Salinactinospora qingdaonensis gen. nov., sp. nov., a halophilic actinomycete isolated from a salt pond

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A novel halophilic, filamentous, actinomycete strain, designated CXB832^T, was isolated from a salt pond in Qingdao, China. Optimal growth occurred at 37 °C, pH 7.0–8.0 and 9–12 % (w/v) NaCl. Strain CXB832^T formed pale yellow to deep yellow branched substrate mycelium without fragmentation. Abundant white aerial mycelium differentiated into long chains of spores and the spores were rod-shaped with smooth surfaces. Strain CXB832^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan, and glucose and xylose as the major whole-cell sugars. The phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phospholipids, glycolipid and unidentified lipids. MK-10(H₈), MK-9(H₈), MK-10(H₂) and MK-10(H₆) were the predominant menaquinones. The major fatty acids were *i*-C_{16:0} (30.71 %), *ai*-C_{17:0} (13.31 %) and C_{16:0} (11.28 %). The G + C content of the DNA was 60.1 mol%. Comparative analysis of 16S rRNA gene sequences showed that the novel strain was most closely related to genera within the family *Nocardiopsaceae*, but formed a separate lineage. The highest sequence similarities were to *Nocardiopsis arabia* DSM 45083^T (95.4 %) and *Haloactinospora alba* DSM 45015^T (94.9 %). On the basis of phenotypic, chemotaxonomic and phylogenetic distinctiveness, strain CXB832^T represents a new genus and novel species in the family *Nocardiopsaceae*, for which the name *Salinactinospora qingdaonensis* gen. nov., sp. nov. is proposed. The type strain of the type species is CXB832^T (=DSM 45442^T=LMG 25567^T).

The family *Nocardiopsaceae* was created, with *Nocardiopsis* as the type genus, by Rainey *et al.* (1996) based on data obtained using a polyphasic taxonomic approach. At present, the family *Nocardiopsaceae* contains seven genera, namely, *Nocardiopsis* (Meyer, 1976), *Thermobifida* (Zhang *et al.*, 1998), *Streptomonospora* (Cui *et al.*, 2001), *Haloactinospora* (Tang *et al.*, 2008), *Marinactinospora* (Tian *et al.*, 2009), *Murinocardiopsis* (Kämpfer *et al.*, 2010) and *Spinactinospora* (Chang *et al.*, 2011). With more than 25 species and subspecies, the genus *Nocardiopsis* is the largest genus in the family *Nocardiopsaceae*. Most strains of the genus *Nocardiopsis* were isolated from hypersaline soils (M.-G. Li *et al.*, 2003; W.-J. Li *et al.*, 2004, 2006; Chen *et al.*, 2008; Yang *et al.*, 2008) and some strains were isolated from marine sediments (Sabry *et al.*, 2004; Kroppenstedt & Evtushenko, 2006; Tian *et al.*, 2009). The four species of the genus *Thermobifida* were isolated from composts or

damp stored hay (Zhang *et al.*, 1998), manure heaps (Kukolya *et al.*, 2002) and a salt mine (Yang *et al.*, 2008). The five species of the genus *Streptomonospora* were isolated from a salt lake (Cui *et al.*, 2001; Cai *et al.*, 2009) and soil (W.-J. Li *et al.*, 2003; Cai *et al.*, 2008). The genera *Haloactinospora*, *Marinactinospora* and *Spinactinospora* contain only one species and the type species of these genera were isolated from a salt lake (Tang *et al.*, 2008) and marine sediments (Tian *et al.*, 2009; Chang *et al.*, 2011). Many strains of the family *Nocardiopsis* have been isolated from saline soils and are halophilic, some of which are strictly halophilic (Tang *et al.*, 2008). A novel strain was isolated from a salt pond in Qingdao, China, designated CXB832^T, and was found to be phylogenetically closely related to members of the genera *Nocardiopsis* and *Haloactinospora*. In the present study, the taxonomic position of strain CXB832^T was determined by using a polyphasic taxonomic approach.

