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Analysis of Secondary Metabolite Synthesis Potential of *Streptomyces fradiae* sf106 Based on the Whole Genome and Non-Target Metabolomics and Exploration of the Biosynthesis of Tylosin

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Abstract: *Streptomyces fradiae* sf106 is a type of actinomycete that can produce abundant secondary metabolites, making it a natural cell factory for drug synthesis. In order to comprehensively understand the genomic profile of *Streptomyces fradiae* sf106 and its potential for producing secondary metabolites, a combination of several methods was used to perform whole-genome sequencing of sf106. The results showed that sf106 is most closely related to *Streptomyces xinghaiensis* S187; the average nucleotide identity and average amino acid identity of sf106 and S187 were more than 96%. The genome size of sf106 is approximately 7300 kb, the GC content is greater than 72%, and more than 6700 coding sequences (CDS) were identified. Analysis of mobile genetic elements revealed the presence of a large number of horizontally transferred genes in *Streptomyces fradiae* sf106, which contribute to microbial diversity. Through antiSMASH prediction, 22 secondary metabolite gene clusters were obtained, which had great potential to generate polyketide metabolites. By examining the data, it was found that the genes contained in cluster 9 were similar to those involved in tylosin synthesis. Non-targeted metabolome sequencing revealed that a total of 1855 identifiable metabolites were produced in the fermentation broth, and the majority of metabolites showed highly significant differences in mean relative abundance between the groups. The identified metabolites were compared against the KEGG compound database to obtain metabolite classifications, mainly including Biological Roles, Phytochemical Compounds, Lipids, and Pesticides. One-way ANOVA indicated that the relative concentration of tylosin differed significantly across all the growth periods, except for the late-logarithmic and stabilization stages. This study provides important basic information on the secondary metabolite research of sf106, which will help us to understand and apply *Streptomyces fradiae* sf106 more comprehensively.

Keywords: *Streptomyces*; whole genome; non-targeted metabolome; secondary metabolites; tylosin

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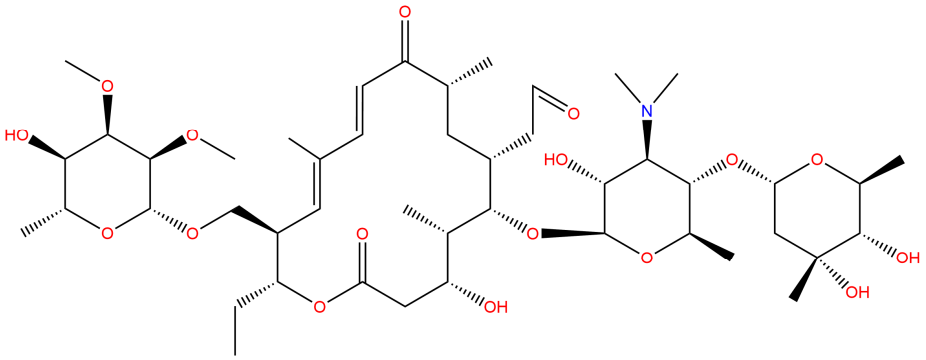
1. Introduction

Streptomyces can produce structurally diverse secondary metabolites and has been extensively studied. More than two-thirds of known antibiotics in nature are discovered and isolated from *Streptomyces* [1–3], such as erythromycin and oxytetracycline, among others [4–7]. The natural products of *Streptomyces* usually exhibit good antibacterial, antiviral, and other biological activities and have been widely used in the fields of animal husbandry, public health, etc., playing an important role in the health of humans and animals. Researchers have developed a large number of antibiotics, antitumor drugs, agricultural and veterinary drugs, etc., using secondary metabolites produced by microorganisms [8]. Although chemically modified derivatives of active natural drugs can improve their physical and chemical properties and biological activities, the discovery of new natural products is still an area worthy of attention.

