NOTE

***Pseudomonas extremorientalis* sp. nov., isolated from a drinking water reservoir**

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On the basis of phenotypic and genotypic characteristics and 16S rDNA sequence analysis, a novel species belonging to the genus *Pseudomonas sensu stricto* was identified. The saprophytic, fluorescent bacterium, designated KMM 3447^T, was isolated from a drinking water reservoir near Vladivostok City, Russia. The novel organism was a Gram-negative, aerobic, rod-shaped bacterium that produced a cyclic depsipeptide with surface-active properties. It degraded casein, but did not degrade gelatin, starch, agar or Tween 80. The bacterium was also haemolytic. Growth of the novel bacterium occurred between 4 and 35 °C. The predominant cellular fatty acids of the novel pseudomonad were C_{16:0}, C_{16:1(n-7)}, C_{18:1(n-7)} and C_{17:0 cyclo}; branched fatty acids were only found in trace amounts. The G+C content of the novel bacterium was 61.0 mol%. 16S rDNA sequence analysis indicated that the novel bacterium had a clear affiliation with *Pseudomonas fluorescens* and species closely related to this recognized pseudomonad. DNA–DNA hybridization experiments showed that the novel bacterium bound at low levels (27–53 %) with the DNA of the type strains of its nearest phylogenetic relatives, namely *Pseudomonas tolaasii*, *Pseudomonas veronii*, *Pseudomonas orientalis* and *Pseudomonas rhodesiae*, indicating that the novel bacterium represented a novel species within the genus *Pseudomonas*, for which the name *Pseudomonas extremorientalis* is proposed; the type strain is KMM 3447^T (= LMG 19695^T).

Keywords: *Proteobacteria*, *Pseudomonas sensu stricto*, *Pseudomonas extremorientalis*

The genus *Pseudomonas* as described by Migula (1894) consisted of a diverse group of bacteria that were common inhabitants of soil and fresh water ecosystems. During the last decade, bacteria belonging to the genus *Pseudomonas* have been studied extensively using polyphasic approaches, and these studies have led to the improved classification of members of this genus (see reviews by De Ley, 1992; Kersters *et al.*, 1996; Moore *et al.*, 1996). Currently, the genus *Pseudomonas sensu stricto* consists of two major

intrageneric clusters – one focussed around *Pseudomonas aeruginosa* and one focussed around *Pseudomonas fluorescens* (Moore *et al.*, 1996). In the last decade, the number of species belonging to the latter cluster has grown due to the characterization of a number of strains isolated from natural mineral waters (Coroler *et al.*, 1996; Elomari *et al.*, 1996; Verhille *et al.*, 1999; Dabboussi *et al.*, 1999).

During the taxonomic investigation of bacterial populations of a drinking water reservoir in the Far East of Russia, we isolated a number of strains that belonged to different genera, including *Aeromonas*, *Vibrio* (Ivanova *et al.*, 2001) and *Pseudomonas*. The aim of this study was to characterize one of the strains isolated from the reservoir, designated KMM 3447^T, which had phenotypic, chemotaxonomic, genetic and phylogenetic characteristics distinct from other recognized

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The GenBank accession number for the 16S rRNA gene sequence of *Pseudomonas extremorientalis* KMM 3447^T is AF405328.

The full phylogenetic tree from which Fig. 1 was taken can be found as supplementary data in IJSEM Online (<http://ijs.sgmjournals.org>).

bacterial species. The results obtained suggest that the water bacterium represents a novel species within the genus *Pseudomonas*, for which the name *Pseudomonas extremorientalis* is proposed.

