Haloechinotherix alba gen. nov., sp. nov., a halophilic, filamentous actinomycete of the suborder Pseudonocardineae

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A novel halophilic, filamentous actinomycete strain, designated YIM 93221T, was isolated from a salt lake in Xinjiang province, north-west China, and subjected to a polyphasic taxonomic characterization. The isolate grew with 9–23 % (w/v) NaCl and did not grow without NaCl. The isolate formed spiny aerial mycelium and did not form spores at maturity. The isolate contained meso-diaminopimelic acid as the diagnostic diamino acid and glucose, glucosamine, mannose and an unknown sugar as the major whole-cell sugars. The phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and an unknown phospholipid. MK-8(H4) was the predominant menaquinone. The major fatty acid was iso-C16 :0. The DNA G+C content was 68.1 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YIM 93221T formed a distinct lineage within the suborder Pseudonocardineae and showed 91.9–94.8 % 16S rRNA gene sequence similarity with members of the suborder Pseudonocardineae. On the basis of the evidence from this polyphasic study, a novel genus and species, Haloechinotherix alba gen. nov., sp. nov., are proposed. The type strain of Haloechinotherix alba is YIM 93221T (DSM 45207T = CCTCC AB 208140T).

Since the first halophilic, filamentous actinomycete, Actinopolyspora halophila, was reported (Gochnauer et al., 1975), it has been known that some filamentous actinomycetes can grow with and tolerate high salt concentrations. However, in recent decades, only a few halophilic actinomycetes have been discovered and cultured, such as the genera Actinopolyspora and Streptomonospora (Cui et al., 2001) and some species belonging to the genera Nocardiopsis, Saccharomonospora and Prauserella. Recently, on the basis of extensive studies on the biological characteristics of halophilic filamentous actinomycetes, an efficient isolation medium, cellulose-casein-multisalts (CCMS) medium (Tang et al., 2008), was designed and used for the isolation of filamentous halophilic actinomycetes from hypersaline environments. By using this method, over 2000 halophilic actinomycete strains, which cannot grow without NaCl and have optimal growth with 10–15 % NaCl, have been isolated from hypersaline soil in Xinjiang province in China. Phylogenetic analysis based on 16S rRNA gene sequences has revealed, in addition to the above-mentioned genera, two new genera, Halactinospora (Tang et al., 2008) and Haloglycomyces (Guan et al., 2009), and three novel species, Amycolatopsis halophila (Tang et al., 2010), Saccharopolyspora halophila (Tang et al., 2009a) and Saccharopolyspora qijiaojingensis (Tang et al., 2009b).

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 93221T is G0366705.

A micrograph of spiny aerial mycelium and a chromatogram of polar lipids of strain YIM 93221T are available as supplementary material with the online version of this paper.
Strain YIM 93221T was isolated from a soil sample collected from Qijiaojing Lake, which is a salt lake in Xinjiang Province, north-west China (43° 27' N 91° 29' E), after 3 weeks of incubation at 37 °C on CCMS medium (Tang et al., 2008). Strain YIM 93221T was maintained on modified International Streptomyces Project (ISP) 4 agar containing 15 % (w/v) NaCl at 4 °C and as a glycerol suspension (20%, v/v) at −20 °C. Biomass for chemical and molecular studies was obtained by cultivation in ISP 4 without agar (15%, w/v, NaCl; pH 7.0) at 37 °C and 150 r.p.m. for 1 week.

Cultural characteristics were determined after incubation for 3–4 weeks by methods given by the ISP (Shirling & Gottlieb, 1966). All media were supplemented with 15 % (w/v) NaCl for growth. The colours of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). Growth was good on inorganic salts-starch agar (ISP 4) and oatmeal agar, moderate on Czapek’s agar and nutrient agar and weak on glycerol/asparagine agar; there was no growth on yeast extract-malt extract agar or potato agar. The aerial mycelium was white and the substrate mycelium was yellow–white (Supplementary Table S1, available in IJSEM Online). No soluble pigments were produced. Morphological characteristics of strain YIM 93221T were observed by light microscopy (model BH2; Olympus) and scanning electron microscopy (JSM 5600LV; JEOL) after incubation on ISP 4 agar and oatmeal agar containing 15 % (w/v) NaCl at 37 °C for 4 weeks. The substrate mycelium was well developed and fragmented into rod-like elements, while the aerial mycelium was ‘hedgehog-like’ or spiny. No spores were formed at maturity (Fig. 1 and Supplementary Fig. S1).

