



Exploration of Secondary Metabolite Production Potential in Actinobacteria Isolated From *Kandelia candel* Mangrove Plant

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Mangroves are extreme inter-tidal environments containing rich microbial communities. Actinobacteria from mangroves have an unprecedented ability to biosynthesize secondary metabolites. Therefore, the secondary metabolite production potential of actinobacteria sourced from mangroves warrants further exploration to determine whether they offer new sources of natural products. For this purpose, we selected a strain of *Mycobacterium saopaulense* (Actinobacteria phylum) from the mangroves in Macau for whole genome sequencing and tandem mass spectrometry analysis. The results showed that the 5,376,881-bp genome from this strain contains 5,391 protein-coding genes and a coding density of 90.53%. The main participating KEGG pathway was “Metabolism”. Altogether, the 81 gene clusters identified in its genome were associated with the production of 23 secondary metabolites. Sixteen of them were classified as antibiotics and three of them as bioactive compounds. Furthermore, two of the predicted secondary metabolites from the fermentation process were clavulanic acid and streptomycin. Both of these antibiotics were initially found to be produced by the *M. saopaulense* strain. This study shows that mangrove-derived actinobacteria have a large number of biosynthetic pathways with the potential to produce a range of biologically active secondary metabolites.

Keywords: *Mycobacterium*, mangrove, whole genome, gene cluster, secondary metabolite, mass spectrometry

INTRODUCTION

The natural products produced by microorganisms are important sources of many drugs in widespread use today for treating various diseases and infectious agents, such as cancer, immunosuppression, bacteria, and viruses (Gullo et al., 2006; Newman and Cragg, 2016). Among these drugs, 75% are natural products derived from actinobacteria, a Gram-positive bacteria with a high genomic G+C content (Abdel-Razek et al., 2020). Actinobacteria produce structurally diverse secondary metabolites such as polyketides, terpenes, macrolides, and alkaloids, which among others have remarkable biological activities (Rateb et al., 2018). However, recent genomics studies have

found that the number of biosynthetic gene clusters in actinobacteria is far greater than the number of compounds that have been obtained from them (Hu et al., 2018; Hu et al., 2019; Hu et al., 2020). Therefore, identifying all of the gene clusters and exploring the potentials of secondary metabolic biosynthesis in actinobacteria will not only help to enrich the resource pool of natural products, but also to provide more lead compounds for drug development.

Our previous work and some other studies found that mangrove-derived actinobacteria contain a higher number of gene clusters and have greater potential to produce more bioactive compounds than those from terrestrial environments (Huang et al., 2016; Pei et al., 2021). In general, actinobacteria isolated from mangroves have a higher number of clusters for secondary metabolites than those obtained from terrestrial habitats (Bentley et al., 2002; Liu et al., 2012; Komatsu et al., 2013; Hu et al., 2018; Hu et al., 2019; Ser et al., 2019; Hu et al., 2020; Ser et al., 2020). Mangroves are unique plant communities that grow in the intertidal zones of tropical and subtropical coasts and are submerged by periodic tides (Collins et al., 2017). Actinobacteria are generally best suited to semi-arid environments (Ghorbani-Nasrabadi et al., 2013); hence, it appears that the actinobacteria that live in mangroves are likely to have unique molecular adaptations because these environments have been characterized by humid, highly acidic and high-salt conditions for long periods of time. As a consequence, mangrove-derived actinobacteria have broad biosynthetic potential and merit further exploration.

Many well-known antibiotics were initially sourced from actinobacterial strains, among which many are from *Streptomyces*, the largest genus in the Actinobacteria phylum (Raja and Prabakaran, 2011). *Streptomyces* species can produce most of the known aminoglycosides, many beta-lactams, non-ribosomal peptides, and a great number of macrolides (Nagarajan et al., 1971; Challis and Ravel, 2000). *Streptomyces*-derived macrolides tend to be nystatin, natamycin and amphotericin B (Linke et al., 1974; Brautaset et al., 2000; Farid et al., 2000). Aminoglycosides that are produced by the *Streptomyces* group include streptomycin, kanamycin, and neomycin (Shier et al., 1969; Distler et al., 1987). However, the secondary bioactive metabolite production capacities of non-*Streptomyces* or rare genera may be completely underestimated. Without doubt, the search for antibiotic production potential in rare actinobacterial species for pharmaceutical development is of great interest (Demain, 2014; Lee et al., 2015; Indupalli et al., 2018; Ser et al., 2018). In common with the findings from our previous study, it was first reported that mangrove-derived non-*Streptomyces* *Mycobacterium* species can produce asukamycin and apramycin antibiotics (Hu et al., 2019), and that *Micromonospora aurantiaca* can produce the kanamycin antibiotic, the yield of which may be greater than that of the original actinobacteria that produced it (Hu et al., 2020). Thus, we propose that other mangrove-derived non-*Streptomyces* species have unknown secondary metabolite production potential worth exploring.

