Demequina globuliformis sp. nov., Demequina oxidasica sp. nov. and Demequina aurantiaca sp. nov., actinobacteria isolated from marine environments, and proposal of Demequinaceae fam. nov.

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Three Gram-stain-positive actinobacterial strains, designated YM24-125T, YM05-1041T and YM12-102T, were isolated from marine environments. Phylogenetic analysis based on 16S rRNA gene sequences indicated that they belonged to the suborder Micrococcineae and were most closely related to members of the genus Demequina with 95.0–98.4 % sequence similarities. Cells of strains YM05-1041T and YM12-102T were rod-shaped, similar to members of the genus Demequina, while cells of strain YM24-125T were cocci to short ovoid rods. All strains grew in the absence of NaCl, but tolerated up to 15 % NaCl. The major menaquinone was demethylmenaquinone DMK-9 (H4) and the major polar lipids were phosphatidylinositol, diphosphatidylglycerol and phosphatidylglycerol. All strains contained straight-chain, iso-branched and anteiso-branched saturated fatty acids. The DNA G+C contents were 65.3–67.3 mol%. The peptidoglycans of strains YM05-1041T and YM12-102T were the A4b type with ornithine while that of strain YM24-125T contained ornithine and lysine as diamino acids. Phylogenetic analyses and differences in physiological and biochemical characteristics indicated that these three isolates represent novel species within the genus Demequina, for which the names Demequina globuliformis sp. nov. (with YM24-125T =NBRC 106265T =KCTC 19747T =MBIC 08349T as the type strain), Demequina oxidasica sp. nov. (with YM05-1041T =NBRC 106264T =KCTC 19746T =MBIC 08346T as the type strain) and Demequina aurantiaca sp. nov. (with YM12-102T =NBRC 106265T =KCTC 19745T =MBIC 08347T as the type strain) are proposed. Based on differences in the quinone system, the presence of demethylmenaquinone DMK-9(H4) and the pattern of 16S rRNA signatures, the three novel strains and current members of the genus Demequina represent a unique family within the suborder Micrococcineae. Therefore, the family Demequinaceae fam. nov. is also proposed.

The genus Demequina was proposed by Yi et al. (2007) and, at the time of writing, consists of three species: Demequina aestuarii JCM 12123T, isolated from a tidal flat sediment (Yi et al., 2007), Demequina lutea DSM 19970T, isolated from a permafrost soil (Finster et al., 2009) and Demequina salines-moris DSM 22060T, isolated from mangrove soil (Matsumoto et al., 2010). The genus Demequina is characterized by the presence of demethylmenaquinone in the quinone system and belongs to the family Cellulomonadaceae within the suborder Micrococcineae, order Actinomycetales of the subclass Actinobacteria (Zhi et al., 2009).

Actinobacteria are considered treasure troves of bioactive secondary metabolites. However, the identities and diversity of actinobacteria in marine environments remain...
Three novel species of *Demequina* gen. nov

poorly resolved. Three strains were isolated during screening of a library of isolates from different habitats in marine environments. Strain YM24-125\textsuperscript{T} was isolated from sea sand collected on Shimohisage (Ehime Prefecture, Japan) using H medium (Supplementary Method, available in IJSEM Online), strain YM05-1041\textsuperscript{T} was isolated from *Zostera marina* Linnaeus on Charatsnai beach in Muroran City (Hokkaido, Japan) using 1/10 MA2216 medium (Difco), while strain YM12-102\textsuperscript{T} was isolated from sea alga collected on Lake Hamana (Shizuoka Prefecture, Japan) using HSV medium (Kageyama et al., 2008). The source homogenate was cultivated at 25 °C for 30 days for isolation of all strains. We elucidated the phylogenetic positions of strains YM24-125\textsuperscript{T}, YM05-1041\textsuperscript{T} and YM12-102\textsuperscript{T} based on 16S rRNA gene sequence. We performed physiological, biochemical and chemotaxonomic analyses to characterize the three novel isolates, which are proposed to represent novel species of the genus *Demequina*. Additionally, with the inclusion of these isolates, we propose the family *Demequinaceae* fam. nov. within the suborder *Micrococccaceae*

The three strains grew well on Luria Bertani (LB) agar (Difco), LB agar supplemented with 2 % (w/v) NaCl (final concentration) and on MA 2216 medium (Difco). The strains were maintained on LB agar supplemented with 2 % NaCl at 30 °C, which was also used for all phenotypic assays unless otherwise stated.