Growth was tested at 5–55 °C (at intervals of 5 °C) on ISP 4 containing 15 % (w/v) NaCl and at 0–30 % (w/v) NaCl (at intervals of 1%) using ISP 4 as the basal medium. Growth was investigated at pH 4.0–10.0 (at intervals of 1 pH unit) using the following buffer systems: 0.1 M citric acid/0.1 M sodium citrate (pH 4.0–5.0), 0.1 M KH2PO4/0.1 M NaOH (pH 6.0–8.0) and 0.1 M NaHCO3/0.1 M Na2CO3 (pH 9.0–10.0). Media and procedures used for determination of physiological features and carbon source utilization were those described by Williams et al. (1989). Enzyme activity and acid production from carbohydrates were determined by using the API ZYM and API 50CH systems (bioMerieux) according to the manufacturer’s instructions. Anaerobic growth was determined using the GasPak anaerobic system (BBL) according to the manufacturer’s instructions. Strain YIM 93221T could grow at 20–45 °C, at pH 4.0–8.0 and with 9–23 % NaCl, but could not grow in the absence of NaCl, showing that strain YIM 93221T was a moderately halophilic actinomycete. The phenotypic and chemotaxonomic characteristics of strain YIM 93221T are distinctly different from those of other genera of the suborder Pseudonocardinae (Table 1). The detailed physiological and biochemical characteristics of strain YIM 93221T are given in the species description.

Isomers of diaminopimelic acid were analysed according to the procedures developed by Hasegawa et al. (1983). The whole-cell sugars were detected by HPLC after pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone (Tang et al., 2009b). Polar lipids were extracted and examined by two-dimensional TLC and identified using described procedures (Minnikin et al., 1984). Menaquinones were isolated according to Minnikin et al. (1984) and separated by atmospheric-pressure photoionization LC-MS (Tang et al., 2008). For fatty acid analysis, strain YIM 93221T was cultured on tryptic soy agar (Difco) containing 15 % NaCl at 37 °C for 7 days. Cellular fatty acid analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI). Genomic DNA of strain YIM 93221T for the determination of G+C content was prepared according to the method of Marmur (1961). The G+C content of the DNA was determined by reversed-phase HPLC of nucleosides according to Mesbah et al. (1989). Strain YIM 93221T contained meso-diaminopimelic acid as the cell-wall diamino acid, with glucose (47.2 %), glucosamine (18.7 %), mannose (11.3 %) and an unknown sugar (11.3 %) as the major whole-cell sugars; minor amounts of ribose (6.9 %) and galactose (4.6 %) were also detected. The phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and one unknown phospholipid (Supplementary Fig. S2). The predominant menaquinone was MK-8(H4) (95.4 %), and a minor amount of MK-8(H6) (4.6 %) was also detected. The cellular fatty acid profile contained major amounts of branched fatty acids and minor straight-chain and methyl fatty acids: iso-C14:0 (1.2 %), C14:0 (1.7 %), iso-C15:0 (1.0 %), C15:0 3-OH (0.8 %), C15:0 (2.7 %), iso-C15:1.
Table 1. Differential phenotypic characteristics of strain YIM 93221T and the most closely related genera of the suborder Pseudonocardineae