With the development of sequencing technology and the decrease in costs, more and more microbiome data have been reported. The development of whole-genome sequencing of microorganisms has allowed us to have a global understanding of the basic situation of bacteria, which has played a significant role in the molecular genetic research of *Streptomyces* [9,10]. There is still great potential to discover new natural products from this genus. In the long process of survival and evolution, microorganisms often take in foreign gene fragments with specific functions and integrate them into their own genomes. These foreign genomic fragments, such as virulence genes, drug resistance genes, and metabolic genes, are called mobile genetic elements, and this phenomenon is called horizontal gene transfer [11]. Horizontal gene transfer breaks the boundaries of kinship and increases microbial diversity. This phenomenon is more common in *Streptomyces* and can help them improve their survival rates and occupy advantageous ecological niches. Common mobile genetic elements include genomic islands [12], prophages [13], CRISPR-Cas [14], and insertion sequences [15]. To develop and utilize secondary metabolites to a greater extent, researchers have explored them from multiple perspectives. For example, efforts have included using *Streptomyces* as a host cell to express exogenous gene clusters, taking advantage of its complex and rich metabolic pathways, unique post-transcriptional modification systems, and antibiotic resistance mechanisms [16–18], which give the genus unique advantages in heterologous expression of secondary metabolite gene clusters [19–22]. These genetic manipulations are based on a clear understanding of the target strain's genome and its metabolites. Therefore, it is common to characterize and produce natural products by utilizing genome editing techniques after obtaining genomic information [23–25]. In addition, analyzing the genomic information of organisms provides information on the inducement of the expression of silent secondary metabolite gene clusters in the chromosome, which can lead to the discovery of natural products that are not expressed under conventional culture conditions [26].

Several active secondary metabolites synthesized by microorganisms have been discovered [27–30], and *Streptomyces* is considered to have great economic value and potential applications. Non-targeted metabolomics is the study of the collection of all metabolites in a cell at a certain point in time. Additionally, it is considered to be the closest to the phenotype of the organism, which is widely used because of its ability to efficiently mine metabolites [31–33]. To further explore the natural product synthesis ability of *Streptomyces fradiae* sf106, a non-targeted metabolomics analysis was conducted on the fermentation broth of *Streptomyces fradiae* sf106 at different time periods. We can preliminarily clarify the potential of metabolite biosynthesis of the strain, as well as the relative expression of metabolites at different periods. Tylosin, also known as Tylan (Figure 1), is a typical 16-membered macrolide antibiotic for veterinary use. Various microorganisms have been found to produce tylosin, but *Streptomyces fradiae* is considered one of the most promising strains for tylosin biosynthesis. Tylosin plays a significant role in combating Gram-positive bacterial infections and promoting animal growth, shortening the breeding cycle of livestock and poultry, improving feed utilization efficiency, and increasing economic benefits. Therefore, it is widely used in the livestock and poultry farming industry. Due to its economic value and application potential, extensive scientific research on tylosin biosynthesis has been conducted since the 1970s [34–37]. The existing research is mainly based on gene editing, mutagenesis, and other technologies to increase the yield of tylosin [38–42]. In the early stages of our laboratory, a strain of *Streptomyces fradiae* named sf106 was preserved (BioProject ID: PRJNA1017371). What is the potential of this strain to produce secondary metabolites? In order to further understand its genomic profile and explore its secondary metabolism potential, we sequenced the whole genome and metabolome of *Streptomyces fradiae* sf106. Additionally, we further analyzed its basic biological information and gained a preliminary understanding of its potential secondary metabolism capabilities. This will be beneficial for further exploring the application potential of this strain at a deeper level, enriching the basic research data

of this strain and providing theoretical support for industrial genetic manipulation of *Streptomyces fradiae* sf106.



tylosin

Figure 1. Tylosin structure.

2. Materials and Methods

2.1. Strain and Culture Conditions

Streptomyces fradiae sf106 was used for this study. Single colonies taken from sf106 on a solid medium of wl-50 were transferred to a TSB liquid medium. The wl-50 medium had the following composition: yeast extract, 1 g/L (Oxoid Ltd., Basingstoke, Britain); soluble starch, 5 g/L (Tianjin Bohuatong Chemical Products Sales Center, Tianjin, China); sodium chloride, 2.5 g/L (Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China); anhydrous magnesium sulfate, 3.6 g/L (Damao Chemical Reagent Factory, Tianjin, China); magnesium chloride hexahydrate, 4.8 g/L (Shanghai Guangnuo Chemical Technology Co., Ltd., Shanghai, China); L-alanine, 0.2 g/L; L-arginine, 0.2 g/L; L-asparagine, 0.5 g/L (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China); and agar, 18 g/L. The medium was sterilized under high pressure at 115 °C for 30 min. *Streptomyces fradiae* sf106 cultures were prepared in 250 mL conical flasks containing 50 mL of the culture medium. The medium was prepared using TSB (tryptic soy broth) with the following composition: yeast extract, 5 g/L (Oxoid Ltd., Basingstoke, Britain); tryptone, 15 g/L (Oxoid Ltd.); soya peptone, 5 g/L (Beijing Aoboxing Bio-Tech Co. Ltd., Beijing, China); and sodium chloride, 5 g/L (Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China). After preparation, the medium was sterilized under high pressure at 121 °C for 30 min and used for the cultivation of sf106. The pictures of sf106 incubation on the solid medium (wl-50) and in the liquid medium (TSB) are attached in the Supplementary File (Figure S1).

