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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 YM24-125^T, YM05-1041^T and YM12-102^T, 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 YM05-1041^T and YM12-102^T were rod-shaped, similar to members of the genus *Demequina*, while cells of strain YM24-125^T 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 (H_4) and the major polar lipids were phosphatidylinositol, diphosphatidylglycerol and phosphatidyglycerol. 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 YM05-1041^T and YM12-102^T were the A4β type with ornithine while that of strain YM24-125^T 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 YM24-125^T =NBRC 106266^T =KCTC 19747^T =MBIC 08349^T as the type strain), *Demequina oxidasica* sp. nov. (with YM05-1041^T =NBRC 106264^T =KCTC 19746^T =MBIC 08346^T as the type strain) and *Demequina aurantiaca* sp. nov. (with YM12-102^T =NBRC 106265^T =KCTC 19745^T =MBIC 08347^T as the type strain) are proposed. Based on differences in the quinone system, the presence of demethylmenaquinone DMK-9(H_4) 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 12123^T, isolated from a tidal flat sediment (Yi *et al.*, 2007), *Demequina lutea* DSM 19970^T, isolated from a permafrost soil (Finster *et al.*, 2009) and *Demequina salsinemoris* DSM 22060^T, 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 *Actinobacteridae* (Zhi *et al.*, 2009).

Actinobacteria are considered treasure troves of bioactive secondary metabolites. However, the identities and diversity of actinobacteria in marine environments remain

Abbreviations: ML, maximum likelihood; MP, maximum parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YM24-125^T, YM05-1041^T and YM12-102^T are AB522643, AB522640 and AB522641, respectively.

Two supplementary figures and four supplementary tables and a supplementary method are available with the online version of this paper.

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poorly resolved. Three strains were isolated during screening of a library of isolates from different habitats in marine environments. Strain YM24-125^T was isolated from sea sand collected on Shimohisage (Ehime Prefecture, Japan) using H medium (Supplementary Method, available in IJSEM Online), strain YM05-1041^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^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^T, YM05-1041^T and YM12-102^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 *Micrococcineae*.

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 microscopy (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_2O_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, L-arabinose, D-mannose, D-xylose, D-glucose, D-fructose, L-rhamnose, maltose monohydrate, sucrose and D-mannitol were used. Production of H_2S was tested using ISP 6 medium (Difco). Menaquinones 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 (Yamasa, 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 amplified 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) from K_{nuc} values (Kimura, 1983), maximum-parsimony (MP; Fitch, 1971) and maximum-likelihood (ML; Felsenstein, 1981). Phylogenetic analyses were carried out by using MEGA version 4.0.2 (Tamura *et al.*, 2007) for NJ and MP, and 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

analysis. 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 phosphatidylinositol, diphosphatidylglycerol and phosphatidyglycerol (Supplementary Fig. S1). All strains contained