A total of four samples of fresh water (each of 500 ml) were collected from different sites of a raw drinking water (which is additionally treated before supply) reservoir near Vladivostok City, in the Far East of Russia in September 1998. The samples were kept at -4°C and were analysed at the laboratory within 24 h of collection. Aliquots (0.1 ml) of the samples were plated onto tryptic soy agar (TSA); the inoculated plates were incubated at 28°C for 24–48 h. A total of 46 environmental strains, presumptively identified as belonging to the genus *Pseudomonas*, were isolated from the plates. Phenotypically, these isolates were related to the fluorescent species of the genus *Pseudomonas* and were tentatively identified as strains of *P. fluorescens* and *Pseudomonas rhodesiae*. One of the 46 pseudomonad-like strains was distinct from the other strains isolated, due to its phenotypic features and its ability to produce a surface-active compound; this strain was chosen for a detailed investigation. All strains were maintained on TSA plates. For long-term storage, pellets of the strains were stored at -80°C in medium containing 4% (w/v) tryptic soy broth (pH 7.2) and 20% (v/v) glycerol.

The cell morphology of KMM 3447^T was determined after growth of the organism for 24 h on TSA (Oxoid) at 28°C . The motility of the organism was determined by examining 18-h-old cultures grown in tryptic soy broth. The ability of KMM 3447^T to produce a fluorescent pigment was tested on King's B medium (King *et al.*, 1954). Physiological and biochemical properties of KMM 3447^T were also examined, including its mode of glucose metabolism (oxidation/fermentation; Hugh & Leifson, 1953), its nitrate-reducing ability (Azegami *et al.*, 1987), its catalase activity, its ability to liquefy gelatin, its arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase activities, its sodium requirement (0, 3, 6 and 8%, w/v, NaCl), its ability to produce indole and H_2S , its ability to hydrolyse starch, Tween 80 and casein, and its ability to produce lecithinase. Unless indicated otherwise, all physiological and biochemical tests were determined according to the methods of Smibert & Krieg (1994).

Growth of KMM 3447^T at different temperatures was determined after growth of the organism in tryptic soy broth for 24 h at 37 and 42°C , and for 3 days at 10 and 4°C . The BIOLOG GN test kit was also used to examine the primary oxidation of 95 carbon compounds, as described previously (Ivanova *et al.*, 1998a, b). The susceptibility of KMM 3447^T to antibacterials was tested by using the conventional diffusion plate technique, using solid King's B medium and discs impregnated with kanamycin (10 µg), ampicillin (10 µg), benzylpenicillin (10 µg), streptomycin (10 µg), erythromycin (15 µg), gentamicin (10 µg),

oxacillin (20 µg), lincomycin (15 µg), carbenicillin (25 µg), vancomycin (30 µg), tetracycline (30 µg), oleandomycin (15 µg) or 0/129 (150 µg).

The haemolytic activity of KMM 3447^T was tested on Merck blood agar (6.5% sheep blood); the haemolytic activity of the organism on mouse erythrocytes and its cytotoxicity on Ehrlich cells were also tested using butanol extracts from the strain. Extracts from bacterial cells from a 100 ml culture of KMM 3447^T were obtained with butanol (100 ml), and the solvent was subsequently evaporated under reduced pressure. The extracts were dissolved in water up to 4 mg ml^{-1} . Mouse blood was diluted three-fold with saline-containing heparin. Erythrocytes were separated by centrifugation at 3000 g for 4 min, washed three times and then resuspended in saline to give a 0.2% erythrocyte suspension. Butanol extracts of the bacterial cells (40 µl) were added to 1 ml of the erythrocyte suspension. The mixture was incubated at room temperature for 30 min with shaking. The tubes were then centrifuged for 5 min at 3000 g and the absorbance of the supernatants was measured at 540 nm to detect released haemoglobin. An erythrocyte suspension in saline was used as the blank. Total haemolysis was achieved by suspending the erythrocytes in distilled water.

Ascites tumour cells of Ehrlich carcinoma (tetraploid strain) were taken 7–8 days after tumour inoculation into stainless white mice of both sexes. The cell suspension was diluted five-fold (v/v) with saline and centrifuged at 3000 g for 10 min; the cells were then resuspended in culture medium 199 containing penicillin and streptomycin (250 U ml^{-1}) to a final concentration of 6–8 million cells ml^{-1} (Sasaki *et al.*, 1985).

