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## **Classification of the gifted natural product producer *Streptomyces roseofaciens* sp. nov. by polyphasic taxonomy — Source link**

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1 **Classification of the gifted natural product producer *Streptomyces***  
2 ***roseofaciens* sp. nov. by polyphasic taxonomy**

3

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18

19

20 **ABSTRACT**

21 A novel verticillate strain of streptomycetes, *Streptomyces* strain MBT76<sup>T</sup>, was  
22 isolated from the QinLing mountains, which harbours more than 40 biosynthetic gene  
23 clusters for natural products. Here we present full taxonomic classification of strain  
24 MBT76<sup>T</sup>, and show that it has chemotaxonomic, genomic and morphological  
25 properties consistent with its classification in the genus *Streptomyces*. Strain  
26 MBT76<sup>T</sup> is part of the cluster of Streptovercillates, a group within the genus  
27 *Streptomyces* that has characteristic whorl-forming spores produced in chains along  
28 the lateral wall of the hyphae. Multi-locus sequence analysis based on five house-  
29 keeping gene alleles showed that MBT76<sup>T</sup> is closely related to *Streptomyces*  
30 *hiroshimensis*. Average Nucleotide Identification (ANI) and Genome to Genome  
31 Distance Calculation (GGDC) of the genomes of strain MBT76<sup>T</sup> and *S. hiroshimensis*  
32 separated them into distinct species. Strain MBT76<sup>T</sup> represents a novel species of  
33 the genus *Streptomyces* for which we propose the name *Streptomyces roseofaciens*  
34 sp. nov. The type strain is MBT76<sup>T</sup> (=NCCB 100637<sup>T</sup> =DSM 106196<sup>T</sup>). The whole  
35 genome of MBT76<sup>T</sup> has 7974 predicted open reading frames and a total genome  
36 size of 8.64 Mb. Further genomic analysis showed that verticillate streptomycetes  
37 lack the sporulation gene *ssgE*, and our data suggest that this is a useful genetic  
38 marker for the spore-chain morphology of the verticillates.

39

40

## 41 INTRODUCTION

42 Actinomycetes are a major source of secondary metabolites, among which  
43 antimicrobial, antifungal and anticancer compounds (1). Of these, the  
44 streptomycetes are an especially rich source of clinical secondary metabolites, and  
45 produce over half of all clinically used antibiotics (2). One of the challenges of  
46 antibiotic discovery is that the potential for secondary metabolite expression of  
47 *Streptomyces* is difficult to determine. Even the model organism *Streptomyces*  
48 *coelicolor*, which has been a topic of research of over 30 years, produces  
49 unexpected secondary metabolites under novel conditions (3). Classification of  
50 prokaryotes is a rapidly changing field, being driven by technological advances like  
51 the current increase in genome sequence information. Even though the field is  
52 continuously leaning more towards the use of molecular techniques, phenotypic data  
53 needs to be collected to draw conclusions on the novelty of a species, for this reason  
54 we use a polyphasic approach (4-7). Classification of prokaryotes is typically based  
55 on genomic and phenotypic data, *i.e.* on polyphasic taxonomy (5, 6, 8). The impact  
56 of 16S rRNA gene sequence and DNA:DNA relatedness values is particularly high in  
57 terms of delineating taxa at the rank of species (4, 9), and has led to strongly  
58 improved classification of taxa belonging to the phylum Actinobacteria (7). Still, the  
59 resolution offered by 16S rRNA sequences is not sufficient for the recognition of new  
60 taxa. The SsgA-like proteins (SALPs) are very good additional markers for the  
61 accurate classification of Actinobacteria, and relate closely to morphological  
62 characteristics (10). The SALPs are unique to morphologically complex  
63 actinobacteria (11), and orchestrate aspects of peptidoglycan synthesis and  
64 remodelling, including cell division and spore maturation (12, 13). The archetypal  
65 SALP is SsgB, which initiates sporulation-specific cell division (13). SsgB shows

66 extremely high conservation (maximum of one aa change) within a genus, while  
67 there is high diversity even between closely related genera, making it a good marker  
68 to help classify genera within the Actinobacteria (10). Members of the genus  
69 *Kitasatospora* have an SsgB orthologue that differs from that of streptomycetes in 4  
70 positions, which is a distance that is sufficient to separate the two genera (14).