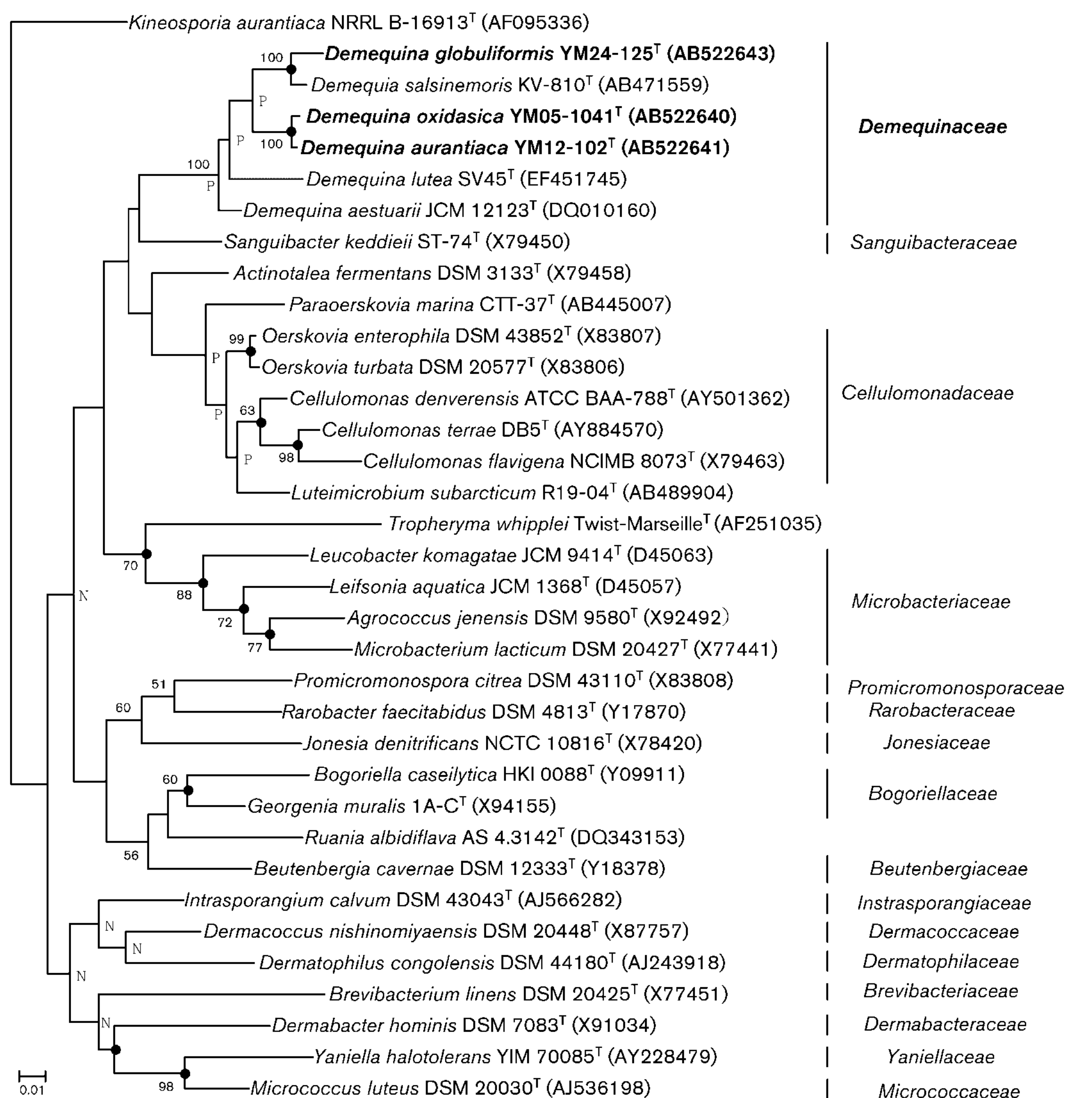

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences showing relationships between the strains studied and representatives of all families in the suborder *Micrococcineae*. Only bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at nodes. 'N' and 'P' indicate branches that were also recovered by using the neighbour-joining and maximum-parsimony treeing algorithms, respectively. Filled circles indicate branches recovered with all three mehods. Numbers in parentheses are GenBank accession numbers. Sequences determined in this study are shown in bold. The sequence of *Kineosporia aurantiaca* NRRL B-16913^T was used as an outgroup reference. Bar, 0.01 nucleotide substitutions per site.

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 A4β 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 globuliformis* sp. nov. (type strain YM24-125^T), *Demequina oxidasica* 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 *Micrococcineae* (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 keddieii* 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 *Micrococcineae* 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 *Paraoerskovia 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 *Micrococcineae* (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 *Micrococcaceae*. Based on these different parameters we propose that the six strains belong to a new family for which we propose the name *Demequinaceae* 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 coccoid-shaped cells and ferment glucose.

Description of *Demequina globuliformis* sp. nov.

Demequina globuliformis (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* globule-shaped).

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-xylose, 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-xylose, 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.

Characteristic	YM24-125^T	YM05-1041^T	YM12-102^T	*Demequina aestuarii* JCM 12123^T	*Demequina lutea* DSM 19970^T	*Demequina salsinemoris* DSM 22060^T
Isolation source	Sea sand	*Zostera marina* Linnaeus	Seaweed	Tidal flat sediment	Permafrost soil	Mangrove soil
Cell morphology	Cocci, short ovoid rod	Rod	Rod	Rod	Rod	Rod
Colony colour	Cream–yellow	Yellow	Orange	Yellow	Yellow	Pale yellow
Oxidase production	+	+	−	−	−	−
Oxygen sensitivity	FAN	SA	FAN	FAN	FAN	SA
NaCl tolerance (%)	15	15	15	15	2	8
Growth at 4 °C	+	+	+	+	+	−
Growth at 37 °C	+	−	−	−	+	−
Fermentation of glucose	+	−	−	−	−	+
Production of acid from:						
Glycerol	W	−	+	−	−	−
D-Arabinose	−	−	−	+	−	−
β-Methyl D-xyloside	−	−	−	−	W	+
Galactose	+	+	+	+	W	+
Mannose	+	−	+	+	+	+
Rhamnose	+	−	+	−	−	+
N-Acetylglucosamine	+	−	−	−	−	−
Amygdalin	+	−	−	+	−	+
Arbutin	+	−	−	+	−	+
Cellobiose	+	+	+	+	+	+
Maltose	+	+	+	W	+	+
Lactose	+	−	−	W	−	−
Sucrose	+	−	+	+	+	−
Trehalose	+	−	−	W	+	−
Starch	−	−	+	−	−	−
Gentiobiose	+	−	+	+	−	+
D-Lyxose	−	−	+	+	−	−
Enzyme production						
Alkaline phosphatase	+	+	+	−	−	−
Esterase(C4)	+	+	+	+	−	W
Esterase lipase(C8)	+	+	+	+	+	−
Valine arylamidase	+	+	+	+	−	−
Cystine arylamidase	+	+	+	+	−	−
Chymotrypsin	+	−	−	+	−	−
Acid phosphatase	+	+	+	+	+	−
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	−	+
α-Galactosidase	+	+	+	−	+	+
β-Galactosidase	+	W	+	+	+	+
β-Glucuronidase	+	W	−	−	−	−
N-Acetyl-*β*-glucosaminidase	+	−	−	−	−	−
Major menaquinone	DMK-9(H_4)	DMK-9(H_4)	DMK-9(H_4)	DMK-9(H_4)[a*]	DMK-9(H_4)[b]	DMK-9(H_4)[c]
Minor menaquinones	DMK-8(H_4), DMK-9(H_2), DMK-9(H_6)	DMK-8(H_4), DMK-9(H_6)	DMK-8(H_4), DMK-9(H_6)	−	−	−
Major polar lipids	PI, DPG, PG	PI, DPG, PG	PI, DPG, PG	PI, DPG, PG	ND[b]	Unknown phospholipids[c]
Cell wall amino acids						
Diamino acid	Orn, Lys	Orn	Orn	Orn[a]	Orn[b]	Orn[c]