Therefore, the aim of this study was to explore the potential of secondary metabolite production of mangrove plant-derived actinobacteria. We selected one actinobacteria species, *Mycobacterium saopaulense*, which was isolated from mangroves,

with which to conduct whole genome sequencing to identify its biosynthetic gene clusters and corresponding compounds. The fermentation products of this *M. saopaulense* strain were analyzed by mass spectrometry to determine which predicted compounds were actually produced. Our results augment current understanding about the secondary metabolite production ability of actinobacteria, and provide possible bacterial-derived resources for the development of natural products.

MATERIALS AND METHODS

Isolation of the *M. saopaulense* Strain

The *M. saopaulense* strain was isolated from a mangrove plant (*Kandelia candel*) in Macau. It shares 99.90% 16S rRNA sequence similarity with *M. saopaulense* EPM 10906^T (Hu et al., 2020). The isolate was stored in 20% glycerol at -80°C in the State Key Laboratory of Quality Research in Chinese Medicine, University of Macau.

DNA Extraction and Whole Genome Sequencing

DNA extraction, library construction and whole genome sequencing were performed in accordance with the methods of a previous study (Hu et al., 2018). Genomic DNA from the *M. saopaulense* strain was extracted using the IANamp Bacteria DNA Kit (TIANGEN Biotech Co. LTD). The library was constructed using the NEBNext Ultra II DNA library Prep Kit. Sequencing was conducted on the Illumina NovaSeq HiSeq 4000 instrument. IBDA was used to assemble and annotate the reads obtained after sequencing. MetaGeneMark (<http://topaz.gatech.edu/GeneMark/>), tRNAscan-SE (<http://trna.ucsc.edu/software/>) and rnammer (<http://www.cbs.dtu.dk/services/RNAmmer/>) were used for gene prediction, tRNA prediction and rRNA prediction, respectively. Gene functions were determined by comparison with the Kyoto Encyclopedia of Genes and Genomes (KEGG, <https://www.genome.jp/kegg/>) database. Mega software was used to construct a phylogenetic tree. The 16S rRNA sequences of the *M. saopaulense* strain were extracted from the annotation results. The top 20 closely related type strains' 16S rRNA sequences were downloaded from the NCBI database. These species were *M. saopaulense* strain EPM10906 (GeneBank accession number: KM973037.1, similarity: 99.90%), *M. abscessus* strain WH572 (GeneBank accession number: KR995262.1, similarity: 99.89%), *M. abscessus* strain WH834 (GeneBank accession number: KR995258.1, similarity: 99.89%), *M. chelonae* strain CH 3/4 (GeneBank accession number: MN577459.1, similarity: 99.78%), *M. saopaulense* strain FMS-15 (GeneBank accession number: MK396591.1, similarity: 99.78%), *M. chelonae* strain JNBP 03213 (GeneBank accession number: LC082312.1, similarity: 99.78%), *Mycobacterium* sp. strain MA45 (GeneBank accession number: MH699146.1, similarity: 99.78%), *Mycobacterium* sp. strain TM-A149 (GeneBank accession number: MH698675.1, similarity: 99.78%), *Mycobacterium* sp. strain TM-A163 (GeneBank accession number: MH698674.1, similarity: 99.78%), *Mycobacterium* sp.