71 The position of the genus *Streptoverticillium* (15) has been a subject of debate  
72 for decades, and it is now generally accepted based on 16S rRNA phylogeny that  
73 members of this group of verticillate, or whorl-forming, *Streptomycetaceae* in fact  
74 belong to the genus *Streptomyces* (10, 16). Exceptionally, while the aerial hyphae of  
75 most streptomycetes develop into a single chain of spores at the apex, the  
76 verticillates produce small chains of spores at multiple sides perpendicular to the  
77 aerial hyphae. The MBT-collection of Actinobacteria isolated from soil from the  
78 QinLing mountains in China display wide phylogenetic and chemical diversity (17).  
79 We recently identified a novel member of the verticillate streptomycetes, namely  
80 *Streptomyces* species MBT76<sup>T</sup>. A screen against the so-called ESKAPE pathogens  
81 (18) followed by genome and natural product mining showed that *Streptomyces* sp.  
82 MBT76<sup>T</sup> is a rich source of natural products, including antibiotics (19). Analysis of the  
83 genome using AntiSMASH (20) identified 44 putative biosynthetic gene clusters  
84 (BGCs) for the secondary metabolites. Further investigation of the natural products  
85 produced by MBT76<sup>T</sup> using NMR-based metabolomics (21) identified a range of  
86 bioactive compounds, including isocoumarins, flavonoids, phenylpropanoids,  
87 siderophores and naphthaquinones (22-25).

88 In this work we wish to establish strain MBT76<sup>T</sup> as a novel species of  
89 *Streptomyces*. MBT76<sup>T</sup> was originally isolated from the QinLing mountains and has  
90 been the subject of several metabolomic studies. Polyphasic taxonomy shows that

91 MBT76<sup>T</sup> is a novel verticillate *Streptomyces* species for which we propose the name  
92 *Streptomyces roseofaciens* sp. nov., the name reflecting its production of a red/pink  
93 pigmented compound.

94

## 95 MATERIAL METHODS

### 96 *Media and growth conditions, strains*

97 *Streptomyces* sp. MBT76<sup>T</sup> was isolated from a soil sample collected from Shandi  
98 Village, the Qinling mountains, Xi'an, Shaanxi Province, China: (34°03'28.1"N, 109°  
99 22'39.0"E) height 660 m (26). MBT76<sup>T</sup> is part of the culture collection at Molecular  
100 Biotechnology, IBL, Leiden University. The reference strain, *Streptomyces*  
101 *hiroshimensis* DSM 40037 was obtained from the DSMZ collection. The strains were  
102 maintained by sub-culturing on ISP-2 and a spore-stock is frozen in glycerol at -80  
103 degrees. Biomass for biochemical tests was harvested from solid ISP-2 medium and  
104 freeze-dried.

105

### 106 *Phylogenetic analysis*

107 The complete 16S rRNA gene sequence (1,416 nucleotides [nt]), isolated from the  
108 genome sequence of *Streptomyces* sp. MBT76<sup>T</sup> (Genbank accession number:  
109 LNBE00000000.1), was submitted to the EzTaxon-e server ([http://eztaxon-](http://eztaxon-e.ezbiocloud.net/)  
110 [e.ezbiocloud.net/](http://ezbiocloud.net/); (27, 28) and aligned with corresponding 16S rRNA gene  
111 sequences of the type strains of the most closely related *Streptomyces* species  
112 using CLUSTALW version 1.8 (29). Phylogenetic trees were generated from the  
113 aligned sequences using the maximum-likelihood (30), maximum-parsimony (31)  
114 and neighbour-joining (32) algorithms drawn from the MEGA 5 and PHYL  
115 software packages (33, 34); an evolutionary distance matrix for the neighbour-  
116 joining analysis was prepared using the Jukes and Cantor model (35). The  
117 topology of the inferred evolutionary trees was evaluated by bootstrap analyses  
118 (36) based on 1,000 resamplings of the maximum-likelihood using MEGA 5

119 software. The root positions of unrooted trees were estimated using the sequence  
120 of *Kitasatospora setae* KM 6054<sup>T</sup>.

121

### 122 *Multilocus Sequence Analysis*

123 Multilocus sequence analysis was based on the method of Labeda (37). The  
124 sequences of *atpD* (ATP synthase F1,  $\beta$ -subunit), *gyrB* (DNA gyrase B subunit),  
125 *recA* (recombinase A), *rpoB* (RNA polymerase  $\beta$ -subunit) and *trpB* (tryptophan  
126 synthase,  $\beta$ -subunit) were extracted from the full genome sequence of strain  
127 MBT76<sup>T</sup>. The sequences of the loci for each strain were concatenated head to tail  
128 and exported in FASTA format, providing a dataset of 33 strains and 2351  
129 positions. Sequences were aligned using MUSCLE (38) and phylogenetic  
130 relationships constructed in MEGA 5.2 (33) using maximum-likelihood based on the  
131 General Time Reversible model (39). The phylogenetic relationships of the strains  
132 were also determined using maximum-parsimony and neighbour-joining analyses.  
133 MLSA evolutionary distances were determined using MEGA 5.2 to calculate the  
134 Kimura 2-parameter distance (40).