Table 1. cont.

Characteristic	YM24-125^T	YM05-1041^T	YM12-102^T	*Demequina aestuarii* JCM 12123^T	*Demequina lutea* DSM 19970^T	*Demequina salsinemoris* DSM 22060^T
Other amino acids	Glu, Ser, Ala	Glu, Ser, Ala, Asp	Glu, Ser, Ala, Asp	Glu, Ser, Ala, Asp, Gly[a]	Glu, Ser, Ala, Asp[b]	Glu, Ser, Ala, Asp[c]
DNA G+C content (mol%)	67.3	65.3	65.4	67[a]	66.2[b]	70-72[c]

*Data taken from: *a*, Yi *et al.* (2007); *b*, Finster *et al.* (2009); *c*, Matsumoto *et al.* (2010).

glucose, fructose, mannose, rhamnose, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, sucrose, trehalose and gentiobiose. Acid is not produced from erythritol, D-arabinose, ribose, L-xylose, adonitol, β-methyl D-xyloside, sorbose, dulcitol, *myo*-inositol, mannitol, sorbitol, α-methyl D-mannoside, α-methyl D-glucoside, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, L-fucose, D- and L-arabitol, gluconate, 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 $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, iso-$C_{14:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. 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(H_4) and the minor menaquinones are DMK-8(H_4), DMK-9(H_2) and DMK-9(H_6). 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 YM24-125^T (=NBRC 106266^T =KCTC 19747^T =MBIC 08349^T), 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 H_2S is not produced. Cells are negative for arginine dihydrolase, urease and indole production. Hydrolysis of starch and gelatin is negative and hydrolysis of aesculin 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, α-galactosidase, α-glucosidase and β-glucosidase, but negative for urease, lipase (C14), *N*-acetyl-β-glucosaminidase, trypsin, chymotrypsin, α-mannosidase and α-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-xylose, 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-xylose, galactose, glucose, fructose, salicin, cellobiose and maltose. Acid is not produced from glycerol, erythritol, D-arabinose, ribose, L-xylose, adonitol, β-methyl D-xyloside, mannose, 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*-acetylglucosamine, inulin, melezitose, raffinose, glycogen, xylitol, D-lyxose, D-tagatose, D- and L-fucose, D-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. The major menaquinone is demethylmenaquinone DMK-9(H_4) and the minor menaquinones are DMK-8(H_4) and DMK-9(H_6). The fatty acids (>1 %) comprise $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, iso-$C_{14:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The cell-wall peptidoglycan contains Orn, Ala, Ser, Glu and Asp in a molar ratio of 0.4 : 1.9 : 0.6 : 1.0 : 0.3. The polar lipids are phosphatidylinositol, diphosphatidylglycerol and phosphatidylglycerol; an unknown compound is also detected. The DNA G+C content of genomic DNA of the type strain is 65.3 mol%.

The type strain is YM05-1041^T (=NBRC 106264^T =KCTC 19746^T =MBIC 08346^T), 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, H_2S is not produced and arginine dihydrolase and indole production are negative. Hydrolysis of starch and gelatin is negative, but hydrolysis of aesculin 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, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase, but negative for lipase (C14), trypsin, chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-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, maltose, 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-α-D-mannoside, methyl-α-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, gluconate, 2-ketogluconate or 5-ketogluconate. The major menaquinone is demethylmenaquinone DMK-9(H_4) and the minor menaquinones are DMK-8(H_4) and DMK-9(H_6). The fatty acids ($>1\%$) comprise $C_{10:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, iso-$C_{14:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The cell-wall peptidoglycan contains Orn, Ala, Ser, Glu and Asp in a molar ratio of 0.8 : 2.1 : 1.1 : 1.0 : 0.6. The major polar lipids are phosphatidylinositol, diphosphatidylglycerol and phosphatidylglycerol; unknown compounds were also detected. The DNA G+C content of genomic DNA of the type strain is 65.4 mol%.

The type strain is YM12-102^T (NBRC 106265^T =KCTC 19745^T =MBIC 08347^T), isolated from seaweed.

Description of *Demequinaceae* fam. nov.

Demequinaceae (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. *Demequinaceae* 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 *Micrococcineae* 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(H_4). The type genus is *Demequina*.

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