Streptomyces scopuliridis sp. nov., a bacteriocin-producing soil streptomycete

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Actinomycete strain RB72\textsuperscript{T} was isolated from woodland bluff soil in northern Alabama, USA, and shown to produce a broad spectrum bacteriocin. Based on morphological and chemotaxonomic characteristics, the strain was determined to belong to the genus Streptomyces. Phylogenetic analysis of the near-complete 16S rRNA gene sequence indicated that it differed from those of the described streptomycetes available in public databases. The distinctive white aerial hyphae and lack of sporulation suggest a deficiency in the \textit{whi} pathway of the organism. A combination of substrate utilization patterns, morphological and chemotaxonomic characteristics and DNA–DNA hybridization results supported the affiliation of strain RB72\textsuperscript{T} to the genus \textit{Streptomyces} and enabled the genotypic and phenotypic differentiation of strain RB72\textsuperscript{T} from closely related reference strains. Strain RB72\textsuperscript{T} therefore represents a novel species of the genus \textit{Streptomyces}, for which the name \textit{Streptomyces scopuliridis} sp. nov. is proposed. The type strain is RB72\textsuperscript{T} (=DSM 41917\textsuperscript{T} = NRRL B-24574\textsuperscript{T}).

The genus \textit{Streptomyces}, with more than 500 species with validly published names, contains the largest number of species of any genus in the domain \textit{Bacteria} (Hain \textit{et al.}, 1997). The genus, first proposed by Waksman & Henrici (1943), includes aerobic, Gram-positive, high G+C content (69–78 mol\%) bacteria. Most members of the genus \textit{Streptomyces} possess L-diaminopimelic acid in the ultrastructure of their peptidoglycan cell wall and produce extensively branching networks of substrate mycelia that give rise to the vertical projection of branching aerial hyphae (Williams \textit{et al.}, 1983; Embley & Stackebrandt, 1994). Maturity of the aerial hyphae typically culminates in a sporulation event, resulting in the formation of chains of uninucleoidal spores from the multinucleoidal, filamentous hyphae (Kwak & Kendrick, 1996). The ejection of aerial hyphae generally requires a minimum of 48 h of substrate mycelium growth, while the maturation of the spores can take an additional 2 to 4 days (Lawlor \textit{et al.}, 1987; Willey \textit{et al.}, 1991; Kieser \textit{et al.}, 2000). Mutations in the regulatory genes guiding this process can result in alterations of phenotype. Mutations in the \textit{bld} cascade and/or the proposed sky pathway cause early termination of aerial hyphae production with the differentiation of the colony arrested at the substrate mycelium growth stage (Claessen \textit{et al.}, 2006). Mutation within the \textit{whi} cascade results in the production of an aerial mycelium that does not generate mature spores and remains white in colour (Willey \textit{et al.}, 1991; Chater, 2001). Bacteriocin production within the genus \textit{Streptomyces} has been previously reported, with bactericidal spectra described as species-specific (Zhang \textit{et al.}, 2003) or genus-specific (Roelants & Naudts, 1964).

In the present study, we isolated strain RB72\textsuperscript{T} from a soil sample collected at Rainbow Bluff, a woodland bluff in Lynn, Alabama. Soil-extract medium, developed from a cold-water extraction of the native soil of the organism supplemented with 10 μg cycloheximide ml\textsuperscript{−1}, 20 μg nalidixic acid ml\textsuperscript{−1} and 100 U catalase ml\textsuperscript{−1}, was seeded with a soil sample suspension and incubated at 25 °C for 14 days (Farris & Olson, 2007). Strain RB72\textsuperscript{T} was selected for its appearance as a characteristic streptomycete colony producing a leathery substrate mycelium and developing aerial hyphae with colony maturity. Colour production within the substrate mycelium and aerial hyphae was evaluated according to the \textit{Colour Harmony Manual} as described by Tresner & Backus (1963) and Shirling & Gottlieb (1966). The isolate was maintained on nutrient slants at 25 °C and as suspensions in nutrient broth (Difco) with glycerol (20 %, v/v) at −20 °C. Biomass for the chemotaxonomic and molecular systematic studies was prepared as described previously (Li \textit{et al.}, 2002). Mannitol soya flour agar (Hobbs \textit{et al.}, 1989) was used for maintenance growth, and nutrient broth with 0.4 % glucose (w/v) was used for biomass growth.

