Oceaniserpentilla haliotis gen. nov., sp. nov., a marine bacterium isolated from haemolymph serum of blacklip abalone

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An aerobic, Gram-negative bacterial isolate, strain DSM 19503^T, was isolated from haemolymph serum of the blacklip abalone *Haliotis rubra*. Cells of strain DSM 19503^T were vibrioid to spiral, motile and were able to pass through sterile filters with a pore size of 0.2 µm, indicating the small width of the bacterium. The isolate was psychrophilic, with the ability to grow at 2–8 °C. Oxidase activity was present, whereas catalase activity was absent. The nearly complete 16S rRNA gene sequence of strain DSM 19503^T was obtained and phylogenetic sequence analysis showed that it formed a distinct genus in the family *Oceanospirillaceae* with highest sequence similarity of 92.9 % to *Oleispira antarctica* RB-8^T. The cellular fatty acid composition was dependent on the growth medium used for cultivation. During growth on seawater agar, the fatty acid composition was most similar to that of *Oleispira antarctica* DSM 14852^T, with mainly C_{16:0} (90.3 %). In contrast, Columbia blood agar/NaCl-grown cells exhibited mainly C_{10:0} 3-OH (11.8 %), C_{12:1} cis5 (8.2 %), C_{16:1} cis9 (29.6 %), C_{16:0} (19.3 %) and C_{18:1} cis9 (13.1 %) fatty acids. On the basis of phenotypic, chemotaxonomic and genotypic data, strain DSM 19503^T is considered to represent a novel species in a new genus for which the name *Oceaniserpentilla haliotis* gen. nov., sp. nov. is proposed. The type strain of *Oceaniserpentilla haliotis* is DSM 19503^T (=LMG 24225^T).

In the last few years, a large number of strains have been isolated from diverse marine environments (Connon & Giovannoni, 2002; Allgaier *et al.*, 2003) and many have been identified as representing novel species in new genera. The blacklip abalone *Haliotis rubra* is endemic mainly to the southern waters of Australia and is one of the most valuable fishery species. Nearly 80 species of abalone are known; they appear in offshore areas worldwide and grow at depths of 0–40 m. In an earlier study, a previously unknown strain of the genus *Vibrio*, LMG 21323^T, was isolated from the gut of *Haliotis rubra* and described as *Vibrio superstes* (Hayashi *et al.*, 2003). This Gram-negative strain was non-motile, facultatively anaerobic and possessed alginate activity.

Abalones were harvested by the fishery industry near Hobart, Tasmania (42° 55' S 147° 20' E), in February 2006. Isolate DSM 19503^T was obtained from haemolymph serum prepared from *Haliotis rubra*. The haemolymph serum was filtered through a sterile filter with a pore size of

0.2 µm and stored at 2–8 °C. After several months, bacterial growth was detected in the filtered haemolymph sera and samples were subjected to microbial investigations. Isolates of strain DSM 19503^T were grown on Columbia blood agar supplemented with 1.5 % NaCl after incubation for 14 days at 2–8 °C as tiny, opaque colonies. The growth period of strain DSM 19503^T could be decreased to 3–4 days incubation at 22 °C by plating on fresh Columbia blood agar supplemented with 1.5 % NaCl. Colonies reached a maximum size of 2–3 mm after 1 week and haemolytic activity was observed around the colonies. Strain DSM 19503^T could also be grown on marine agar 2216 (Difco). When grown on Columbia blood agar and suspended in NaCl-supplemented seawater medium, the isolate exhibited weak reactions for utilization of Tweens 40 and 80 after 9 days of incubation at 22 °C, using the Biolog substrate panel. Using API ID 32 GN and API ID 20 NE test kits (bioMérieux), the strain utilized L-alanine, L-proline, L-arabinose, caproic acid, 3-hydroxybutyric acid and potassium 5-keto-D-gluconate after incubation of the test kits for 96 h at 22 °C. Cellular morphology and motility of the isolate were observed by using

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain DSM 19503^T is AM747817.

phase-contrast microscopy (Fig. 1). The novel isolate was Gram-negative, vibrioid to spiral, non-spore-forming, about 1.8–3.5 µm long by 0.2–0.6 µm wide and highly motile. Oxidase activity was detected, whereas catalase activity was absent. For long-term preservation, cryopreservation at below –80 °C using Microbank vials (Prolab) was feasible. In contrast, lyophilized cells in 10 % skimmed milk lost viability.

