Saccharopolyspora spongiae sp. nov., a novel actinomycete isolated from the marine sponge Scopalina ruetzleri (Wiedenmayer, 1977)

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Abstract

A novel marine actinomycete, designated strain CMAA 1452T, was isolated from the sponge Scopalina ruetzleri collected from Saint Peter and Saint Paul Archipelago, in Brazil, and subjected to a polyphasic taxonomic investigation. The organism formed a distinct phylectic line in the Saccharopolyspora 16S rRNA gene tree and had chemotaxonomic and morphological properties consistent with its classification in this genus. It was found to be closely related to Saccharopolyspora dendranthemae KLBMP 1305T (99.5% 16S rRNA gene sequence similarity) and shared similarities of 99.3, 99.2 and 99.0% with ‘Saccharopolyspora endophytiica’ YIM 61095, Saccharopolyspora triptyergyi YIM 65359T and ‘Saccharopolyspora pathumthaniensis’ S582, respectively. DNA–DNA relatedness values between the isolate and its closest phylogenetic neighbours, namely S. dendranthemae KLBMP 1305T, ‘S. endophytiica’ YIM 61095 and S. triptyergyi YIM 65359T, were 53.5, 25.8 and 53.2%, respectively. Strain CMAA 1452T was also distinguished from the type strains of these species using a range of phenotypic features. On the basis of these results, it is proposed that strain CMAA 1452T (=DSM 103218T=NRRL B-65384T) merits recognition as the type strain of a novel Saccharopolyspora species, Saccharopolyspora spongiae sp. nov.

The genus Saccharopolyspora belongs to the family Pseudonocardiae and was first described by Lacey and Goodfellow [1] to accommodate an actinomycte isolated from sugar cane bagasse. The genus currently encompasses aerobic, Gram-positive, non-acid-fast organisms which form branched substrate mycelium that may fragment into rod-shaped and/or coccoid structures [2]. In some species, the substrate hyphae remain intact or are partially transformed into chains of spores. Aerial mycelium, when present, generally differentiates into bead-like chains of spores containing a smooth sheath [3]. Members of the genus Saccharopolyspora are also characterized by the presence of meso-diaminopimelic acid in the cell wall, arabinose and galactose as the diagnostic sugars in the whole-cell hydrolysates, fatty acid profiles comprised mainly of iso- and anteiso-branched chain compounds, phospholipid pattern III (phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylethanolamine; sensu Lechevalier et al. [4]) and tetra-hydrogenated menaquinone with nine isoprene units (MK-9(H4)) as the predominant isoprenologue, but lack mycolic acids [5–7]. The DNA G+C contents of members of the genus fall within the range 66–77 mol% [2].

The genus encompasses 27 recognized species of which 17 were identified in the last 10 years. Most of these species were isolated from soil samples [8–15], saline lakes [16–21] and endophytic associations [22–25]. Furthermore, some members of Saccharopolyspora have been recovered from symbiotic associations with marine sponges, but polyphasic taxonomic studies have rarely been described [26–30]. At the time of writing, only one isolate from the marine sponge Haliclona sp. was the subject of a taxonomic analysis and consequently described as a novel species, namely Saccharopolyspora ceubensis [31]. Recently, interest in the genus Saccharopolyspora has increased due to its biotechnological appeal, mainly as a source of unique biocompounds [26, 28, 29, 32–34]. However, despite this scientific and economic importance, only erythromycin and spinosyn produced by Saccharopolyspora erythraea and Saccharopolyspora spinosa, respectively, have reached the market [35, 36]. The present study is a part of our...
investigations into the diversity of actinomycetes associated with marine sponges from Saint Peter and Saint Paul Archipelago, Brazil [37]. In this study, a halotolerant actinomycete, designated strain CMAA 1452, was isolated. Based on data from the present polyphasic taxonomic study, the isolate deserves to be considered to represent a novel species of the genus *Saccharopolyspora*.

