Streptomyces sodiiphilus sp. nov., a novel alkaliphilic actinomycete

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An alkaliphilic actinomycete, strain YIM 80305^T, which was isolated from a muddy sample in Chaka salt lake, Qinghai Province of China, was characterized using a polyphasic approach. The isolate produced light-yellow substrate and yellow–white aerial mycelia on most tested media. Optimum pH for growth was 9·0–10·0 with scant growth at pH 7·0. Results showed that strain YIM 80305^T was obligately Na^+-dependent, and showed sensitivity to K^+. The DNA G+C content was 70·5 mol%. 16S rRNA gene sequence analysis together with these characteristics consistently assigned strain YIM 80305^T to the genus *Streptomyces*. It formed a distinct clade based on analyses of the almost-complete and 120-nucleotide variable γ region of the 16S rRNA gene. It could be differentiated by phenotypic and genotypic analysis from all the *Streptomyces* species whose names have been validly published. On the basis of polyphasic evidence, *Streptomyces sodiiphilus* sp. nov. is proposed. The type strain is YIM 80305^T (=CCTCC AA 203015^T=CIP 107975^T).

There are many alkaliphilic actinomycetes in highly alkaline environments, such as soda lakes and saline-alkaline lakes (Groth *et al.*, 1997; Jones *et al.*, 1998; Duckworth *et al.*, 1998). Mikami *et al.* (1982) first reported alkaliphilic actinomycetes, and some taxonomic data and applications for alkaliphilic actinomycetes were reported subsequently (Groth *et al.*, 1997; Duckworth *et al.*, 1998). Alkaliphilic actinomycetes can produce many alkaline enzymes (Horikoshi, 1999) and bioactive substances, such as antibiotics (Tsujibo *et al.*, 1988, 1990) and enzyme inhibitors (Bahn *et al.*, 1998), and they also have typical metabolites and wide exploitation in industries.

Alkaliphilic actinomycetes that thrive in alkaline environments have typical nutrient requirements, cultural conditions and physiological properties. Up to now, there have been several reports on the physiology and energetics of alkaliphilic bacteria (Krulwich *et al.*, 2001; Yumoto, 2002), while there are few reports on alkaliphilic actinomycetes. Thus, studies on the physiology of alkaliphilic actinomycetes are urgently required to exploit this microbial resource of great potential.

The effect of Na_2CO_3, which is usually used to regulate pH when cultivating alkaliphilic actinomycete strains, NaOH, KOH and K_2CO_3 on the growth of some alkaliphilic actinomycete isolates, including strain YIM 80305^T, was determined. The results showed that strain YIM 80305^T had special physiological characteristics; thus it was classified further using a polyphasic approach.

Strain YIM 80305^T was isolated from a muddy saline–alkaline soil sample collected near Chaka salt lake, Qinghai Province, China, using soil-extract agar (pH 10·0). The isolate was cultivated on yeast extract/malt extract agar (ISP medium 2, pH 9·0) at 28 °C. Modified ISP medium 2 was used as basic medium for pH and other physiological tests; the pH was regulated to pH 9·0 by using autoclaved Na_2CO_3 and the cultivation temperature was 28 °C unless stated otherwise. The following buffers were used: pH 6·0, 7·0 and 8·0–0·1 M KH_2PO_4/0·1 M NaOH; pH 9·0 and 10·0–0·1 M $NaHCO_3$/0·1 M Na_2CO_3; pH 11·0–0·05 M Na_2HPO_4/0·1 M NaOH; and pH 12·0–0·2 M KCl/0·2 M NaOH. Strain YIM 80305^T was incubated in liquid ISP medium 2 for 2–3 weeks. After the basic medium was sterilized, the pH was regulated to pH 6·0, 7·0, 8·0, 9·0, 10·0, 11·0 or 12·0 using autoclaved KOH, K_2CO_3, NaOH or

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Na_2CO_3 before pouring the medium onto plates. A further test was carried out by adding 1·0, 2·0 or 3·0 % (w/v) NaCl to the basic medium and the pH was regulated by using autoclaved KOH or K_2CO_3. The inoculated plates were cultivated for 2–3 weeks.

