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Characterization of Streptomyces nymphaeiformis sp. nov., and Its Taxonomic Relatedness to Other Polyhydroxybutyrate-Degrading Streptomycetes

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Abstract:	A polyhydroxybutyrate (PHB)-degrading actinomycete, strain SFB5A T , was identified as a species of Streptomyces based on its membrane fatty acid profile and the presence of LL-diaminopimelic acid in the cell wall. It formed sporulating mycelia on most agar media, but flat or wrinkled, moist colonies on trypticase soy agar. Spores were smooth, cylindrical, and borne on long, straight to flexuous chains. It produced a light brown diffusible pigment, but not melanin. Comparison of genomic digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) values indicated that strain SFB5A T was related to Streptomyces litmocidini JCM 4394 T , Streptomyces vietnamensis GIMV4.0001 T , Streptomyces nashvillensis JCM 4498 T , and Streptomyces tanashiensis JCM 4086 T , plus eleven other species. However, the dDDH and ANI values were well below the species differentiation thresholds of <70% and <95%, respectively; also, multilocus sequence analysis distances exceeded the species threshold of 0.007. Moreover, strain SFB5A T differed from the other species in pigmentation and its ability to catabolize arabinose. Strain SFB5A T and 11 of its 15 closest relatives degraded PHB and have genes for extracellular, short-chain-length denatured polyhydroxyalkanoate depolymerases. These enzymes from strain SFB5A T and its closest relatives had a type 1 catalytic domain structure, while those from other relatives had a type 2 structure, which differs from type 1 in the position of a consensus histidine in the active site. Thus, phenotypic and genotypic differences suggest that strain SFB5A T represents a new species of Streptomyces, for which we propose the name Streptomyces nymphaeiformis sp. nov. The type strain is SFB5A T (= NRRL B-65520 T = DSM 112030 T).

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Does this article report on work with humans or animals?	No
Does this article include details (names, initials, hospital numbers), images, or videos relating to an individual person?	No

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Characterization of *Streptomyces nymphaeiformis* sp. nov., and its taxonomic relatedness to other polyhydroxybutyrate-degrading streptomycetes

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1.4 Keyword

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The IMG Genome ID and GenBank/EMBL/DDBJ accession numbers for the genome of strain SFB5A^T are 2863412751 and NZ_JACHJY000000000, respectively; its NCBI Bioproject, Biosample, and Sequence Read Archive (SRA) numbers are PRJNA581032, SAMN13190080, and SRX7669706, respectively. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SFB5A^T is MH392705.3. The IMG Genome ID and GenBank/EMBL/DDBJ accession numbers for the genome of *S. cinereoruber* subsp. *cinereoruber* NRRL ISP-5012^T are 2834008668 and JACIFQ000000000, respectively; its NCBI Bioproject, Biosample, and SRA numbers are PRJNA581031, SAMN13165722, and SRX7669712.

Seven supplementary figures and six tables are available with the online version of this article. The Supplementary Materials file is shown at the end of this version of the article.

ABSTRACT

A polyhydroxybutyrate (PHB)-degrading actinomycete, strain SFB5A^T, was identified as a species of *Streptomyces* based on its membrane fatty acid profile and the presence of LL-diaminopimelic acid in the cell wall. It formed sporulating mycelia on most agar media, but flat or wrinkled, moist colonies on trypticase soy agar. Spores were smooth, cylindrical, and borne on long, straight to flexuous chains. It produced a light brown diffusible pigment, but not melanin. Comparison of genomic digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) values indicated that strain SFB5A^T was related to *Streptomyces litmocidini* JCM 4394^T, *Streptomyces vietnamensis* GIMV4.0001^T, *Streptomyces nashvillensis* JCM 4498^T, and *Streptomyces tanashiensis* JCM 4086^T, plus eleven other species. However, the dDDH and ANI values were well below the species differentiation thresholds of <70% and <95%, respectively; also, multilocus sequence analysis distances exceeded the species threshold of 0.007. Moreover, strain SFB5A^T differed from the other species in pigmentation and its ability to catabolize arabinose. Strain SFB5A^T and 11 of its 15 closest relatives degraded PHB and have genes for extracellular, short-chain-length denatured polyhydroxyalkanoate depolymerases. These enzymes from strain SFB5A^T and its closest relatives had a type 1 catalytic domain structure, while those from other relatives had a type 2 structure, which differs from type 1 in the position of a consensus histidine in the active site. Thus, phenotypic and genotypic differences suggest that strain SFB5A^T represents a new species of *Streptomyces,* for which we propose the name *Streptomyces nymphaeiformis* sp. nov. The type strain is SFB5A^T (= NRRL B-65520^T = DSM 112030^T).

Introduction

The genus *Streptomyces* consists of Gram positive, filamentous soil and water bacteria with ~70% G + C content of the genomic DNA that form abundant reproductive spores and produce a variety of antibiotics. The genus is broad, containing 682 species with validly published and correct names to date (http://www.bacterio.net/streptomyces.html). On most agar media, streptomycetes initially form a substrate mycelium which later differentiates to produce a sporulating aerial mycelium, giving colonies a dry, powdery appearance. *Streptomyces* species have a type I cell wall containing the LL isomer of diaminopimelic acid [1] and large amounts of straight chain, iso-branched, and anteiso-branched saturated C14-C18 membrane fatty acids, but lack mycolic acids [2].

Streptomycetes efficiently degrade biological polymers such as: agar, starch, cellulose, chitin, and xylan by means of specific hydrolases [3]. Poly(3-hydroxyalkanoates) [PHAs] are water-insoluble polymers of R-3-hydroxyalkanoic acid monomers produced by many bacteria for storage of carbon and energy [4]. They represent a biodegradable alternative to petroleum-based plastics for certain applications. Native, intracellular PHAs (nPHAs) are amorphous, but when removed from the cell convert to partially crystalline, denatured PHAs (dPHAs) [5]. Short-chain-length dPHAs (dPHA$_{scl}$) consist of monomers with 3 to 5 carbon atoms, the most common of which is poly(3-hydroxybutyrate) (PHB) [5]. Other common dPHA$_{scl}$ are poly(3-hydroxyvalerate) [PHV] and heteropolymers such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), containing 3-hydroxybutyrate and 3-hydroxyvalerate monomers in various proportions. Medium-chain-length PHAs (dPHA$_{mcl}$) consist of monomers containing 6 or more carbon atoms. PHA producers use intracellular PHA depolymerases (i-PHA depolymerases) to degrade and catabolize intracellular

reserves of nPHAs. In contrast, environmental microbes catabolize dPHAs after first degrading them to their monomers via extracellular (e-dPHA) depolymerases [5].

In situ soil studies done in 1993 showed that numerous *Streptomyces* species degrade PHB and PHBV [6]. Accordingly, e-dPHA depolymerases specific for $dPHA_{scl}$ or $dPHA_{mcl}$ have been detected in cultures of many streptomycetes, and some purified and characterized [7-22]. The PHA Depolymerase Engineering Database (http://www.ded.uni-stuttgart.de/) [23] was constructed by using 28 seed sequences of enzymes with known PHA depolymerase activity to identify other putative PHA depolymerases in GenBank, and currently includes 24 homologous families of e-$dPHA_{scl}$ depolymerases. All known e-$dPHA_{scl}$ depolymerase primary amino acid sequences have: 1) a signal peptide; 2) a catalytic domain with a consensus "lipase box" pentapeptide (G-X1-S-X2-G) and a conserved histidine (thought to serve as an "oxyanion hole"); 3) a fibronectin type III- or threonine-rich linker domain; and 4) a C-terminal substrate-binding domain [5]. Two types of catalytic domains exist in e-$dPHA_{scl}$ depolymerases: in type 1 enzymes, the conserved histidine precedes the lipase box pentapeptide in the primary sequence; in type 2 enzymes it follows the lipase box pentapeptide [5]. Sixteen homologous families of type 1 enzymes have been identified, including five from *Streptomyces* spp. Eight homologous families of type 2 enzymes are listed, with only one from the genus *Streptomyces*.

Although the phylogeny of dPHA-degrading bacteria in general has been investigated [24], to our knowledge, no phylogenetic study focusing on $dPHA_{scl}$-degrading streptomycetes has been published to date. A $dPHA_{scl}$-degrading bacterium (strain SFB5A^T), tentatively identified as a *Streptomyces* species, was previously isolated and its e-$dPHA_{scl}$ depolymerase purified and characterized [18]. Based on data presented in this study, we propose that this strain represents a new species of the genus *Streptomyces.* We also investigate its phylogenetic relatedness to other $dPHA_{scl}$-degrading streptomycetes.

Isolation and Ecology

Strain SFB5A^T was previously isolated from decaying hardwood mulch located underneath a common boxwood shrub (*Buxus sempervirens*) in Harrisonburg, Virginia, USA (GPS coordinates 38.439475, -78.872423; altitude, 414 m) [18]. Briefly, a sample of the mulch was taken from approximately 1 cm below the surface. A 1.0 g portion was added to 100 mL of sterile saline solution and shaken vigorously. After large debris was allowed to settle for 1 min, the supernatant liquid was serially diluted 10-fold, and 100 µL samples thereof plated onto a defined mineral salts agar medium (SNC) [25] overlaid with SNC agar containing 0.2% w/v PHB (SNC-PHB). The strain was selected based on its ability to produce a clearing zone in the turbid PHB overlay after 2 days of incubation at 30°C and purified by streaking on SNC-PHB and trypticase soy agar (TSA) plates. It was deposited at the Agricultural Research Service (ARS) Culture Collection, Peoria, Illinois, USA with accession number NRRL B-65520^T and at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany with accession number DSM 112030^T.

All other *Streptomyces* species used for laboratory work in this study were obtained from ARS, except for *Streptomyces vietnamensis* DSM 41927^T and *Streptomyces wedmorensis* DSM 41676^T, which were obtained from DSMZ. Spore suspensions of all streptomycetes were prepared as described [26] and stored at -80°C as 25% v/v glycerol stocks.

16S RNA Phylogeny

Genomic DNAs from strain SFB5A^T and *S. cinereoruber* subsp. *cinereoruber* NRRL ISP-5012^T were isolated by the cetyltrimethylammonium bromide procedure [26], except that RNase A was included at a final concentration of 40 µg/mL in the TE25S buffer. The 16S rRNA gene of strain SFB5A^T was amplified from genomic DNA by PCR using universal primers pA (5'-AGAGTTTGATCCTGGCTCAG-3') and pH (5'-AAGGAGGTGATCCAGCCGCA-3') [27]. The ~1,500 base pair (bp) amplicon was purified by agarose gel electrophoresis and extraction with a Zymoclean™ Gel DNA Recovery Kit (Zymo Research, Irvine, California, USA). The DNA was ligated together with a pCR™4-TOPO® vector using the TOPO® TA Cloning® Kit for Sequencing (Life Technologies, Grand Island, NY, USA) and introduced into competent cells of *Escherichia coli* DH5α by transformation [28]. Plasmid DNA from positive transformants was sequenced using M13 forward and M13 reverse primers, pA and pH primers, and internal *Streptomyces*-specific primers: StrepB, StrepE, and StrepF [29]. Sequencing was performed by Eurofins MWG Operon (Louisville, Kentucky, USA). Overlapping sequences were assembled into a contig with the CAP3 Sequence Assembly Program [30] (available at: http://doua.prabi.fr/software/cap3). A total of 1,515 bp of clear sequence from the 16S rRNA gene of strain SFB5A^T was obtained by PCR and verified by comparison to the 16S rRNA gene sequence predicted from the genome.

The nearly complete 16S rRNA gene (1515 bp) from strain SFB5A^T was analyzed for similarities to those of other bacteria using the EZBioCloud 16S-based ID app (available at https://www.ezbiocloud.net/identify) [31]. Subsequent evolutionary analysis of the top 25 sequence matches was completed using MEGA X software, version 10.0.5 [32]. Sequences were first aligned with ClustalW, and a phylogenetic tree was constructed using the maximum likelihood method and Tamura-Nei model [33]. MEGA-X was also used to generate phylogenetic trees by the neighbor-joining [34] and maximum parsimony methods [35] for comparison. Bootstrap analysis with 1000 replicates was used to infer consensus trees in all three methods [36].

The search of the sequence of the 16S rRNA gene (1515 bp) from strain SFB5A^T against the EZBioCloud database revealed the highest similarity to sequences from *Streptomyces cinereoruber* subsp*. cinereoruber* NBRC 12756^T, *Streptomyces viridobrunneus* LMB 20317^T, and *Streptomyces showdoensis* NBRC 13417^T. Similarities among the 16S rRNA sequences for these and other organisms are shown in Table 1. A maximum likelihood phylogenetic tree constructed from the top 25 sequence matches placed strain SFB5A^T in a monophyletic clade consisting of *S. cinereoruber* subsp. *cinereoruber* NBRC 12756^T, *S. violaceorectus* NBRC 13102^T, *S. bikiniensis* NRRL B-1049^T, and *S. vietnamensis* GIMV4.001^T, and to a broader clade also containing *S. showdoensis* NBRC 13417^T and *S. viridobrunneus* LMB 20317^T (Fig. 1). These clades are respectively similar to clades 46 and 45 identified by Labeda *et al*., using neighbor joining analysis of 16S rRNA sequences [37]. Similar phylogenetic placement of strain SFB5A^T was obtained by the maximum parsimony method (not shown). However, neighbor joining analysis (Fig. S1) suggested inclusion of strain SFB5A^T in a clade containing *S. cinereoruber* subsp. *cinereoruber*, *S. viridobrunneus,* and *S. showdoensis*, with *S. violaceorectus, S. bikiniensis,* and *S. vietnamensis* in a neighboring clade.

Phylogenomic Comparisons

Whole-genome shotgun (WGS) sequencing was performed on genomic DNA isolated from strain SFB5A^T and *S. cinereoruber* subsp. *cinereoruber* NRRL ISP-5012^T, as part of the Genomic Encyclopedia of Type Strains, Phase III (KMG-III) study by the Joint Genome Institute, United States Department of Energy [38]. Illumina standard shotgun libraries were constructed and sequenced using the Illumina NovaSeq S4 platform [39]. Raw Illumina sequence was quality filtered using BBTools (available at http://jgi.doe.gov/data-and-tools/bb-tools/). Paired end sequencing of the strain SFB5A^T library (2 x 151 bp) generated 9.8 x 10^6 quality filtered reads; final draft assembly resulted in 36 contigs in 32 scaffolds totaling 9.23 Mbp in length, with a mapped coverage of 157.2X. Paired end sequencing of the *S. cinereoruber* subsp. *cinereoruber* library (2 x 151 bp) generated 2.5 x 10^7 quality filtered reads; final draft assembly resulted in 24 contigs in 23 scaffolds, totaling 7.46 Mbp in length, with a mapped coverage of 199.4X. The genome of strain SFB5A^T contained 72.09% G+C, 9.23 Mbp, 8,476 protein-coding genes, and 87 functional RNA genes, including 77 tRNA genes.

Pairwise average nucleotide identities (ANI) among FastA genome sequences of strain SFB5A^T and selected organisms were determined with the EZBioCloud ANI Calculator [40] (available at: https://www.ezbiocloud.net/tools/ani) using the OrthoANI algorithm [41]. The genomes of *S. violaceorectus, S. viridobrunneus,* and the type strain of *S. showdoensis* (NBRC 13417^T) were not available, although the genome of *S. showdoensis* ATCC 15227 (not a type strain) has been sequenced.

Phylogenomic analysis and computation of digital DNA-DNA hybridization values (dDDH) were done with the Type Strain Genome Server (TYGS) [42], available at https://tygs.dsmz.de. The genome of strain SFB5A^T was compared with those of all available type strain genomes via the MASH algorithm [43]. The ten type strains with the smallest MASH distances were chosen, and an additional set of ten closely related type strains was determined via the 16S rDNA gene sequences. These were extracted from the query genomes using RNAmmer [44] and each sequence was subsequently BLASTed [45] against the 16S rDNA gene sequences of all available type strains. This was used as a proxy to find the 50 best matching type strains and to subsequently calculate precise distances using the Genome BLAST Distance Phylogeny approach (GBDP) under the algorithm 'coverage' and distance formula d5 [46]. These distances were finally used to determine the 10 closest type strain genomes for each of the user genomes. For the phylogenomic inference, all pairwise comparisons among the set of genomes were conducted using GBDP and accurate intergenomic distances inferred under the algorithm 'trimming' and distance formula d5 [46]. 100 distance replicates were calculated each. dDDH values and confidence intervals were calculated using the recommended settings of the Genome-to-Genome Distance Calculator (GGDC) 2.1 [46]. The resulting intergenomic distances were used to infer a balanced minimum evolution tree with branch support via FASTME 2.1.6.1 including SPR postprocessing [47]. Branch support was inferred from 100 pseudo-bootstrap replicates each. The trees were rooted at the midpoint [48] and visualized with PhyD3 [49]. After an initial run with TYGS to identify the closest matches to strain SFB5A^T, a new search was done but was restricted to the top 15 genomic matches plus an outgroup, *Kitasatospora setae* B-16185^T, and a phylogenomic tree constructed from the data.

