Miniimonas arenae gen. nov., sp. nov., an actinobacterium isolated from sea sand

Harumi Ue,1 Yoshihide Matsuo,2 Hiroaki Kasai3 and Akira Yokota1

Correspondence
Harumi Ue
Harua@aol.jp

1Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-Ku, Tokyo 113-0032, Japan
2Suntory Holdings Limited, R&D Planning Division, 1-1-1 Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618-8503, Japan
3Marine Biosciences Kamaishi Research Laboratory, Kitasato University, 3-75-1, Heita, Kamaishi, Iwate 026-0001, Japan

A Gram-positive, non-motile, coccoid- to rod-shaped, non-spore-forming bacterium, designated strain YM18-15T, was isolated from sea sand and studied using a polyphasic taxonomic approach. Strain YM18-15T grew under both aerobic and anaerobic conditions. The cell-wall peptidoglycan type was A4\(^b\) and ornithine was the diagnostic diamino acid. The polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and an unknown phospholipid, MK-8(H4) was the major menaquinone and the predominant fatty acids were anteiso-C\(_{15}\) : 0 and C\(_{16}\) : 0. The DNA G + C content was 74.2 mol%. High 16S rRNA gene sequence similarities (96.3–97.3 %) were found with the sequences of the type strains of the three genera of the family Beutenbergiaceae. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YM18-15T formed a clade with Serinibacter salmoneus, Salana multivorans and Beutenbergia cavernae. Strain YM18-15T differed from these three type strains in chemotaxonomic characteristics and in 16S rRNA gene signature nucleotides. Based on genetic and chemotaxonomic evidence, it is suggested that strain YM18-15T represents a novel species of a new genus within the family Beutenbergiaceae, for which the name Miniimonas arenae gen. nov., sp. nov. is proposed. The type strain of the type species is YM18-15T (\(^{\text{T}}\) = NBRC 106267\(^\text{T}\) = KCTC 19750\(^\text{T}\) = MBIC 08348\(^\text{T}\)).

The family Beutenbergiaceae within the suborder Micrococcineae was proposed by Zhi et al. (2009). The family Beutenbergiaceae contains three genera, Salana (von Wintzingerode et al., 2001), Beutenbergia (Groth et al., 1999) and Serinibacter (Hamada et al., 2009). Each of these genera is represented by only one species. Salana multivorans DSM 13521\(^\text{T}\) was isolated from an anaerobic bioreactor, Beutenbergia cavernae DSM 12333\(^\text{T}\) was isolated from a cave and Serinibacter salmoneus DSM 21801\(^\text{T}\) was isolated from the intestinal tract of a fish. In the present study, we describe a bacterium, strain YM18-15\(^\text{T}\), which was isolated from sea sand in Teguma fishing harbour in Nagasaki Prefecture, Japan. It is proposed that strain YM18-15\(^\text{T}\) represents a novel species in a new genus within the family Beutenbergiaceae.

Isolation was performed on H medium (for composition, see Supplementary Method in IJSEM Online) at 25 °C for 30 days. Strain YM18-15\(^\text{T}\) was maintained as glycerol suspensions (20 %, v/v) at –80 °C. Biomass for phenotypic tests was obtained from Luria–Bertani (LB) broth (Difco) by incubating at 30 °C for 1 week. Biomass for chemical and molecular systematic studies was obtained by culturing the strain in IL8 medium (10 g lactose, 10 g polypeptone, 15 g yeast extract, 10 g NaCl), except for the cells used for the preparation of fatty acids. For quantitative analysis of whole-cell fatty acids, cells were grown on tryptic soy agar (TSA) at 30 °C for 7 days. Gram staining and tests for motility were performed with the Favor G (Nissui) and API M media (bioMérieux), respectively, according to the manufacturers’ instructions.

Morphology was observed using light microscopy (ECLIPSE E600; Nikon). Colony morphology and colour were observed on LB agar (Difco) at 30 °C for 1 week. Growth was tested at 5, 10, 15, 20, 25, 28, 35, 37, 45 and 50 °C on LB agar. Experiments investigating tolerance to NaCl were carried out using LB broth as the basal medium. The following NaCl concentrations (w/v) were tested: 0.5, 2, 5, 8, 10, 15 and 20 %. For determination of the optimal pH for growth, cells were grown on LB agar at pH 5–11 at 30 °C for 3 weeks. The ability of the novel strain to use sole

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YM18-15T is AB522642.

Supplementary material is available with the online version of this paper.