2.2. Genomic DNA Extraction

Streptomyces fradiae sf106 was streaked on a TSB liquid medium and cultured at 28 °C for approximately 96 h at 150 rpm. The cell biomass was harvested after 10 min centrifugation at 12,000 *g* (approximately 11,000 rpm). The genomic DNA of *Streptomyces fradiae* sf106 was extracted using a bacterial DNA extraction kit (Wizard Genomic DNA Purification Kit, Madison, WI, USA) according to the manufacturer's protocol. Purified genomic DNA was quantified by a TBS-380 fluorometer (Turner BioSystems Inc., Sunnyvale, CA, USA). High-quality DNA (OD260/280 = 1.8~2.0, >1 μg) was used to conduct further research (Table S1).

2.3. Library Construction and Genome Sequencing

The genomic DNA was sequenced using a combination of the PacBio RS II Single Molecule Real-Time (SMRT) and Illumina sequencing platforms. The Illumina data were used to evaluate the complexity of the genome.

For Illumina sequencing, at least 1 μg genomic DNA was used for each strain in the sequencing library construction. DNA samples were sheared into 400–500 bp fragments using a Covaris M220 Focused Acoustic Shearer, and the resulting sequencing libraries were purified according to the manufacturer's protocol. Illumina sequencing libraries were prepared from the sheared fragments using the NEXTflex™ Rapid DNA-Seq Kit (Bioo Scientific, Austin, TX, USA). Briefly, the 5′ prime ends were first end-repaired and phosphorylated. Next, the 3′ ends were A-tailed and ligated to sequencing adapters. The third step was to enrich the adapter-ligated products using PCR. The prepared libraries then were used for paired-end Illumina sequencing (2 × 150 bp) on an Illumina HiSeq X Ten machine.

For Pacific Biosciences sequencing, an aliquot of 15 μg DNA was spun in a Covaris g-TUBE (Covaris, Woburn, MA, USA) at 6000 RPM for 60 s using an Eppendorf 5424 centrifuge (Eppendorf, Hamburg, Germany). The DNA fragments were then purified, end-repaired, and ligated with SMRT bell sequencing adapters following the manufacturer's recommendations (Pacific Biosciences, Menlo Park, CA, USA). The resulting sequencing libraries were purified three times using 0.45 × volumes of Agencourt AMPure XP beads (Beckman Coulter Genomics, MA, USA) following the manufacturer's recommendations. Next, a ~10 kb insert library was prepared and sequenced on one SMRT cell using standard methods.

2.4. Genome Assembly and Annotation

The data generated with the PacBio and Illumina platforms were used for bioinformatics analysis. All the analyses were performed using the free online platform Majorbio Cloud Platform (http://cloud.majorbio.com (accessed on 10 January 2021)) from Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). Glimmer [43] was used for CDS prediction, tRNA-scan-SE [44] was used for tRNA prediction, and Barrnap was used for rRNA prediction. The predicted CDSs were annotated according to the NR, Swiss-Prot, Pfam, GO, COG, and KEGG databases using sequence alignment tools, such as BLAST, Diamond, and HMMER. Briefly, each set of query proteins was aligned with the databases, and annotations of best-matched subjects (e-value < 10^{-5}) were obtained for gene annotation.

2.5. Metabolite Extraction

A total of 200 μL of the liquid sample was extracted using an 800 μL methanol: acetonitrile (1:1, v/v) solution. The mixture was then sonicated at 40 kHz for 30 min at 5 °C. The samples were placed at −20 °C for 30 min to precipitate proteins. After centrifugation at 13,000 g (approximately 11,063 rpm) at 4 °C for 15 min, the supernatant was carefully transferred to new microtubes and evaporated to dryness under a gentle stream of nitrogen. For UHPLC–MS/MS analysis, the samples were reconstituted in 120 μL of a loading solution of acetonitrile: water (1:1, v/v) via brief sonication in a 5 °C water bath. The extracted metabolites were spun for 15 min at 13,000 g (approximately 11,000 rpm) at 4 °C on a bench-top centrifuge, and the cleared supernatant was transferred to sample vials for LC–MS/MS analysis. As a part of the system conditioning and quality control process, a pooled quality control (QC) sample was prepared by mixing equal volumes of all samples.

