The microbiome of a shell mound: ancient anthropogenic waste as a source of *Streptomyces* degrading recalcitrant polysaccharides

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Abstract

Metagenome amplicon DNA sequencing and traditional cell culture techniques are helping to uncover the diversity and the biotechnological potential of prokaryotes in different habitats around the world. It has also had a profound impact on microbial taxonomy in the last decades. Here we used metagenome 16S rDNA amplicon sequencing to reveal the microbiome composition of different layers of an anthropogenic soil collected at a shell mound Sambaqui archeological site. The Sambaqui soil microbiome is mainly composed by phyla *Acidobacteria*, *Rokubacteria*, *Proteobacteria* and *Thaumarchaeota*. Using culture-dependent analysis we obtained few *Streptomyces* isolates from the Sambaqui soil. One of the isolates, named *Streptomyces* sp. S3, was able to grow in minimal medium containing recalcitrant polysaccharides including chitin, xylan, carboxymethylcellulose or microcrystalline cellulose as sole carbon sources. The activities of enzymes degrading these compounds were confirmed in cell free supernatants. The genome sequence revealed not only an arsenal of genes related to polysaccharides degradation but also biosynthetic gene clusters which may be involved in the production of biotechnologically interesting secondary metabolites.

Keywords Biodiversity · 16S sequencing · Streptomyces · Hydrolase

Introduction

Microbiomes constitute an important source for the prospection of industrially relevant enzymes and bioactive metabolites such as antibiotics or chemotherapeutic drugs. For instance, most of the antibiotics used in the clinics nowadays are still the fruits of valuable prospection of *Streptomyces* strains from the environment, mostly from soil (Liu et al. 2013).

The recent advances in metagenomic 16S rDNA amplicon sequencing and whole metagenome DNA sequencing is helping to uncover the microbial diversity and their biotechnological potential at an unprecedented scale (Nayfach et al. 2020; Thompson et al. 2017). Global efforts such as the earth’s microbiome project is providing valuable data on how microbial diversity is distributed around the globe and how microbial taxa correlate in similar environments in distant locations (Stevens et al. 2017). In addition to these high-tech platforms, microbial diversity can be accessed using traditional culture isolation techniques. Quite remarkably, traditional methods are still very effective in the discovery of novel bacterial strains with biotechnological potential (Wilson et al. 2014).

Brazil hosts a significant portion of the world's biodiversity and the Brazilian territory constitute an untapped source for the prospection of novel microbes. Still, most of the Brazilian biomes are only marginally explored in terms of microbiome composition (Pylo and Roesch 2017). Our research group is combining metagenomics and traditional cell culture techniques to uncover the microbiome in yet
to be explored Brazilian environments such as the pristine Amazon forest air (Souza et al. 2019) and the mangrove soil (Huergo et al. 2018).

Mangroves are particularly interesting habitats for microbial prospection, given the unique chemical characteristics and diel tidal variations which affect salinity, oxygen, pH and nutrient availability (Alongi 2014). Nevertheless, there are very few studies on Brazilian mangrove microbiome (Andreote et al. 2012; Ceccon et al. 2019; Huergo et al. 2018; Imchen and Kumavath 2020; Mendes and Tsai 2018).

The high primary productivity of mangrove estuaries provides plenty of availability of sea food. Not surprisingly, mangrove estuaries were colonized by ancient groups of fisher–hunters–collectors, vestiges of these ancestral human activities are preserved in several archaeological sites on the coast of Brazil which are named Sambaqui sites (Oki-mura and Eggers 2005). Sambaqui sites consist mostly of molluscs’ shells which are likely to be the waste products of meals eaten by groups of hunter–fishers–collectors that lived in the area between 2000 and 10,000 years ago. The Sambaqui anthropogenic soil are typically arranged in layers with varying composition that are likely to reflect the occupation of these sites by different groups (Fig. 1), with the bottom layers representing the debris of the most ancient groups.

Given their unique characteristics the Sambaqui sites constitute unique niches which may host interesting uncharacterized microbes. Here we used 16S rRNA gene ampli-con sequencing and culture techniques to provide the first characterized microbes. Here we used 16S rRNA gene amplification to explore these sites and provide the first characterized microbes. Here we used 16S rRNA gene amplification to explore these sites and provide the first characterized microbes. Here we used 16S rRNA gene amplification to explore these sites and provide the first characterized microbes.

