Streptomyces osmaniensis sp. nov., isolated from soil

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A novel actinomycete, designated strain OU-63^T, was isolated from garden soil collected on the campus of Osmania University in Hyderabad, southern India. The strain was found to have morphological and chemotaxonomic characteristics typical of the genus *Streptomyces*. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain belonged to the genus *Streptomyces*, and was related most closely to *Streptomyces chartreusis* NBRC 12753^T (98.6 % similarity). However, the mean level of DNA–DNA relatedness between the two strains was only 25 ± 1.73 %. Based on DNA–DNA relatedness, morphological and phenotypic data, strain OU-63^T could be distinguished from the type strains of phylogenetically related species. It is therefore considered to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces osmaniensis* sp. nov. is proposed. The type strain is OU-63^T (=CCTCC AA209025^T =PCM 2690^T).

The family *Streptomycetaceae*, which comprises the genera *Streptomyces*, *Kitasatospora* and *Streptacidiphilus*, was proposed by Waksman & Henrici (1943) and its description was emended by Kim *et al.* (2003). At the time of writing, the genus *Streptomyces* comprises over 550 recognized species (Euzéby, 2009). *Streptomyces* species are aerobic actinomycetes and most of them are able to form an extensively branched substrate mycelium and aerial hyphae that typically differentiate into chains of spores. Members of the genus have LL-diaminopimelic acid in the cell-wall peptidoglycan, but no characteristic sugars (wall chemotype I *sensu* Lechevalier & Lechevalier, 1970) and possess DNA rich in G+C (Williams *et al.*, 1983; Manfio *et al.*, 1995). The streptomycetes, producers of more than half of the 10 000 documented bioactive compounds (for example antibiotics, enzymes, inhibitors and pharmacologically active agents), have offered over 50 years of interest to industry and academia (Anderson & Wellington, 2001; Berdy, 2005). In the course of a screening programme for antibiotics, a novel strain, OU-63^T, was isolated from a garden soil sample collected on Osmania University campus, Hyderabad, India, and was

provisionally assigned to the genus *Streptomyces* by using chemotaxonomic and morphological properties. A polyphasic taxonomic investigation based on a combination of phenotypic and genotypic characteristics revealed that strain OU-63^T represents a novel species of the genus *Streptomyces*.

Strain OU-63^T was isolated by using a modification of the protocol developed by Atalan *et al.* (2000) on actinomycetes isolation agar (Himedia) supplemented with cycloheximide (50 µg ml⁻¹) and nystatin (25 µg ml⁻¹). The agar was seeded with the soil suspension and incubated for 14 days at 28 °C (Olson, 1968). The new isolate was maintained on oatmeal agar slants [International *Streptomyces* Project (ISP) medium 3; Shirling & Gottlieb, 1966] at 4 °C and as glycerol suspensions (20 %, v/v) at –20 °C.

The morphological characteristics of strain OU-63^T were examined by light and scanning electron microscopy of 14-day-old cultures on ISP medium 3 (Williams & Davies, 1967). The coverslip technique (Zhou *et al.*, 1998; Kawato & Shinobu, 1959) was used to observe hyphae and spore chains by light microscopy. Spore-chain morphology and spore-surface ornamentation were studied by examining gold-coated, dehydrated specimens with a scanning electron microscope (JSM 5600; JEOL). Growth and cultural characteristics were observed on various media after incubation at 28 °C for 14 days. A range of physiological

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain OU-63^T is FJ613126.

A table detailing the growth and cultural characteristics of strain OU-63^T on various media is available as supplementary material with the online version of this paper.

and biochemical properties was tested as described by Shirling & Gottlieb (1966) and Williams *et al.* (1983).

The isomer of diaminopimelic acid and whole-cell sugars were analysed following the procedures developed by Hasegawa *et al.* (1983) and Lechevalier & Lechevalier (1980). Menaquinones were extracted and purified according to Collins (1985) and were analysed by HPLC. Fatty acid analysis was performed by GC according to the transmethylation technique described by Lepage & Roy (1984). The DNA G+C content of strain OU-63^T was determined by using the thermal denaturation method (Mandel & Marmur, 1968).