135

### 136 *Whole Genome analysis*

137 The average nucleotide identity (ANI) between the genomes of MBT76 (GenBank  
138 Accession number: NZ\_LNBE01000001.1) and *S. hirosimensis* (GenBank  
139 Accession number: NZ\_JOFL01000001.1) was determined using the OrthoANlu  
140 algorithm available as an online tool on EZbiotaxon (41). The digital DNA–DNA  
141 hybridization (dDDH) values between the genomes were calculated using the  
142 genome-to-genome distance calculator, GGDC 2.0 available at <http://ggdc.dsmz.de>.  
143 For dDDH, a cut-off value of 70% is used. (42). The ANI was calculated using the



144 ANI calculator on EzBiocloud using the orthoANlu algorithm (43). For ANI, a general  
145 cut-off value of 95-96% was used.

146

#### 147 *Sequence alignment and phylogenomic analysis.*

148 To find all SsgA-like proteins (SALPs) for the strains of interest, refseq annotated  
149 protein files were downloaded from NCBI of three verticillate strains of which a full  
150 genome sequence was available: *S. hiroshimensis* (NZ\_JOFL01000001.1), *S.*  
151 *cinnamoneus* (NZ\_MOEP01000440.1) and *S. mobaraensis* (NZ\_AORZ01000001.1).  
152 *S. coelicolor* (NC\_003888.3), *S. griseus* (NC\_010572.1) and *S. lividans* TK24  
153 (NZ\_GG657756.1) were added as reference strains. All genes for SALPs were  
154 obtained from the genome sequences of these strains by a BLAST search with low  
155 cut-off (e-value  $10^{-5}$ ) using the SALPs from *S. coelicolor* as the queries. To verify that  
156 all hits found were true SALPs, a second BLAST search was performed, using the  
157 output hits to interrogate the genome sequence of *S. coelicolor* M145. All hits whose  
158 reciprocal best hits were again SALPs were used for further phylogenetic analysis.  
159 Positive hits were then aligned using MUSCLE (38). Phylogenetic trees were  
160 generated using maximum-likelihood algorithms with default parameters as  
161 implemented in MEGA version 5 (33) The tree reliability was estimated by  
162 bootstrapping with 1,000 replicates (36). Secondary metabolite biosynthetic gene  
163 clusters were assessed using the antiSMASH 4.0 server (44).

164

#### 165 *Chemotaxonomy and morphology*

166 MBT76<sup>T</sup> was examined for chemotaxonomic and morphological properties  
167 considered to be typical of the genus *Streptomyces* (45). The arrangement of aerial  
168 hyphae and spore chains were observed on oatmeal agar (ISP medium 3; (46))

169 after 14 days at 28°C. Spore chain morphology and spore surface ornamentation  
170 were detected by examining gold-coated, dehydrated specimens by Scanning  
171 Electron Microscopy (JEOL JSM-7600F instrument) (47). Cultural characteristics of  
172 the isolate were determined on ISP 1-7 media (46) following incubation at 28°C for  
173 14 days. Standard protocols were used to detect the isomers of diaminopimelic  
174 acid (48), menaquinones and polar lipids (49), and whole organism sugars (48).  
175 Cellular fatty acids were extracted, methylated and analysed by gas  
176 chromatography (Hewlett Packard, model 6890) following the recommended  
177 procedure of the Sherlock Microbial Identification System (50). The resultant fatty  
178 acid methyl esters were identified and quantified using the MIDI ACTINO 1  
179 database (version 6.10).

180

### 181 *Phenotypic tests*

182 *Streptomyces* sp. MBT76<sup>T</sup> and *S. hiroshimensis* DSM 40037<sup>T</sup> were examined for  
183 biochemical, degradative and physiological properties, in duplicate, using media  
184 and methods described by Williams et al. (51) and known to be of value in the  
185 systematics of Streptomycetes (45). The enzyme profile of the strain was  
186 determined using API ZYM strips (BioMerieux) following the manufacturer's  
187 instructions; a standard inoculum equivalent to 5.0 on the McFarland scale (52)  
188 was used to inoculate the API ZYM strips.

189

## 190 **RESULTS AND DISCUSSION**

191 Strain MBT76<sup>T</sup> has cultural and morphological properties typical of *Streptomyces*  
192 (45). *Streptomyces* sp. MBT76<sup>T</sup> was isolated from a Qinling mountain soil sample  
193 (26) and produces many natural products that are activated in response to specific

194 environmental cues (17). The metabolomic potential was assessed by NMR-  
195 metabolomics (24) and genomics (23). Strain MBT76<sup>T</sup> shows verticillate sporulation  
196 with smooth spores (Figure 1). The strain grows moderately well on most ISP-media,  
197 and well on ISP2, 3 and 4 (Table 1), producing mostly pink pigments, characteristic  
198 of representatives of the red-pigmented verticillates (53).

