Streptomyces coacervatus sp. nov., isolated from the intestinal tract of *Armadillidium vulgare*

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A Gram-staining-positive bacterium, designated AS-0823^T, which formed spiral spore chains on the aerial mycelium, was isolated from the intestinal tract of *Armadillidium vulgare*, a small terrestrial crustacean commonly found on the ground around houses in Japan. 16S rRNA gene sequence analysis showed that the isolate belonged to the genus *Streptomyces* and was most closely related to *Streptomyces longisporus* ISP 5166^T (98.6% 16S rRNA gene sequence similarity), *Streptomyces curacoi* NBRC 12761^T (98.4%) and *Streptomyces griseoruber* NBRC 12873^T (98.4%). The affiliation of strain AS-0823^T to the genus *Streptomyces* was supported by chemotaxonomic data: iso-C_{16:0}, anteiso-C_{15:0}, C_{16:0}, iso-C_{15:0} and anteiso-C_{17:0} as the major cellular fatty acids, LL-diaminopimelic acid as the characteristic diamino acid in the peptidoglycan and the absence of mycolic acids. DNA–DNA hybridization and physiological and biochemical analysis supported the differentiation of strain AS-0823^T from *S. longisporus* JCM 4395^T. Therefore, strain AS-0823^T represents a novel species, for which the name *Streptomyces coacervatus* sp. nov. is proposed. The type strain is AS-0823^T (=IFM 11055^T =DSM 41983^T =JCM 17138^T).

The genus *Streptomyces* includes nearly 600 species with validly published names (<http://www.bacterio.cict.fr/s/streptomyces.html>). Streptomycetes are aerobic, Gram-positive actinomycetes, of which most form an extensively branched substrate mycelium and aerial hyphae that typically differentiate into chains of spores (Anderson & Wellington, 2001). Streptomycetes are a rich source of novel bioactive compounds. To find new strains able to produce useful bio-products, we attempted to isolate strains from unusual environments. We isolated a novel *Streptomyces* strain, designated AS-0823^T, from the intestinal tract of *Armadillidium vulgare* (common pill woodlouse), a small terrestrial crustacean commonly found on the ground around houses in Japan that characteristically curls into a ball when threatened. The culture broth of strain AS-0823^T showed antifungal activity against *Candida albicans* and *Aspergillus niger*.

After collection of *Armadillidium vulgare* in Chiba City, Japan, the intestinal tract of the animal was dissected and washed. The tract contents were spread over brain heart

infusion (BHI) agar (Difco) containing 2% glucose and 2% glycine and incubated at 28 °C. A colony of strain AS-0823^T was obtained after 7 days. The isolate grew well on all tested International *Streptomyces* Project (ISP) media (ISP 2–7; Shirling & Gottlieb, 1966), yeast-starch agar (Luedemann, 1971) and Bennett's agar (Jones, 1949) when incubated at 28 °C for 2 weeks. Particularly good growth was observed on ISP 4 and yeast-starch agar at 25–30 °C. Cell morphology was observed under a light microscope (Nikon) at ×400 magnification after incubation on each agar at 28 °C. On all media, strain AS-0823^T produced a cream substrate mycelium and a cream aerial mycelium with white spores by day three. When cultured on agar or in liquid medium for longer than 2 weeks, the formation of an intracellular purple pigment was observed. In contrast, the closest phylogenetic relative, *Streptomyces longisporus* JCM 4395^T, produced a white substrate mycelium and a white aerial mycelium with white spores in all tested ISP media and grew well on yeast-starch agar and Bennett's agar at 25–30 °C; no pigment was formed during culture for several weeks. Observation with a scanning electron microscope (S-3400N; Hitachi; Kageyama *et al.*, 2004a, b) revealed that strain AS-0823^T produced a sparse aerial mycelium and spiny spores in spiral spore chains (Fig. 1), which contrasted clearly with the smooth spores in *rectiflexibiles* spore chains of *S. longisporus* (Al-Bari

Abbreviations: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IFM 11055^T is AB500703.

Fig. 1. Scanning electron micrograph of strain AS-0823^T, showing sparse aerial mycelium and spiral chains of spiny spores. The isolate was cultured on yeast-starch agar at 28 °C for 20 days. Bar, 5 μm.

et al., 2005). These characteristics apparently differentiate the two strains. Strain AS-0823^T was Gram-staining-positive using the method of Murray *et al.* (1994).

