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Saccharopolyspora cebuensis sp. nov., a novel actinomycete isolated from a Philippine sponge (*Porifera*)

Sheila Marie Pimentel-Elardo,[1,2] Loralie P. Tiro,[2] Lubomir Grozdanov[1] and Ute Hentschel[1]

Correspondence
Ute Hentschel
ute.hentschel@mail.uni-wuerzburg.de

[1]Research Center for Infectious Diseases, University of Würzburg, Röntgenring 11, D-97070 Würzburg, Germany

[2]College of Pharmacy, University of San Carlos, Cebu City 6000, Philippines

The taxonomic status of a marine actinomycete isolated from the sponge *Haliclona* sp. collected from Cebu, Philippines, was established using both phenotypic and genotypic data. Strain SPE 10-1^T exhibited chemotaxonomic and morphological characteristics that were consistent with those of members of the genus *Saccharopolyspora*. It showed a strict requirement for salt and is the first obligate marine bacterium of the genus *Saccharopolyspora* to be isolated. The principal isoprenoid quinone detected was MK-9(H_4). The fatty acid pattern consisted mainly of terminally branched iso and anteiso fatty acids. The DNA G+C content was 72.6 mol%. Analysis of the 16S rRNA gene sequence supported affiliation of the strain with the genus *Saccharopolyspora*; the type strain of *Saccharopolyspora gregorii* was the closest phylogenetic relative (96 % sequence similarity). Sequence similarities of strain SPE 10-1^T to other type strains of this genus were 93–95 %. It is proposed that strain SPE 10-1^T should be classified in the genus *Saccharopolyspora* as a representative of *Saccharopolyspora cebuensis* sp. nov. The type strain of *Saccharopolyspora cebuensis* is SPE 10-1^T (=DSM 45019^T=CIP 109355^T).

The genus *Saccharopolyspora* was first described by Lacey & Goodfellow (1975) and at present comprises ten species with validly published names: *Saccharopolyspora hirsuta* (Lacey & Goodfellow, 1975), *Saccharopolyspora erythraea* (Labeda, 1987), *Saccharopolyspora taberi* (Korn-Wendisch *et al.*, 1989), *Saccharopolyspora gregorii* (Goodfellow *et al.*, 1989), *Saccharopolyspora hordei* (Goodfellow *et al.*, 1989), *Saccharopolyspora rectivirgula* (Korn-Wendisch *et al.*, 1989), *Saccharopolyspora spinosa* (Mertz & Yao, 1990), *Saccharopolyspora spinosporotrichia* (Zhou *et al.*, 1998), *Saccharopolyspora flava* (Lu *et al.*, 2001) and *Saccharopolyspora thermophila* (Lu *et al.*, 2001). Members of this genus are aerobic, Gram-positive, non-acid-fast organisms with substrate hyphae that either fragment into rod-shaped elements, do not fragment or are transformed partially into chains of spores (Korn-Wendisch *et al.*, 1989). They lack mycolic acid, but contain *meso*-diaminopimelic acid, arabinose and galactose in the cell wall and predominantly tetrahydrogenated menaquinones with nine isoprene units. The DNA G+C contents for type strains of species of the genus fall within the range 67–74 mol% (Embley *et al.*, 1987; Goodfellow *et al.*, 1989; Korn-Wendisch *et al.*, 1989).

In this study, cultivation and characterization of strain SPE 10-1^T, a marine sponge isolate that exhibits properties consistent with its assignment to the genus *Saccharopolyspora*, are described.