Fatty acid analysis was performed on the isolate and two reference strains using cells grown on seawater agar and on NaCl-supplemented Columbia blood agar for 10 days at 22 °C. Growth of *Oleispira antarctica* DSM 14852^T was only obtained on seawater agar, whereas *Marinomonas vaga* ATCC 27119^T failed to grow on seawater agar and instead grew only on NaCl-supplemented Colombia blood agar. Saponification with 15 % NaOH in 50 % methanol, acid methylation with 6 M HCl in 50 % methanol and extraction of fatty acid methyl esters were performed as described by Sasser (1990). The fatty acid methyl ester extracts were analysed by GC (Hewlett Packard model 6890) equipped with a 5 % phenyl-methyl-silicone capillary column and a flame ionization detector. The identity of the fatty acids was verified by GC-MS (Hewlett Packard model 5890 series II equipped with a 5 % phenyl-methyl-silicone capillary column and a model 5972 MS detector). The chromatographic conditions used were described previously (Lipski & Altendorf, 1997). The positions of double bonds were verified by analysing the dimethyl-disulfide adducts of the fatty acid methyl esters. The positions of hydroxy and methoxy groups and double bonds are given from the carboxyl group of the fatty acid molecule

according to the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (1977).

The fatty acid composition of cells grown on Columbia blood agar differed from that of cells cultivated on seawater agar. Large amounts (62.7 % of total fatty acids) of C_{10:0} 3-OH (11.8 %), C_{12:1} cis5 (8.2 %), C_{16:1} cis9 (29.6 %) and C_{18:1} cis9 (13.1 %) were found in cells grown on Columbia blood agar (Table 1). When grown on seawater agar, the principal saturated fatty acids were C_{14:0} (1.4 %), C_{16:0} (90.3 %) and C_{18:0} (2.9 %), representing more the fatty acid synthesis pathways of isolate DSM 19503^T. The fatty acid profile of strain DSM 19503^T cultivated on seawater agar was highly similar to that of the reference strain *Oleispira antarctica* DSM 14852^T, with palmitic acid as the dominant compound and C_{10:0} 3-OH as hydroxyl fatty acid.

For 16S rRNA gene sequence analysis, cells of strain DSM 19503^T were lysed with the PrepMan Ultra preparation reagent (Applied Biosystems). Amplification of the 16S rRNA gene was performed using the PCR-MicroSeq Full Gene 16S rDNA PCR kit (Applied Biosystems). Amplified PCR products were purified using the QIAquick PCR Purification kit (Qiagen). Sequencing reactions were carried out using the MicroSeq Full Gene 16S rDNA sequencing kit (Applied Biosystems). Sequencing products were purified with the DyeEx 2.0 spin kit (Qiagen).

Table 1. Fatty acid compositions (%) of *Oceaniserpentilla haliotis* DSM 19503^T and the reference strains *Oleispira antarctica* DSM 14852^T and *Marinomonas vaga* ATCC 27119^T

Strains/growth conditions: 1, *Oceaniserpentilla haliotis* DSM 19503^T grown on seawater agar; 2, *Oceaniserpentilla haliotis* DSM 19503^T grown on blood/NaCl agar; 3, *Oleispira antarctica* DSM 14852^T grown on seawater agar; 4, *Marinomonas vaga* ATCC 27119^T grown on blood/NaCl agar. ND, Not detected.

Fatty acid	1	2	3	4
C _{10:0}	0.1	2.7	0.8	3.7
C _{10:0} 3-methoxy	ND	0.3	ND	0.2
C _{10:0} 3-OH	1.0	11.8	1.0	18.7
C _{12:1} cis5	1.2	8.2	2.3	3.0
C _{12:0}	0.4	2.2	1.5	4.2
C _{12:0} 3-OH	ND	ND	1.5	0.2
C _{14:0}	1.4	0.6	1.6	1.7
C _{16:1} cis9	1.3	29.6	7.0	16.7
C _{16:1} trans9	ND	0.6	1.3	0.5
C _{16:1} cis11	ND	0.3	0.1	0.1
C _{16:0}	90.3	19.3	80.9	9.4
C _{17:0}	0.1	0.3	0.1	ND
C _{18:2} cis9,12	ND	1.3	0.9	0.4
C _{18:1} cis9	0.6	13.1	ND	0.4
C _{18:1} cis11	0.2	3.6	0.1	38.9
C _{18:1} trans11	ND	ND	ND	0.4
C _{18:0}	2.9	4.8	0.8	0.9
C _{19:0}	ND	0.4	ND	ND

Fig. 1. Light micrograph of cells of strain DSM 19503^T grown on Columbia blood agar with 1.5 % NaCl for 14 days at 22 °C. Bar, 2 µm.