A multilocus sequence analysis (MLSA) was done with strain SFB5A^T and the top 15 genomic matches from dDDH analysis, plus the outgroup, *Kitasatospora setae* B-16185^T. Five housekeeping gene sequences: *atpD, gyrB, recA, rpoB* and *trpB,* were obtained from GenBank for each organism (see Table S1 for sequence accession numbers). End length disparities were manually trimmed, and

the sequences were then concatenated head to tail in-frame. Subsequent evolutionary analysis was completed using MEGA X, version 10.0.5 [32]. Concatenated sequences were aligned with ClustalW, and a phylogenetic tree was constructed using the maximum likelihood method and Tamura-Nei model [33] with 1000-replicate bootstrap analysis [36]. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). Phylogenetic trees were also constructed by the neighbor-joining [34] and maximum parsimony methods [35] for comparison.

The phylogenomic tree constructed with dDDH values via the TYGS platform (Fig. 2) displayed many of the same species as with the 16S rRNA analysis in Fig. 1, but with a different clustering. Strain SFB5A^T most closely matched with *S. litmocidini,* and these two strains were contained within a broader clade consisting of *S. vietnamensis, S. tanashiensis, S. nashvillensis* and *S. wedmorensis.* The phylogenomic tree placed *S. cinereoruber* subsp. *cinereoruber*, the closest match from 16S rRNA sequence data, in a more distantly related group. The trends within the dDDH values, ANI values, and 16S rDNA sequence similarity percentages (Table 1) roughly reflected the structure of this phylogenomic tree but did not correlate well with either genome size or %GC content difference.

A maximum likelihood tree constructed with the MLSA results (Fig. 3) showed a similar clustering of species as in the phylogenomic tree (Fig. 2). Strain SFB5A^T clustered with *S. litmocidini, S. vietnamensis, S. tanashiensis, S. nashvillensis* and *S. wedmorensis,* with the closest relative being *S. vietnamensis.* However, this cluster also included *S. bikiniensis, S. cinereoruber* subsp. *cinereoruber,* and *S. showdoensis*, which were placed in a more distantly related group by dDDH analysis (Fig. 2). Similar trees were obtained by neighbor joining and maximum parsimony analysis (not shown). Although *S. tanashiensis* and *S. nashvillensis* grouped with strain SFB5A^T, the branch for these two species was quite long, with pairwise distances versus strain SFB5A^T and the other organisms >0.28 (Table S2). Inspection of alignments showed considerable sequence disparity for the *rpoB* genes from these two bacteria versus the other organisms (coincidentally, these two *rpoB* genes are listed as identical in GenBank). When the MLSA was performed without *rpoB*, a similar clustering of species was obtained (Fig. S2), but the pairwise distances for *S. tanashiensis* and *S. nashvillensis* were reduced to <0.1.

The recommended species cutoff thresholds for ANI and dDDH are 95 to 96 % [50] and 70% [51], respectively. All ANI values among strain SFB5A^T and its relatives were below 95% (84.0 to 92.7%) and all dDDH values well below 70% (23.6 to 44.6%) (Table 1). Furthermore, the MSLA distances for strain SFB5A^T versus the other streptomycetes were considerably greater than the commonly accepted species level threshold of 0.007 [52], ranging from 0.022 to 0.280 (Table S2). Therefore, these genomic data collectively suggest that strain SFB5A^T is a separate species from the other strains being compared. Its four closest relatives based on ANI and dDDH values are *S. litmocidini*, *S. vietnamensis*, *S. nashvillensis*, and *S. tanashiensis*.

Physiology and Chemotaxonomy

International Streptomyces Project (ISP) media [53] and mannitol soy flour (MSF) agar [26] were prepared as described. Trypticase soy agar (TSA) was obtained from Smith River Biologicals, Ferrum, Virginia, USA. All *Streptomyces* cultures were incubated at 30°C unless otherwise indicated. Characterization and coloration of mycelial growth, sporulation, and pigments were assessed using 14-day-old cultures on ISP and other media as described [53], except that colors were referenced

against the RAL color code [54], as prescribed by Wink [55]. Morphologies of substrate mycelia were viewed by direct observation of colony edges on ISP 2 (yeast extract-malt extract) agar plate cultures using bright-field light microscopy. Spore morphologies were determined by gently pressing sterile 22 x 22 mm glass coverslips onto the surface of ISP 3 (oatmeal agar) plate cultures, followed by *in situ* fixation with methanol, Gram staining, and viewing with bright-field light microscopy. Fine structure of spores from strain SFB5A^T and *S. wedmorensis* DSM 41676^T was determined by scanning electron microscopy (EM) on angled coverslips inserted into ISP 3 agar plate cultures [56]. Coverslips were removed after 14 days, frozen at -80°C, lyophilized overnight, and sputter coated with gold using a Cressington 108 auto unit, (90 sec coating, 20 mA) under argon. Scanning EM was done with a LEO 1430VP microscope (5-15kV, working distance 7-13 mm, spot size 380-400 µm, probe current 84-120 pA, scan speed 6).

Temperature range was determined by growth on TSA slants incubated at 7, 15, 20, 30, 35, 37, 40, 45, and 50 °C. Salt tolerance was evaluated by growth on TSA plates (basal NaCl concentration 0.5% w/v) amended with additional NaCl, yielding final concentrations of 0.5, 3.0, 5.5, 8.0, and 10.5 % w/v. The pH range for growth was determined in ISP 2 (yeast extract-malt extract) broth [53] adjusted with HCl or NaOH to pH values ranging from 2 to 13 [29]. Utilization of soluble carbon sources (1% w/v) was tested using ISP 9 carbon utilization medium [53]. Degradation of chitin or PHB was evaluated on SNC agar plates (85 mm diameter, 20 mL of medium) overlaid with an SNC agar overlay (6 mL) containing 0.2% w/v PHB or 0.14% chitin. Degradation of the polymers was evidenced by growth and formation of clearing zones, monitored daily for 14 days. Production of H_2S was determined with lead acetate strips suspended 1 cm above the tips of nutrient agar slant cultures; a positive test was indicated by browning of the strip after 7 days [57]. Other standard microbiological tests were done essentially as described [57]. Antibiosis testing was performed using the agar plug diffusion method [58] with 6 mm diameter plugs cut from heavily inoculated, 7-day old ISP 2 agar cultures of the *Streptomyces* strains. Antibiosis was tested on ISP 2 agar plates against: *Bacillus cereus* ATCC 11778*, Bacillus subtilis* ATCC 6051*, Escherichia coli* K12 ATCC 10798, *Pseudomonas aeruginosa* ATCC 15442*, Salmonella typhimurium* ATCC 14028*, Staphylococcus aureus* ATCC 6538, and *Candida parasilosis* ATCC 7330. Bacteriophage typing of selected *Streptomyces* species was performed using bacteriophage BRock, which was previously isolated using strain SFB5A^T as the host [59]. Bacterial inocula were spread onto TSA plates with cotton swabs. Aliquots (1 µL) of a high titer lysate of BRock were then applied to the plates, which were then incubated at 30°C and monitored daily for up to 5 days for confluent lysis at the point of spotting in the surrounding lawn of bacterial growth.

Diaminopimelic acid isomers were analyzed using the method of Hasegawa *et al.* [60]. Sugars in whole cell hydrolysates of were determined by the method of Staneck and Roberts [61] except that staining was done with aniline-phosphoric acid reagent [62]. Cells for sugar analysis were grown in nutrient broth, washed twice with deionized water, and lyophilized. Polar lipids were extracted and analyzed by 2-dimensional TLC as described by Nguyen *et al.* [63]. Fatty acid methyl esters of cell membrane lipids were prepared from washed, lyophilized cells grown overnight in tryptic soy broth at 30°C with shaking, and analyzed using gas chromatography (FAME-Direct method) [64] by Microbial ID, Inc., Newark, Delaware, USA.

Morphological and biochemical testing of strain SFB5A^T confirmed that it was a member of the genus *Streptomyces,* as previously proposed [18]. It exhibited a typical streptomycete morphology on agar plate media. It grew and sporulated well on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), tyrosine agar (ISP 7), and SNC-PHB, but less so on inorganic salts-starch agar (ISP 4) and glycerol-asparagine agar (ISP 5) (Table S3). It grew well but did not sporulate on peptone-

yeast extract-iron agar (ISP 6) and TSA, forming moist, above-surface colonies lacking the typical powdery appearance of a sporulating mycelium. Colonies on these two media were often circular and wrinkled or flat with slightly raised edges, resembling water lily pads. A light brown, soluble pigment was formed on many of the media tested, but melanin was not produced on any media, including the melanin test media ISP 6 and ISP 7 [53]. Growth occurred from pH 5 to 12, 15° to 37°C, and with NaCl concentrations up to 5.5% w/v. Strain SFB5A^T was positive for catalase, oxidase, and H_2S production. The vegetative mycelium of strain SFB5A^T on ISP 3 agar showed branched hyphae, many 50 µm or more in length, without verticils (Fig. 4A). Spore chains were straight to flexuous (rectiflexibiles), some with greater than 50 spores (Fig. 4B). Spores measured about 1 x 2 µm and were smooth, cylindrical, and slightly tapered at the ends (Fig. 4C).

The cell wall of strain SFB5A^T contained the LL isomer of diaminopimelic acid (Fig. S3), indicating a type I cell wall characteristic of the genus *Streptomyces* [1]. Whole cell hydrolysates contained glucose and ribose (Fig. S3). Polar lipids included phosphatidylethanolamine, two phosphoglycolipids, plus small amounts of two unidentified aminolipids and four other unidentified lipids (Fig. S4). The major fatty acids (>1% of total) were straight chain, iso-branched, and anteiso-branched saturated C12-C18, including (from greatest to least): 15:0-anteiso, 17:0-anteiso, 16:0-iso, 15:0-iso, 17:0-iso, 16:0, 14:0-iso, and 17:0-cyclo (Table S4). Also detected were smaller amounts of straight chain or branched saturated C12, C13, C15, C17, and C18 plus C16-C18 straight chain or branched unsaturated fatty acids.

Cultural, morphological, and physiological characteristics of strain SFB5A^T were compared to those of eight streptomycetes identified by phylogenetic and phylogenomic analysis. As experimentally determined for strain SFB5A^T and *S. wedmorensis* (Fig. 4), the spore chains of the other species are all reported to have rectiflexibiles morphology, with smooth, cylindrical spores (see Table 2 for references). The effects of temperature, pH, and NaCl concentration on growth of all the organisms were similar. However, only *S. nashvillensis* grew at 45°C. The temperature minimum for five of the organisms, including strain SFB5A^T, was 15°C, but 21 °C for the others. All but *S. showdoensis, S. tanashiensis,* and *S. violaceorectus* tolerated up to 5.5% w/v NaCl, and *S. wedmorensis* did not grow at pH 5. All species formed sporulating mycelia with shades of gray, white, or green on ISP 3 (Table 2). The results with strain SFB5A^T for hydrolytic reactions (casein, chitin, esculin, gelatin, starch, Tween-80, and urea) and standard microbiological tests (citrate utilization, H_2S production, and nitrate reduction) were similar to those of its phylogenetic neighbors (Table 2), as were its fatty acid profile (Table S4) and antibiosis profile (Table S5). However, its pigmentation patterns were notably different. Strain SFB5A^T was one of four strains that did not produce melanin on ISP 6 agar (Table 2). As observed by Zhu *et al*. [65], *S. vietnamensis* secreted a blue to violet pigment on ISP 3 (Table 2) and several other media (Table S3), while strain SFB5A^T formed a light brown pigment on most media. Furthermore, on ISP4 medium, *S. litmocidini* formed a soluble violet pigment and *S. tanashiensis* a light green-brown pigment (Table S3). The carbon utilization pattern of strain SFB5A^T was similar to that of the other species, but it was one of only two that catabolized arabinose, of three that used raffinose, and of five that used carboxymethyl cellulose (Table 2). Interestingly, only strain SFB5A^T and its four closest neighbors by ANI and dDDH comparisons (Table 1) were susceptible to lysis by bacteriophage BRock, which was originally isolated using strain SFB5A^T as host [59].

e-dPHA$_{scl}$ Depolymerase Comparisons

Strain SFB5A^T was previously isolated based on its ability to degrade PHB [18]. The ability to degrade PHB appears to be widespread within the genus *Streptomyces* [6], and in fact, thirteen of the 18 phylogenetic cohort species from Table 1 at least partially degraded PHB (Fig. S5). Strain SFB5A^T, *S. nashvillensis*, *S. tanashiensis, S. gardneri,* and *S. violaceorectus* did so the most rapidly, exhibiting full clearing zones after only 2 days. Strains that did not degrade PHB after 14 days included *S. bikiniensis*, *S. narbonensis*, *S. purpureus, S. showdoensis*, and *S. vietnamensis.* All 18 strains grew well on SNC agar containing 0.5% glucose (not shown), suggesting that lack of PHB degradation was not due to the inability to grow on the defined medium, SNC.

Degradation of PHB by strain SFB5A^T is catalyzed by its e-dPHA$_{scl}$ depolymerase, which has previously been purified and characterized and its corresponding gene cloned and sequenced [18]. Its predicted amino acid sequence aligns best with catalytic domain type 1, homologous family 11 e-dPHA$_{scl}$ depolymerases catalogued in the PHA Depolymerase Engineering Database [23]. As of the last update in 2009, this family lists 33 enzymes from the phylum *Proteobacteria* and six from the phylum *Actinobacteria*, but only three from *Streptomyces* species. As of this writing, 562 genomes of *Streptomyces* species have been sequenced (https://www.ncbi.nlm.nih.gov/genome/browse#!/overview/streptomyces), presenting the opportunity to search for additional e-dPHA$_{scl}$ depolymerases within this genus. Thus, the e-dPHA$_{scl}$ depolymerase protein sequence from strain SFB5A^T (Genbank accession number WP_184931656) was used as the query in a blastp search [66] (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) of the refseq_select_prot database versus all *Streptomyces* genomes (txid1883), excluding uncultured and environmental samples. This strategy restricted the search to taxa with official nomenclature. A distance tree was then constructed from the search results using the fast minimum evolution method and Kimura protein distance model [67], with a maximum sequence difference of 0.85. To identify the catalytic domain type of proteins identified in the defined blastp searches, the FASTA amino acid sequences of the hits were then used in a BLAST search against the PHA Depolymerase Engineering Database (http://www.ded.uni-stuttgart.de/) [23], with an expect value threshold of 10^{-10}. The catalytic domain type was taken to be the consensus type from the top 10 hits in each search.

This search produced 100 hits with maximum scores ≥414, identity ≥50.8%, E-values <10^{-154}, and coverage ≥ 89% (Table S6). Three of the streptomycetes most closely related to strain SFB5A^T by dDDH analysis: *S. nashvillensis, S. tanashiensis,* and *S. litmocidini*, had among the highest percent identities (83.3, 82.3, and 78.7, respectively), with 100% coverage and were closely clustered with strain SFB5A^T in a distance tree constructed with the data (Fig. S6). All the enzymes identified were determined to be catalytic domain type 1 e-dPHA$_{scl}$ depolymerases (not shown). Thus, this particular category of e-dPHA depolymerases appears to be common among streptomycetes.