Morphological and physiological tests were performed as follows. Gram-staining was performed using a Favor G Nissui kit (Nissui Pharmaceutical). Cellular morphology was observed using light microscope (ECLIPSE E600; Nikon). Motility was determined using API M medium (bioMérieux). Anaerobic growth was examined using a disposable anaerobic chamber (Anaeromate-P; Nissui Pharmaceutical). The temperature range for growth was evaluated on the basis of colony formation on LB agar plates supplemented with 2 % NaCl incubated at 4, 5, 10, 15, 20, 25, 28, 35, 37, 42, 45 and 50 °C. The pH range for growth was evaluated using agar plates with pH ranging from 5 to 11 in 0.5 pH unit increments. To test salt tolerance, LB agar containing various concentrations of NaCl (1.5, 2, 5, 8, 10, 15 and 20 %, w/v) was used to evaluate growth. Catalase and oxidase activities were determined by bubble formation in a 3 % H\textsubscript{2}O\textsubscript{2} solution and using cytochrome oxidase test paper (Nissui Pharmaceutical), respectively. Nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase, aesculin hydrolysis and the fermentation of glucose were tested in duplicate using the API 20NE kit (bioMérieux) according to the manufacturer’s instructions, with the exception that cell suspensions were supplemented with 2 % NaCl (w/v). Several other enzyme activities and acid production were assayed in duplicate using the API ZYM and API 50CH kits (bioMérieux), respectively. API 50CH and API ZYM tests were evaluated after 48 h of incubation at 30 °C and 24 h of incubation at 30 °C, respectively. Decomposition of adenine, L-tyrosine, casein, hypoxanthine and xanthine was tested according to Gordon et al. (1974) after 3 weeks of incubation at 30 °C. Carbon utilization was assayed using ISP medium 9 (Difco) supplemented with NaCl (2 %, w/v) and 1 % carbon source after 3 weeks of incubation as described by Pridham & Gottlieb (1948). As carbon sources, D-ribose, D-arabinose, D-mannose, D-xylene, D-glucose, D-fructose, D-rhamnose, maltose monohydrate, sucrose and D-mannitol were used. Production of H\textsubscript{2}S was tested using ISP 6 medium (Difco). Menaquiones were extracted using the procedure described by Collins (1985) and analysed by LC-MS as described by Ogiso et al. (2008) without the addition of phosphoric acid to the elution buffer. Polar lipids were extracted using the procedure described by Minnikin et al. (1984) and analysed by LC-MS. Molecular species were identified from retention times and mass spectra of LC-MS. Cellular fatty acids were identified with the Sherlock Microbial Identification System (MIDI) after strains were grown on tryptic soy agar (TSA; Becton Dickinson) for 3 days at 30 °C. Genomic DNA was prepared according to the method of Suzuki et al. (1999) and the DNA G+C content was quantified by HPLC analysis using a DNA-GC kit (Yamasita, Chiba, Japan). Cell walls were prepared by the methods described by Schleifer & Kandler (1972) and amino acids in the acid hydrolysates of the cell walls were identified by TLC (Harper & Davis, 1979), and by HPLC as their phenylthiocarbamoyl derivatives (Yokota et al., 1993). DNA–DNA hybridizations were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki et al. (1989). Hybridizations were performed using five replications and after the highest and lowest value for each sample were excluded, the mean was calculated and reported as the DNA–DNA relatedness value.