The antibacterial activity of the butanol extracts of KMM 3447^T was determined by using the agar-diffusion assay, based on the method described by Barry (1980).

The surface-active compounds of KMM 3447^T were obtained by their extraction from 3 l of culture supernatant using 2-butanol (centrifugation for 20 min at 5000 r.p.m. at 15°C), after cultivation of the organism in tryptic soy broth for 24 h at 25°C on a rotary shaker (120 r.p.m.). The solvent was subsequently evaporated and the resulting residue was fractionated by flash chromatography on silica using a gradient of ethyl acetate in ethanol. Fractions 4:1 and 3:1, which showed surface-active properties, were combined, evaporated, dissolved in chloroform/ethanol (3:1) and further crystallized from ethanol [with a yield of $75\text{ mg peptide (1 culture medium)}^{-1}$]. To test the surface properties of the peptide, a simple drop assay (Hildebrand, 1989) was performed. Matrix-assisted laser desorption/ionization analysis of the compound revealed that it had ion peaks at m/z 1126 ($M^+\text{H}^+$), 1148 ($M^+\text{Na}^+$) and 1164 ($M^+\text{K}^+$); the IR spectrum was indicative of the presence of amide carbonyls (1660 and 1538 cm^{-1}) and a conjugated

ester-carbonyl (1745 cm^{-1}) in the compound. The ^1H -NMR spectrum contained three resonances that were characteristic for peptides (amide-aromatic, $\delta = 7.6\text{--}10.45$; $\alpha\text{-CH}$, $\delta = 4.1\text{--}4.7$; side-chain protons, $\delta = 0.8\text{--}2.0$). Amino-acid-sequence analysis of the isolated peptide was performed as described previously (Kalinovskaya *et al.*, 1995). Briefly, the peptide was *N*-permethylated according to Hacomori's methylation procedure with 10% NaOH in CH_3OH at room temperature for 16 h. The derivatives obtained by using this procedure were analysed by electron-impact MS (EIMS, LKB 9000s) using an ionization voltage of 50 V, a collector current of 60 μA and a vaporizing temperature of 200–220 $^\circ\text{C}$. The results revealed six amino acids to be present in the methylated derivatives at a ratio of Glx:Ile:(Leu)₃:(Ser)₂:Thr:Val, which corresponded to 954/955 Da. The last fragment, 171/170 Da, contained 10 carbon, two hydrogen and two oxygen atoms. The presence in the ^{13}C -NMR spectrum of the ester-amide carbonyl ($\delta = 169.9$), nine CO–NH-carbonyls, one diagnostic carbonyl at $\delta = 176.3$ (most likely belonging to $\gamma\text{-NOOH}$ Glu), nine $\alpha\text{-CH}$ atoms ($\delta = 53.9\text{--}63.7$) and two carbonyl-methines ($\delta = 68.9$ and 69.2) and nine doublet signals of amide protons in the ^1H -NMR spectrum, confirmed the presence of nine amino acids in the peptide. The lack of two wide singlet signals at $\delta = 6.78$ and 7.24 in the ^1H -NMR spectrum of the methylated derivatives indirectly indicated the presence of Glu, not Gln, in the peptide. Analysis of the distortionless enhanced polarization transfer (DEPT) spectrum of the purified compound detected 16 methine, 15 methylene, two of which were the CH_2OH of serine ($\delta = 62.8$ and 63.8), and 12 methyl groups. The above data allowed us to estimate the molecular mass of the purified surface-active compound as being 1125 Da, and indicated the presence of a depsipeptide cycle and a hydroxy-decanoyl fragment within the molecule.