Strain SPE 10-1^T was isolated from the marine sponge *Haliclona* sp. on M1 agar. M1 medium, which is specifically designed for the isolation of marine actinomycetes, is composed of the following [per litre artificial seawater (ASW)]: 10 g starch, 4 g yeast extract and 2 g peptone (Mincer *et al.*, 2002). ASW contains the following salts (l^{-1}): 23.477 g NaCl, 10.64 g $MgCl_2.6H_2O$, 3.917 g Na_2SO_4, 1.102 g $CaCl_2$, 0.664 g KCl, 0.192 g $NaHCO_3$, 0.096 g KBr, 0.026 g H_3BO_3, 0.024 g $SrCl_2$ and 0.03 g NaF (Lyman & Fleming, 1940). The sponge was collected by scuba diving off Maribago waters (GPS: 10° 17′ 0.97″ N 124° 00′ 01.8″ E), Cebu, Philippines, in February 2003. Mechanical separation of the sponge tissue and bacterial isolation were performed as described by Pimentel-Elardo *et al.* (2003).

Growth on M1 broth was tested at 10, 15, 20, 25, 30, 37, 45 and 55 °C. Strain SPE 10-1^T was able to grow between 15 and 37 °C, with optimal growth at 25–30 °C. Colonies displayed chalky-white mycelia with brownish soluble

Abbreviation: ASW, artificial seawater.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SPE 10-1^T is EF030715.

pigment on M1 agar. Cultures of strain SPE 10-1^T grown in M1 broth for 7–14 days appeared yellowish-brown to brown in colour. Strain SPE 10-1^T was capable of growing in ISP2 medium (Shirling & Gottlieb, 1966) (l^{-1}: 4 g yeast extract, 8 g malt extract and 4 g glucose) in ASW, as well as in Zobell marine medium (Oppenheimer & ZoBell, 1952) (1 g yeast extract l^{-1}, 5 g peptone l^{-1}, 250 ml distilled water, 750 ml ASW). Strain SPE 10-1^T did not grow in ISP2 medium without ASW. No growth was observed on M1 agar plates incubated in an anaerobic jar. M1 media supplied with different amounts of ASW or NaCl were used to test for the requirement for seawater and salt tolerance. Growth was possible in regular strength M1 (100 % ASW) and in M1 containing 75, 50 and 25 % ASW, but not 0 % ASW. Growth was also possible when regular strength ASW was replaced with 12.5, 10.0, 7.5 or 5.0 % NaCl in distilled water. Growth was poor in M1 with 2.5 % NaCl and growth was not observed without NaCl or with 15 % NaCl. From these data, it is concluded that strain SPE 10-1^T has a strict requirement for salt, which suggests that it is an obligate marine bacterium. Furthermore, strain SPE 10-1^T was able to grow in M1 liquid medium supplemented with the antibiotics (100 μg ml^{-1}) gentamicin and kanamycin, but not with rifampicin, penicillin, streptomycin, lincomycin, vancomycin, oxacillin, chloramphenicol, ampicillin or tetracycline.

For phenotypic testing, API identification kits (bioMérieux) were used. Strain SPE 10-1^T was grown in M1 agar for 72 h and resuspended in ASW. Data on growth on different carbon sources were obtained using the API CH system and are given in the species description.

Table 1. Selected physiological properties of strain SPE 10-1^T and the type strains of the species of the genus *Saccharopolyspora*