strain TM-A160 (GenBank accession number: MH698673.1, similarity: 99.78%), *M. chelonae* subsp. *bovis* strain QIA-37 (GenBank accession number: NR158073.1, similarity: 99.67%), *M. abscessus* strain JCM 6390 (GenBank accession number: LC149865.1, similarity: 99.67%), *Mycobacterium* sp. G1 (GenBank accession number: LC637768.1, similarity: 99.67%), *Mycobacterium* sp. G2 (GenBank accession number: LC637767.1, similarity: 99.67%), *M. abscessus* strain AF7 (GenBank accession number: MW332182.1, similarity: 99.67%), *M. abscessus* strain AF6 (GenBank accession number: MW332181.1, similarity: 99.67%), *M. abscessus* strain WH750 (GenBank accession number: KR995261.1, similarity: 99.67%), *M. abscessus* strain WH999 (GenBank accession number: KR995259.1, similarity: 99.67%), *Mycobacterium* sp. strain TM-B145 (GenBank accession number: MH698713.1, similarity: 99.57%), *M. chelonae* strain JNBP 03238 (GenBank accession number: LC082323.1, similarity: 99.57%) and *Escherichia coli* strain U 5/41 (GeneBank accession number: NR 024570.1). Online anti-SMASH (<https://antismash.secondarymetabolites.org>) software was used to profile the biosynthetic gene clusters and secondary metabolites.

Fermentation Culture and Tandem Mass Spectrometry (MS/MS) Analysis

The fermentation culture and chemical analysis were conducted in accordance with Hu et al. (2018). ISP2 medium, which was selected as the culture medium, contained 4.0 g/L yeast, 10.0 g/L malt extract, 4.0 g/L glucose and 20.0 g/L agar, and pH 7.2. The stored isolate was streaked out onto ISP2 solid medium, and cultured at 28°C for four days. A single colony was selected and inoculated into 20 mL of ISP2 liquid medium and shaken (28°C,

7 days, 250 rpm). Ethyl acetate (60mL) was added to the fermentation products, the culture was centrifuged, and the supernatant was collected and dried at 60°C. The extracts were dissolved in 3mL of methanol for chemical analysis. The 4000 Q Trap MS/MS instrument was used to identify the fermentation products. The full scan mode was used first for detecting compounds falling into the 100–1500 m/z range under the positive ion mode. The conditions were set as follows: capillary voltage, 3.0 kV; cone voltage, 20V; 0.6S per spectrum. A full secondary scan, which was conducted to detect the fragments of ions, was compared with the Human Metabolome Database (HMDB, <https://hmdb.ca/>) to finally determine the compound.

RESULTS AND DISCUSSION

Phylogenetic Analysis

A phylogenetic analysis of the isolated strain to illustrate its evolutionary relationship with the top 20 closely related type strains (Figure 1). The 16S sequences based phylogenetic tree showed that the *M. saopaulense* strain and *M. abscessus* strain WH572, *M. abscessus* strain WH999, *M. abscessus* strain WH750, *M. abscessus* strain AF6, *M. abscessus* strain AF7, *Mycobacterium* sp. G2, *Mycobacterium* sp. G1, *M. abscessus* strain JCM 6390, *M. chelonae* subsp. *bovis* strain QIA-37, *Mycobacterium* sp. strain TM-A160, *Mycobacterium* sp. strain TM-A163, *Mycobacterium* sp. strain TM-A149, *Mycobacterium* sp. strain MA45, *M. chelonae* strain JNBP 03213, *M. saopaulense* strain FMS-15, *M. abscessus* strain WH834, *M. saopaulense* strain EPM10906 and *M. chelonae* strain CH 3/4 were located on the



FIGURE 1 | Comparison of 16S rRNA sequences from the *M. saopaulense* strain with 20 other orthologous sequences. *Escherichia coli* was used as the root of the phylogenetic tree. The method of neighbor-joining was used to construct the tree. The number of bootstrap replications was set to 1000.

same branch in the tree, suggesting that they should have a close relationship, but had a relatively long distance from *Mycobacterium* sp. strain TM-B145 and *M. chelonae* strain JNBP 03238. It is reasonable that the *M. saopaulense* strain and 2 type strains of *Mycobacterium* sp. strain TM-B145 and *M. chelonae* strain JNBP 03238 with low 16S sequence similarity (99.57%) were located on the different branch in the tree. But the relationship between the *M. saopaulense* strain and the other type strains did not show the closest phylogenetic distance in combination with the highest 16S sequence similarity, which might result from that the sequences used to construct the tree were obviously short (1300bp-1500bp).