Tissue samples from the marine sponge *Scopalina ruetzleri* were collected at depths between 20 and 30 m at East Face (0° 54’ 57” N 29° 20’ 41” W) of the Saint Peter and Saint Paul Archipelago in the Atlantic Ocean in August 2013. Strain CMAA 1452 was isolated from a ground sponge tissue suspension on M1 agar medium [38] containing artificial seawater (ASW: 33 g Red Sea Salt l⁻¹; Red Sea), amended with cycloheximide and nystatin (each at 25 μg ml⁻¹) and incubated at 25 °C for 4 weeks. The strain was purified and maintained on ISP 2 agar medium [39] at 4 °C and as a cell suspension in 20 % (v/v) glycerol at −80 °C. Biomass for chemical and molecular systematic studies was harvested by centrifugation and washed twice in distilled water.

The phylogenetic position of strain CMAA 1452 was determined by 16S rRNA gene sequence analysis. Genomic DNA extraction, PCR amplification and 16S rRNA gene sequencing were achieved following Kim et al. [40]. The almost-complete 16S rRNA gene sequence (1486 bp) was aligned using MEGA version 6 software [41] against corresponding sequences of closely related type strains of *Saccharopolyspora* species retrieved from the GenBank database using the EzTaxon-e server [42]. Phylogenetic trees were inferred by using the maximum-likelihood [43], maximum-parsimony [44] and neighbour-joining [45] tree-making algorithms drawn from the MEGA 6 package. The evolutionary distance matrix for the neighbour-joining analysis was generated using the Tamura-Nei parameter (TN93+G+I) model [46]. The appropriate nucleotide substitutions for the maximum-likelihood analysis were selected by the Bayesian Information Criterion (BIC) using the MEGA version 6 software and found to follow the General Time-Reversible (GTR+G+I) model. Topologies of the resultant trees were evaluated by bootstrap analysis [47] based on 1000 replicates. The percentage of G+C content in the genomic DNA was determined by the method of Gonzalez and Saiz-Jimenez [48], using two individual plates and three wells as replicates.

It can be seen from Fig. 1 that strain CMAA 1452 was placed within the *Saccharopolyspora flava* 16S rRNA gene subclade. The isolate formed a new phyletic line closely associated with *Saccharopolyspora dendaranthema* KLBMP 1305, although this result was supported by a bootstrap value of only 50 %. However, it seems likely that the exact phylogenetic position of isolate CMAA 1452 will be clarified only after closely related strains are described (see also Fig. S1, available with the online Supplementary Material). Isolate CMAA 1452 shared highest 16S rRNA gene sequence similarity with *S. dendaranthema* KLBMP 1305, *Saccharopolyspora endophytica* YIM 61095, *Saccharopolyspora tripterygii* YIM 65359, and *Saccharopolyspora pathumthaniensis* S582, namely 99.5, 99.3, 99.2 and 99.0 %, respectively; these values corresponded to 8–14 nt differences at 1486 sites. The corresponding 16S rRNA gene sequence similarities with the remaining type strains assigned to the genus *Saccharopolyspora* were below 97.0 %. The DNA G+C content of strain CMAA 1452 was 64.8 mol %.

Reciprocal DNA–DNA pairing assays were performed between isolate CMAA 1452 and its closest phylogenetic neighbours, namely *S. dendaranthema* DSM 46699, *S. endophytica* DSM 45425 and *S. tripterygii* DSM 45269 at the DSMZ. Cells were disrupted by using a Constant Systems TS 0.75 KW disruptor (IUL Instruments) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion et al. [49]. DNA–DNA hybridization was performed as described by De Ley et al. [50] under consideration of the modifications described by Huss et al. [51] 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) at 70 °C.

The DNA–DNA relatedness values between isolate CMAA 1452 and *S. dendaranthema* KLBMP 1305, *S. endophytica* YIM 61095 and *S. tripterygii* YIM 65359 were 53.5±2.3, 25.8±4.2 and 53.2±3.1 %, respectively; these values were well below the 70 % cut-off point recommended for assigning a bacterial strain to the same genomic species [52]. The hybridization data corroborated those found by Zhang et al. [25] and Simna et al. [53] in which some *Saccharopolyspora* species share high 16S rRNA gene sequence similarities but moderate DNA–DNA relatedness and therefore reinforce the necessity to use polyphasic approaches for correct taxonomic placement of newly isolated *Saccharopolyspora* members.