Strains: 1, SPE 10-1^T; 2, *S. gregorii* DSM 44324^T; 3, *S. spinosporotrichia* DSM 44350^T; 4, *S. spinosa* DSM 44228^T; 5, *S. erythraea* DSM 40517^T; 6, *S. hirsuta* subsp. *hirsuta* DSM 43463^T; 7, *S. hordei* DSM 44065^T; 8, *S. rectivirgula* DSM 43747^T; 9, *S. flava* AS4.1520^T; 10, *S. thermophila* AS4.1511^T; 11, *S. taberi* DSM 43856^T. Data for strain SPE 10-1^T are from this study; data for all other strains are from Lu *et al.* (2001), except for data on hypoxanthine, DNA G+C content and D-mannitol utilization which are from Goodfellow *et al.* (1989), Labeda (1987), Lacey & Goodfellow (1975), Mertz & Yao (1990) and Zhou *et al.* (1998). BF, Buff; BR, brown; C, colourless; G, grey; O, orange; P, pink; R, red; W, white; Y, yellow; +, positive; −, negative; NA, no aerial mycelium; ND, not determined. All *Saccharopolyspora* strains were positive for utilization of D-fructose, glycerol and D-mannose as sole carbon source.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Utilization of:											
L-Arabinose	+	+	−	+	+	−	+	−	−	−	−
D-Galactose	+	+	+	−	+	+	+	+	+	+	+
D-Lactose	+	−	−	−	−	+	+	+	+	+	+
Maltose	+	+	+	−	+	+	+	+	+	+	+
D-Mannitol	−	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	−	+	+	+	+	+	+	+
L-Rhamnose	+	+	+	−	+	+	+	+	+	+	+
Sucrose	+	+	+	−	+	+	+	+	+	+	+
D-Xylose	+	+	+	−	+	+	+	+	+	−	+
Temperature range for growth (°C)	15–37	10–35	28–37	15–37	20–42	25–50*	20–60	37–63*	28–37	45–55	20–45
Nitrate reduction	−	−	−	+	+	−	−	+	+	−	+
NaCl tolerance (%)	2.5–12.5†	13	<3	<11	<5	<7	<13	<10	7	7	7
Degradation of:											
Adenine	−	−	−	−	+	+	+	−	+	+	+
Casein	−	+	+	−	−	+	+	−	−	−	+
Chitin	−	−	−	−	+	−	+	−	−	−	+
Hypoxanthine	−	+	+	+	+	+	+	+	+	−	+
Tyrosine	+	+	−	+	+	+	+	+	−	+	+
Colour of mycelia:											
Aerial	W‡	W–Y	W–G	W–P	P–BRG–W	W	W–Y	W-light P	W	W	NA
Substrate	W‡	C–BF	BR–R	G–OY–BR	OY–RBR	C–BF	C–BF	Y–O	Y	C–BF	C–Y
DNA G+C content (mol%)	72.6	74.0	70.4	ND	71.1	71.5	72.0	70.4	67.0	73.1	70.8

*The temperature data from Lu *et al.* (2001) for the type strains differ slightly from those of Korn-Wendisch *et al.* (1989) who reported a temperature range of 20–50 °C for *S. hirsuta* and 37–60 °C for *S. rectivirgula* species.

†Strain SPE 10-1^T is the only known isolate with a strict requirement for salt.

‡Note that the ISP2 medium was supplemented with ASW and that mycelial colour may vary depending on media composition.

Strain SPE 10-1^T was positive for the following enzymes using the API ZYM system: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase. The ability to degrade macromolecules was determined using the method of Korn-Wendisch *et al.* (1989). The strain was able to degrade tyrosine, but not adenine, casein, chitin or hypoxanthine. Furthermore, strain SPE 10-1^T stained Gram-positive and was catalase-positive (standard hydrogen peroxide reaction). Strain SPE 10-1^T tested negative for oxidase activity and for reduction of nitrate to nitrite using established procedures (Gordon, 1967; MacFaddin, 1980). The organism could be distinguished from the type strains of other species of the genus *Saccharopolyspora* by using a combination of phenotypic properties (Table 1).

Determination of the following diagnostic cell-wall components and G+C content of the genomic DNA were performed by the DSMZ, Braunschweig, Germany. Established procedures were used to determine diagnostic diaminopimelic acid isomers and the predominant sugars of the whole organism (Staneck & Roberts, 1974). Quinone analysis was carried out as described by Kroppenstedt (1985). The presence of mycolic acids was investigated following the procedure of Minnikin *et al.* (1975). Polar lipids were extracted and analysed following the integrated procedure of Minnikin *et al.* (1984). The fatty acid content was determined by GC using MIDI software. The genomic DNA G+C content was determined by HPLC (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984). The strain contained *meso*-diaminopimelic acid as the wall diamino acid. The diagnostic sugars arabinose and galactose were present; glucose and ribose were also found. A menaquinone with a tetrahydrogenated-isoprenoid side chain of nine units [MK-9(H_4)] was the principal isoprenoid quinone. Small amounts of MK-8(H_4) and MK-10(H_4) were also found. Mycolic acids were not detected. The phospholipid pattern comprised phosphatidylcholine, phosphatidylethanolamine, phosphatidylmethylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. Two unknown glycolipids were also found. The fatty acid pattern was composed mainly of terminally branched iso and anteiso fatty acids, but small amounts of diagnostic 10-methyl-branched fatty acids were found and no 2-OH fatty acids were detected. The chemical properties of strain SPE 10-1^T are consistent with its assignment to the genus *Saccharopolyspora*.