Morphological features were observed on ISP medium 2 under different conditions (pH 7·0, pH 9·0 and pH 9·0 with 3 % NaCl) for 3–4 weeks with an Olympus BH-2 microscope and by scanning electron microscopy (JSM-5600LV; JEOL). Media and procedures used for cultural characteristics, physiological and biochemical features and carbon source utilization were those described by Shirling & Gottlieb (1966) and Locci (1989), except that pH was regulated to pH 9·0 using autoclaved Na_2CO_3. Growth temperature range of strain YIM 80305^T was determined on modified ISP medium 2 (pH 9·0) and inoculated plates were incubated at 4, 10, 20, 28, 37, 45, 55 or 65 °C for 1–2 weeks. NaCl tolerance of strain YIM 80305^T was determined by adding 0, 3, 5, 7, 10 or 15 % (w/v) NaCl to the basic medium, followed by incubation for 3–4 weeks.

Cell-wall amino acids were purified and analysed by the methods of Jiang *et al.* (2001). The procedure of Lechevalier & Lechevalier (1980) was used for analysis of whole-cell sugar hydrolysates. Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1984). Menaquinones were determined using the procedures of Collins (1985) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acid analysis was performed as described by Sasser (1990).

The genomic DNA of strain YIM 80305^T was extracted and purified by using the method of Marmur (1961). The DNA G+C content of strain YIM 80305^T was measured using the thermal denaturation method (Marmur & Doty, 1962).

Extraction of genomic DNA, amplification of the 16S rRNA gene and sequencing were done as described by Cui *et al.* (2001). Reference strains were chosen from BLAST (Altschul *et al.*, 1997) search results. Phylogenetic analysis was performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983). The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Morphological observation of a 21-day culture of strain YIM 80305^T grown on yeast extract/malt extract agar (ISP medium 2) (pH 9·0 or pH 9·0 with 3 % NaCl) revealed that strain YIM 80305^T had typical characteristics of the genus *Streptomyces*. Aerial mycelium and substrate mycelium were well-developed and not fragmented. Long or short chains of spores were straight to flexuous and spores were non-motile (Fig. 1). The control for strain YIM 80305^T grown on ISP medium 2 at pH 7·0 produced very little aerial mycelium (data not shown). For cultural characteristics, strain YIM 80305^T developed well on most media including Czapek's agar medium, oatmeal agar (ISP medium 3), glycerol/asparagine agar (ISP medium 5) and yeast extract/malt extract (ISP medium 2). It showed poor growth on nutrient agar. No growth was observed on inorganic salt/starch agar (ISP medium 4). No diffusible pigments were produced except on nutrient agar medium (pale orange–yellow).

