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Streptomyces axinellae sp. nov., isolated from the Mediterranean sponge *Axinella polypoides* (Porifera)

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An actinomycete strain, isolated from the marine sponge *Axinella polypoides* collected from Banyuls-sur-Mer, France, was characterized using a polyphasic approach. Based on its chemotaxonomic and morphological characteristics, strain Pol001^T belongs to the genus *Streptomyces*. The strain is characterized by LL-diaminopimelic acid in the cell wall, menaquinones MK-9(H_4, H_6, H_8) and a DNA G+C content of 71.0 mol%. It forms a separate phyletic line based on phylogenetic analyses of the nearly complete 16S rRNA gene sequence. Strain Pol001^T could be differentiated from other closely related *Streptomyces* species with validly published names by phenotypic and genotypic analysis. DNA–DNA hybridization between strain Pol001^T and closely related reference strains further confirmed that strain Pol001^T represents a novel taxon of the genus *Streptomyces*. Therefore, it is proposed that strain Pol001^T represents a novel species in the genus *Streptomyces*, *Streptomyces axinellae* sp. nov.; the type strain is Pol001^T (=DSM 41948^T =CIP 109838^T).

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) for aerobic, spore-forming actinomycetes. These Gram-positive bacteria have distinct features such as extensively branching substrate and aerial mycelia, a high DNA G+C content (69–78 mol%) and the presence of LL-diaminopimelic acid (LL-A_2pm) and the absence of characteristic sugars in the cell wall (Anderson & Wellington, 2001). The genus *Streptomyces* currently contains more than 500 species and subspecies with validly published names, making this genus the largest in the domain *Bacteria* (Hain *et al.*, 1997). In this study, cultivation and characterization of strain Pol001^T, which exhibits properties that are consistent with its assignment to the genus *Streptomyces*, are described.

Strain Pol001^T was isolated from the Mediterranean sponge *Axinella polypoides*. The sponge was collected by scuba diving offshore from Banyuls-sur-Mer, France (GPS: 42° 29′ N 03° 08′ E) in May 2003. Mechanical separation of the sponge tissue and storage of bacterial cells were performed as described by Fieseler *et al.* (2004). M1 medium, containing [per litre artificial seawater (ASW)] 10 g starch, 4 g yeast extract and 2 g peptone (Mincer *et al.*, 2002), was used to cultivate the strain. ASW contained the following salts (l^{-1}): NaCl, 23.477 g; $MgCl_2 . 6H_2O$, 10.64 g; Na_2SO_4, 3.917 g; $CaCl_2$, 1.102 g; KCl, 0.664 g; $NaHCO_3$, 0.192 g; KBr, 0.096 g; H_3BO_3, 0.026 g; $SrCl_2$, 0.024 g; and NaF, 0.03 g (Lyman & Fleming, 1940).

Cultural characteristics were observed on a number of standard media (Shirling & Gottlieb, 1966). Strain Pol001^T grew well on a variety of standard International *Streptomyces* Project (ISP) agar media after incubation at 30 °C for 21 days: yeast-malt extract (ISP2), oatmeal (ISP3) and peptone-yeast extract-iron (ISP6). A diffusible pigment was observed only on tyrosine agar (ISP7) (Table 1). Grey aerial and white substrate mycelia were abundant on ISP2 medium. The strain was further examined for a range of phenotypic and physiological properties using standard procedures (Gottlieb, 1961; Shirling & Gottlieb, 1966; Korn-Wendisch *et al.*, 1989). Strain Pol001^T was positive for gelatin liquefaction, but negative for melanin production, starch hydrolysis, nitrate reduction and hydrogen sulfide production. The strain was able to degrade casein, but not adenine, chitin or hypoxanthine. Growth in ISP2 broth was tested at 20, 25, 30, 37, 42 and 55 °C. Strain Pol001^T was able to grow at 20–37 °C, with optimum growth at 30 °C. ISP2 media supplied with different amounts of NaCl and ASW were used to test for salt tolerance and seawater requirements. Growth was possible in 0, 2.5, 5.0 and 7.5 % NaCl, but not in 10.0, 12.5 or 15.0 % NaCl, with optimal growth at 0–2.5 % NaCl.

Abbreviations: A_2pm, diaminopimelic acid; ASW, artificial seawater.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Pol001^T is EU683612.