General Genome Features and Function Annotations

The *de novo* assembly showed that the full length genome from the *M. saopaulense* strain was 5,376,881-bp long with an average read length of 21,085 bp, and a G+C content of 63.91%. A total of 6,881,851 reads and 255 scaffolds were generated. The genome contains 5,391 protein-coding genes, 72 tRNA genes, 3 rRNA genes, and has a coding density of 90.53%. The average coding sequence length was 903 bp. In the KEGG database, 80.44% of the genes were annotated as “Metabolism”, and their main metabolic pathways involved “global and overview maps”, “carbohydrate metabolism”, and “amino acid metabolism” (Figure 2).

Identification of Gene Clusters and Secondary Metabolites

Altogether, 81 biosynthetic gene clusters were identified in the genome from the *M. saopaulense* strain; these were linked to bacteriocin, ectoine, fatty acid, indole, nonribosomal peptides (NRPS), polyketide synthase (PKS), saccharide and others. Worth noting is the possibility that the 54 gene clusters in the genome that fall within the putative-type category encode unique

natural products (Dahal et al., 2021). These gene clusters putatively produce 23 known secondary metabolites (Table 1), from which 16 are antibiotics, three are bioactive compounds, leaving four others. Until now, no known antibiotics have been found to be produced by *M. saopaulense* sp., but the genome results suggest that the mangrove-derived *M. saopaulense* strain’s genome has huge potential to biosynthesize antibiotics. With the exception of mycobactin, which is produced by actinobacteria in the *Mycobacterium* genus, the other predicted antibiotics are derived from non-*Mycobacterium* genera, such as *Actinomadura*, *Amycolatopsis*, *Saccharothrix*, *Salinispora*, *Streptomyces*, and *Micromonospora* (Table 1). This indicates that the extreme environment of mangroves acts not only to enrich the gene clusters of the actinobacteria that live there, but has also enabled them to evolve different biosynthetic pathways.

Detecting Fermentation Products in the *M. saopaulense* Strain.

To move beyond genome prediction, fermentation and mass spectrometry experiments were performed to determine which of the secondary metabolites predicted by genomic analysis were actually produced by the *M. saopaulense* strain. The secondary metabolites were detected using the same previously reported methods (Hu et al., 2018; Hu et al., 2019; Hu et al., 2020).

We first calculated the theoretical m/z values of the 23 predicted secondary metabolites displayed in Table 2. An MS/MS system was used to separate the secondary metabolites produced by the *M. saopaulense* strain for analysis. By comparing the theoretical m/z values with the mass spectrometry’s full scan atlas, the compounds with molecular ion peaks close to or consistent with the theoretical values were selected. Eight predicted secondary metabolites were detected in the full-scan mode (Table 2). We then conducted a secondary

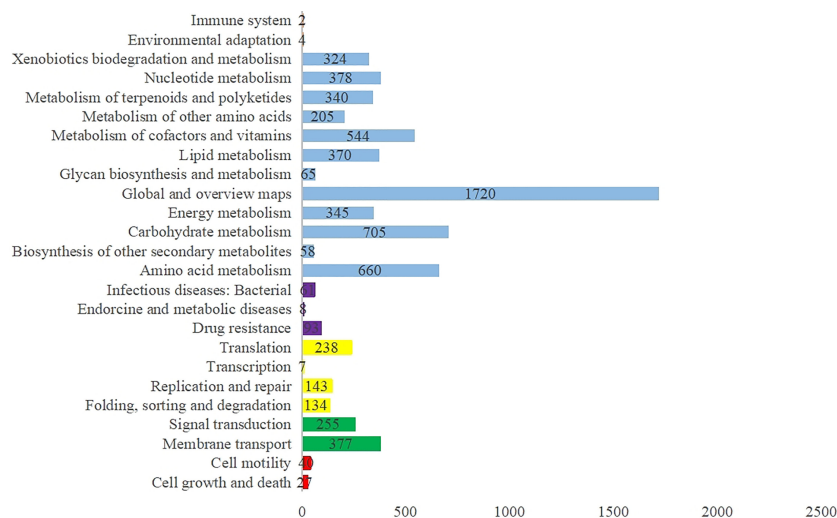


FIGURE 2 | Distribution of KEGG pathways in the *M. saopaulense* strain’s genome, showing the top five KEGG Orthology (KO) categories in the assigned sequences, which include organismal systems (orange), metabolism (blue), human diseases (purple), genetic information processing (yellow), environmental information processing (green), and cellular process (red).