The morphological characteristics of strain RB72\textsuperscript{T} were examined using light and scanning electron microscopy of
colonies grown on mannitol soya flour agar, nutrient agar with 0.4% glucose (w/v), yeast extract-malt extract agar [International Streptomyces Project (ISP) medium 2; Shirling & Gottlieb, 1966] and oatmeal agar (ISP medium 3) after 7, 14 and 21 days at 25 °C. The overspill method of Hopwood (1960) was used to observe hyphal characters by phase-contrast light microscopy with a Nikon Eclipse E600 microscope equipped with a Spot RT Colour imaging system (version 3.4 imaging software; Diagnostic Instruments). For high-resolution scanning electron microscopy, agar blocks containing mycelium were fixed with osmium tetroxide (1%, w/v, in 0.1 M cacodylate buffer, pH 7.2) for 2 h, passed through increased concentrations of acetone (25, 50, 75, 90 and 100%) and dried to critical point with a Denton DCP-1 critical point drying apparatus. The dried samples were mounted on graphite-coated aluminium stubs, coated with gold/palladium alloy by a Technics Hummer sputter coater, and examined with a Hitachi S2500 scanning electron microscope.

Colony morphology of strain RB72 T was observed on several standard media [ISP2, ISP3, inorganic salts-starch agar (ISP4), glycerol-asparagine agar (ISP5)] after 14 days of incubation at 25 °C. Examination of strain RB72 T for a range of biochemical and physiological characters was as described by Shirling & Gottlieb (1966), Williams et al. (1983) and Kämpfer et al. (1991). Tolerance to salt, temperature and pH was tested on nutrient agar with 0.4% (w/v) glucose plates incubated for 7–14 days.

Liquid cultures of strain RB72 T, Streptomyces hachijoensis NRRL B-3106 T and Streptomyces kentuckensis NRRL B-1831 T were grown under identical conditions (nutrient broth with 0.4% , w/v, glucose, 225 r.p.m., 30 °C) until late exponential phase (8 days), washed, lyophilized and whole-cell fatty acid profiles determined for triplicate samples following standard protocols (Sasser, 2001) except that fatty acids were identified by co-elution with known standards and mass spectral analysis of their methyl and picolinyl esters (Christie, 1998).

Genomic DNA was extracted from biomass of actively growing cultures on nutrient agar supplemented with 0.4% glucose (w/v) as described by Olson et al. (2002). PCR amplification using universal primers 24f and 1492r was performed as described by Farris & Olson (2007). Amplified fragments were ligated into pCR2.1 cloning vector (TA cloning kit; Invitrogen) and used to transform Escherichia coli DH10B (Invitrogen) according to the manufacturer’s instructions. Plasmids with inserts of the correct size were sequenced at the Macrogen (Korea) sequencing facility. Genomic DNA isolated from strain RB72 T using the method of Bollet et al. (1991) was sent to the HudsonAlpha Genomic Services Lab (Huntsville, AL) for Illumina Genome Analyzer IIx sequencing.

16S rRNA gene sequence data were aligned using Sequencher version 4.5 (Gene Codes) and relatedness to gene sequences of type strains of characterized species of the genus Streptomyces was determined via NCBI BLAST searches (Altschul et al., 1997). The reference sequences and strain RB72 T sequence (GenBank accession number EF657884) were aligned in BioEdit Sequence Alignment Editor, version 7.0.5.3 (Hall, 1999), using CLUSTAL W (Thompson et al., 1994). The neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony algorithms of PAUP* version 4.0b 10 (Swofford, 2002) were used to infer the phylogenetic relatedness of the sequences. The method of Kimura (1980) was used to generate evolutionary distance matrices for the neighbour-joining algorithm. Tree topologies were calculated by bootstrap analyses based on 1000 resamplings.

