Marinococcus luteus sp. nov., a halotolerant bacterium isolated from a salt lake, and emended description of the genus *Marinococcus*

Yun Wang,^{1†} Lan-Lan Cao,^{1,2†} Shu-Kun Tang,³ Kai Lou,¹ Pei-Hong Mao,⁴ Xiang Jin,⁴ Cheng-Lin Jiang,³ Li-Hua Xu³ and Wen-Jun Li³

Correspondence

Wen-Jun Li

wjli@ynu.edu.cn or

liact@hotmail.com

¹Xinjiang Institute of Microbiology, Xinjiang Academy of Agricultural Science, Urumqi, Xinjiang 830091, PR China

²College of Prataculture and Environment Sciences, Xinjiang Agricultural University, Urumqi, Xinjiang 830052, PR China

³The Key Laboratory for Microbial Resources of the Ministry of Education, PR China, and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China

⁴Institute of Ion Beam Biotechnology, College of Physics Science and Technology, Xinjiang University, Urumqi, Xinjiang 830008, PR China

A Gram-positive, aerobic, motile, coccoid, orange-pigmented bacterium, designated strain YIM 91094^T, was isolated from a salt lake sample collected from Barkol Lake in Xinjiang Province, north-west China. The strain was able to grow at pH 6.0–8.0 (optimal growth at pH 7.0), at 10–37 °C (optimal growth at 28 °C) and in the presence of 0–25 % (w/v) NaCl [optimal growth in the presence of 10–15 % (w/v) NaCl]. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain YIM 91094^T was affiliated with the genus *Marinococcus* and exhibited levels of sequence similarity of 99.2 % to *Marinococcus halotolerans* YIM 70157^T and 99.7 % to *Marinococcus halophilus* DSM 20408^T. However, it showed moderately low levels of DNA–DNA relatedness with the above type strains (56.0 and 57.5 %, respectively). The peptidoglycan type of strain YIM 91094^T was A1 γ , with *meso*-diaminopimelic acid as the diagnostic diamino acid. MK-7 was the predominant menaquinone and anteiso-C_{15:0} (49.9 % of the total) and anteiso-C_{17:0} (29.6 %) were the major cellular fatty acids. The DNA G + C content was 48.7 mol%. Strain YIM 91094^T possessed chemotaxonomic markers that were consistent with its classification in the genus *Marinococcus*. On the basis of the data presented, strain YIM 91094^T is considered to represent a novel species of the genus *Marinococcus*, for which the name *Marinococcus luteus* sp. nov. is proposed. The type strain is YIM 91094^T (=KCTC 13214^T=CCTCC AA 208014^T). An emended description of the genus *Marinococcus* is provided.

The genus *Marinococcus* was established by Hao *et al.* (1984, 1985) with the description of two species, *Marinococcus halophilus* and *Marinococcus albus*. Two additional species have been described, *Marinococcus hispanicus* (Marquez *et al.*, 1990) and *Marinococcus halotolerans* (Li *et al.*, 2005). *M. albus* and *M. hispanicus* have been subsequently reclassified as *Salinicoccus hispanicus* (Ventosa *et al.*, 1993) and *Salimicrobium album* (Yoon *et al.*, 2007), respectively. Members of the genus *Marinococcus* are Gram-positive cocci with MK-7 as the predominant isoprenoid quinone and *meso*-diaminopimelic acid

as the diagnostic diamino acid of the cell-wall peptidoglycan. In the present study, we describe a Gram-positive, coccoid bacterium, designated strain YIM 91094^T, isolated from a salt lake in China. The isolate was considered to be closely related to members of the genus *Marinococcus* based on 16S rRNA gene sequence comparisons. The results of a polyphasic characterization of strain YIM 91094^T are presented.

Strain YIM 91094^T was isolated from a soil sample collected from Barkol Lake (GPS coordinates of the sampling site are 43° 37' 04" N 92° 46' 37" E). The surface water of Barkol Lake is highly saline, with a total salts concentration of 215–263 g l⁻¹. Salts include those of Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻, HCO₃⁻ and CO₃²⁻. A 2 g soil sample was suspended in 18 ml sterile water (10 %

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 91094^T is FJ214659.

