Actinoalloteichus spitiensis sp. nov., a novel actinobacterium isolated from a cold desert of the Indian Himalayas

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An actinobacterial strain, RMV-1378^T, isolated from a cold desert of the Indian Himalayas, was subjected to polyphasic taxonomic characterization. The strain formed branching, non-fragmenting vegetative hyphae and did not produce diffusible pigments. Neither aerial mycelium nor spore formation was observed. The G+C content of the DNA was 72.0 mol%. The strain had chemotaxonomic characteristics typical of the genus *Actinoalloteichus* and was closely related (99.3% 16S rRNA gene sequence similarity) to *Actinoalloteichus cyanogriseus*, currently the only *Actinoalloteichus* species with a validly published name. However, the results of DNA–DNA hybridization experiments showed 51.9% relatedness with the type strain of *A. cyanogriseus*. On the basis of the above data and the physiological and biochemical distinctiveness of RMV-1378^T (=MTCC 6194^T=JCM 12472^T=DSM 44848^T), this strain should be classified as the type strain of a novel species of *Actinoalloteichus*, for which the name *Actinoalloteichus spitiensis* sp. nov. is proposed.

The genus *Actinoalloteichus* was first described by Liu *et al.* (1984) but the name of the genus was not validly published. Subsequently, Itoh *et al.* (1987) reported the morphological and chemotaxonomic characteristics of the genus, and its phylogenetic position was reported by Tamura & Hatano (1998) and Stackebrandt *et al.* (1997). The genus name was finally validly published by Tamura *et al.* (2000). At present, this genus accommodates only one species, *Actinoalloteichus cyanogriseus*. In this paper, we describe the polyphasic characterization of strain RMV-1378^T and propose it as the type strain of a novel species.

Strain RMV-1378^T was isolated from a soil sample collected from the Lahaul–Spiti Valley, a cold desert of the Indian Himalayas, by using a dilution plating technique on actinomycetes isolation agar (sodium caseinate, 0.2%; asparagine, 0.01%; sodium propionate, 0.4%; dipotassium phosphate,

0.05%; magnesium sulphate, 0.01%; ferrous sulphate, 0.0001%; agar, 1.5%; pH 8.1) and maintained as glycerol stocks at –70 °C. The type strain of *A. cyanogriseus* (MTCC 6195^T) was obtained from the Microbial Type Culture Collection, Chandigarh, India.

Cultural, physiological and biochemical characteristics (Table 1) of strain RMV-1378^T were examined by using standard procedures (Yokota *et al.*, 1993; Gordon *et al.*, 1974). Some tests were re-examined in this study as indicated in Table 1. To check growth at different pH values between 8.0 to 11.0, CM broth (yeast extract, 0.4%; malt extract, 1.0%; glucose, 0.4%) with different pH values along with a biological buffer system were used (a solution of Na₂CO₃, using 25% as stock, was used for pH adjustment). Freeze-dried cells for chemotaxonomic analyses were prepared after growth of the strains for 4 days at 30 °C in shake flasks containing trypticase soy broth. The whole-cell sugars were analysed by using the HPLC method of Mikami & Ishida (1983) and the diaminopimelic acid isomer was determined as described by Stanek & Roberts (1974). Phospholipids and menaquinones were extracted and analysed as described by Minnikin *et al.* (1984). The absence of mycolic acids was confirmed by TLC (Minnikin & Goodfellow, 1976). The *N*-acyl type of the muramic

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RMV-1378^T is AY426714.

Chemotaxonomic characteristics of strain RMV-1378^T and the type strain of *A. cyanogriseus* are available in a supplementary table in IJSEM Online.

Table 1. Differential characteristics for strain RMV-1378^T and the type strain of *A. cyanogriseus*

+, Positive; −, negative; W, weakly positive.

Characteristic	Strain RMV-1378 ^T	<i>A. cyanogriseus</i> MTCC 6195 ^T
Presence of aerial mycelium and spores	—	+
Utilization of:		
D-Glucose	—	+
Salicin	+	—
Sucrose	+	—
D-Maltose	—	+
D-Xylose	—	+
L-Rhamnose	—	+
D-Sorbitol	—	+
D-Raffinose	+	—
Sodium citrate	+	—
Sodium succinate	+	—
Decomposition of casein	+	—*
Growth in 7 % NaCl	—	+
Hydrolysis of starch	+	w*
Pigmentation in ISP-6	Absent	Black*
Menaquinone MK-9(H ₂) content (%)	Absent	9*
DNA G + C content (mol%)	72.0	72.5*

*Data from Tamura *et al.* (2000).

acid in the cell wall was determined by using the method of Uchida & Aida (1984). Fatty acid methyl ester analysis was performed by using GLC according to the instructions of the Microbial Identification System (MIDI) (Sasser, 1990).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene sequence, sequencing of the PCR products and determination of the phylogenetic position were carried out as described previously (Nakajima *et al.*, 1999). DNA-DNA hybridization was performed by using the membrane filter method (Tourova & Antonov, 1987) as

described by Reddy *et al.* (2000). The G + C content of the genomic DNA was determined using the HPLC method as described by Tamaoka & Komagata (1984).

