Antonie van Leeuwenhoek (2013) 103:367–373
DOI 10.1007/s10482-012-9816-x

ORIGINAL PAPER

Streptomyces bullii sp. nov., isolated from a hyper-arid Atacama Desert soil

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Received: 28 July 2012 / Accepted: 12 September 2012 / Published online: 26 September 2012

Abstract A *Streptomyces* strain isolated from a hyper-arid Atacama Desert soil was characterised using a polyphasic taxonomic approach. The strain, designated C2^T, had chemical and morphological properties typical of the genus *Streptomyces*. The isolate formed a branch in the *Streptomyces* 16S rRNA gene tree together with the type strain of *Streptomyces chromofuscus* and was also loosely related to *Streptomyces fragilis* NRRL 2424^T. DNA:DNA relatedness values between the isolate and its two phylogenetic neighbours showed that it formed a distinct genomic species. The strain was readily distinguished from these organisms using a combination of morphological and phenotypic data. Based on the genotypic and phenotypic results, isolate C2^T represents a novel species in the genus *Streptomyces*, for which the name *Streptomyces bullii* sp. nov. is proposed. The type strain is C2^T (=CGMCC 4.7019^T = KACC 15426^T).

Keywords *Streptomyces* · Polyphasic taxonomy

The GenBank accession number for the 16S rRNA gene sequence of *Streptomyces bullii* C2^T is HE 591384.

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Introduction

The genus *Streptomyces*, which encompasses nearly 600 species with validly published names (Euzéby J 2012; http://www.bacterio.cict.fr/s/streptomcesa.html), remains a rich source of novel pharmaceutically important compounds, notably antibiotics (Goodfellow and Fiedler 2010). It is, however, important in the search for new therapeutic compounds to screen phylogenetically novel streptomycetes to avoid the costly rediscovery of known compounds from common *Streptomyces* species (Antony-Babu and Goodfellow 2008). To this end, we are currently focusing on the isolation of streptomycetes from hyper- and extreme hyper-arid Atacama Desert soils on the premise that these extreme habitats will contain novel isolates which, in turn, will lead to novel chemistry. To date, we have described two new *Streptomyces* species from Atacama Desert soils (Santhanam et al. 2012a, b) and shown that two additional isolates produce new ansamycin and 22-membered macrolactones that express a range of antibacterial and antitumour antibiotics (Nachtigall et al. 2011; Rateb et al. 2011a, b). Another putatively novel *Streptomyces* strain isolated from Chilean highland soil of the Atacama Desert produces novel aminobenzoquinones, the

abenquines, which show inhibitory activity against bacteria and dermatophytic fungi (Schulze et al. 2011). The present study was undertaken to establish the taxonomic status of a *Streptomyces* strain, isolate C2^T, which had been isolated from an Atacama Desert soil and shown to form a distinct phyletic line in the 16S rRNA gene tree with *Streptomyces chromofuscus* as its nearest neighbour (Okoro et al. 2009). A polyphasic taxonomic study showed that isolate C2^T belonged to a new *Streptomyces* species, *S. bullii* sp. nov.

Materials and methods

Organisms, maintenance and culture conditions

Strain C2^T was isolated on glucose-yeast extract agar supplemented with antifungal antibiotics and rifampicin (Athalye et al. 1981) after incubation at 28 °C for 14 days following inoculation with a suspension of soil taken from the Chaxa de Laguna, Salar de Atacama of the Atacama Desert (Okoro et al. 2009). The isolate and the type strains of *S. chromofuscus* and *S. fragilis* were maintained on modified Bennett's agar (Jones 1949) slopes and as suspensions of hyphal fragments and spores in 20 % (v/v) glycerol at −20 °C. Biomass for the molecular systematic and most of the chemotaxonomic studies on isolate C2^T was scraped from 14 day-old modified Bennett's agar plates incubated at 28 °C and washed twice in distilled water; biomass for the chemotaxonomic analyses was freeze dried and that for the molecular systematic work stored at −20 °C. Biomass for the fatty acid analysis was harvested from yeast extract-malt extract broth (Shirling and Gottlieb 1966) after 3 days at 25 °C.

Chemotaxonomy and morphology

The isolate was examined for chemotaxonomic and morphological properties typical of the genus *Streptomyces* (Kämpfer 2012). The arrangements of hyphae and spore chains were detected on oatmeal agar (International *Streptomyces* Project [ISP] medium 3; Shirling and Gottlieb 1966) after 14 days at 28 °C, using the coverslip technique of Kawato and Shinobu (1959). Spore chain arrangement and spore surface ornamentation were observed by examining gold-coated dehydrated specimens, taken from the oatmeal agar plate and under a scanning electron microscope (Cambridge Stereoscan 240 instrument), as described by O'Donnell et al. (1993). Cultural characteristics were determined using ISP media (Shirling and Gottlieb 1966) after incubation at 28 °C for 14 days. Standard procedures were used to detect the isomers of diaminopimelic acid (Hasegawa et al. 1983) and menaquinones (Collins et al. 1985). Cellular fatty acids were extracted, methylated and analysed by gas chromatography (model 6890; Hewlett Packard) and identified using version 5 of the Sherlock Microbial Identification System (MIDI) and the ACTINO database (Sasser 1990).