Strain CXB832^T was isolated and maintained on starch-casein agar medium (pH 7.5) supplemented with 15 % (w/v) NaCl at 37 °C for 3 weeks. The composition of the starch-casein agar medium was (distilled water 1⁻¹): 10 g starch, 0.3 g casein, 2 g KNO₃, 0.05 g MgSO₄ · 7H₂O, 2 g K₂HPO₄, 0.02 g CaCO₃, 0.01 g FeSO₄ and 15 g agar. After primary isolation and purification by the streak plate method, the isolates were preserved both on slants of International

Abbreviations: DPG, diphosphatidylglycerol; ISP, International *Streptomyces* Project; PC, phosphatidylcholine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PME, phosphatidylmethylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CXB832^T is GU253338.

Supplementary figures and a sequence alignment are available with the online version of this paper.

Streptomyces Project medium 4 agar (ISP 4; Shirling & Gottlieb, 1966) supplemented with 10 % (w/v) NaCl at 4 °C and in 20 % (v/v) glycerol at –80 °C.

All the media were supplemented with 10 % (w/v) NaCl for the observation of growth. Strain CXB832^T grew well on ISP 2, ISP 3, ISP 4 and ISP 5 agars (Shirling & Gottlieb, 1966), Czapek solution agar (Waksman, 1961), nutrient agar (Difco) and potato agar (Waksman, 1961). The novel strain did not produce diffusible pigments on any of the media tested. Micromorphology was observed by light microscopy (BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) using cells incubated for 7, 14, 21 and 28 days on ISP 2 agar supplemented with 10 % (w/v) NaCl. The colours of the substrate and aerial mycelia were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). The aerial and substrate mycelia were well developed on most media tested, producing a pale yellow to deep yellow substrate mycelium that carried white aerial hyphae on ISP 4 agar supplemented with 10 % (w/v) NaCl. The aerial mycelium formed long chains of spores at maturity that were rod-shaped with smooth surfaces; they were non-motile (Fig. 1a, b). Substrate mycelium was extensively branched with non-fragmenting hyphae (Fig. 1c).

Physiological characteristics, including temperature and pH ranges for growth and tolerance to NaCl, were tested using ISP 4 as the basal medium. Growth was tested at 4, 16, 20, 28, 37, 40, 43, 46, 49, 50, 51 and 52 °C on ISP 4 agar with 10 % (w/v) NaCl. For tolerance experiments involving concentrations of NaCl between 0 and 25 % (at intervals of 1 %), ISP 4 was used as the basal medium. The pH range for growth was investigated between pH 4.0 and 11.0 at intervals of 1.0 pH unit using the buffer system described by Xu *et al.* (2005). Carbon-source utilization for growth was performed as described by Shirling & Gottlieb (1966). Tests for hydrolysis of cellulose, gelatin, starch and Tweens 20, 40, 60 and 80, nitrate reduction, utilization of urea, milk coagulation and peptonization and production of H₂S and melanin were performed with 10 % (w/v) NaCl as described by Gonzalez *et al.* (1978). Antibiotic susceptibility was examined according to Groth *et al.* (2004) using antibiotic discs on ISP 4 agar with 10 % (w/v) NaCl. *Haloactinospora alba* DSM 45015^T (the type species of the genus *Haloactinospora*) obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) was used as a reference strain and cultured under the same conditions. The detailed physiological properties of the novel strain are given in the genus and species description (see below) and are presented in Table 1.

Biomass for chemotaxonomic studies was obtained by centrifugation after cultivation in ISP 4 broth with 10 % (w/v) NaCl for 7 days at 180 r.p.m. at 37 °C. For fatty acid analyses, cells were grown on tryptic soy agar (Difco) containing 10 % (w/v) NaCl at 180 r.p.m. at 37 °C. For analysis of amino acids and sugars in the cell-wall

Fig. 1. Scanning electron micrographs showing the long spore chains and spores of strain CXB832^T after incubation on ISP 2 with 10 % NaCl for 28 days at 37 °C. Bars, 1 μm.

