Streptomyces hypolithicus sp. nov., isolated from an Antarctic hypolith community

Marilize Le Roes-Hill,^{1†} Jeffrey Rohland,² Paul R. Meyers,² Don A. Cowan³ and Stephanie G. Burton^{1†}

Correspondence
Stephanie G. Burton
BurtonS@cput.ac.za

¹Department of Chemical Engineering, University of Cape Town, Private Bag X3, Rondebosch, 7701 Cape Town, South Africa

²Department of Molecular and Cell Biology, University of Cape Town, Private Bag X3, Rondebosch, 7701 Cape Town, South Africa

³Institute for Microbial Biotechnology and Metagenomics, Department of Biotechnology, University of the Western Cape, Bellville, 7535 Cape Town, South Africa

As part of an enzyme-screening programme, an actinobacterium, strain HSM#10^T, was isolated from a sample collected from the base of a translucent quartz rock in Miers Valley, eastern Antarctica. The isolate produced branching vegetative mycelium that was characteristic of filamentous actinobacteria. The chemotaxonomic characteristics of the strain suggested that HSM#10^T should be classified as a member of the genus *Streptomyces*. Furthermore, phylogenetic analysis based on 16S rRNA gene sequences showed that the strain was closely related to members of the genus *Streptomyces*, which supports the classification of this strain within the family *Streptomycetaceae*. Phenotypic and phylogenetic results allowed strain HSM#10^T to be differentiated from known streptomycetes. DNA–DNA hybridization data also showed that strain HSM#10^T could be differentiated from its nearest phylogenetic neighbours *Streptomyces chryseus* DSM 40420^T (53.55 ± 3.15 % DNA relatedness), *Streptomyces helveticus* DSM 40431^T (38.75 ± 2.75 %), *Streptomyces flavidovirens* DSM 40150^T (30.7 ± 2.90 %) and *Streptomyces albidochromogenes* DSM 41800^T (33.9 ± 0.10 %). Therefore, the name *Streptomyces hypolithicus* sp. nov. is proposed, with HSM#10^T (=DSM 41950^T =NRRL B-24669^T) as the type strain.

A hypolith can be defined as an organism that inhabits the microenvironment located under translucent quartz rocks (hypolithons) in either cold or hot deserts. Such rocks typically provide protection from direct sunlight, serve as a refuge against physical (aolian) disturbance and trap moisture as a source of water. Most hypoliths are photosynthetic micro-organisms belonging to the cyanobacteria (Cockell & Stokes, 2004). The unique micro-environments under translucent quartz rocks serve as a rich source of potentially novel microbes and, hence, potentially novel biocatalysts that can be used in biotransformation reactions that require extreme conditions (Thomas, 2005).

†Present address: Biocatalysis and Technical Biology Research Group, Cape Peninsula University of Technology, Bellville Campus, PO Box 1906, Bellville, 7535 Cape Town, South Africa.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HSM#10^T is EU196762.

Growth characteristics of strain HSM#10^T and closely related strains on different agar media are available as supplementary material with the online version of this paper.

As part of a screening programme for oxidative enzyme-producing actinobacteria, a novel actinobacterium was isolated from samples collected from the base of translucent quartz rocks in Miers Valley in the McMurdo Dry Valleys region of eastern Antarctica.

Hypolithic community samples were collected by one of us (D.A.C.) from around the base of translucent quartz rocks. Samples were stored below 0 °C, transported on dry ice and stored at –80 °C until processed.

Strain HSM#10^T was isolated on starch-casein-nitrate agar (Atlas, 2004) using a standard soil isolation technique: 0.1 g sample was suspended in 1 ml sterile distilled water and the mixture was vortexed for 5 min and allowed to settle for 1 min. A 100-fold dilution of the suspension was plated in triplicate. Strain HSM#10^T was isolated after incubation at room temperature (22 °C) for 21 days. Following isolation, strain HSM#10^T was maintained on International *Streptomyces* Project medium 7 agar (ISP 7; Shirling & Gottlieb, 1966).

The morphological characteristics of strain HSM#10^T were determined using standard methods (Locci, 1989). The

isolate was grown on ISP 4 agar (Shirling & Gottlieb, 1966) for 21 days at 22 °C and morphology was observed under a light microscope and by scanning electron microscopy. Growth characteristics of HSM#10^T were also observed on Czapek solution agar (Atlas, 2004) and Middlebrook 7H9 agar (Becton Dickinson; supplemented with 10 mM glucose and with the ADC enrichment omitted) and compared with those of its closest phylogenetic neighbours.

