# *Microbacterium halotolerans* sp. nov., isolated from a saline soil in the west of China

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A Gram-positive short rod isolated from a saline soil in China was characterized using a polyphasic approach. This actinobacterium grew over a wide salinity range [0–15 % NaCl, 0–20 % KCl and 0–30 % $MgCl_2.6H_2O$ (w/v); optimum concentrations for growth were 5 % NaCl, 5–10 % KCl, 10 % $MgCl_2.6H_2O$]. The optimum growth temperature and pH were 28–30 °C and 7·0–8·0, respectively. Chemotaxonomic features (peptidoglycan-type B2$\beta$ with glycolyl residues; major menaquinones MK-10 and MK-11; predominating iso- and anteiso-branched cellular fatty acids; DNA G+C content 66·5 mol%) placed this organism within the genus *Microbacterium*. 16S rRNA gene sequence analysis confirmed this classification of the strain, but showed that it was distinct from its nearest neighbours. It formed a separate branch with type strains of the genus *Microbacterium*, and also shared low sequence similarity with them (<96 %). Based on phenotypic and molecular taxonomic results, it is proposed that the unknown isolate should be classified as a novel species in the genus *Microbacterium*, for which the name *Microbacterium halotolerans* sp. nov. is proposed. The type strain is YIM 70130$^T$ (=KCTC 19017$^T$=CIP 108071$^T$).

The genus *Microbacterium* was established by Orla-Jensen (1919) and comprises a diverse collection of Gram-positive, non-spore-forming rods. The description of the genus was emended by Collins *et al.* (1983). More recently, the genus *Microbacterium* was emended again to unite the genera *Microbacterium* and *Aureobacterium* (Takeuchi & Hatano, 1998). Members of the genus *Microbacterium* can be isolated from a wide range of environmental habitats, including plants, soil, water, steep liquor, milk products and humans. Here, we describe the phenotypic and genotypic properties of a novel *Microbacterium* strain isolated from a saline habitat in the west of China.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 70130$^T$ is AY376165.

Detailed phenotypic characteristics and a phylogenetic dendrogram of strain YIM 70130$^T$ and other *Microbacterium* species are available as supplementary material in IJSEM Online.

The strain used in this study was isolated from a saline soil from Qinghai Province, and is designated YIM 70130$^T$. The strain was isolated using modified glycerol/asparagine agar (Shirling & Gottlieb, 1966) (ISP5 medium) supplemented with 15 % (w/v) KCl. The capacity for anaerobic growth was tested in a polystyrene jar using Anaerocult A (Merck) to create an anaerobic atmosphere. For chemotaxonomic and molecular systematic analysis, shake cultures were grown in modified trypticase soy broth (supplemented with 10 %, w/v, KCl, pH 7·0) at 28 °C for 1 week before harvesting by centrifugation (10 000 r.p.m. for 10 min; model J2-21 centrifuge; Beckman). Stocks of the strain were maintained routinely at 4 °C on modified trypticase soy agar slants containing 5–10 % KCl and as glycerol suspensions (20 %, v/v) at −20 °C.

Cell and colony morphology were studied by light (Olympus microscope BH-2) and electron microscopy (JEOL JEM-1010). Gram-staining and the KOH test were performed as described by Moaledj (1986). Motility of cells was studied by examining colony morphology on Luria–Bertani swarming agar (0·3 %, w/v). Growth at different temperatures, salt (NaCl, KCl and $MgCl_2.6H_2O$) concentrations and pH

values was investigated as described by Tang *et al.* (2003). Growth temperatures employed ranged from 4 to 55 °C. Tolerance to NaCl, KCl and $MgCl_2.6H_2O$ was tested at concentrations between 0 and 30 % (w/v) in combination with ISP5 medium. Growth was also tested at pH 5·0–13·0.

Biochemical and physiological tests were performed using the methods described by Shirling & Gottlieb (1966). Oxidase activity was investigated by examining oxidation of 1 % benzidine (Deibel & Ewans, 1960) and tetramethyl-*p*-phenylenediamine (Tarrand & Groschel, 1982). Catalase activity was determined by bubble formation in a 3 % (v/v) hydrogen peroxide solution. Acid production from carbohydrates was determined as described by Leifson (1963). Substrate utilization and enzyme activities of the studied strains were determined using an API Coryne system and API ID 32 E test kits (bioMérieux) according to the manufacturer's instructions.

