Streptomyces hyderabadensis sp. nov., an actinomycete isolated from soil

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A novel actinomycete, designated strain OU-40^T, was isolated from farm soil collected from the Hyderabad region of Andhra Pradesh, southern India. The strain was found to have morphological and chemotaxonomic characteristics typical of species of the genus *Streptomyces*. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain OU-40^T belonged to the genus *Streptomyces*, and was related most closely to *Streptomyces pactum* NBRC 13433^T (99.0 % sequence similarity), *Streptomyces olivaceus* NBRC 12805^T (99.0 %) and *Streptomyces parvulus* NBRC 13193^T (98.8 %). Strain OU-40^T could be distinguished from the type strains of its closest phylogenetic relatives based on levels of DNA–DNA relatedness and comparison of morphological and phenotypic data. It is therefore concluded that strain OU-40^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces hyderabadensis* sp. nov. is proposed. The type strain is OU-40^T (=CCTCC AA 209024^T =PCM 2692^T).

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) to accommodate aerobic, spore-forming actinomycetes. At the time of writing, the genus comprises nearly 550 recognized species (Euzéby, 2009). Most species of the genus *Streptomyces* are able to form an extensively branched substrate mycelium and are also able to produce aerial hyphae that typically differentiate into chains of spores. Species of the genus *Streptomyces* 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, such as antibiotics, enzymes, inhibitors and pharmacologically active agents, have offered over 50 years of interest to industry and academia (Anderson & Wellington, 2001; Berdy, 2005). During the course of our screening programme for antibiotics, strain OU-40^T was isolated from a soil sample collected from farm soil, Hyderabad region, Andhra Pradesh, southern India, and was provisionally assigned to the genus *Streptomyces* based on its chemotaxonomic

and morphological properties. A polyphasic taxonomic investigation based on a combination of phenotypic and genotypic characteristics revealed that strain OU-40^T represents a novel species of the genus *Streptomyces*.

Strain OU-40^T was isolated by using a modified 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 at 28 °C for 14 days (Olson, 1968). The isolate was maintained on oatmeal agar slants (International *Streptomyces* Project medium 3, ISP 3) at 4 °C and as glycerol suspensions (20 %, v/v) at –20 °C.

The morphological characteristics of strain OU-40^T were examined by light and scanning electron microscopy of 21-day-old cultures on oatmeal agar (Williams & Davies, 1967). The coverslip technique (Zhou *et al.*, 1998; Kawato & Shinobu, 1959) was used to observe hyphae and spore chain morphology by light microscopy. Spore chain morphology and spore surface ornamentation were studied by examining gold-coated dehydrated specimens with a Jeol model JSM 5600 scanning electron microscope. Cultural characteristics were observed on a number of agar media following incubation at 28 °C for 14 days.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain OU-40^T is FM998652.

Strain OU-40^T was examined for a range of physiological and biochemical properties as described by Shirling & Gottlieb (1966) and Williams *et al.* (1983). Colony colour was determined with reference to Kelly (1964).

Cells for most of the chemotaxonomic studies were obtained after incubation at 28 °C for 1 week in shake flasks of ISP 2 broth. 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. Analyses of fatty acids were performed by GC by using the transmethylation technique described by Lepage & Roy (1984). The isolate was grown for 7 days with shaking at 28 °C in modified Bennett's broth, both the supernatant and mycelial mass of a culture were extracted with ethyl acetate and acetone, and antimicrobial activity was determined via the paper disc diffusion method (Bauer *et al.*, 1966).

The genomic DNA of strain OU-40^T was isolated by using the Kirby extraction method as described by Kieser *et al.* (2000). Amplification of the 16S rRNA gene was carried out as described by Edwards *et al.* (1989). Pairwise sequence similarities were calculated by using a global alignment algorithm, which was implemented with the EzTaxon server (Chun *et al.*, 2007). Phylogenetic analyses were performed by using the software package MEGA 4.0 (Tamura *et al.*, 2007) after multiple alignment of the sequence data with CLUSTAL_X (Thompson *et al.*, 1997). Distances were calculated by using distance options according to Jukes & Cantor (1969) and clustering was performed by using the neighbour-joining method (Saitou & Nei, 1987). Minimum-evolution (Rzhetsky & Nei, 1992) and maximum-parsimony (Eck & Dayhoff, 1966) trees were generated by means of methods contained within the MEGA 4.0 software package. Bootstrap analyses were used to evaluate the tree topology based on 1000 resamplings (Felsenstein, 1985). The DNA G + C content of strain OU-40^T was determined by using the thermal denaturation method (Mandel & Marmur, 1968).