Standard physiological tests were performed as described by Locci (1989). ISP media were prepared as described by Shirling & Gottlieb (1966). Antibiotic resistance was determined by incorporation of antibiotics into Bennett's medium agar plates (Atlas, 2004) at the recommended concentrations (Locci, 1989). Physiological characteristics were determined after growth at 22 °C (unless otherwise stated) for the recommended incubation periods. All carbon sources for carbon utilization tests were filter-sterilized and tested at the concentrations recommended by Locci (1989) and Shirling & Gottlieb (1966). Staining was performed using the standard Gram staining technique.

Freeze-dried cells used in chemotaxonomic tests were obtained from a 500 ml ISP 2 culture of strain HSM#10^T, which was cultivated on an orbital shaker at 22 °C for 14 days. The diaminopimelic acid isomer and whole-cell sugar pattern were determined as described by Hasegawa *et al.* (1983) except that freeze-dried cells were used instead of colonies from agar plates.

The 16S rRNA gene was amplified by PCR using the bacterial 16S rRNA gene primers F1 and R5 (Cook & Meyers, 2003). PCR conditions were as described by Cook & Meyers (2003). The amplified DNA was purified for sequencing using a MSB Spin PCRapace PCR purification kit (Invitex). For phylogenetic analysis, reference strains chosen from BLAST results (Altschul *et al.*, 1997), as well as type strains from other genera within the family *Streptomycetaceae*, were used. The top hits from the identity analysis of the EzTaxon server (Chun *et al.*, 2007) were also included. For the construction of phylogenetic trees, the software package MEGA version 3.1 (Kumar *et al.*, 2004; <http://www.megasoftware.net/>) was used. Unrooted phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), minimum evolution and maximum-parsimony methods (Takahashi & Nei, 2000) and evaluated by bootstrap resampling (1000 replications).

DNA–DNA hybridization was performed between strain HSM#10^T and the following strains: *Streptomyces chryseus* DSM 40420^T, *Streptomyces helveticus* DSM 40431^T, *Streptomyces flavidovirens* DSM 40150^T and *Streptomyces albidochromogenes* DSM 41800^T. Analysis was performed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). DNA was isolated using a French pressure cell (Thermo Spectronic) and 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) [incorporating the modifications described by Huß *et al.* (1983)] using a 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).

From scanning electron microscopy (Fig. 1), it is evident that the strain produces smooth spores with straight chain morphology. Light microscopy analysis showed that growth appeared branched with *Rectiflexibiles*-type spore chain morphology.

Chemotaxonomic characteristics of strain HSM#10^T suggest that this strain should be classified as a member of the genus *Streptomyces*. LL-Diaminopimelic acid was detected in its peptidoglycan and whole-cell hydrolysates yielded no diagnostic sugars (Lechevalier & Lechevalier, 1970).

A 1499 bp 16S rRNA gene sequence was obtained for strain HSM#10^T. A BLAST search revealed similarities of 98.1 % to *S. chryseus* NBRC 13377^T and *S. helveticus* NBRC 13382^T and 97.7 % to *S. albidochromogenes* NBRC 101003^T and *S. flavidovirens* NBRC 13039^T. These values are low enough to suggest that strain HSM#10^T represents a distinct species (Keswani & Whitman, 2001; Stackebrandt & Ebers, 2006). Furthermore, DNA–DNA hybridization studies showed that strain HSM#10^T represents a distinct genomic species; it shared low DNA relatedness values with the type strains of its closest phylogenetic neighbours, i.e. *S. chryseus* DSM 40420^T (53.55 ± 3.15 % DNA relatedness), *S. helveticus* DSM 40431^T (38.75 ± 2.75 %), *S. flavidovirens* DSM 40150^T (30.7 ± 2.90 %) and *S. albidochromogenes* DSM 41800^T (33.9 ± 0.10 %). These values are well below the 70 % DNA relatedness cut-off value recommended by Wayne *et al.* (1987) and it is therefore proposed that strain HSM#10^T represents a novel species within the genus *Streptomyces*. A phylogenetic tree of streptomycete strains, the type strains

Fig. 1. Scanning electron micrograph of cells of strain HSM#10^T grown on ISP 4 agar at 22 °C for 21 days. The straight spore chain morphology and smooth spores are clearly visible. Bar, 2 µm.

Fig. 2. Unrooted phylogenetic tree based on 16S rRNA gene sequences obtained by the neighbour-joining method showing the position of strain HSM#10^T among its phylogenetic neighbours and representatives of the other genera belonging to the family Streptomycetaceae. The sequence of *Streptosporangium roseum* DSM 43021^T was used as an outgroup. All sequences were edited to the longest common region (1460 bp). GenBank sequence accession numbers are given in parentheses. Numbers at nodes show bootstrap values (%). Asterisks indicate clades that were conserved when the neighbour-joining, minimum evolution and maximum-parsimony methods were used to construct the phylogenetic tree.

of the type species of the genera *Kitasatospora* and *Streptacidiphilus* and strain HSM#10^T (Fig. 2) showed that strain HSM#10^T clustered with members of the genus *Streptomyces*.