Strain KMM 3447^T, isolated from a drinking water reservoir, had all of the phenotypic characteristics of the fluorescent pseudomonads. The bacterium was Gram-negative, aerobic, non-spore-forming, oxidase- and catalase-positive, rod-shaped, 0.8–1.0 μm in diameter and 2.0–3.5 μm in length with a single polar flagellum. Colonies were uniformly round, 3–6 mm in diameter, circular, regular, convex, smooth and slightly whitish after incubation of the organism for 48 h on TSA. Cells produced a fluorescent pigment on King's B medium, but did not produce phenazine pigments on King's A medium. The bacterium did not accumulate poly- β -hydroxybutyrate as an intracellular reserve product and did not have an arginine dihydrolase system, but it reduced nitrate to nitrite, was positive for arginine dihydrolase and lecithinase activities, was haemolytic on blood agar and tolerated up to 6% NaCl in the culture medium. The temperature range for growth of KMM 3447^T was 4–35 $^\circ\text{C}$, with an optimum temperature of 25 $^\circ\text{C}$; no growth was detected at 42 $^\circ\text{C}$. The pH range of growth was 6.0–10.0, with optimum growth at pH 7.0. KMM

3447^T decomposed casein. Gelatin, Tween 80, starch, agar and chitin were not hydrolysed by the bacterium, and indole and acetoin were not produced by it. Of the 95 carbohydrates tested using the BIOLOG GN test kit, KMM 3447^T did not oxidize D-glucose, melibiose, lactose, adonitol, L-rhamnose, L-fucose, myo-inositol, α -cyclodextrin, gentiobiose, *N*-acetyl-D-galactosamine, i-erythritol, D-raffinose, α -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, hydroxy-L-proline, L-ornithine, L-phenylalanine, phenylethylamine, putrescine nor 2,3-butanediol. The antibacterial susceptibility profile of KMM 3447^T was essentially similar to those of *Pseudomonas tolaasii*, *Pseudomonas veronii*, *P. rhodesiae* and *Pseudomonas orientalis*. KMM 3447^T was susceptible to gentamicin, kanamycin, streptomycin and tetracycline, and was resistant to ampicillin, benzylpenicillin, carbenicillin, lincomycin and polymyxin. Anti-tumour and antimicrobial activities of butanol extracts of KMM 3447^T against *Staphylococcus aureus* CIP 103594^T, *Escherichia coli* ATCC 15034, *Proteus vulgaris* IFO 3851^T, *Enterococcus faecium* CIP 104105, *Bacillus subtilis* ATCC 6051^T and *Candida albicans* KMM 455 were not detected.

One of the characteristic features of KMM 3447^T was its ability to produce a cyclic depsipeptide with surface-active properties similar to those of viscosin (Hiramoto *et al.*, 1970) and the white-line-inducing principle (WLIP) compound (Mortishire-Smith *et al.*, 1991), though only further structural investigations can clarify the identity and structural differences, if any, of these peptides. We also screened the type strains of *P. veronii*, *P. rhodesiae* and *P. orientalis* for their ability to produce surface-active compounds. Our results showed that the cells of *P. veronii* and *P. orientalis* contained low-molecular-mass peptides with surface-active properties, but neither the cells nor supernatants of *P. rhodesiae* contained similar substances. Members of the genus *Pseudomonas* produce a wide variety of extracellular low-molecular-mass compounds, including siderophores, antibiotics, toxins, some enzymes and peptides. For example, strains of *P. fluorescens* are known producers of cyclic lipopeptides with surface-active, antimicrobial (against *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare*) and antifungal properties, namely tensin (Nielsen *et al.*, 2000) and viscosinamide (Nielsen *et al.*, 1999). Viscosinamide belongs to a family of cyclic depsipeptides and is closely related to viscosin (Hiramoto *et al.*, 1970), which was isolated from '*Pseudomonas viscosa*' (Kochi *et al.*, 1951) and *P. fluorescens* bv. II (Hildebrand, 1989). Viscosinamide and viscosin only differ at their second amino acid – D-glutamic acid is present in viscosin, whereas D-glutamine is present in viscosinamide. One of the other related compounds isolated from '*Pseudomonas reactans*', WLIP (Mortishire-Smith *et al.*, 1991), had an antipodal fifth amino-acid residue (L-Leu) compared with that found in viscosin (D-Leu). A new series of compounds – termed the massetolides – were isolated

