# *Sinococcus qinghaiensis* gen. nov., sp. nov., a novel member of the order *Bacillales* from a saline soil in China

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A Gram-positive, non-spore-forming isolate, designated YIM 70212$^T$, was isolated from a hypersaline soil sample collected from Qinghai, north-west China. Cells of the isolate were orange-pigmented, motile cocci with multiple flagella. A polyphasic taxonomic investigation was carried out on the isolate. The organism grew at 10–45 °C and pH 7·5–11·0, with optimum growth at 28 °C and pH 8·0–9·5. Strain YIM 70212$^T$ grew optimally in the presence of 10 % NaCl, KCl or $MgCl_2.6H_2O$ and growth was observed in 1–25 % NaCl, KCl or $MgCl_2.6H_2O$. The peptidoglycan type was A1$\gamma$. Ribose and minor amounts of galactose were detected as the whole-cell sugars. MK-5 was the only menaquinone. The major cellular fatty acids were ai-$C_{15:0}$ (52·4 %) and ai-$C_{17:0}$ (26·5 %). The DNA G+C content was 47·0 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YIM 70212$^T$ formed a distinct lineage within the order *Bacillales* and was most closely related to members of the genus *Marinococcus*, showing 16S rRNA gene sequence similarity levels of 91·0–91·4 %. Based on the high 16S rRNA gene sequence divergence and differences in phenotypic characteristics, it is proposed that the unknown strain be classified in a novel genus and species with the name *Sinococcus qinghaiensis* gen. nov., sp. nov.; the type strain of *Sinococcus qinghaiensis* is YIM 70212$^T$ (=KCTC 3943$^T$=DSM 17008$^T$).

Recent studies on hypersaline soils in Qinghai Province in north-west China have revealed the presence of a considerable diversity of organisms, constituting moderately halophilic as well as halotolerant bacteria (Li *et al.*, 2004a, 2005a, b, c; Zhang *et al.*, 2005). In this paper, the results are reported of a polyphasic study of another hitherto unknown moderately halophilic strain, designated YIM 70212$^T$, which was isolated from a hypersaline soil. The strain grew optimally in media that contained 10 % KCl, NaCl or $MgCl_2.6H_2O$. Based on phylogenetic and phenotypic evidence, particularly chemotaxonomic data, the isolate could not be placed in any known genus. It is therefore proposed that the unknown bacterium represents a novel species in a new genus.

Strain YIM 70212$^T$ was isolated from a hypersaline soil sample using the dilution plating method. The sample was collected from a region where there are numerous saline lakes with pH values of 7·0–11·5. According to the composition of the soil, which was rich in KCl, a modified SG medium (Sehgal & Gibbons, 1960) was designed as the isolation medium. It contained (g $l^{-1}$): KCl, 250; Casamino acids, 7·5; yeast extract, 10·0; trisodium citrate, 3·0; NaCl, 2·0; $MgCl_2.6H_2O$, 2·0; $MgSO_4.7H_2O$, 1·0; $FeSO_4.7H_2O$, 0·05; and $MnSO_4.7H_2O$, 0·0002. KCl was sterilized separately and then added to the medium. The plate was incubated at 28 °C for 2 weeks. The isolate was maintained on ISP2 agar slants that contained 10 % (w/v) KCl at 4 °C and as glycerol suspensions (20 %, w/v) at −20 °C. Biomass for chemical and molecular systematic studies was obtained from enrichment agar plates of ISP2 medium supplemented with 10 % (w/v) KCl and incubated at 28 °C for about 4–5 days.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 70212$^T$ is DQ168584.

A table showing the fatty acid composition of strain YIM 70212$^T$ and related species and TEMs of cells of strain YIM 70212$^T$ are available as supplementary material in IJSEM Online.

Morphology and motility of cells grown for 12–48 h on ISP2 agar medium supplemented with 10 % KCl (w/v) were examined by light microscopy (model BH 2; Olympus) and electron microscopy (JEM-1010; JEOL). For transmission electron microscopy (TEM) observation, cells were negatively stained with 1 % (w/v) phosphotungstic acid after air-drying. Motility and flagellar arrangement were analysed using semi-solid agar and the staining method of Leifson (1960), followed by light microscopy (model BH 2; Olympus) and TEM. Gram staining was carried out using the standard Gram reaction. The colony colour of the isolate grown on ISP2 agar supplemented with 10 % (w/v) KCl was determined by comparing the cultures with the most suitable colour chips from the ISCC-NBS colour charts (Kelly, 1964). Growth at different temperatures and pH values was investigated as described by Xu *et al.* (2005), but using ISP2 as basic medium. Tolerance of chlorides of sodium, potassium, magnesium and calcium (at 1, 3, 7, 10, 13, 15, 20, 25, 28 and 30 %) was tested. Metabolic properties were determined using API ID 32E test kits (bioMérieux) according to the manufacturer's instructions, except that microbial suspensions were prepared using sterilized distilled water with 5 % KCl. Other physiological and biochemical tests were performed as described previously (Li *et al.*, 2004b, c, 2005a).