2.6. (UHPLC–MS/MS) Analysis

The instrument platform for the LC–MS analysis was the UHPLC-Q Exactive HF-X system from Thermo Fisher Scientific (Waltham, MA, USA).

Chromatographic conditions: The chromatographic column was an ACQUITY UPLC HSS T3 (100 mm × 2.1 mm i.d., 1.8 μm, Waters, Milford, MA, USA). The mobile phases consisted of 0.1% formic acid in water: acetonitrile (95:5, v/v) (solvent A) and 0.1% formic acid in acetonitrile: isopropanol: water (47.5:47.5:5, v/v) (solvent B). The sample injection volume was 3 μL, and the flow rate was set to 0.4 mL/min. The column temperature was maintained at 40 °C.

MS conditions: The mass spectrometric data were collected using a Thermo UHPLC-Q Exactive Mass Spectrometer equipped with an electrospray ionization (ESI) source operating in either positive or negative ion mode. The optimal conditions were set as follows: heater temperature, 425 °C; capillary temperature, 325 °C; sheath gas flow rate, 50 arb; aux gas flow rate, 13 arb; ion-spray voltage floating (ISVF), −3500 V in negative mode and 3500 V in positive mode; normalized collision energy, 20–40–60 V. The full MS resolution was 60,000, and the MS/MS resolution was 7500. Data acquisition was performed in Data Dependent Acquisition (DDA) mode. Detection was carried out in a mass range of 70–1050 m/z.

2.7. Data Preprocessing and Annotation

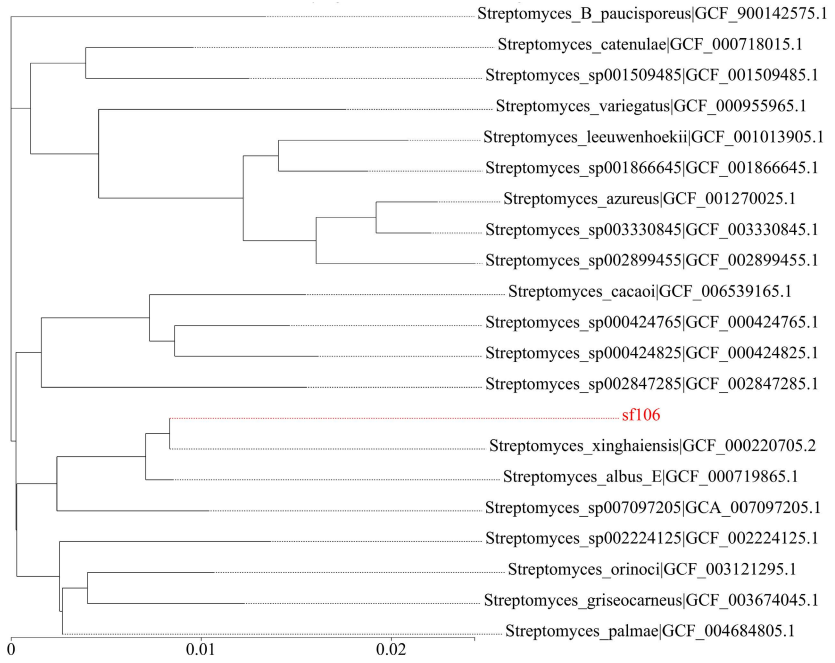
After the mass spectrometry detection was completed, the raw data of LC/MS were preprocessed by Progenesis QI 2.4 (Waters Corporation, Milford, MA, USA) software. At the same time, the metabolites were searched and identified, and the main databases were the Human Metabolome Database (HMDB, http://www.hmdb.ca/ (accessed on 25 August 2022)) [45], Metlin (https://metlin.scripps.edu/ (accessed on 25 August 2022)) [46], and the Majorbio Database. After the database search, the data were uploaded to the Majorbio cloud platform (https://cloud.majorbio.com (accessed on 25 August 2022)) for further analysis. At least 80% of the metabolic features detected in any set of samples were retained. After filtering, minimum metabolite values were imputed for specific samples in which the metabolite levels fell below the lower limit of quantitation, and each feature was normalized by sum. In order to reduce errors caused by sample preparation and instrument instability, the response intensity of the sample mass spectrum peaks was normalized by the sum normalization method, and a normalized data matrix was obtained. At the same time, variables with a relative standard deviation (RSD) greater than 30% in the QC samples were removed, and $\log_{10}$ transformation was performed in order to obtain the final data matrix for subsequent analysis.