Table 1. Cellular fatty acid compositions of *P. extremorientalis* KMM 3447^T and its phylogenetically related saprophytic, fluorescent species isolated from mineral waters

Strains: 1, *P. extremorientalis* KMM 3447^T; 2, *P. rhodesiae* CIP 104664^T; 3, *P. tolaasii* LMG 2342^T; 4, *P. veronii* CIP 04663^T; 5, *P. orientalis* CIP 105540^T. Values are given as mean percentages of the total fatty acid content. The values were obtained after growth of the strains at 28 °C.

Fatty acid	1	2	3	4	5
Saturated					
C _{12:0}	1.4	3.0	1.6	1.6	2.3
C _{14:0}	0.4	0.3	0.4	0.8	0.4
C _{15:0}	0.5	0.1	0.4	0.3	0.2
C _{16:0}	37.0	32.8	36.0	32.9	30.4
C _{17:0}	0.9	0.2	0.8	0.3	0.3
C _{18:0}	1.2	1.2	1.7	2.0	1.1
Unsaturated					
C _{16:1(n-7)}	8.7	26.5	17.0	23.6	32.0
C _{18:1(n-7)}	14.0	23.0	17.2	22.6	25.3
Branched					
C _{15:0 iso}	—	—	—	0.6	—
C _{17:0 iso}	0.1	0.6	0.7	0.6	0.5
C _{19:0 iso}	0.2	0.3	0.3	0.3	0.4
Hydroxy					
C _{10:0-3OH}	0.4	0.3	0.3	0.3	0.3
C _{12:0-3OH}	0.2	0.6	0.6	0.5	0.5
Cyclo					
C _{17:0 cyclo}	31.3	8.7	20.6	11.0	4.9
C _{19:0 cyclo}	2.1	1.9	2.2	2.4	0.7

from two unidentified marine strains belonging to the genus *Pseudomonas* (Gerard *et al.*, 1997). These compounds differed from viscosin by one of the amino-acid units and/or by the fatty acid moiety. Gerard *et al.* (1997) reported that the massetolide-producing strains were often able to incorporate amino acids artificially at various positions in the peptides, thereby producing new types of massetolides. It is also of interest to mention that *P. tolaasii* produces a number of extracellular compounds that are toxic to the cultivated mushroom *Agaricus bisporus*, and these compounds include a heat-stable lipopeptide (tolasin) (Rainey *et al.*, 1991). The observed structural diversity of the surface-active peptides produced by the pseudomonads may be a reflection of their important eco-physiological functions, but these functions remain to be understood and explained (Peypoux *et al.*, 1999).

Analysis of the fatty acid methyl ethers (FAMES) of KMM 3447^T was performed by using GLC as described previously (Svetashev *et al.*, 1995). The FAME profile of KMM 3447^T was specific to the genus *Pseudomonas*, and contained the characteristic C_{10:0-3OH} and C_{12:0-3OH} fatty acids of *sensu stricto* pseudomonads (Oyaizu & Komagata, 1983). Since

Table 2. DNA relatedness among the tested *Pseudomonas* species

Strains: 1, *P. extremorientalis* KMM 3447^T; 2, *P. rhodesiae* CIP 104664^T; 3, *P. tolaasii* LMG 2342^T; 4, *P. veronii* CIP 104663^T; 5, *P. orientalis* CIP 105540^T.