The family Beutenbergiaceae within the suborder Micrococcineae was proposed by Zhi et al. (2009). The family Beutenbergiaceae contains three genera, Salana (von Wintzingerode et al., 2001), Beutenbergia (Groth et al., 1999) and Serinibacter (Hamada et al., 2009). Each of these genera is represented by only one species. Salana multivorans DSM 13521\(^\text{T}\) was isolated from an anaerobic bioreactor, Beutenbergia cavernae DSM 12333\(^\text{T}\) was isolated from a cave and Serinibacter salmoneus DSM 21801\(^\text{T}\) was isolated from the intestinal tract of a fish. In the present study, we describe a bacterium, strain YM18-15\(^\text{T}\), which was isolated from sea sand in Teguma fishing harbour in Nagasaki Prefecture, Japan. It is proposed that strain YM18-15\(^\text{T}\) represents a novel species in a new genus within the family Beutenbergiaceae.

Isolation was performed on H medium (for composition, see Supplementary Method in IJSEM Online) at 25 °C for 30 days. Strain YM18-15\(^\text{T}\) was maintained as glycerol suspensions (20 %, v/v) at –80 °C. Biomass for phenotypic tests was obtained from Luria–Bertani (LB) broth (Difco) by incubating at 30 °C for 1 week. Biomass for chemical and molecular systematic studies was obtained by culturing the strain in IL8 medium (10 g lactose, 10 g polypeptone, 15 g yeast extract, 10 g NaCl), except for the cells used for the preparation of fatty acids. For quantitative analysis of whole-cell fatty acids, cells were grown on tryptic soy agar (TSA) at 30 °C for 7 days. Gram staining and tests for motility were performed with the Favor G (Nissui) and API M media (bioMérieux), respectively, according to the manufacturers’ instructions.

Morphology was observed using light microscopy (ECLIPSE E600; Nikon). Colony morphology and colour were observed on LB agar (Difco) at 30 °C for 1 week. Growth was tested at 5, 10, 15, 20, 25, 28, 35, 37, 45 and 50 °C on LB agar. Experiments investigating tolerance to NaCl were carried out using LB broth as the basal medium. The following NaCl concentrations (w/v) were tested: 0.5, 2, 5, 8, 10, 15 and 20 %. For determination of the optimal pH for growth, cells were grown on LB agar at pH 5–11 at 30 °C for 3 weeks. The ability of the novel strain to use sole

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YM18-15T is AB522642.

Supplementary material is available with the online version of this paper.
carbon sources was tested with International *Streptomyces*
Project (ISP) medium 9 (Difco) supplemented with 1 %
(w/v) carbon source for 3 weeks. The activity of several
enzymes was determined by using API ZYM (bioMérieux)
and checked after 24 h. Several physiological tests were
performed using the API 20NE kit (bioMérieux). Acid
production under anaerobic conditions was assayed using
API 20A (bioMérieux). API tests were performed twice.
The oxidase reaction was tested by using cytochrome
oxidase test paper (Nissui Pharmaceutical Co.). Catalase
activity was determined based on production of bubbles
after the addition of a drop of 3 % \( \text{H}_2\text{O}_2 \). All phenotypic
assays (except analysis of the temperature range) were
carried out at 30 °C, which was in the range of optimal
growth temperatures for the strain.

Menaquinones were extracted using the procedures
described by Collins *et al.* (1977) and analysed by LCMS
as described by Ogiso *et al.* (2008), but without adding
phosphoric acid to the elution buffer. Polar lipids were
extracted according to Minnikin *et al.* (1984) and analysed
by TLC. The purified cell wall was obtained by using the
method described by Schleifer & Kandler (1972). Amino
cells in cell-wall hydrolysates were analysed by two-
dimensional ascending TLC on cellulose plates (Merck)
by using the solvent systems of Harper & Davis (1979) and by
HPLC as their phenylthiocarbamoyl derivatives with a
Wakopak WS-PTC column (Wako Pure Chemical
Industries) (Yokota *et al.* 1993). Analysis of the whole-
cell fatty acid pattern was performed by using the MIDI
system (Microbial ID, Inc.). Mycolic acids were analysed by
the method of Minnikin *et al.* (1975). The acyl type of the
muramic acid in the cell wall was determined according to
the method described by Uchida *et al.* (1999). Analysis
of sugars from whole-cell hydrolysates was performed using
the procedures described by Staneck & Roberts (1974).
DNA was prepared according to the method of Suzuki
*et al.* (1999). The DNA base composition of strain YM18-
15\( ^T \) was determined by reversed-phase HPLC using a
DNA-GC kit (Yamas) according to the method of