The 16S rRNA gene sequence was analysed as described by Kageyama *et al.* (2004a, b) using strains of related species of the genus *Streptomyces* that were selected using EzTaxon (Chun *et al.*, 2007). Phylogenetic analysis was performed using MEGA4 (Tamura *et al.*, 2007) after multiple alignment of data using CLUSTAL X version 2.0 (Thompson *et al.*, 1994). Genetic distances were calculated using distance options according to the Kimura two-parameter model, Kimura & Ohta, 1972) and clustering was determined with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods and evaluated using bootstrap values based on 1000 replications (Felsenstein, 1992; Fig. 2). The 16S rRNA gene sequence of strain AS-0823^T was a continuous stretch of 1496 bp. Sequence similarity calculations indicated that the closest relatives of the isolate were *S. longisporus* ISP 5166^T (98.6 % 16S rRNA gene sequence similarity), *Streptomyces curacoi* NBRC 12761^T (98.4 %) and *Streptomyces griseoruber* NBRC 12873^T (98.4 %, Chun *et al.*, 2007).

Analysis of the isomer of diaminopimelic acid (A₂pm) was performed using hydrolysates of whole bacterial cells by TLC (Staneck & Roberts, 1974). The whole-cell sugar pattern was analysed using whole-cell hydrolysates as described elsewhere (Lechevalier & Lechevalier, 1980; Miyadoh, 2001). Mycolic acids were analysed by TLC (Minnikin *et al.*, 1980) and GC (Springer *et al.*, 1993; Klatte *et al.*, 1994). For analysis of fatty acids, cells were cultured

on 0.5 % glucose, 0.5 % starch and half-strength WL Nutrient Medium (Difco Laboratories) at 27 °C for 48 h. Fatty acid methyl esters were prepared and analysed using the standard Microbial Identification System (MIDI) as described elsewhere (Klatte *et al.*, 1994; Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The fatty acid profile comprised iso-C_{16:0} (22.5 %), anteiso-C_{15:0} (19.1 %), C_{16:0} (12.0 %), iso-C_{15:0} (11.7 %), anteiso-C_{17:0} (10.3 %), iso-C_{17:0} (6.7 %), iso-C_{14:0} (4.2 %), C_{15:0} (3.5 %), C_{14:0} (1.2 %), iso-C_{17:1} (1.1 %), anteiso-C_{17:1} (0.5 %), cyclo-C_{17:0} (0.4 %), iso-C_{16:1} (0.4 %) and iso-C_{18:0} (0.3 %). Other results of the chemotaxonomic analysis are given in the species description. The chemotaxonomic characteristics were consistent with the assignment of strain AS-0823^T to the genus *Streptomyces*.

The DNA G+C content of strain AS-0823^T was determined by HPLC (Tamaoka & Komagata, 1984) and was calculated to be 73.5 mol%, which supported the affiliation of the isolate to the order *Actinomycetales*. DNA–DNA hybridization was performed by the photobiotin method as described by Ezaki *et al.* (1989). DNA–DNA relatedness between strain AS-0823^T and *S. longisporus* JCM 4395^T was 15.5 ± 0.5 % (SD, *n*=2), which was well below the cut-off point of 70 % for species delineation that was recommended by Wayne *et al.* (1987).

Because *S. curacoi* has synonymy with *S. coeruleorubidus* (Labeda & Lyons, 1991; also see <http://www.jcm.riken.go.jp/>), phenotypic characterization of strain AS-0823^T was performed alongside *S. coeruleorubidus* JCM 4359^T as well as *S. longisporus* JCM 4395^T and *S. griseoruber* JCM 4642^T. Carbon source utilization tests were performed as described by Shirling & Gottlieb (1966). Inhibitory concentrations of NaCl were determined as described by Tresner *et al.* (1968), using ISP 4 as the basal medium. Nitrate reduction was tested using nitrate broth (Eiken Chemical Co.) according to the manufacturer's instructions. Hydrogen sulfide production was determined as described by Tresner & Danga (1958). The results are presented in Table 1. Strain AS-0823^T could be distinguished from *S. longisporus* JCM 4395^T and the other type strains by utilization of several carbon sources, growth temperature, inhibitory concentration of NaCl, effect of pH on growth, nitrate reduction, melanin and hydrogen sulfide production and mycelium colour (Kämpfer *et al.*, 1991).