Transmission electron microscopy of ultrathin sections of strain SPE 10-1^T was performed as described previously (Pimentel-Elardo *et al.*, 2003). Scanning electron microscopy was performed as described by Scheuermayer *et al.* (2006). Strain SPE 10-1^T exhibited morphological properties characteristic of members of the genus *Saccharopolyspora*, forming extensively branched substrate mycelia that fragmented into rod-shaped elements (Fig. 1a). Scanning electron microscopy showed hyphae bearing short chains of spores, as well as single spore cells (Fig. 1b). The spores were round to oval and the surface was smooth. Light microscopy of colonies confirmed the presence of spores in aerial mycelia (data not shown).

16S rRNA gene amplification, cloning and sequencing were performed according to the methods of Hentschel *et al.* (2001) using the universal primers 27f and 1492r (Lane, 1991). An almost complete 16S rRNA gene sequence (1483 nt) was generated for the strain and it was compared with those of species of the genus *Saccharopolyspora* as its closest neighbours, as well as those from representatives of genera from the family *Pseudonocardiaceae*. The sequences were then aligned using CLUSTAL W and phylogenetic analysis was performed using the ARB software (Strunk *et al.*, 2000). Phylogenetic tree construction was performed using the neighbour-joining algorithm with bootstrap values based on 100 replications (Fig. 2). Phylogenetic analysis revealed that strain SPE 10-1^T had highest sequence similarity (96 %) with the type strain of *S. gregorii* and 93–95 % similarity with the type strains of all other species of this genus. Taken together, the phenotypic and genotypic data obtained in this study show clearly that strain SPE 10-1^T

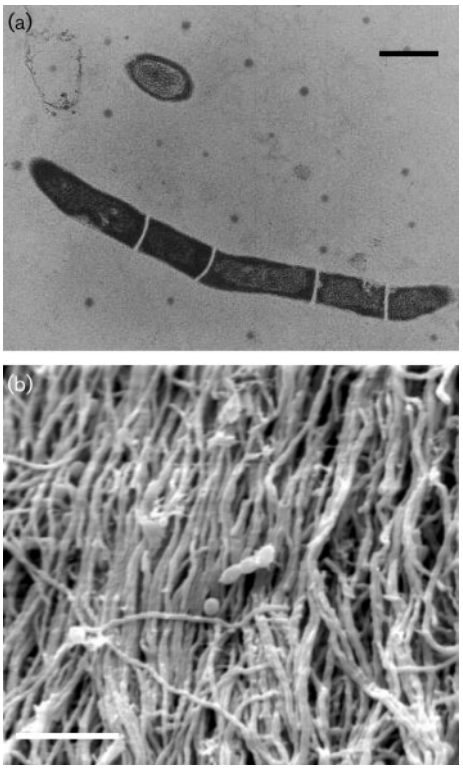

Fig. 1. Hyphae and spores of strain SPE 10-1^T grown in M1 broth (a, transmission electron microscopy, bar, 0.5 μm) and on M1 agar (b, scanning electron microscopy, bar, 5 μm) at 30 °C for 7 days.