Characteristic	1	2	3	4	5
G + C content (mol %)	61.0	61.4	61.7	61.4	61.8
Hybridization with DNA from KMM 3447 ^T (%)	100	53	53	27	45

there were no FAME data available for the species that were phylogenetically most closely related to KMM 3447^T, the FAME patterns for *P. tolaasii*, *P. veronii*, *P. rhodesiae* and *P. orientalis* were obtained (Table 1). Four fatty acid components, C_{16:0}, C_{16:1(n-7)}, C_{18:1(n-7)} and C_{17:0 cyclo}, were found to be the main fatty acid components of the five species tested (which included *P. extremorientalis*); the rare hydroxy fatty acids C_{10:0-3OH} and C_{12:0-3OH} were present among the minor components. The proportion of unsaturated and cyclo fatty acids varied significantly among the five species tested and represent species-differentiating characteristics. For example, the amount of the C_{16:1(n-7)} and C_{18:1(n-7)} fatty acids present in the different species was lowest for KMM 3447^T (23%) and highest for *P. orientalis* (57.3%), in contrast to the cyclo fatty acids, which were present at 33.9 and 5.7%, respectively, in the two species.

DNA was isolated from the tested *Pseudomonas* species according to the method of Marmur (1961). The G + C content of the DNA of KMM 3447^T was 61.0 mol%, as determined by using the thermal denaturation method of Marmur & Doty (1962). For DNA–DNA hybridization experiments, *P. tolaasii* LMG 2342^T, *P. rhodesiae* CIP 104664^T, *P. veronii* CIP 104663^T and *P. orientalis* CIP 105540^T were obtained from the Culture Collection of the Laboratorium voor Microbiologie Universiteit Gent (LMG; Ghent, Belgium) and the Collection de l'Institut Pasteur (CIP; Institut Pasteur, France). All of the reference strains were routinely cultured on TSA plates. DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were recorded as described by Marmur & Doty (1962) and De Ley *et al.* (1970). The reassociation temperature was 70 °C. The ΔT_m was the difference between the T_m of the homoduplex and the T_m of the heteroduplex and ranged from 3 to 4 °C. The genetic similarity of KMM 3447^T with the four type strains of the genus *Pseudomonas* tested here (Table 2) ranged from 27 to 53%. Based on the generally accepted criterion for the definition of genomic species of Wayne *et al.* (1987) – i.e. the grouping of bacterial strains into species based on a DNA–DNA hybridization value of $\geq 70\%$ with ΔT_m values of < 5 °C – KMM 3447^T

warrants separate species status within the genus *Pseudomonas*.

DNA from KMM 3447^T was prepared by using the Promega Wizard genomic DNA extraction kit according to the manufacturer's instructions. The prepared DNA (100 ng) was used for a PCR amplification of the small-subunit rRNA gene of KMM 3447^T, as described previously (Sawabe *et al.*, 1998a, b). The PCR protocol used consisted of an initial denaturation step at 94 °C for 180 s, an annealing step at 55 °C for 60 s and an extension step at 72 °C for 90 s; the thermal profile consisted of 30 cycles. The amplification primers used in this study gave a 1.5 kb long PCR product, which corresponded to positions 25–1521 based on the *E. coli* numbering system. The PCR product was purified by using the Promega Wizard PCR preps DNA purification kit and directly sequenced by using a *Taq* FS dye terminator sequencing kit (ABI), according to the manufacturers' instructions. DNA sequencing was performed with an Applied Biosystems model 373S automated DNA sequencer; nine primers were used for sequencing (Sawabe *et al.*, 1998a).