Materials and methods

Sampling and culturable bacteria analysis

The Sambaqui Boguacu is located at the margin of the Boguacu river in a place called Ilha do Casqueiro, Guaratuba Bay estuary, Paraná, Brazil (25° 55′11″ S/48° 37′39″ W). This Sambaqui is 7 m high and is likely to date between 10,000 and 2000 years ago (Huergo et al. 2018) (Fig. 1). Samples were collected in a dry region of the site not subject to sea tide or river flooding. Samples were collected in the morning on 25/10/2016 in a rainy day during low tide, local temperature ranged from 20 to 23 °C during sampling. Samples were retrieved from three usually different stratification profiles of the Sambaqui layers, namely samples B, CM and S, from bottom to top, respectively (Fig. 1). These different stratification layers are formed and visible from the river margin of the 7 m high Sambaqui site due to natural erosion caused by the Boguacu river. About 2 g of sediment were removed from between 10 and 20 cm within the interior of Sambaqui profile using a sterile spoon. Samples were immediately stored in a sterile tube. Approximately one hour after sampling, 1 g sample of the sediment was diluted with 9 ml of sterile saline (10⁻¹ dilution), vortexed for 5 min and allowed to stand for 5 min for dense material to settle down. The supernatant was used to prepare a 10⁻² dilution in saline, of which 100 μl were spread on LB agar containing cycloheximide 50 μg ml⁻¹ to inhibit the growth of fungi. Plates were incubated aerobically at 30°C for 5 days. The colonies developed were further replicated and stored in glycerol 50% at −20 °C at the culture collection of Setor Litoral, Federal University of Parana (UFPR), Matinhos—PR, Brazil.

Some isolates were subjected to partial 16S rRNA gene sequencing as described previously (Stets et al. 2013). Isolates were taxonomically identified by comparing the DNA sequences against the 16 S ribosomal RNA gene sequences (Bacteria and Archaea) database at NCBI, using blastn (Altschul et al. 1990). All sequences were deposited in GenBank under the accession numbers MT019671, MT019672, MT019673 and MT019674.

Total DNA extraction and 16 S rRNA gene amplicon Illumina sequencing

Total DNA extraction from the three Sambaqui soil layers were performed using Power Soil DNA Kit (MOBIO). The DNA recovered from the kit was precipitated with ethanol 80%, dried and all the genetic material was used as template for PCR amplification as described previously (Souza et al. 2019). Briefly, the V4 region of the 16 S gene was PCR amplified in 10 μl reactions using: all dried template DNA; 1 μM primer 515 F; 1 μM primer 806R (which includes the barcode for each sample); and 5 μl KlenTaq DV ReadyMix (Sigma-Aldrich). The PCR cycles were 94 °C 3 min, 18 × (94 °C 45 s, 50 °C 30 s, 68 °C 1 min), 72 °C 10 min. Amplicons were sequenced in a MiSeq platform using MiSeq 500v2 Reagent Kit (Illumina) pair-end reads (2x of 250 bp).
Sample sequences were imported and demultiplexed in QIIME2 (Bolyen et al. 2019), then inputted into the plugin DADA2 (Callahan et al. 2016) on QIIME2 to filter low-quality data, denoising sequences, remove chimeras, join forward and reverse reads, and dereplicate sequences in amplicon sequence variants (ASV). After all steps,
non-chimeric sequences were generated and reduction in sequence numbers per samples were observed as follow: sample B, from 45,305 to 30,874 (31.8 %) and sample S, from 133,303 to 108,230 (18.8 %). A naïve Bayesian classifier was used through q2-feature-classifier QIIME2 plugin to classify sequences against a pre-trained classifiers on SILVA v.128 16 S rRNA 515 F/806R gene-region database clustered at 99% identity (Pedregosa et al. 2011; Quast et al. 2013), available at https://docs.qiime2.org/2020.11/data-resources (MD5: 28105eb0f-1256bf38b9bb310c701dc4e). The 16S rDNA ampli-con sequences were deposited in NCBI study numbers PRJNA605351, SRS6116179 and SRS6116180.