The peptidoglycan structure was provided by the DSMZ identification service. Qualitative analyses of amino acids and peptides in peptidoglycan hydrolysates were carried out as described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose was applied instead of paper chromatography. Quantitative analysis of amino acids in the total hydrolysate was performed by GC and GC-MS as described by MacKenzie (1987) and Groth *et al.* (1996). The N terminus of the interpeptide bridge was determined by dinitrophenylation according to Schleifer (1985).

Methods for the determination of cell-wall sugars, menaquinone patterns and fatty acids analysis followed those of Li *et al.* (2004a, b) and Chen *et al.* (2004).

The genomic DNA of the bacterium was extracted and purified by the method of Marmur (1961). The DNA G+C content of strain YIM 70130$^T$ was measured by the thermal denaturation method of Marmur & Doty (1962).

The 16S rRNA gene sequence of the isolate was amplified by PCR using conserved primers close to the 3′ and 5′ ends of the gene, as described by Cui *et al.* (2001). Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001) following multiple alignment of data using CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distances (distance options according to the Kimura two-parameter model; Kimura, 1980, 1983) and clustering were calculated with the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985).

Strain YIM 70130$^T$ is a Gram-positive, aerobic, non-motile, non-spore-forming, short rod (Fig. 1). Colonies are white, circular, convex with entire margins, shiny, moist and approximately 1–2 mm diameter after 48 h growth at 28 °C.

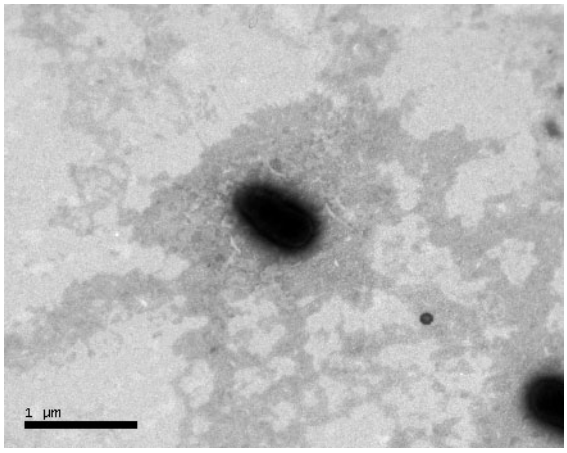


**Fig. 1.** Electron micrograph of strain YIM 70130$^T$ grown on modified ISP5 agar medium supplemented with 10 % (w/v) KCl for 2 days at 28 °C. Bar, 1 μm.

Physiological and biochemical characteristics, metabolic properties and substrate utilization results of strain YIM 70130$^T$ are given in detail in a table available as supplementary material in IJSEM Online and in the species description below.

Strain YIM 70130$^T$ contained ornithine, alanine, glycine, homoserine (Hsr), hydroxy-glutamic acid (Hyg) and small amounts of glutamic acid in the total hydrolysate of the peptidoglycan. From these data, we concluded that the peptidoglycan type was B2$\beta$ (Schleifer & Kandler, 1972), {Gly}[L-Hsr]D-Glu(Hyg)–Gly–D-Orn, with Gly at position 1 and L-Hsr at position 3 of the peptide subunit (type B6; DSMZ, 2001). Its cell-wall sugars contained ribose, galactose, glucose and trace amounts of xylose. The predominant

**Table 1.** Fatty acid composition of strain YIM 70130$^T$

| Fatty acid | Content (%) |
|---|---|
| anteiso-$C_{13:0}$ | 0·12 |
| iso-$C_{14:0}$ | 0·99 |
| $C_{14:0}$ | 0·18 |
| anteiso A $C_{15:1}$ | 0·75 |
| iso-$C_{15:0}$ | 1·19 |
| anteiso-$C_{15:0}$ | 39·91 |
| $C_{15:0}$ | 0·10 |
| iso G $C_{16:1}$ | 0·34 |
| iso-$C_{16:0}$ | 23·40 |
| $C_{16:0}$ | 1·97 |
| anteiso A $C_{17:1}$ | 0·31 |
| iso-$C_{17:0}$ | 0·67 |
| anteiso-$C_{17:0}$ | 30·07 |

menaquinones were MK-10 and MK-11. The cellular fatty acid composition of strain YIM 70130$^T$ is given in Table 1.