An approximately 1500 bp fragment of the 16S rRNA gene was amplifed from extracted DNA using universal primers 27F and 1492R (Escherichia coli numbering system; Brosius et al., 1978) and sequenced. Sequence similarity searches were performed using GenBank BLASTN and the EzTaxon server (http://www.eztaxon.org; Chun et al., 2007). Sequences of closely related taxa were retrieved and aligned using the CLUSTAL X program (Thompson et al., 1983) and, if necessary, the alignment was corrected manually. Phylogenetic analysis was performed using three tree-making algorithms, namely neighbour-joining (NJ; Saitou & Nei, 1987) for ML tree was calculated by PHYML (version 2.4.4; Guindon & Gascuel, 2003) for ML. The ML tree was calculated by using GTR (gamma distribution and invariable sites) as the substitution model. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. Similarity values were calculated using MEGA 4.0.2 software. Signature nucleotides present within 16S rRNA genes were identified using the identical sequences aligned for the phylogenetic
The positions of the identified signature nucleotides on the secondary structure of the *E. coli* 16S rRNA molecule were then determined (Brosius et al., 1978).

The morphological, physiological, and biochemical characteristics of strains YM24-125^T^, YM05-1041^T^, and YM12-102^T^ are included in the species descriptions. The phylogenetic tree generated, which was based on maximum-likelihood-, neighbour-joining- and maximum-parsimony-generated comparisons of the 16S rRNA gene sequences, revealed that the three strains belonged to the suborder *Micrococcineae* within the order *Actinomycetales* (Fig. 1). The strains were most closely related to the genus *Demequina* (95.0–98.4% similarities), with YM24-125^T^, YM05-1041^T^ and YM12-102^T^ showing similarity to *D. aestuarii* (96.3–97.0%), *D. lutea* (95.0–96.5%) and *D. salsinemoris* (96.9–98.4%). The 16S rRNA gene sequence similarities among the three isolates and the known species of the genus *Demequina* are shown in Supplementary Table S1.

The major polar lipids of the three isolates were phosphatidylglycerol, diphosphatidylglycerol and phosphatidylinositol (Supplementary Fig. S1). All strains contained...
Three novel species of Demequina gen. nov

straight-chain saturated, iso-branched and anteiso-branched saturated fatty acids with anteiso-C_{15:0} (52.06–55.77 %), anteiso-C_{17:0} (7.58–9.8 %) and C_{16:0} (8.27–18.33 %) representing the major fatty acids (Supplementary Table S2). The fatty acid profiles of the isolates discriminated them from the other members of the genus Demequina. The DNA G+C content was 65.3–67.3 mol%. The peptidoglycan of strains YM05-1041^T and YM12-102^T was of the A4 type with Orn as the diamino acid, while strain YM24-125^T contained Orn and Lys as diamino acids. Although the cells of strains YM05-1041^T and YM12-102^T were rods similar to those of the type species of the genus Demequina, the cells of strain YM24-125^T were cocci to short ovoid rods (Supplementary Fig. S2). The differences in physiological and biochemical characteristics among these isolates (YM24-125^T, YM05-1041^T and YM12-102^T), D. aestuarii JCM 12123^T, D. lutea DSM 19970^T and D. salsinemoris DSM 22060^T are summarized in Table 1. Based on the phenotypic characteristics, the three isolates could be distinguished from each other and the other taxa of the genus Demequina. For further verification of the taxonomic positions of YM24-125^T, YM05-1041^T and YM12-102^T, DNA–DNA hybridizations were performed with the members of the genus Demequina with a 16S rRNA gene sequence dissimilarity below 3 %. Strain YM24-125^T exhibited relatively low DNA–DNA relatedness values with respect to D. salsinemoris DSM 22060^T (10.2 %). Strains YM05-1041^T and YM12-102^T exhibited relatively low DNA–DNA relatedness values with D. aestuarii JCM 12123^T (3.0 % and 7.3 %) and D. salsinemoris DSM 22060^T (5.7 % and 5.8 %), indicating that they are different at the species level (Wayne et al., 1987). Strains YM05-1041^T and YM12-102^T formed a single cluster in the phylogenetic tree (Fig. 1) and were 99.6 % similar to each other (Supplementary Table S1). DNA–DNA hybridization experiments showed low values (50.6 %) for relatedness between strains YM05-1041^T and YM12-102^T. There were also differences in oxidase activity and growth under anaerobic conditions between these strains. These observations support an affiliation of strains YM05-1041^T and YM12-102^T to different species. Comparison of the characteristics of the genus Demequina with those of the nearest genera are summarized in Supplementary Table S3. Based on the results of phylogenetic analyses and biochemical and physiological properties, the bacterial isolates examined here represent three novel species of the genus Demequina. The names Demequina globiformis sp. nov. (type strain YM24-125^T), Demequina oxidascia sp. nov. (type strain YM05-1041^T) and Demequina aurantiaca sp. nov. (type strain YM12-102^T) are proposed.