Strain CMAA 1452 was examined to establish whether it had a chemotaxonomic profile typical of *Saccharopolyspora* members. Standard procedures were used to determine the isomers of diaminopimelic acid [54], whole-organism sugars [55], polar lipids and predominant menaquinone [56]. Biomass for fatty acid methyl ester analysis was produced from trypticase soy agar (TSA; Difco) after growth at 28 °C ±4.2 and 53.2±3.1 %, respectively; these values were well below the 70 % cut-off point recommended for assigning a bacterial strain to the same genomic species [52]. The hybridization data corroborated those found by Zhang et al. [25] and Simna et al. [53] in which some *Saccharopolyspora* species share high 16S rRNA gene sequence similarities but moderate DNA–DNA relatedness and therefore reinforce the necessity to use polyphasic approaches for correct taxonomic placement of newly isolated *Saccharopolyspora* members.

The organism was found to contain *meso*-diaminopimelic acid, arabinose and galactose in whole-cell hydrolysates (wall chemotype IV and type A sugar pattern *sensu* Lechevalier and Lechevalier [5]), a complex phospholipid pattern consisting of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylcholine, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylinositol mannosides, phosphatidylinositol, unknown glycolipids, an unknown phospholipid and an unknown lipid (phospholipid pattern type PI1 [58]) (Fig. S2), but lacked mycolic
Acid. A tetra-hydrogenated menaquinone with nine isoprene units (MK-9[H₄]) was the major isoprenologue in strain CMAA 1452ᵀ at 82.95% of the total, whilst minor amounts of MK-9[H₂] and MK-9 were also detected (15.55 and 2.5%, respectively). Fatty acid analysis revealed that MK-9[H₄] (13.26%), summed feature 9 (C₁₅:0 10-methyl and/or C₁₇:1ω9c; 11.29%) and anteiso-C₁₇:0 (10.65%) (Table 1). These chemical features are also in line with the classification of isolate CMAA 1452ᵀ in the genus *Saccharopolyspora* [2].

Cultural characteristics were determined using standard ISP media [39] and on potato-dextrose agar, Czapek’s agar and nutrient agar after incubation at 28°C for 21 days. Spore ornamentation and arrangement were observed on gold-coated dehydrated specimens using a scanning electron microscope (Phenom-World) [59]. Tests of temperature tolerance (4, 10, 15, 20, 25, 28, 30, 37, 40 and 45°C), pH (4.0–10.0) and NaCl concentrations (0, 3, 5, 7, 10, 12 and 15%, w/v) were checked using ISP 2 culture medium after growth at 28°C for 8 days. Degradation and assimilation of various carbon sources were checked using the procedures described by Kurup and Schmitt [60], Gordon et al. [61] and Williams et al. [62]. Enzymatic activities were determined using API ZYM strips (bioMérieux), according to the manufacturer’s instructions.

Isolate CMAA 1452ᵀ was an aerobic, Gram-stain-positive, non-acid-alcohol-fast actinomycete that formed extensively, but not fragmented, branched substrate mycelia. The isolate...
grow relatively well on most of the tested media (exception was found for ISP 3 and ISP 7 agar media) but no soluble pigments were observed (Table S1). The colour of the vegetative mycelium was predominantly pale yellow whereas the aerial mycelium was white on ISP 2 medium but sparse or absent on the other tested media. The aerial mycelium was predominantly pale yellow whereas the vegetative mycelium was predominantly white on ISP 2 medium at 28°C for 21 days (Fig. 2).

