Streptomyces iranensis sp. nov., isolated from soil

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A novel streptomycete, designated strain HM 35^T, was isolated from soil in Isfahan city, Iran. Strain HM 35^T produced a branched substrate mycelium and aerial hyphae that developed into short, compact, spiral spore chains with grey rugose spores at the tips of the aerial hyphae. On some media, these spirals coalesced into dark masses of spores with age. Whole-cell hydrolysates of strain HM 35^T contained LL-diaminopimelic acid, glucose and ribose. Phospholipids detected were phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidylinositol mannosides, hydroxy-phosphatidylethanolamine, lyso-phosphatidylethanolamine and hydroxy-lyso-phosphatidylethanolamine. MK-9(H_4), MK-9(H_6) and MK-9(H_8) were the predominant menaquinones. The major fatty acids were iso- and anteiso-branched components. The chemotaxonomic characteristics of the novel isolate matched those described for members of the genus *Streptomyces*. Based on 16S rRNA gene sequence analysis, strain HM 35^T showed highest similarity to *Streptomyces rapamycinicus* NRRL 5491^T (99.2 %), *Streptomyces violaceusniger* DSM 40563^T (99.1 %), *Streptomyces javensis* DSM 41764^T (99.1 %) and *Streptomyces yogyakartensis* DSM 41766^T (99.1 %). The novel strain formed a distinct monophyletic line within the 16S rRNA gene sequence tree. The level of DNA–DNA relatedness between strain HM 35^T and the type strain of *S. rapamycinicus* was 72.7 %. Strain HM 35^T showed the typical morphology found among members of the *S. violaceusniger*/*Streptomyces hygroscopicus* group but could be clearly differentiated from closely related species based on other phenotypic markers. Phenotypic and genotypic data thus indicate that strain HM 35^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces iranensis* is proposed. The type strain is HM 35^T (=DSM 41954^T=CCUG 57623^T).

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) to accommodate aerobic, spore-forming actinomycetes. The genus comprises Gram-positive bacteria that have a high DNA G+C content (69–73 mol%), contain LL-diaminopimelic acid in the peptidoglycan and lack diagnostic sugars in whole-cell hydrolysates. More than 500 *Streptomyces* species have been described, the largest number of any bacterial genus (Euzéby, 2009). Although molecular systematic data show that the genus is clearly over-speciated (Lanoot *et al.*, 2004), other polyphasic studies based on a combination of genotypic and phenotypic features continue to identify novel species and indicate that the genus *Streptomyces* as a whole is under-speciated (Kim & Goodfellow, 2002). Members of novel *Streptomyces* species are in demand as a source of new, commercially significant, bioactive compounds (Berdy, 1995; Fiedler *et al.*, 2005).

The Microbial Biotechnology Laboratory of the University of Tehran has been interested in isolating actinomycetes from Iranian soils, with the purpose of selecting strains with potential for biotechnological application.

The aim of the present study was to classify a *Streptomyces*-like strain, designated HM 35^T, based on a polyphasic analysis. To determine its taxonomic position, morphological, physiological, chemotaxonomic and molecular genetic data were analysed. On this basis, it is proposed that strain HM 35^T represents a novel species of the genus *Streptomyces*.

Strain HM 35^T was isolated from a rhizosphere soil sample (pH 8.0 and salinity 1.9 %) taken at a depth of 10 cm in

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HM 35^T is FJ472862.

Isfahan city, Iran. The soil sample was treated with 1.5 % phenol for 30 min (Hayakawa *et al.*, 1991) before being applied to GAC agar (Nonomura & Ohara, 1971), supplemented with cycloheximide (100 μg ml^{-1}), by the dilution plating method. Strain HM 35^T was isolated after 3 weeks incubation at 28 °C. Reference strains were obtained from the DSMZ. These strains were maintained on International *Streptomyces* Project (ISP) 2 agar slants at 4 °C and as 20 % (v/v) glycerol suspensions at −20 °C.