Phenotypic characteristics of strain HSM#10^T and the two phylogenetically closest neighbours [*S. chryseus* NRRL B-12347^T (=DSM 40420^T) and *S. helveticus* NRRL B-12365^T

(=DSM 40431^T)] are shown in Table 1 and Supplementary Table S1 (available in IJSEM Online). Additional physiological characteristics are given in the species description.

Based on the polyphasic taxonomic analysis described, strain HSM#10^T is proposed as a representative of a novel species of the genus *Streptomyces*, *Streptomyces hypolithicus* sp. nov.

Table 1. Physiological characteristics of strain HSM#10^T and related strains of the genus *Streptomyces*

Strains: 1, strain HSM#10^T; 2, *S. chryseus* NRRL B-12347^T; 3, *S. helveticus* NRRL B-12365^T. –, Negative; +, positive; w, weakly positive; vw, very weakly positive. Unless otherwise indicated, data were obtained in this study. All three strains show smooth spore surfaces and show cream substrate mycelium when grown on ISP 4 medium.

Characteristic	1	2	3
Spore chain morphology	Straight	Spirals*	Spirals/straight to flexuous*
Aerial mycelium (ISP 4)	Off-white	White	White
Growth at 37 °C	–	vw	vw
Growth in the presence of:			
0.1 % 2-Phenylethanol	–	+	vw
0.3 % 2-Phenylethanol	–	vw	–
Degradation of:			
Allantoin	–	–	vw
L-Tyrosine	–	+	–
Xanthine	–	vw	–
Sole carbon source utilization			
(+) -L-Arabinose	–	–	w
Fructose	–	w	–
(+) -D-Galactose	+	+	w
(–) -D-Mannitol	–	w	–
Raffinose	–	w	–
Salicin	w	+	–
Sucrose	–	w	–
(+) -D-Xylose	–	+	w

*Data from Locci (1989).

Description of *Streptomyces hypolithicus* sp. nov.

Streptomyces hypolithicus (hy.po.li'thi.cus. N.L. masc. adj. *hypolithicus* belonging to a hypolith community).

Good growth occurs on Czapek solution agar (produces white substrate and aerial mycelia); no growth is observed on Middlebrook 7H9 agar. Good growth is observed on ISP 2, but no sporulation is observed after 14 days of growth. Very good growth is observed on ISP 4 with cream substrate and off-white aerial mycelia. No diffusible pigments are produced on ISP 5, but good growth with beige substrate and sparse white aerial mycelia is observed. No melanin production is detected on ISP 6 or ISP 7 after 14 days of growth. Very good growth on ISP 7. Very weak growth is observed in the presence of 3 % NaCl and 0.1 % phenol, but no growth is observed in the presence of 5 % NaCl, 0.0001 % crystal violet, 0.1 % 2-phenylethanol or 0.01 % NaN_3 . Growth is observed in the presence of penicillin G (10 IU ml^{-1}), but not in the presence of cephaloridine (100 $\mu\text{g ml}^{-1}$), lincomycin (100 $\mu\text{g ml}^{-1}$), neomycin (50 $\mu\text{g ml}^{-1}$), rifampicin (50 $\mu\text{g ml}^{-1}$) or streptomycin (100 $\mu\text{g ml}^{-1}$). Growth occurs at 22 °C, with very weak growth at 30 °C; no growth is observed at 37 °C or at pH 4.3. L-Arginine, L-asparagine, potassium nitrate, L-serine and L-threonine are utilized as sole nitrogen sources; L-histidine, L-4-hydroxyproline, L-phenylalanine and L-valine are utilized weakly, but DL- α -amino-*n*-butyric acid, L-cysteine and L-methionine are not utilized. Uses glucose and (+)-D-galactose as sole carbon sources (with weak growth on salicin), but does not utilize (+)-L-arabinose, (–)-D-fructose, *myo*-inositol, (–)-D-mannitol, raffinose, sucrose or (+)-D-xylose. Degrades adenine, arbutin, casein, aesculin, gelatin and hypoxanthine, but not allantoin, cellulose, guanine, starch, tyrosine, urea, xanthine or xylan.

The type strain is strain HSM#10^T (=DSM 41950^T =NRRL B-24669^T), isolated from hypolith community samples collected from the base of translucent quartz rocks in Miers Valley in the McMurdo Dry Valleys region of eastern Antarctica.

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