hydrolysate, wet biomass was well suspended in iso-propanol/water (1 : 1, v/v). For analysis of polar lipids and menaquinones, wet biomass was freeze-dried. Analysis of whole-cell sugars and amino acids in the cell-wall hydrolysate, polar lipids and menaquinones was carried out by the Identification Service of the DSMZ. Analysis of whole-cell sugars was performed according to the procedure described by Becker *et al.* (1965). Amino acids in the cell-wall hydrolysate were analysed by the method described by Schleifer & Kandler (1972). Menaquinones were extracted and analysed according to Collins *et al.* (1979) and Groth *et al.* (1996). Polar lipids extracted by the method of Minnikin *et al.* (1979) were identified by two-dimensional TLC as described by Collins & Jones (1980). The analysis of fatty acids was performed as described by Sasser (1990) and the results were compared with the database of fatty acids in the Microbial Identification System (MIDI) (TSBA6; version, 6.0B library). The whole-cell hydrolysates of strain CXB832^T contained *meso*-diaminopimelic acid as the diagnostic cell-wall peptidoglycan and the whole-cell sugars were glucose, xylose with traces of ribose and arabinose. Phospholipids (see Fig. S1 in IJSEM Online) comprised

Table 1. Differentiating phenotypic and chemotaxonomic characteristics of strain CXB832^T and related genera of the family *Nocardiopsaceae*

Taxa: 1, strain CXB832^T; 2, *Haloactinospora* (data from this study and Tang *et al.*, 2008); 3, *Nocardiopsis* (Hozzein & Goodfellow, 2008); 4, *Spinactinospora* (Chang *et al.*, 2011); 5, *Marinactinospora* (Tian *et al.*, 2009); 6, *Thermobifida* (Yang *et al.*, 2008); 7, *Murinocardiopsis* (Kämpfer *et al.*, 2010); 8, *Streptomonospora* (Cai *et al.*, 2008). Cell walls of all the taxa contain *meso*-diaminopimelic acid. Glu, glucose; Xyl, xylose; Rib, ribose; Gal, galactose; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PIM, phosphatidylinositol mannoside; PME, phosphatidylmethylethanolamine; PL, phospholipids; GL, glycolipids; L1, unidentified lipids. NT, not tested; i, iso; ai, anteiso.

Characteristic	1	2	3	4	5	6	7	8
Isolation source	Salt pond	Salt lake	Sand dune soil	Marine sediment	Marine sediment	Salt mine	Indoor walls	Hypersaline soil
Spore surface	Smooth	Smooth	Smooth	Spinous	Wrinkled	Smooth	No aerial mycelium	NT
Growth conditions								
NaCl range (% w/v)	1–23	9–21*	0–15	1–15	0–5	0–10	0–11	5–20
NaCl optimum (% w/v)	9–12	15*	5	3–8	0–1	NT	NT	10
Temperature range (°C)	37	37*	28–30	37	28	45	28	37
Maximum temperature (°C)	50	45*	40	44	55	50	34	45
Diagnostic sugars	Glu, Xyl	Gal, Rib	Gal, Glu	Rib, Glu	None	Gal, Xyl, Glu	None	Gal
Predominant menaquinones	MK-10(H ₂ , H ₆ , H ₈), MK-9(H ₈)	MK-10(H ₈), MK-11(H ₄ , H ₆ , H ₈)	MK-10(H ₄ , H ₆)	MK-10(H ₆ , H ₈), MK-9(H ₈)	MK-11(H ₈ , H ₁₀), MK-10(H ₈)	MK-10(H ₄ , H ₆ , H ₈)	MK-10(H ₄ , H ₈), MK-11(H ₄), MK-12(H ₂)	MK-10(H ₈ , H ₆), MK-11(H ₈)
Diagnostic phospholipids	DPG, PG, PL, GL, L1	DPG, PG, PC, PIM	PC, PME, DPG, PG, PI, PIM, GL, PL	PG, PC, DPG, PI, PL	DPG, PC, PG, PIM, PI, PL	DPG, PME, PC, PI, PG, PL	PC, PG, PI, DPG, PL, GL	DPG, PG, PC, PIM, PI, PL
Major fatty acids (>10 %)	i-C _{16:0} , ai-C _{17:0} , C _{16:0}	ai-C _{17:0} , i-C _{16:0} *	i-C _{16:0} , 10-methyl-C _{18:0} , ai-C _{17:0}	i-C _{16:0} , ai-C _{17:0} , C _{18:0}	i-C _{16:0} , i-C _{16:1} G, 10-methyl-C _{18:0}	i-C _{16:0} , ai-C _{17:0}	i-C _{16:0} , ai-C _{17:0} , C _{18:1} ω _{9c}	i-C _{16:0} , ai-C _{17:0} , 10-methyl-C _{18:0} , 10-methyl-C _{17:0}