However, none of the other phylogenetic neighbors from Table 1 were represented in the distance tree of e-dPHA$_{scl}$ depolymerases (Fig. S6). To further explore the reason for this, each *Streptomyces* species in Table 1 was used in a search of the NCBI protein database (https://www.ncbi.nlm.nih.gov/protein/) with the term "depolymerase" to identify proteins annotated as e-dPHA depolymerases; unfortunately, this strategy could not be used for *S. violaceorectus* and *S. viridobrunneus*, since their genomes have not been sequenced. The FASTA sequences of these proteins were then aligned against that of the e-dPHA$_{scl}$ depolymerase of strain

SFB5A^T using blastp. The catalytic domain type was determined as above by searching against the PHA Depolymerase Engineering Database. The homologous family of a sequence was determined by searching for the accession numbers of the top 2 hits within the different families in the database. Finally, a distance tree (Fig. S7) was constructed with the search results as described above.

Again, the enzymes from *S. nashvillensis, S. tanashiensis, S. litmocidini,* and *S. xantholiticus* showed the highest scores, percent identities, and coverage (Table 3) and formed a cluster (cluster 1 in Fig. S7). The e-dPHA$_{scl}$ depolymerases of these species and strain SFB5A^T were all of catalytic domain type 1 and homologous family 11. However, both the scores (33.9-305) and coverage (21 to 75%) for the other hits were substantially lower. The enzymes from *S. bikiniensis, S. cinereoruber*, *S. exfoliatus* (3 enzymes), *S. gardneri, S. lateritius, S. venezuelae* (3 enzymes), and *S. wedmorensis* formed two clusters (cluster 2 and 3) and were classified as catalytic domain type 2. This might account for their lower scores and why they were not represented in the tree in Fig. S5. A further cluster (cluster 4) consisted of additional enzymes from *S. nashvillensis, S. tanashiensis,* and *S. venezuelae,* all of which were of catalytic domain type 1 but of homologous family 10. Furthermore, the cluster 4 enzymes were considerably smaller than that of strain SFB5A^T (246 to 306 amino acid residues versus 501), and their search scores were much lower than their cluster 1 counterparts. The reason for the structural differences among catalytic domain type 1 and 2 enzymes and the various homologous families is unknown, but may reflect variations in function, reaction mechanism, or substrate specificity.

No potential e-dPHA$_{scl}$ depolymerases could be found for *S. narbonensis*, *S. showdoensis,* and *S. vietnamensis* in the NCBI protein database. Instead, additional blastp searches of their genomes versus the enzyme from strain SFB5A^T (not shown) returned generic descriptions such as: fibronectin type III domain-containing protein, glycoside hydrolase family 18 protein, cellulose binding domain containing protein, tannase/feruloyl esterase, chitinase, and hypothetical proteins. The apparent absence of e-dPHA$_{scl}$ depolymerases would explain why these three bacteria did not degrade PHB (Fig. S5). On the other hand, *S. purpureus* and *S. bikiniensis* did not degrade PHB (Fig. S5), even though they both had putative type 2 e-dPHA$_{scl}$ depolymerases (Fig. S7), albeit with very poor blastp scores (Table 3). The reason for this discrepancy is unclear but could be due to uncertainty in genomic annotation, lack of transcriptional induction of their e-dPHA$_{scl}$ depolymerase genes under the conditions used, or perhaps to mutations in their promoter regions.

As previously noted, phylogenomic analysis suggested that strain SFB5A^T is most closely related to *S. litmocidini*, *S. tanashiensis*, *S. nashvillensis*, and *S. vietnamensis*. However, this phylogeny did not fully correlate with e-dPHA$_{scl}$ depolymerase classification. For example, *S. vietnamensis* did not degrade PHB and lacks an e-dPHA$_{scl}$ depolymerase gene in its genome. Furthermore, several of the other close phylogenomic neighbors (Table 1) had type 2 e-dPHA$_{scl}$ depolymerases instead of type 1 found in strain SFB5A^T, failed to degrade PHB, or apparently lacked e-dPHA$_{scl}$ depolymerase. Thus, differences in dDDH values, ANI values, MLSA distances, pigmentation patterns, sugar utilization, and e-dPHA$_{scl}$ depolymerase classification all argue that strain SFB5A^T represents a new species within the genus *Streptomyces,* for which we propose the name *Streptomyces nymphaeiformis* sp. nov.

Proposal of *Streptomyces nymphaeiformis* sp. nov.

Streptomyces nymphaeiformis sp. nov. [nym.phae.i.for'mis. L. fem. n. *nymphaea*, water lily; L. suff. -*formis* (from L. fem. n. *forma*, shape); N.L. masc. adj. *nymphaeiformis*, referring to resemblance of colonies on trypticase soy agar to water lily pads].

Aerobic, Gram-positive, filamentous rods. Good to moderate growth is obtained on ISP 1, ISP 2, ISP 3, ISP 5, ISP 6, ISP 7, and trypticase soy agar (TSA) media but poor growth on ISP 4 agar. Forms a sporulating mycelium with shades of gray, brown, or olive on most media. Substrate mycelium appears shades of brown to yellow when viewed from the underside. Spores are about 1 x 2 µm, smooth, cylindrical, and slightly tapered at the ends. They are borne on straight to flexuous chains, often with 50 or more spores per chain. Does not sporulate on TSA or ISP 6 but forms moist, flat or wrinkled colonies often with lipped edges. A light brown diffusible pigment, but not melanin, is produced on most agar media. Positive for amylase, oxidase, catalase, urease, gelatin liquefaction, esculin hydrolysis, Tween-80 hydrolysis, utilization of citrate, and H_2S production. Growth in defined medium is obtained with D-glucose, D-fructose, sucrose, D-raffinose, D-arabinose, D-xylose and carboxymethylcellulose; but no growth with myo-inositol, D-mannitol, or L-rhamnose. Grows on and hydrolyzes polyhydroxybutyrate and chitin. Growth in complex media occurs at pH 5 to 12, temperatures from 15° to 37°C, and NaCl concentrations from 0.5 to 5.5% w/v. Slight antibiosis is exhibited against *Bacillus cereus*, but not against *Candida parapsilosis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium,* or *Staphylococcus aureus.* Whole cell hydrolysates contain LL-diaminopimelic acid (LL-DAP), glucose, and ribose. Major polar lipids include phosphatidylethanolamine and phosphoglycolipids. The major fatty acids include 15:0-anteiso, 17:0-anteiso, 16:0-iso, 15:0-iso, 17:0-iso, 16:0, 14:0-iso, and 17:0-cyclo. The G + C content of the genomic DNA is 72.0%.

The type strain, SFB5A^T (=NRRL B-65520^T, =DSM 112030^T), was isolated from decaying hardwood mulch in Harrisonburg, Virginia, USA. The IMG Genome ID and GenBank/EMBL/DDBJ accession numbers for the genome of strain SFB5A^T are 2863412751 and NZ_JACHJY000000000, respectively; its NCBI Bioproject, Biosample, and Sequence Read Archive (SRA) numbers are PRJNA581032, SAMN13190080, and SRX7669706, respectively. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SFB5A^T is MH392705.3.

AUTHOR STATEMENTS

1.6 Authors and contributors

[A section on authorship and contributions using the CRediT taxonomy from CASRAI: https://casrai.org/credit/]

1.7 Conflicts of interest

The authors declare that there are no conflicts of interest.

1.8 Funding information

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1.9 Ethical approval (not applicable)

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ABBREVIATIONS

PCR, polymerase chain reaction

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FIGURES AND TABLES

Fig. 1. Maximum likelihood phylogenetic tree prepared with 25 sequences of 16S rRNA genes from *Streptomyces* species showing significant similarity to that of strain SFB5A^T. *Kitasatospora setae* NRRL B-16185^T was used as an outgroup. GenBank accession numbers for the sequences are shown in parentheses. The tree with the highest log likelihood (-2998.29) is shown. The percentage of trees (>50%) in which the associated taxa clustered together is shown next to the branches (1000 bootstrap replicates). There were 1450 positions in the final dataset. Bar: 0.01 substitutions per site.

Fig. 2. Phylogenomic tree inferred with GBDP distances calculated from genome sequences of strain SFB5A^T and other genomes from Table 1. Genome assembly accession numbers for the strains are as in Table 1. The branch lengths are scaled in terms of GBDP distance formula d5 [46]. The numbers are GBDP pseudo-bootstrap support values > 60 % from 100 replications. The average branch support was 96.3 %, and the δ-statistic [68] was 0.184. *Kitasatospora setae* NRRL B-16185^T was used as an outgroup. Bar: 0.01 substitutions per site.

Fig. 3. Maximum likelihood phylogenetic tree inferred with results of MLSA analysis of strain SFB5A^T and 16 other genomes from Table 1. Genes analyzed were *atpD*, *gyrB*, *recA*, *rpoB,* and *trpB* (see Table S1 for accession numbers). The tree with the highest log likelihood (-41257.12) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were 8442 total positions in the final dataset. Bar: 0.05 substitutions per site.

Fig. 4. Micrographs of cells of strain SFB5A^T and *S. wedmorensis* DSMZ 41676^T grown on agar media for 14 days. **A,** substrate mycelium of strain SFB5A^T viewed directly from an ISP 2 plate culture, bright-field illumination, 100X magnification, bar = 50 µm; **B,** Gram stain of spores from an ISP 3 716 plate culture of strain SFB5A^T, bright-field illumination, 1000X magnification, bar = 10 µm; **C,** scanning electron micrograph of gold coated spores from an ISP 3 culture of strain SFB5A^T, bar = 2 µm; **D**, scanning electron micrograph of gold coated spores from an ISP 3 culture of *S. wedmorensis* DSM 41676^T, bar = 2 µm. Since the spore morphology of *S. wedmorensis* DSM 41676^T was not available in the literature, it was determined experimentally in this study.

723 **Table 1.** Phylogenomic and phylogenetic data for strain SFB5A^T and related *Streptomyces* species. *Kitasatospora setae* NRRL B-16185^T was used as an
724 outgroup. All are type strains except *S. showdoensis* ATCC 15227. Coverage for the 16S rRNA data was > 98.0 %. dDDH values were obtained with formula
725 d4 [46]. % G + C content difference is with respect to the genome of strain SFB5A^T. The genomes of *Streptomyces viridobrunne*us LMG20317^T,
726 *Streptomyces showdoensis* NBRC 13417^T, and *Streptomyces violaceorectus* NBRC 13102^T have not been sequenced. Values in parentheses for MLSA pairwise
727 similarity were determined without *rpoB* in the analysis.

Organism	**GenBank Assembly Accession Number**	**Genome size (bp)**	**dDDH (%)**	**ANI (%)**	**MLSA pairwise similarity (%)**	**16S rRNA pairwise similarity (%)**	**% G + C Content Difference**
Streptomyces sp. SFB5A^T	GCA_014203895.1	9,225,538	-	-	-	-	-
Streptomyces litmocidini JCM 4394^T	GCA_014649755	7,944,220	44.6 ± 2.6	91.42	96.42 (95.4)	99.03	0.68
Streptomyces vietnamensis GIMV4.0001^T	GCA_000830005.1	9,153,777	44.0 ± 2.6	91.30	97.78 (97.2)	99.22	0.1
Streptomyces nashvillensis JCM 4498^T	GCA_014650095	8,581,463	42.9 ± 2.5	91.06	71.98 (96.9)	99.24	0.19
Streptomyces tanashiensis JCM 4086^T	GCA_014648895	8,981,402	42.6 ± 2.5	90.89	71.85 (96.6)	99.31	0.14
Streptomyces wedmorensis NRRL 3426^T	GCA_000716445.1	9,380,839	35.2 ± 2.5	88.00	96.15 (95.50)	98.83	0.02
Streptomyces venezuelae ATCC 10712^T	GCA_000253235.1	8,226,158	34.1 ± 2.5	87.55	94.72 (92.81)	98.96	0.39
Streptomyces cinereoruber subsp. *cinereoruber* NRRL ISP-5012^T	GCA_014197485.1	7,463,914	34.0 ± 2.5	87.36	95.61 (94.50)	99.65	0.97
Streptomyces exfoliatus NRRL B-2924^T	GCA_000718175.1	8,618,209	34.0 ± 2.5	87.60	95.96 (95.40)	98.90	0.37
Streptomyces bikiniensis NRRL B-1049^T	GCA_000716465.1	7,390,663	33.9 ± 2.5	87.45	95.17 (93.80)	99.17	0.98
Streptomyces narbonensis JCM 4147^T	GCA_014649015	7,582,412	33.5 ± 3.0	87.28	95.28 (94.31)	99.10	0.19
Streptomyces gardneri JCM 4375^T	GCA_014655085	9,054,543	33.3 ± 2.5	87.29	95.42 (94.66)	99.10	0.43
Streptomyces showdoensis ATCC 15227	GCA_001008345.1	8,146,620	28.9 ± 2.5	84.76	95.64 (94.48)	99.51	0.94
Streptomyces lateritius JCM 4389^T	GCA_014649715	7,688,656	27.5 ± 3.5	83.74	94.69 (94.16)	98.96	0.74
Streptomyces purpureus KA281, ATCC 21405^T	GCA_000384175.1	7,456,034	25.7 ± 2.4	82.12	92.40 (90.22)	98.55	0.57
Streptomyces xantholiticus JCM 4863^T	GCA_014651015	7,751,065	23.5 ± 2.4	79.88	78.09 (65.96)	97.94	1.6
Kitasatospora setae NRRL B-16185^T	GCA_000716965.1	8,560,950	21.1 ± 2.3	75.74	85.04 (82.69)	96.06	2.21
Streptomyces viridobrunneus LMG 20317^T	Not available	-	-	-	-	99.59	-

Streptomyces showdoensis NBRC 13417^{T}	Not available	-	-	-	-	99.40	-728
Streptomyces violaceorectus NBRC 13102^{T}	Not available	-	-	-	-	99.31	-729

730

731

Table 2. Differential phenotypic characteristics of strain SFB5A^T and selected phylogenetic neighbors identified by genomic comparisons. All characteristics were determined experimentally in this study except spore morphologies, most of which were obtained from the references listed. Strain designations: **SFB5A**, strain SFB5A^T; ***S. lit.***, *S. litmocidini* NRRL B-3635^T; ***S. viet.***, *S. vietnamensis* DSM 41927^T; ***S. tan.***, *S. tanashiensis* NRRL B-1692^T; ***S. nash.***, *S. nashvillensis* NRRL B-2606^T; ***S. wed.***, *S. wedmorensis* DSM 41676^T, ***S. sho.***, *S. showdoensis* NRRL B-12430^T; ***S. vio.***, *S. violoaceorectus* NBRC 13102^T; ***S. vir.***, *S. viridobrunneus* NRRL B-24332^T. See Table S3 for cultural characteristics on other media.

Characteristic†	SFB5A	*S. lit.*	*S. viet.*	*S. tan.*	*S. nash.*	*S. wed.*	*S. sho.*	*S. vio.*	*S. vir.*
Growth ranges:									
Temperature range (°C)	15-37	15-40	21-40	21-37	21-45	21-37	15-40	15-37	15-40
pH range	5-12	5-12	5-12	5-12	5-12	6-12	5-12	5-12	5-11
NaCl tolerance (% w/v)	5.5	5.5	5.5	3.0	5.5	5.5	3.0	3.0	5.5
Cultural Characteristics (on medium indicated):									
Sporulation (ISP3)	Good	Good	Moderate	Good	Moderate	Moderate	Moderate	Poor	Moderate
Mycelium color (ISP3)	Reed green	Moss gray	Cream	Olive gray	Clean room white	Olive gray	Gray white	Gray white	Olive green
Reverse color (ISP3)	Olive brown	Golden yellow	Olive brown	Ivory	Clean room white	Olive gray	Zinc yellow	Zinc yellow	Olive brown
Diffusible pigment (ISP3)	Light brown	Light brown	Violet-blue	Light brown	None	None	None	None	Olive brown
Melanin production (ISP6)	-	+	+	+	-	-	+	-	+
Utilization of:									
D-Arabinose	+	-	+	-	-	-	-	-	-
D-Raffinose	+	-	-	+	-	-	+	-	-
L-Rhamnose	-	-	-	+	-	+	-	-	-
Carboxymethylcellulose	+	+	-	+	+	-	-	-	+
Other tests*:									
Gelatin hydrolysis	+	+	+	+	-	+	+	+	+
H_2S production	+	+	+	w	+	+	+	+	w
NO_3^- reduction	+	w	+	w	-	+	+	w	-
Lysis with BRock bacteriophage	+	+	+	+	+	-	-	-	-
Reference for hyphal and spore morphologies	This study	[69]	[65]	[70]	[71]	This study	[72]	[71]	[55]

†All strains had straight to flexuous hyphae and smooth spores as mentioned in the references shown. All strains were positive for utilization of citrate, glucose, fructose, sucrose, and xylose but negative for myo-inositol and D-mannitol. All strains hydrolyzed casein, chitin, esculin, starch, Tween-80, and urea.

