Another Brick in the Wall: the role of the actinobacterial cell wall in antibiotic resistance, phylogeny and development

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CHAPTER 2

Polyphasic classification of the gifted natural product producer *Streptomyces roseifaciens* sp. nov.

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ABSTRACT

A polyphasic study was designed to establish the taxonomic status of a *Streptomyces* strain isolated from soil from the QinLing Mountains, Shaanxi Province, China, and found to be the source of known and new specialized metabolites. Strain MBT76^T was found to have chemotaxonomic, cultural and morphological properties consistent with its classification in the genus *Streptomyces*. The strain formed a distinct branch in the *Streptomyces* 16S rRNA gene tree and was closely related to the type strains of *Streptomyces hiroshimensis* and *Streptomyces mobaraerensis*. Multi-locus sequence analyses based on five conserved house-keeping gene alleles showed that strain MBT76^T is closely related to the type strain of *S.hiroshimensis*, as was the case in analysis of a family of conserved proteins. The organism was also distinguished from *S. hiroshimensis* using cultural and phenotypic features. Average Nucleotide Identity and digital DNA-DNA hybridization values between the genomes of strain MBT76^T and *S. hiroshimensis* DSM 40037^T were 88.96 and 28.4+/-2.3%, respectively, which is in line with their assignment to different species. On the basis of this wealth of data it is proposed that strain MBT76^T (=DSM 106196^T = NCCB 100637^T), be classified as a new species*, Streptomyces roseifaciens* sp.nov.

Strain MBT76^T is an actinomycete isolated from a soil sample taken from the QinLing mountains in China. Many actinobacteria isolated from this niche turned out to be rich sources of bioactive compounds effective against multi-drug resistant bacterial pathogens (Zhu *et al.*, 2014b). Based on its genome sequence, MBT76 was positioned within the genus *Streptomyces* (Wu *et al.*, 2016). *Streptomyces* sp. MBT76^T is a gifted strain that produces various novel antibiotics and siderophores (Gubbens *et al.*, 2017, Wu *et al.*, 2017a, Wu *et al.*, 2017b, Wu *et al.*, 2016), its genome contains at least 44 biosynthetic gene clusters (BGCs) for specialized metabolites as identified by antiSMASH (Blin *et al.*, 2017). The importance of validly naming novel industrially important streptomycetes is often overlooked despite improvements in the classification of the genus *Streptomyces* (Kämpfer, 2012, Labeda *et al.*, 2017, Labeda *et al.*, 2012) and adherence to the rules embodied in the International Code of Nomenclature of Prokaryotes (Parker *et al.*, 2015).

Actinobacteria are Gram-positive often filamentous bacteria that are a major source of bioactive natural products (Hopwood, 2007, Barka *et al.*, 2016). The genus *Streptomyces*, the type genus of the family *Streptomycetacae* within the actinobacteria (Waksman & Henrici, 1943), encompasses over 700 species with valid names (http://www.bacterio.net/streptomyces.html), many of which have been assigned to multi- and single-membered clades in *Streptomyces* 16S rRNA gene trees (Labeda *et al.*, 2012, Kämpfer, 2012). Despite being the largest genus in the domain *Bacteria,* a steady stream of new *Streptomyces* species are being proposed based on combinations of genotypic and phenotypic features (Kumar & Goodfellow, 2010, Goodfellow *et al.*, 2017). It is particularly interesting that multi-locus sequence analyses (MLSA) of conserved house-keeping genes are providing much sharper resolution of relationships between closely related *Streptomyces* species than corresponding 16S rRNA gene sequence studies (Labeda *et al.*, 2017, Rong & Huang, 2014). Labeda and his colleagues observed correlations between certain morphological traits of streptomycetes and phylogenetic relationships based on MLSA data, as exemplified by the clustering of whorl-forming (verticillate) species (formerly *Streptoverticillium*) into a single well supported clade. Similarly, the sequences of highly conserved proteins (SALPS) have been used to resolve relationships between morphologically complex actinobacteria, including streptomycetes and closely related taxa classified in the family *Streptomycetaceae* (Girard *et al.*, 2013, Traag & van Wezel, 2008).

The aim of the present study was to establish the taxonomic status of *Streptomyces* sp. MBT76^T using a polyphasic approach. The resultant data show that the strain forms the nucleus of a novel verticillate *Streptomyces* species for which we propose the name *Streptomyces roseifaciens* sp.nov.