An approximately 1500 bp fragment of the 16S rRNA gene
was amplified from the extracted DNA by using bacterial
universal primers 27F and 1492R (Escherichia coli number-
ing system; Weisburg *et al.*, 1991) specific to the 16S rRNA
gene. A sequence similarity search was conducted using
GenBank BLASTN (National Center for Biotechnology
Information). Sequences of closely related taxa were retrieved
and aligned using the CLUSTAL_X program (Thompson
*et al.*, 1997) and the alignment was corrected manually.
Aligned sequences were analysed by using MEGA 4.0.2
software (Tamura *et al.*, 2007). For neighbour-joining
analysis (Saitou & Nei, 1987), genetic distances were
calculated using Kimura’s two-parameter model (Kimura,
1983). Clustering with the neighbour-joining (Saitou &
Nei, 1987) and maximum-parsimony (Fitch, 1971) meth-
ods was determined by using bootstrap values based on
1000 replications (Felsenstein, 1985). Similarity values were
calculated using the MEGA software.

The components of the peptidoglycan of strain YM18-15\( ^T \)
were Ala, Ser, Glu and Orn and were found in a molar ratio of
0.4:0.5:1.0:0.4 and the peptidoglycan type was A4\( \beta \)
(Schleifer & Kandler, 1972) with an interpeptide bridge of
Orn–Glu. The fatty acid pattern was of the iso- and anteiso-
branched and straight-chain saturated type. Major fatty
acids were anteiso-C\(_{15:0}\) (46.7 %) and C\(_{16:0}\) (23.2 %) and
minor fatty acids were anteiso-C\(_{17:0}\) (9.4 %), C\(_{14:0}\) (5.4 %),
iso-C\(_{16:0}\) (3.8 %), iso-C\(_{15:0}\) (3.4 %), iso-C\(_{14:0}\) (2.8 %), C\(_{15:0}\)
(2.3 %) and iso-C\(_{17:0}\) (1.5 %). The detailed fatty acid
composition of strain YM18-15\( ^T \) is given in Supple-
mental Table S1 (see IJSEM Online). The isoprenoid
quinones were menaquinones of type MK-8(H\(_4\)) (88.5 %),
MK-8 (7.0 %), MK-8(H\(_2\)) (2.7 %) and MK-8(H\(_6\)) (1.8 %).
The polar lipids consisted of phosphatidylinositol, phos-
phatidylglycerol, diphosphatidylglycerol and an unknown
phospholipid. The DNA G + C content was 74.2 mol%.

Phylogenetic analysis based on the neighbour-joining
method showed that strain YM18-15\( ^T \) was positioned
within the family *Beutenbergiaceae* of the suborder
*Micro-
coccineae* and joined the phylogenetic lineage of *Serini-
bacter salmoneus* DSM 21801\( ^T \) (Fig. 1). The relative
position of strain YM18-15\( ^T \) was also confirmed in the
maximum-parsimony tree. The BLAST sequence similarity
result based on the 16S rRNA gene sequence revealed that
strain YM18-15\( ^T \) was closely related to *Serinibacter
salmoneus* DSM 21801\( ^T \) (sequence similarity 97.3 %),
*Salana multivorans* DSM 13521\( ^T \) (96.3 %) and *B. cavernae*
DSM 12333\( ^T \) (97.1 %) of the family *Beutenbergiaceae*.

The differences in cell morphology and chemotaxonomic
characteristics between strain YM18-15\( ^T \) and members of
the genera *Serinibacter, Salana* and *Beutenbergia* are shown
in Table 1. The most noticeable differences were in the polar
lipids. The peptidoglycan type of the cell wall was A4\( \beta \)
(containing Orn) for strain YM18-15\( ^T \), but A4\( \alpha \) (containing
Lys) for the genera *Serinibacter and Beutenbergia*. The
combination of major fatty acids (anteiso-C\(_{15:0}\) and C\(_{16:0}\))
for strain YM18-15\( ^T \) did not match those of the genera
*Salana* or *Beutenbergia*. The results of detailed test reactions
are presented in the species description and Supplementary
Table S2 (available in IJSEM Online). The pattern of the 16S
rRNA gene sequence signature nucleotides is shown in
Supplementary Table S3. The pattern of 16S rRNA signature
nucleotides consisted of nucleotides at positions 140:223
(G–C), 589:650 (U–A), 610 (U), 612:628 (C–G), 616:624
(G–C), 839:847 (C–A) and 863 (A), which indicated that
strain YM18-15\( ^T \) did not belong to any recognized genus
within the family *Beutenbergiaceae*, having signature nucle-
otide differences from *Serinibacter salmoneus* DSM 21801\( ^T \)
(three positions), *Salana multivorans* DSM 13521\( ^T \) (three
positions) and *B. cavernae* DSM 12333\( ^T \) (four positions).
The pattern of 16S rRNA gene signature nucleotides of strain
YM18-15\( ^T \) perfectly matched that given in the description of
the family *Beutenbergiaceae* as reported by Hamada *et al.*
(2009). Strain YM18-15T and the genera *Beutenbergia*, *Salana* and *Serinibacter* had an A–G at position 131:231, not a C–G as reported by Zhi et al. (2009). From the differences in its chemotaxonomic and genotypic properties, strain YM18-15T is considered to represent a novel genus of the family *Beutenbergiaceae*, for which the name *Miniimonas arenae* gen. nov., sp. nov. is proposed.

