Kitasatospora sampliensis sp. nov., a novel actinobacterium isolated from soil of a sugar-cane field in India

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Polyphasic characterization of an actinomycete strain VT-36^T isolated from a sugar-cane field soil sample collected in Punjab State, India, revealed that the strain belongs to the genus *Kitasatospora*. The strain's chemotaxonomic characters and G+C content of DNA (76.5 mol%) were typical of members of the genus. Analysis of the 16S rRNA gene sequence supported the generic affiliation of the strain and showed that its closest phylogenetic relative was *Kitasatospora putterlickiae* F18-98^T (=DSM 44665^T) (98.3 % 16S rRNA gene sequence similarity). The similarities with type strains of all other *Kitasatospora* species were in the range 95.1–97.0 %. The results of DNA–DNA hybridization showed 54 % relatedness of the isolate and *K. putterlickiae* F18-98^T. Based on the above data and the phenotypic differences from *K. putterlickiae* and other *Kitasatospora* species, it is proposed that the isolate should be classified as the type strain of a novel species, *Kitasatospora sampliensis* sp. nov., with strain VT-36^T (=MTCC 6546^T=DSM 44898^T=JCM 13010^T) as the type strain.

At the time of writing, the genus *Kitasatospora* (Omura *et al.*, 1982; Zhang *et al.*, 1997) was composed of 19 species with validly published names, including the recently described *Kitasatospora putterlickiae*, *K. arboriphila*, *K. gansuensis*, *K. nipponensis*, *K. paranensis*, *K. terrestris* (Groth *et al.*, 2003, 2004) and *K. viridis* (Liu *et al.*, 2005).

Strain VT-36^T was isolated from a sugar-cane field soil sample collected at Sampli village, Punjab state, India, by using *Streptomyces* agar (1.0 % malt extract, 0.4 % yeast extract, 0.4 % glucose, 0.2 % calcium carbonate and 1.2 % agar) complemented with cycloheximide (25 µl ml⁻¹) and amphotericin B (10 µl ml⁻¹) at 28 °C. The pure culture was maintained as a glycerol suspension (10 % v/v) of spores and mycelial fragments at –70 °C. The reference strain *K. putterlickiae* MTCC 6643^T was obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India.

Strain VT-36^T was grown on *Streptomyces* agar for morphological and physiological characteristics. Morphological properties were examined according to the methods reported by Williams *et al.* (1983). Growth and sporulation were observed on standard media-like actinomycetes isolation agar (0.2 % sodium caseinate, 0.01 % asparagine,

0.4 % sodium propionate, 0.05 % dipotassium phosphate, 0.01 % magnesium sulphate, 0.0001 % ferrous sulphate and 1.5 % agar, pH 8.1) and other media as detailed in Table 1. Aerial spore mass colour, pigmentation of substrate mycelium and the production of diffusible pigments were examined on diagnostic media as described by Williams *et al.* (1989), following incubation at 28 °C for 10 days. Growth on sole carbon sources were examined on carbon utilization agar and melanin production was examined on peptone/yeast extract/iron agar (ISP-6) and tyrosine agar (ISP-7) (Shirling & Gottlieb, 1966; Williams *et al.*, 1983, 1989). Tolerance to sodium chloride was examined on modified Bennett's agar at concentrations up to 10 %. Physiological tests and susceptibility to antibiotics were examined by the method described by Groth *et al.* (2004) by using antibiotic discs (HiMedia). For scanning electron microscopy, samples were processed as mentioned previously (Richard & Wilson, 1993).

Freeze-dried cells for chemotaxonomic analysis were prepared following growth of the strains in shake flasks of tryptic soy broth for 4 days at 30 °C. For analysis of fatty acid methyl esters, the cells were grown on tryptic soy broth medium at 30 °C for 36 h. The diagnostic cell wall amino acids were determined as described by Stanek & Roberts (1974) and whole-cell sugars were determined as described by Mikami & Ishida (1983). Polar lipids were extracted and analysed by methods described by Minnikin *et al.* (1984). Menaquinones were analysed as described by Collins *et al.*

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Kitasatospora sampliensis* VT-36^T is AY260167.

Table 1. Growth and culture characteristics of strain VT-36^T and *K. putterlickiae*

Growth and culture characteristics for *K. putterlickiae* grown on ISP media 2–5 were taken from Groth *et al.* (2004). + + +, Very good growth; + +, moderate growth.