*w, weak reaction

740 **Table 3.** Comparison of the amino acid sequences of e-PHA$_{scl}$ depolymerases from strain SFB5A^{T} and its phylogenomic cohort. The accession
741 number for the enzyme from strain SFB5A is WP_184931656. See Materials and Methods for details of the analysis.

Taxonomic Name	Max Score	Total Score	Query Coverage (%)	E value	% Identity	Length	Accession number	Catalytic Domain Type
Streptomyces tanashiensis JCM 4086^{T}	778	778	100	0	83.27	497	WP_189801336.1	1
Streptomyces nashvillensis JCM 4498^{T}	769	769	100	0	82.27	497	WP_190105687.1	1
Streptomyces litmocidini JCM 4394^{T}	721	721	100	0	78.69	497	WP_190158237.1	1
Streptomyces xantholiticus JCM 4863^{T}	305	385	70	2.00E-102	54.03	421	WP_189885961.1	1
Streptomyces venezuelae ATCC 10712^{T}	164	259	75	4.00E-48	62.5	490	ALO13326.1	2
Streptomyces wedmorensis NRRL 3426^{T}	139	234	75	5.00E-39	55.32	488	WP_033209340.1	2
Streptomyces gardneri JCM 4375^{T}	137	232	74	8.00E-38	57.45	578	WP_190133566.1	2
Streptomyces exfoliatus NRRL B-2924^{T}	118	209	75	1.00E-31	61.33	488	WP_198540439.1	2
Streptomyces venezuelae ATCC 14585	48.1	48.1	16	4.00E-09	35.16	244	QES42386.1	2
Streptomyces venezuelae ATCC 10712^{T}	48.1	87.4	35	5.00E-09	35.16	354	WP_190329368.1	2
Streptomyces lateritius JCM 4389^{T}	45.1	74.3	38	8.00E-08	30	547	WP_158992956.1	2
Streptomyces venezuelae ATCC 10712^{T}	42.4	42.4	21	3.00E-07	31.48	287	WP_150221098.1	1
Streptomyces exfoliatus NRRL B-2924^{T}	42.4	72.8	36	6.00E-07	30.61	544	WP_137991574.1	2
Streptomyces venezuelae ATCC 10712^{T}	42	42	16	6.00E-07	32.67	536	WP_150163299.1	2
Streptomyces venezuelae ATCC 10712^{T}	42	68.5	37	7.00E-07	32.67	536	WP_015038468.1	2
Streptomyces venezuelae ATCC 10712^{T}	41.6	41.6	16	7.00E-07	32.67	536	WP_150500144.1	2
Streptomyces exfoliatus NRRL B-2924^{T}	41.2	67.8	37	1.00E-06	31.68	547	WP_030552286.1	2
Streptomyces venezuelae ATCC 10712^{T}	40.4	40.4	22	1.00E-06	30.97	306	WP_150186535.1	1
Streptomyces bikiniensis NRRL B-1049^{T}	39.7	67	50	3.00E-06	31.68	536	WP_030212439.1	2
Streptomyces venezuelae ATCC 10712^{T}	38.1	38.1	21	9.00E-06	29.36	306	WP_150172905.1	1
Streptomyces cinereoruber subsp. *cinereoruber* NRRL ISP 5012^{T}	37.4	67	50	2.00E-05	29.7	536	WP_152371356.1	2
Streptomyces purpureus KA281, ATCC 21405^{T}	37	37	16	3.00E-05	29.59	531	WP_189199456.1	2
Streptomyces nashvillensis JCM 4498^{T}	35.8	35.8	48	3.00E-05	25.7	246	WP_190104955.1	1
Streptomyces tanashiensis JCM 4086^{T}	33.9	33.9	21	1.00E-04	26.85	246	WP_189807001.1	1

Figure 1

Figure 2

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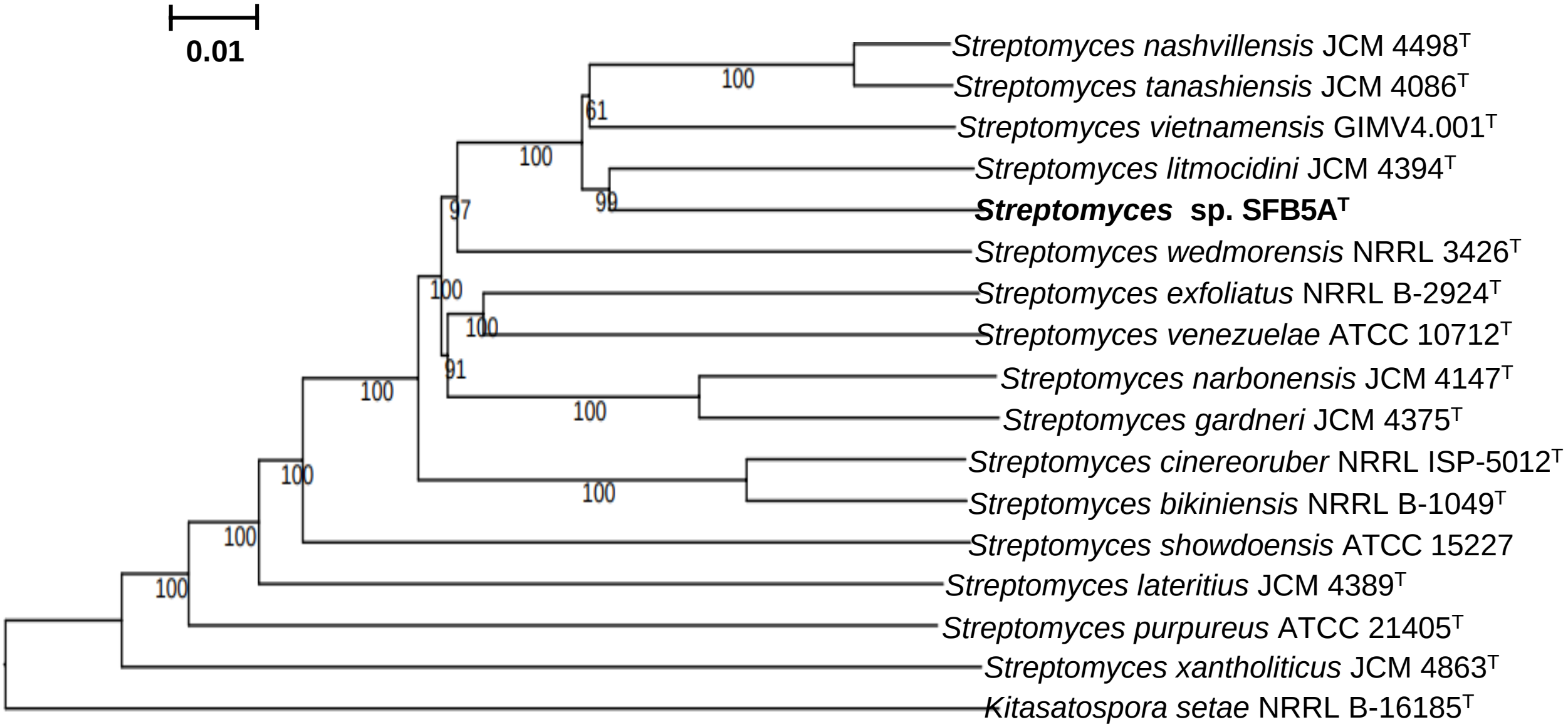

Figure 3

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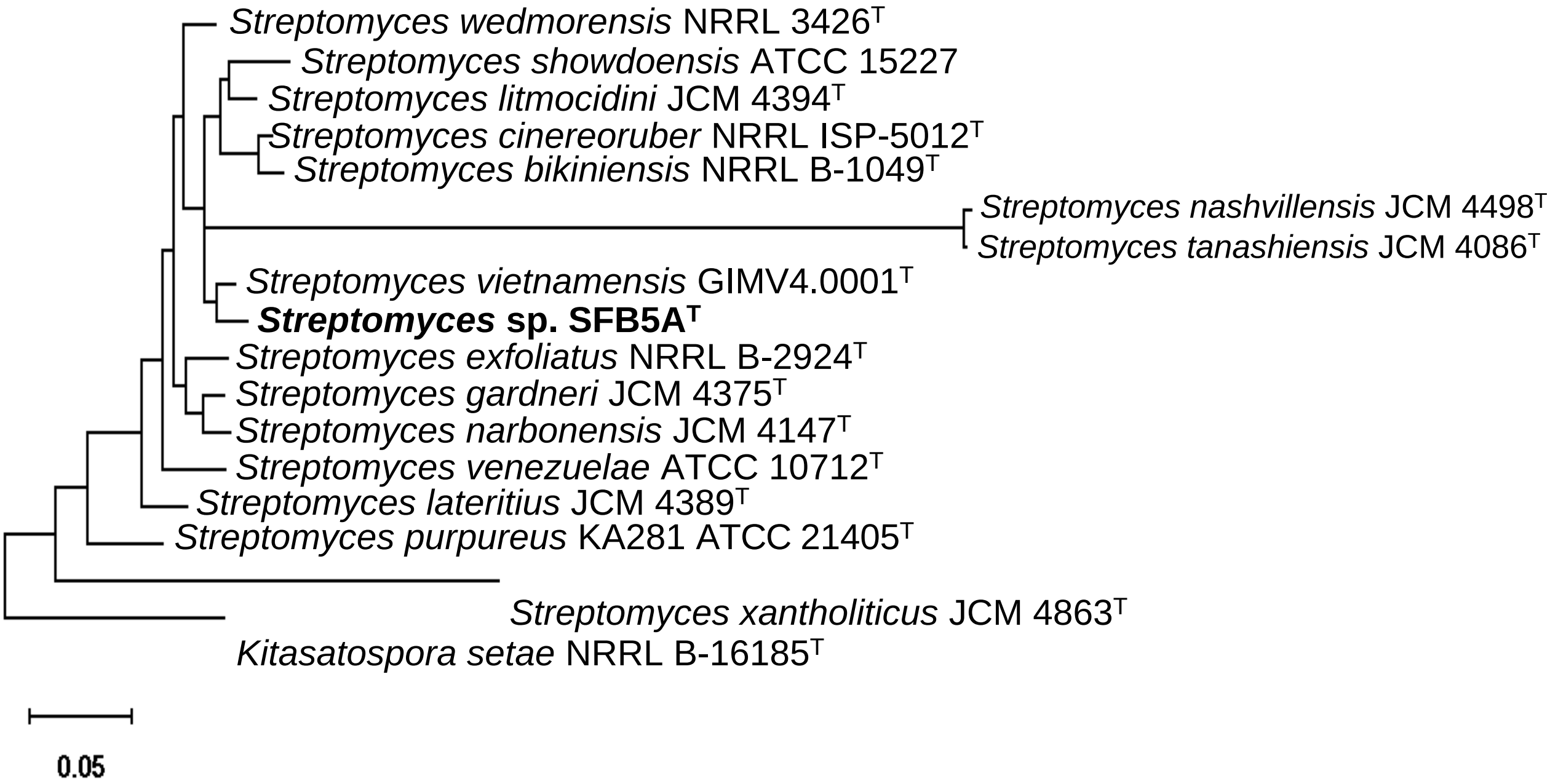
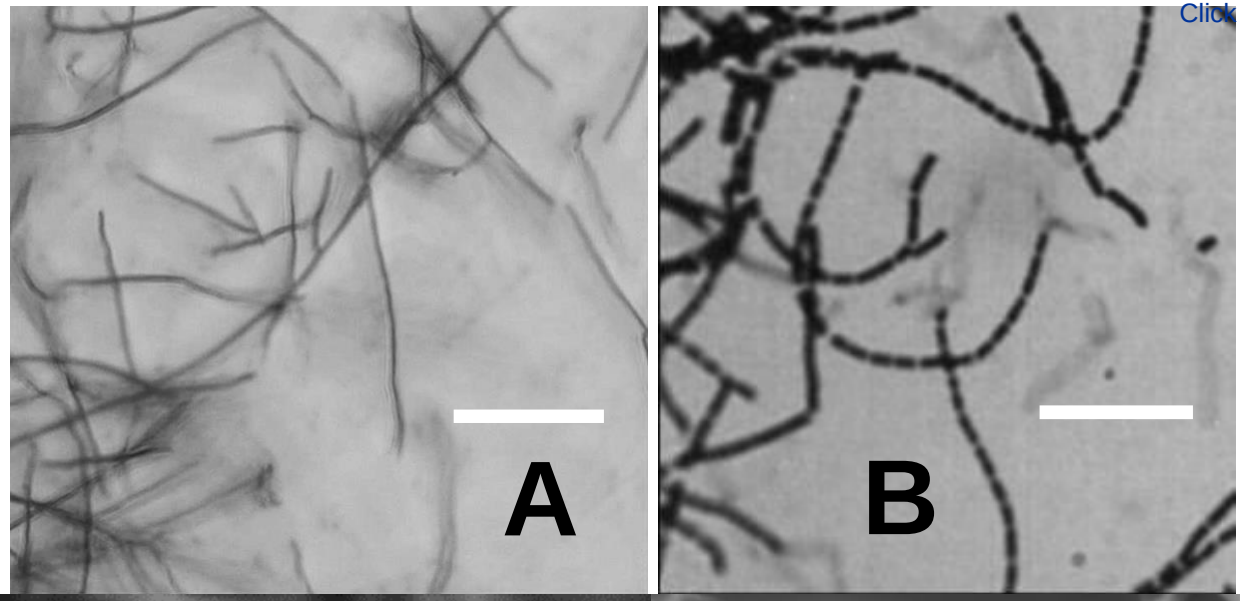
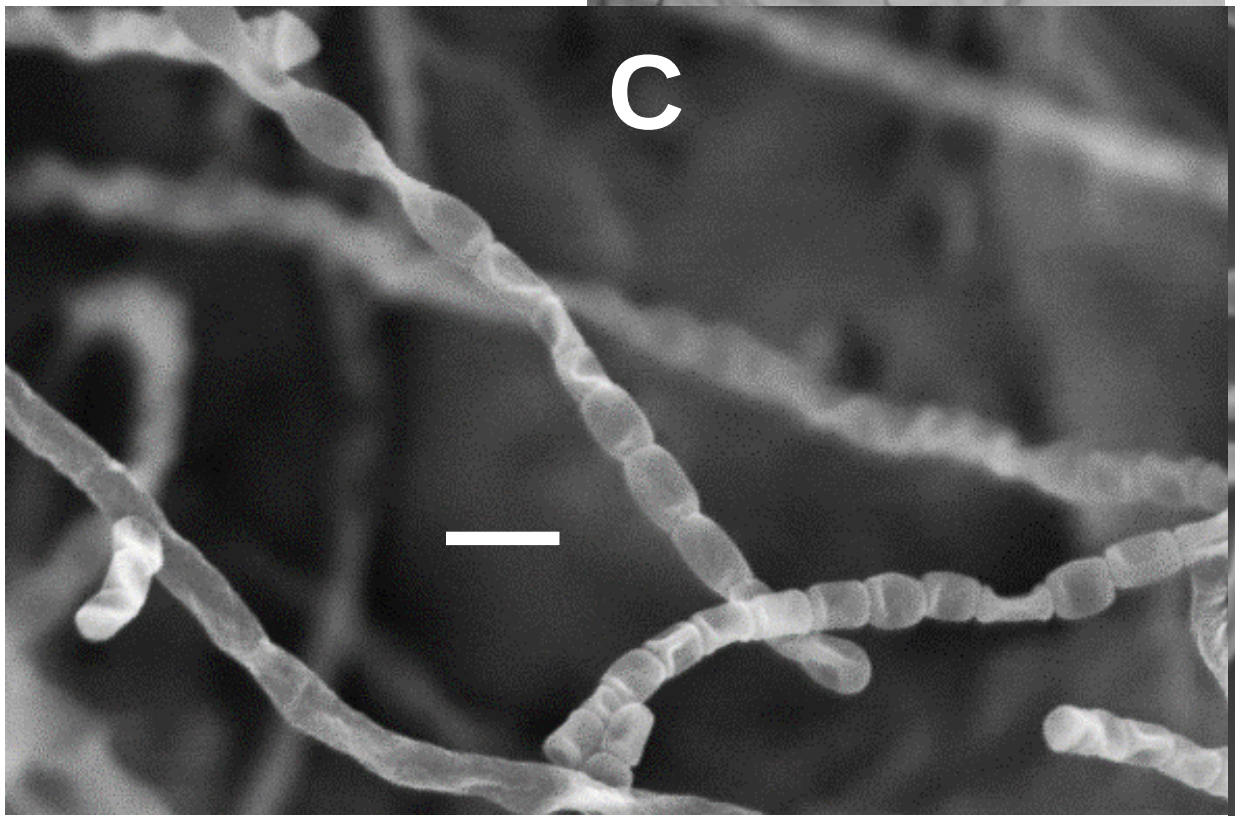
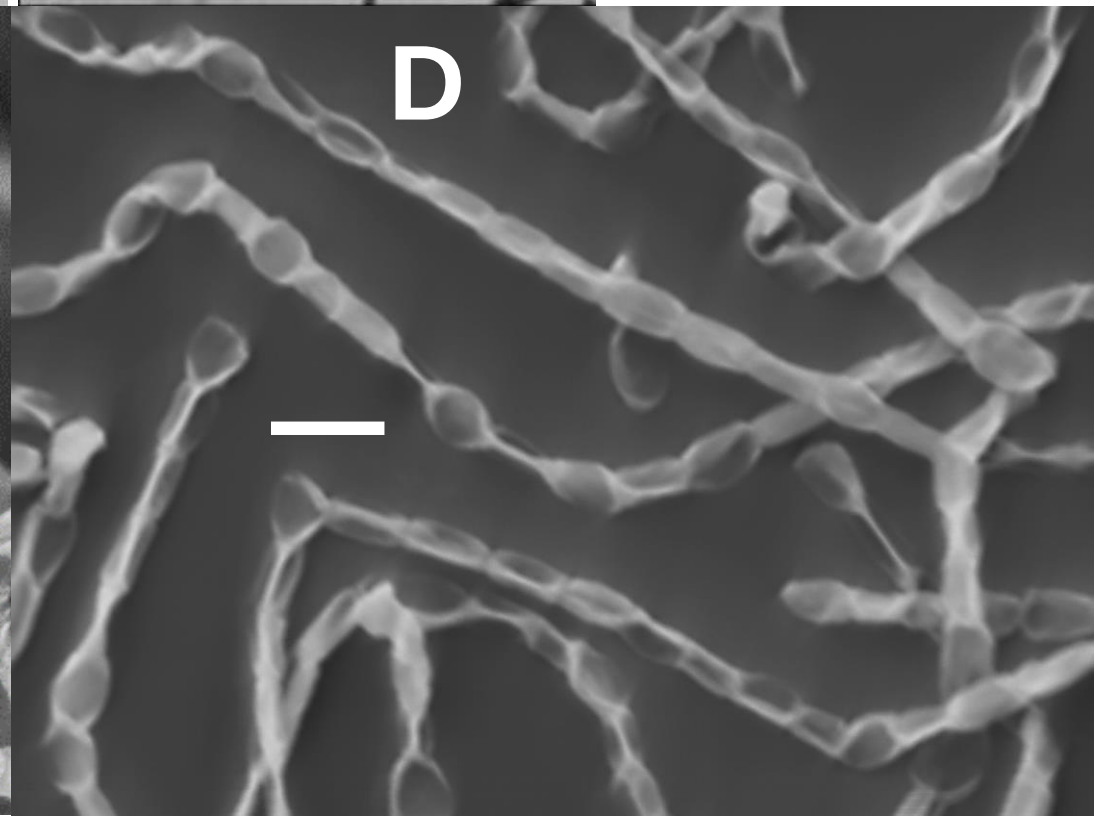


