Paraoerskovia marina gen. nov., sp. nov., an actinobacterium isolated from marine sediment

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A Gram-positive-staining, facultatively anaerobic bacterial strain, CTT-37^T, was isolated from a marine sediment sample collected from Tottori city, located on the shore of the Sea of Japan. A 16S rRNA gene sequence comparison indicated that the isolate represents a novel clade that clusters with members of the families *Cellulomonadaceae* and *Sanguibacteraceae*. Strain CTT-37^T shared maximum 16S rRNA gene sequence similarity of 96.4 % with *Oerskovia paurometabola* DSM 14281^T and 96.2 % with *Oerskovia enterophila* DSM 43852^T. The DNA–DNA hybridization value between strain CTT-37^T and *O. enterophila* JCM 7350^T was 10–12 %. The following chemotaxonomic characteristics of strain CTT-37^T were markedly different from those of strains in the genus *Oerskovia*. The cell wall contained L-serine in the peptidoglycan interpeptide bridge. The predominant menaquinone was MK-9 (H_4); other quinones detected were MK-9 and MK-9(H_2). The only polar lipid was phosphatidylglycerol and the G+C content of the DNA was 70 mol%. Differences in phenotypic characteristics and large phylogenetic distances between strain CTT-37^T and all members of the genus *Oerskovia* supported the classification of CTT-37^T within a new genus and species, for which the name *Paraoerskovia marina* gen. nov., sp. nov. is proposed. The type strain of *Paraoerskovia marina* is CTT-37^T (=NBRC 104352^T =DSM 21750^T).

The family *Cellulomonadaceae* was proposed by Stackebrandt & Prauser (1991) and its description was emended by Stackebrandt *et al.* (1997). The genera *Actinotalea* (Yi *et al.*, 2007), *Cellulomonas* (Bergey *et al.*, 1923) and *Oerskovia* (Prauser *et al.*, 1970) and the genus *Tropheryma* (La Scola *et al.*, 2001), the legitimacy of which is currently under question, represent this family. The genera *Oerskovia* and *Cellulomonas* are phylogenetically close to each other and form a single radiation in a 16S rRNA-gene-sequence-based tree (Stackebrandt *et al.*, 2002; Yi *et al.*, 2007). The union of the genera *Cellulomonas* and *Oerskovia* in a redefined genus *Cellulomonas* was proposed by Stackebrandt *et al.* (1980, 1982). However, the genus *Oerskovia* was later re-established based on a polyphasic taxonomic study (Stackebrandt *et al.*, 2002). During our studies on marine bacteria isolated from sediment samples in Japan, a novel strain was isolated that clusters with the members of the families *Cellulomonadaceae* and *Sanguibacteraceae* but represents a new genus.

Strain CTT-37^T was isolated from a marine sediment sample collected from a beach at Tottori city, located on the shore of the Sea of Japan (35° 31′ N 134° 05′ E). The sediment sample was suspended and serially diluted in sterile artificial seawater (Naigai Chemicals) and an aliquot of each dilution was spread on half-strength marine agar [HSMA; 19 g Bacto marine broth 2216 (Difco), 17 g artificial seawater salts (Naigai Chemicals) and 15 g agar, dissolved in 1 l distilled water]. The plates were incubated at 28 °C for 7 days. Strain CTT-37^T formed small, regular, creamy yellow-coloured colonies on HSMA plates after 3–5 days of incubation. The strain was preserved at −80 °C in artificial seawater supplemented with 20 % glycerol (v/v).

Prepman Ultra (Applied Biosystems) was used to prepare template DNA for 16S rRNA gene amplification. The gene was amplified by using a universal primer set (27f and 1492r; Brosius *et al.*, 1978) and sequenced directly using a

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CTT-37^T is AB445007.

The detailed cellular fatty acid profile of strain CTT-37^T is available as supplementary material with the online version of this paper.