Streptomyces sp. MBT76^T was isolated from a soil sample (depth 10-20 cm), collected from Shandi Village in the QinLing mountains, Shaanxi Province, China (34˚03'28.1"N, 109˚ 22'39.0"E) at an altitude of 660 m (Zhu *et al.*, 2014b). The soil sample (1 g) was enriched with 6% yeast extract broth (Hayakawa & Nomomura, 1989) and incubated at 37^{0}C for 2 h in a shaking incubator. 0.1 mL aliquots of 10^{-2} to 10^{-4} dilutions of the resultant preparations were spread over selective agar plates (Zhu *et al.*, 2014b) supplemented with nystatin (50 μg/ml) and nalidixic acid (10 mg/ml), that were incubated at 30^{0}C for 4 days. The colony of the test strain was subcultured onto Soy Flour Mannitol agar (SFM) (Kieser *et al.*, 2000). The isolate and *Streptomyces hiroshimensis* DSM 40037^T were maintained on yeast extract-malt extract agar slopes (International *Streptomyces* Project medium [ISP 2]

(Shirling & Gottlieb, 1966)) at room temperature and as suspensions of spores and hyphae in 20%, v/v glycerol at -20^{0}C and -80^{0}C. Biomass for the chemotaxonomic and molecular systematic studies was cultured in shake flasks (180 rpm) of ISP 2 broth after incubation at 30^{0}C for 2 days and washed with distilled water, cells for the detection of the chemical markers were freeze-dried and then stored at room temperature.

The test strain was examined for chemotaxonomic and morphological properties known to be of value in *Streptomyces* systematics (Kämpfer, 2012, Goodfellow *et al.*, 2017). Spore chain arrangement and spore surface ornamentation were determined following growth on oatmeal agar (ISP 3 (Shirling & Gottlieb, 1966)) for 14 days at 28^{0}C, by scanning electron microscopy on a JEOL JSM-7600F instrument (Piette *et al.*, 2005). Key chemotaxonomic markers were sought using standard chromatographic procedures; the strain was examined for isomers of diaminopimelic acid (A_2pm) (Hasegawa *et al.*, 1983), menaquinones and polar lipids (Collins *et al.*, 1985) and whole-organism sugars (Hasegawa *et al.*, 1983). In turn, cellular fatty acids were extracted, methylated and analysed by gas-chromatography (Hewlett Packard, model 6890) using the Sherlock Microbial Identification System (Sasser, 1990) and the ACTINO version 6 database.

Strain MBT76^T was found to have chemotaxonomical and morphological properties consistent with its classification in the genus *Streptomyces* (Kämpfer, 2012). The organism formed branched substrate hyphae that carried filaments bearing short chains of oval to cylindrical, smooth-surfaced spores arranged in verticils (Fig. 1).

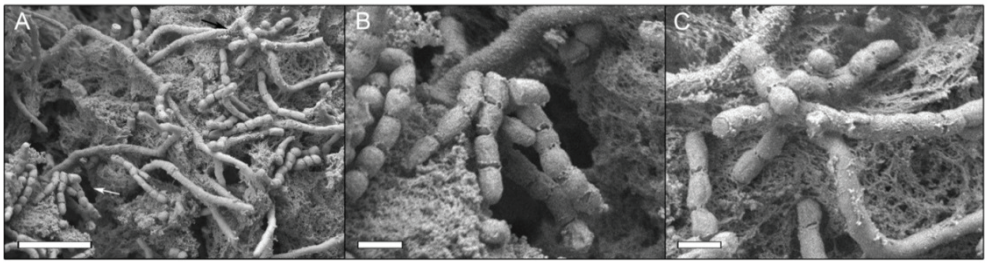

Figure 1. Scanning electron micrograph from a 14-day old culture of *Streptomyces* MBT76^T grown on an ISP-3 agar plate showing the presence of smooth, round to cylindrical verticillate spores. A shows a full overview, the white and black arrows refer to the respective magnifications B and C. Scale bars 1 μM.

Whole-organism hydrolysate of the strain was rich in *LL*-diaminopimelic acid, glucose, mannose and ribose, the isoprenologues were composed of octahydrogenated menaquinone with nine isoprene units (MK-9[H8]) (47%) and lesser amounts of MK-9[H6] (8%) and MK-9[H4] (3%). The polar lipid pattern consisted of diphospatidylglycerol, glycophospholipid, phosphatidylethanolamine, phosphatidylinositol, and an unknown compound, as shown in Fig. S1. The cellular fatty acids of the organism contained major proportions (>10%) of *anteiso*- $C_{15:0}$ (34.40%), and *anteiso*- $C_{17:0}$ (10.92%), lower proportions (i.e. <10%) of *iso*-$C_{14:0}$ (8.28%), *iso*-$C_{15:0}$ (5.11%), *iso*-$C_{16:0}$ (7.99%), *anteiso*-$C_{16:0}$ (2.54%), $C_{16:1}$ ω9 (2.84%), $C_{16:0}$ (5.64%), $C_{18:1}$ ω9 (8.93%), $C_{20:11}$ ω11 (4.53%) and summed features $C_{18:2}$ ω9,12/ $C_{18:0}$ (8.81%).