Strain CMAA 1452\textsuperscript{T} could be distinguished from the type strain of its nearest neighbour, *S. dendranthema*, based on a combination of phenotypic properties, notably by its ability to degrade casein, cellulose, chitin and aesculin, and ability to grow on D-galactose and sorbitol, but not on dextrin, D-ribose or D-xylene as sole carbon sources, ability to use L-asparagine, L-histidine, L-lysine, L-serine or L-valine as sole nitrogen sources, and by its capacity to produce acid from cellulose, D-fructose, D-galactose, maltose or xylose (Table 2). The new isolate also degraded xanthine, hypoxanthine, tyrosine, starch, Tween 80, xylan and uric acid, but not adenine or urea. D-Arabinose, maltose, L-rhamnose and xylitol were used as sole carbon sources, but not L-arabinose, lactose, D-mannose or raffinose. It produced acid phosphatase, alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, \( \beta \)-galactosidase, chymotrypsin, \( \alpha \)-glucosidase, \( \beta \)-glucosidase, cystine arylamidase, esterase (C4), esterase lipase (C8), lucine arylamidase, N-acetyl-\( \beta \)-glucosaminidase and trypsin, but not \( \alpha \)-fucosidase, \( \alpha \)-galactosidase, \( \alpha \)-mannosidase, \( \beta \)-glucosidase or lipase (C14) as determined by APY ZYM tests.

The results of the present study demonstrated that strain CMAA 1452\textsuperscript{T} has morphological, chemotaxonomic and molecular genetic properties in line with its classification in the genus *Saccharopolyspora* [2]. The isolate can be distinguished from the type strain of *S. dendranthema* using DNA–DNA relatedness, 16S rRNA gene sequence and phenotypic data. It can also be concluded from the genotypic and phenotypic data that the isolate CMAA 1452\textsuperscript{T} can be distinguished readily from other members of the *S. flava* 16S rRNA gene subclade. It is therefore proposed that the isolate CMAA 1452\textsuperscript{T} be recognized as representing a novel *Saccharopolyspora* species, namely *Saccharopolyspora spongiae* sp. nov.

### Table 1. Comparison of cellular composition of strain CMAA 1452\textsuperscript{T} and the type strains of its closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Component</th>
<th>Strains</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10%)</td>
<td>iso-C(\text{16} _\text{a}) (26.2 %)</td>
</tr>
<tr>
<td></td>
<td>iso-C(\text{15} _\text{a}) (20.1 %)</td>
</tr>
<tr>
<td></td>
<td>iso-C(\text{17} _\text{a}) (13.3 %)</td>
</tr>
<tr>
<td></td>
<td>anteiso-C(\text{17} _\text{a}) (10.3 %)</td>
</tr>
<tr>
<td>Menaquinones</td>
<td>Arabinose, galactose, ribose</td>
</tr>
<tr>
<td></td>
<td>MK-9[H(<em>{1})], MK-9[H(</em>{2})], MK-9</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>64.8</td>
</tr>
</tbody>
</table>

**Fig. 2.** Scanning electron micrograph of spore chains of strain CMAA 1452\textsuperscript{T} after growth on ISP 2 medium at 28°C for 21 days. Bar, 2 \( \mu \)m.
Aerobic, Gram-stain-positive, non-acid-alcohol-fast actinomycete that forms an extensively branched substrate mycelium, which carries aerial hyphae that differentiate into short, straight or flexuous chains of smooth-surfaced spores on ISP 2 medium. Diffusible pigments are not produced. Growth occurs at 15–30 °C (optimum 25 °C), pH 6.0–10.0 (optimum pH 7) and in the presence of 0–7.0 % (w/v) NaCl (optimum 3 %). Degradates casein, chitin, aesculin, xanthine, hypoxanthine, starch, tyrosine, cellulose, xylan, uric acid and Tween 80, but not adenine or urea. D-Arabinose,
D-galactose, maltose, L-rhamnose, sorbitol and xyitol are used as sole carbon sources for energy and growth, but not L-arabinose, dextrin, lactose, D-mannose, raffinose, D-ribose or D-xylose (at 1.0%, w/v). Additional phenotypic and chemotaxonomic properties are cited in the main text and in Tables 1 and 2. Chemotaxonomic properties are typical of the genus Saccharopolyspora.

The type strain, CMAA 1452\(^T\) (=DSM 103218\(^T\)=NRRL B-65384\(^T\)), was isolated from the marine sponge Scopalinia ruetzleri, collected from St. Peter and St. Paul archipelago, Brazil. The G+C content of the DNA of the type strain is 64.8 mol%. The species description is based on a single strain and hence serves as the description of the type strain.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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