Screening for bacteria isolates that degrade recalcitrant polysaccharides

Isolates were tested for the ability to grow in solid agar M9 minimum medium (Green et al. 2012) containing ammonium chloride 20 mM as nitrogen source and 1% (w/v) of glucose, xylan, colloidal chitin, carboxymethyl cellulose or cellulose microcrystalline as carbon sources. Control agar plates without extra carbon source was also used to evaluate the ability of the isolates to use agar as carbon source. Isolates were streaked on M9 media plates and incubated for 5 days at 30 °C. The ability to use each carbon source was based on the visual inspection of the amount of biomass after 5 days at 30 °C. The ability to degrade xylan, colloidal chitin and carboxymethyl cellulose was confirmed by the formation of degradation halo after staining the plates with congo red 0.1% (w/v) and de-staining with NaCl 1 mol/l.

To confirm the ability of Streptomyces sp. S3 to degrade recalcitrant polysaccharides, one isolated colony from LA medium was inoculated into 200 ml of M9 liquid medium containing xylan, carboxymethyl cellulose, microcrystalline cellulose or filter paper (Whatman n°1 cut in small pieces) 1% (w/v) as sole carbon source. The flasks were incubated for 7 days in a rotary shaker at 30 °C and 120 rpm. Bacterial growth was accessed by visual inspection of bacterial biomass, by staining the cell-substrate matrix clumps for protein with Bradford reagent and/or by electronic microscopy analysis of the cell-substrate matrix clumps. To verify the presence of secreted hydrolytic enzymes, the supernatant was separated from the cells and insoluble substrates by centrifugation at 5000×g for 10 min. A fraction of 10 µl of the supernatant was spotted onto a plate containing solid agar M9 medium with 1% (w/v) xylan or carboxymethyl as substrate. After incubation at 30 °C for 30 min enzymatic activity was confirmed by the formation of degradation halo after staining the plates with congo red.

Scanning electron microscopy

The samples were fixed with 2% paraformaldehyde, dehydrated in ascending ethanol series, and the critical point was reached in a Bal-Tec CPD-030 with carbon dioxide. The gold plating was obtained in a Balzers SCD-030. SEM analyses were performed in a scanning electron microscope JEOL-JSM 6360 L at the Electron Microscopy Center of UFPR, Curitiba, PR. Brazil.

Genome sequencing and analysis

Total DNA extraction from the isolate Streptomyces sp. S3 were obtained from cells cultured in LB agar medium and extracted using Power Soil DNA Kit (MOBIO). The whole-genome sequence was carried out using Nextera DNA Library Preparation Kit and paired-end sequencing in the Illumina MiSeq platform. Raw reads quality was checked by FastQC (Andrews 2010). DNA sequence was de novo assembled using Spades (Bankevich et al. 2012). The draft genome was annotated using Prokka (Seemann 2014) and RAST (Overbeek et al. 2014). The draft genome sequence assembly was deposited in NCBI Assembly database under the identifier ASM1304450v1. Genome blast phylogeny was directly retrieved from NCBI.

The identification of carbohydrate-active enzymes were performed by comparing the putative proteome of Streptomyces sp. S3 against the latest (available on January 2020) CAZy database (Cantarel et al. 2009) using BlastP (Altschul et al. 1990). Proteins were considered as putative carbohydrate-active enzymes when the alignment score with a CAZY database entry had evalue ≤ 5−100 and the fraction of hit (CAZY) covered in the alignment was ≥ 0.4. Analysis of secondary metabolite biosynthesis gene clusters were performed using antiSMASH (Blin et al. 2019) and phylogenetic analysis were performed using autoMLST (Alanjary et al 2019).

Cell culture collection deposit

The isolate Streptomyces sp. S3 was deposited at CMRP WDCM1240—Microbiological Collections of Paraná Network—Federal University of Parana at code number CMRPS5229.

Results

In this study we focused on an archaeological Sambaqui site located in the Boguaçú River which is part of the Guaraçuba bay estuary (Fig. 1). The soil of the Sambaqui Boguaçú is arranged in three visually distinct layers, with varying composition, that are likely to reflect the time of occupation of...
this site by different groups (Fig. 1C). To assess the microbial diversity within the Sambaqui-Boguaçu samples of the three visually different layers were collected and subjected to microbial cultivation approach and to metagenome microbial biodiversity survey.

**Prokaryotic biodiversity using 16 S rDNA amplicon sequencing**

Total DNA was extracted from each of the three Sambaqui layers (Fig. 1C). The medium layer, namely CM, did not yield enough DNA for this analysis and was not considered for further analysis. The extracted metagenomic DNA from the top layer, namely S, and the bottom layer, namely B, were used as template for PCR amplification of the V4 region of the 16S rRNA gene and subjected to next generation Illumina sequencing using primers and methodology described previously (Huergo et al. 2018).