The cultural properties of strain HM 35^T were evaluated according to the guidelines of the ISP as described by Shirling & Gottlieb (1966). Colours of the aerial and substrate mycelia were determined by comparing the culture with chips from the ISCC–CNBS colour charts (Kelly, 1964).

The intact arrangement of aerial hyphae and spore chains was observed on ISP 2 agar after 14 days at 28 °C by using the coverslip technique (Kawato & Shinobu, 1959). Spore chain morphology and spore surface ornamentation were examined by scanning electron microscopy (CEM902A; Zeiss).

Strain HM 35^T showed good growth on ISP 2, ISP 3, ISP 4 and ISP 5 media, with yellow–brownish substrate mycelia and beige, grey, grey and white aerial mycelia, respectively. No pigments were released into the medium.

Strain HM 35^T produced a well-developed substrate mycelium and an aerial mycelium with short, compact, spiral spore chains with rugose spore ornamentation (Fig. 1). On some media, these spirals coalesced into dark masses of spores with age. This phenomenon is often observed in species belonging to the *Streptomyces violaceusniger*/*Streptomyces hygroscopicus* group (Shirling & Gottlieb, 1972).

Assimilation of carbon sources at a final concentration of 1 % (w/v) was tested by using ISP 9 as the basal medium (Shirling & Gottlieb, 1966). Utilization of nitrogen sources, decomposition of organic compounds, degradation activity and enzyme activity were determined as described by Williams *et al.* (1983, 1989).

Strain HM 35^T was able to grow at 13–37 °C but not at 10 or 40 °C. It was able to grow in the presence of 2.5 % NaCl but not in the presence of 5 % NaCl. Strain HM 35^T grew well at pH 6–11 but did not show any growth at pH 4 or 12.

Biomass for chemical and molecular systematic studies was obtained by cultivation for 6 days in shaken flasks (200 r.p.m.) by using trypticase soy broth (TSB) medium (pH 7.2) at 28 °C. Cells were harvested by centrifugation and washed twice with distilled water. Analyses of amino acids and sugars were carried out according to the methods of Staneck & Roberts (1974). Menaquinones and polar lipids were extracted following the procedure of Minnikin *et al.* (1984). Polar lipids and menaquinones were analysed by TLC (Minnikin *et al.*, 1984) and HPLC (Kroppenstedt, 1982, 1985), respectively. Fatty acids were analysed according to the method of Sasser (1990).

Whole-cell hydrolysates of strain HM 35^T contained LL-diaminopimelic acid as the diamino acid in the peptidoglycan. Glucose and ribose were the only sugars found (cell wall type I of Lechevalier & Lechevalier, 1980). Menaquinone analysis by HPLC revealed MK-9(H_4), MK-9(H_6) and MK-9(H_8) as predominant components. The polar lipids were phosphatidylethanolamine, hydroxyphosphatidylethanolamine, lyso-phosphatidylethanolamine, hydroxy-lyso-phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides (phospholipid pattern type II of Lechevalier *et al.*, 1977). This pattern matched those found in recognized *Streptomyces* species (Kämpfer, 2006; Kroppenstedt & Evtushenko, 2006). Fatty acid analysis revealed mainly saturated iso- and anteiso-branched compounds. Small amounts of 2-hydroxy fatty acids were also found. The principal fatty acids were iso-$C_{15:0}$ (21 %), iso-$C_{17:0}$ (14 %), anteiso-$C_{15:0}$ (14 %), iso-$C_{16:0}$ (13 %) and anteiso-$C_{17:0}$ (12 %). The qualitative and quantitative combination of fatty acids is diagnostic for species of the genus *Streptomyces*, corresponding to fatty acid pattern 2c of Kroppenstedt (1985, 1992). All chemotaxonomic properties of strain HM 35^T were consistent with its classification in the genus *Streptomyces* (Kämpfer, 2006; Kroppenstedt & Evtushenko, 2006).