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Table 1. Cultural characteristics of strain Pol001^T on various agar media

Medium	Growth	Diffusible pigment	Aerial mycelium	Substrate mycelium
Yeast-malt extract (ISP2)	Abundant	None	Grey	White
Oatmeal (ISP3)	Abundant	None	Dark brown	Light green
Inorganic salts-starch (ISP4)	Poor	None	Yellow–orange	White
Glycerol-asparagine (ISP5)	Moderate	None	White	Light yellow
Peptone-yeast extract-iron (ISP6)	Abundant	None	Light pink	White
Tyrosine (ISP7)	Moderate	Red	Light pink	Light green
Czapek's medium	Abundant	None	Grey to black	Light green
Nutrient agar	Abundant	None	White	White

Growth was also possible in ISP2 medium with 25, 50, 75 and 100 % ASW. Furthermore, strain Pol001^T was able to grow in medium supplemented (100 μg ml^{-1}) with the antibiotics ampicillin, chloramphenicol, nalidixic acid, penicillin and rifampicin, but not with erythromycin, gentamicin, kanamycin or vancomycin. Utilization of different carbon sources was tested using Biolog SF-P2 following the manufacturer's instructions (Biolog); data are given in the species description. The morphological, physiological and biochemical characteristics of strain Pol001^T and phylogenetically related *Streptomyces* species are shown in Table 2.

Determination of the genomic DNA G+C content and diagnostic cell-wall components was performed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. The G+C content of the genomic DNA of strain Pol001^T, determined by HPLC (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984), was 71.0 mol%. Established procedures were used to determine the diagnostic isomer of A_2pm and the predominant sugars of the whole organism (Staneck & Roberts, 1974). Strain Pol001^T contained LL-A_2pm in the cell wall. Analysis of the whole-cell sugar composition revealed the presence of glucose and ribose, as well as traces of mannose. Quinone analysis was carried out as described by Kroppenstedt (1985). A menaquinone with a hexahydrogenated-isoprenoid side chain of nine units, MK-9(H_6), was found as the principal isoprenoid quinone. Two additional quinones with nine isoprene units [MK-9(H_4, H_8)] were also found. Polar lipids were extracted and analysed following the integrated procedure of Minnikin *et al.* (1984). Strain Pol001^T contained the following polar lipids: diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, unidentified phospholipids, phosphoglycolipids, glycolipids and an aminolipid. The fatty acid composition was determined by GC using MIDI software. Strain Pol001^T contained the following fatty acids: iso-$C_{16:0}$ (30.78 %), anteiso-$C_{15:0}$ (17.77 %), iso-$C_{15:0}$ (12.03 %), anteiso-$C_{17:0}$ (9.80 %), iso-$C_{16:1}$ (6.92 %), iso-$C_{14:0}$ (5.77 %) and iso-$C_{17:1}$ (4.58 %).

Table 2. Selected physiological properties that differentiate strain Pol001^T from closely related *Streptomyces* species

Strains: 1, Pol001^T; 2, *S. sclerotialus* DSM 43032^T; 3, *S. rimosus* subsp. *rimosus* DSM 40260^T; 4, *S. niger* DSM 43049^T; 5, *S. olivaceiscleroticus* DSM 40595^T. Colour of mycelium and reverse side of colony and production of diffusible and melanoid pigments were compared using growth on ISP2 medium. All strains had smooth spore surfaces, utilized glucose, mannitol and fructose as carbon sources and lacked melanoid pigments. Data for reference strains were taken from Shirling & Gottlieb (1968a, b, 1972). Abbreviations: SP, *Spirales*; RA, *Retinaculiaperti*; G, grey; W, white; Y, yellow; R, red; B, brown; L, light; D, dark; +, positive; −, negative; d, doubtful.