NaCl) and mixed. Soil particles were allowed to sediment, the liquid phase was diluted 10^3 -fold and 100 μ l samples were spread onto the surface of each cultivation plate and incubated for 1 week at 37 °C. Modified ISP 5 medium was used for selective isolation, and comprised (per litre distilled water): 1 g L-asparagine, 10 g glycerol, 5 g yeast extract, 1 g K_2HPO_4 , 5 g KNO_3 , 100 g NaCl and 15 g agar. The pH of the medium was adjusted to 7.5. Colonies were picked and repeatedly restreaked onto modified ISP 5 medium until purity was confirmed. Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (approximately 150 r.p.m.) by using modified ISP 5 medium [10% (w/v) NaCl] at 28 °C for about 1 week. For analysis of fatty acid methyl esters, cell mass of strain YIM 91094^T was obtained from agar plates after incubation for 2 days on trypticase soy agar (Difco) containing 10% NaCl at 28 °C. *M. halophilus* DSM 20408^T and *M. halotolerans* YIM 70157^T were obtained from the DSMZ (the German Collection of Microorganisms and Cell Cultures) and Yunnan Institute of Microbiology (China), respectively. They were used as reference strains for DNA–DNA hybridizations and other parallel phenotypic experiments.

Morphological observations under a transmission electron microscope (model H-800; Hitachi) were performed after the strain had grown to exponential phase on modified ISP 5 medium. The Gram reaction was determined by the KOH lysis method (Cerny, 1978). Cell motility was confirmed by the presence of turbidity throughout the tube including semi-solid medium (Leifson, 1960). Methyl red and Voges–Proskauer tests were performed as described by Smibert & Krieg (1981). The temperature range for growth was determined by incubating cells for 1 week on modified ISP 5 medium at 4–55 °C. The pH growth range was investigated between pH 4.0 and 10.0 at intervals of 1 pH unit, by using the buffer system described by Xu *et al.* (2005). Tolerance to chlorides of sodium, potassium and magnesium was tested by using the same basal medium, with the above at concentrations of 0–30% (w/v). Catalase activity was determined based on production of bubbles after the addition of a drop of 3% H_2O_2 . Oxidase activity was observed by oxidation of tetramethyl-*p*-phenylenediamine. Hydrolysis of casein, starch, and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Additional metabolic properties were tested by means of the API ID 32E, API 50CH and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Strips were inoculated with a heavy bacterial suspension in a 10% salts solution.

Cells of strain YIM 91094^T were aerobic, Gram-positive cocci. Optimum growth occurred in the presence of 10–15% NaCl, at pH 7.0 and at 28 °C on modified ISP 5 medium. Growth was weak in the absence of NaCl and was not observed with only KCl or $MgCl_2 \cdot 6H_2O$. Strain YIM 91094^T was able to grow over a wide NaCl concentration range (0–25%, w/v) on modified ISP 5 medium, in contrast to the results for *M. halophilus* DSM 20408^T and

M. halotolerans YIM 70157^T. Table 1 provides a comparison of the taxonomic features of strain YIM 91094^T and the two recognized species of the genus *Marinococcus*.

The peptidoglycan was purified and cell-wall amino acids and peptides in cell-wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates by using the solvent systems of Schleifer & Kandler (1972). Cellular fatty acids were identified as described by Sasser (1990) by using the Microbial Identification System (MIDI). Menaquinones were extracted and purified according to the methods of Minnikin *et al.* (1984) and were analysed by HPLC (Kroppenstedt, 1982). Polar lipids were extracted, examined by two-dimensional TLC and identified by using standard procedures (Minnikin *et al.*, 1984).

The peptidoglycan contained *meso*-diaminopimelic acid, which is characteristic of the genus *Marinococcus*. The predominant menaquinone of strain YIM 91094^T was MK-7 (85.4%); menaquinone MK-9(H_4) (14.5%) was found as a minor component. The major polar lipids of strain YIM 91094^T were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol mannosides, phosphatidylinositol, two unknown phosphoglycerolipids and an unknown phospholipid, the same pattern as reported for *M. halophilus* DSM 20408^T. The polar lipid profile of *M. halotolerans* YIM 70157^T, by contrast, lacked phosphatidylethanolamine and an unknown phosphoglycerolipid. The cellular fatty acid profile of strain YIM 91094^T comprised anteiso- $C_{15:0}$ (49.9% of the total), anteiso- $C_{17:0}$ (29.6%), iso- $C_{15:0}$ (5.9%), $C_{16:0}$ (5.0%), iso- $C_{17:0}$ (4.4%) and iso- $C_{16:0}$ (2.6%). The above features are consistent with the chemotaxonomic description of the genus *Marinococcus*.