Phylogenetic analyses inferred from ML, NJ and MP based on 16S rRNA gene sequences revealed that the six strains representing the genus Demequina (three novel isolates and three previously described species) formed a distinct monophyletic clade with 100 % bootstrap support, forming a deep branch, clearly distinguished from other families within the suborder Micrococccinae (Fig. 1). 16S rRNA gene sequence similarities between these six strains and the type species in the neighbouring families were 93.3–94.4 % for Sanguibacter keddiic ST-74^T in the family Sanguibacteraceae and 91.4–92.2 % for Cellulomonas flavigena NCIMB 8073^T in the family Cellulomonadaceae. The 16S rRNA gene sequence similarities between the six strains and the type species of neighbouring genera unassigned to a family within the suborder Micrococccinae were 93.3–94.8 % for Actinotalea fermentans DSM 3133^T, 92.2–92.9 % for Luteimicrobium subarcticum DSM 22413^T and 92.1–93.4 % for Paraeroskvia marina DSM 21750^T. The patterns of 16S rRNA signature nucleotides for the six strains do not match those of any of the 15 known families within the suborder Micrococccinae (Supplementary Table S4). In addition, the major quinone of the six strains is demethylmenaquinone DMK-9 (H_4), which is distinct from all other families of the suborder Micrococccinae. Based on these different parameters we propose that the six strains belong to a new family for which we propose the name Demeinquaceae fam. nov.

Emended description of the genus Demequina Yi et al. 2007

The description is based on that given for Demequina aestuarii by Yi et al. (2007).

Some species have coccioid-shaped cells and ferment glucose.

Description of Demequina globiformis sp. nov.

Demequina globiformis (glo.bu.li.for.mis. L. dim. n. globulus a small sphere, globule; L. adj. suffix -formis -is -e (from L. n. forma figure, shape, appearance), -like in the shape of; N.L. masc. adj. globuliformis globe-like).

Gram-stain-positive, non-motile, non-spore-forming, facultatively anaerobic, oxidase and catalase-positive. Cells are cocci to short ovoid rods, 0.50–1.00 μm in diameter or 0.50–1.00 μm in length and 0.50–0.95 μm in width. Colonies are cream–yellow on LB plates. H_2S is not produced. Indole production and hydrolysis of starch and gelatin are negative. Hydrolysis of aesculin is positive. Negative for arginine dihydrolase and urease. The temperature range for growth is 4–37 °C with optimum growth occurring at 30 °C. The pH range for growth is 5–11 with optimum growth occurring between pH 7.5 and 8. Based on API ZYM, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase, but negative for lipase (C14), trypsin, α-mannosidase and α-fucosidase. Decomposition of adenine, L-tyrosine, casein, hypoxanthine and xanthine is negative. Can also utilize L-arabinose, D-mannose, D-xylene, D-glucose, D-fructose, L-rhamnose, maltose monohydrate, glucose and sucrose as sole carbon source, but cannot utilize D-mannitol. Acid is produced from L-arabinose, D-xylene, galactose,
Table 1. Different characteristics of the novel strains and the type strains of three species of the genus *Demequina*