199

## 200 **16S rRNA and MLSA trees**

201 When the 16S rRNA gene of strain MBT76 is compared with sequences of  
202 neighbouring *Streptomyces*, it was found to be closely related to *Streptomyces*  
203 *hiroshimensis* (99.37%), *Streptomyces mobaraensis* (99.24%) and *Streptomyces*  
204 *cinnamoneus* (99.17%). 16S rRNA gene sequence similarities with the remaining  
205 strains fell within the range of 99.10 to 98.13% (Figure 2). The results of the multi-  
206 locus sequence analysis (MLSA), based on five house-keeping genes head to tail,  
207 are shown in Figure 3. Isolate MBT76<sup>T</sup> forms a well-supported clade with the type  
208 strain of *S. hiroshimensis*<sup>T</sup>, as the strain morphologically fits in a clade with red-  
209 pigmented verticillate streptomycetes of which the morphology and pigmentation  
210 clusters very well with MLSA-based phylogeny (53-55). The MLSA evolutionary  
211 distances between isolate MBT76 and other verticillate strains in the same clade are  
212 shown in Table 3. Strain MBT76 showed an MLSA distance greater than 0.007 with  
213 all phylogenetically near species, supporting the proposal that this strain represents  
214 a new species (Table 2) (56).

215 To validate that strain MBT76<sup>T</sup> and *S. hiroshimensis* are separate species, *in*  
216 *silico* DNA:DNA hybridisation (DDH) studies were performed using two different  
217 methods, both the genome-to-genome distance calculator (GGDC) (57-59) and  
218 Average Nucleotide Identity (ANI)(41). Comparisons with the genome of

219 *Streptomyces* strain MBT76<sup>T</sup> with *S. hirosimensis*<sup>T</sup> yielded GGDC values of  
220 28.40%± 2.3 %, confirming that the strain is genetically separated from the type  
221 strain of *S. hirosimensis* (Table S1). The orthoANIu value of MBT76<sup>T</sup> and *S.*  
222 *hirosimensis* is 88.96 (Table S2). Both the GGDC and ANI values are well below  
223 the cut-off point for prokaryotic species delineation. The genomic DNA G+C  
224 composition of strain MBT76<sup>T</sup> is 71.9%. Interestingly, *S. cinnamoneus* and *S.*  
225 *mobaraensis* are known to produce elfamycin-type antibiotics of the kirromycin class  
226 that target elongation factor EF-Tu, and the antiSMASH data of MBT76<sup>T</sup> and *S.*  
227 *hirosimensis* shows that these two strains are potential kirromycin producers as  
228 well (60, 61).

229

### 230 **Taxonomic classification based on SALP phylogeny**

231 The developmental genes encoding members of the family of SsgA-like proteins  
232 (SALPs) serve as good predictive markers for morphological differences between  
233 Actinobacteria (10). Considering the obvious difference in spore-chain positioning  
234 between verticillates, which form small spore chains at multiple position along the  
235 lateral wall, and canonical streptomycetes that form long spore chains at the tips of  
236 the aerial hyphae, we compared the SALP proteins. Of these, SsgA, SsgB, SsgD,  
237 SsgE and SsgG are conserved within the streptomycetes, while various additional  
238 members are encountered with a strain- or clade-specific distribution. As verticillate  
239 streptomycetes we chose *Streptomyces* sp. MBT76<sup>T</sup>, *S. hirosimensis*, *S.*  
240 *cinnamoneus*, and *S. mobaraensis*, and as non-verticillate *S. coelicolor*, *S. lividans*  
241 and *S. griseus* (Figure 4). The *ssgA* gene is a useful phylogenetic marker for the  
242 further subclassification of members of the genus *Streptomyces* (10). The SsgA-  
243 based branch confirms the close correlation between MBT76<sup>T</sup> and *S. hirosimensis*<sup>T</sup>.

244 As mentioned above, the SsgB sequence of streptomycetes is invariable, except for  
245 amino acid residue 128. Streptomycetes that carry a threonine at position 128 (T128)  
246 sporulate in submerged cultures, while those carrying a glutamine (Q128) do not.  
247 Like all verticillates, strain MBT76 has an SsgB sequence that is consistent with  
248 classification within the genus *Streptomyces*. The specific variant is SsgB T128  
249 variant, suggesting that the verticillates sporulate in submerged cultures. Preliminary  
250 experiments confirmed this ability for strain MBT76 (not shown).

251 Interestingly, while all verticillate *Streptomyces* also have orthologues of *ssgD*  
252 and *ssgG*, they lack an orthologue of *ssgE*, which is fully conserved in non-  
253 verticillate streptomycetes. Importantly, *ssgE* deletion mutants of *S. coelicolor* fail to  
254 produce long spore-chains, with instead single spores and occasional short spore  
255 chains formed, suggesting that SsgE plays a role in spore-chain length and  
256 morphogenesis (12). Verticillate streptomycetes produce short spore chains along  
257 the lateral wall of the aerial hyphae (Figure 1), and the lack of *ssgE* may directly  
258 correlate to this different mode of sporulation. Taken together, the absence of *ssgE*  
259 could be a useful taxonomic marker for verticillate streptomycetes, and it will be very  
260 interesting to see which other genetic differences may correlate to the sporulation  
261 phenotype seen in members of this clade.