The phylogenetic position of strain YIM 91094^T was determined based on 16S rRNA gene sequence analysis. The 16S rRNA gene was amplified by using oligonucleotide primers complementary to highly conserved regions of bacterial 16S rRNA genes. The forward and reverse primers were 27F and 1492R (Reysenbach *et al.*, 2000). The purified PCR product was sequenced by means of the ABI PRISM BigDye Terminator cycle sequencing kit and an Applied Biosystems model 310 automatic DNA sequencer. Species related closely to strain YIM 91094^T were identified by performing sequence database searches via the BLAST program. Sequence data for related species were retrieved from the GenBank/DDBJ/EMBL database. Phylogenetic analysis was performed by using the CLUSTAL_X program (Thompson *et al.*, 1997) and the alignment was corrected manually. Phylogenetic analyses were performed with the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Nucleotide substitution rates (K_{nuc} values) were calculated and phylogenetic trees were constructed by using the neighbour-joining method (Saitou & Nei, 1987) with MEGA version 3.0 (Kumar *et al.*, 2004). The topology of the phylogenetic tree

Table 1. Differential characteristics between strain YIM 91094^T and recognized species of the genus *Marinococcus*

Taxa: 1, YIM 91094^T; 2, *M. halotolerans* YIM 70157^T; 3, *M. halophilus* DSM 20408^T. Data for reference strains are from Hao *et al.* (1984), Li *et al.* (2005) and the present study. All are Gram-positive cocci. All have cell-wall type based on meso-diaminopimelic acid and MK-7 as the predominant menaquinone. Positive for catalase, hydrolysis of aesculin and acid production from glucose. Negative for: methyl red and Voges–Proskauer tests; indole, melanin and H₂S production; hydrolysis of starch, and Tweens 20 and 80; and oxidase, ornithine decarboxylase and arginine dihydrolase. Positive for β -glucosidase, β -galactosidase and lipase. All are able to utilize D-galactose, D-glucose, D-mannitol, D-mannose and salicin as sole carbon source, but not adonitol, D-arabitol or inositol. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Optimal NaCl range (% w/v)	10–15	10	5–15
Growth without salts	w	+	–
pH range	6.0–8.0	6.5–9.0	6.0–10.0
Nitrate reduction	–	+	–
Hydrolysis of:			
Casein	–	–	+
Gelatin	+	–	+
Enzyme activities			
L-Aspartic arylamidase	+	–	–
Lysine decarboxylase	–	–	+
α -Galactosidase	+	–	+
α -Maltosidase	+	+	–
Acid production from:			
Fructose	–	–	+
D-Mannitol	–	+	+
Carbon-source utilization			
D-Arabinose	+	–	–
Dextrin	+	+	–
D-Fructose	–	+	+
D-Mannose	+	+	–
Maltose	–	+	+
L-Rhamnose	+	–	–
D-Sorbitol	+	–	–
Major fatty acids (>10 %)			
anteiso-C _{15:0}	49.9	37.4	45
anteiso-C _{17:0}	29.6	21.1	30
iso-C _{16:0}			14
Menaquinone system	MK-7, MK-9(H ₄)	MK-7, MK-6	MK-7
DNA G + C content (mol%)	48.7	48.5	46.4

was evaluated by the bootstrap resampling method based on 1000 replicates (Felsenstein, 1985).

An almost-complete 16S rRNA gene sequence (1479 bp) of strain YIM 91094^T was obtained. Fig. 1 shows the relationship between the novel strain and its nearest phylogenetic relatives. Levels of 16S rRNA gene sequence similarity between strain YIM 91094^T and the type strains of *M. halophilus* and *M. halotolerans* were 99.7 and 99.2 %, respectively. In the phylogenetic tree based on the neighbour-joining algorithm, strain YIM 91094^T clustered with the type strains of *M. halophilus* and *M. halotolerans* (Fig. 1). The results of 16S rRNA gene sequence comparison clearly demonstrated that strain YIM 91094^T was a member of the genus *Marinococcus*. The topologies of phylogenetic trees built by using the maximum-likelihood

and maximum-parsimony algorithms were similar to that of the neighbour-joining tree (data not shown).

Genomic DNA was extracted and purified according to the methods described by Li *et al.* (2007). The DNA base composition was estimated by HPLC (Mesbah *et al.*, 1989). The genomic G + C content of the DNA of strain YIM 91094^T was 48.7 mol%. Levels of DNA–DNA relatedness were determined according to the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992). DNA–DNA hybridization experiments were performed in triplicate. Levels of DNA–DNA relatedness between strain YIM 91094^T and *M. halophilus* DSM 20408^T and *M. halotolerans* YIM 70157^T were 56.0 and 57.5 %, respectively, below the 70 % cut-off point recommended for assignment of strains to the same genomic species (Wayne *et al.*, 1987).