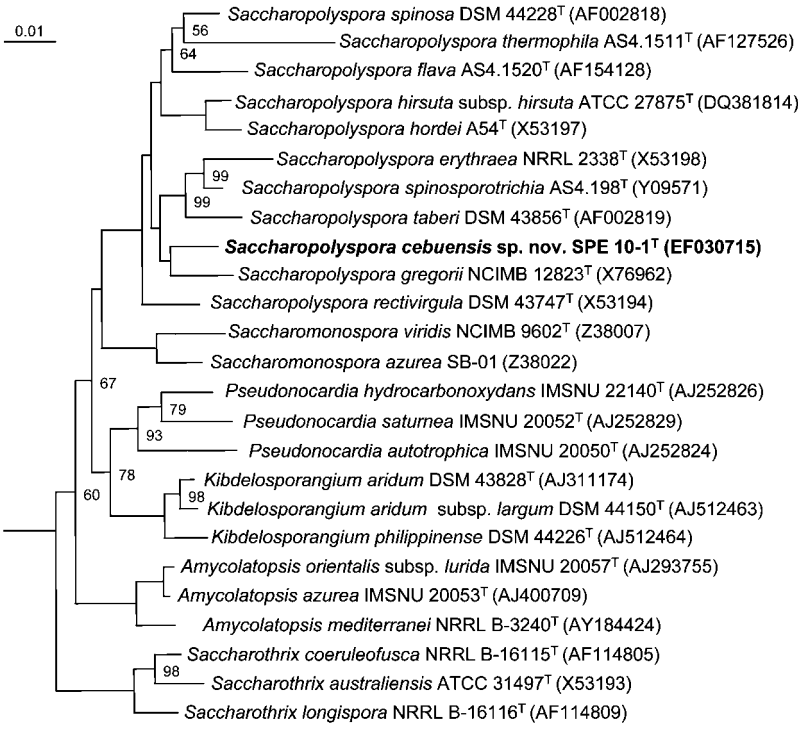

Fig. 2. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences of strain SPE 10-1^T and representative strains of members of the family *Pseudonocardiaceae* and related taxa. *Escherichia coli* (GenBank accession no. DQ360844) was used as outgroup (not shown). Numbers at the nodes indicate the levels of bootstrap support based on 100 resampled datasets; only values greater than 50 % are shown. Bar, 0.01 substitutions per nucleotide position.

represents a novel and obligate marine species within the genus *Saccharopolyspora*.

Description of *Saccharopolyspora cebuensis* sp. nov.

Saccharopolyspora cebuensis (ce.bu′en.sis. N.L. fem. adj. *cebuensis* pertaining to the province of Cebu in the Philippines where the type strain was collected).

Cells are Gram-positive, aerobic and form extensively branched white mycelium fragmenting into rod-shaped elements and aerial hyphae bearing short chains of spores. Brown diffusible pigment is observed. Growth is possible at 15–37 °C and in ISP2 or M1 media containing 2.5–12.5 % NaCl or 25–100 % ASW. Does not grow anaerobically. Utilizes a variety of organic compounds such as glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, D-adonitol, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, *N*-acetylglucosamine, amygdalin, aesculin, D-cellobiose, maltose, D-lactose, sucrose, trehalose, inulin, raffinose, starch, glycogen, gentiobiose, D-fucose, D-arabitol and potassium gluconate as sole carbon sources. Able to degrade tyrosine, but not adenine, casein, chitin or hypoxanthine. Negative for oxidase and nitrate reduction, but positive for catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase. Cell wall contains *meso*-diaminopimelic acid as the diagnostic diamino acid. Whole cell hydrolysate contains galactose and arabinose. Major menaquinone is MK-9(H_4). Lacks mycolic acids. Fatty acid pattern consists of iso, anteiso and 10-methyl-branched fatty acids. The DNA G+C content of the type strain is 72.6 mol%.

The type strain, SPE 10-1^T (=DSM 45019^T=CIP 109355^T), was isolated from the Philippine sponge *Haliclona* sp.

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