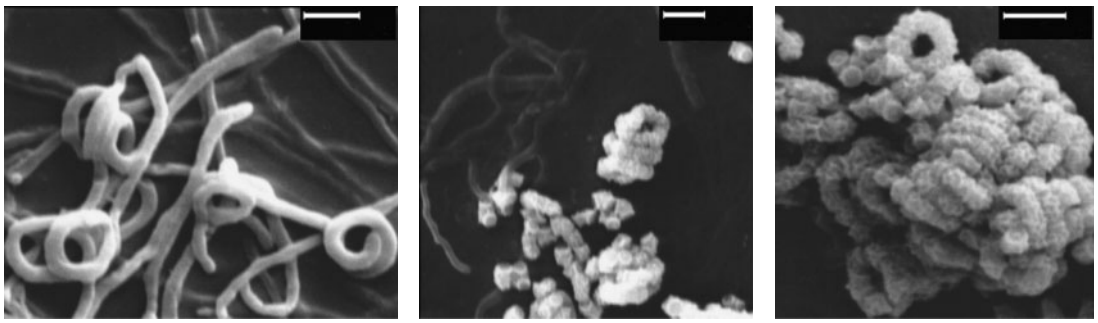

Fig. 1. Scanning electron micrographs of cells of strain HM 35^T after growth on ISP 2 medium for 14 days at 28 °C. Bars, 2 μm.

Genomic DNA was extracted with a DNA extraction kit (JetFlex). PCR-mediated amplification of the 16S rRNA gene was performed by using primers 10-30F (5′-GAG-TTTGATCCTGGCTCA-3′) and 1500R (5′-AGAAAGG-AGGTGATCCAGCC-3′) as described by Rainey *et al.* (1996). Purification of PCR products was carried out by using a DNA purification kit (Qiagen). The DNA G+C base content of strain HM 35^T was determined by HPLC (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984). DNA–DNA hybridization experiments with strain HM 35^T and related strains were carried out according to the thermal renaturation method. DNA was isolated by using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977).

DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) by using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with *in situ* temperature probe (Varian).

Phylogenetic trees based on almost-complete nucleotide sequences were inferred by using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP software package (Felsenstein, 1993). The resultant tree topologies were evaluated in a bootstrap analysis (Felsenstein, 1993) based on 1000 resamplings from the neighbour-joining dataset, by using the PHYLIP package (Felsenstein, 1993). The root position of the unrooted neighbour-joining tree was estimated by using *Streptomyces phaeochromogenes* DSM 40073^T as the outgroup.

Based on 16S rRNA gene sequences, strain HM 35^T was related most closely to members of the genus *Streptomyces*, in particular to *Streptomyces rapamycinicus* NRRL 5491^T (99.2 % similarity), *S. violaceusniger* DSM 40563^T (99.1 %), *Streptomyces yogyakartensis* DSM 41766^T (99.1 %) and *Streptomyces javensis* DSM 41764^T (99.1 %). These similarity values are lower than those found for some other individual pairs of *Streptomyces* species. The phylogenetic position of strain HM 35^T among the type strains of closely related *Streptomyces* species is shown in Fig. 2. The taxonomic position of strain HM 35^T was supported by all of the tree-making algorithms used.

DNA–DNA hybridizations were carried out between strain HM 35^T and the four type strains that showed highest levels of 16S rRNA gene sequence similarity. Levels of DNA–DNA relatedness between strain HM 35^T and *S. rapamycinicus* NRRL 5491^T, *S. violaceusniger* DSM 40563^T, *S. javensis* DSM 41764^T and *S. yogyakartensis* DSM 41766^T were 72.7±4.2, 26.7±3.4, 26.5±2.2 and 21.6±4.5 %, respectively (mean±SD of two determinations). The level of relatedness between strain HM 35^T and *S. rapamycinicus* NRRL 5491^T is at the borderline for species differentiation by this method whereas those for the other three type