BigDye Terminator v 3.1 Cycle Sequencing kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). In a BLAST search (Altschul *et al.*, 1990) against the sequences available in the DDBJ, *Oerskovia turbata* NCIMB 10587^T was identified to be the closest relative of strain CTT-37^T. Thus, pairwise comparison of the 16S rRNA gene sequences of strain CTT-37^T and the type strains of species in the genera *Oerskovia*, *Cellulomonas* and *Sanguibacter* was performed using the Needleman–Wunsch alignment algorithm (Needleman & Wunsch, 1970; http://www.ebi.ac.uk/emboss/align/). Strain CTT-37^T shared highest sequence similarities of 96.4–96.0 % with members of the genus *Oerskovia*, while the members of the genus *Oerskovia* shared sequence similarities greater than 99 % with each other. The sequence similarity between CTT-37^T and the genera *Cellulomonas* and *Sanguibacter* was lower (93–95 %). The sequences of strain CTT-37^T and the type strains shown in Fig. 1 were aligned using the CLUSTAL_X program (Thompson *et al.*, 1997), and phylogenetic trees were constructed by the neighbour-joining (NJ; Saitou & Nei, 1987) and maximum-likelihood (ML; Adachi & Hasegawa, 1996) algorithms. The robustness of the tree topology was evaluated by bootstrap analysis using 1000 and 100 resamplings of the sequences (Felsenstein, 1985) for the NJ and ML methods, respectively. Strain CTT-37^T clustered with the members of the families *Cellulomonadaceae* and *Sanguibacteraceae* (Fig. 1). An almost identical branching pattern was obtained with the ML method (not shown). However, the bootstrap values supporting these branches were very low, and it is difficult to determine the closest relative based on the phylogenetic tree. Therefore, based on the results of BLAST analysis, the members of the genus *Oerskovia* were considered to be the closest phylogenetic neighbours of strain CTT-37^T. To determine whether strain CTT-37^T has the conserved 16S rRNA signatures of the genus *Oerskovia* or *Cellulomonas* (Stackebrandt *et al.*, 2002), the 16S rRNA gene sequences of strain CTT-37^T and members of the two genera and a

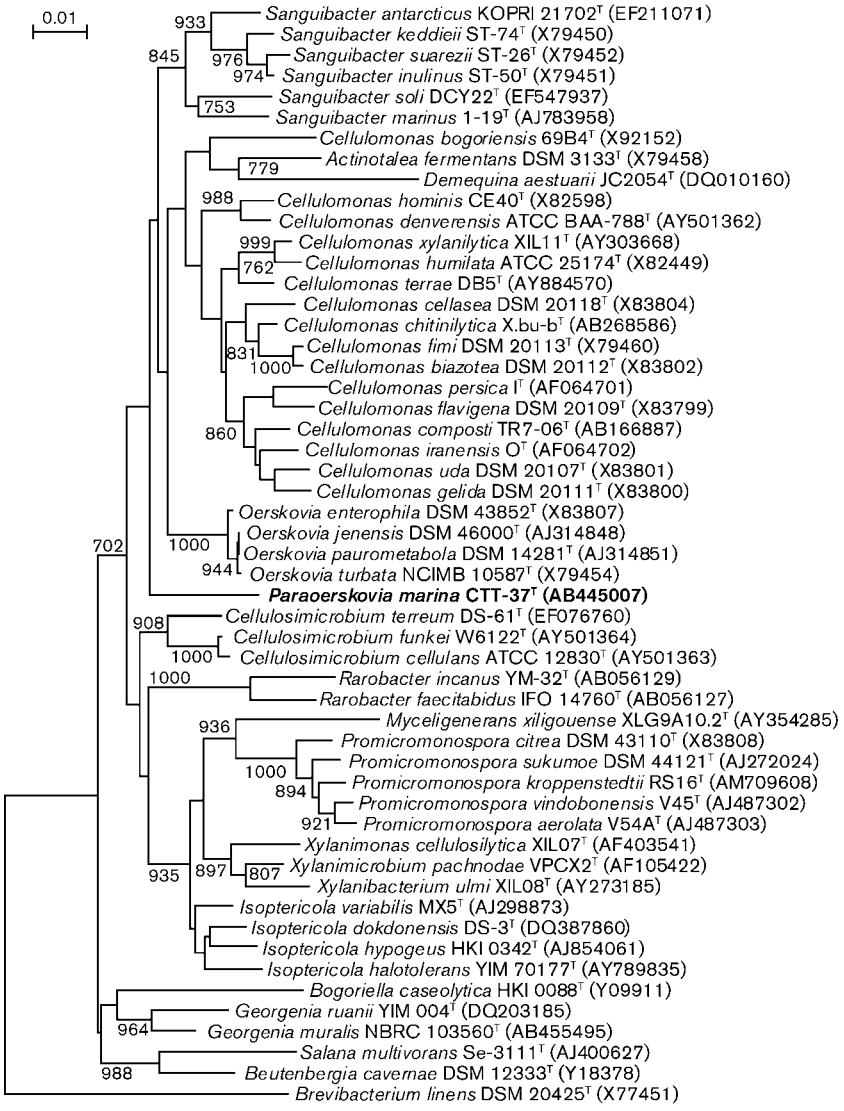

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequence comparisons showing the position of strain CTT-37^T and members of related genera. Bootstrap values from 1000 replications are indicated at branches when the values are significant (>700). The sequence of *Brevibacterium linens* DSM 20425^T was used as an outgroup. Bar, 0.01 K_{nuc}.

sequence from *Escherichia coli* (unidentified strain; GenBank accession no. V00348) were aligned manually. Although the 16S rRNA gene sequence of the novel strain was most similar to those of *Oerskovia* strains, it shared many conserved 16S rRNA positions with members of the genus *Cellulomonas* (Table 1).