The amplicon sequence variants (ASV) were taxonomically classified; 13.8% and 9.3% of the ASV were classified as Archaea in S and B samples, respectively. Strikingly, despite being visually different (Fig. 1C), the samples S and B had similar biodiversity profiles at Phylum level (Fig. 2). The most represented Phylum were Acidobacteria, Rokubacteria, Proteobacteria and the Archaea Thaumarchaeota (Fig. 2). The most represented identified family was the methylotrophic Methylomirabilaceae (~10% of the reads in both samples) (Fig. S1). This family was reported to be abundant in paddy fields, an anaerobic habitat typically rich in methane (Ghashghavi et al. 2019). The Sambaqui site explored in this study was built over a mangrove which is also considered a habitat with high methane production, this may explain the abundance of the methylotrophic Methylomirabilaceae in our samples. Most of the remaining sequences could not be classified at Family (Fig. S1) or lower taxonomic levels. These data

![Microbial taxonomical distribution at phylum level of the S and B samples from Boguaçu Sambaqui, based on metagenome 16S rDNA amplicon sequencing. The figure was generated using QUIIME2](image)
support that the Sambaqui site hosts unique poorly characterized microorganisms.

**Bacteria isolation and selection for growth in recalcitrant polysaccharides**

All the three different layers of Sambaqui samples were diluted in saline and the $10^{-2}$ dilution was plated in LB agar medium. The samples from the layers B and CM did not yield any CFU, while sample S produced 8 CFU, namely S1–S8. The isolates S3 and S8 showed a similar colony morphology and produced a dark pigment in LB agar plates or LB liquid medium (Fig. S2). The isolates were evaluated for their ability to growth in M9 minimum medium containing glucose, agar, colloidal chitin, microcrystalline cellulose (MCC), carboxymethyl cellulose (CMC) or xylan as carbon sources. All the isolates were able to grow using glucose as carbon source while using ammonium as nitrogen source (Table 1). Interestingly, the isolates S3 and S8 showed little growth in M9-glucose agar plates without a nitrogen source (Table 1). Furthermore, the isolates S3 and S8 grew well in LB agar prepared using sea water indicating halophilic properties (not shown).

The isolates S3 and S8 were able to growth in M9 agar containing different recalcitrant carbohydrates as carbon source including agar, chitin, CMC, MCC and xylan (Table 1 and Fig. S3). Given that S3 and S8 were able to grow using agar as sole carbon source, we consider that the growth detected in the presence of other polysaccharides could be sustained by agar degradation instead. However, the degradation of chitin, CMC and xylan could be confirmed by staining the agar plates with congo red which showed typical polysaccharide degradation halo (Fig. S3C).

To provide a taxonomic classification for the isolates the 16 S rRNA gene of the isolates S3, S5, S6, and S8 were PCR amplified and subjected to partial sequencing. All sequences showed 100% identity to various *Streptomyces* when searched against specialized 16 S rRNA NCBI database. Multiple sequence alignment of the 16 S rRNA gene revealed two groups of sequences, one includes the sequences of S3 and S8 which were identical in pair wise comparison. The sequences of isolates S5 and S6 were also identical in pair wise comparison. The two different groups of isolates (S3-S8 and S5-S6) showed 4 base differences within the 16S rRNA V4/V5, 98.5% identity (Fig. S4). Despite belonging to *Streptomyces* genus these two groups of isolates have completely different capacity to use recalcitrant polysaccharides as carbon source in M9 media (Table 1).

The isolate *Streptomyces* sp. S3 was subjected to further characterization. This bacterium was able to growth aerobically in M9 liquid medium containing the soluble polysaccharides xylan and CMC as the only carbon source. *Streptomyces* sp. S3 formed cell pellets in these media (Fig. 3A and B). Scanning electron microscopy analysis of these pellets showed the formation of typical *Streptomyces* mycelium (Fig. 3). Interestingly, the morphology of the mycelium was different in medium containing xylan or CMC. When using CMC as carbon source, the mycelium was covered with an apparent extracellular matrix (Fig. 3). We suspect that this cell surface adhering material may be formed by an extracellular complex of CMC and enzymes involved in CMC degradation.