Characteristic	1	2	3	4	5
Spore-chain morphology	SP	SP	SP, RA	SP	SP
Colour of aerial mycelium	GW	LYR	R or W	YG	BG
Reverse side of colony	W	YB	GY	DB	GY
Production of diffusible pigment	−	−	−	+	+
Utilization of:					
L-Arabinose	−	+	+	+	+
Inositol	−	+	+	+	+
Raffinose	−	+	+	+	+
Rhamnose	+	+	−	+	+
Sucrose	−	+	−	+	+
Xylose	+	+	d	+	+

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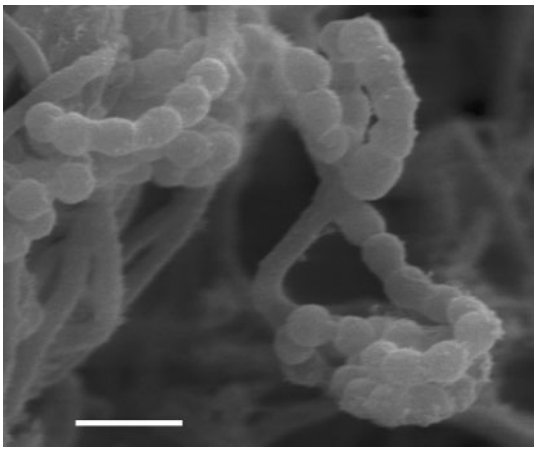

Fig. 1. Scanning electron micrograph of strain Pol001^T grown on ISP2 at 30 °C for 21 days. Bar, 2 μm.

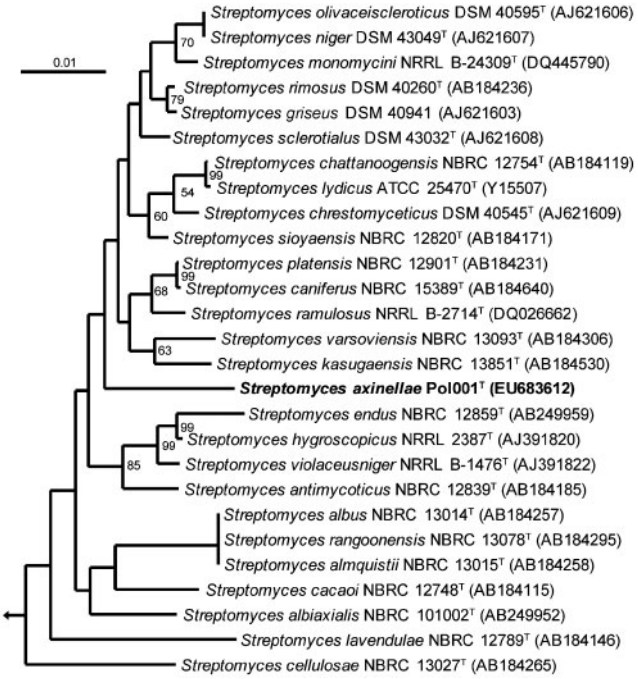

Fig. 2. Neighbour-joining tree of strain Pol001^T and representative species of the genus *Streptomyces* based on nearly complete (1422 nt) 16S rRNA gene sequences. Numbers at nodes indicate levels of bootstrap support based on 1000 resampled datasets. Only values greater than 50 % are shown. The arrow points to the outgroup, *Salinispora tropica* CNB-536 (GenBank accession no. AY040618). Bar, 0.01 substitutions per nucleotide position.

Scanning electron microscopy of strain Pol001^T was performed as described previously (Scheuermayer *et al.*, 2006). Strain Pol001^T exhibited morphological properties that were characteristic of *Streptomyces* species, forming extensively branched substrate and aerial mycelia without fragmentation. The morphology of the spores and spore chains was described according to Pridham *et al.* (1958). Scanning electron microscopy revealed long spiral chains of spores with a smooth surface (Fig. 1). The spores were elliptical in shape and 0.8–0.9 μm in length.

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 (1422 nt) was generated for the strain and compared to sequences of members of the genus *Streptomyces* with validly published names. The sequences were then aligned using CLUSTAL W and phylogenetic analysis was performed using the ARB software (Ludwig *et al.*, 2004). Phylogenetic tree construction was performed using the neighbour-joining algorithm with bootstrap values based on 1000 replications (Fig. 2). Phylogenetic analysis revealed that strain Pol001^T exhibited highest sequence similarities with *Streptomyces sclerotialus* DSM 43032^T (97.61 %), *Streptomyces rimosus* subsp. *rimosus* DSM 40260^T (97.47 %), *Streptomyces niger* DSM 43049^T (97.20 %) and *Streptomyces olivaceiscleroticus* DSM 40595^T (97.20 %).