A 16S rRNA gene sequence (1,416 nucleotides [nt]) taken from the genome

sequence of *Streptomyces* sp. MBT76^T (Genbank accession number: LNBE00000000.1) was compared with corresponding sequences of the type strains of closely related *Streptomyces* species using the Eztaxon server (Yoon *et al*., 2017a). The resultant sequences were aligned using CLUSTALW version 1.8 (Thompson *et al.*, 1994)1994 and phylogenetic trees generated using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) algorithms taken from MEGA 7 software package (Tamura *et al*., 2011, Guindon & Gascuel, 2003, Kumar *et al*., 2016); an evolutionary distance matrix for the neighbour-joining analysis was prepared using the model of Jukes and Cantor (1969) (Jukes & Cantor, 1969). The topologies of the inferred evolutionary trees were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1,000 repeats. The root positions of unrooted trees were estimated using the sequence of *Kitasatospora setae* KM 6054^T (Genbank accession number: AP010968) .

Streptomyces sp. MBT76^T formed a distinct phyletic line in the *Streptomyces* 16S rRNA gene tree (Fig. 2; see also Fig. S2-S3). It was found to be most closely related to the type strains of *Streptomyces hiroshimensis* (Witt & Stackebrandt, 1990, Shinobu, 1955), *Streptomyces mobaraensis* (Witt & Stackebrandt, 1990, Nagatsu & Suzuki, 1963) and *Streptomyces cinnamoneus* (Witt & Stackebrandt, 1990, Benedict *et al.*, 1952) sharing 16S rRNA gene sequence similarities with them of 99.37% (9 nt differences), (99.24%) (= 11 nt differences) and 99.17% (=12 nt differences), respectively. The corresponding 16S rRNA gene sequence similarities with the remaining strains fell within the range 98.13 to 99.10%. The test strain was also found to form a distinct phyletic line in the analysis based on the maximum-parsimony and neighbour-joining algorithms.