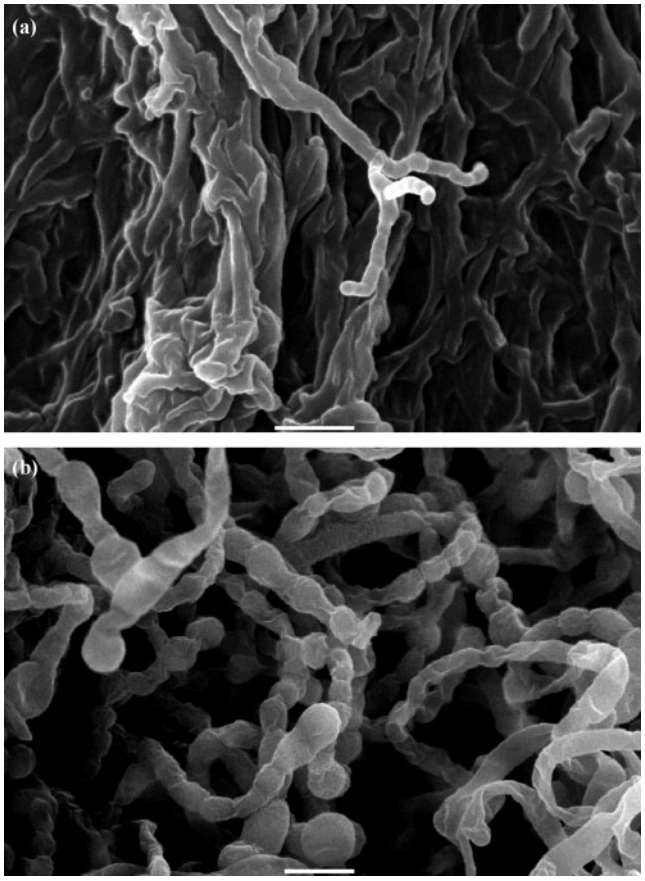

Fig. 1. Scanning electron micrographs of the spore chains of *S. sodiiphilus* YIM 80305^T grown on yeast extract/malt extract agar (ISP medium 2) at 28 °C for 21 days at pH 10·0 (a) or at pH 8·0–9·0 supplemented with 5 % NaCl (b). Bars, 2 μm.

The cell wall of strain YIM 80305^T contained LL-diaminopimelic acid and glycine, indicating that strain YIM 80305^T was of chemotype I (Lechevalier & Lechevalier, 1970a, b). The whole-cell hydrolysates mainly contained galactose and glucose but no diagnostic sugars. The predominant menaquinones were MK-9(H_4) (13 %), MK-9(H_6) (68 %) and MK-9(H_8) (19 %), and the diagnostic phospholipid was phosphatidylethanolamine. The major fatty acid components were ai-$C_{15:0}$ (16·47 %), ai-$C_{17:0}$ (13·30 %) and i-$C_{16:0}$ (31·32 %). Thus, chemotaxonomic and phenotypic

Table 1. Influence of different alkaline compounds on the growth of strain YIM 80305^T

Na_2CO_3 showed no obvious effect on the growth of YIM 80305^T at pH 7·0–12·0. Symbols: +, growth; −, no growth.

Compound	pH					
	7·0	8·0	9·0	10·0	11·0	12·0
KOH	+	+	−	−	−	−
K_2CO_3	+	+	−	−	−	−
NaOH	+	+	+	+	+	−

data showed that strain YIM 80305^T should be assigned to the genus *Streptomyces*.

Strain YIM 80305^T could grow between pH 7·0 and 12·0, and its optimal pH was 9·0–10·0. KOH, K_2CO_3, NaOH and Na_2CO_3 had different effects on its growth (Table 1). KOH and K_2CO_3 showed obvious inhibition of the growth of strain YIM 80305^T, and it only grew at pH 7·0–8·0 with them, while NaOH and Na_2CO_3 showed no obvious effect on growth. However, when 1, 2 or 3 % NaCl was added to the basic medium using KOH and K_2CO_3 to regulate pH, the pH range for the growth of YIM 80305^T was increased: it grew at pH 7·0–11·0 when using KOH and at pH 7·0–9·0 with K_2CO_3. Small amounts of NaCl could promote the growth of YIM 80305^T. It was interesting that strain YIM 80305^T showed a wider pH range for growth on the basic medium using KOH than that using K_2CO_3 when adding 1·0, 2·0 or 3·0 % NaCl. All the results showed that YIM 80305^T was obligately dependent on Na^+, especially in highly alkaline media, but that it showed sensitivity to K^+ in highly alkaline media. The optimum growth temperature and NaCl concentration are 28 °C and 3 % (w/v), respectively.

The almost-complete 16S rRNA gene sequence (1489 nt) for the novel strain was aligned manually with corresponding almost-complete sequences of representative *Streptomyces* species retrieved from the GenBank, EMBL and DDBJ databases by using BLAST (Altschul *et al.*, 1997). Phylogenetic analyses based on a dataset consisting of 1452 unambiguous nucleotides at positions 45 to 1496 (*Escherichia coli* numbering; Brosius *et al.*, 1978) showed that the novel isolate falls into one distinct subclade with two other species, *Streptomyces albus* subsp. *albus* (GenBank/EMBL/DDBJ accession no. AJ621602) (97·6 % sequence similarity) and *Streptomyces armeniacus* (GenBank/EMBL/DDBJ accession no. AB018094) (96·0 % sequence similarity). The phylogenetic tree based on the 16S rRNA gene sequences of strain YIM 80305^T and the most closely related type strains of the genus *Streptomyces* is shown in Fig. 2.

