Actinocatenispora rupis sp. nov., isolated from cliff soil, and emended description of the genus *Actinocatenispora*

Seong Hae Seo and Soon Dong Lee

Correspondence
Soon Dong Lee
sdlee@cheju.ac.kr

Department of Science Education, Cheju National University, Jeju 690-756, Republic of Korea

A novel actinomycete, designated strain CS5-AC17^T, was isolated from cliff soil in the Republic of Korea. Cells of the organism were aerobic and catalase- and oxidase-negative. Substrate mycelium was well developed and was pale to strong yellow. Spore chains were borne on rudimentary aerial mycelium. The spores (0.4–0.5×0.9–1.6 µm) were smooth surfaced and cylindrical. Growth occurred at 25–42 °C, at pH 5.1–12.1 and in the presence of up to 4 % NaCl. Phylogenetic analyses based on 16S rRNA gene sequences indicated that the isolate was closely related to members of the genus *Actinocatenispora* in the family *Micromonosporaceae*. The phylogenetic neighbours were *Actinocatenispora thailandica* TT2-10^T (97.7 % 16S rRNA gene sequence similarity) and *Actinocatenispora sera* KV-744^T (97.6 %). The diagnostic diamino acid in the cell-wall peptidoglycan was *meso*-diaminopimelic acid. The major respiratory quinones were MK-9(H₄), MK-9(H₆) and MK-9(H₈). The polar lipids contained phosphatidylethanolamine and phosphatidylinositol with a small amount of phosphatidylglycerol. The DNA G+C content was 74.3 mol%. DNA–DNA relatedness values between strain CS5-AC17^T and its phylogenetically closest relatives were 6.1–7.1 % (*A. thailandica* DSM 44816^T) and 21.5–27.6 % (*A. sera* NRRL B-24477^T). On the basis of the phenotypic features and the DNA–DNA hybridization data, strain CS5-AC17^T (=DSM 45178^T=NRRL B-24660^T) represents a novel species of the genus *Actinocatenispora*, for which the name *Actinocatenispora rupis* sp. nov. is proposed.

The genus *Actinocatenispora* was originally proposed by Thawai *et al.* (2006) to encompass *Actinocatenispora thailandica*, which bears spore chains on aerial mycelium, as a member of the family *Micromonosporaceae*. Another species, *Actinocatenispora sera*, which produces aerial spores directly from the substrate mycelium, has subsequently been added to the genus (Matsumoto *et al.*, 2007). The aim of this study is to describe the classification and identification of a mycelium-forming actinomycete.

Strain CS5-AC17^T was isolated from soil from a cliff of Mara Island, Jeju, Republic of Korea. Bacterial isolation and maintenance were performed as described previously (Lee, 2006). For phenotypic comparisons and DNA–DNA hybridizations, *A. thailandica* DSM 44816^T and *A. sera* NRRL B-24477^T were grown on yeast extract-malt extract agar (ISP 2; Shirling & Gottlieb, 1966) at 30 °C.

Growth on various culture media was investigated by using ISP 2, oatmeal agar (ISP 3), inorganic salts-starch agar

(ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6), tyrosine agar (ISP 7) (Shirling & Gottlieb, 1966) and oatmeal-nitrate agar (Prauser & Bergholz, 1974). Cultural characteristics were recorded after incubation for 14 days at 30 °C. Cell morphology was observed with a scanning electron microscope (JSM-6500; JEOL) and cells grown for 14 days at 30 °C. After fixation with 1 % osmium tetroxide, specimens were dehydrated and critical-point-dried as described previously (Lee, 2006).

Growth on ISP 2 was tested at 4, 10, 25, 30, 37 and 42 °C and at pH 4.1–12.1 (at intervals of 1.0 pH unit); for pH experiments, the pH of the medium was adjusted after autoclaving with 6 M HCl or 10 M NaOH. Tolerance of NaCl for growth was examined on ISP 2 supplemented with 1–9 % (w/v) NaCl. The results were observed and recorded after incubation for 2 weeks at 30 °C. Oxidase activity was investigated by assessing the oxidation of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride. Gram staining was performed as described by Collins *et al.* (1995). The following tests were performed as described by Lee (2006): nitrate reduction, catalase and urea activities, gelatin liquefaction and degradation of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CS5-AC17^T is AM980986.