Data from this study unless indicated. +, Positive; −, negative; w, weak reaction; ND, no data available. FAN, Facultive anaerobe; SA, strict aerobe; PI, phosphatidylinositol; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>YM24-125&lt;sup&gt;T&lt;/sup&gt;</th>
<th>YM05-1041&lt;sup&gt;T&lt;/sup&gt;</th>
<th>YM12-102&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Demequina aestuarii</em></th>
<th><em>Demequina lutea</em>&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>Demequina salinemoris</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Sea sand</td>
<td>Zostera marina Linnaeus</td>
<td>Seaweed Tidal flat sediment</td>
<td>Permafrost soil Mangrove soil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colony morphology</td>
<td>Cocci, short ovoid rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
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<tr>
<td>Oxidase production</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>−</td>
<td>−</td>
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<td>NaCl tolerance (%)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>2</td>
<td>8</td>
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<tr>
<td>Growth at 4 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Growth at 37 °C</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<td>Fermentation of glucose</td>
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<td>+</td>
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<td>Enzyme production</td>
<td>Alkaline phosphatase</td>
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<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>Esterase(C4)</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>−</td>
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<tr>
<td>Esterase lipase(C8)</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Cystine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Chymotrypsin</td>
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<td>Acid phosphatase</td>
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<td>Naphthol-AS-BI-</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>−</td>
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<td>phosphohydrolase</td>
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<td>N-Acetyl-β-glucosaminidase</td>
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<td>Major menaquinone</td>
<td>DMK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>DMK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>DMK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>DMK-9(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DMK-9(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DMK-9(H&lt;sub&gt;4&lt;/sub&gt;)&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Minor menaquinones</td>
<td>DMK-8(H&lt;sub&gt;4&lt;/sub&gt;), DMK-9(H&lt;sub&gt;2&lt;/sub&gt;), DMK-9(H&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>DMK-8(H&lt;sub&gt;4&lt;/sub&gt;), DMK-9(H&lt;sub&gt;2&lt;/sub&gt;), DMK-9(H&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>DMK-8(H&lt;sub&gt;4&lt;/sub&gt;), DMK-9(H&lt;sub&gt;2&lt;/sub&gt;), DMK-9(H&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>−</td>
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<td>−</td>
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<tr>
<td>Major polar lipids</td>
<td>PI, DPG, PG</td>
<td>PI, DPG, PG</td>
<td>PI, DPG, PG</td>
<td>PI, DPG, PG</td>
<td>PI, DPG, PG</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt; Unknown phospholipids&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Cell wall amino acids</td>
<td>Orn, Lys</td>
<td>Orn</td>
<td>Orn</td>
<td>Orn&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Orn&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Orn&lt;sup&gt;c&lt;/sup&gt;</td>
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Table 1. cont.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>YM24-125T</th>
<th>YM05-1041T</th>
<th>YM12-102T</th>
<th>Demequina aestuarii</th>
<th>Demequina lutea DSM 19970T</th>
<th>Demequina salinisemoris DSM 22060T</th>
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<tr>
<td>Other amino acids</td>
<td>Glu, Ser, Ala</td>
<td>Glu, Ser, Ala, Asp</td>
<td>Glu, Ser, Ala, Asp</td>
<td>Glu, Ser, Ala, Asp, Gly</td>
<td>Glu, Ser, Ala, Asp</td>
<td>Glu, Ser, Ala, Asp</td>
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<td>DNA G + C content (mol%)</td>
<td>67.3</td>
<td>65.3</td>
<td>65.4</td>
<td>67a</td>
<td>66.2b</td>
<td>70-72c</td>
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*Data taken from: a, Yi et al. (2007); b, Finster et al. (2009); c, Matsumoto et al. (2010).*