Isolation of DNA was performed according to the method of Tripathi & Rawal (1998). Amplification of the 16S rRNA gene of strain OU-63^T by PCR was carried out as described by Lane (1991). The amplified product was directly sequenced on a 3100 automatic DNA sequencer (Applied Biosystems). The 16S rRNA gene sequence of strain OU-63^T was manually aligned with the corresponding sequences of representative *Streptomyces* species retrieved from the DDBJ, EMBL and GenBank databases. The program MEGA version 2.1 (Kumar *et al.*, 2001) was used for both multiple alignment and phylogenetic analysis. Phylogenetic analyses were performed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed with the neighbour-joining method from an evolutionary distance matrix generated according to the coefficient of Jukes & Cantor (1969). The topologies of the phylogenetic trees were evaluated by bootstrap analysis (Felsenstein, 1985) with 1000 replicates.

The level of DNA–DNA relatedness between strain OU-63^T and *Streptomyces chartreusis* NBRC 12753^T was determined by using the dot-blot hybridization method (Chung *et al.*, 1999) and a simple fluorimetric method for estimation of DNA–DNA relatedness based on thermal denaturation temperatures (Gonzalez & Saiz-Jimenez, 2005). Genomic DNA (200 and 400 ng as homoduplex and heteroduplex DNA, respectively) was used for melting curve analysis in a 10- μ l reaction volume with a final 1 $\times$ concentration of SYBR green. Analyses of the melting curves were performed by using initial denaturation for 10 min and renaturation for 5 min, followed by heating from 20 to 90 °C (2 °C min⁻¹). Melting curves were plotted in triplicate. ΔT_m values between homologous and hybrid DNA of 5 °C or higher were considered as indicative of different bacterial species.

The chemical and morphological properties of strain OU-63^T were consistent with its assignment to the genus *Streptomyces* (Williams *et al.*, 1989; Manfio *et al.*, 1995). The organism formed extensively branched substrate mycelium and aerial hyphae which carried spiny-surfaced cylindrical spores in spiral chains (Fig. 1). The cultural characteristics of strain OU-63^T on various standard media are given in Supplementary Table S1, available in IJSEM Online. The strain contained LL-diaminopimelic acid in the cell-wall

Fig. 1. Scanning electron micrograph of cells of strain OU-63^T grown on oatmeal agar for 14 days at 28 °C. Bar, 2 μ m.

peptidoglycan and no characteristic sugars (cell-wall chemotype I of Lechevalier & Lechevalier, 1970). It contained MK-9 (H₆, H₈ and H₄) as the predominant isoprenologue. The predominant fatty acids were iso-C_{16:0} (23 %), iso-C_{15:0} (17 %), iso-C_{17:0} (13 %), anteiso-C_{15:0} (12 %), anteiso-C_{17:0} (10 %), C_{16:0} (7 %) and iso-C_{14:0} (5 %), consistent with the fatty acid profiles obtained for recognized *Streptomyces* species. The G+C content of the DNA of strain OU-63^T was 71.6 mol%.

The partial 16S rRNA gene sequence (1418 nt) of strain OU-63^T was determined in this study. Primary analysis with sequences of representatives of the family *Streptomycetaceae* confirmed that strain OU-63^T was related closely to members of the genus *Streptomyces*. A neighbour-joining tree (Fig. 2) based on 16S rRNA gene sequences indicated that strain OU-63^T formed a tight cluster with *Streptomyces chartreusis* NBRC 12753^T (98.6 % sequence similarity), albeit with only moderate bootstrap support (54 %). The position of strain OU-63^T in the phylogenetic tree was unaffected by the choice of tree-making algorithm or outgroup strains used. Relatively high levels of 16S rRNA gene sequence similarity were also found between strain OU-63^T and *Streptomyces resistomycificus* NBRC 12814^T (98.1 %), '*Streptomyces argillaceus*' JCM 4975 (98.1 %) and *Streptomyces bungsensis* NRRL B-24305 (98.0 %). Strain OU-63^T showed levels of 16S rRNA gene sequence similarity lower than 98.0 % to the type strains of other recognized *Streptomyces* species.

Strain OU-63^T could be distinguished from all of its close phylogenetic relatives based on a combination of phenotypic properties (Table 1). The level of DNA–DNA relatedness between strain OU-63^T and *Streptomyces chartreusis* NBRC 12753^T was 25 $\pm$ 1.73 % (mean $\pm$ SEM of three determinations), which is well below the 70 % cut-off for recognition of genomic species (Wayne *et al.*, 1987), thus suggesting that the strain should be considered as representing a separate species. DNA–DNA hybridization studies were not carried out between strain OU-63^T and

Fig. 2. Neighbour-joining phylogenetic tree showing the relationship between strain OU-63^T and representative members of the genus *Streptomyces* based on 1401 unambiguously aligned nucleotides of 16S rRNA gene sequence. Numbers at nodes are bootstrap support percentages based on 1000 sampled datasets; only values >50% are shown. Asterisks indicate the branches that were also found in the maximum-parsimony tree. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential characteristics between strain OU-63^T and the type strains of related *Streptomyces* species

Strains: 1, OU-63^T; 2, *S. chartreusis* NBRC 12753^T; 3, *S. resistomycificus* NBRC 12814^T; 4, *S. prunicolor* NRRL B-12281^T. +, Positive; –, negative; ND, no data. Data for reference type strains were taken from Shirling & Gottlieb (1968, 1972) and Williams *et al.* (1983).