Morphology and motility of cells grown on HSMA plates for 3–5 days at 28 °C were examined under a light microscope. Gram-staining was performed as described by Smibert & Krieg (1981). The method described by Buck (1982) was also used for the differentiation of Gram-positive and Gram-negative bacteria. Catalase activity was tested by mixing cells from colonies grown on HSMA plates with 3 % (v/v) hydrogen peroxide on a glass slide while oxidase activity was tested by spotting the cells on a cytochrome oxidase strip (Nissui Pharmaceuticals). Absorption spectra (260–700 nm) of acetone extracts of cells were recorded spectroscopically to investigate the presence of carotenoid-type pigments. Growth at different temperatures (4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 42 and 45 °C) was assessed on HSMA plates, while growth at different pHs was examined in marine broth with the pH adjusted with either HCl (pH 5 and 6) or NaOH (pH 8, 9 and 10). Growth at high NaCl concentrations [4, 5, 6, 7 and 8 % (w/v) NaCl] was tested in marine broth. The requirement for NaCl for growth was tested on IFO 802 (Dasman *et al.*, 2002) and ISP2 (International *Streptomyces* project; Shirling & Gottlieb, 1966) media.

Methods described elsewhere in detail (Smibert & Krieg, 1981; Khan *et al.*, 2007) were used to test the abilities of the strain to hydrolyse agar, carrageenan (type I; Sigma), casein, cellulose, chitin, CM-cellulose (high-viscosity; Sigma), gelatin and starch. The ability of the strain to produce acid from various carbon sources was tested with the API 50 CH system (bioMérieux) following the instructions of the manufacturer, except that the inoculum was prepared in a 1 : 1 mixture of CHB medium (bioMérieux) and artificial seawater. The API strips were incubated at 28 °C for 3–4 days before test results were scored. Biochemical and physiological properties such as the presence of different enzymes were tested using the commercially available API ZYM and API Coryne systems (bioMérieux) according to the instructions of the manufacturer. Susceptibility to different antibiotics was tested on HSMA agar plates using commercially available antibiotic discs (Nissui Pharmaceutical). For fatty acid analysis, the strain was grown on marine agar for 3–4 days at 28 °C. Fatty acid profiles were determined using the Sherlock microbial identification system (MIDI) according to the manufacturer's protocol (Sasser, 1990). Respiratory quinones were analysed using the protocol of Nakagawa & Yamasato (1993). Cell-wall amino acids were determined as described by Tamura *et al.* (1994). Polar lipids were extracted and examined by two-dimensional TLC (Minnikin *et al.*, 1984). Exponentially growing cells in marine broth were used to prepare the genomic DNA as described previously (Minamisawa, 1990), and the HPLC method of Mesbah *et al.* (1989) was used to determine the G+C content of the DNA sample. The G+C content of strain CTT-37^T was 70 mol%. The fluorometric method of Ezaki *et al.* (1989) was used for DNA–DNA hybridization in 50 % (v/v) formamide at 53 °C. The DNA–DNA hybridization value between strain CTT-37^T and *O. enterophila* JCM 7350^T was 10–12 %.

Table 1. Comparison of 16S rRNA signature nucleotides for strain CTT-37^T and members of the genera *Oerskovia* and *Cellulomonas*

Positions are numbered according to the sequence of *E. coli*. Data for reference genera were taken from Stackebrandt *et al.* (2002). R, Purine (G, A); Y, pyrimidine (C, U).

Position	Strain CTT-37^T	*Oerskovia*	*Cellulomonas*
18 : 917	?*–G	?*–G	?*–A
139 : 224	U–G	A–U	U, A–G
146 : 176	G–C	G–U	G–C
185 : 192	A–C	C–G	R–Y
186 : 192	G–C	U–G	C–G, G–C
601 : 637	G–C	G–C	G–U
602 : 636	G–U	G–U	C–G
612 : 628	U–G	U–A	Y–G
614 : 626	A–U	A–U	G–C, C–G
1120 : 1153	C–G	U–A	C–G
1438 : 1463	G–C	G–U	G–C

*Position 18 not known.

Cells of strain CTT-37^T were rods, 0.4–0.6 μm wide and 1.0–1.6 μm long, stained Gram-positive and were non-motile. Morphological, biochemical and physiological characters of strain CTT-37^T are listed in the species and genus descriptions and in Table 2.