Sugar analysis of the whole-cell hydrolysate was carried out as described by Staneck & Roberts (1974). The diaminopimelic acid isomer was identified in the whole-cell hydrolysate (4 M HCl, 100 °C, 16 h) by the method of Rhuland *et al.* (1955). Analyses of polar lipids (two-dimensional TLC) and menaquinones (HPLC, MS) was done according to published procedures (Monciardini *et al.*, 2003). Analysis of the cellular fatty acid pattern followed described methods (Miller, 1982) using the MIDI system (Microbial ID).

Extraction and amplification of genomic DNA for 16S rRNA gene sequence analysis were carried out as described by Xu *et al.* (2003). Multiple alignments with sequences of a broad selection of related species of the order *Bacillales* and calculations of levels of sequence similarity were carried out using CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree (Fig. 1) was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from $K_{nuc}$ values (Kimura, 1980, 1983). Topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The DNA G+C content of strain YIM 70212$^T$ was determined using the thermal denaturation method (Marmur & Doty, 1962).

Cells of strain YIM 70212$^T$ were aerobic, Gram-positive, non-spore-forming, motile cocci with multiple flagella. Colonies of YIM 70212$^T$ were orange, circular, lubricious and opaque on most tested agar media. The strain grew optimally in ISP2 medium supplemented with 10 % (w/v) KCl at 28 °C and pH 7·5–9·0. KCl could be replaced by NaCl or $MgCl_2.6H_2O$; the concentration ranges for growth in KCl, NaCl and $MgCl_2.6H_2O$ were the same (1–25 %). Strain YIM 70212$^T$ could utilize maltose, mannitol, glucose, mannose, fructose, galactose, sucrose, cellobiose and trehalose as carbon sources, but not adonitol, arabinose, arabitol, rhamnose, inositol or sorbitol. Acid was produced from glucose, maltose, sucrose, cellobiose and trehalose. The peptidoglycan type of strain YIM 70212$^T$ was A1$\gamma$, based on *meso*-diaminopimelic acid as diagnostic diamino acid. The phospholipids contained phosphatidylglycerol and diphosphatidylglycerol. In addition, some unidentified lipid components were detected (one phospholipid, one glycolipid and two aminoglycolipids). The only menaquinone was MK-5. The major fatty acids were ai-$C_{15:0}$ (52·4 %) and ai-$C_{17:0}$ (26·5 %).

Comparison of the almost-complete 16S rRNA gene sequence (1531 bp) of strain YIM 70212$^T$ with sequences of a wide range of related type strains revealed closest phylogenetic relatedness to *Marinococcus halophilus* DSM 20408$^T$ (91·4 % sequence similarity) and *Marinococcus halotolerans* YIM 70157$^T$ (91·0 % sequence similarity). A distance matrix dendrogram is shown in Fig. 1.

The results of 16S rRNA gene sequence comparisons clearly demonstrated that strain YIM 70212$^T$ represents a member of the order *Bacillales* and is most closely related to members of the genus *Marinococcus*. However, strain YIM 70212$^T$ clearly differed from its closest phylogenetic neighbours of the genus *Marinococcus* by the number of flagella, acid production from several carbohydrates, hydrolysis of gelatin and casein and enzyme activities, as well as in the menaquinone composition and fatty acid profiles (see Table 1; see also Supplementary Table S1 available in IJSEM Online).

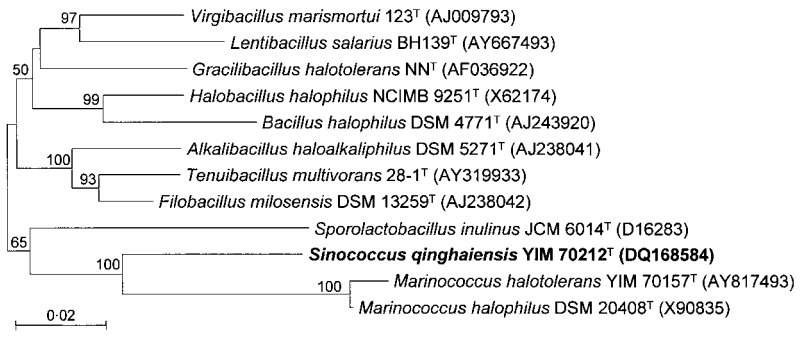


**Fig. 1.** Neighbour-joining tree showing phylogenetic relationships based on 16S rRNA gene sequences of strain YIM 70212$^T$ and other related taxa. Bootstrap values (50 % and above) are shown in percentages of 1000 replicates. *Brevibacillus brevis* JCM 2503$^T$ was used as an outgroup (not shown). Bar, 0·02 changes per nucleotide position.