The 16S rRNA gene sequence of strain RMV-1378^T generated in this work (1476 bases) was aligned, using the CLUSTAL_X program (Thompson *et al.*, 1997), with the 16S rRNA gene sequences of closely related strains (retrieved from the GenBank/EMBL/DDBJ databases); the alignment was corrected manually. A sequence-similarity search was done using the BLASTN program of GenBank (Altschul *et al.*, 1997). For the neighbour-joining analysis (Saitou & Nei, 1987), distances between sequences were calculated using Kimura's two-parameter model (Kimura, 1980). A bootstrap analysis was performed to assess the confidence limits of the branching (Felsenstein, 1985).

Strain RMV-1378^T showed a high level of 16S rRNA gene sequence similarity (99.3 %) to the sole member of the genus *Actinoalloteichus*, *A. cyanogriseus* (Fig. 1); sequence similarities to all other species (with validly published names) of related genera were below 95 %. The DNA–DNA hybridization experiments revealed 51.9 % relatedness (the mean of 50.1 and 53.9 %) between RMV-1378^T and the type strain of *A. cyanogriseus*, which is well below the 70 % value recommended for the delineation of bacterial species (Wayne *et al.*, 1987). Strain RMV-1378^T exhibited good growth on *Streptomyces* agar (malt extract, 1.0 %; yeast extract, 0.4 %; glucose, 0.4 %; calcium carbonate, 0.2 %; agar, 1.2 %). Neither aerial mycelium nor spore formation was observed on the media tested (ISP media 1, 2, 3, 4, 5, 6, 7 and 9, *Streptomyces* agar and actinomycetes isolation agar). No soluble pigment was observed on the above media. Greyish colonies developed on all media tested. Detailed phenotypic characteristics are presented in the species description. Strain RMV-1378^T differs substantially from *A. cyanogriseus* in terms of phenotypic characteristics (Table 1). Most of the chemotaxonomic data confirmed that strain RMV-1378^T belongs to the genus *Actinoalloteichus* (see Supplementary Table S1 available in IJSEM Online). The cell-wall peptidoglycan contained *meso*-diaminopimelic acid as a diagnostic amino acid. The whole-cell sugars comprised rhamnose, ribose, mannose, galactose and glucose. The acyl type of the

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene (1476 bases) sequences, showing the phylogenetic relationship between strain RMV-1378^T and other close relatives, including *A. cyanogriseus*. *Streptomyces bottropensis* was used as an outgroup. Bootstrap values (expressed as percentages of 100 replications) greater than 50% are given at the nodes. Bar, 1% sequence variation.

glycan chain of peptidoglycan was acetyl. The major menaquinone was MK-9(H₄) (82 %); MK-10(H₄) (9 %), MK-8(H₄) (5 %) and MK-9(H₆) (2 %) were also detected. The predominant cellular fatty acids were iso-branched C_{16:0} (33 %) and C_{15:0} (17 %) fatty acids and anteiso-branched C_{15:0} (7 %) and C_{17:0} (8 %) fatty acids. The phospholipid profile consisted of phosphatidylmonoethylethanolamine, phosphatidylinositol, phosphatidylglycerol, phosphatidylinositolmannosides and diphosphatidylglycerol.

On the basis of a combination of the genotypic and phenotypic data, strain RMV-1378^T is very clearly distinguishable from its phylogenetically closest relative, *A. cyanogriseus*. Therefore strain RMV-1378^T represents a novel species of the genus *Actinoalloteichus*, for which we propose the name *Actinoalloteichus spitiensis* sp. nov.

Description of *Actinoalloteichus spitiensis* sp. nov.

Actinoalloteichus spitiensis (spi.ti.en'sis. N.L. masc. adj. *spitiensis* pertaining to Spiti Valley, located in the Indian Himalayas, where the type strain was isolated).

Gram-positive, non-acid-fast, aerobic actinobacterium with branching, non-fragmenting vegetative hyphae. No aerial mycelium or spores produced. Positive for utilization of D-mannitol, D-raffinose, sucrose, salicin, sodium citrate and sodium succinate as sole carbon sources. Negative for utilization of D-glucose, D-maltose, *myo*-inositol, D-fructose, D-arabinose, D-xylose, L-rhamnose, D-mannose and D-sorbitol as sole carbon sources. Positive for decomposition of casein and negative for decomposition of urea. No growth occurs on MacConkey agar or in Sabouraud dextrose broth. Positive for hydrolysis of starch and negative for hydrolysis of hippurate and aesculin. Tolerates up to 2 % NaCl and grows at temperatures between 20 and 37 °C, with an optimum temperature of 25 °C; cannot grow at 15 or 42 °C. Growth occurs at initial pH values between 6 and 11, the optimum being pH 8.0. Chemotaxonomic characteristics correspond to those typical for the genus *Actinoalloteichus*. Contains major amounts of iso-branched C_{16:0} (33.0 %) and C_{15:0} (17.0 %) fatty acids and anteiso-branched C_{15:0} (7.0 %) and C_{17:0} (8.0 %) fatty acids. Contains a large amount of MK-9(H₄) (82 %) and does not contain MK-9(H₂). The G + C content of the DNA is 72.0 mol%.

The type strain, RMV-1378^T (=MTCC 6194^T=JCM 12472^T=DSM 44848^T), was isolated from soil 3600 m above sea level, at Rangrik Village in Spiti Valley, Himachal Pradesh, India.

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