TABLE 1 | Predicted gene clusters and their corresponding secondary metabolites in the *M. saopaulense* strain.

Type	Location	Category	Predicted compound	Similarity (%)	Reference strain
Putative	41964-55766	Antibiotic	Asukamycin	4	<i>Streptomyces nodosus</i> subsp. <i>asukaensis</i> (GQ926890.1)
Putative	227156-242587		BE-7585A	7	<i>Amycolatopsis orientalis</i> subsp. <i>vinearia</i> (HM055942.1)
Putative	185870-220588		Calcimycin	27	<i>Streptomyces chartreusis</i> NRRL 3882 (HM452329.1)
Putative	648-26404		Clavulanic acid	8	<i>Streptomyces clavuligerus</i> ATCC 27064 (DS570624.1)
Other	1-38408		Enduracidin	4	<i>Streptomyces fungicidicus</i> (DQ403252.1)
Putative	527964-545974		Kjianimicin	4	<i>Actinomadura kijaniata</i> (EU301739.1)
Putative	482852-491108		Kosinostatin	3	<i>Micromonospora</i> sp. TP-A0468 (JN038178.1)
Putative	10189-19773		Meridamycin	5	<i>Streptomyces</i> sp. NRRL 30748 (DQ351275.1)
NRPS	1-25792		Mycobactin	50	<i>Mycobacterium tuberculosis</i> H37Rv (AL123456.3)
Putative	342927-366860		Nosiheptide	15	<i>Streptomyces actuosus</i> (FJ438820.1)
T2PKS-	497091-		Pimaricin	11	<i>Streptomyces natalensis</i> (AJ278573.1)
Fatty acid	5421127				
Putative	27592-55301		Rifamycin	3	<i>Amycolatopsis mediterranei</i> S699 (AF040570.3)
Putative	386387-400838		Salinomycin	6	<i>Streptomyces albus</i> (DQ630728.1)
Putative	858982-882748		Streptomycin	2	<i>Streptomyces griseus</i> subsp. <i>griseus</i> NBRC 13350 (AP009493.1)
Other	107309-131171		Thiolactomycin	100	<i>Salinispora pacifica</i> DSM 45543 (KT282100.1)
Putative	62842-69164		Thiolutin	8	<i>Saccharothrix algeriensis</i> (KM114209.1)
Indole-NRPS	794627-851996	Bioactive compound	Cladoniamide	61	<i>Streptomyces uncialis</i> (JN165773.1)
NRPS	111098-164042		Nocobactin	75	<i>Nocardia farcinica</i> (CP031418.1)
Putative	14327-30757		Divergolide	6	<i>Streptomyces</i> sp. W112 (KF765440.1)
Putative	157085-165223	Others	Amychelin	12	<i>Streptomyces</i> sp. AA4 (GG657746.1)
Ectoine	1-7958		Ectoine	75	<i>Streptomyces anulatus</i> (AY524544.1)
Saccharide-NRPS	62158-133033		Glycopeptidolipid	100	<i>Mycobacteroides chelonae</i> (AM231612.1)
NRPS	350350-383115		Glycopeptidolipid	10	<i>Mycobacteroides chelonae</i> (AM231612.1)
T1PKS-NRPS	14813-76493		Glycopeptidolipid	20	<i>Mycobacteroides chelonae</i> (AM231612.1)
Other	1-35881		Glycopeptidolipid	10	<i>Mycobacteroides chelonae</i> (AM231612.1)
T1PKS	9276-55548		Glycopeptidolipid	13	<i>Mycobacteroides chelonae</i> (AM231612.1)
Saccharide-NRPS	1-64770		Glycopeptidolipid	26	<i>Mycobacteroides chelonae</i> (AM231612.1)
Putative	248037-262363		Sioxanthin	25	<i>Salinispora tropica</i> CNB-440 (CP000667.1)