DNA–DNA hybridization experiments were conducted by using the dot-blot hybridization method (Chung *et al.*, 1999) and a simple fluorimetric method was used to estimate levels of DNA–DNA relatedness based on thermal denaturation temperatures (Gonzalez & Saiz-Jimenez, 2005; Reddy *et al.*, 2010). Genomic DNA (200 and 400 ng as homoduplex and heteroduplex DNA, respectively) was used for melting curve analysis in a 10 µl reaction volume with a final 1 × concentration of SYBR green. Analyses of the melting curves were performed by using 10 min initial denaturation and 5 min renaturation, followed by melting curve analysis from 20 to 90 °C (ramping at 2 °C min⁻¹). Melting curve analyses were performed in triplicate. ΔT_m values between homologous and hybrid DNA of 5 °C or higher were considered as corresponding to distinct microbial species.

The chemical and morphological properties of strain OU-40^T were consistent with its assignment to the genus *Streptomyces* (Williams *et al.*, 1989; Manfio *et al.*, 1995). The strain formed extensively branched substrate mycelia and aerial hyphae which carried smooth-surfaced oval spores in long straight chains (Fig. 1). The cultural characteristics of strain OU-40^T on various standard media are given in Table 1. The aerial mycelium of strain OU-40^T varied from white to grey on different media, and substrate mycelium from brown to light yellow. No diffusible pigments were produced and no melanin was observed on peptone yeast extract iron agar or tyrosine agar.

The strain contained LL-diaminopimelic acid in the peptidoglycan of the cell wall and no characteristic sugars, indicating that it exhibits wall chemotype I (Lechevalier & Lechevalier, 1970). It contained MK-9 (H₆, 68 %; H₈, 21 %; H₄, 11 %) as the predominant isoprenologues. The major fatty acids were iso-C_{16:0} (17 %), iso-C_{15:0} (19 %), iso-C_{17:0} (15 %), anteiso-C_{15:0} (14 %), anteiso-C_{17:0} (9 %), C_{16:0} (8 %) and iso-C_{14:0} (5 %), a profile that is consistent with those for species of the genus *Streptomyces*. The G + C content of the DNA of strain OU-40^T was 70.2 mol%.

Fig. 1. Scanning electron micrographs showing the morphology of cells of strain OU-40^T grown on oatmeal agar for 21 days at 28 °C. (a) Smooth oval-shaped spores; bar, 1 µm. (b) Long straight chains; bar, 5 µm.

Table 1. Growth and cultural characteristics of strain OU-40^T

+++ , Good growth; ++ , moderate growth; + , poor growth.

Agar medium	Growth	Colour of mycelium	
		Aerial	Substrate
Yeast extract malt extract (ISP 2)	+++	Grey	Brown
Oatmeal (ISP 3)	++	Grey/white	Brown
Inorganic salts/starch (ISP 4)	+++	Grey	Pale yellow
Glycerol/asparagine (ISP 5)	++	Dark grey	Light yellow
Tyrosine (ISP 7)	++	Grey	Light brown
Czapek–Dox	++	Whitish grey	Brown
Nutrient agar	+	White	Light yellow
Modified Bennett's	+++	Grey	Yellow

The phenotypic characteristics of strain OU-40^T were also consistent with its placement in the genus *Streptomyces*. An almost-complete 16S rRNA gene sequence of strain OU-40^T (1402 nt) was determined in this study. A phylogenetic tree was reconstructed based on 16S rRNA gene sequences to show the relationship between strain OU-40^T and related species of the genus *Streptomyces* (Fig. 2). Strain OU-40^T formed a distinct phyletic line together with the type strains of *Streptomyces pactum*, *Streptomyces parvulus* and *Streptomyces olivaceus*. Strain OU-40^T shared highest 16S rRNA gene sequence similarity with *Streptomyces pactum* NBRC 13433^T (99.0%), *Streptomyces olivaceus* NBRC 12805^T (99.0%), which corresponds to 14 nt differences out of 1402, and 98.8% similarity with *Streptomyces*

parvulus NBRC 13193^T (16 nt differences out of 1402). Differential phenotypic characteristics between strain OU-40^T and its closest phylogenetic relatives are given in Table 2.