We verified whether the isolate produced bioactive-substance(s) with anti-fungal activities. The isolate was cultured in WL Nutrient Medium containing 0.1 % glycerol and 1 % soluble starch at 28 °C for 3 days. A methanol extract of the cells was assayed using the paper disc agar diffusion method (NCCLS, 1995) with *Aspergillus niger* IFM 5368 and *C. albicans* ATCC 90028 as reference strains. A biologically active

Fig. 2. Neighbour-joining phylogenetic tree, based on 1436 bp 16S rRNA gene sequences, showing the relationships between *Streptomyces coacervatus* sp. nov. IFM 11055^T and closely related strains of species of the genus *Streptomyces*. Bootstrap values based on 1000 replications are shown at branch nodes. Asterisks indicate that the corresponding nodes were recovered in trees generated with the maximum-parsimony algorithm. Bar, 5 substitutions per 1000 nucleotide positions.

substance was separated further using a silica gel column. The UV spectrum of the active fraction showed peaks of absorbance at 318–321, 333–337 and 350–354 nm in methanol, which suggested that the bioactive compound contained a pentane molecular structure (Okami *et al.*, 1954).

Phylogenetic, chemotaxonomic and phenotypic analysis demonstrated that strain AS-0823^T belongs to the genus *Streptomyces* but is not a member of any of the recognized species in the genus. Therefore, strain AS-0823^T is classified in a novel species, for which the name *Streptomyces coacervatus* sp. nov. is proposed.

Table 1. Characteristics differentiating strain AS-0823^T from closely related type strains of species of the genus *Streptomyces*

Strains: 1, *Streptomyces coacervatus* sp. nov. AS-0823^T; 2, *S. longisporus* JCM 4395^T; 3, *S. coeruleorubidus* JCM 4359^T; 4, *S. griseoruber* JCM 4642^T. All data were taken from this study. All strains degrade adenine, hypoxanthine, starch and gelatin and grow well on L-asparagine and L-cysteine (1%, w/v) as sole nitrogen sources, on D-fructose, sucrose, L-rhamnose and inositol as sole carbon sources, at 20 °C and at pH 6–9. None of the strains produce diffusible pigment on ISP 5. ++, Good growth; +, growth; –, no growth.

Characteristic	1	2	3	4
Sole carbon sources				
L-Arabinose	++	–	++	+
D-Xylose	++	++	+	–
Raffinose	++	++	++	–
D-Mannitol	++	++	++	–
Growth at:				
10 °C	+	–	–	–
28 °C	++	++	+	++
37 °C	++	++	+	+
Growth with:				
4% NaCl	++	++	++	–
7% NaCl	–	–	+	–
Growth at (ISP 4):				
pH 5	–	–	+	–
pH 10	–	+	+	+
pH 11	–	–	+	+
Nitrate reduction	+	–	++	–
Melanin production on:				
ISP 6	–	–	+	++
ISP 7	–	+	–	+
H ₂ S production	–	–	+	++
Mycelium colour (ISP 4)*				
Aerial	C	W	T	YW
Substrate	C	W	BR	P
Diffusible pigment on:*				
ISP 6	None	None	BL	BR

*BL, Black; BR, brown; C, cream; P, pink; T, turquoise; YW, yellowish white.

Description of *Streptomyces coacervatus* sp. nov.

Streptomyces coacervatus (co.a.cer.va'tus. L. masc. part. adj. *coacervatus* heaped together, heaped up, collected in a mass, referring to the colony morphology on agar plates).

Aerobic, Gram-staining-positive actinomycete that forms an extensively branched substrate mycelium and sparse aerial hyphae that differentiate into spiral chains of spores. Growth occurs at 10–37 °C, at pH 6–9 and with 4% NaCl. Good growth occurs on all tested ISP media (ISP 2–7), yeast-starch agar and Bennett's agar at 28 °C. Cream aerial mycelia are formed on all media. Purple pigments are formed during cultivation at 28 °C for 2 weeks. Contains LL-diaminopimelic acid, but not meso-diaminopimelic acid, mycolic acid or characteristic sugars in the cell wall (wall chemotype I *sensu* Lechevalier & Lechevalier, 1980). The fatty acids are iso-C_{16:0}, anteiso-C_{15:0}, C_{16:0}, iso-C_{15:0}, anteiso-C_{17:0}, iso-C_{17:0}, iso-C_{14:0}, C_{15:0}, C_{14:0}, iso-C_{17:1}, anteiso-C_{17:1}, cyclo-C_{17:0}, iso-C_{16:1} and iso-C_{18:0}. Degrades adenine, hypoxanthine, starch and gelatin, reduces nitrate and utilizes L-arabinose, D-xylose, raffinose, D-mannitol, D-fructose, sucrose, L-rhamnose and inositol. The DNA G+C content of the type strain is 73.5 mol%.

The type strain is AS-0823^T (=IFM 11055^T =DSM 41983^T =JCM 17138^T), isolated from the intestinal tract of *Armadillidium vulgare* in Chiba City, Japan.

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