glucose, fructose, mannose, rhamnose, N-acetylgluco-amine, amygdalin, arbutin, asesulin, salicin, cellobiose, maltose, lactose, sucrose, trehalose and gentiobiose. Acid is not produced from erythritol, D-arabinose, ribose, L-xylose, adonitol, β-methyl D-xylolside, sorbose, dulcitol, myo-inositol, mannitol, sorbitol, α-methyl D-mannoside, α-methyl D-glucoside, melibiose, inulin, melizitose, raffinose, starch, glycon, xylitol, turanose, D-lyxose, D-tagatose, L-fucose, D- and L-arabitol, glucorona, 2-ketogluconate or 5-ketogluconate. Acid is weakly produced from glycerol. The predominant cellular fatty acids are of the anteiso-branched and straight-chain types. The fatty acids (>1%) comprise C14:0, C15:0, C16:0, C17:0, iso-C14:0, iso-C15:0, iso-C16:0, anteiso-C15:0 and anteiso-C17:0. The components of the peptidoglycan are Lys, Orn, Ala, Ser and Glu in a molar ratio of 0.3 : 0.4 : 1.1 : 1.8 : 1.0. The major menaquinone is demethylmenaquinone DMK-9(H4) and the minor menaquinones are DMK-8(H4), DMK-9(H2) and DMK-9(H6). Nitrate reduction to nitrite is negative. Fermentation of glucose is positive. Can grow in the presence of 15% sodium chloride. The major polar lipids are phosphatidylinositol, diphosphatidylglycerol and phosphatidylglycerol; unknown compounds were also detected. The G+C content of genomic DNA of the type strain is 67.3 mol%.

The type strain is YM24-125T (=NBRC 106266T =KCTC 19747T =MBIC 08349T), isolated from sea sand.

**Description of Demequina oxidasica sp. nov.**

*Demequina oxidasica* [o.xi.da’si.ca. N.L. neut. n. oxidasum oxidase; L. suff. -icus -a -um suffix used in adjectives with the sense of belonging to, pertaining to; N.L. fem. adj. oxidasica pertaining to oxidase (pertaining to the ability of this species to produce oxidase)].

Cells are non-motile, Gram-stain-positive, short rods, 0.3–0.7 μm in width and 0.6–1.5 μm in length. Colonies are yellow on LB plates. Temperature range for growth is 4–30 °C with optimum growth occurring between 28 and 30 °C. The pH range for growth is 6–10 with optimum growth occurring at pH 6.5. Cells grow optimally in the absence of salt but tolerate up to 15% NaCl (w/v). Cells are obligately aerobic and oxidase- and catalase-positive. Nitrate reduction to nitrite is negative and H2S is not produced.

Cells are negative for arginine dihydrolase, urease and indole production. Hydrolysis of starch and gelatin is negative and hydrolysis of ascinin is positive. Fermentation of glucose is negative. Based on API ZYM, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucosidase and β-glucosidase, but negative for urease, lipase (C14), N-acetyl-β-glucosaminidase, trypsin, chymotrypsin, α-mannosidase and x-fucosidase. Decomposition of adenine, L-tyrosine, casein, hypoxanthine and xanthine is negative. Weakly positive for β-galactosidase and β-glucuronidase. Can utilize L-arabinose, D-mannose, D-xylolose, D-glucose, D-fructose, L-rhamnose, maltose monohydrate, glucose and sucrose as sole carbon source but cannot utilize D-mannitol. Acid is produced from L-arabinose, D-xylolose, galactose, glucose, fructose, salicin, cellobiose and maltose. Acid is not produced from glycerol, erythritol, D-arabinose, ribose, L-xylene, adonitol, β-methyl D-xylolside, rhamnose, mannitol, amygdalin, arbutin, lactose, melibiose, sucrose, trehalose, starch, gentibiose, D-turanose sorbose, dulcitol, myo-inositol, sorbitol, α-methyl D-mannoside, α-methyl D-glucoside, N-acetylgluconosamine, inulin, melizitose, raffinose, glycogen, xylitol, D-lyxose, D-tagatose, D- and L-fucrose, D-arabitol, glucorona, 2-ketogluconate or 5-ketogluconate. The major menaquinone is demethylmenaquinone DMK-9(H4) and the minor menaquinones are DMK-8(H4) and DMK-9(H6).