Molecular systematics

Genomic DNA was extracted from isolate C2^T biomass and PCR-mediated amplification of the 16S rRNA gene of the purified product was achieved, as described by Kim and Goodfellow (2002). The resultant almost complete 16S rRNA gene sequence (1452 nucleotides [nt]) was submitted to the EzTaxon server (http://eztaxon-e.ezbiocloud.net/; Kim et al. 2012) and aligned with 16S rRNA gene sequences of the most closely related *Streptomyces* species using CLUSTAL W version 1.8 software (Thompson et al. 1994). Phylogenetic trees were generated using the maximum-parsimony (Fitch 1971), minimum-evolution (Rzhetsky and Nei 1992) and neighbour-joining (Saitou and Nei 1987) algorithms drawn from the MEGA 5 package (Tamura et al. 2011); an evolutionary distance matrix for the neighbour-joining analysis was prepared using the Jukes and Cantor (1969) model. The robustness of the inferred tree topologies was evaluated after 1,000 bootstrap replicates (Felsenstein 1985) of the neighbour-joining data using MEGA 5 software. The root position in the neighbour-joining tree was inferred by using *S. megasporus* NBRC 14749^T (accession number AB184617) as a outgroup.

DNA:DNA relatedness studies were carried out, in triplicate, between isolates C2^T and *S. chromofuscus* NRRL B-12175^T and *S. fragilis* NRRL 2424^T respectively, using the fluorometric microplate method and biotinylated probe DNA (Ezaki et al. 1989) with modifications by Rong and Huang (2010). The genomic DNA G+C content of the isolates was determined by using the HPLC method of Mesbah et al. (1989).

Phenotypic tests

Isolate C2^T, *S. chromofuscus* NRRL B-12175^T and *S. fragilis* NRRL 2424^T were examined for a diverse battery of phenotypic properties using media and methods described by Williams et al. (1983). The isolate was also screened for additional tests drawn from those used by Williams and his colleagues.

Results and discussion

Isolate C2^T showed a range of chemotaxonomic and morphological properties consistent with its classification in the genus *Streptomyces* (Kämpfer 2012). It formed an extensively branched substrate mycelium which carried aerial hyphae that differentiated into smooth surfaced spores borne in spiral chains (Fig. 1). The organism grew well on all of the ISP media showing a range of aerial spore mass and substrate mycelial pigments (Table 1). It contained major amounts of LL-diaminopimelic acid in whole-organism hydrolysates; produced hexa- and octa-hydrogenated menaquinones with nine isoprene units in a ratio of 4:6, and had a fatty acid profile rich in iso-$C_{15:0}$ (14.2 %), anteiso-$C_{15:0}$ (18.0 %), iso-$C_{16:0}$ (10.8 %), iso-$C_{17:0}$ (5.8 %) and anteiso-$C_{17:0}$ (11.9 %) components. The DNA G+C content of the isolate was 73.0 mol %.

Comparison of the almost complete 16S rRNA gene sequence of the isolate with corresponding sequences of phylogenetically related species with validly published names showed that it formed a branch in the *Streptomyces* 16S rRNA gene tree with the type strain of *S. chromofuscus*, a relationship supported by all of the tree- making algorithms and a bootstrap value of 70 % (Fig. 2). The 16S rRNA gene similarity between these organisms was 99.1 %, a value which corresponded to 12 nt differences at 1,452 locations. Isolate C2^T was also loosely associated with the type strain of *S. fragilis*; these strains shared a 16S rRNA gene similarity of 98.9 %, a value equivalent to 15 nt differences at 1,452 sites. The taxonomic integrity of the isolate was also supported by the DNA:DNA relatedness data. It shared DNA:DNA homology values of 37.8 ± (3.18) % and 13.2 ± (0.65) % respectively with the type strains of *S. chromofuscus* and *S. fragilis*. These values are clearly well below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne et al. 1987).