*Data from this study for *Haloactinospora alba*. This strain was grown under the same culture conditions as strain CXB832^T.

diphosphatidylglycerol, phosphatidylglycerol, phospholipids, glycolipid and unidentified lipids. The menaquinones consisted of MK-10(H₈) (32 %), MK-9(H₈) (29 %), MK-10(H₂) (20 %), MK-10(H₆) (17 %) with traces of MK-6(H₆) and MK-10(H₄). The fatty acid profile contained i-C_{16:0} (30.71 %), ai-C_{17:0} (13.31 %), C_{16:0} (11.82 %), C_{18:0} (9.43 %), i-C_{18:0} (6.56 %), 10-methyl-C_{18:0} (6.08 %), C_{18:1}ω7c and/or C_{18:1}ω6c (4.02 %), 10-methyl-C_{17:0} (3.17 %), C_{16:0} 2-OH (2.73 %), i-C_{17:0} (2.63 %), C_{17:0} (2.63 %), C_{18:1}ω9c (2.52 %), ai-C_{15:0} (1.47 %), C_{14:0} (1.19 %), C_{17:0} iso ω9c and/or 10-methyl-C_{16:0} (1.14 %) and i-C_{14:0} (0.56 %).

The G+C content of the chromosomal DNA was determined according to the methods described by Mesbah *et al.* (1989) using reverse-phase HPLC. The G+C content of strain CXB832^T was 60.1 mol%.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene were conducted as described by Li *et al.* (2007). The 16S rRNA gene sequence was aligned and compared with available sequences in the GenBank/EMBL/DDJB database using BLAST searches. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using

the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Sequences were aligned using CLUSTAL_X 1.8 (Thompson *et al.*, 1997) (the sequence alignment is available as supplementary material in IJSEM Online). The phylogenetic trees were constructed using the neighbour-joining and maximum-likelihood methods with Kimura 2-state parameter model analyses implemented in the MEGA version 5 program (Tamura *et al.*, 2007). In each case, bootstrap values were calculated based on 1000 replicates.

BLAST results for the 16S rRNA gene sequence of strain CXB832^T showed that its closest relatives were members of genera *Nocardiopsis* and *Haloactinospira* in the family *Nocardiopsaceae*, with the highest gene sequence similarity of 95.4 % with *Nocardiopsis arabia* DSM 45083^T and 94.9 % with *Haloactinospira alba* DSM 45015^T, respectively. In the neighbour-joining (Fig. 2) and maximum-likelihood trees (Fig. S2) based on the 16S rRNA gene sequences of representatives of all genera in family *Nocardiopsaceae*, strain CXB832^T formed a distinct lineage, which showed the evolutionary divergence between strain CXB832^T and the previously described genera in this family.

The phenotypic and chemotaxonomic characteristics of the novel strain could be readily distinguished from the closest

Fig. 2. Phylogenetic dendrogram obtained by neighbour-joining analysis based on 16S rRNA gene sequences showing the position of strain CXB832^T and its phylogenetic neighbours. Numbers at nodes are bootstrap values based on 1000 resamplings (only values >50 % are indicated). Bar, 1 % sequence divergence.

phylogenetic genera, *Nocardiopsis* and *Haloactinospora*, by the maximum NaCl concentration for growth, the diagnostic sugars, the predominant menaquinones and the diagnostic phospholipids. The maximum NaCl concentration for growth of strain CXB832^T was 23 % (w/v), but members of the genera *Nocardiopsis* and *Haloactinospora* are unable to grow at this concentration. The diagnostic sugars of strain CXB832^T were glucose and xylose, which are different from those found for the other two genera. The predominant menaquinones for strain CXB832^T were MK-10(H₂,H₆,H₈) and MK-9(H₈), but MK-10(H₂) and MK-9(H₈) are absent from members of the genera *Nocardiopsis* and *Haloactinospora*. The diagnostic phospholipids of the genera *Nocardiopsis* and *Haloactinospora* are phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIM) and phosphatidylmethylethanolamine (PME), but none of these phospholipids were present in strain CXB832^T. Other characteristics that differentiated strain CXB832^T from members of the other seven genera of the family *Nocardiopsaceae* are shown in Table 1. Thus, based on the above phenotypic and genotypic results, strain CXB832^T represents a novel genus for which the name *Salinactinospora* gen. nov. is proposed. The type species of the genus is *Salinactinospora qingdaonensis* sp. nov.