Medium	Strain VT-36 ^T			<i>K. putterlickiae</i> MTCC 6643 ^T		
	Growth	Aerial mycelium	Colony reverse	Growth	Aerial mycelium	Colony reverse
Potato dextrose agar	+ + +	Dark grey	Pale grey	+ + +	Grey	Yellow–brown
ISP-1	+ + +	Dark grey	Pale grey	+ + +	Grey	Yellow–brown
ISP-2	+ + +	Dark grey	Dark grey	+ + +	Dark grey	Dark brown
ISP-3	+ + +	Dark grey	Pale grey	+ + +	Grey	Yellow–brown
ISP-4	+ + +	Dark grey	Grey	+ + +	Dark grey	Beige, pale grey
ISP-5	+ +	Pale grey	Cream	+ + +	Dark grey	Dark brown
ISP-6	+ +	Absent	Grey	+ +	Absent	Grey
ISP-7	+ +	Pale grey	Grey	+ +	Grey	Cream
Streptomyces agar	+ + +	Dark grey	Dark brown	+ + +	Grey	Yellow–brown
Actinomycetes agar	+ +	Pale grey	Grey	+ +	Cream	Cream
Sabouraud dextrose agar	+ + +	Dark grey	Dark grey	+ + +	Grey	Yellow–brown

(1984). The *N*-acyl type of muramic acid in the cell wall was determined by the method of Uchida & Aida (1984). Absence of mycolic acid was demonstrated by TLC (Minnikin & Goodfellow, 1976). Fatty acid methyl esters were extracted and analysed as described by Pandey *et al.* (2002). Chemo-taxonomic characters are given in the species description.

Genomic DNA extraction, amplification and sequencing of the 16S rRNA gene and phylogenetic analyses were performed as described earlier by Mayilraj *et al.* (2005). DNA–DNA hybridization was performed using the membrane filter method (Tourova & Antonov, 1987). The G + C content of genomic DNA was determined spectrophotometrically (Lambda 35; Perkin Elmer) using the thermal denaturation method (Mandel & Marmur, 1968).

The results of the polyphasic study placed isolate VT-36^T in the genus *Kitasatospora*. The organism formed an extensively branched substrate and aerial mycelium with smooth-surfaced spores. The colours of the aerial and substrate mycelium on different media are given in Table 1. The almost complete (1481 nt) 16S rRNA gene sequence of the strain VT-36^T was determined and analysis revealed high similarity with other members of the genus *Kitasatospora*. It was evident from the phylogenetic tree (Fig. 1) that isolate VT-36^T forms a cluster along with *K. putterlickiae*. It showed closest sequence similarity with *K. putterlickiae* (98.3 %) followed by *K. arboriphila* (97 %) and *K. azatica* (97 %). The 16S rRNA gene sequence similarity of strain VT-36^T to the type strains of other *Kitasatospora* species was less than 97 % (95.1–96.7 %). Genomic relatedness as shown by DNA–DNA hybridization of strain VT-36^T with the most closely related species *K. putterlickiae* was 54 %, which is distinctly lower than the 70 % cut-off point recommended for the delineation of bacterial species (Wayne *et al.*, 1987). Determination of DNA–DNA relatedness between strain VT-36^T and other type strains of *Kitasatospora* species was

not carried out as the 16S rRNA gene sequence similarities did not exceed 97 %, the threshold value for the delineation of bacterial species (Stackebrandt & Goebel, 1994). Strain VT-36^T differed from *K. putterlickiae* and other species of this genus in some phenotypic characters (Tables 1 and 2). The fatty acid methyl ester profile matches qualitatively that of the genus *Kitasatospora*. However, in the present study, under similar growth conditions (tryptic soy broth medium, 30 °C for 36 h), VT-36^T showed quantitative differences from *K. putterlickiae*. Fatty acid content in VT-36^T and *K. putterlickiae* was iso-C_{15:0}, 20.1 and 14.3 %; iso-C_{16:0},

Fig. 1. Neighbour-joining tree based on 16S rRNA gene (1481 nt) sequences showing the phylogenetic relationship between *K. samplensis* MTCC 6546^T and other related species of the genus *Kitasatospora*. *Streptomyces coelicolor* was used as an outgroup. Bootstrap values greater than 500 are given at the nodes. Bar, 0.5 % sequence difference.

Table 2. Phenotypic characteristics that separate strain VT-36^T from closely related *Kitasatospora* species

Strains: 1, VT-36^T; 2, *K. putterlickiae* DSM 44665^T; 3, *K. nipponensis* DSM 44787^T; 4, *K. azatica* DSM 41650^T; 5, *K. arboriphila* DSM 44785^T; 6, *K. phosalacinea* DSM 43860^T; 7, *K. kifunensis* DSM 41654^T; 8, *K. paranensis* DSM 44788^T; 9, *K. terrestris* DSM 44789^T; 10, *K. cystarginea* DSM 41680^T. +, Positive; –, negative; (+), weak; ND, not determined. Data for reference strains were taken from Groth *et al.* (2004).