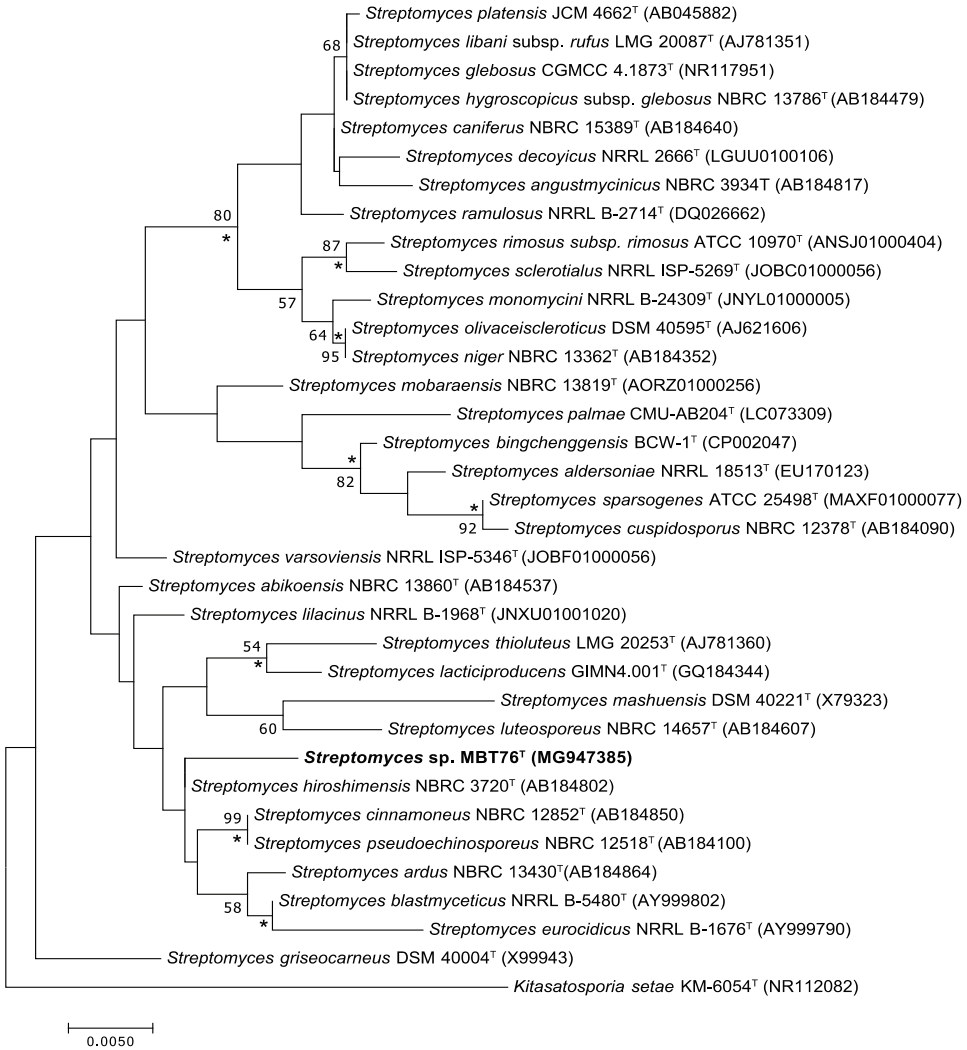

Figure 2. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences, showing relationships between isolate MBT76^T and the type strains of closely related *Streptomyces* species. Asterisks indicate branches of the tree that were also recovered using the neighbour-joining and maximum-parsimony tree-making algorithms. Numbers at the nodes indicate levels of bootstrap based on an analysis of 1,000 sampled datasets, only values above 50% are given. The root position of the tree was determined using *Kitasatospora setae* KM-6054^T. GenBank accession numbers are given in parentheses. Scale bar, 0.005 substitutions per nucleotide position.

The partial sequences of five house-keeping genes: *atpD* (encoding ATP synthase F1, β-subunit), *gyrB* (for DNA gyrase B subunit), *recA* (for recombinase A), *rpoB* (for RNA polymerase β-subunit) and *trpB* (for tryptophan synthase, β-subunit) were drawn from the full genome sequence of strain MBT76^T and from corresponding sequences on the *Streptomyces* type strains used to generate the 16S rRNA gene tree (Fig. 3; sequences presented in Table S1). The multilocus sequence analysis was based on the procedure described by Labeda (Labeda, 2011), the sequences of the protein loci of the strains were concatenated head-to-tail and exported in FASTA format, yielding a dataset of 33 strains and 2351 positions. The sequences were inferred using MUSCLE (Edgar, 2004) and phylogenetic relationships defined using the maximum-likelihood algorithm from MEGA 7 software (Tamura *et al.*, 2011, Kumar *et al.*, 2016) based on the General Time Reversible model (Nei & Kumar, 2000). The topology of the inferred tree was evaluated in a bootstrap analysis as described above. Phylogenetic trees were also generated using the maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) algorithms. Pairwise distances between the sequences of each locus were established using the two parameter model (Kimura, 1980). Strain pairs showing MLSA evolutionary distances <0.007 were taken to be conspecific as determined by Rong and Huang (Rong & Huang, 2012)validating the MLSA scheme for systematics of the whole genus, a value that corresponds to the 70% DNA-DNA threshold recommended for the discrimination of prokaryotic species (Wayne *et al.*, 1987).

MLSA have clarified relationships between closely related streptomycetes, thereby reflecting the strong phylogenetic signal provided by partial sequences of single copy house-keeping genes (Labeda, 2011, Labeda *et al.*, 2017, Labeda *et al.*, 2012, Rong & Huang, 2012). In the present study all of the verticillate-forming streptomycetes fell into a single clade that is sharply separated from associated clades composed of strains that form spores in straight, looped or spiral chains (Fig. 3). Strain MBT76^T and the type strain of *S. hiroshimensis* were found to form a distinct phyletic line supported by all of the tree-making algorithms and a 100% bootstrap value. It can also be seen from Figure 3 that these strains are at the periphery of a well-supported branch composed of an additional eight *Streptomyces* type strains that produce verticillate spore chains. The discovery that the strain can be separated from its closest phylogenetic neighbours by MLSA distances well above 0.007 threshold (Table 1) indicates that it forms a distinct phyletic line within the evolutionary radiation of the genus *Streptomyces* (Rong & Huang, 2014). The results of this study underpin those presented by Labeda et al. (Labeda *et al.*, 2017) by showing that streptomycetes which produce verticillate spore chains form a recognizable group in the *Streptomyces* gene tree that can be equated with the genus "*Streptoverticillium"* (Baldacci & Locci, 1974, Baldacci *et al.*, 1966).