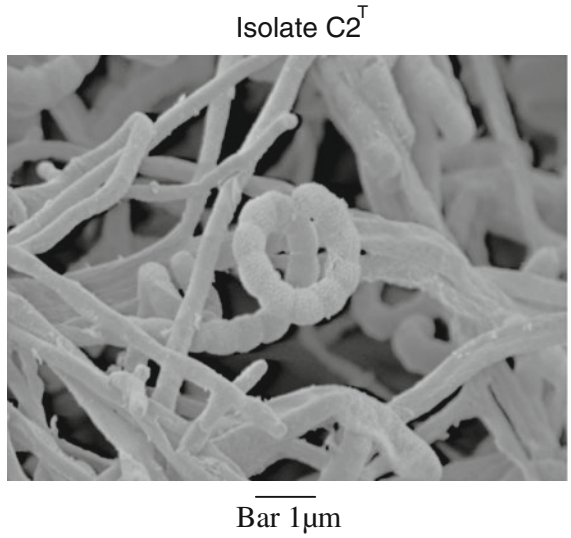

Fig. 1 Scanning electron micrograph of isolate C2^T on oatmeal agar after 14 days at 28 °C showing smooth surfaced spores in spiral chains

Labeda et al. (2012) considered that 16S rRNA gene sequence and phenotypic data, especially morphological features, such as spore colour, spore ornamentation and spore surface ornamentation, provide a reliable way for assigning new species to the genus *Streptomyces*. It is, therefore, significant that isolate C2^T can be distinguished from the type strains of its nearest phylogenetic neighbours using a combination of phenotypic properties that include aerial spore mass colour and spore surface ornamentation (Table 2). Thus, isolate C2^T, unlike *S. chromofuscus* NRRL B-12175^T and *S. fragilis* NRRL 2424^T, degrades xanthine, uses amygdalin, D-galactose, *meso*-inositol, inulin, D-lactose and D-sucrose as sole carbon sources, and glycine, L-*iso*leucine, DL-methionine, DL-*nor*leucine, L-*nor*valine and L-valine as sole nitrogen sources. In contrast, the two established type strains, unlike isolate C2^T, hydrolyse aesculin, produce hydrogen sulphide and reduce nitrate. Consequently, isolate C2^T is proposed as a new *Streptomyces* species, *S. bullii* sp. nov. The results of the additional tests carried out on isolate C2^T are cited in the species description. It is encouraging that very good congruence was found between the phenotypic data acquired for the *S. chromofuscus* and *S. fragilis* strains and corresponding results reported in the numerical taxonomic study of Williams et al. (1983).

Table 1 Growth and cultural characteristics of strain C2^T on standard agar media after incubation for 14 days at 28 °C

Media	Growth	Substrate mycelium colour	Aerial spore mass colour	Diffusible pigment
Glycerol asparagine agar (ISP 5)	+++	Dark brown	Dark gray	Brown
Inorganic salts-starch agar (ISP 4)	++	Mauve	Grayish red	None
Oatmeal agar (ISP 3)	+++	Reddish brown	Whitish gray	None
Peptone -yeast extract -iron agar (ISP 6)	+++	Brown	Grayish white	Dark brown
Tryptone-yeast extract agar (ISP 1)	++	Brown	White	None
Tyrosine agar (ISP 7)	++	Black	Gray	Dark brown
Yeast extract -malt extract agar (ISP 2)	+++	Dark brown	Dark grayish white	Brown

+++, abundant; ++, moderate

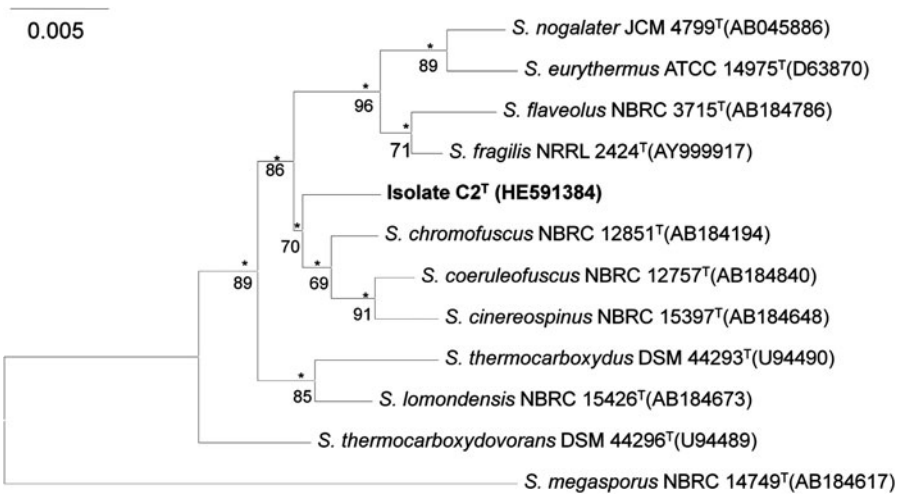

Fig. 2 Neighbour-joining tree based on nearly complete 16S rRNA gene sequences (1,452 nt) showing relationships between isolate C2^T and closely related *Streptomyces* species. *Asterisks* indicate branches of the tree that were also found using the maximum-parsimony and minimum-evolution tree-making algorithms. *Numbers* at the nodes are percentage bootstrap values based on 1,000 resampled datasets. *Bar* 0.005 substitutions per nucleotide position

Description of *Streptomyces bullii* sp. nov.