Qualitative analyses for presence of active extracellular enzymes degrading xylan and CMC were carried out by placing 10 µl of each cell culture supernatant over M9 solid medium containing either xylan or CMC. After 30 min incubation at 30 °C, the plates were stained with congo red to detect substrate degradation. As expected, degradation of xylan was readily detected using the supernatant of cells cultured in xylan (Fig. S5A, spot 1). On the other hand, and quite surprisingly, only a faint CMC degradation halo

| Isolate | Glucose | Agar | Chitin | MCC | CMC | Xylan | Glucose without nitrogen |
|---------|---------|------|-------|-----|-----|-------|-------------------------|
| S1      | +++     | −    | −     | −   | −   | −     | −                       |
| S2      | +       | −    | −     | −   | −   | −     | −                       |
| S3      | +++     | +    | ++    | +   | +++ | +++   | +                       |
| S4      | +       | −    | −     | −   | −   | −     | −                       |
| S5      | +++     | −    | −     | −   | −   | −     | −                       |
| S6      | +++     | −    | −     | −   | −   | −     | −                       |
| S7      | +       | −    | −     | −   | −   | −     | −                       |
| S8      | +++     | +    | ++    | +   | +++ | +++   | +                       |

*aAll media contain agar 15 g/L and 1 g/L of the indicated carbon source. Ammonium chloride 20 mM as nitrogen source except when indicated. + indicates the visual amount of biomass observed after growth (+ low, ++ medium, +++ high)
was detected using the supernatant of cells cultured in CMC (Fig. S5A, spot 3). The amount of protein in the culture supernatants were below the limit of detection and could not be determined. Hence, at this point, we cannot infer if the differences in xylan vs. CMC degradation halo are caused by differences in total enzymes load and/or specific enzyme activities.

The ability of *Streptomyces* sp. S3 to grow aerobically in M9 liquid medium carrying insoluble MCC or filter paper as sole carbon sources was also evaluated. Even though the cells did not appear as pellets as observed when growing with CMC or xylan, it was possible to confirm microbial biomass development adhered to the insoluble MCC by staining the remaining insoluble substrate for protein using the Bradford reagent (not shown). Furthermore, it was possible to detect active xylan and CMC degrading extracellular enzymes from the *Streptomyces* sp. S3 supernatant of cells cultured in MCC (Fig. S5A, spot 3). The supernatant from MCC growing cells produced a more intense CMC degradation halo than those assayed with the supernatant of cells growing in CMC itself (Fig. S5B, compare spots 3 and 4).

**Genome analysis**

A draft genome sequence of *Streptomyces* sp. S3 was obtained comprising 569 contigs (40,558 N50), with a total
of 10,992,946 bp with G + C content of 70.55%. Genome annotation revealed 9844 protein coding sequences. The Streptomyces sp. S3 genome was used to perform phylogenetic analysis against reference organisms using Automated Multi-Locus Species (auto MLSST) (Alanjary et al. 2019). The phylogenetic tree revealed that Streptomyces antibioticus DSM40234 is the closest type strain to Streptomyces sp. S3 (Fig. S6) with a MASH distance of 0.1064 and estimated average nucleotide identity of 0.8936 (Table S1). S. antibioticus was originally isolated from soil and is well known for its potential to produce several bioactive compounds, including several antibiotics. There are strains of S. antibioticus isolated from marine environments which may explain the halophilic properties of the Streptomyces S3 isolate (Sharma et al. 2019; Waksman and Woodruff 1941; Wang et al. 2017; Xu et al. 2011). Automated genome blast-based phylogeny retrieved from NCBI indicated that soil environmental isolate Streptomyces sp. WM6386 (Blodgett et al. 2016) as the closest relative (Fig. S7).

In order to identify biosynthetic gene clusters (BGC) the Streptomyces sp. S3 genome was subjected to antiSMASH analysis (Blin et al. 2019) which predicted 22 BGCs, totaling 464,995 bp (4.2% of the genome), including: 4 terpene (76,408 bp); 1 polyketide synthase (PKS; 32,694 bp); 1 polyketide synthase (PKS)/nonribosomal peptide synthetase (NRPS) hybrid (68,067 bp); 6 NRPS among other (220,742 bp) (Table S2). Out of these, only 5 showed similarities ≥ 0.7 to known BGC including those involved in the biosynthesis of the terpenes albaflavenone, geosmin, ectoine and hopene. A BGC for melanin production was also found, suggesting that melanin be responsible for the black pigmentation of Streptomyces sp. S3 when cultured in LB medium (Fig. S2). Indeed, two putative tyrosinases containing secretion N-terminal signal peptides were identified in the annotated genome (not shown). A BGC involved in ectoine production was identified (Table S2). Ectoine is produced by halophilic and halotolerant microorganisms to prevent osmotic stress in highly saline environments (Reshetnikov et al. 2011). The production of ectoine may explain the halotolerant behavior of Streptomyces sp. S3.