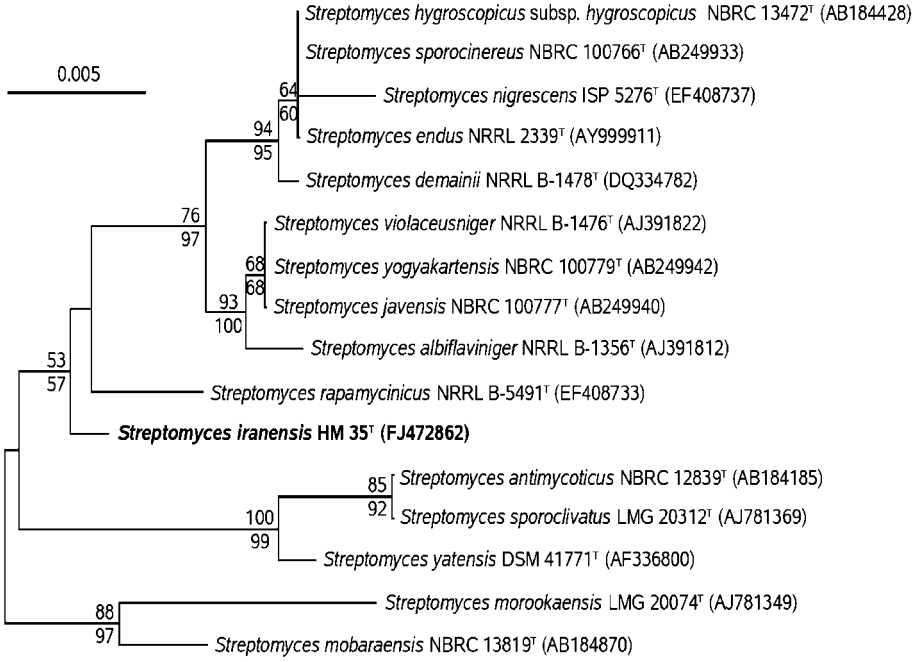

Fig. 2. Phylogenetic tree showing the position of strain HM 35^T among the type strains of its closest related species in the genus *Streptomyces* inferred from 1406 aligned characters of the 16S rRNA gene sequence under the maximum-likelihood criterion. Numbers at nodes are bootstrap support values based on 1000 replicates inferred from the maximum-likelihood (above) and neighbour-joining data (below); only values >50 % are shown. Bar, 5 substitutions per 1000 nt.

strains are well below the generally recognized 70 % cut-off point (Wayne *et al.*, 1987; Stackebrandt & Ebers, 2006). However, it has been shown that a DNA–DNA relatedness value of 80 % might be a more appropriate threshold for defining species in the genus *Streptomyces* than the accepted value of 70 % (Labeda, 1998). Strain HM 35^T could be differentiated from all recognized *Streptomyces* species by several genotypic characteristics and based on its placement in a different branch in the phylogenetic tree (Fig. 2). This separate position of strain HM 35^T is supported by phenotypic characteristics, which show significant differences to those of the type strains of closely related *Streptomyces* species (Table 1). For example, strain HM 35^T has rugose ornamented spores, and the spore mass does not become black and moist when mature. In addition, strain HM35^T does not produce light-yellow diffusible pigment. There are differences in utilization of adonitol, inositol and cysteine. It can also be differentiated based on ability to degrade compounds such as aesculin, adenine, allantoin and pectin. Physiological and chemotaxonomic experiments showed that strain HM 35^T and *S. rapamycinicus* NRRL 5491^T differ significantly with regard to growth temperature (growth at 10 °C) and NaCl tolerance (growth in the presence of 2.5 % NaCl). Strain HM 35^T contained MK-9(H_4), MK-9(H_6) and MK-9(H_8) whereas *S. rapamycinicus* DSM 41530^T contained 41 % MK-9(H_6), 35 % MK-9(H_4), 8 % MK-9(H_2) and 8 % MK-9(H_8) as major menaquinones (data not shown). Phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, phosphatidylinositol mannosides, lyso-phosphatidylethanolamine and hydroxy-lyso-phosphatidylethanolamine were detected in strain HM 35^T whereas *S. rapamycinicus* DSM 41530^T contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. Unusually, the diagnostic compound phosphatidylethanolamine was absent in *S. rapamycinicus* DSM 41530^T. The major fatty acids of strain HM 35^T were iso-$C_{15:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$; those for *S. rapamycinicus* are iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{14:0}$ and $C_{16:0}$.