262

### 263 **Chemotaxonomy**

264 To further classify *Streptomyces* sp. MBT76<sup>T</sup> and compare it to other  
265 streptomycetes, we performed chemotaxonomy based on chemical composition of  
266 the cell wall. A whole-cell hydrolysate of the strain was rich in LL-diaminopimelic  
267 acid, glucose, mannose and ribose. The predominant menaquinone (>25%) was  
268 MK9H8 (47%). The cellular fatty acids contained large proportions (>10%) of *anteiso-*

269 C<sub>15:0</sub> (34.40%), and *anteiso*- C<sub>17:0</sub> (10.92%). Lower proportions (i.e. <10%) of *iso*-  
270 C<sub>14:0</sub> (8.28%), *iso*-C<sub>15:0</sub> (5.11%), *iso*-C<sub>16:0</sub> (7.99%), *anteiso*-C<sub>16:0</sub> (2.54%), C<sub>16:1</sub> ω<sub>9</sub>  
271 (2.84%), C<sub>16:0</sub> (5.64%), C<sub>18:1</sub> ω<sub>9</sub> (8.93%), C<sub>20:11</sub> ω<sub>11</sub> (4.53%) and summed features  
272 C<sub>18:2</sub> ω<sub>9,12</sub>/C<sub>18:0</sub> (8.81%). Polar lipids are diphosphatidylglycerol,  
273 phosphatidylethanolamine, phosphatidylinositol, Glycophosphatidylinositol, and an  
274 unknown lipid. Growth characteristics and phenotypic properties are summarized in  
275 Tables 1 and 2. Growth characteristics of *S. hiroshimensis* are summarized in Table  
276 S3.

277

### 278 **Description of *Streptomyces roseofaciens* sp. nov.**

279 *Streptomyces roseofaciens* (*ro.se.o.fa'ci.ens* L.adj.roseus.rosy; L.V.facie, make  
280 N.L.part.adj. roseofaciens, making rosy).

281 Aerobic, Gram-positive actinomycete which forms a branching substrate mycelium  
282 that carries aerial mycelium which forms verticillate chains with 3-5 smooth spores  
283 per chain. Substrate mycelium is pink on ISP1,2,3,4,5,7. Grows from 20 to 50°C,  
284 optimally at ~30°C, from pH 5.0 to pH 11, optimally at pH ~7, and in the presence of  
285 2% NaCl. L-tyrosinase, hypoxanthine and casein are hydrolysed. Degrades starch  
286 and gelatine. Uses glucose, sucrose and inositol as sole carbon source. The strain is  
287 positive for acid and alkaline phosphatase, α-cysteine arylamidase, α-chymotrypsin,  
288 esterase (C4), esterase lipase (C8), α- and β-glucosidase, α-mannosidase, *N*-acetyl-  
289 β-glucosaminidase, naphthol-AS-B1-phosphatase, trypsin and valine arylamidase, but  
290 negative for α-fucosidase, α- and β-galactosidase and β-glucoronidase (API-ZYM  
291 tests). The diagnostic amino acid in the peptidoglycan is LL-diaminopimelic acid,  
292 whole cell hydrolysates contain glucose, mannose and ribose. The predominant  
293 menaquinones is MK9(8). Polar lipids are diphosphatidylglycerol,

294 phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides,  
295 and an unknown lipid. The major fatty acids are anteiso- C<sub>15:0</sub>, and anteiso-C<sub>17:0</sub>. The  
296 strain has 44 putative secondary metabolite biosynthetic gene clusters predicted by  
297 antiSMASH 4.0 and has been analysed extensively by NMR- and MS-based  
298 metabolomics. The digital DNA G+C composition of the type strain is 71.9%.  
299 The type strain (=NCCB 100637<sup>T</sup> =DSM 106196<sup>T</sup>) was isolated from the QinLing  
300 mountains in the Shaanxi Province, China at an altitude of 660m, with permission.  
301 The species description is based on a single strain and hence serves as a  
302 description of the type strain. The GenBank accession number for the assembled  
303 genome of *Streptomyces roseofaciens* is GCA\_001445655.1.