DNA–DNA relatedness experiments were performed between strain RB72 T and two closely related strains, Streptomyces hachijoensis NRRL B-3106 T and Streptomyces kentuckensis NRRL B-1831 T (= Streptomyces netropsis; Hatano et al. 2003), using the fluorometric method described by Gonzalez & Saiz-Jimenez (2005). Briefly, strains RB72 T, Streptomyces hachijoensis NRRL B-3106 T and Streptomyces kentuckensis NRRL B-1831 T were grown in either nutrient broth (Difco) or SYZ (15 g soluble starch, 2 g yeast extract, 4 g N2 amine, 2 g glucose, 1 l deionized H2O; pH 6.2) medium. Genomic DNA was isolated from the above strains using the method of Bollet et al. (1991). The purified genomic DNA samples possessed A260/A280 ratios between 1.8 and 2.0. Homoduplex and heteroduplex DNA–DNA hybridizations were performed as described by Gonzalez & Saiz-Jimenez (2005) using a Tm of 82.7 °C. Thermal denaturation experiments contained 0.2 μg duplex DNA μl−1, 0.1× SSC (pH 8.0) and SYBR Green nucleic acid stain diluted 1:100 000. Melting curve analysis was performed using a MyiQ Real-time PCR Detection System (Bio-Rad). Tm values for homoduplex and heteroduplex genomic DNA solutions were calculated as the temperatures corresponding to a 50% decrease in fluorescence. ΔTm values were calculated as the difference between the Tm of the heteroduplex genomic DNA solution and the Tm of the reference strain homoduplex genomic DNA solution.

The organism exhibited a range of chemotaxonomic and phenotypic characters typical of the members of the genus Streptomyces (Table 1 and Supplementary Table S1, available in IJSEM Online). Strain RB72 T formed an extensively branched substrate mycelium and aerial hyphae on several standard growth media (Supplementary Figs S1 and S2). The organism produced white aerial hyphae with no spores and a golden brown substrate mycelium on all standard morphological media tested with the exception of ISP2, on which the extent of the aerial hyphae formation was reduced and the substrate mycelium did not produce pigment. Sporulation of the aerial hyphae was not detected after 14 days, and the aerial hyphae remained white in colour, typical of other Streptomyces strains that do not sporulate (Hopwood et al., 1970; Chater, 1972, 1993; Ainsa et al., 2000; Gehring et al., 2000). Interestingly, analysis of the genomic sequencing failed to identify highly conserved (within the genus Streptomyces) primers for the bacterial signal recognition particle receptor FsY, which has been
Table 1. Comparison of morphological, cultural and physiological characteristics of strain RB72<sup>T</sup> and related species of the genus Streptomyces

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology and pigmentation</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Aerial mass on oatmeal agar</td>
<td>White</td>
<td>Beige</td>
<td>Red–white</td>
</tr>
<tr>
<td>Spore-chain arrangement</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Spore surface</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Melanin production</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Production of diffusible pigments</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Growth on sole carbon</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>sources (1 %, w/v)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>–</td>
<td>d</td>
<td>–</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Raffinose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Melibiose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Adonitol</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Dextrin</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Inulin</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

shown to regulate sporulation in *Streptomyces coelicolor* through interaction with *whiH* (Shen et al., 2008). These results suggest a deficiency (or silent transcription) in the *whi* pathway of the organism.