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain YIM 91094^T among related taxa. Numbers at branch points are bootstrap values (percentages of 1000 resamplings; only values >50 % are shown). The sequence of *Alicyclobacillus acidocaldarius* DSM 446^T was used as outgroup. Bar, 2 % sequence divergence.

The morphological and chemotaxonomic characteristics of the new isolate were consistent with its assignment to the genus *Marinococcus*. A number of physiological and chemotaxonomic characteristics that can be used to distinguish strain YIM 91094^T from its nearest phylogenetic neighbours are presented in Table 1. On the basis of the phenotypic, chemotaxonomic, phylogenetic and DNA–DNA hybridization data presented, we consider that strain YIM 91094^T represents a novel species of the genus *Marinococcus*, for which the name *Marinococcus luteus* sp. nov. is proposed. Additionally, as a consequence of newly reported characteristics not included in the original description of *M. luteus* YIM 91094^T, the description of the genus *Marinococcus* (Hao *et al.*, 1985) needs to be emended.

Emended description of *Marinococcus* Hao *et al.* 1985

The genus is as described by Hao *et al.* (1985), with the following emendation and modifications. Positive for catalase, hydrolysis of aesculin and acid production from glucose. Methyl red and Voges–Proskauer tests are negative. The major cellular fatty acids are anteiso-*C*_{15:0} and anteiso-*C*_{17:0}. The polar lipid profile comprises diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol mannosides, phosphatidylinositol, an unknown phosphoglycolipid and an unknown phospholipid or phosphatidylethanolamine. The G+C content of the genomic DNA is in the range 46.4–48.7 mol%.

Description of *Marinococcus luteus* sp. nov.

Marinococcus luteus (lu'te.us. L. masc. adj. *luteus* orange, referring to the colour of the colonies of the type strain).

Cells are Gram-positive, aerobic, non-spore-forming, motile cocci about 0.1–0.5 µm in size. Colonies are circular, tangerine-coloured, convex and opaque on

modified ISP 5 medium. Grows over a wide range of NaCl concentration (0–25 %, w/v), with optimal growth in the presence of 10–15 % (w/v) NaCl. Growth is weak in the absence of NaCl; no growth is observed in the presence of only KCl or MgCl₂ · 6H₂O. Growth occurs at 10–37 °C and at pH 6.0–8.0; optimal growth at 28 °C and pH 7.0. Catalase-positive. Negative for oxidase, nitrate reduction, milk peptonization and coagulation, and methyl red and Voges–Proskauer tests. Indole, melanin and H₂S are not produced. Negative for lysine and ornithine decarboxylases and arginine dihydrolase. Casein, cellulose, starch, urea, and Tweens 20, 40, 60 and 80 are not hydrolysed. Positive for gelatin liquefaction. In the API ZYM system, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, cystine arylamidase, trypsin, β-glucosidase, β-galactosidase and α-galactosidase, but negative for valine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Acid is produced from aesculin, L-arabinose, D-glucose, glycerol, L-rhamnose and D-xylose (API 50CHB). The following compounds are utilized as sole carbon, nitrogen and energy sources: D-arabinose, L-arginine, dextrin, D-galactose, D-glucose, L-lysine, D-mannose, D-mannitol, L-proline, raffinose, L-rhamnose, D-ribose, sucrose, salicin, D-sorbitol, trisodium citrate and D-xylose. The following are not utilized: adenine, adonitol, L-asparagine, D-arabitol, cellobiose, dulcitol, D-fructose, glycerol, glycine, L-histidine, hypoxanthine, inositol, lactose, maltose, xylitol, L-serine, sodium acetate, sodium propionate, trehalose, L-threonine, L-tyrosine and xanthine. The predominant isoprenoid quinone is MK-7; MK-9(H₄) is present as a minor component. The polar lipid profile comprises diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol mannosides, phosphatidylinositol, two unknown phosphoglycolipids and an unknown phospholipid. The main

cellular fatty acids are anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{15:0}, C_{16:0}, iso-C_{17:0} and iso-C_{16:0}.

The type strain, YIM 91094^T (=KCTC 13214^T=CCTCC AA 208014^T), was isolated from Barkol Lake in Xinjiang Province, north-west China. The DNA G+C content of the type strain is 48.7 mol%.

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