Scanning electron micrographs of growth of strain CS5-AC17^T are available as supplementary material with the online version of this paper.

casein, aesculin, hypoxanthine, starch, DL-tyrosine and xanthine. Utilization of various carbohydrates was determined by using ISP 9 (Shirling & Gottlieb, 1966), with each filter-sterilized carbon source at a final concentration of 1 % (w/v). Degradation of chitin (0.5 %, w/v), elastin (0.4 %, w/v) and Tween 80 (1 %, v/v) was examined using ISP 2 as the basal medium. Hydrolysis of DNA was tested on DNase test agar (Difco).

Chemotaxonomic characteristics of strain CS5-AC17^T were determined by using freeze-dried biomass obtained from cultures grown in YMG broth (0.4 % yeast extract, 1 % malt extract and 0.4 % glucose) for 3 days at 30 °C with shaking. Analyses of the isomer of diaminopimelic acid and the acyl type of the muramic acid were performed according to the methods of Stanek & Roberts (1974) and Uchida & Aida (1984), respectively. Whole-cell sugars were analysed by GC as described by Saddler *et al.* (1991). Menaquinones were determined by HPLC (Kroppenstedt, 1985). Analysis of polar lipids by TLC was performed as described by Minnikin *et al.* (1977). The G + C content of the DNA was determined by the method of Mesbah *et al.* (1989). For cellular fatty acid analysis, strain CS5-AC17^T,

A. thailandica DSM 44816^T and *A. sera* NRRL B-24477^T were grown on ISP 2 for 7 days at 30 °C. Fatty acid methyl esters were extracted and analysed by using the Microbial Identification System version 6 (MIDI, Inc.), according to the manufacturer's instructions.

Chromosomal DNA was extracted and purified according to the method of Hopwood *et al.* (1985). The 16S rRNA gene of strain CS5-AC17^T was amplified by PCR using the universal primers 27f (5'-AGAGTTTGATCMTGGCT-CAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT-3'). The reaction mixture for PCR (final volume 100 µl) contained 50 ng genomic DNA, 2 U DyNAzyme, 1 × DyNAzyme buffer (Finnzymes), 0.2 mM dNTPs and 2 µM each primer. The PCR cycling protocol was as follows: an initial denaturation step at 95 °C for 5 min, 30 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 3 min and a final extension step at 72 °C for 20 min. The resultant PCR product was purified using Wizard PCR Preps DNA Purification System (Promega) and subjected to direct sequencing by using an ABI PRISM BigDye Terminator cycle sequencing kit and an automatic DNA sequencer (3730xl; Applied Biosystems).

Table 1. Differential characteristics of strain CS5-AC17^T and the type strains of the genus *Actinocatenispora*

Strains: 1, *Actinocatenispora rupis* sp. nov. CS5-AC17^T (data from this study); 2, *A. sera* NRRL B-24477^T (Matsumoto *et al.*, 2007); 3, *A. thailandica* DSM 44816^T (Thawai *et al.*, 2006). All of the strains utilized D-glucose as a sole carbon source but not D-fructose. +, Positive; w, weak; –, negative.

Characteristic	1	2	3
Growth at/with:			
42 °C	+	–	–
pH 4.5	–	–	+
pH 12.1	+	–	+
7 % NaCl	–	–	+
Nitrate reduction	–	+	+
Gelatin liquefaction	+	–	w
Utilization of:			
L-Arabinose	+	–	–
Cellobiose	+	–	+
Glycerol	+	–	+
myo-Inositol	+	–	+
D-Mannitol	+	–	+
Melibiose	–	–	+
Raffinose	–	–	+
L-Rhamnose	+	+	–
Trehalose	+	–	+
D-Xylose	–	+	+
Menaquinones	MK-9(H _{4,6,8})	MK-9(H ₄)	MK-9(H _{4,6})
Whole-cell sugars*	Ara, Glc, Rha, Rib, Xyl	Ara, Gal, Xyl	Ara, Gal, Glc, Man, Rib, Xyl
Phospholipid(s)†	PE, PG, PI	PE	DPG, PE, PI, PIM, UP
DNA G + C content (mol%)	74.3	72	72

*Ara, Arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose.

†DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; UP, unidentified ninhydrin-negative phospholipid.

Table 2. Cellular fatty acid compositions of strain CS5-AC17^T and the type strains of the genus *Actinocatenispora*

Strains: 1, *A. rupis* sp. nov. CS5-AC17^T; 2, *A. sera* NRRL B-24477^T; 3, *A. thailandica* DSM 44816^T. Values are percentages of total fatty acids. Data were taken from this study; values in parentheses were taken from Thawai *et al.* (2006) and Matsumoto *et al.* (2007). Fatty acids less than 1% were omitted; —, not detected. ai, Anteiso-branched; i, iso-branched; ECL, equivalent chain-length.