304

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308

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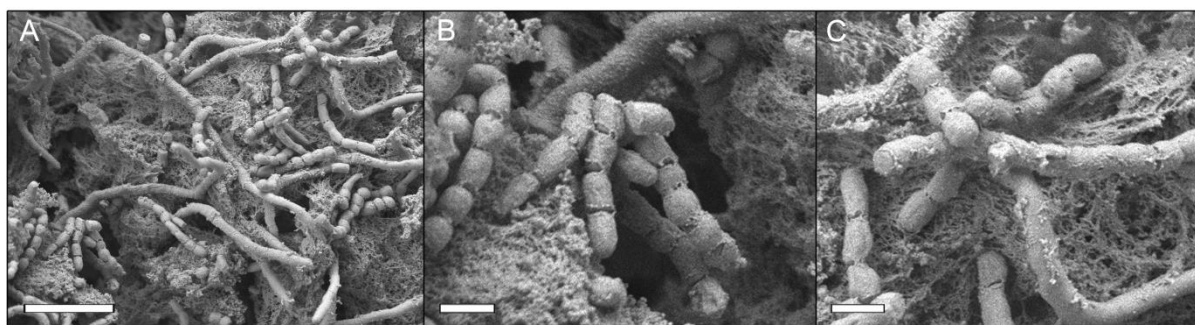
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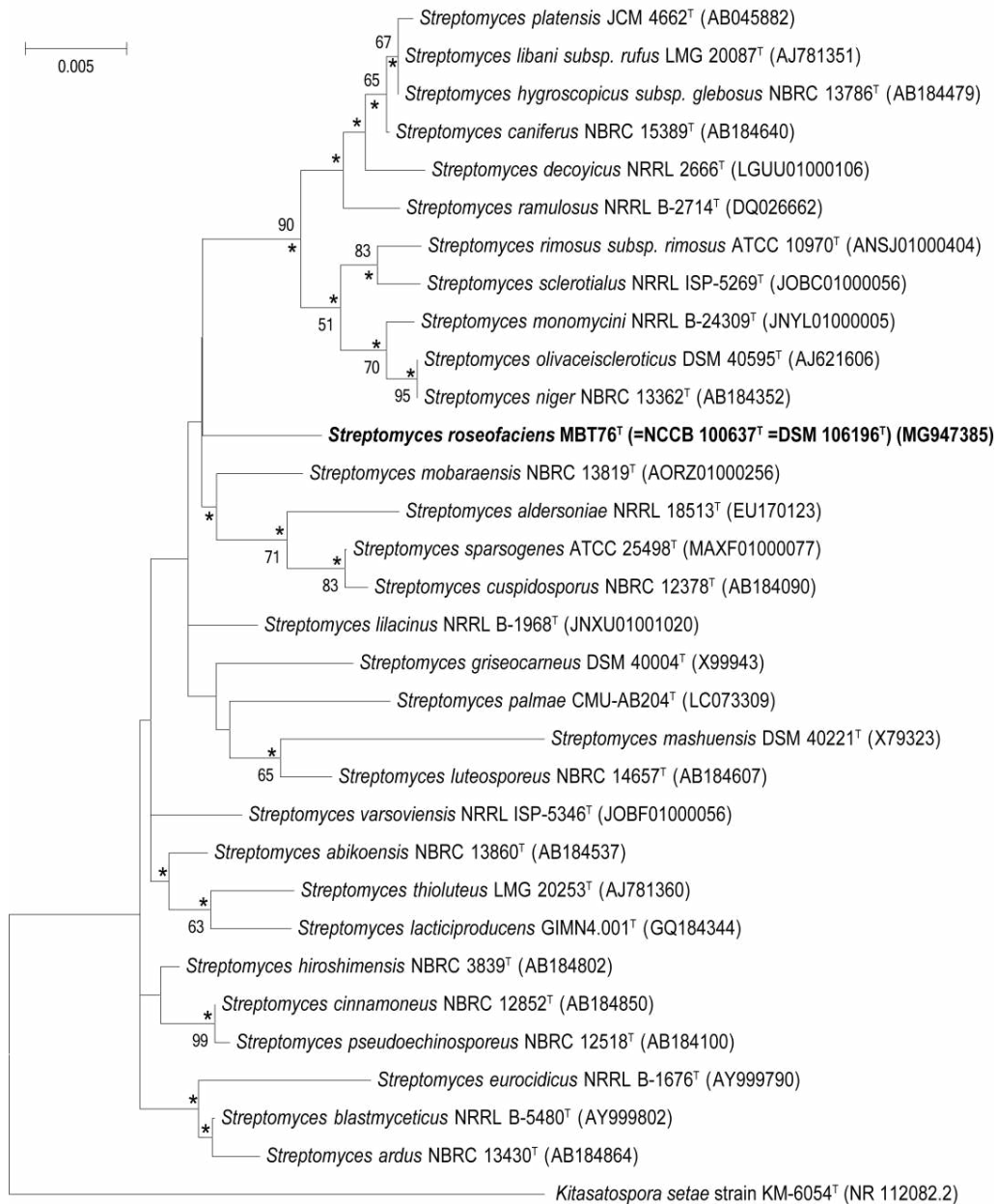
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475 **Figure 1:** Scanning electron micrographs from a 14-day old culture of *Streptomyces*  
476 MBT76<sup>T</sup> grown on ISP-3 agar plates. Images B and C are enlargements of image A.  
477 MBT76<sup>T</sup> produces smooth, verticillate spores of +/- 1  $\mu$ M in size. Bars: A: 5  $\mu$ M; B, C:  
478 1  $\mu$ M.