The variable γ region sequences (positions 158 to 277) of the 16S rRNA gene from 452 known *Streptomyces* species obtained from the DDBJ databases and from strain YIM 80305^T were aligned. Analysis of γ region sequences showed that strain YIM 80305^T was grouped into a branch with

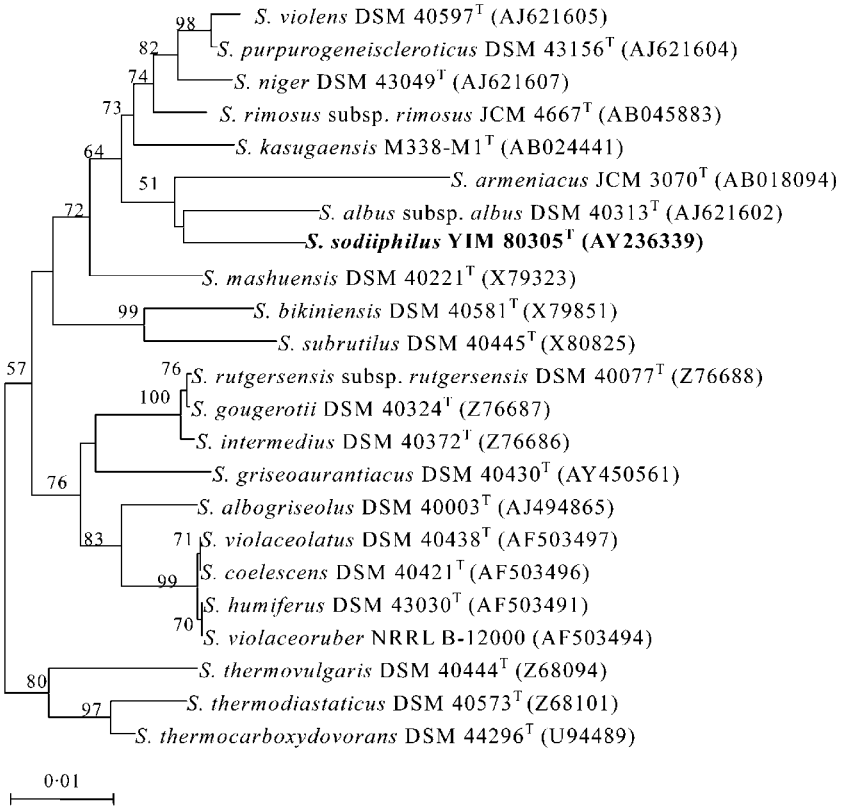

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of YIM 80305^T and related strains. Only bootstrap values above 50 %, expressed as percentages of 1000 replications, are shown at the branch points. Bar, 0·01 substitution per nucleotide position.

strains of recognized *Streptomyces* species, including *Streptomyces rimosus* ISP 5260^T, *Streptomyces ochraceiscleroticus* ISP 5594^T, *Streptomyces olivaceus* JCM 4066, *Streptomyces violens* ISP 5597^T, *Streptomyces purpurogeneiscleroticus* ISP 5271^T and *Streptomyces niger* ISP 5302^T. Although strain YIM 80305^T had almost the same sequence of the variable γ region as those strains, it had broad phenotypic differences (Table 2). The renaturation rates of genomic fragments from pairs of strains were determined spectrophotometrically with a model 1601 UV spectrophotometer equipped with a Thermoelectric Cell Temperature Controller (Shimadzu) according to the previously described methods (De Ley *et al.*, 1970; Huss *et al.*, 1983), and the low DNA–DNA relatedness (all below 40 %) between strain YIM 80305^T and the related type strains also confirmed that they are different genomic species.

Thus, polyphasic data show that strain YIM 80305^T represents a novel species of the genus *Streptomyces*, for which we propose the name *Streptomyces sodiiphilus* sp. nov.

Description of *Streptomyces sodiiphilus* sp. nov.