Figure 4







Supplemental Material to accompany:

Characterization of *Streptomyces nymphaeiformis* sp. nov., and its taxonomic relatedness to other polyhydroxybutyrate-degrading streptomycetes

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Figure S1. Neighbor-joining phylogenetic tree prepared with 25 sequences of 16S rRNA genes from *Streptomyces* species showing significant similarity to that of strain SFB5A^T. *Kitasatospora setae* NRRL B-16185^T was used as an outgroup. GenBank accession numbers for the sequences are shown in parentheses. The optimal tree with the sum of branch length = 0.11162893 is shown. The percentage (>50%) of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. There were 1450 total positions in the final dataset. Bar: 0.01 substitutions per site.

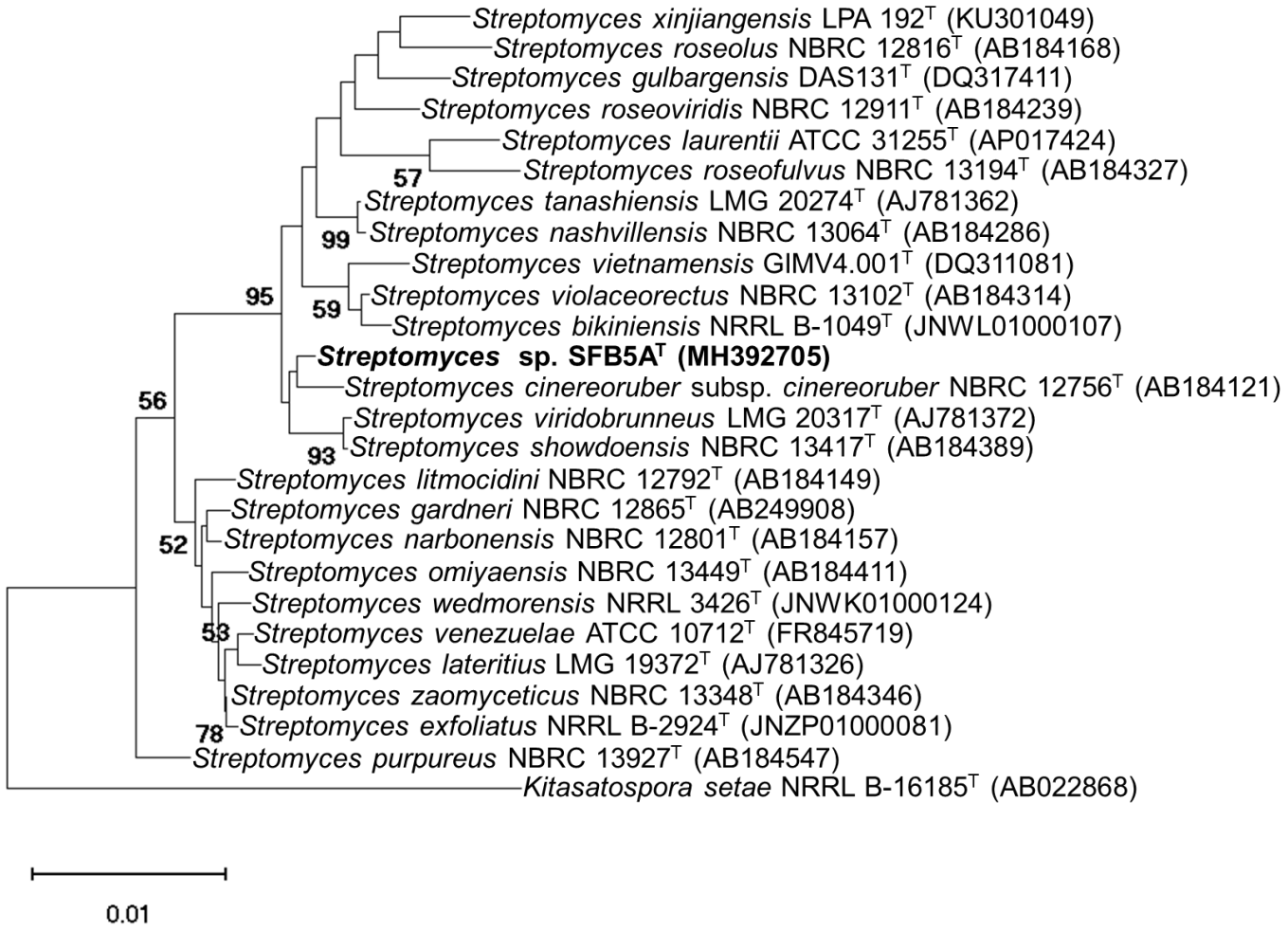

Figure S2. Maximum likelihood phylogenetic tree inferred with results of MLSA analysis of strain SFB5A^T and 16 other genomes from Table 1; genes analyzed were *atpD*, *gyrB*, *recA*, and *trpB*, with *rpoB* excluded; see Table S1 for accession numbers. The tree with the highest log likelihood (- 23883.67) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 17 concatenated nucleotide sequences. There were 5110 positions in the final dataset. Bar: 0.5 substitutions per site.

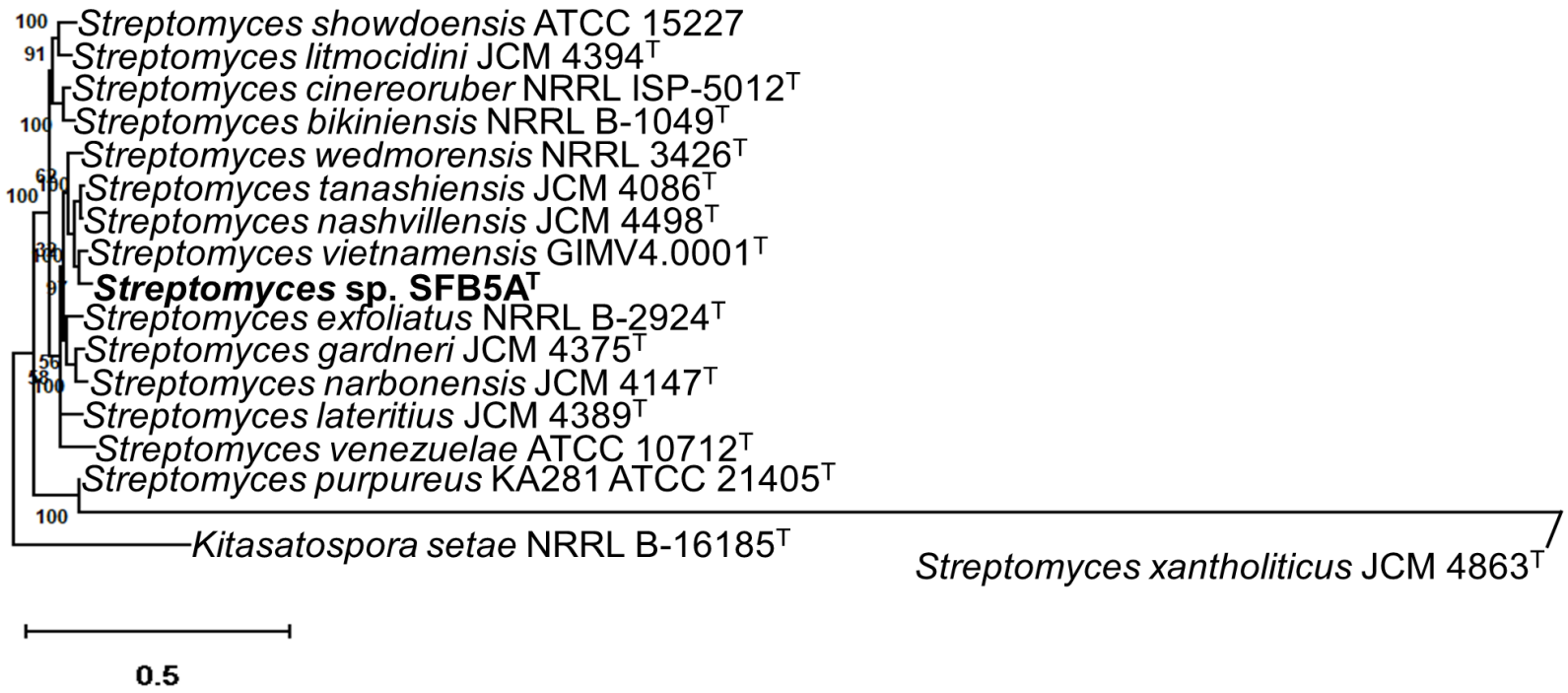

Figure S3. Identification of whole cell sugars and diaminopimelic acid (DAP) isomer in whole cell hydrolysates of strain SFB5A^{T}. **A,** whole cell sugar analysis;lanes: **1, 2, and 3,** standards (g=galactose, m=mannose, gl=glucose, a=arabinose, r=ribose), **4,** hydrolysate from strain SFB5A^{T}, **5,** hydrolysate from *S. vietnamensis* GIMV4.0001^{T}. **B,** DAP analysis; lanes: **1,** hydrolysate from strain SFB5A^{T}; **2,** LL-DAP and meso-DAP standard mixture (Sigma- Aldrich, St. Louis, Missouri, USA).

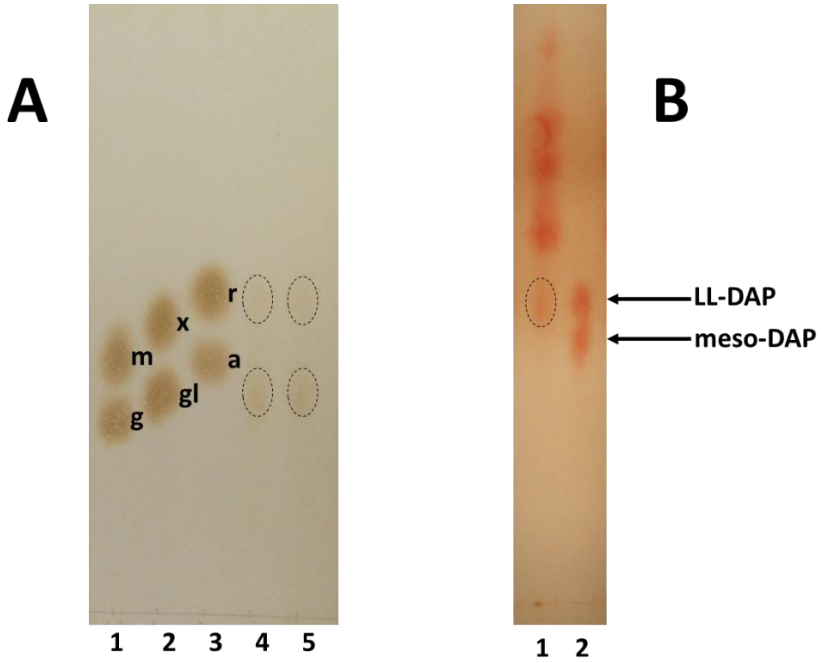

Figure S4. Two-dimensional TLC of polar lipids extracted from strain SFB5A^T. Panels: **A** = phospholipids (molybdenum blue); **B** = nitrogen containing phospholipids (ninhydrin); **C** = total lipids (phosphomolybdic acid); **D** = glycolipids (α-naphthol); **E**, stained with molybdenum blue, then Dragendorff's reagent. Lipid symbols: **PGL,** phosphoglycolipid; **PE,** phosphatidylethanolamine; **UA1, UA2,** unknown aminolipids; **UL1-4,** unknown lipids. **o**, spotting origin.