Data for reference genera were obtained from Goodfellow et al. (1989), Korn-Wendisch et al. (1989), Korn-Wendisch et al. (1995), Tang et al. (2008) and Tian et al. (2009). +, Positive; −, negative; +/−, some species are positive and some are negative.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain YIM 93221T</th>
<th>Sciscionella</th>
<th>Thermocrispum</th>
<th>Saccharopolyspora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial mycelium</td>
<td>Abundant</td>
<td>Sparse</td>
<td>Abundant</td>
<td>Abundant</td>
</tr>
<tr>
<td>Spiny aerial mycelium</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>Spores</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>Growth in:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% NaCl</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>20% NaCl</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+/−</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>20–45</td>
<td>10–37</td>
<td>20–62.5</td>
<td>10–63</td>
</tr>
<tr>
<td>Whole-cell sugar type</td>
<td>C</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Major whole-cell sugars*</td>
<td>Glc, Man, GlcN, UK</td>
<td>Ara, Gal, Glc</td>
<td>Ara, Man, Glc</td>
<td>Ara, Gal</td>
</tr>
<tr>
<td>Predominant menaquinone</td>
<td>MK-8(H4)</td>
<td>MK-9(H4)</td>
<td>MK-9(H4)</td>
<td>MK-9(H4)</td>
</tr>
<tr>
<td>Phospholipids†</td>
<td>DPG, PG, PE, PI, PIM, PL</td>
<td>DPG, PC, PE, PME, PI, PL</td>
<td>PE, OH-PE, PI</td>
<td>DPG, PC, PE, PI, PIM</td>
</tr>
<tr>
<td>Major fatty acid(s) (&gt;10%)‡</td>
<td>i-C16:0</td>
<td>i-C16:0, i-C16:2-OH</td>
<td>i-C16:0, 69</td>
<td>i-C15:0, i-C16:0, i-C17:0</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>68.1</td>
<td>69–73</td>
<td>66.3–74</td>
<td></td>
</tr>
</tbody>
</table>

*Ara, Arabinose; Gal, galactose; Glc, glucose; GlcN, glucosamine; Man, mannose; UK, unknown sugar.
†DPG, Diphosphatidylglycerol; OH-PE, hydroxy-phosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, phospholipid; PME, phosphatidylethanolamine.
‡i, Iso-branched.

(2.3%), iso-C16:0 (43.5%), C16:0 (8.6%), 10-methyl C16:0 (5.6%), iso-C17:0 (0.8%), anteiso-C17:0 (1.6%), C17:1ω8c (6.1%), C17:1ω6c (2.6%), C17:0 (3.5%), 10-methyl C17:0 (3.1%), iso-C18:0 (0.8%), C18:1ω9c (2.7%), C18:0 (1.8%), 10-methyl C18:0 (2-tuberculostearic acid; 0.6%) and C16:1ω7c and/or iso-C15:0 2-OH (8.1%). The G+C content of the DNA was 68.1 mol%.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Li et al. (2007). Multiple alignments with sequences from the most closely related members of the suborder Pseudonocardineae and calculations of sequence similarity were carried out using the EzTaxon server 2.1 (Chun et al., 2007). Phylogenetic analyses were performed using three tree-making algorithms: neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971). A phylogenetic tree was constructed using the neighbour-joining method from K_{sub} values (Kimura, 1980) using MEGA version 4.0 (Tamura et al., 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM 93221T falls within the radius of the suborder Pseudonocardineae and has the highest sequence similarity to Saccharopolyspora rosea IMMIB L-1070T (GenBank accession no. AM992060; 94.8%). The sequence similarity between strain YIM 93221T and members of other genera of the suborder Pseudonocardineae was 91.9–94.8%.

In the phylogenetic tree based on the neighbour-joining algorithm, strain YIM 93221T and Sciscionella marina SC500231T (GenBank accession no. EU503139) formed a distinct clade supported by a high bootstrap value (66%). This relationship was supported by the other tree-making methods used in this study (Fig. 2). All of the above data confirmed that strain YIM 93221T should be assigned to the suborder Pseudonocardineae.