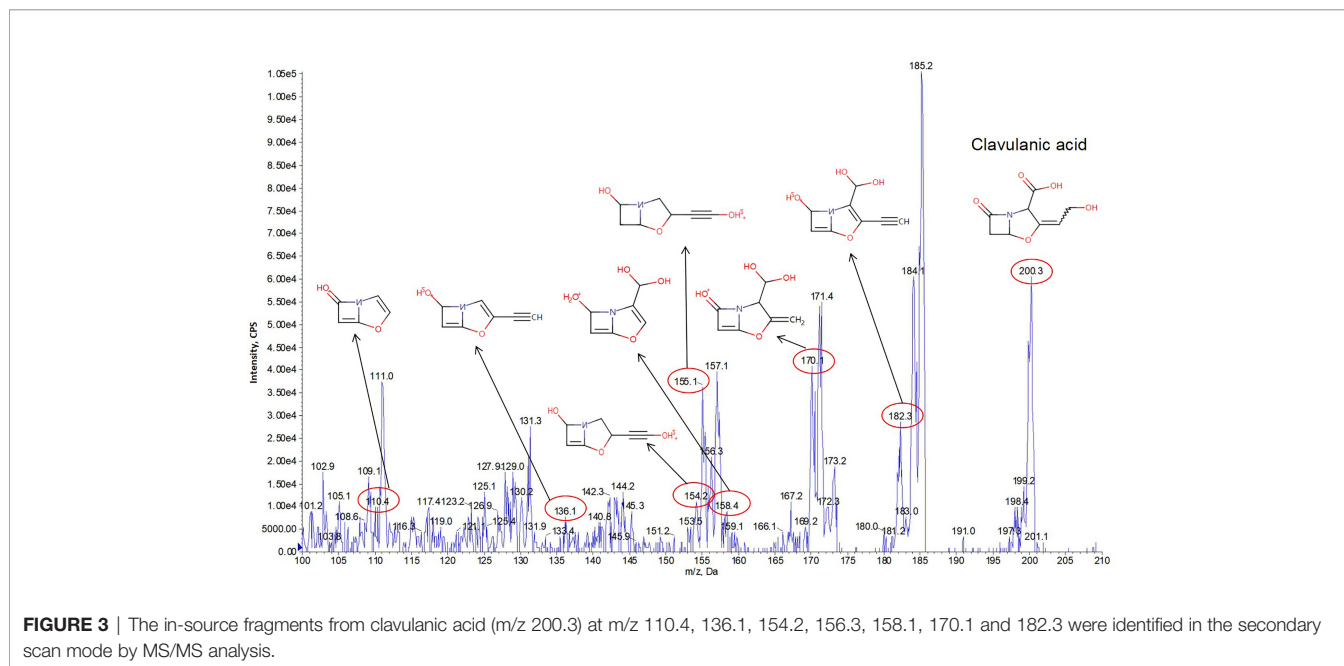
mass spectrometry scan of the eight secondary metabolites, and the fragments' information we obtained was compared with that in the HMDB to further determine the identities of the tested compounds. **Table 2** shows that a compound with a molecular ion at 200.3 m/z was identified in the spectrum from the first scan, indicating that it could be clavulanic acid. Its in-source fragments at m/z 110.4, 136.1, 154.2, 156.3, 158.1, 170.1 and 182.3 identified by the secondary scanning mode (**Figure 3**) matched the MS/MS spectrum (20V, positive) of clavulanic acid from the HMDB. **Table 2** also shows that the compound with a molecular ion at m/z 582.5, which was identified in the spectrum from the first scan, could be streptomycin. Its in-source fragments at m/z 202.1, 261.3, 371.4 and 522.4, which were identified in the secondary scan mode (**Figure 4**), matched the reference MS/MS spectrum (20V, positive) in the HMDB. However, the fragment information of kijanimicin, mycobactin, pimaricin, thiolactomycin, thiolutin and ectoine was not identified in the secondary scan mode. Hence, the tentative production of clavulanic acid and streptomycin is suggested by the MS spectra but needs to be confirmed by isolation and characterization of the pure compound (LC-MS), bioassays and quantification of the compounds.