Major fatty acids detected in strain CTT-37^T were ai-$C_{15:0}$, $C_{16:0}$, ai-$C_{17:0}$ and $C_{18:0}$ (Supplementary Table S1, available in IJSEM Online). The cell-wall type is A4α, with acetylated muramic acid residues. The peptidoglycan interpeptide bridge of the novel strain is characterized by the presence of L-serine, which is not found in members of the genera *Oerskovia* (Stackebrandt *et al.*, 2002) and *Cellulomonas* (Kang *et al.*, 2007). The novel strain also lacks L-ornithine and L-threonine, which are respectively found in *Cellulomonas* and *Oerskovia*. Mannose and galactose are the main sugars in the cell wall. The only polar lipid is phosphatidylglycerol and the major isoprenoid quinone was tetrahydrogenated menaquinone-9.

The results of the chemotaxonomic characterizations described in Table 2 and differences in 16S rRNA signature sequences (Table 1) clearly indicate that this strain represents a novel lineage. Therefore, the name *Paraoerskovia marina* gen. nov., sp. nov. is proposed.

Table 2. Differential characteristics of strain CTT-37^T and its phylogenetic neighbours

Data for *Oerskovia* and *Cellulomonas* were taken from Kang *et al.* (2007), Stackebrandt *et al.* (2002), Yi *et al.* (2007) and Prauser *et al.* (1970). NK, Not known.

Characteristic	Strain CTT-37^T	*Oerskovia*	*Cellulomonas*
Peptidoglycan type	A4α	A4α	A4β
Interpeptide bridge	L-Lys–L-Ser–D-Glu	L-Lys–L-Thr–D-Asp or L-Lys–L-Thr–D-Glu	L-Orn–D-Asp or L-Orn–D-Glu
Major polar lipid(s)*	PG	PG, DPG, PI	NK
β-Glucuronidase	+	−	NK

*DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol.

Description of *Paraoerskovia* gen. nov.

Paraoerskovia (Pa.ra.oer.sko′vi.a. Gr. prep. *para* beside; N.L. fem. n. *Oerskovia* a bacterial genus name; N.L. fem. n. *Paraoerskovia* beside or close to *Oerskovia*).

Cells stain Gram-positive and are non-motile, facultatively anaerobic rods. Catalase- and oxidase-positive. Do not produce aerial mycelium. Major fatty acids are ai-$C_{15:0}$, $C_{16:0}$, ai-$C_{17:0}$ and $C_{18:0}$. The interpeptide bridge contains L-serine and D-glutamic acid. The cell wall is A4α type with acetylated muramic acid residues. The only polar lipid is phosphatidylglycerol. The major quinone is menaquinone MK-9(H_4). The G+C content of the DNA of the type strain of the type species is 70 mol%. The type species is *Paraoerskovia marina*.

Description of *Paraoerskovia marina* sp. nov.

Paraoerskovia marina (ma.ri′na. L. fem. adj. *marina* of the sea, marine).

Displays the following characteristics in addition to those given in the genus description. Cells are 0.4–0.6 μm wide and 1.0-1.6 μm long. Forms creamy yellow-coloured colonies, 1–2 mm in diameter, on HSMA or ISP2 plates after 3–5 days of incubation at 28 °C. Carotenoid-type pigments are present. Growth occurs at 10–35 °C (optimum at 28 °C) and pH 6.0–10.0 (optimum pH 7.0–8.0). Growth occurs at 0–8 % NaCl (w/v). According to the API Coryne system, positive for the reduction of nitrate, pyrazinamidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and urease and negative for pyrrolidonyl arylamidase, alkaline phosphatase, β-galactosidase and gelatin hydrolysis. According to the API Coryne system, utilizes D-glucose, maltose and xylose and does not utilize lactose, mannitol or ribose. Sucrose and glycogen are utilized weakly. With the API ZYM system, positive for arylamidase, α-glucosidase and leucine, weakly positive for esterase and lipase and negative for all other enzymes included in the API ZYM system. With the API 50CH system, produces acid from starch, L-arabinose, cellobiose, aesculin ferric citrate, D-fructose, D-galactose, gentiobiose, D-glucose, glycogen, glycerol, lactose, maltose, D-mannose, D-ribose, D-xylose, sucrose, salicin and trehalose; acid production from amygdalin, D-lyxose, L-rhamnose and turanose is weak. Does not produce acid from the other 28 compounds included in the API 50CH system. Positive for the degradation of CM-cellulose and starch and negative for the degradation of casein, cellulose, chitin and gelatin. Sensitive to vancomycin (50 μg) and resistant to bacitracin (10 μg), gentamicin (30 μg), kanamycin (30 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), nystatin (100 IU) and streptomycin (10 μg). Menaquinones MK-9 and MK-9(H_2) are detected in addition to the major menaquinone MK-9(H_4). Cell-wall sugars are mannose and galactose.

The type strain is CTT-37^T (=NBRC 104352^T =DSM 21750^T), isolated from a sediment sample collected from a beach on the coast of Tottori city.

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