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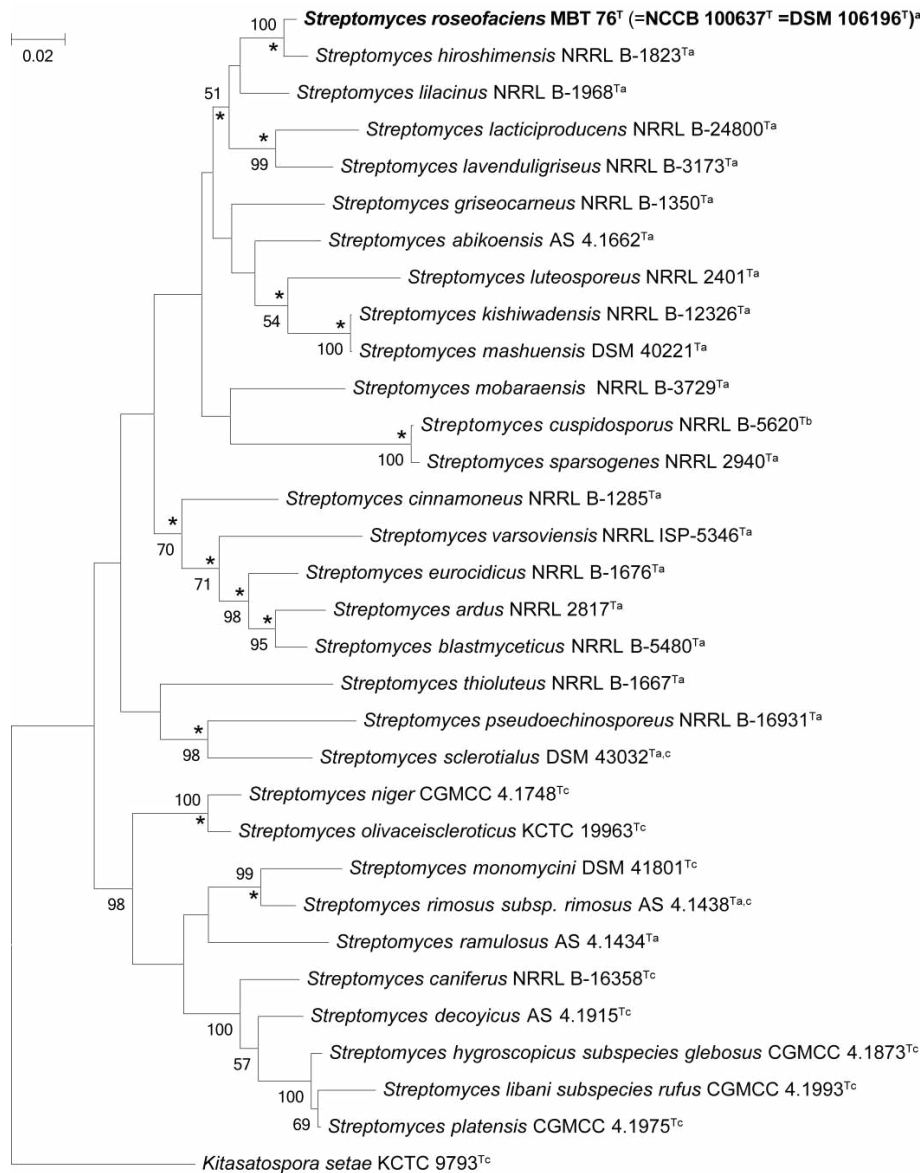
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483 **Figure 2.** Maximum-likelihood phylogenetic tree (30) based on 16S rRNA gene  
484 sequences. The tree shows relationships between isolate MBT76 and the type  
485 strains of closely related *Streptomyces* species. Asterisks indicate branches of the  
486 tree that were also recovered using the neighbour-joining (32) and maximum-  
487 parsimony (62) tree-making algorithms. Numbers at the nodes indicate levels of  
488 bootstrap based on a maximum likelihood analysis of 1,000 sampled datasets, only  
489 values above 50% are given. The root position of the tree was determined using  
490 *Kitasatospora setae* KM-6054<sup>T</sup>. GenBank accession numbers are given in  
491 parentheses. Scale bar, 0.005 substitutions per nucleotide position.