Fig. 2. Dendrogram of 16S rRNA gene sequence relatedness showing the phylogenetic position of strain *Oceaniserpentilla haliotis* DSM 19503^T and most closely related type strains within the *Oceanospirillaceae*. Numbers at branching points refer to bootstrap values (1000 resamplings). Bar, genetic distance of 1 nucleotide substitution per 100 nucleotides.

Analysis of the sequencing reactions was performed with the 3100-Avant Genetic Analyzer (Applied Biosystems). The nearly complete 16S rRNA gene sequence (1475 bp) of isolate DSM 19503^T was obtained by sequencing both strands at least twice. Initial sequence comparisons against the 16S rRNA gene sequences available in the Ribosomal Database Project (Maidak *et al.*, 2001) and the FASTA33 database (Pearson, 1990) indicated that the strain belongs to the class *Gammaproteobacteria*, with highest sequence similarity of 97.5% to an undescribed gammaproteobacterial isolate Gp_4_7.1 (GenBank accession no. AM117931) and a similarity of 92.9% to the next related type strain, which was *Oleispira antarctica* RB-8^T (AJ426420).

A phylogenetic tree based on 16S rRNA gene sequences of type strains with highest nucleotide similarity was constructed using the program MEGA (Tamura *et al.*, 2007). Neighbour-joining analysis revealed that strain DSM 19503^T formed a branch together with *Oleispira antarctica* RB-8^T (bootstrap value of 99%) within the family *Oceanospirillaceae* (Fig. 2). Generally, the threshold of sequence similarity to separate species based on the 16S rRNA gene is 97% (Stackebrandt & Goebel, 1994); the phylogenetic distance of the novel isolate from *Oleispira antarctica* suggests that the isolate represents a novel species in a new genus within the family *Oceanospirillaceae*, *Oceaniserpentilla haliotis* gen. nov., sp. nov.

Description of *Oceaniserpentilla* gen. nov.

Oceaniserpentilla (O.ce.a.ni.ser.pen.til'la. L. masc. n. *oceanus* the ocean; L. fem. n. *serpens* -tis a snake; N.L. fem. n. *serpentilla* a small snake; N.L. fem. n. *Oceaniserpentilla* small snake of the ocean, indicating shape and origin).

Gram-negative, motile, obligately aerobic, vibrioid to spiral, non-spore-forming cells. Oxidase activity is present, whereas catalase activity is absent. A member of the family *Oceanospirillaceae*. The type species is *Oceaniserpentilla haliotis*.

Description of *Oceaniserpentilla haliotis* sp. nov.

Oceaniserpentilla haliotis (ha.li.o'tis. N.L. n. *Haliotis* scientific name of a genus; N.L. gen. n. *haliotis* of

Haliotis, referring to the isolation of the type strain from *Haliotis rubra*).

Cells are about 1.8–3.5 µm long by 0.2–0.6 µm wide and highly motile. Grows on seawater agar or Columbia blood agar with 1.5% NaCl and in abalone haemolymph serum. Psychrophilic, with optimal growth at 2–8 °C. Cultivation time to the appearance of tiny colonies of about 0.5 mm in diameter is 10–14 days. Growth can be adapted to 22 °C. Weak reactions for utilization of Tweens 40 and 80. Utilizes L-alanine, L-proline, L-arabinose, caproic acid, 3-hydroxybutyric acid and potassium 5-keto-D-gluconate. The cellular fatty acid composition is highly dependent on the growth medium. When grown on seawater agar, the principal saturated fatty acids are C_{14:0} (1.4%), C_{16:0} (90.3%) and C_{18:0} (2.9%), whereas Columbia blood agar/NaCl-grown cells exhibit mainly C_{10:0} 3-OH (11.8%), C_{12:1 cis5} (8.2%), C_{16:1 cis9} (29.6%), C_{16:0} (19.3%) and C_{18:1 cis9} (13.1%).

The type strain is DSM 19503^T (=LMG 24225^T), isolated after filtration of abalone haemolymph serum through a filter with a pore size of 0.2 µm. The haemolymph serum was isolated from the blacklip abalone *Haliotis rubra* harvested near Hobart, Tasmania. No bacterial species with a validly published name shows more than 92.9% 16S rRNA gene sequence similarity to the type strain.

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