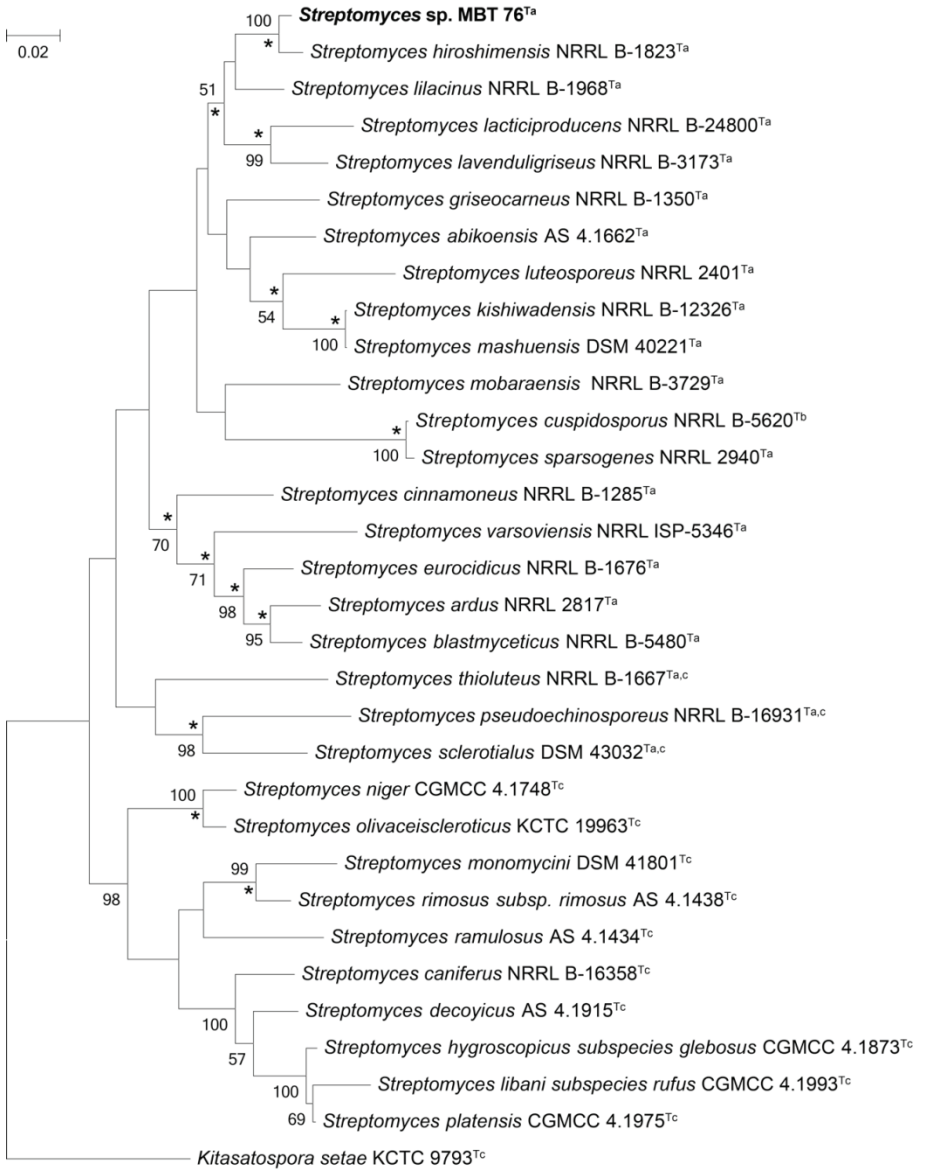

Figure 3. Phylogenetic tree inferred from concatenated partial sequences of house-keeping genes *atpD, gyrB, recA, rpoB* and *trpB* using the maximum-likelihood algorithm, based on the general time reversible model. The final dataset consisted of 2351 positions and 33 strains. Asterisks indicate branches of the tree that were recovered using the maximum-parsimony and neighbor-joining algorithms. Percentages at the nodes represent levels of bootstrap support from 1,000 resampled datasets with values with less than 60% not shown. Streptomyces morphology: [a]: verticillate spore chains. [b]: not determined [c]: *Streptomyces* with canonical (apical) spore chains.

Table 1. MLSA distances between strain MBT76^T and the type strains of closely related *Streptomyces* species.

	Strain	MLSA (Kimura 2-parameter) distance										
	1	2	3	4	5	6	7	8	9	10	11	12
1	Strain MBT76^T	-										
2	S. abikoensis AS 4.1662^T	**0.056**										
3	S. cuspidosporus NRRL B-5620^T	**0.097**	0.128	0.117								
4	S. griseocarneus NRRL B-1350^T	**0.059**	0.121	0.067	0.075							
5	S. hiroshimensis NRRL B-1823^T	**0.014**	0.114	0.084	0.070	0.063						
6	S. kishiwadensis NRRL B-12326^T	**0.062**	0.106	0.093	0.080	0.068	0.107					
7	S. lacticiproducens NRRL B-24800^T	**0.065**	0.106	0.089	0.090	0.083	0.112	0.078				
8	S. lavenduligriseus NRRL B-3173^T	**0.055**	0.115	0.090	0.086	0.079	0.117	0.077	0.052			
9	S. lilacinus NRRL B-1968^T	**0.038**	0.109	0.075	0.081	0.054	0.115	0.060	0.066	0.109		
10	S. luteosporeus NRRL 2401^T	**0.079**	0.106	0.080	0.084	0.092	0.104	0.066	0.088	0.117	0.074	
11	S. mashuensis DSM 40221^T	**0.062**	0.106	0.093	0.081	0.069	0.107	0.001	0.078	0.104	0.060	
12	S. sparsogenes NRRL 2940^T	**0.100**	0.130	0.119	0.123	0.112	0.133	0.102	0.108	0.122	0.097	0.102