**Table 1.** Phenotypic characteristics that differentiate strain YIM 70212$^T$ from closely related taxa

Strains: 1, YIM 70212$^T$; 2, *M. halophilus* DSM 20408$^T$; 3, *M. halotolerans* YIM 70157$^T$. Data were taken from this and previous studies (Hao *et al.*, 1984; Li *et al.*, 2005b).

| Characteristic | 1 | 2 | 3 |
|---|---|---|---|
| Flagella | Multiple | One or two | One or two |
| Acid production from: | | | |
| Fructose | − | + | − |
| Cellobiose | + | − | − |
| Mannitol | + | − | + |
| Maltose | + | − | − |
| Hydrolysis of: | | | |
| Gelatin | − | + | − |
| Casein | + | + | − |
| Enzyme activities | | | |
| Lysine decarboxylase | − | + | + |
| α-Galactosidase | − | + | − |
| α-Glucosidase | + | + | − |
| α-Maltosidase | + | − | + |
| Predominant menaquinone | MK-5 | MK-7 | MK-7 |
| Major fatty acids (>10 % of the total) | ai-$C_{15:0}$ (52·4 %), ai-$C_{17:0}$ (26·5 %) | ai-$C_{15:0}$ (45 %), ai-$C_{17:0}$ (30 %), i-$C_{16:0}$ (14 %) | ai-$C_{15:0}$ (37·4 %), ai-$C_{17:0}$ (21·1 %) |

Additionally, isolate YIM 70212$^T$ formed a separate clade next to *M. halotolerans* and *M. halophilus* (Fig. 1). Therefore, based on the above phenotypic and genotypic data, it is proposed that isolate YIM 70212$^T$ should be classified as a representative of a novel genus and species, for which the name *Sinococcus qinghaiensis* gen. nov., sp. nov. is proposed.

## Description of *Sinococcus* gen. nov.

*Sinococcus* (Si.no.coc′cus. M.L. gen. n. *Sinae* of China; N.L. masc. n. *coccus* from Gr. n. *kokkos* a grain or berry; N.L. masc. n. *Sinococcus* coccus-shaped microbe isolated from places in China).

Cells are Gram-positive, non-spore-forming, motile cocci with multiple flagella. Strictly aerobic and Gram-positive. Catalase-positive and oxidase-negative. The peptidoglycan type is A1$\gamma$ (*meso*-diaminopimelic acid, directly cross-linked). Major cellular fatty acids are ai-$C_{15:0}$ and ai-$C_{17:0}$. The menaquinone is MK-5. The G+C content of genomic DNA is about 47 mol%. The type species is *Sinococcus qinghaiensis*.

## Description of *Sinococcus qinghaiensis* sp. nov.

*Sinococcus qinghaiensis* (qing.hai.en′sis. N.L. masc. adj. *qinghaiensis* pertaining to Qinghai, a province of north-west China).

Displays the following properties in addition to those given in the genus description. Cell diameter is about 0·8–1·0 μm (see Supplementary Fig. S1 in IJSEM Online). Colony colour on most tested media is orange. Colonies are circular, opaque and approximately 1·5–1·8 mm in diameter after 24 h at 28 °C. The optimum concentration of KCl for growth is 10 % (w/v) (KCl can also be replaced by $MgCl_2.6H_2O$ or NaCl). Optimum growth occurs at pH 8·0–9·5 and 28 °C. Grows in 1–25 % KCl, $MgCl_2.6H_2O$ and NaCl. Positive for lipase, $\beta$-glucosidase, $\beta$-galactosidase, α-glucosidase and casein hydrolysis, but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, α-galactosidase, α-maltosidase, urease, *N*-acetylglucosaminidase, nitrate reduction, gelatin liquefaction, ammonia production, methyl red and Voges–Proskauer tests, milk peptonization and coagulation, growth on cellulose, $H_2S$ and melanin production and starch hydrolysis. Maltose, mannitol, glucose, mannose, fructose, galactose, sucrose, cellobiose and trehalose can be utilized as carbon sources; adonitol, arabinose, arabitol, rhamnose, inositol and sorbitol cannot be utilized. Acid is produced from glucose, maltose, sucrose, cellobiose and trehalose. The major whole-cell wall sugar is ribose; galactose is present in minor amounts. Polar lipids contain diphosphatidylglycerol, phosphatidylglycerol and some unidentified components, including one phospholipid, one glycolipid and two aminoglycolipids. The fatty acid profile contains ai-$C_{15:0}$ (52·4 %), ai-$C_{17:0}$ (26·5 %), i-$C_{16:0}$ (7·2 %), $C_{16:0}$ (4·8 %), i-$C_{14:0}$ (2·8 %), $C_{16:1}\omega11c$ alcohol (2·4 %) and $C_{16:1}\omega7c$ alcohol (1·6 %).

The type strain is strain YIM 70212$^T$ (=KCTC 3943$^T$=DSM 17008$^T$), isolated from a saline soil sample collected

from Qinghai in north-west China. The DNA G+C content of the type strain is 47·0 mol%.

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