the major respiratory lipoquinone present is ubiquinone 9 (Q9) and the major phospholipids are phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. The fatty acids present include 3-OH 10:0, 12:0, 2-OH 12:0, 3-OH 12:0, 14:0, 16:1 ω 7c, 16:1 ω 7t, 16:0, cyclo 17:0, 18:1, 18:1 ω 7c and 18:0. The level of 14:0 does not exceed 1.5 %. The 3-OH 10:0 and 2-OH 12:0 are ester-linked, while the 3-OH 12:0 is amide-linked.

The type strain is IpA-2^T (= DSM 18327^T = CCUG 53115^T).

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