Strain YIM 93221T was different from members of other genera of the suborder Pseudonocardineae in some morphological and physiological properties (Table 1): strain YIM 93221T was a halophilic actinobacterium that could grow with 23% NaCl but not without NaCl, whereas members of the genera Sciscionella, Thermocrispum and Saccharopolyspora (except Saccharopolyspora halophila and Saccharopolyspora qijiaojingensis) are non-halophilic actinomycetes that can grow without NaCl but not with 15% NaCl. Moreover, strain YIM 93221T exhibited some chemotaxonomic differences from the genera Sciscionella, Thermocrispum and Saccharopolyspora: strain YIM 93221T had type C as the whole-cell sugar type (no diagnostic sugar), MK-8(H4) as the predominant menaquinone and phospholipids containing phosphatidylglycerol (no phosphatidylcholine), whereas members of the genera Sciscionella, Thermocrispum and Saccharopolyspora have type A as the whole-cell sugar type (arabinose and/or galactose as the diagnostic sugars), MK-9(H4) as the predominant menaquinone and phospholipids containing phosphatidylcholine (no phosphatidylglycerol). Therefore, on the basis of phenotypic and phylogenetic differentiation of the new isolate, we propose that strain YIM 93221T represents a novel species of a new genus, Haloechinothrix alba gen. nov., sp. nov.
Description of *Haloechinothrix* gen. nov.

*Haloechinothrix* (Ha.lo.e.chi.no’thrix. Gr. n. *halos* salt; Gr. n. *echinos* hedgehog; Gr. fem. n. *thrix* hair; L.N. fem. n. *Haloechinothrix* halophilic, hedgehog-like filament, referring to halophilic filamentous actinomycetes with spiny aerial mycelium).

Gram-staining-positive, strictly aerobic, moderately halophilic, filamentous actinomycetes. Substrate mycelium fragments into rod-like elements and does not form chains of spores at maturity. Spiny aerial mycelium. The whole-cell hydrolysates contain meso-diaminopimelic acid as the cell-wall diamino acid. Glucose, glucosamine, mannose and an unknown sugar are the major whole-cell sugars. The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and an unknown phospholipid. The predominant menaquinone is MK-8(H4). The major fatty acid is iso-C16 : 0. The G+C content of the genomic DNA is about 68 mol%. The type species is *Haloechinothrix alba*.

Description of *Haloechinothrix alba* sp. nov.


Exhibits the following properties in addition to those given in the genus description. Aerial mycelium is white and substrate mycelium is yellow–white on most tested media. No diffusible pigments are produced. Grows at 20–45 °C (optimum 37 °C), at pH 4.0–8.0 (optimum pH 7.0) and with 9–23 % (w/v) NaCl (optimum 15 % NaCl). Starch and Tweens 20, 40, 60 and 80 are degraded, but aesculin, casein, cellulose, chitin and urea are negative. For gelatin liquefaction, nitrate reduction, milk peptonization and coagulation and H2S and melanin production. With API ZYM, positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase and naphthol-AS-BI-phosphohydrolase, but negative for alkaline phosphatase, lipase (C14), α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, N-acetyl-β-glucosaminidase, trypsin and α-chymotrypsin. Utilizes D-mannose, rhamnose, trehalose, erythritol, dulcitol, xylitol, starch, trisodium citrate, L-lysine, alanine, L-arginine, L-asparagine, glycine, L-histidine, L-proline, L-serine, L-threonine, L-tyrosine and hypoxanthine as sole carbon or nitrogen sources, but not D-arabinose, cellobiose, D-fructose, galactose, D-glucose, lactose, maltose, raffinose, D-ribose, sucrose, D-xylitol, glycerol, inositol, mannitol, sorbitol, sodium acetate, sodium propionate, adenine, methionine, L-phenylalanine or xanthine. With API 50CH, does not produce acid. The DNA G+C content of the type strain is 68.1 mol%.

The type strain is YIM 93221T (=DSM 45207T =CCTCC AB 208140T), isolated from a salt lake in Xinjiang province, north-west China.

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References