Fatty acid (%)	1	2	3
Saturated fatty acids			
C _{16:0}	2.6	2.1 (—)	2.9 (—)
C _{18:0}	2.3	2.8 (—)	2.5 (—)
Branched fatty acids			
i-C _{14:0}	6.8	3.6 (—)	2.5 (—)
i-C _{15:0}	3.8	15.7 (14.2)	7.3 (20.2)
i-C _{16:0}	39.2	37.9 (21.6)	29.4 (26.2)
i-C _{16:1} G	12.8	7.0 (—)	11.1 (—)
i-C _{17:0}	1.0	5.7 (15.5)	5.6 (11.3)
ai-C _{15:0}	8.2	4.1 (—)	5.5 (—)
ai-C _{17:0}	15.2	12.3 (24.6)	19.7 (19.3)
ai-C _{17:1} A	3.1	2.1 (—)	6.4 (—)
Hydroxy fatty acids			
i-C _{14:0} 3-OH	—	2.3 (9.2)	1.9 (—)
C _{14:0} 2-OH	—	—	1.4 (—)
Summed features*			
i-C _{17:1} I/ai-C _{17:1} B	—	2.1	—
C _{18:1} ω9c/ω12t/ω7c	—	—	1.0
Unknown ECL 15.665	—	—	1.2

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System.

A partial 16S rRNA gene sequence (1408 nt) of strain CS5-AC17^T was subjected to a preliminary BLAST search against the GenBank database, which revealed that the isolate was closely related to members of the genus *Actinocatenispora* in the family *Micromonosporaceae*. The CLUSTAL X program (Thompson *et al.*, 1997) was used to perform multiple alignments with 16S rRNA gene sequences from strain CS5-AC17^T and related taxa. Phylogenetic analyses were performed with the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A neighbour-joining tree was developed from evolutionary distances that were calculated by using the model of Jukes & Cantor (1969). Bootstrap analysis was performed as described by Felsenstein (1985) with 1000 neighbour-joining datasets.

Strain CS5-AC17^T grew well on ISP 2 and oatmeal-nitrate agar, moderately well on ISP 3 and ISP 6 and poorly on ISP 4, ISP 5 and ISP 7. Substrate mycelium (0.2 µm in diameter) was well developed and pale to strong yellow in colour. Supplementary Fig. S1 (available in IJSEM Online) shows scanning electron micrographs of growth of strain CS5-AC17^T after 3–4 weeks at 30 °C. Rudimentary aerial mycelium (0.4 µm in diameter) was formed only on ISP 3

Fig. 1. Neighbour-joining tree showing the position of strain CS5-AC17^T within the radiation of the family *Micromonosporaceae*, based on an alignment of 1408 nucleotides from the 16S rRNA gene sequences. Bootstrap values ($\geq 50\%$) based on 1000 resamplings are shown at branch nodes. Asterisks indicate branches that were also recovered by both the maximum-likelihood and maximum-parsimony methods. Bar, 0.01 substitutions per nucleotide position.

and oatmeal-nitrate agar. Spore chains developed on aerial mycelium and contained fewer than six spores; the spores were cylindrical (0.4–0.5 × 0.9–1.6 µm) and smooth-surfaced. Hyphal swellings (0.5 µm in diameter) on tips of the vegetative mycelium were also observed on ISP 5 and 6 but seemed to be smaller than the spores (0.8–1.5 µm) of *Micromonospora* strains (Kawamoto, 1989). Diffusible pigments were not produced on any of the media tested. Data for physiological and biochemical properties are given in the species description and Table 1.

Whole-cell hydrolysates of strain CS5-AC17^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid and glucose, rhamnose, ribose, arabinose and xylose as the whole-cell sugars. Galactose was not detected, in contrast to the type strains of the genus *Actinocatenispora*. The menaquinone profile contained MK-9(H₄), MK-9(H₆) and MK-9(H₈) (peak ratio 34:38:28). The polar lipids were phosphatidylethanolamine and phosphatidylinositol with a small amount of phosphatidylglycerol. The DNA G+C content of strain CS5-AC17^T was 74.3 mol%. The fatty acid profile of CS5-AC17^T was represented by iso- and anteiso-branched acids, with small amounts of saturated components, and the predominant fatty acids were iso-C_{16:0}, anteiso-C_{17:0} and iso-C_{16:1} G. The cellular fatty acid profiles of the organism and the type strains of the genus *Actinocatenispora* are given in Table 2.