Description of *Salinactinospora* gen. nov.

Salinactinospora (Sa.lin.ac.ti.no'spo.ra. N.L. adj. *salinus* saline; Gr. n. *actis actinos* a ray; Gr. n. *spora* a seed; N.L. fem. n. *Salinactinospora* an actinomycete originating from a saline habitat).

Gram-positive-staining, aerobic, moderately halophilic, filamentous actinomycetes. The aerial mycelium forms long chains of spores. Spores are rod-shaped with smooth surfaces. Substrate mycelium is extensively branched with non-fragmenting hyphae. No diffusible pigments are produced. The whole-cell hydrolysates contain *meso*-diaminopimelic acid, glucose and xylose. The predominant menaquinones are MK-10(H₂), MK-10(H₆), MK-10(H₈) and MK-9(H₈). The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phospholipids, glycolipid and unidentified lipids. The major fatty acids are *i*-C_{16:0}, *ai*-C_{17:0} and C_{16:0}. The G + C content of the genomic DNA is 60.1 mol%.

Description of *Salinactinospora qingdaonensis* sp. nov.

Salinactinospora qingdaonensis (qing.da.o.nen'sis. N.L. fem. adj. *qingdaonensis* pertaining to Qingdao, a city in the coastal region of East China, from which the type strain was isolated).

Displays the following properties in addition to those described for the genus. Good growth on ISP 2, ISP 3, ISP 4 and ISP 5 agars, Czapek solution agar, nutrient agar and potato agar supplemented with 10 % (w/v) NaCl. The colour of the aerial mycelium is white and the substrate

mycelium is pale yellow to deep yellow on ISP 4 agars supplemented with 10 % (w/v) NaCl. Growth occurs between 16 and 50 °C, but not at 4 or 51 °C. Grows at pH 6.0–9.0 and with 1–23 % (w/v) NaCl, with optimum growth at pH 7.0–8.0 and 37 °C with 9–12 % (w/v) NaCl. Utilizes α -cyclodextrin, dextrin, glycogen, inulin, mannan, L-arabinose, D-arabitol, arbutin, cellobiose, D-galactose, D-glucose, lactose, lactulose, maltose, maltotriose, D-mannose, melibiose, raffinose, L-rhamnose, sucrose, salicin, D-ribose, D-sorbitol, turanose, xylitol and inosine as sole carbon sources, but D-fructose, L-fucose, inositol, D-galacturonic acid, D-mannitol, melezitose, stachyose, D-xylose, D-tagatose and D-psicose are not utilized. Susceptible to (μ g per disc): norfloxacin (10), vancomycin (30), amoxicillin (10), minocycline (30) and erythromycin (15). Resistant to (μ g per disc): penicillin (10), oxacillin (1), ampicillin (10), carbenicillin (100), amikacin (30), gentamicin (10), kanamycin (30), neomycin (30), midecamycin (30), ofloxacin (5), norfloxacin (10), polymyxin B (300), furazolidone (300), clindamycin (2), netilmicin (30), ciprofloxacin (5), tobramycin (10), trimethoprim (5), lincomycin (2) and streptomycin (10). Tests for hydrolysis of starch and cellulose, melanin production, H₂S production and gelatin liquefaction are negative, whereas tests for Tweens 20, 40, 60 and 80, nitrate reduction, utilization of urea, milk coagulation and milk peptonization are positive. The fatty acid profile contained mainly *i*-C_{16:0}, *ai*-C_{17:0} and C_{16:0}.

The type strain, CXB832^T (=DSM 45442^T=LMG 25567^T), was isolated from a salt pond in Qingdao, China. The DNA G + C content of the type strain is 60.1 mol%.

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