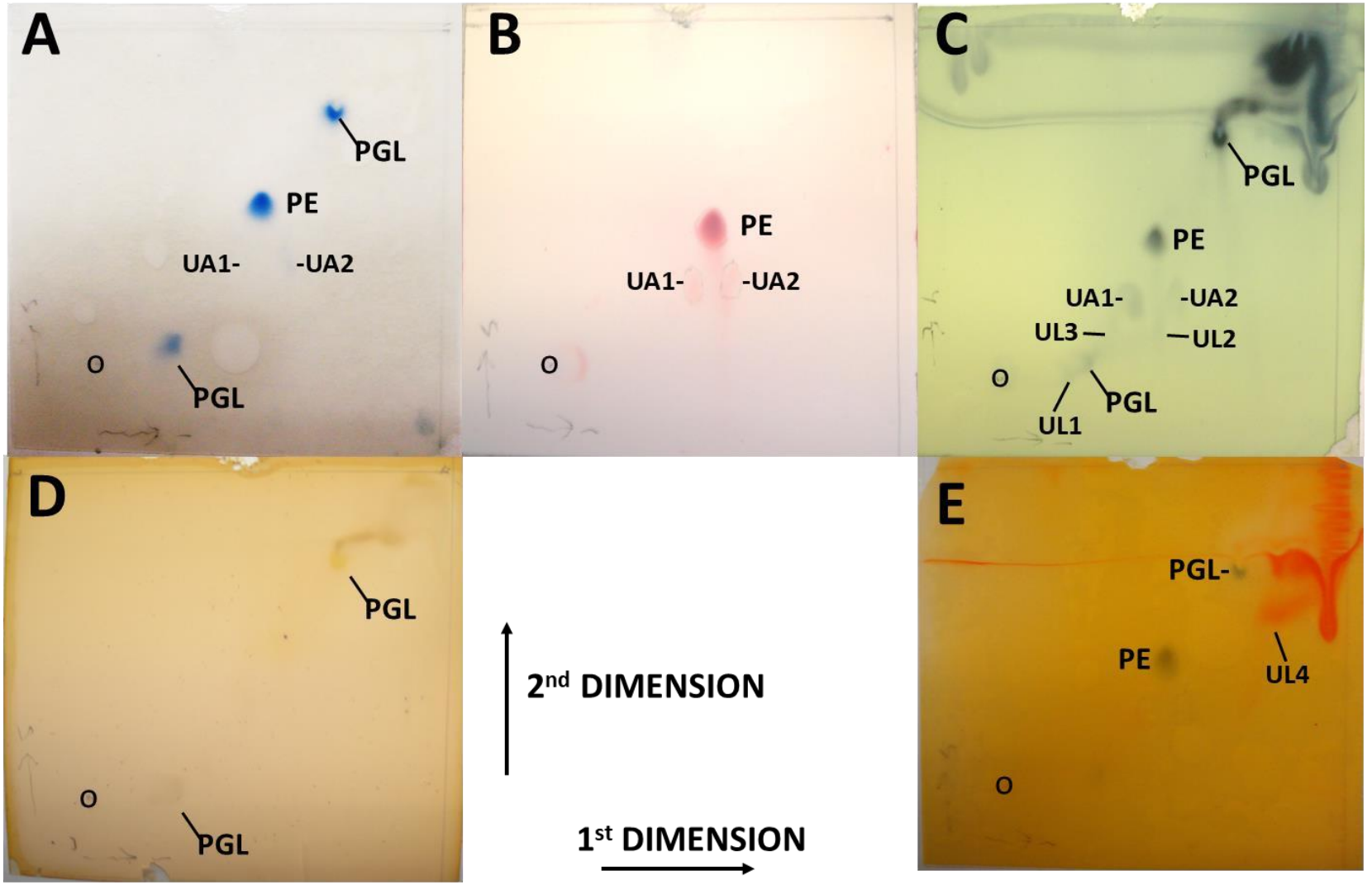

Figure S5. Degradation of PHB by strain SFB5A^T and its phylogenomic cohort after 7 and 14 days of incubation at 30°C. Strain abbreviations: **5A,** strain SFB5A^T; **LIT**, *S. litmocidini* NRRL B-3635^T; **VIET**, *S. vietnamensis* DSM 41927^T; **NASH**, *S. nashvillensis* NRRL B-2606^T; **TAN**, *S. tanashiensis* NRRL B- 1692^T; **WED**, *S. wedmorensis* DSM 41676^T; **VEN**, *S. venezuelae* ATCC 10712^T; **CIN**, *S. cinereoruber* subsp. *cinereoruber* NRRL ISP-5012^T; **EXF**, *S. exfoliatus* NRRL B-2924^T; **BIK**, *S. bikiniensis* NRRL B-1049^T; **NAR**, *S. narbonesis* JCM 4147^T; **GAR**, *S. gardneri* JCM 4375^T; **SHO**, *S. showdoensis* ATCC 15227; **LAT**, *S. lateritius* JCM 4389^T; **PUR**, *S. purpureus* KA281, ATCC 21405^T; **VIO,** *S. violaceorectus* NRRL B-12181; **VIR,** *S. viridibrunneus* NRRL B-12430; **XAN**, *S. xantholiticus* JCM 4863^T. An interpretation of the level of degradation is shown next to each strain abbreviation: -, no degradation; ±, possible degradation; +, partial degradation; +2, full clearing.

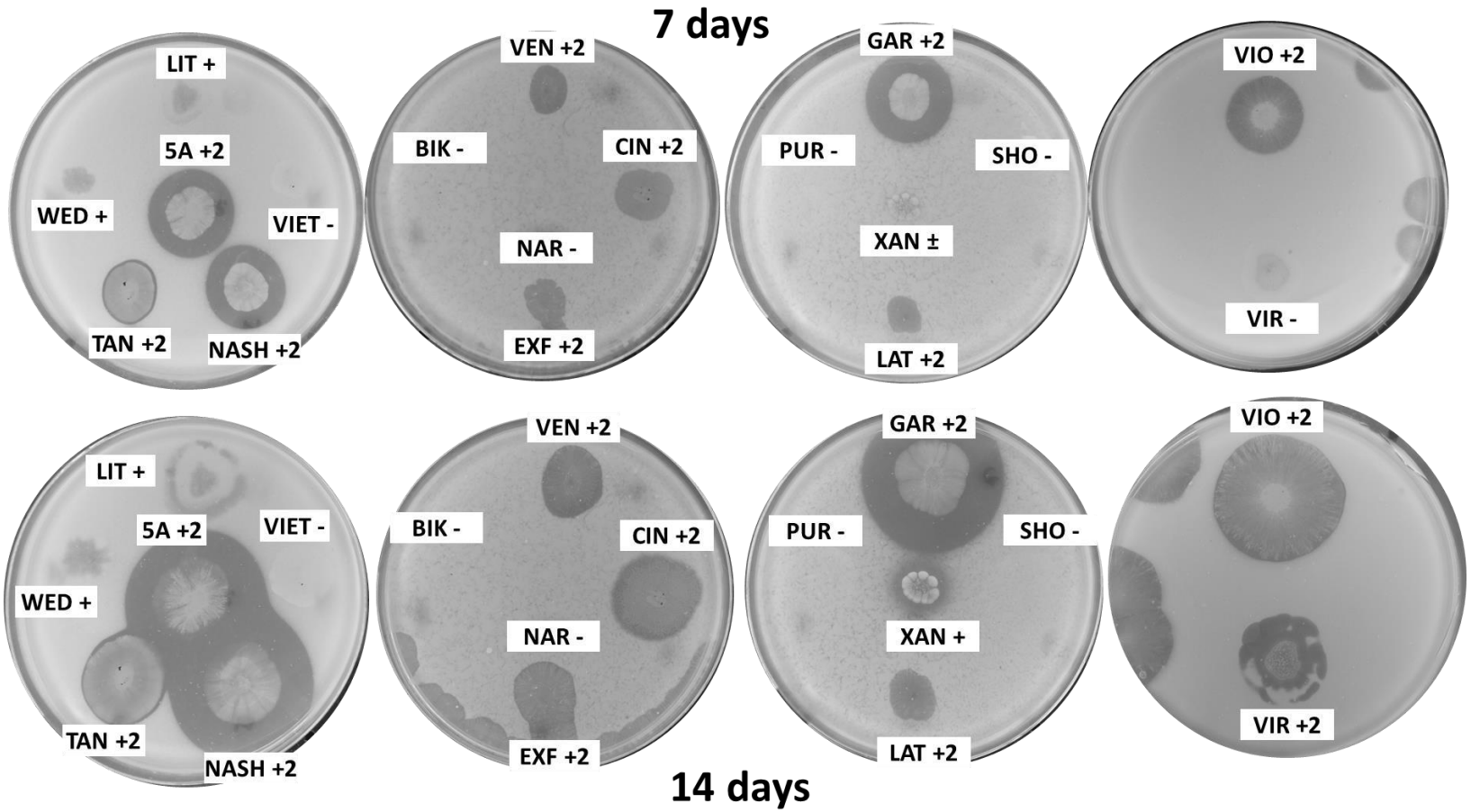

Figure S6. Fast minimum evolution phylogenetic tree constructed by blastp searches with the e-PHA depolymerase sequence from strain SFB5A^{T} (Genbank accession number WP_184931656.1) versus *Streptomyces* genomes (NCBI txid1883). Genbank accession numbers are shown after the organism names. Bar, 0.01 substitutions per site.

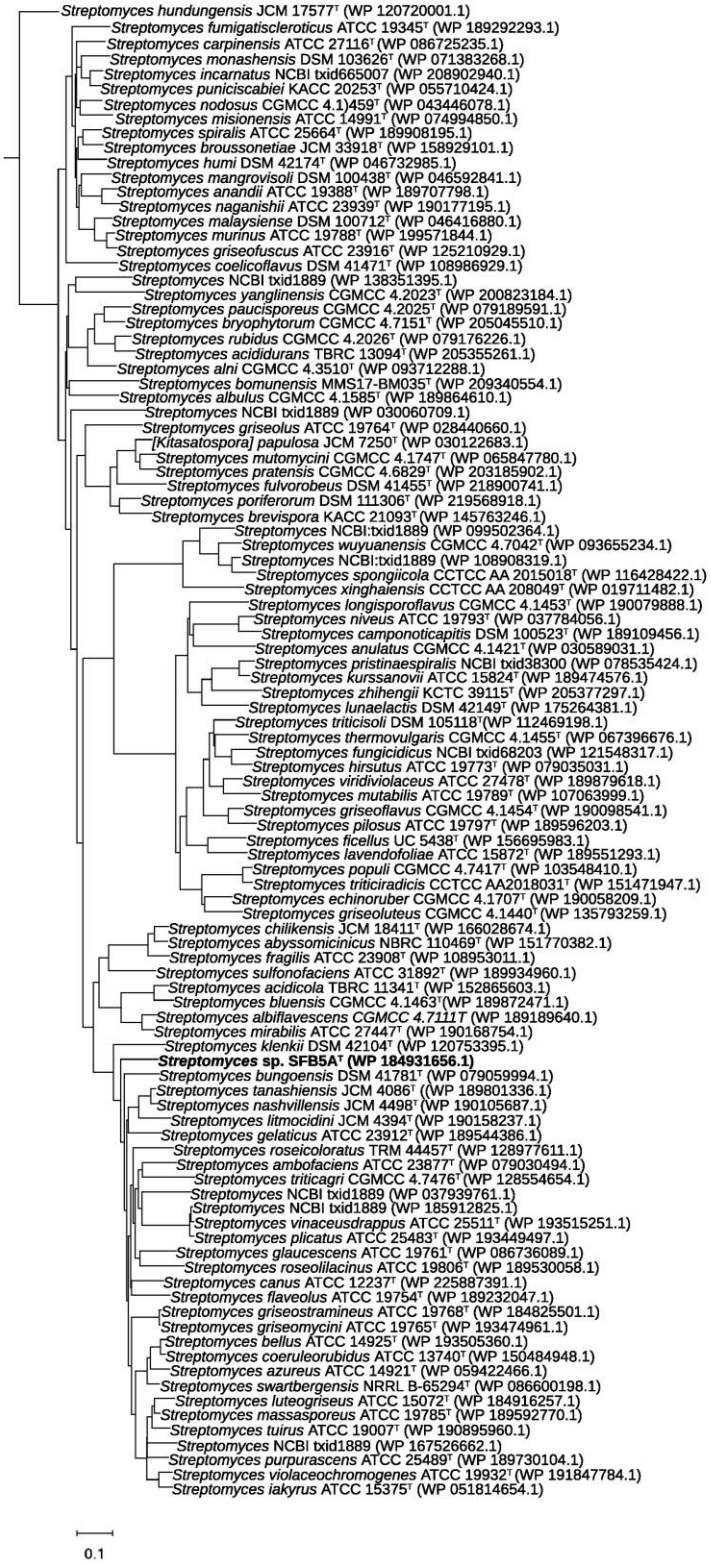

Figure S7. Fast minimum evolution phylogenetic tree constructed with results of blastp searches with the e-PHA depolymerase sequence from strain SFB5A^T (Genbank accession number WP_184931656.1) versus PHB depolymerases identified for its phylogenomic cohort. Genbank accession numbers are shown after the organism names. Bar, 0.21 substitutions per site. Enzyme clusters and the type of catalytic domain identified are shown in brackets and bold type.

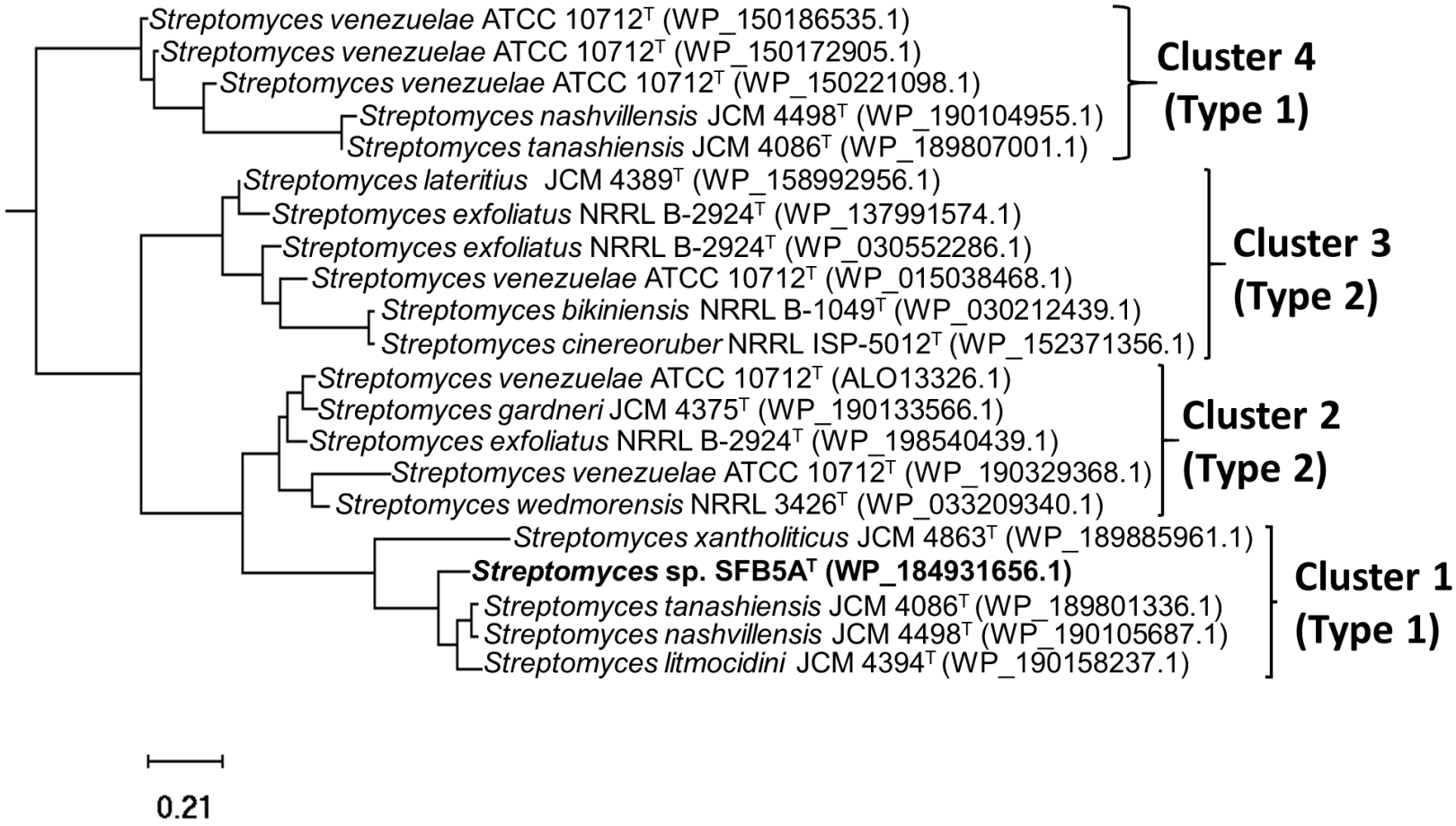

Table S1. Accession numbers for genes used in MLSA. The accession numbers are for NCBI reference protein sequences. Corresponding nucleotide sequences were downloaded from the datasets provided for each.