DNA–DNA hybridization experiments were carried out between strain OU-40^T and *S. parvulus* NBRC 13193^T, *S. pactum* NBRC 13433^T and *S. olivaceus* NBRC 12805^T. Mean ($\pm$ SD) levels of DNA–DNA relatedness between strain OU-40^T and the type strains of *S. parvulus*, *S. pactum* and *S. olivaceus* were 40 ± 1.2 , 35.6 ± 0.8 and 25 ± 1.6 %, respectively, significantly lower than the 70% recommended cut-off for recognition of a genomic species (Wayne *et al.*, 1987). The ΔT_m values obtained via the thermal denaturation method between the homologous (*S. parvulus* NBRC 13193^T, *S. pactum* NBRC 13433^T, *S. olivaceus* NBRC 12805^T) and hybrid (strain OU-40^T with *S. parvulus* NBRC 13193^T, OU-40^T with *S. pactum* NBRC 13433^T and OU-40^T with *S. olivaceus* NBRC 12805^T) DNA were 10, 8 and 7.5 °C, respectively. A ΔT_m value of 5 °C or higher is considered to indicate separate bacterial species (Anderson & Wellington, 2001; Wayne *et al.*, 1987; Rossello-Mora & Amann, 2001), and thus these data corroborate the findings of dot-blot analysis. Based on the genotypic and phenotypic evidence presented, strain OU-40^T is considered to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces hyderabadensis* sp. nov. is proposed.

Description of *Streptomyces hyderabadensis* sp. nov.

Streptomyces hyderabadensis (hy.de.ra.bad.en'sis. N.L. masc. adj. *hyderabadensis* pertaining to the Hyderabad

Fig. 2. Unrooted neighbour-joining tree (Saitou & Nei, 1987) based on 16S rRNA gene sequences showing the phylogenetic positions of strain OU-40^T and the type strains of related species of the genus *Streptomyces*. Asterisks indicate branches that were also found by using the minimum-evolution (Rzhetsky & Nei, 1992) and maximum-parsimony (Eck & Dayhoff, 1966) tree-making algorithms. Numbers at nodes are bootstrap percentages based on a neighbour-joining analysis of 1000 resampled datasets; only values ≥ 30 % are shown. Bar, 0.01 substitutions per nucleotide position.

Table 2. Differential phenotypic characteristics between strain OU-40^T and its closest phylogenetic relatives in the genus *Streptomyces*

Strains: 1, OU-40^T; 2, *S. pactum* NBRC 13433^T; 3, *S. olivaceus* NBRC 12805^T; 4, *S. parvulus* NBRC 13193^T. All data are from the present study.

Characteristic	1	2	3	4
Spore-chain morphology	Straight	Spirales	Rectiflexibiles	Spirales
Spore surface	Smooth	Hairy	Smooth	Smooth
Production of diffusible pigments	—	+	—	+
Degradation of:				
Casein	—	+	+	+
DNA	—	+	—	+
Utilization of carbon sources				
L-Arabinose	+	—	+	+
Fructose	—	+	+	+
Rhamnose	+	—	+	+
Raffinose	—	—	+	—
Sucrose	+	+	—	+
Xylose	+	—	+	—
Lactose	+	—	+	+
Utilization of nitrogen sources				
Cysteine	+	—	+	—
Isoleucine	+	+	—	+
Glycine	+	+	—	—
Growth at/with:				
45 °C	—	—	—	+
7 % NaCl	—	—	+	+
pH 5	—	+	+	—
pH 11	+	+	—	+

region of Andhra Pradesh, southern India, from where the type strain was isolated).

Aerobic, Gram-positive actinomycete that forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long straight spore chains. Spores are oval in shape with smooth surfaces. A brown substrate mycelium and a grey aerial spore mass are formed on glucose-yeast extract/malt agar (ISP 2). Melanin pigments are not produced on peptone yeast extract/iron agar or tyrosine agar, and no diffusible pigment is produced on all media tested. Growth occurs well at 20 and 37 °C but not at 10 °C. Grows at pH 6–11 and in the presence of 5 % NaCl. Metabolizes hypoxanthine, tyrosine, urea, xanthine, gelatin, guanine, starch and Tween 80 but not casein, DNA, xylan or keratin. Galactose, glucose, mannitol, mannose, raffinose, arabinose, xylose, ribose, cellobiose, lactose, sucrose, maltose, trehalose, sodium acetate, sodium succinate and sodium pyruvate are used as sole carbon sources for energy and growth, but not fructose, rhamnose or cellulose. Proline, cysteine, histidine, phenylalanine, isoleucine and glycine are metabolized as sole nitrogen sources. Growth occurs in the presence of penicillin G (10 IU ml⁻¹), rifampicin (50 µg ml⁻¹) and ampicillin (30 µg ml⁻¹), but not in the presence of streptomycin (50 µg ml⁻¹), kanamycin (30 µg ml⁻¹) or novamycin (30 µg ml⁻¹). The predominant menaquinones are MK-9

(H₆, 68 %; H₈, 21 %; H₄, 11 %). The major 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}. Active against plant pathogenic moulds *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* and also against bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The genomic DNA G+C content of the type strain is 70.2 mol%.

The type strain, OU-40^T (=CCTCC AA 209024^T =PCM 2692^T), was isolated from farm soil collected from the Hyderabad region of Andhra Pradesh, southern India.

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