To assess the potential for carbohydrate degradation the predicted proteome of Streptomyces S3 were compared with the enzymes deposited at the CAZy database using blastP. This analysis revealed a total of 459 enzymes comprising: 247 Glycosyl Hydrolase; 99 Glycosyl transferases; 25 Polysaccharide lyases; 31 Carbohydrate esterases; 5 Auxiliary activity; 121 Carbohydrate-binding modules (Table S3). 214 (46.6%) of these putative CAZy enzymes were annotated as hypothetical proteins by Prokka while some enzymes were annotated as putative Chitinases, xylanases and cellulases (Table S3). These data provide additional support that Streptomyces S3 is well suited for recalcitrant polysaccharide degradation and a potential source of biotechnologically relevant enzymes.

**Discussion**

Despite global efforts to uncover the microbial diversity and biotechnological potential there are still untapped sources to explore specially in environments where less sampling efforts were concentrated. In this study we provide the first microbiome analysis of the anthropogenic soil layers of an archeological Sambaqui site located in the South of Brazil. By using metagomic DNA extraction and 16 S rDNA amplification sequencing we identified Acidobacteria (~19%), Rokubacteria (~17%), Proteobacteria (~16%) and Thaumarchaeota (~9%) as the most representative phylum in different layers of the Sambaqui soil. Quite remarkably, most of the reads could not be classified at lower taxonomic level suggesting that this site contains unique previously undescribed microbes (Fig. 2 and Fig. S1). We have analysed previously the microbiome of the pristine mangrove soil and mangrove soil affected by the eroding debris at this Sambaqui site. The microbiome composition in the mangrove soil at the flooding level was remarkably different with Proteobacteria (~49%), Chloroflexi (~12%), and Bacteroidetes (~6%) being the dominant taxa (Huergo et al. 2018).

Using culture techniques, a low number of CFU was obtained from just one of the soil layers using the rich medium for cell culture (Fig. S2). Such poor recovery is likely to reflect the fact that the Sambaqui soil is poor in organic matter despite abundant carbonaceous shell debris. Nevertheless, few Streptomyces could be isolated which may reflect the fact that Streptomyces can survive in dormant spores and/or because Streptomyces typically can use a range of substrates as carbon sources including recalcitrant polysaccharides. In fact, the isolated Streptomyces sp. S3 was able to use agar, chitin, CMC, MCC and xylan as carbon sources and showed residual growth even in the absence of a nitrogen source (Table 1). The presence of enzyme degrading recalcitrant polysaccharides was confirmed by assaying the enzymatic activity in cell culture supernatant (Fig. S5) and corroborated by genome analysis of Streptomyces sp. S3 (Table S3). Interestingly, several studies on different Streptomyces support that some strains have the ability to degrade cellulotic biomass (Takasuka et al. 2013; Lim et al. 2015; Pinheiro et al. 2017). Studies are under way to identify the enzymes responsible for CMC and MCC degradation in Streptomyces sp. S3.

The genome analysis of Streptomyces sp. S3 indicated that S. antibioticus is the closest type strain (Figs. S1 and S6). However, the low nucleotide identity ANI suggest that Streptomyces sp. S3 may constitute a novel species. The genome data also support that Streptomyces sp. S3 may
produce secondary metabolites of biotechnological importance (Fig. S2). This is expected since its closest relative \textit{S. antibioticus} is a well-known antibiotic producer (Sharma et al. 2019).

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Author contributions LH, MC, MVG, MG collected and processed the samples; VB and EB processed the samples for Illumina sequencing; EMG and TT performed the microscopy analysis; AP analyzed the samples; VB and EB processed the samples for Illumina sequencing. RR, LH, ES, LC and FP, project and lab management. LH wrote the manuscript with inputs form all authors.

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Data availability Materials and data are available upon request.

Code availability Not applicable.

Declarations

Conflict of interest Nothing to declare.

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