**Description of *Miniimonas* gen. nov.**

*Miniimonas* (Mi.ni.mo'nas. L. adj. minius cinnabar-red, vermilion; L. fem. n. monas a unit, monad; N.L. fem. n. *Miniimonas* vermilion monad, referring to the colour of the cell mass).

Gram-positive, non-motile, coccoid- to rod-shaped, oxidase-negative and catalase-positive. Growth occurs under both aerobic and anaerobic conditions (i.e. facultatively anaerobic). The menaquinone is MK-8(H4). The fatty acid pattern is of the iso- and anteiso-branched and straight-chain saturated type. The major fatty acids are anteiso-C15:0 and C16:0. The cell-wall peptidoglycan contains Orn, Ser, Ala and Glu. The whole-cell sugars are galactose, xylose and ribose. Mycolic acids are absent. The acyl type of muramic acid is acetyl. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and one unknown phospholipid. Phylogenetically, the genus *Miniimonas* belongs to the family *Beutenbergiaceae* of the suborder *Micrococcineae* of the class *Actinobacteria*. The type species is *Miniimonas arenae*.

**Description of *Miniimonas arenae* sp. nov.**

*Miniimonas arenae* (a.re'na.e. L. gen. n. arenae of sand, isolated from sea sand).

Displays the following properties in addition to those given in the genus description. Rod-shaped cells are 0.6–3.7 μm in length. Cells exhibit a rod-coccus cycle. The diameters of
the cocci are 1.0–1.7 μm. Growth occurs weakly at 25–30 °C. The pH range for growth is 5–11, with optimum growth at pH 7–7.5. Cells grow in the absence of NaCl, but tolerate up to 5 % NaCl (w/v). When grown aerobically for 7 days on LB agar or IL8 medium agar, the type strain forms a vermilion-coloured cell mass. Does not form spores. Cells form circular and smooth colonies that are 1–4 mm diameter after 7 days at 30 °C on LB agar. Nitrate is reduced to nitrite. H2S is not produced. Hydrolysis of starch is negative. The type strain forms vermilion-coloured colonies. Based on API ZYM tests, cells are positive for esterase lipase (C8), leucine arylamidase, naphthol AS-BI-phosphohydrolase, β-galactosidase, β-glucosidase and β-glucosidase, but negative for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Cells are weakly positive for esterase (C4) and acid phosphatase. Utilizes the following substrates as sole carbon sources: L-arabinose, D-mannose, D-xyllose, D-glucose, D-fructose, L-rhamnose and maltose monohydrate, but does not utilize D-mannitol. Anaerobic formation of acid from API 20A is positive for glucose, sucrose, maltose, D-xyllose, L-arabinose, D-mannose, raffinose, L-rhamnose and trehalose, weakly positive for cellulobiose and negative for D-mannitol, lactose, salicin, glycerol, melezitose and D-sorbitol. Based on API 20A tests, cells are anaerobically positive for the hydrolysis of aesculin, but negative for the hydrolysis of gelatin and for the production of indole, arginine dihydrolase and urease. The peptidoglycan type, polar lipids, fatty acid pattern and major menaquinone are as given in the genus description.

The type strain, YM18-15T (=NBRC 106267T=KCTC 19750T=MBIC 08348T), was isolated from sea sand in Teguma fishing harbour in Nagasaki Prefecture, Japan. The G+C content of the genomic DNA is 74.2 mol%.


The description of the family is as emended by Hamada et al. (2009), but the 16S rRNA gene sequence signature nucleotide pattern contains an A–G at position 131:231, not a C–G (Zhi et al., 2009). The family contains the type genus **Beutenbergia** (Groth et al., 1999), as well as the genera **Salana** (von Wintzingerode et al., 2001), **Serinibacter** (Hamada et al., 2009) and **Miniimonas**.

**Acknowledgements**

We would like to thank Atsuko Katsuta for technical assistance with isolation and preservation of the strain and Dr Ritsuko Katahira for LCMS analysis of quinones. We are grateful to Dr J. P. Euzéby (Ecole Nationale Vétérinaire, Toulouse, France) for his support with nomenclatural problems. This work was supported in part by a research grant (2009–2011) from the Institute for Fermentation, Osaka, Japan.

**References**