ORGANISM	***atpD***	***gyrB***	***recA***	***rpoB***	***trpB***
Streptomyces sp. SFB5A^T	WP_116158817.1	WP_221518124.1	WP_181924483.1	WP_116159375.1	WP_116161516.1
Streptomyces litmocidini JCM 4394^T	WP_190154770.1	WP_123453557.1	WP_190159171.1	WP_190157477.1	WP_190157089.1
Streptomyces vietnamensis GIMV4.0001^T	WP_041131258.1	WP_041130165.1	WP_041131563.1	WP_041130747.1	WP_041128740.1
Streptomyces nashvillensis JCM 4498^T	WP_190101279.1	WP_190106386.1	WP_190106528.1	WP_189800406.1	GGY13592.1
Streptomyces tanashiensis JCM 4086^T	WP_189799850.1	WP_189804964.1	WP_189804724.1	WP_189800406.1	GGS85911.1
Streptomyces wedmorensis NRRL 3426^T	WP_033206795.1	WP_017240389.1	WP_033208492.1	WP_033202006.1	WP_017238749.1
Streptomyces venezuelae ATCC 10712^T	WP_150216177.1	WP_015034851.1	WP_015036606.1	CCA57631.1	WP_150219178.1
Streptomyces cinereoruber subsp. cinereoruber NRRL ISP-5012^T	WP_062752183.1	WP_062753758.1	WP_062751894.1	WP_062752790.1	WP_062756551.1
Streptomyces exfoliatus NRRL B-2924^T	WP_137993316.1	WP_030549768.1	WP_030217661.1	WP_024755374.1	WP_030547266.1
Streptomyces bikiniensis NRRL B-1049^T	WP_030218857.1	WP_030206730.1	WP_030221368.1	WP_030208961.1	WP_030205408.1
Streptomyces narbonensis JCM 4147^T	WP_189509314.1	WP_189508755.1	GGW09329.1	WP_189511709.1	WP_189505946.1
Streptomyces gardneri JCM 4375^T	WP_024761910.1	WP_055642461.1	WP_055641007.1	WP_055641866.1	WP_141296339.1
Streptomyces showdoensis ATCC 15227^T	WP_046909152.1	WP_046908690.1	KT385423.1	WP_046908216.1	WP_046907043.1
Streptomyces lateritius JCM 4389^T	WP_073808569.1	WP_073811630.1	WP_073819720.1	WP_158989353.1	WP_189599232.1
Streptomyces purpureus KA281, ACC 21405^T	WP_019889509.1	WP_211231204.1	WP_019889926.1	WP_019888710.1	WP_189200596.1
Streptomyces xantholiticus JCM 4863^T	WP_189887562.1	GGW44792.1	WP_189892625.1	WP_189886884.1	WP_189884230.1
Kitasatospora setae NRRL B-16185^T	WP_014138096.1	WP_033257672.1	WP_014138431.1	WP_014136318.1	WP_014135191.1

Table S2. Pairwise distance matrix for MLSA**.** Strain abbreviations: **5A,** strain SFB5A^{T}; ***S. lit.***, *S. litmocidini* NRRL B-3635^{T}; ***S. viet***, *S. vietnamensis* DSM 41927^{T}; **S. nash.**, *S. nashvillensis* NRRL B-2606^{T}; ***S. tan.***, *S. tanashiensis* NRRL B-1692^{T}; ***S. wed.***, *S. wedmorensis* DSM 41676^{T}; ***S. ven.***, *S. venezuelae* ATCC 10712^{T}; ***S. cin.***, *S. cinereoruber* subsp. *cinereoruber* NRRL ISP-5012^{T}; ***S. exf.***, *S. exfoliatus* NRRL B-2924^{T}; ***S. bik.***, *S. bikiniensis* NRRL B-1049^{T}; ***S. nar.***, *S. narbonesis* JCM 4147^{T}; ***S. gar.***, *S. gardneri* JCM 4375^{T}; ***S. sho.***, *S. showdoensis* ATCC 15227; ***S. lat.***, *S. lateritius* JCM 4389^{T}; ***S. pur.***, *S. purpureus* KA281, ATCC 21405^{T}; ***S. vio.,*** *S. violaceorectus* NRRL B-12181; ***S. vir.,*** *S. viridibrunneus* NRRL B-12430; ***S. xan.***, *S. xantholiticus* JCM 4863^{T}; ***K. setae***, *Kitasatospora setae* NRRL B-16185^{T}. Table A is with all five genes used in the analysis; Table B is with *rpoB* omitted from the analysis.

A.

	SFB5A	*S. bik.*	*S. cin.*	*S. exf.*	*S. gar.*	*S. lat*	*S. nar.*	*S. nash*	*S. pur.*	*S. sho.*	*S. tan.*	*S. ven.*	*S. viet.*	*S. wed.*	*S. xan.*	*K. setae*
SFB5A																
S. bik.	0.0483															
S. cin.	0.0440	0.0172														
S. exf.	0.0404	0.0508	0.0457													
S. gar.	0.0458	0.0475	0.0486	0.0333												
S. lat.	0.0531	0.0532	0.0559	0.0483	0.0455											
S. lit.	0.0358	0.0379	0.0343	0.0425	0.0475	0.0578										
S. nar.	0.0472	0.0490	0.0516	0.0316	0.0213	0.0481	0.0494									
S. nash	0.2802	0.3003	0.2961	0.2875	0.2883	0.2994	0.2858	0.2965								
S. pur.	0.0760	0.0723	0.0708	0.0764	0.0778	0.0728	0.0730	0.0803	0.3360							
S. sho.	0.0436	0.0478	0.0441	0.0479	0.0521	0.0614	0.0380	0.0530	0.2950	0.0756						
S. tan.	0.2815	0.3003	0.2959	0.2858	0.2901	0.2997	0.2845	0.2953	0.0049	0.3351	0.2928					
S. ven.	0.0528	0.0517	0.0528	0.0482	0.0451	0.0549	0.0545	0.0465	0.3049	0.0794	0.0592	0.3035				
S. viet.	0.0222	0.0441	0.0388	0.0377	0.0388	0.0544	0.0297	0.0428	0.2754	0.0749	0.0466	0.2752	0.0492			
S. wed.	0.0385	0.0413	0.0437	0.0393	0.0300	0.0444	0.0426	0.0311	0.2832	0.0758	0.0514	0.2820	0.0464	0.0306		
S. xan.	0.2191	0.2192	0.2169	0.2144	0.2174	0.2131	0.2218	0.2188	0.5336	0.1992	0.2222	0.5324	0.2193	0.2196	0.2201	
K. setae	0.1496	0.1405	0.1436	0.1492	0.1484	0.1472	0.1463	0.1516	0.3995	0.1516	0.1498	0.3983	0.1494	0.1500	0.1513	0.2597

B.

	SFB5A	***S. bik.***	***S. cin.***	***S. exf.***	***S. gar.***	***S. lat***	***S. nar.***	***S. nash***	***S. pur.***	***S. sho.***	***S. tan.***	***S. ven.***	***S. viet.***	***S. wed.***	***S. xan.***	***K. setae***
SFB5A																
S. bik.	0.0619															
S. cin.	0.0546	0.0229														
S. exf.	0.0455	0.0612	0.0552													
S. gar.	0.0534	0.0573	0.0607	0.0446												
S. lat.	0.0584	0.0560	0.0602	0.0494	0.0454											
S. lit.	0.0459	0.0491	0.0426	0.0509	0.0603	0.0677										
S. nar.	0.0569	0.0603	0.0668	0.0396	0.0282	0.0490	0.0639									
S. nash	0.0313	0.0593	0.0527	0.0404	0.0429	0.0541	0.0410	0.0532								
S. pur.	0.0978	0.0904	0.0873	0.0959	0.0973	0.0933	0.0939	0.1037	0.0988							
S. sho.	0.0552	0.0558	0.0493	0.0540	0.0605	0.0683	0.0398	0.0646	0.0494	0.0938						
S. tan.	0.0336	0.0600	0.0531	0.0392	0.0454	0.0548	0.0398	0.0526	0.0071	0.0987	0.0476					
S. ven.	0.0719	0.0712	0.0745	0.0655	0.0601	0.0625	0.0777	0.0629	0.0645	0.1047	0.0780	0.0636				
S. viet.	0.0276	0.0567	0.0487	0.0419	0.0475	0.0569	0.0385	0.0541	0.0272	0.0948	0.0550	0.0274	0.0679			
S. wed.	0.0450	0.0471	0.0555	0.0495	0.0418	0.0463	0.0577	0.0452	0.0371	0.0977	0.0636	0.0365	0.0631	0.0389		
S. xan.	0.3404	0.3378	0.3352	0.3277	0.3326	0.3273	0.3407	0.3357	0.3412	0.3122	0.3429	0.3396	0.3410	0.3368	0.3368	
K. setae	0.1731	0.1628	0.1660	0.1710	0.1685	0.1697	0.1715	0.1734	0.1713	0.1760	0.1699	0.1703	0.1739	0.1713	0.1711	0.4085

Table S3. Cultural characteristics of strain SFB5A^T and selected phylogenetic neighbors on agar media after 14 days. G, growth (+4, lush; +3, abundant; +2, moderate; +1, poor, 0, no visible growth); S, sporulation; M, sporulating mycelium color (or colony description); RC, reverse color of mycelium (not determined if no sporulation occurred); SP, soluble pigment; N/A, could not be determined because of masking pigments, medium opacity, or lack of growth or sporulation. RAL color names are shown. All data were determined experimentally in this study. Strains: **SFB5A,** strain SFB5A^T; ***S. lit.***, *S. litmocidini* NRRL B-3635^T; ***S. viet***., *S. vietnamensis* DSM 41927^T; ***S. nash***., *S. nashvillensis* NRRL B-2606^T; ***S. tan***., *S. tanashiensis* NRRL B-1692^T; ***S. wed***., *S. wedmorensis* DSM 41676^T; ***S. cin.***, *S. cinereoruber* subsp. *cinereoruber* NRRL ISP-5012^T; ***S. exf***., *Streptomyces exfoliatus* NRRL B-2924^T; ***S. bik.***, *S. bikiniensis* NRRLB-1049^T; ***S. sho.***, *S. showdoensis* NRRLB-12430^T; ***S. vio.***, *S. violaceorectus* NBRC 13102^T; ***S. vir.***, *S. viridobrunneus* NRRLB-24332^T

Medium, characteristic		SFB5A	*S. lit.*	*S. viet.*	*S. nash.*	*S. tan.*	*S. wed.*	*S. cin.*	*S. exf.*	*S. bik.*	*S. sho.*	*S. vio.*	*S. vir.*
ISP2	G	+4	+4	+4	+4	+3	+4	+3	+4	+4	+3	+2	+2
	S	Good	Good	Good	Poor	Good	Good	Good	Moderate	Good	Moderate	Moderate	Poor
	M	Reed green	Cement gray	Olive gray	Signal white	Olive gray	Olive gray	Reed green	Light pink	Olive green	Cream	Pebble gray	Olive grey
	RC	Clay brown	Sepia brown	Gray blue	Ivory	Clay brown	Sand yellow	Brown red	Sand yellow	Chocolate brown	Maize yellow	Honey yellow	Green brown
	SP	Light brown	Olive brown	Violet-blue	None	Brown	None	Olive brown	None	Olive brown	None	None	Green brown
ISP3	G	+4	+3	+3	+2	+2	+3	+3	+4	+3	+3	+2	+3
	S	Good	Good	Moderate	Poor	Good	Good	Good	Moderate	Good	Moderate	Poor	Moderate
	M	Reed green	Moss gray	Cream	Clean room white	Olive gray	Olive gray	Reed green	Light pink	Olive green	Grey white	Gray white	Olive green
	RC	Olive brown	Golden yellow	Olive brown	Clean room white	Ivory	Olive gray	Red violet	Ivory	Honey yellow	Zinc yellow	Zinc yellow	Olive brown
	SP	Light brown	Light brown	Violet-blue	None	Brown	None	None	None	None	N/A	None	Olive brown
ISP4	G	+1	+3	+2	+3	+3	+2	+2	+3	+1	+1	+1	+1
	S	Moderate	Moderate	Moderate	Good	Good	Moderate	Moderate	Moderate	Poor	Poor	None	Poor
	M	Olive grey	Olive gray	Cream	Gray white	Olive gray	Olive gray	Reed green	Traffic white	Concrete gray	N/A	Signal white	Olive grey
	RC	N/A	Olive gray	Light ivory	Clean room white	Olive gray	Ivory	N/A	Ivory	Ivory	N/A	Signal white	N/A
	SP	None	Gray-violet	None	None	Green brown	None	None	None	None	None	None	None
ISP5	G	+2	+2	+2	+2	+3	+3	+3	+2	+3	+2	+2	+1
	S	Good	Moderate	Moderate	Moderate	Poor	None	Good	Poor	Moderate	Moderate	Moderate	Very poor
	M	Concrete grey	Cement gray	Gray white	Gray white	Moist	N/A	Beige red	Traffic white	Moss gray	Cream	Signal white	N/A
	RC	Honey yellow	Ochre brown	Purple violet	Ivory	N/A	Sand yellow	Purple red	Ivory	Olive brown	Zinc yellow	Honey yellow	N/A
	SP	Light brown	Black	Violet-blue	None	None	Light brown	None	None	None	None	Light brown	Green brown
ISP6	G	+2	+3	+3	+3	+3	+4	+2	+4	+2	+2	+2	+1
	S	None	None	Poor	None	None	None	Moderate	None	Poor	None	Moderate	None
	M	N/A	Moist	Olive gray	Moist	Moist	Moist	Umbra grey	Sand yellow	Signal White	N/A	Signal white	N/A
	RC	Ivory	N/A	Brown gray	N/A	N/A	N/A	N/A	Sand yellow	N/A	N/A	Honey yellow	N/A
	SP	Light brown	Melanin	Melanin	None	Melanin	None	Melanin	Light brown	Melanin	Melanin	Light brown	Melanin
ISP7	G	+4	+4	+4	+3	+4	+3	+4	+4	+4	+3	+3	+2
	S	Good	Good	Good	Good	Good	Good	Good	Good	Good	Moderate	Moderate	Moderate
	M	Concrete grey	Pebble gray	Olive gray	Clean room white	Moss gray	Olive gray	Reed green	Ivory	Moss gray	Cream	Signal white	Cream
	RC	Olive brown	Sepia brown	Gray blue	Ivory	Sepia brown	Olive brown	Purple violet	Brown beige	Chocolate brown	Maize yellow	Olive brown	N/A
	SP	Light brown	Gray	Violet-black	None	Melanin	None	Concrete grey	Light brown	None	Olive brown	None	Melanin
TSA	G	+3	+3	+3	+3	+3	+4	+2	+4	+2	+2	+2	+1
	S	None	None	None	Poor	None	None	Moderate	None	Moderate	Poor	Poor	None
	M	Moist	Moist	Green beige	Signal White	Moist	Moist	Cream	Moist	Pebble grey	N/A	Cream	N/A
	RC	N/A	N/A	Green beige	Ivory	N/A	N/A	Orange brown	N/A	Olive brown	N/A	Cream	Beige
	SP	Light brown	Light brown	Blue green	None	Light brown	None	Olive brown	None	Ochre brown	Olive brown	None	Olive brown
MSF	G	+2	+2	0	+3	0	+2	+3	+2	+2	+2	+2	+1
	S	Moderate	Moderate	N/A	Good	N/A	Poor	Good	Moderate	Moderate	Moderate	Poor	Poor
	M	Pebble grey	Gray white	N/A	Clean room white	N/A	Light ivory	Sand yellow	Traffic white	Pebble gray	Pebble grey	Signal white	Olive brown
	RC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	SP	Light brown	None	N/A	None	N/A	None	Olive brown	None	Ochre brown	Brown	None	Brown

Table S4. Fatty acid profiles of strain SFB5A^T and selected relatives. Numbers are the percentages of the total fatty acids detected; -, either not detected or not reported. Organism abbreviations: **SFB5A,** strain SFB5A^T; ***S. lit.,*** *S. litmocidini* NRRL B-3635^T; ***S. viet.,*** *S. vietnamensis* DSM 41927^T; ***S. nash.,*** *S. nashvillensis* NRRLB-2606^T**;** ***S. tan.,*** *S. tanashiensis* NRRL B-1692^T; ***S. wed.,*** *S. wedmorensis* DSM 41676^T; ***S. cin.,*** *S. cinereoruber* subsp. *cinereoruber* NRRLISP-5012^T; ***S. bik.,*** *S. bikiniensis* NRRLB-1049^T; ***S. exf.,*** *Streptomyces exfoliatus* NRRLB-2924^T.
*, >1% for at least one of the strains shown.