Referencing with genomic information, the gene cluster of clavulanic acid was composed of core biosynthetic genes, additional biosynthetic genes, transport-related genes, regulatory genes and some other genes. The core biosynthetic genes with the length of 2,520 nt

were responsible for the functions of beta-lactam synthetase and clavaminic synthase. The additional biosynthetic genes, transport-related genes, regulatory genes and some other genes were related to the functions of hypothetical protein, carboxyethylarginine synthetase, proclavaminic amidinohydrolase, glutamate N-acetyltransferase 2, oligopeptide-binding lipoprotein, transcription activator, clavaldehyde dehydrogenase, cytochrome P450-SU2, ferredoxin, beta-lactamase, efflux protein, acetyltransferase, oligopeptide-binding lipoprotein, conserved hypothetical protein, glycy clavaminic synthase, penicillin-binding protein PBP, penicillin-binding protein, cytochrome P450 hydroxylase, RNA polymerase sigma factor SigL, histidine kinase, two-component system response regulator and transcriptional regulator. Besides, the core biosynthetic genes, additional biosynthetic genes, transport-related genes and some other genes were observed in the gene cluster of streptomycin. The core biosynthetic genes with the length of 2,505 nt were associated with the functions of putative dTDP-dihydrostreptose-streptidine-6-phosphate dihydrostreptosyltransferase and putative streptomycin-6-phosphate phosphatase. The functions of rest genes were related to putative NAD(P)-dependent oxidoreductase, putative ABC-type multidrug transport system ATPase and permease component, putative ABC-type multidrug transport system ATPase and permease component, putative L-glutaminy-2-N-methyltransferase, StsF protein, putative ATP:scyllo-inosamine phosphotransferase, putative aminosugar-converting enzyme, putative L-glutamine:scyllo-inosose aminotransferase, putative NADH:N-amidino-scyllo-

TABLE 2 | Theoretical and detected m/z values for potential secondary metabolites from the *M. saopaulense* strain.

Category	Predicted compound	Theoretical MH ⁺ (m/z)	Detected MH ⁺ (m/z)
Antibiotic	Asukamycin	521.22878	
	BE-7585A	848.27672	
	Calcimycin	524.27551	
	Clavulanic acid	200.05590	200.3
	Enduracidin	2388.91205	
	Kijanimicin	1317.67388	1317.5
	Kosinostatin	632.23700	
	Meridamycin	822.53621	
	Mycobactin	592.26132	592.5
	Nosiheptide	1222.15566	
	Pimaricin	666.31202	666.3
	Rifamycin	698.31711	
	Salinomycin	751.49909	
	Streptomycin	582.27350	582.5
Thiolactomycin	211.07873	211.3	
Thiolutin	229.00999	229.0	
Bioactive compound	Cladoniamide	460.17644,426.21541	
	Nocobactin	734.43347	
	Divergolide	550.24409	
Others	Amychelin	739.28933	
	Ectoiole	143.08205	143.2
	Glycopeptidolipid	1155.76313	
	Sioxanthin	727.45738	

**FIGURE 3** | The in-source fragments from clavulanic acid (m/z 200.3) at m/z 110.4, 136.1, 154.2, 156.3, 158.1, 170.1 and 182.3 were identified in the secondary scan mode by MS/MS analysis.

inosamine oxidoreductase, putative L-alanine:N-amidino-3-keto-scylo-inosamine aminotransferase, putative glucose-1-phosphate thymidyltransferase, putative N-amidionstreptamine 6-phosphotransferase, putative scylo-inosamine-4-phosphate amidinotransferase, putative dTDP-4-dehydrorhamnose 3,5-epimerase, putative dTDP-4-dehydrorhamnose reductase, putative dTDP-glucose 4,6-dehydratase, putative glucose-1-phosphate thymidyltransferase, streptomycin biosynthesis operon regulator, streptomycin 6-phosphotransferase, scylo-inosamine-4-phosphate

amidinotransferase, StrF protein, StrG protein, putative NAD:myo-inositol oxidoreductase, putative N-methyl-L-glucosamine biosynthetic aminotransferase and putative oxidoreductase.