A neighbour-joining tree (Fig. 1), based on 16S rRNA gene sequences, showed that strain CS5-AC17^T formed a distinct

clade at the base of the *A. thailandica*–*A. sera* cluster, with bootstrap support of 100 %. The branching pattern was also found in the trees of maximum-parsimony and maximum-likelihood methods (not shown). The closest relatives were *A. thailandica* TT2-10^T (97.7 % 16S rRNA gene sequence similarity) and *A. sera* KV-744^T (97.6 %). Levels of 16S rRNA gene sequence similarity of strain CS5-AC17^T with the other recognized species of the family *Micromonosporaceae* ranged from 92.5 to 94.4 %.

DNA–DNA hybridization experiments were performed using the initial renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983). DNA relatedness values between strain CS5-AC17^T and the phylogenetically closest relatives were 6.1–7.1 % (*A. thailandica* DSM 44816^T) and 21.5–27.6 % (*A. sera* NRRL B-24477^T). These values are distinctly lower than the 70 % threshold value proposed to delineate a genospecies (Wayne *et al.*, 1987) and show that strain CS5-AC17^T belongs to a different species of the genus *Actinocatenispora*.

Strain CS5-AC17^T is distinguishable from both *A. sera* NRRL-B24477^T and *A. thailandica* DSM 44816^T with respect to its abilities to grow at 42 °C and to utilize L-arabinose as a sole carbon source and its inability to reduce nitrate to nitrite or to utilize D-xylose. The isolate differs chemotaxonomically from the type strains of the genus *Actinocatenispora* in having MK-9(H₈) as a major menaquinone and phosphatidylglycerol as a polar lipid. Other phenotypic features that differentiate strain CS5-AC17^T from the type strains of the genus *Actinocatenispora* are given in Table 1.

On the basis of the phenotypic features and the DNA–DNA hybridization data, strain CS5-AC17^T is considered to represent a novel species of the genus *Actinocatenispora*, for which the name *Actinocatenispora rupis* sp. nov. is proposed.

Emended description of the genus *Actinocatenispora* Thawai *et al.* 2006

The description is as given by Thawai *et al.* (2006) with the following amendments. Spore chains are borne on the aerial mycelium or produced directly from the vegetative mycelium. Characteristic whole-cell sugars are arabinose and xylose. The presence of galactose as a whole-cell sugar is variable, depending on the strain. MK-9(H₄) is a major menaquinone; the presence of MK-9(H₆) and MK-9(H₈) is strain-dependent. The predominant fatty acids are iso- and anteiso-methyl branched acids. The DNA G + C content is 72.0–74.3 mol%.

Description of *Actinocatenispora rupis* sp. nov.

Actinocatenispora rupis (ru'pis. L. gen. n. *rupis* of a cliff, referring to the site from which the type strain was isolated).

Aerobic, Gram-staining-positive, catalase- and oxidase-negative. Vegetative mycelium is well developed and pale to

strong yellow in colour. Spore chains are borne on rudimentary aerial mycelium. The spores are smooth-surfaced and cylindrical (0.4–0.5 × 0.9–1.6 µm). Hyphal swellings are observed on the tips of the vegetative mycelium. Soluble pigments are not produced on any of the tested media. Growth occurs at 25–42 °C (optimum 37 °C) and pH 5.1–12.1 (optimum pH 5.1–9.1). Tolerates up to 4 % NaCl. Degrades DNA, elastin, aesculin, casein, Tween 80 and chitin, but not cellulose, starch, hypoxanthine, tyrosine, urea or xanthine. Utilizes D-arabinose, dextran, D-galactose, lactose, maltose, D-mannose, methyl α-D-glucoside, sucrose, adonitol, *meso*-erythritol, D-sorbitol and citrate as sole carbon sources, but not inulin, melezitose, methyl α-D-mannoside, salicin, L-sorbose, dulcitol, D-xylitol, acetate, benzoate, formate, malate, succinate or tartrate. Does not reduce nitrate to nitrite. Other physiological properties are given in Table 1. *meso*-Diaminopimelic acid is the diagnostic diamino acid in the cell-wall peptidoglycan. The major menaquinones are MK-9(H₄), MK-9(H₆) and MK-9(H₈). The whole-cell sugars are glucose, rhamnose, ribose, arabinose and xylose. The diagnostic polar lipids are phosphatidylethanolamine, phosphatidylinositol and phosphatidylglycerol. The predominant fatty acids are iso-C_{16:0}, anteiso-C_{17:0} and iso-C_{16:1} G. The DNA G + C content of the type strain is 74.3 mol%.

The type strain, CS5-AC17^T (=DSM 45178^T=NRRL B-24660^T), was isolated from soil taken from a cliff on Mara Island, Jeju, Republic of Korea.

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