In addition to the characters in Table 1, strain RB72<sup>T</sup> reduced nitrate to nitrite and growth occurred at sodium chloride concentrations of 4 and 7 %, but not at 10 or 13 % (w/v). Growth occurred at pH 6.0–11.0 (optimum, pH 7.0) and 15–37 °C (optimum, 25 °C). Strain RB72<sup>T</sup> hydrolysed adenine, casein, aesculin, gelatin, hypoxanthine, l-tyrosine, starch and xanthine but not cellulose. Strain RB72<sup>T</sup> demonstrated a broad spectrum of bacteriocytic activity. The purified bacteriocin (data not shown) was active against the Gram-positive bacteria *Streptomyces avermitilis MA-4680*<sup>T</sup>, *Streptomyces coelicolor* A3(2), *‘Streptomyces lividans’* 66, *Streptomyces venezuelae* NRRL-ISP 5230<sup>T</sup>, *Nocardia salmonicida* NRRL B-2778<sup>T</sup>, *Nocardia vaccinii* NRRL WC-3500<sup>T</sup>, *Rhodococcus marinonascens* DSM 43752<sup>T</sup>, *Bacillus megaterium* ATCC 14581<sup>T</sup>, *Bacillus subtilis* 168, *Staphylococcus aureus* FDA209, *Streptococcus pyogenes* ATCC 12489, *Enterococcus faecalis* ATCC 29212 and *Micrococcus luteus* strain 85W0996, and the Gram-negative bacteria *Escherichia coli* DH10B and *Klebsiella pneumoniae* ATCC 13883<sup>T</sup>.
hybridization/thermal denaturation experiments, chemo-
taxonomic characters, broad spectrum bacteriocin pro-
duction and lack of sporulation that set it apart from
other described species of the genus *Streptomyces*. For
strain RB72^T^, we propose the name *Streptomyces scopu-
liridis* sp. nov.

**Description of Streptomyces scopuliridis sp. nov.**

*Streptomyces scopuliridis* (scop.ul.i’rid.is. L. masc. n. *scopulus* cliff, bluff, crag; L. gen. n. *Iridis* of or belonging
to the goddess of the rainbow; N.L. gen. n. *scopuliridis* from
a rainbow cliff, referring to the location of isolation,
Rainbow Bluff, a woodland bluff in Lynn, Alabama).

Aerobic, Gram-positive, non-motile, non-spore-forming
actinomycete. Substrate and aerial mycelia are produced;
however, the aerial hyphae fail to undergo the sporulation
process. The substrate and aerial hyphae branch exten-
sively, and the aerial hyphae remain white upon matura-
tion. The reverse side of the substrate mycelium produces a
golden brown pigment on ISP3, ISP4 and ISP5 media. In
addition to the characters described in Table 1, nitrate is
reduced to nitrite and growth occurs at sodium chloride
concentrations of 4–7 % (w/v), at pH 6.0–11.0 and at
temperatures of 15–37 °C. Hydrolyses adenine, casein,
aesculin, gelatin, hypoxanthine, L-tyrosine, starch and
xanthine, but not cellulose. The four most abundant fatty
acids are iso-C16 : 0, iso-C 17 : 1 (v)8, iso-C 15 : 0 and anteiso-
C15 : 0 and the G+C content of the genomic DNA of the
type strain is 70.3 mol%. Produces a broad spectrum
bacteriocin with activity against Gram-positive and Gram-
negative bacteria.

The type strain, RB72^T^ (=DSM 41917^T^ =NRRL
B-24574^T^), was isolated from a soil sample collected from
Rainbow Bluff, a woodland bluff in Lynn, Alabama.

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among Gram-positive bacteria, is essential for sporulation in

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corrig., sp. nov., nom. rev.

Int J Syst Evol Microbiol

Streptomyces violaceusniger

Streptomyces ruanii

and

Streptomyces mordarskii

ensis

sp. nov., comb. nov., Streptomyces himastatinicus sp. nov., Streptomyces mordarskii sp. nov., Streptomyces rapamycinicus sp. nov. and Streptomyces ruanii sp. nov. Int J Syst Evol Microbiol 58, 1369–1378.