The SsgA-like proteins (SALPs) have recently been proposed as phylogenetic markers for the accurate classification of Actinobacteria (Girard *et al.*, 2013). Members of the SALP protein family are typically between 130 and 145 amino acids (aa) long, and are unique to morphologically complex actinobacteria (Traag & van Wezel, 2008); they coordinate cell division and spore maturation (Noens *et al.*, 2005, Willemse *et al.*, 2011). SsgB shows extremely high conservation within a genus, while there is high diversity even between closely related genera (Girard *et al.*, 2013). Genes encoding SALPs were drawn from the genomes of strains MBT76^T, *S. cinnamoneus* (NZ_MOEP01000440.1), *S. mobaraensis* (NZ_AORZ01000001.1) and *S. hiroshimensis* (NZ_JOFL01000001.1) and from those of non-verticillate reference organisms, namely "*Streptomyces coelicolor"* A3(2) (NC_003888.3), *S. griseus* subspecies *griseus* NBBC 13350^T (NC_010572.1) and "*Streptomyces lividans"* TK24 (NZ_GG657756.1). A second BLAST search was undertaken based on a low cut-off value (e-value 10^{-5}) to interrogate the genome sequence of "*S. coelicolor"* M145 (NC_003888.3) to verify that the initial hits were *bona fide* SALPs. Sequences showing their best reciprocal hits against SALPs were aligned using MUSCLE (Edgar, 2004) and trees generated using the maximum-likelihood algorithm with default parameters as implemented in MEGA 7 software (Tamura *et al.*, 2011), the robustness of the resultant trees was checked in bootstrap analyses (Felsenstein, 1985) based on 1000 replicates.

The maximum-likelihood tree (Fig. 4) shows that all of the strains have genes that encode for the cell division proteins SsgA, SsgB, SsgD and SsgG (Noens *et al.*, 2005, Traag & van Wezel, 2008). It is also evident that the SsgB-protein, which mediates sporulation-specific division in *Streptomyces* strains (Willemse *et al.*, 2011) encodes for identical proteins in both the verticillate and reference strains. The sequences of the SALP proteins, SsgA and SsgG, underpin the close relationship between the test strain and *S. hiroshimensis* and separate them from the type strains of *S. cinnamoneus* and *S. mobaerensis*. It is particularly interesting that the verticillate strains lack an orthologue of SsgE, which is fully conserved in non-verticillate streptomycetes. SsgE proteins are considered to have a role in morphogenesis and the length of spore chains in "*S. coelicolor"* (Noens *et al.*, 2005). Further comparative studies are needed to determine whether the absence of SsgE in the genomes of verticillate streptomycetes is correlated to their different mode of sporulation.

Strain MBT76^T and *S. hiroshimensis* DSM 40037^T were examined for cultural and phenotypic properties known to be of value in the systematics of the genus *Streptomyces* (Williams *et al.*, 1983, Goodfellow *et al.*, 2017). The cultural properties were recorded from tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic-salt starch, glycerol-asparagine, peptone- yeast extract-iron and tyrosine agar (ISP media 1-7, (Shirling & Gottlieb, 1966)) plates following incubation as 28^{0}C for 14 days. Aerial and substrate mycelium colours and those of diffusible pigments were determined by comparison against colour charts (Kelly, 1964). The strains grew well on all of the media forming a range of pigments (Table 2). In general, strain MBT76^T produced a pink aerial spore mass, dark red substrate mycelia and pale brown diffusible pigments, black melanin pigments were formed on ISP 6 agar. In contrast, *S. hiroshimensis* formed a white aerial spore mass, cream, pink or white substrate mycelia and, when produced, a brown diffusible pigment, it also formed melanin pigments on ISP 6 agar.

The enzyme profiles for the test strain and *S. hiroshimensis* were determined using API-ZYM kits (BioMerieux) and a standard inoculum corresponding to 5 on the Mc Farland scale (Murray *et al.*, 1999) and by following the protocol provided by the manufacturer. Similarly, a range of biochemical, degradative and physiological properties were acquired using media and methods described previously (Williams *et al.*, 1983). Identical results were obtained for all of the duplicate cultures.