Characteristic	1	2	3	4
Aerial spore mass on oatmeal agar*	GB	BG	G	PY
Spore-chain arrangement†	S	S	S, RF	RF
Spore-surface ornamentation	Spiny	Spiny	Smooth	Smooth
Melanin production	+	+	+	–
Degradation of:				
Starch	+	+	–	+
Xanthine	+	+	–	+
Casein	–	+	+	+
Gelatin	+	+	+	–
DNA	–	ND	ND	+
Utilization of carbon sources (1%, w/v)				
Mannose	+	+	–	+
Sorbitol	+	–	+	–
Cellulose (0.1%, w/v)	+	–	–	–
Xylose	+	+	–	+
Lactose	+	+	–	–

*BG, Bluish grey; G, grey; GB, greyish blue; PY, pale yellow.

†RF, Rectiflexibles; S, spirales.

other *Streptomyces* species, as pairwise 16S rRNA gene sequence similarities were low ($\leq 98\%$) (Sembiring *et al.*, 2000; Kim & Goodfellow, 2002; Manfio *et al.*, 2003). The ΔT_m value obtained from the thermal denaturation method between the homologous (*Streptomyces chartreusis* NBRC 12753^T) and hybrid (*Streptomyces chartreusis* NBRC 12753^T and strain OU-63^T) DNA was 7.5 °C. A ΔT_m value of 5 °C or higher is considered to indicate a separate bacterial species (Wayne *et al.*, 1987; Rosselló-Mora & Amann, 2001). Based on the genotypic and phenotypic evidence, strain OU-63^T is therefore considered to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces osmaniensis* sp. nov. is proposed.

Description of *Streptomyces osmaniensis* sp. nov.

Streptomyces osmaniensis (os.ma.ni.en'sis. N.L. masc. adj. *osmaniensis* pertaining to Osmania University, the source of the soil from which the type strain was isolated).

Aerobic, Gram-stain-positive, non-motile actinomycete that forms extensively branched substrate mycelium and aerial hyphae that differentiate into spiral chains of spiny-surfaced spores. The aerial mycelium is grey and the substrate mycelium appears pale yellow on ISP medium 4. Melanin pigments are formed on peptone–yeast extract iron agar (ISP medium 6) and tyrosine agar (ISP medium 7). Growth occurs at 15–40 °C and pH 6–10 but not at 45 °C or at pH 4 or 11. Growth occurs in the presence of 0.1% (w/v) phenol and 4% (w/v) NaCl, but not in the presence of 7% (w/v) NaCl. Degrades starch, gelatin, xanthine, hypoxanthine, L-tyrosine, urea and Tween 80, but not casein, DNA or xylan. Utilizes several compounds as sole carbon sources, including L-arabinose, cellobiose, cellulose (0.1%), fructose, D-galactose, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, raffinose, L-rhamnose, D-sorbitol, sucrose, xylose, acetate, pyruvate and succinate. Utilizes L-cysteine, L-histidine, L-isoleucine, L-phenylalanine and L-proline as sole nitrogen sources. Resistant to penicillin G (10 IU ml⁻¹), neomycin (50 µg ml⁻¹) and rifampicin (50 µg ml⁻¹), but sensitive to kanamycin (30 µg ml⁻¹), novamycin (30 µg ml⁻¹) and streptomycin (100 µg ml⁻¹). The predominant fatty acids are iso-C_{16:0}, iso-C_{15:0}, iso-C_{17:0}, anteiso-C_{15:0}, anteiso-C_{17:0}, C_{16:0} and iso-C_{14:0}. Shows antimicrobial activities against *Escherichia coli* MTCC 739, *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96, *Aspergillus niger* MTCC 282 and *Candida albicans* MTCC 27.

The type strain, OU-63^T (=CCTCC AA209025^T =PCM 2690^T), was isolated from a soil sample collected from Osmania University, Hyderabad, India.

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