The type strain is YM05-1041T (=NBRC 106264T =KCTC 19746T =MBIC 08346T), isolated from *Zostera marina* Linnaeus.

**Description of Demequina aurantiaca sp. nov.**

*Demequina aurantiaca* (au.ran.ti.a’ca. N.L. fem adj. aurantiaca orange-coloured).
Cells are non-motile, Gram-stain-positive, short rods, 0.2–0.6 μm in width and 0.3–1.2 μm in length. Colonies are orange on LB plates. Temperature range for growth is 4–30 °C with optimum growth at 28–30 °C. The pH range for growth is 6–9 with optimum growth at pH 6–6.5. Cells grow optimally in the absence of salt but tolerate up to 15% NaCl (w/v). Cells are facultatively anaerobic, urease negative, oxidase-negative and catalase-positive. Nitrate reduction to nitrite is negative, H2S is not produced and methyl red test is positive. Hydrolysis of starch and gelatin is negative, but hydrolysis of asucin is positive. Fermentation of glucose is negative. Based on API ZYM, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, x-galactosidase, β-galactosidase, β-glucosidase and β-glucosidase, but negative for lipase (C14), trypsin, chymotrypsin, β-glucuronidase, N-acetyl-β-glucosaminidase, x-mannosidase and x-fucosidase. Decomposition of adenine, L-tyrosine, casein, hypoxanthine and xanthine is negative. Can also utilize L-arabinose, D-mannose, D-xylose, galactose D-glucose, D-fructose, L-rhamnose, maltose monohydrate and sucrose as sole carbon source, but cannot utilize D-mannitol. Acid is produced from glycerol, L-arabinose, D-xylose, glucose, fructose, mannose, rhamnose aesculin, salicin, cellobiose, malate, sucrose, starch, gentiobiose and D-lyxose. Acid is not produced from erythritol, D-arabinose, ribose, L-xylose, adonitol, methyl-β-D-xyloside, sorbose, dulcitol, myo-inositol, mannitol, sorbitol, methyl-β-D-mannoside, methyl-β-D-glucoside, N-acetylglucosamine, amygdalin, arbutin, lactose, melibiose, trehalose, inulin, melezitose, raffinose, glycogen, xylitol, turanose, D-tagatose, D- and L-fucose, D- and L-arabitol, glucose, 2-ketogluconate or 5-ketogluconate. The major menaquinone is demethylmenaquinone DMK-9(H4) and the minor menaquinones are DMK-8(H4) and DMK-9(H6). The type genus is Demequina demethylmenaquinone DMK-9(H4). The type strain is YM12-102T (NBRC 106265T =KCTC 19745T =MBIC 08347T), isolated from seaweed.

Description of Demequinaeae fam. nov.

Demequinaeae (De.me.qui.na’ce.a.e. N.L. fem. n. Demequina type genus of the family, -aceae ending to denote a family: N.L. fem. pl. n. Demequinaeae the Demequina family). On the basis of 16S rRNA gene sequence analysis and combinations of genotypic, phenotypic and chemotaxonomic characteristics, the family is a member of the suborder Micrococccaeae with the following nucleotide sequence characteristics: 120 (A), 131 : 231 (C-G), 196 (A), 342 : 347 (C-G), 444 : 490 (A-U), 580 : 761 (C-G), 602 : 636 (C-G), 670 : 736 (A-U), 822 : 878 (G-C), 823 : 877 (G-C), 826 : 874 (C-G), 827 (U), 843 (U), 950 : 1231 (U-A), 1047 : 1210 (G-C), 1109 (C), 1145 (G), 1309 : 1328 (G-C), 1361 (G) and 1383 (C). The predominant quinone is demethylmenaquinone DMK-9(H4). The type genus is Demequina.

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References