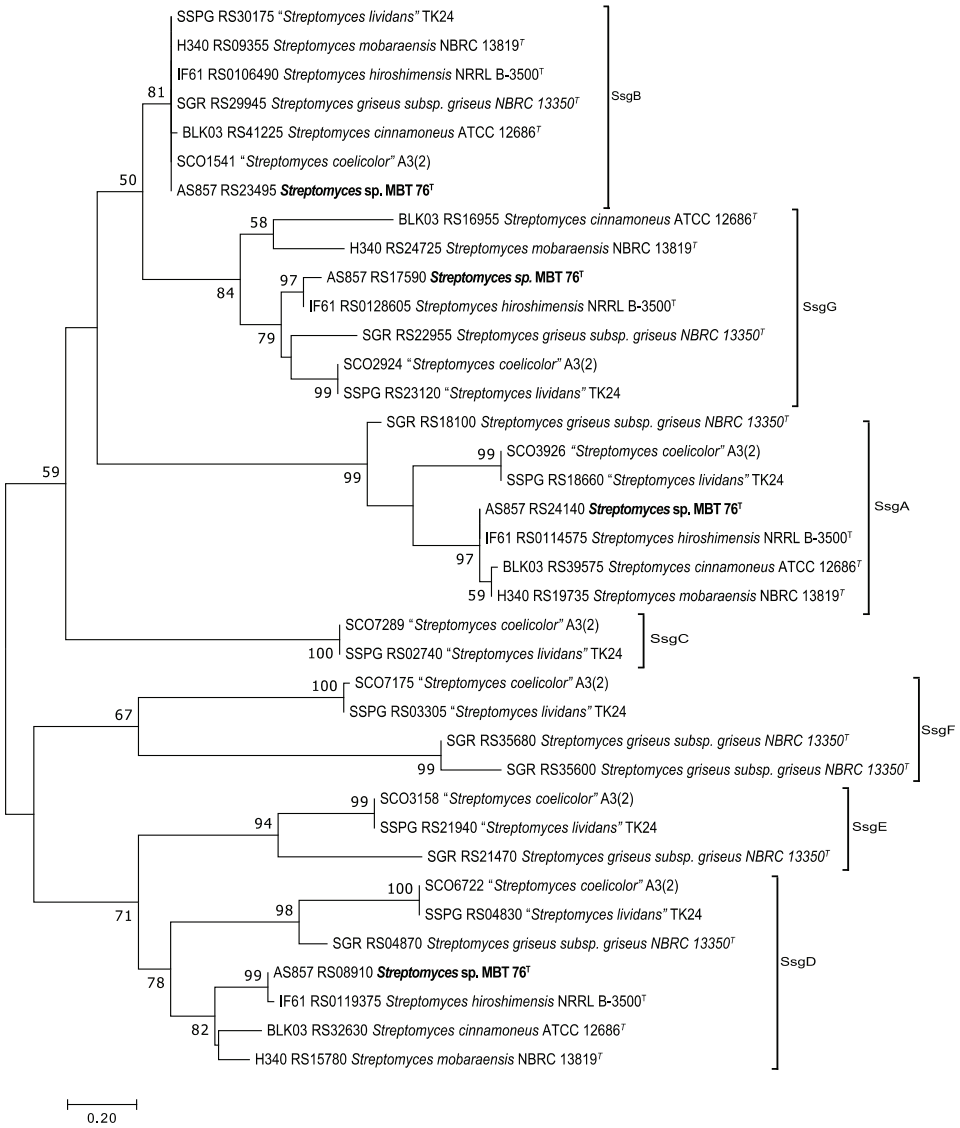

Figure 4. A composite maximum-likelihood tree showing the relationships between strain MBT76^T, the type strains of *S. cinnamoneus*, *S. hiroshimensis*, *S. mobaraensis* and reference strains "*S. coelicolor*", "*S. lividans*" and *S. griseus,* based on the sequences of SALP proteins.

The full genome sequence of strain MBT76T (GenBank accession number GCF_001445655) was elucidated using Illumina sequencing. The sequences assembled into 18 contigs, giving a total genome size of 8.64 Mb with a G+C content of 72.1%, with an N50 of 2,514,044 and a 200x genome coverage. The genome is predicted to encode 73 RNAs and 7,598 proteins. Gene functions were distributed among different classes using the RAST annotation tool (Fig. S4) (Aziz *et al.*, 2008). A total number of 44 secondary metabolites are predicted by antiSMASH 4.2.0 (Blin *et al.*, 2017), as shown in Table S2. Several genomic metrics are now available to distinguish between orthologous genes of closely related prokaryotes, including the calculation of average nucleotide identity (ANI) and digital DNA-DNA hybridization values (Yoon *et al.*, 2017b, Meier-Kolthoff *et al.*, 2013). In the present study, ANI and dDDH values were determined from the genomes of strain MBT76^T and *S. hiroshimensis* DSM 40037^T using the ortho-ANIu algorithm from Ezbiotaxon (Yoon *et al.*, 2017b) and the genome-to-genome distance calculator (GGDC 2.0) at http://ggdc.dsmz.de. The dDDH value between the genomes of the two strains was 28.4% ± 2.3 %, a result well below the 70% threshold for assigning strains to the same species (Wayne *et al.*, 1987), the digital DNA G+C value recorded for strain MBT76^T was 71.9 mol%. Similarly, a low ANI value of 88.96 was found between the two organisms, a result well below the threshold used to delineate prokaryote species (Richter & Rossello-Mora, 2009, Chun & Rainey, 2014).