As reported, clavulanic acid, which was first found to be produced by *S. clavuligerus*, is an antibiotic with a weak antibacterial effect and has been used on several livestock species (Putecova et al., 2021). It has the important ability of inhibiting β -lactamase, and is therefore an appropriate supplement for use with other β -lactam antibiotics, such as amoxicillin and ticarcillin, and

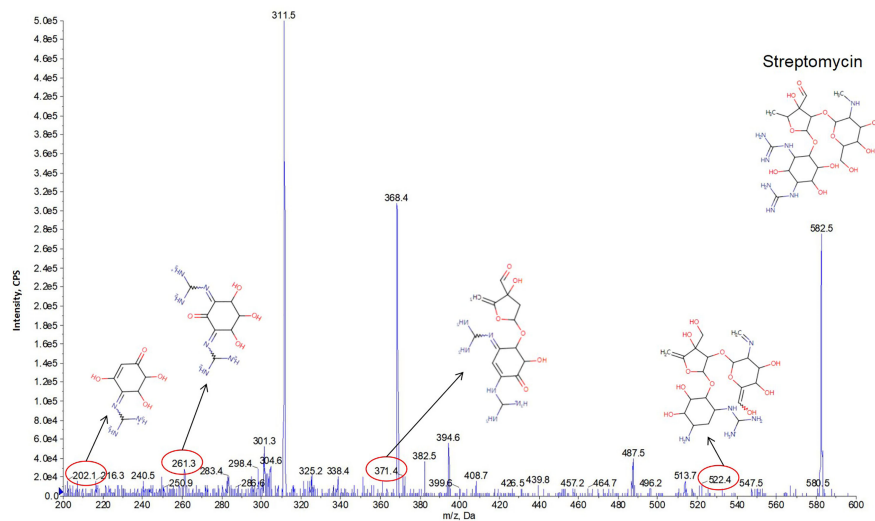


FIGURE 4 | The in-source fragments from streptomycin (m/z 582.5) at m/z 202.1, 261.3, 371.4 and 522.4 were identified in the secondary scan mode by MS/MS analysis.

it can enhance their antibacterial effects (Putecova et al., 2021; Shin et al., 2021). The commercial drugs, AugmentinTM and TimentinTM, which are mixtures of clavulanic acid and β -lactam antibiotics, have been prescribed in many countries (Elander, 2003). Many studies have attempted to identify ways of increasing clavulanic acid production. For example, adding glycerol to bacterial cultures can enhance clavulanic acid production by approximately two-fold (Chen et al., 2003). Furthermore, stimulating the expression of additional copies of the glycerol utilization operon was found to increase the yield of clavulanic acid by approximately 4.5-fold. Further supplementation with glycerol was found to enhance clavulanic acid production by about 7.5-fold (Banos et al., 2009). Hence, the *M. saopaulense* strain is another promising clavulanic acid-producing with the potential to be utilized as an alternative strain to produce clavulanic acid identified herein. Our *M. saopaulense* strain was also found to produce streptomycin, an antibiotic first isolated from *S. griseus* in 1943 and subsequently submitted for clinical trial in 1994. Streptomycin is notable for its effective control of tuberculosis in humans, and with its broad spectrum antimicrobial effect it has been widely used in clinical settings (Waksman, 1953; Pawar et al., 2019). This study is the first to report that a strain of *M. saopaulense* can produce streptomycin. The fact that actinobacteria isolated from mangrove swamps have unknown antibiotic producing abilities, which makes them worthy of immediate examination.

Our results confirm that *M. saopaulense* derived from the mangrove environment has a huge gene pool related to the biosynthesis of secondary metabolites. To our best knowledge, this is the first study predicted the production of clavulanic acid and streptomycin by *M. saopaulense*. Thus, it appears that the mangrove environment hosts Actinobacteria capable of producing bioactive secondary metabolites that can be used in biotechnology applications such as antibiotic production.

CONCLUSIONS

In this study, a strain of *M. saopaulense* sp. isolated from the mangrove environment was successfully investigated using a combination of whole genome sequencing and mass spectrometric analysis. The outstanding biosynthetic secondary metabolite-producing potential of mangrove-derived actinobacteria was underestimated before *M. saopaulense* sp. was first found to produce clavulanic acid and streptomycin antibiotics. Thus, the results of this study further augment research on the biosynthetic properties of actinobacteria from extreme mangrove environments, which are undoubtedly promising sampling sites for obtaining bacteria to produce secondary metabolites for medicinal use.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repository and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

DH and SML designed the study. DH and MKM conducted the experiment and analyzed the data. DH, KL and MKM wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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