Based on a combination of morphological, molecular, chemical and physiological data, we consider that strain HM 35^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces iranensis* sp. nov. is proposed.

Table 1. Differential characteristics between strain HM 35^T and closely related *Streptomyces* species

Strains: 1, HM 35^T; 2, *S. rapamycinicus* NRRL 5491^T; 3, *S. violaceusniger* DSM 40563^T; 4, *S. javensis* DSM 41764^T; 5, *S. yogyakartensis* DSM 41766^T. +, Positive; −, negative. All strains produce aerial mycelium, form grey colonies on ISP 3 and have a spiral spore chain morphology.

Characteristic	1	2	3	4	5
Spore ornamentation	Rugose	Rugose	Smooth*	Rugose†	Rugose†
Diffusible pigment	−	+	−	−	−
Utilization as sole carbon source					
Adonitol	+	−	−	−	−
Cysteine	−	+	−	+	+
Inositol	+	+	+	+	−
D-Lactose	+	+	+	−	−
Propionate	+	+	−	−	−
L-Rhamnose	+	+	+	−	−
Ribose	+	+	+	−	+
Sorbitol	+	+	−	−	−
Sucrose	+	+	−	+	+
D-Xylose	+	+	+	−	−
Degradation of:					
Aesculin	+	−	+	+	−
Adenine	−	+	+	+	+
Allantoin	+	−	−	+	+
Pectin	−	+	−	−	−
Growth at/with:					
10 °C	−	+	−	−	−
40 °C	−	−	+	−	+
2.5 % NaCl	+	−	−	+	−

*Data from Shirling & Gottlieb (1972).
†Data from Sembiring *et al.* (2000).

Description of *Streptomyces iranensis* sp. nov.

Streptomyces iranensis (i.ran.en′sis. N.L. masc. adj. *iranensis* referring to Iran, the country from where the type strain was isolated).

Aerobic, Gram-positive actinomycete which forms an extensive, branched substrate mycelium and aerial hyphae with short, compact, spiral spore chains and rugose spores (0.8–1.2 μm). A yellowish-grey to dark-grey spore mass is formed on starch-mineral and oat-meal agars. Colonies on other ISP media are beige to dirty yellow as a result of a lack of spore production. Optimal growth occurs at 28 °C and at pH 6–9. Grows well in the presence of 0–2.5 % NaCl. L-Arabinose, galactose, glycerol, inositol, mannose, mannitol, melibiose, raffinose, rhamnose and xylose can be used as sole carbon sources for growth. Tyrosine and valine are utilized as sole nitrogen sources. Degrades Tween 80, xylan and casein but not hippurate or oxalate. The DNA G+C content of the type strain is 71.1 mol%. Whole-cell hydrolysates contain LL-diaminopimelic acid but no diagnostic sugars. Menaquinones are MK-9(H_4), MK-9(H_6) and MK-9(H_8). Polar lipids are phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, phosphatidylinositol mannosides, lyso-phosphatidylethanolamine and hydroxy-lyso-phosphatidylethanolamine. Major fatty acids (>1 % of the total) are iso-$C_{15:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{17:1}$, $C_{16:0}$, $C_{16:1}\omega9c$, anteiso-$C_{17:1}$, iso-$C_{14:0}$, iso-$C_{16:1}$ and $C_{17:0}$ cyclo. Small amounts of 2-hydroxy fatty acids are also present. Mycolic acids are absent.

The type strain, HM 35^T (=DSM 41954^T=CCUG 57623^T), was isolated from rhizosphere soil taken at a depth of 10 cm in Isfahan city, Iran.

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