The 16S rDNA sequence of KMM 3447^T was automatically and then manually aligned by reference to a database of 35 000 already-aligned bacterial 16S rDNA sequences. Phylogenetic trees were constructed according to three different methods [neighbour-joining (BIONJ), maximum-likelihood and maximum-parsimony analysis]. For the neighbour-joining analysis, a distance matrix was calculated by using the Kimura two-correction parameter. Bootstrap analysis was done with 500 replications, using BIONJ and the Kimura two-correction parameter. BIONJ analysis was done according to Gascuel (1997), and maximum-likelihood and maximum-parsimony analyses were done using the PHYLIP package (Felsenstein, 1993). The phylogenetic trees were drawn using NJPLOT (Perrière & Gouy, 1996) and the CLARIS DRAW software for Apple Macintosh. The nucleotide sequence of the 16S rRNA gene of KMM 3447^T was deposited in GenBank under accession no. AF405328. The domains used to construct the phylogenetic trees were the regions of the small-subunit rDNA sequences available for the pseudomonads and excluded positions likely to show homoplasy. When bacterial sequences from different phyla or genera were included in the phylogenetic analyses, it was clear that the novel species KMM 3447^T did belong to the genus *Pseudomonas*. The domains in these extended analyses were more (genus determination) or less (phylum determination) extended, since the number of positions that can be properly aligned decreases and homoplasy increases with the depth of the phylogenetic tree. For these reasons a detailed phylogenetic tree that analyses the position of a species within a genus cannot usually be properly presented with outgroups (unless some outgroups are so closely related that the entire sequence is orthologous and with little suspected homoplasy). One should note that rooting a clade with outgroups is of

Fig. 1. Phylogenetic position of *P. extremorientalis* KMM 3447^T within the γ -Proteobacteria. Unrooted tree obtained by using the BIONJ algorithm, the Kimura two-correction parameter for distance calculations and bootstrap analysis. Bootstrap values (expressed as a percentage of 500 replications) are only indicated for those branches that were also found upon maximum-likelihood ($P < 0.01$) and maximum-parsimony analyses. The full phylogenetic tree from which this tree was taken can be found as supplementary data in IJSEM Online (<http://ijs.sgmjournals.org>).

importance only when one wants to derive theoretical considerations concerning some traits and to define, for example, which of these traits are ancestral and which are derived.

Fig. 1, which is the relevant part of a complete dendrogram given as supplementary data in IJSEM Online (<http://ijs.sgmjournals.org>), contains only those species of the genus *Pseudomonas* for which the entire 16S rDNA sequences were available. The sequence regions used in the analysis correspond to positions 57–1282 (based on *E. coli* numbering) of the KMM 3447^T sequence.

16S rDNA sequence analysis revealed that KMM 3447^T is a member of the γ -Proteobacteria, and clearly indicated that this organism should be included in the genus *Pseudomonas*. The closest phylogenetic relatives of KMM 3447^T were two 16S rDNA sequences for the type strain of *P. tolaasii*, with which KMM 3447^T formed a robust clade (Fig. 1). Other sequences that had close percentages of similarity with KMM 3447^T, such as *P. veronii* and *P. rhodesiae* (99.4–99.2%), and *P. orientalis* (99%), did not form a robust clade with KMM 3447^T. As KMM 3447^T and *P. tolaasii* (as well as the other related species) were distinct species according to DNA–DNA hybridization data (Table 2), KMM 3447^T is deemed to represent a novel species within the genus *Pseudomonas*, a result which is consistent with the distinct phenotypic characteristics of this novel strain. KMM 3447^T should therefore be recognized as a novel species within the genus *Pseudomonas*, which is distinct from *P. tolaasii*, even though these two species clearly form a distinct subclade within the saprophytic, fluorescent pseudomonads (Fig. 1).