Streptomyces bullii (bul' li. i. N.L. gen. masc. n. *bullii*, of Bull, named after Alan T. Bull in recognition of his pioneering work on actinobacterial diversity in Atacama Desert soils.

Aerobic, gram-positive, non- acid-alcohol-fast actinomycete which forms an extensively branched substrate mycelium that bears aerial hyphae which carry spiral chains of smooth surfaced spores (0.7 × 0.9 μm). Growth occurs from 10 to 37 °C, between pH 4–11, but not in the presence of 10 % (w/v) NaCl. Melanin pigments are formed on peptone-yeast extract-iron and tyrosine agars. Arbutin is hydrolysed. Adenine, chitin, gelatin, Tween 40, uric acid and xylan are degraded, but not elastin or Tween 20. D-arabinose, D-glucose, D-melibiose and D-ribose are used as sole carbon sources for energy and growth (at 1 %, w/v), but not butane 1,4 diol, methanol or xylitol (at 1 %, v/v). Sodium pyruvate is used as a sole carbon source, but not sodium acetate (both at 0.1 %, w/v), L-ornithine and L-phenylalanine are used as sole nitrogen sources, but not L-aminobutyric acid, L-histidine or L-tryptophan. Additional phenotypic properties are cited in the text and in Tables 1 and 2. Chemotaxonomic properties are typical of the genus. The DNA G+C content is 73.0 mol %.

The type strain, C2^T (=CGMCC 4.7019^T = KACC 15426^T) was isolated from soil taken from the Chaxa de Laguna, Salar de Atacama in the Atacama Desert. The species description is based on a single strain and hence serves as a description of the type strain.

Table 2 Phenotypic properties which distinguish isolate C2^T from the type strains of *S. chromofuscus* and *S. fragilis*

Characteristic	Isolate C2^T	*S. chromofuscus* NRRL B-12175^T	*S. fragilis* NRRL 2424^T
Spore chain morphology	Spiral chains	Spiral[a]	Open loops to spirals[a]
Spore surface ornamentation	Smooth	Spiny[a]	Smooth[a]
Aerial spore mass colour on Oatmeal agar	Gray	Gray[a]	Red[a]
Biochemical tests			
Aesculin hydrolysis	−	+	+
Nitrate reduction	−	+	+
Hydrogen sulphide production	−	+	+
Degradation tests			
Cellulose	+	−	+
DNA	+	+	−
Guanine	+	−	−
Hypoxanthine	+	+	−
Keratin	+	−	+
RNA	+	+	−
Tween 80	−	+	+
Tyrosine	+	+	−
Xanthine	+	−	−
Growth on sole carbon sources			
At 1.0 %, w/v			
D-Arabitol	+	−	+
Arbutin	+	+	−
Amygdalin	+	−	−
D-Fructose	+	+	−
D-Galactose	+	−	−
Glycerol	+	+	−
Glycogen	+	−	−
meso-Inositol	+	−	−
Inulin	+	−	−
D-Lactose	+	−	−
D-Maltose	+	+	−
D-Mannitol	+	+	−
D-Mannose	+	+	−
D-Raffinose	+	−	−
D-Salicin	−	+	+
D-Sucrose	+	−	−
At 0.1 %, w/v			
Sodium malonate	+	−	−
Growth on sole nitrogen source (1 %, w/v)			
L-Alanine	+	−	+
Glycine	+	−	−
L-isoleucine	+	−	−
DL-Methionine	+	−	−
DL-*nor*leucine	+	−	−
L-*Nor*valine	+	−	−

Table 2 continued

Characteristic	Isolate C2[T]	*S. chromofuscus* NRRL B-12175[T]	*S. fragilis* NRRL 2424[T]
L-Proline	+	−	+
L-Threonine	+	−	+
L-Valine	+	−	−
Growth in the presence of NaCl			
4 %, w/v	+	+	−
7 %, w/v	+	−	−
Growth at 10 °C	+	−	−

All of the strains degraded adenine, casein, starch and xylan and, grew on L-serine, D-trehalose and D-xylose, but not xylitol as a sole carbon source or L-serine as a sole nitrogen source

+, positive; −, negative

[a] Data taken from Labeda et al. (2012). All the remaining results were determined in this study

Acknowledgments M. G. thanks the Leverhulme Trust for an Emeritus Fellowship and Y. H. and X. R. are grateful for support from the National Science Foundation of China (Grant 31100003). We are all indebted to Dr. Hang-Yeon Weon (Korea Agricultural Culture Collection, Suwon, Korea) for the fatty acid data.

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