DNA–DNA hybridization between strain Pol001^T and closely related strains selected on the basis of their 16S rRNA gene sequence similarities was carried out by the DSMZ Identification Service. Levels of DNA–DNA relatedness between strain Pol001^T and the type strains of four closely related species were as follows (means of two values): *S. sclerotialus* DSM 43032^T, 26.8 %; *S. olivaceiscleroticus* DSM 40595^T, 16.9 %; *S. niger* DSM 43049^T, 8.75 %; and *S. rimosus* subsp. *rimosus* DSM 40260^T, 8.65 %. The hypothesis for the species concept in the genus *Streptomyces* is that strains of the same species have DNA relatedness >70 % (with a ΔT_m of <5 °C) (Anderson & Wellington, 2001; Wayne *et al.*, 1987). The low DNA–DNA relatedness values further confirmed that strain Pol001^T can be considered a novel taxon.

Strain Pol001^T can be differentiated clearly from all other species of the genus *Streptomyces* with validly published names based on a combination of phenotypic characteristics and genotypic data. It is concluded that Pol001^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces axinellae* sp. nov. is proposed.

Description of *Streptomyces axinellae* sp. nov.

Streptomyces axinellae (a.xi.nel′lae. N.L. gen. n. *axinellae* of *Axinella*, referring to the isolation of the type strain from the marine sponge *Axinella polypoides*).

Aerobic, Gram-positive actinomycete that forms extensively branched non-fragmenting substrate and aerial mycelia. Spores have a smooth surface and occur in spiral chains. No diffusible pigments are produced except on ISP7 medium. Growth occurs at 20–37 °C (optimum 30 °C) and in ISP2 medium containing 0–7.5 % NaCl (optimum 0–2.5 %) or 0–100 % ASW. Utilizes a variety of organic compounds as carbon

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sources including *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-glucosamine, *N*-acetyl-L-glutamic acid, L-alaninamide, L-alanine, L-alanyl glycine, D-arabitol, cellobiose, dextrin, D-fructose, D-galactose, gentiobiose, D-gluconic acid, α-D-glucose, L-glutamic acid, glycerol, DL-α-glycerol phosphate, α-D-lactose, L-malic acid, D-mannitol, D-mannose, propionic acid, L-rhamnose, D-ribose, L-serine, Tweens 40 and 80 and D-xylose, but not acetic acid, adenosine, AMP, D-alanine, amygdalin, L-arabinose, arbutin, L-asparagine, 2,3-butanediol, α- or β-cyclodextrin, 2′-deoxyadenosine, D-fructose 6-phosphate, L-fucose, D-galacturonic acid, α-D-glucose 1-phosphate, D-glucose 6-phosphate, glycogen, glycyl L-glutamic acid, α-, β- or γ-hydroxybutyric acids, *p*-hydroxyphenylacetic acid, inosine, *myo*-inositol, inulin, α-ketoglutaric acid, α-ketovaleric acid, lactamide, L-lactic acid, D-lactic acid methyl ester, lactulose, D-malic acid, maltose, maltotriose, mannan, melezitose, melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl D-glucose, methyl α-D-glucoside, methyl β-D-glucoside, methyl α-D-mannoside, palatinose, D-psicose, putrescine, L-pyroglutamic acid, pyruvic acid, pyruvic acid methyl ester, raffinose, salicin, sedoheptulosan, L-serine, D-sorbitol, stachyose, succinamic acid, succinic acid, succinic acid monomethyl ester, sucrose, D-tagatose, thymidine, TMP, trehalose, turanose, uridine, UMP or xylitol. Positive for gelatin liquefaction, but negative for melanin production, starch hydrolysis, nitrate reduction and hydrogen sulfide production. Able to degrade casein, but not adenine, chitin or hypoxanthine. The cell wall contains LL-A_2pm. Whole-cell hydrolysates contain glucose, ribose and traces of mannose. Major menaquinone is MK-9(H_6). Phospholipid pattern consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, some unidentified phospholipids, phosphoglycolipids, glycolipids and an aminolipid. Fatty acid pattern consists of iso-$C_{16:0}$, anteiso-$C_{15:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:1}$, iso-$C_{14:0}$ and iso-$C_{17:1}$.

The type strain is Pol001^T (=DSM 41948^T =CIP 109838^T), isolated from the Mediterranean sponge *Axinella polypoides*. The DNA G+C content of the type strain is 71.0 mol%.

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