KMM 3447^T can be easily distinguished from its phylogenetically and genetically related species by a

Table 3. Characteristics that differentiate *P. extremorientalis* from its phylogenetically related species and from other fluorescent species isolated from mineral waters

Species: 1, *P. extremorientalis*; 2, *P. fluorescens* bv. I; 3, *P. fluorescens* bv. II; 4, *Pseudomonas gessardii*; 5, *Pseudomonas libanensis*; 6, *Pseudomonas migulae*; 7, *P. rhodesiae*; 8, *P. tolaasii*; 9, *P. veronii*. Data are from this study and studies by Elomari *et al.* (1996), Coroler *et al.* (1996), Dabboussi *et al.* (1999) and Verhille *et al.* (1999). All strains were Gram-negative, oxidase-, catalase- and arginine dihydrolase-positive, did not produce pyocyanin, grew from 4 to 35 °C and assimilated D-mannose. +, Positive; –, negative; D, 11–89% of all strains positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Production of:									
Lipase	–	D	–	D	–	–	–	+	–
Gelatinase	–	+	+	+	ND	–	–	+	D
Amylase	–			–		–	–	–	–
Caseinase	+	ND	ND	ND	ND	–	–	–	–
Lecithinase	+	+	D	D	+	+	+	+	+
Nitrate reduction	+	–	+	D	–	+	–	–	+
Haemolysis	+	ND	ND	–	–	–	–	+	–
Growth in 6% NaCl	+	–	–	–	–	–	+	–	
Assimilation of:									
D-Galactose	+	+	+	D	+	+	+	–	+
D-Arabinose	+	+	+	–	+	+	+	+	+
D-Ribose	+	+	+	ND	+	+	+	ND	ND
Sucrose, maltose	+	+	+	–	–	–	–	+	+
myo-Inositol	–	D	+	+	+	–	+	+	+
D-Trehalose	+	+	+	+	D	+	+	+	D
D-Sorbitol	+	+	+	–	+	–	+	+	+
Glycerol	+	+	+	+	ND	+	+	–	+
α-Aminobutyrate	+	–	–	+	+	+	+	+	ND
Xylose	+	+	D	–	+	+	+	+	+
D-Glucose	–	+	+	+	+	+	+	+	+
Melibiose, lactose	–	+	+	–	–	–	–	+	–
D-Mannitol	+	+	+	+	+	–	–	–	+
Adonitol	–	+	–	+	–	–	–	–	–
Rhamnose	–	–	D	–	–	–	–	+	–

number of phenotypic and chemotaxonomic features (see Tables 1 and 3). Therefore, KMM 3447^T represents a novel species within the genus *Pseudomonas*, for which the name *Pseudomonas extremorientalis* is proposed.

Description of *Pseudomonas extremorientalis* sp. nov.

Pseudomonas (ex.trem.or.i.en.ta'lis. L. fem. adj. *extrema* outward, external; L. fem. adj. *orientalis* Eastern; N.L. fem. adj. *extremorientalis* from the region of isolation, the Far East of Russia).

Chemo-organotroph with respiratory metabolism. Other phenotypic and biochemical characteristics of KMM 3447^T can be found within the text and in Tables 1 and 3. Oxidizes dextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, D-fructose, D-galactose, *m*-inositol, α-lactose, α-D-lactose lactulose, maltose, D-mannitol, D-mannose, D-methyl glucoside, psicose, D-

sorbitol, sucrose, D-trehalose, tyranose, xylitol, methyl pyruvate, mono-methyl succinate, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid γ-lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, itaconic acid, 2-oxobutyric acid, 2-oxoglutaric acid, 2-oxovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, L-leucine, L-proline, L-pyroglytamic acid, D-serine, L-serine, L-threonine, DL-camitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, 2-aminoethanol, glycerol, α-DL-glycerol phosphate, glucose 1-phosphate. The predominant cellular fatty acids of KMM 3447^T are C_{16:0}, C_{16:1(n-7)}, C_{18:1(n-7)} and C_{17:0 cyclo}. Branched fatty acids are only found in trace amounts. Isolated from a drinking (raw) water reservoir. Clinical significance is unknown. The

G+C content of the DNA is 61.0 mol%. The type strain of *P. extremorientalis* is KMM 3447^T (= LMG 19695^T).

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