Fatty acid	SFB5A	*S. lit.*	*S. viet.*	*S. nash.*	*S. tan.*	*S. wed.*	*S. cin.*	*S. bik.*	*S. exf.*
Major amounts*									
15:0 anteiso	28.32	28.17	26.57	32.7	31.62	33.17	22.52	32.58	33.06
17:0 anteiso	19.81	14.33	14.28	18.53	14.29	18.77	4.6	12.26	21.28
16:0 iso	16.81	13.17	12.97	8.95	13.46	7.12	31.05	16.49	7.84
15:0 iso	11.5	12.6	17.78	9.87	13.73	16.29	9.93	15.74	10.93
17:0 iso	8.38	7.61	11.01	8.88	9.09	12.31	2.16	6.00	9.74
16:0	5.65	9.43	10.01	10.06	8.45	6.59	2.16	4.05	11.28
14:0 iso	2.51	3.26	2.56	1.43	2.99	1.35	19.79	3.93	1.53
17:0 cyclo	1.56	1.05	0.89	0.75	1.03	-	-	-	-
17:1 anteiso c or w9c	0.92	1.18	-	1.73	0.93	-	-	2.01	-
17:0	0.77	0.62	1.37	0.49	0.99	1.04	-	-	-
16:0 9- or 10-methyl	0.74	-	-	-	-	-	-	2.3	-
16:1 iso h	0.38	-	-	0.61	0.2	-	2.68	-	-
16:1 w6c or 7c	0.27	2.03	-	3.11	0.67	-	-	-	-
15:0	-	-	2.56	-	-	2.24	3.83	2.21	1.87
16:1 cis 9	-	-	-	-	-	1.13	1.27	2.44	1.51
Others									
18:0 iso	0.82	0.48	-	0.23	0.49	-	-	-	-
14:0	0.38	0.75	-	0.56	0.51	-	-	-	0.97
13:0 anteiso	0.27	0.73	-	0.10	0.15	-	-	-	-
18:1w6c/w7c	0.27	0.1	-	0.09	0.19	-	-	-	-
13:0 iso	0.20	2.54	-	0.14	0.22	-	-	-	-
18:0	0.11	0.27	-	0.12	0.09	-	-	-	-
17:1 w8c	0.08	0.19	-	0.20	0.16	-	-	-	-
15:1 w6c	0.05	0.04	-	0.09	-	-	-	-	-
12:0 iso	0.04	0.23	-	-	-	-	-	-	-

Table S5. Antibiosis of strain SFB5A^T and phylogenetically related streptomycetes against seven test microbes. +, inhibition ring around agar plug ≥ 1.0 mm; -, inhibition ring ≤ 1.0 mm. Streptomycetes are listed on the top line: **SFB5A,** strain SFB5A^T; ***S. lit.***, *S. litmocidini* NRRL B-3635^T; ***S. viet***., *S. vietnamensis* DSM 41927^T; ***S. nash***., *S. nashvillensis* NRRL B-2606^T; ***S. tan***., *S. tanashiensis* NRRL B-1692^T; ***S. wed***., *S. wedmorensis* DSM 41676^T; ***S. cin.***, *S. cinereoruber* subsp. *cinereoruber* NRRL ISP-5012^T; ***S. bik.***, *S. bikiniensis* NRRL B-1049^T; ***S. exf***., *Streptomyces exfoliatus* NRRL B-2924^T, ***S. sho.***, *S. showdoensis* NRRL B-12430^T; ***S. vio.***, *S. violoaceorectus* NBRC 13102^T; ***S. vir.***, *S. viridobrunneus* NRRL B-24332^T

Test Microbe	SFB5A	*S. lit.*	*S. viet.*	*S. nash.*	*S. tan.*	*S. wed.*	*S. cin.*	*S. bik.*	*S. exf.*	*S. sho.*	*S. vio.*	*S. vir.*
Bacillus cereus ATCC 11778	+	+	+	+	+	+	+	+	+	-	-	+
Bacillus subtilis ATCC 6051	-	+	+	-	+	-	+	-	-	+	-	-
Candida parapsilosis ATCC 7330	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli K12 ATCC 10798	-	-	-	-	-	-	-	+	-	-	-	-
Pseudomonas aeruginosa ATCC 15442	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella typhimurium ATCC 14028	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus ATCC 6538	-	-	+	-	-	-	+	+	-	-	-	+

Table S6. Results of blastp searches with the e-dPHA$_{scl}$ depolymerase sequence from strain SFB5A^{T} (Genbank accession number WP_184931656.1) versus *Streptomyces* genomes (NCBI txid1883). All hits were described as PHB depolymerase family esterases by blastp and were all identified as catalytic domain type 1 e-dPHA$_{scl}$ depolymerases as described in Materials and Methods. The source organisms and the protein accession numbers are hyperlinked to the NCBI Taxonomy and Protein databases, respectively.

Source Organism	Max Score	Query Coverage (%)	E value	% Identity	Length (# of amino acids)	Protein Accession #
Streptomyces tanashiensis JCM 4086^{T}	778	100	0	83.27	497	WP_189801336.1
Streptomyces nashvillensis JCM 4498^{T}	769	100	0	82.27	497	WP_190105687.1
Streptomyces griseostramineus ATCC 19768^{T}	753	100	0	79.68	495	WP_184825501.1
Streptomyces griseomycini ATCC 19765^{T}	736	100	0	80.08	495	WP_193474961.1
Streptomyces ambofaciens ATCC 23877^{T}	736	100	0	77.05	497	WP_079030494.1
Streptomyces bungoensis DSM 41781^{T}	721	99	0	80.40	495	WP_079059994.1
Streptomyces litmocidini JCM 4394^{T}	721	100	0	78.69	497	WP_190158237.1
Streptomyces gelaticus ATCC 23912^{T}	717	98	0	81.05	495	WP_189544386.1
Streptomyces canus ATCC 12237^{T}	713	91	0	78.91	456	WP_225887391.1
Streptomyces flaveolus ATCC 19754^{T}	706	100	0	77.78	497	WP_189232047.1
Streptomyces bellus ATCC 14925^{T}	704	100	0	78.84	492	WP_193505360.1
Streptomyces luteogriseus ATCC 15072^{T}	699	99	0	74.40	493	WP_184916257.1
Streptomyces tuirus ATCC 19007^{T}	698	93	0	78.16	492	WP_190895960.1
Streptomyces NCBI txid1889	682	94	0	76.27	508	WP_037939761.1
Streptomyces NCBI txid1889	675	98	0	72.03	498	WP_185912825.1
Streptomyces vinaceusdrappus ATCC 25511^{T}	672	98	0	71.43	498	WP_193515251.1
Streptomyces plicatus ATCC 25483^{T}	670	98	0	71.43	498	WP_193449497.1
Streptomyces glaucescens ATCC 19761^{T}	670	89	0	77.06	487	WP_086736089.1
Streptomyces massasporeus ATCC 19785^{T}	662	89	0	79.56	475	WP_189592770.1
Streptomyces azureus ATCC 14921^{T}	656	100	0	77.09	493	WP_059422466.1
Streptomyces coeruleorubidus ATCC 13740^{T}	655	100	0	78.84	493	WP_150484948.1
Streptomyces roseolilacinus ATCC 19806^{T}	642	94	0	75.00	546	WP_189530058.1

Streptomyces acidicola TBRC 11341^{T}	636	95	0	69.10	495	WP_152865603.1
Streptomyces mangrovisoli DSM 100438^{T}	633	98	0	67.00	484	WP_046592841.1
Streptomyces spiralis ATCC 25664^{T}	630	95	0	68.18	488	WP_189908195.1
Streptomyces albiflavescens CGMCC 4.7111T	628	91	0	70.87	480	WP_189189640.1
Streptomyces humi DSM 42174^{T}	627	97	0	65.31	488	WP_046732985.1
Streptomyces mirabilis ATCC 27447^{T}	621	91	0	70.87	481	WP_190168754.1
Streptomyces swartbergensis NRRL B-65294^{T}	614	75	0	80.37	376	WP_086600198.1
Streptomyces chilikensis JCM 18411^{T}	610	89	0	67.77	493	WP_166028674.1
Streptomyces carpinensis ATCC 27116^{T}	609	91	0	66.67	484	WP_086725235.1
Streptomyces abyssomicinicus NBRC 110469^{T}	607	89	0	67.70	493	WP_151770382.1
Streptomyces fragilis ATCC 23908^{T}	604	89	0	67.04	493	WP_108953011.1
Streptomyces griseolus ATCC 19764^{T}	603	89	0	70.58	493	WP_028440660.1
Streptomyces anandii ATCC 19388^{T}	599	89	0	69.49	491	WP_189707798.1
Streptomyces coelicoflavus DSM 41471^{T}	598	89	0	68.07	487	WP_108986929.1
Streptomyces fumigatiscleroticus ATCC 19345^{T}	595	89	0	68.37	490	WP_189292293.1
Streptomyces monashensis DSM 103626^{T}	593	95	0	65.84	491	WP_071383268.1
[Kitasatospora] papulosa JCM 7250^{T}	590	95	0	64.77	496	WP_030122683.1
Streptomyces broussonetiae JCM 33918^{T}	586	89	0	67.93	488	WP_158929101.1
Streptomyces nodosus CGMCC 4.1459^{T}	585	89	0	67.79	487	WP_043446078.1
Streptomyces incarnatus NCBI txid665007	585	91	0	69.06	488	WP_208902940.1
Streptomyces poriferorum DSM 111306^{T}	582	89	0	68.44	493	WP_219568918.1
Streptomyces misionensis ATCC 14991^{T}	581	92	0	66.52	488	WP_074994850.1
Streptomyces paucisporeus CGMCC 4.2025^{T}	575	89	0	68.44	502	WP_079189591.1
Streptomyces naganishii ATCC 23939^{T}	573	91	0	68.63	488	WP_190177195.1
Streptomyces rubidus CGMCC 4.2026^{T}	572	89	0	66.96	512	WP_079176226.1
Streptomyces mutomycini CGMCC4.1747^{T}	571	90	0	65.42	496	WP_065847780.1
Streptomyces alni CGMCC 4.3510^{T}	571	90	0	68.86	509	WP_093712288.1
Streptomyces malaysiense DSM 100712^{T}	571	98	0	67.20	489	WP_046416880.1
Streptomyces puniciscabiei KACC 20253^{T}	571	89	0	68.60	488	WP_055710424.1
Streptomyces bryophytorum CGMCC 4.7151^{T}	567	98	0	65.32	503	WP_205045510.1

Streptomyces NCBI txid1889	566	89	0	65.03	498	WP_030060709.1
Streptomyces hundungensis JCM 17577^T	561	89	0	63.78	519	WP_120720001.1
Streptomyces murinus ATCC 19788^T	561	98	0	64.79	489	WP_199571844.1
Streptomyces pratensis CGMCC 4.6829^T	561	90	0	65.86	496	WP_203185902.1
Streptomyces brevispora KACC 21093^T	560	90	0	65.04	488	WP_145763246.1
Streptomyces NCBI txid1889	560	96	0	64.45	484	WP_138351395.1
Streptomyces acididurans TBRC 13094^T	560	89	0	65.12	517	WP_205355261.1
Streptomyces roseicoloratus TRM 44457^T	554	79	0	79.82	446	WP_128977611.1
Streptomyces griseofuscus ATCC 23916^T	550	98	0	64.59	489	WP_125210929.1
Streptomyces NCBI txid1889	540	71	0	75.00	362	WP_167526662.1
Streptomyces violaceochromogenes ATCC 19932^T	536	68	0	76.90	339	WP_191847784.1
Streptomyces yanglinensis CGMCC 4.2023^T	535	90	0	62.78	535	WP_200823184.1
Streptomyces fulvorobeus DSM 41455^T	530	90	0	63.05	486	WP_218900741.1
Streptomyces purpurascens ATCC 25489^T	520	68	0	77.84	339	WP_189730104.1
Streptomyces iakyrus ATCC 15375^T	517	64	0	78.50	322	WP_051814654.1
Streptomyces NCBI:txid1889	516	89	3.00E-179	59.33	473	WP_099502364.1
Streptomyces bomunensis MMS17-BM035^T	515	90	3.00E-178	66.59	505	WP_209340554.1
Streptomyces klenkii DSM 42104^T	495	59	3.00E-173	77.33	320	WP_120753395.1
Streptomyces anulatus CGMCC 4.1421^T	481	90	5.00E-165	55.73	507	WP_030589031.1
Streptomyces wuyuanensis CGMCC 4.7042^T	479	90	6.00E-165	55.60	467	WP_093655234.1
Streptomyces triticisoli DSM 105118^T	474	90	5.00E-162	54.85	512	WP_112469198.1
Streptomyces niveus ATCC 19793^T	472	93	2.00E-161	54.56	506	WP_037784056.1
Streptomyces pristinaespiralis NCBI txid38300	471	92	3.00E-161	54.29	497	WP_078535424.1
Streptomyces thermovulgaris CGMCC 4.1455^T	469	91	2.00E-160	54.13	488	WP_067396676.1
Streptomyces griseoluteus CGMCC4.1440^T	467	89	9.00E-160	57.21	492	WP_135793259.1
Streptomyces viridiviolaceus ATCC 27478^T	466	89	1.00E-159	56.10	473	WP_189879618.1
Streptomyces longisporoflavus CGMCC 4.1453^T	467	89	1.00E-159	55.65	508	WP_190079888.1
Streptomyces triticagri CGMCC 4.7476^T	461	58	2.00E-159	73.20	357	WP_128554654.1
Streptomyces NCBI:txid1889	464	89	7.00E-159	56.22	467	WP_108908319.1
Streptomyces echinoruber CGMCC4.1707^T	460	89	5.00E-157	57.11	494	WP_190058209.1

Streptomyces ficellus UC 5438^{T}	474	89	2.00E-156	56.42	943	WP_156695983.1
Streptomyces sulfonofaciens ATCC 31892^{T}	452	63	2.00E-156	71.25	332	WP_189934960.1
Streptomyces griseoflavus CGMCC4.1454^{T}	457	91	1.00E-155	53.91	512	WP_190098541.1
Streptomyces camponoticapitis DSM 100523^{T}	456	91	2.00E-155	54.35	480	WP_189109456.1
Streptomyces spongiicola CCTCC AA 2015018^{T}	454	90	5.00E-155	55.38	467	WP_116428422.1
Streptomyces pilosus ATCC 19797^{T}	454	99	2.00E-154	51.08	512	WP_189596203.1
Streptomyces bluensis CGMCC 4.1463^{T}	445	67	3.00E-153	66.77	340	WP_189872471.1
Streptomyces xinghaiensis CCTCC AA 208049^{T}	450	90	4.00E-153	55.16	492	WP_019711482.1
Streptomyces mutabilis ATCC 19789^{T}	447	86	1.00E-152	54.59	433	WP_107063999.1
Streptomyces fungicidicus NCBI txid68203	438	90	2.00E-148	51.87	476	WP_121548317.1
Streptomyces lunaelactis DSM 42149^{T}	439	89	2.00E-148	53.44	491	WP_175264381.1
Streptomyces kurssanovii ATCC 15824^{T}	434	88	3.00E-147	53.59	444	WP_189474576.1
Streptomyces populi CGMCC 4.7417^{T}	435	95	5.00E-147	52.50	493	WP_103548410.1
Streptomyces hirsutus ATCC 19773^{T}	434	90	2.00E-146	50.77	510	WP_079035031.1
Streptomyces zhihengii KCTC 39115^{T}	432	91	3.00E-146	52.83	494	WP_205377297.1
Streptomyces lavendofoliae ATCC 15872^{T}	436	86	9.00E-146	55.50	608	WP_189551293.1
Streptomyces triticiradicis CCTCC AA2018031^{T}	419	89	7.00E-141	53.22	494	WP_151471947.1
Streptomyces albulus CGMCC 4.1585^{T}	409	63	5.00E-139	65.63	334	WP_189864610.1