It can be concluded from the chemotaxonomic, cultural, morphological and phylogenetic data that strain MBT76^T belongs to the genus *Streptomyces*. It can be distinguished from the type strain, *S. hiroshimensis*, its closest phylogenetic neighbour using genotypic and phenotypic procedures, notably by low ANI and dDDH values. Consequentially, strain MBT76^T should be recognised as a new *Streptomyces* species for this we propose the name *Streptomyces roseofaciens* sp.nov.

Table 2. Growth and cultural characteristics of strain MBT76^T and *Streptomyces hiroshimensis* DSM 40037^T after incubation at 30˚C for 14 days.

	Growth	Aerial spore mass colour	Substrate mycelium colour	Diffusible pigment
Strain MBT76^T				
Glycerol- asparagine agar (ISP 5)	+++	Pink	Dark red	None
Inorganic salts-starch agar (ISP 4)	+++	Pink	Pink	None
Oatmeal agar (ISP 3)	+++	Pink	Red	Pale brown
Peptone-yeast extract- iron agar (ISP-6)	++	Pink	Grey	Black
Tryptone-yeast extract agar (ISP 1)	+++	Pink	Dark red	Pale brown
Tyrosine agar (ISP 7)	+++	Grey	Dark red	Pale brown
Yeast extract-malt extract agar (ISP 2)	+++	Pink	Dark red	Pale brown
S.hiroshimensis DSM 40037^T				
Glycerol- asparagine agar (ISP 5)	+++	White	Pink	None
Inorganic salts-starch agar (ISP 4)	+++	White	White	None
Oatmeal agar (ISP 3)	+++	Pink	Pink	Brown
Peptone-yeast extract- iron agar (ISP 6)	++	None	Grey	Black
Tryptone-yeast extract agar (ISP 1)	+++	White	Cream	Brown
Tyrosine agar (ISP 7)	+++	White	Pink	None
Yeast extract-malt extract agar (ISP 2)	+++	White	Cream	Brown

+++abundant growth. ++, very good growth

Table 3. Phenotypic properties that distinguish strain MBT76 ^{T} from *S.hiroshimensis* DSM 40037^{T}

Characteristics	**Strain MBT76^{T}**	***S. hiroshimensis* DSM 40037^{T}**
Cultural characteristics on yeast extract-malt extract agar		
Aerial spore mass	Pink	White
Substrate mycelium	Dark red	Cream
Diffusible pigment	Pale brown	Brown
API ZYM tests:		
α-Chymotrypsin	+	-
β- Glucosidase	+	-
Lipase (C14)	+	-
α -Mannosidase	+	-
Trypsin	+	-
Degradation of:		
Xanthine	-	+
Growth on sole carbon source		
Sucrose	+	-
Fructose	-	+
Growth in presence of:		
3% w/v sodium chloride	-	+

Description of *Streptomyces roseifaciens* sp. nov.

Streptomyces roseifaciens (ro.se.i.fa'ci.ens L. masc. adj. roseus [italic type] rosy; L. pres. part. faciens [italic type] producing; N.L. part. adj. roseifaciens [italc type] producing rosy colour). Aerobic, Gram-stain positive actinobacterium which forms an extensively branched substrate mycelium that carries long straight filaments bearing at more or less regular intervals branches arranged in verticils. Each branch of the verticils produces at its apex short chains of 3-5 spores with smooth surfaces. Grows well on all ISP media. A red substrate mycelium, a pink aerial spore mass and a pale brown diffusible pigment are produced on oatmeal agar. Grows from 20-50˚C, optimally at ~30˚C, from pH 5.0 to pH 11, optimally at pH ~7, and in the presence of 2% NaCl. Produces acid and alkaline phosphatase, α-chymotrypsin, α-cysteine arylamidase, esterase (C4), esterase lipase (C8), *N*-acetyl-β-glucosaminidase, α- and β-glucosidase, α-mannosidase, naphthol-AS-B1-phosphatase, trypsin and valine arylamidase, but not α-fucosidase, α- or β-galactosidase or β-glucoronidase (API-ZYM tests). Degrades casein, gelatin, hypoxanthine, starch and L-tyrosine. Glucose, inositol and sucrose are used as sole carbon sources. Additional phenotype properties are given in Tables 1 and 2. Major fatty acids are anteiso-$C_{15:0}$, and anteiso-$C_{17:0}$, the predominant menaquinone is MK-9 (H8), the polar lipid profile contains diphospatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, glycophospholipid, and an unidentified lipid, the DNA G+C composition is 71.9 mol% and the genome size 8.64 Mbp. The genome contains 44 biosynthetic gene clusters many of which encode for unknown specialized metabolites.

The type strain MBT76^T (=NCCB 100637^T =DSM 106196^T) was isolated from a soil sample from the QinLing mountains, Shaanxi Province, China. The species description is based on a single strain and hence serves as a description of the type strain. The GenBank accession number for the assembled genome of *Streptomyces roseifaciens* is GCA_001445655.1.

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Conflicts of interest

The authors declare that they have no conflict of interest.