Characteristic	1	2	3	4	5	6	7	8	9	10
Spore chain morphology*	RF	RF	RF, RA, S	RF	RF, RA, S	RF	RF, S	RF	RF, RA, S	S
Formation of melanin pigment	+	+	–	–	+	–	+	+	+	–
Degradation of casein	–	+	+	+	+	+	+	+	+	ND
Liquefaction of gelatin	–	+	+	+	+	+	–	+	+	–
Hydrolysis of potato starch	–	–	+	+	+	+	+	+	+	+
Peptonization of milk	(+)	+	+	+	+	+	–	+	+	+
Nitrate reduction	+	+	–	+	+	+	–	+	+	–
Growth on sole carbon sources:										
L-Arabinose	+	–	–	+	+	+	+	+	+	(+)
D-Fructose	+	(+)	(+)	+	+	+	–	–	+	(+)
D-Mannitol	+	–	–	–	–	–	+	–	–	–
D-Raffinose	+	–	–	–	–	+	–	–	+	–
L-Rhamnose	+	–	–	–	–	+	–	+	(+)	–
D-Sucrose	+	(+)	(+)	+	–	+	+	–	(+)	–
D-Xylose	(+)	–	–	+	+	+	+	+	+	–
myo-Inositol	+	–	–	–	–	–	–	–	–	+
Growth in/at:										
10 °C	–	(+)	+	(+)	–	+	(+)	(+)	–	–
35 °C	+	+	(+)	(+)	+	+	–	+	+	+
37 °C	+	+	–	–	+	+	–	+	+	+
40 °C	–	–	–	–	(+)	+	–	–	+	+
42 °C	–	–	–	–	–	+	–	–	–	–
2.0 % NaCl	+	+	+	–	+	+	+	+	+	+
2.5 % NaCl	+	+	(+)	–	–	+	+	+	+	+
3.0 % NaCl	–	+	–	–	–	–	–	+	(+)	–
3.5 % NaCl	–	+	–	–	–	–	–	+	–	–
pH 9.0	(+)	+	–	–	–	+	+	+	+	+
pH 9.5	–	+	–	–	–	+	–	–	–	–
Susceptibility to antibiotics:										
Ampicillin (10 µg)	+	+	–	+	–	+	(+)	(+)	–	ND
Lincomycin hydrochloride (2 µg)	+	–	–	–	–	–	–	(+)	–	ND
Methicillin (5 µg)	+	(+)	(+)	+	–	–	(+)	(+)	–	ND
Norfloxacin (10 µg)	(+)	–	–	(+)	–	–	–	–	(+)	ND
Novobiocin (5 µg)	+	+	+	+	+	–	+	+	+	ND
Penicillin G (10 IU)	+	+	–	+	–	+	–	–	–	ND
Polymyxin B (300 IU)	(+)	(+)	(+)	+	+	(+)	–	+	+	ND
Sulfonamide (200 µg)	–	–	(+)	+	–	–	(+)	(+)	–	ND

*RF, *Rectiflexibiles*; RA, *retinaculiaperti*; S, *spirales*.

16.48 and 15.5; iso-C_{17:0}, 12.5 and 10.2; iso-C_{16:0}, 10.9 and 15.5; anteiso-C_{15:0}, 9.6 and 4.8; anteiso-C_{17:0}, 9.4 and 7.1; 9-methyl-C_{16:0}, 6.3 and 8.8; C_{16:1ω7c}, 4.9 and 8.6 %, respectively. Based on the phenotypic and genotypic data, strain VT-36^T could be clearly distinguished from its closest phylogenetic relative, *K. putterlickiae*. We, therefore, conclude that strain VT-36^T should be assigned as a novel species of the genus *Kitasatospora*, for which we propose the name *Kitasatospora sampliensis* sp. nov.

Description of *Kitasatospora sampliensis* sp. nov.

Kitasatospora sampliensis (sam.pli.en'sis. N.L. fem. adj. *sampliensis* pertaining to Sampli village, Punjab State, India, where the type strain was isolated).

Aerobic, Gram-positive and non-acid-fast actinobacterium. Produces pale-grey, dark-grey or dark-grey–brown substrate mycelium and a pale-grey or dark-grey aerial mycelium on

almost all ISP media. Dark-brown or dark-grey soluble pigments are formed on ISP-2, ISP-3, ISP-6, ISP-7, *Streptomyces* agar, actinomycetes isolation agar and Sabouraud dextrose agar. Melanin is produced on peptone/yeast extract/iron agar and tyrosine agar. Spore chain morphology is *rectiflexibiles* with 10 or more smooth-surfaced spores per chain. Nitrate is reduced to nitrite, casein is not degraded, starch is not hydrolysed and gelatin is not liquefied. Can grow in NaCl at a concentration up to 2.5%, but not at 3.0% or above. Optimum growth is observed at pH 7.0–8.0 and at 30 °C. Positive for utilization of L-arabinose, L-rhamnose, D-fructose, D-mannitol, D-raffinose, D-sucrose and D-xylose as sole carbon and energy sources. Cell wall contains both *meso*- and *LL*-diaminopimelic acid. The *N*-acyl type of muramic acid of the peptidoglycan is acetyl. Major fatty acids are C_{16:0} (16.48%), iso-C_{15:0} (20.07%), iso-C_{16:0} (10.94%), iso-C_{17:0} (12.54%), anteiso-C_{15:0} (9.55%) and anteiso-C_{17:0} (9.36%). Whole-cell sugars are galactose, glucose, mannose and ribose. The polar lipids are phosphatidylinositol, phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositolmannosides. The major menaquinone is MK-9 (H₆). The G + C content of the DNA of the type strain is 76.5 mol%.

The type strain, VT-36^T (= MTCC 6546^T = DSM 44898^T = JCM 13010^T), was isolated from a sugar-cane field soil sample collected from Sampli village, Punjab state, India.

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