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493

494 **Figure 3.** Phylogenetic tree based on MLSA analysis. *Streptomyces roseofaciens*

495 (bold) is a part of a clade consisting of verticillate *Streptomyces*. Maximum-likelihood

496 phylogenetic tree inferred from partial sequences of the house-keeping genes *atpD*,

497 *gyrB*, *recA*, *rpoB* and *trpB* in MEGA 6.0. The analysis involved 33 nucleotide

498 sequences with a total of 2351 positions in the final dataset. Neighbour-joining and

499 maximum-parsimony models in Mega6.0 and conserved branches in all methods are

500 marked with an *asterisk*. Percentages at the nodes represent levels of bootstrap

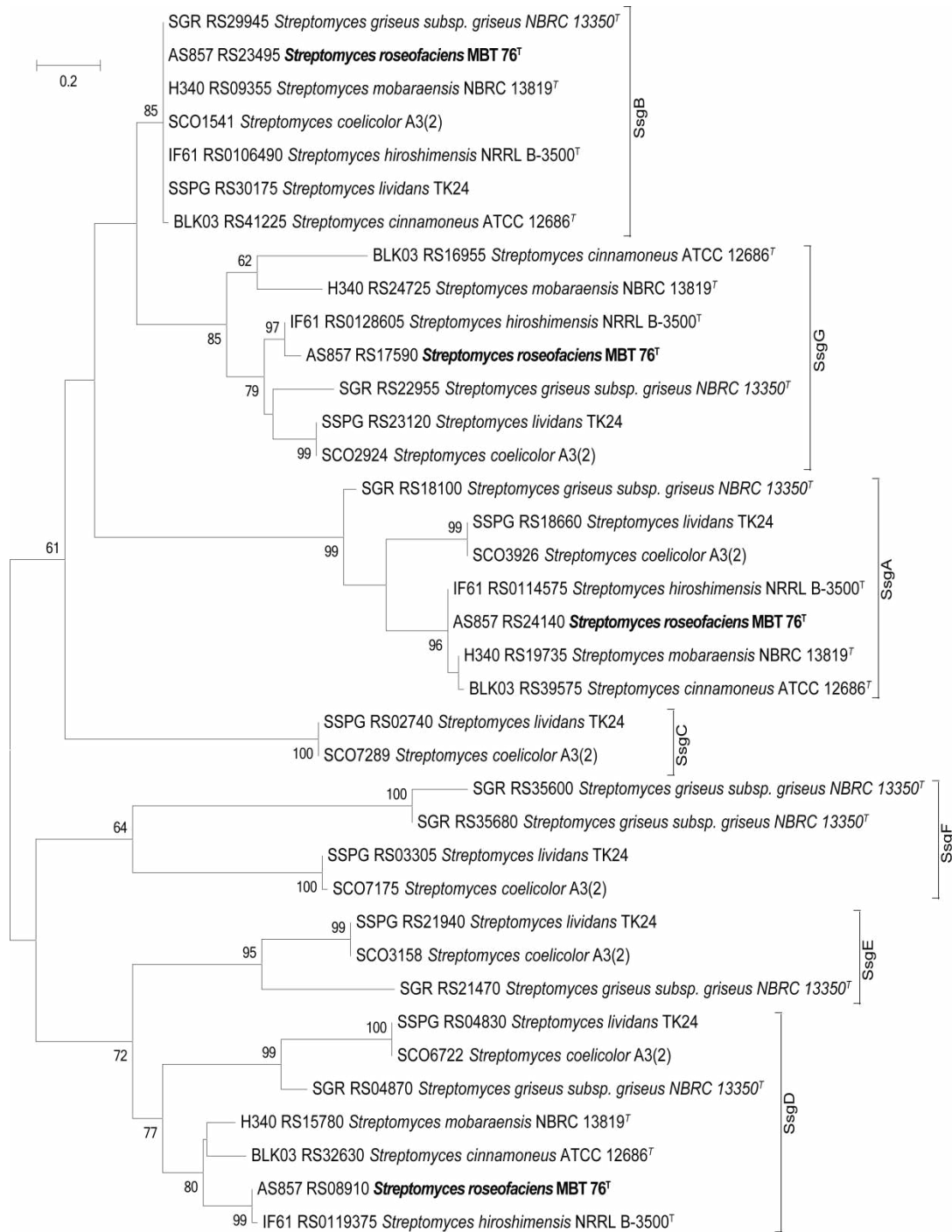
501 support from 1,000 resampled datasets with values with less than 60% not shown.

502 *Streptomyces* morphology: a: verticillate spore chains. b: not determined c:

503 *Streptomyces* with canonical (apical) spore chains.

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508 **Figure 4. Phylogenetic tree based on SALP sequences.** Maximum-likelihood  
 509 phylogenetic tree of SALP protein sequences in *S. roseofaciens*, *S. hirosimensis*,  
 510 *S. cinnamoneus*, *S. mobaraensis*, *S. coelicolor*, *S. lividans* and *S. griseus*. The  
 511 locus-ID of each SALP is indicated in the respective branch.

512