To ascertain its phylogenetic position, the almost-complete 16S rRNA gene sequence (1492 nt) for strain YIM 70130$^T$ was determined. A phylogenetic tree (available as supplementary material in IJSEM Online) demonstrated that the isolate is a member of the genus *Microbacterium*, and that it formed an independent phyletic line within a monophyletic subclade. 16S rRNA gene sequence similarity values of the isolate to all of the *Microbacterium* species ranged from 93·8 % (*Microbacterium imperiale*) to 96·3 % (*Microbacterium kitamiense*). It is clear from phylogenetic analyses based on nearly complete 16S rRNA gene sequences that the isolate belongs to the genus *Microbacterium* and represents a distinct phyletic line that can be equated to a separate genomic species (Stackebrandt & Goebel, 1994).

Strain YIM 70130$^T$ shares some chemotaxonomic features with members of the genus *Microbacterium*; i.e. the cell-wall peptidoglycan type is B2$\beta$, major menaquinones are MK-10 and MK-11, iso- and anteiso-branched cellular fatty acids predominate and the DNA G+C content is 66·5 %. However, it shows considerable phenotypic and genomic differences with other recognized species of the genus *Microbacterium*. We propose that strain YIM70130$^T$ represents a novel species of the genus *Microbacterium*, *Microbacterium halotolerans* sp. nov.

### Description of *Microbacterium halotolerans* sp. nov.

*Microbacterium halotolerans* (ha.lo.to′le.rans. Gr. n. *halos* salt; L. part. *tolerans* tolerating; N.L. part. adj. *halotolerans* referring to the ability of the organism to tolerate high salt concentrations).

Aerobic, Gram-positive, non-motile, non-spore-forming, short rod. Colonies are white, circular, convex with entire margins, shiny, moist and approximately 1–2 mm diameter after 72 h at 28 °C. Can grow in ISP5 medium containing 0–15 % NaCl, 0–20 % KCl or 0–30 % $MgCl_2.6H_2O$; optimum concentrations for growth are 5 % NaCl, 5–10 % KCl, 10 % $MgCl_2.6H_2O$. Optimum growth temperature and pH are 28–30 °C and 7·0–8·0, respectively. Catalase-positive and oxidase-negative. Tweens 20 and 80, gelatin, casein and starch are not decomposed. Voges–Proskauer test, $H_2S$ production and indole production are negative. Nitrate is not reduced to nitrite. Activities for lipase, $\beta$-glucosidase, $\beta$-galactosidase and $\alpha$-maltosidase are positive. Negative for ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, urease, $\alpha$-galactosidase, *N*-acetyl-$\beta$-glucosaminidase, $\beta$-glucosidase, $\beta$-glucuronidase, $\alpha$-maltosidase and L-aspartic arylamidase. Uses the following compounds as sole carbon and energy sources: glucose, mannitol, mannose, ribose, fructose, sucrose, maltose, galactose, arabinose, starch, cellobiose, lactose and xylose. Amygdalin, salicin, dextrin, galacturonate, 5-ketogluconate, L-arabitol, trehalose, rhamnose, inositol, sorbitol, malonate and adonitol can not be assimilated. Acid is produced from glucose, mannitol, mannose, ribose, fructose, maltose and xylose. The peptidoglycan type is B2$\beta$, {Gly}[L-Hsr]D-Glu(Hyg)–Gly–D-Orn, with glycolyl residues. Cell sugars contain ribose, galactose, glucose and trace amounts of xylose. The predominant menaquinones are MK-10 and MK-11. The major cellular fatty acids are anteiso-$C_{15:0}$ (39·91 %), anteiso-$C_{17:0}$ (30·07 %) and iso-$C_{16:0}$ (23·40 %). The DNA G+C content is 63·8 mol%.

The type strain, YIM 70130$^T$ (=KCTC 19017$^T$=CIP 108071$^T$), was isolated from saline soil collected from Qinghai Province in the west of China.

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