Streptomyces sodiiphilus (so.di.i′phi.lus. N.L. n. *sodium -i*; Gr. adj. *philos* loving; N.L. adj. *sodiiphilus* sodium ion-loving, referring to the characteristic of Na^+-dependent growth).

Aerobic and Gram-positive. Both vegetative and aerial hyphae are well-developed and not fragmented. Long or short chains of spores are straight to flexuous and spores are non-motile. No diffusible pigments are produced except on nutrient agar medium (pale orange–yellow). Sodium acetate and rhamnose can be used as sole carbon sources for growth, but not most other carbon sources, such as lactose, maltose, fructose, xylose, ribose, arabinose, sucrose, glucose, galactose, sodium citrate, cellobiose, cellubinose, raffinose, mannitol, sorbitol, glycerol and starch. Positive for gelatin liquefaction and nitrate reduction, but negative for urease, melanin production, starch hydrolysis, H_2S production, milk coagulation and milk peptonization. Cell wall contains LL-diaminopimelic acid and glycine. Whole-cell hydrolysates mainly contain galactose and glucose and no diagnostic sugars. Predominant menaquinones are MK-9(H_4) (13 %), MK-9(H_6) (68 %) and MK-9(H_8) (19 %), and the diagnostic phospholipid is phosphatidylethanolamine. Major fatty acid components are ai-$C_{15:0}$ (16·47 %), ai-$C_{17:0}$ (13·30 %) and i-$C_{16:0}$ (31·32 %). Grows optimally at 28 °C and in ISP medium 2 with 3 % NaCl and pH 9·0–10·0. DNA G+C content is 70·5 mol%.

The type strain, YIM 80305^T (=CCTCC AA 203015^T=CIP 107975^T), was isolated from a soil sample collected from Chaka salt lake, Qinghai Province, China.

Table 2. Phenotypic properties that separate strain YIM 80305^T from most-related *Streptomyces* species based on analyses of almost-complete and variable γ region 16S rRNA gene sequences

Strains: 1, YIM 80305^T; 2, *S. rimosus* ISP 5260^T; 3, *S. ochraceiscleroticus* JCM 4801^T, ISP 5594^T; 4, *S. olivaceus* JCM 4066; 5, *S. violens* ISP 5597^T; 6, *S. purpurogeneiscleroticus* ISP 5271^T; 7, *S. niger* ISP 5302^T; 8, *S. albus* subsp. *albus* DSM 40313^T; 9, *S. armeniacus* JCM 3070^T. Abbreviations: R, red; G, grey; GW, grey–white; Y, yellow; W, white; S, smooth; WS, wrinkled surface; ST, straight; RA, *Retinaculiaperti*; RF, *Rectiflexibiles*; SP, *Spirales*. Symbols: +, utilized; −, not utilized; V, variable; d, doubtful; ND, not determined. For *S. violens* ISP 5597^T, aerial mycelium was absent on yeast extract/malt extract agar (ISP medium 2), oatmeal agar, inorganic salt/starch agar and glycerol/asparagine agar, and thus no spore was borne; see Shirling & Gottlieb (1972). Data for reference type strains were taken from Shirling & Gottlieb (1968, 1969, 1972).

Characteristic	1	2	3	4	5	6	7	8
Colony colour on ISP medium 2	GW	R to W	Y	G	−	W	GW	W or Y
Spore surface	WS	S	S	S	−	S	S	S
Spore-chain morphology	ST to RA	SP to RA	SP	RF	−	SP	SP	SP
Production of diffusible pigment	+	−	V	−	−	+	V	−
Melanoid pigment	+	−	−	−	−	−	−	−
Utilization of:								
Glucose	−	+	+	+	+	+	+	+
Arabinose	−	+	+	+	+	+	+	V
Mannitol	−	+	+	+	+	+	+	+
Fructose	−	+	+	+	+	+	+	+
Rhamnose	+	−	+	+	+	+	+	−
Sucrose	−	V	+	V	+	+	+	−
Raffinose	−	+	+	V	+	+	+	V
Inositol	−	+	+	+	+	+	+	−
Xylose	−	D	+	+	+	+	+	+

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