3. Results

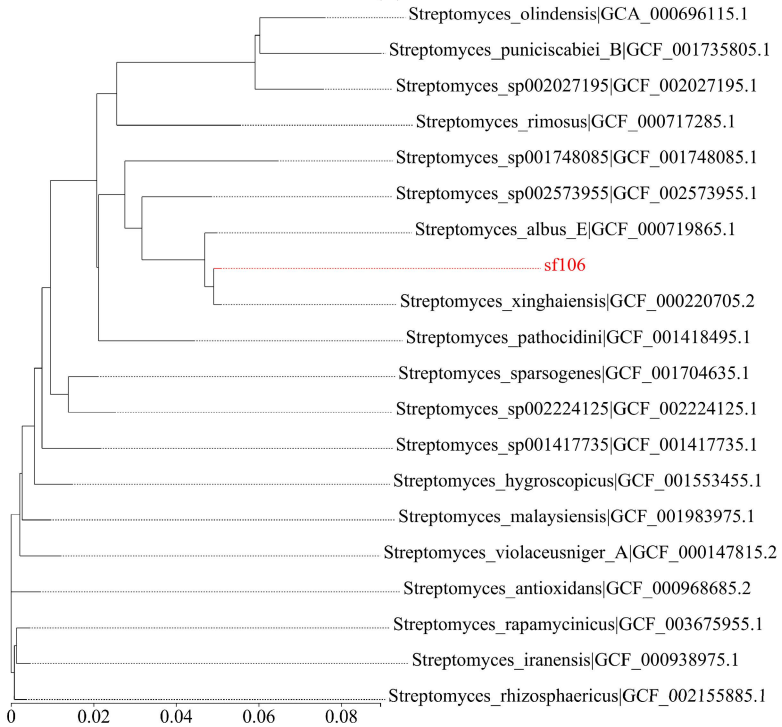
3.1. Systematic Evolution Analysis of Streptomyces fradiae sf106

Following the whole-genome sequencing of *Streptomyces fradiae* sf106, the 19 bacterial strains that were most closely related at the genus level were selected on the basis of their 16S rRNA sequences. The neighbor-joining (NJ) method was employed to construct a phylogenetic tree utilizing MEGA 6.0 software (Figure 2a). The analysis revealed that *Streptomyces fradiae* sf106 exhibited the closest phylogenetic relationship with *Streptomyces xinghaiensis*. Additionally, a species-level phylogenetic tree was constructed on the basis of the housekeeping gene sequence of *Streptomyces fradiae* sf106 (Figure 2b), further corroborating its closest relationship with *Streptomyces xinghaiensis*. Previous studies have identified *Streptomyces fradiae* sf106 to be the same strain as *Streptomyces xinghaiensis* [47,48].

Average nucleotide identity (ANI) is a method used to measure the similarity between genomic sequences by comparing the average percentage identity of nucleotide sequences. Average amino acid identity (AAI) is a method used to measure the similarity between protein-coding sequences by comparing the average percentage identity of amino acid sequences. It is similar to ANI but focuses on the functional similarity of proteins rather than the overall genomic similarity. If two genomes have AAI and ANI values above 95%, they are considered to belong to the same species. ANI/AAI analysis is mainly used to assess the phylogenetic relationship between species at the whole-genome level. These analyses are simple, quick, and accurate. In this study, the closely related *Streptomyces xinghaiensis* S187 (GCA_000220705.2) and sf106 were selected for ANI/AAI analysis. The results showed that the average nucleotide identity (ANI) between *Streptomyces fradiae* sf106 and *Streptomyces xinghaiensis* S187 was 96.13% (Figure 3a), and the average amino acid identity (AAI) was 96.34% (Figure 3b).



(**a**)



(**b**)

Figure 2. (**a**) Phylogenetic tree based on 16S rRNA. (**b**) Phylogenetic tree based on housekeeping genes. Through a comparison with the local database, based on 31 housekeeping genes (*dnaG*, *frr*, *infC*, *nusA*, *pgk*, *pyrG*, *rplA*, *rplB*, *rplC*, *rplD*, *rplE*, *rplF*, *rplK*, *rplL*, *rplM*, *rplN*, *rplP*, *rplS*, *rplT*, *rpmA*, *rpoB*, *rpsB*, *rpsC*, *rpsE*, *rpsI*, *rpsJ*, *rpsK*, *rpsM*, *rpsS*, *smpB*, *tsf*), the closest 19 strains at the species level were selected, and the NJ (neighbor-joining) method was used to construct the phylogenetic tree with MEGA 6.0 software.

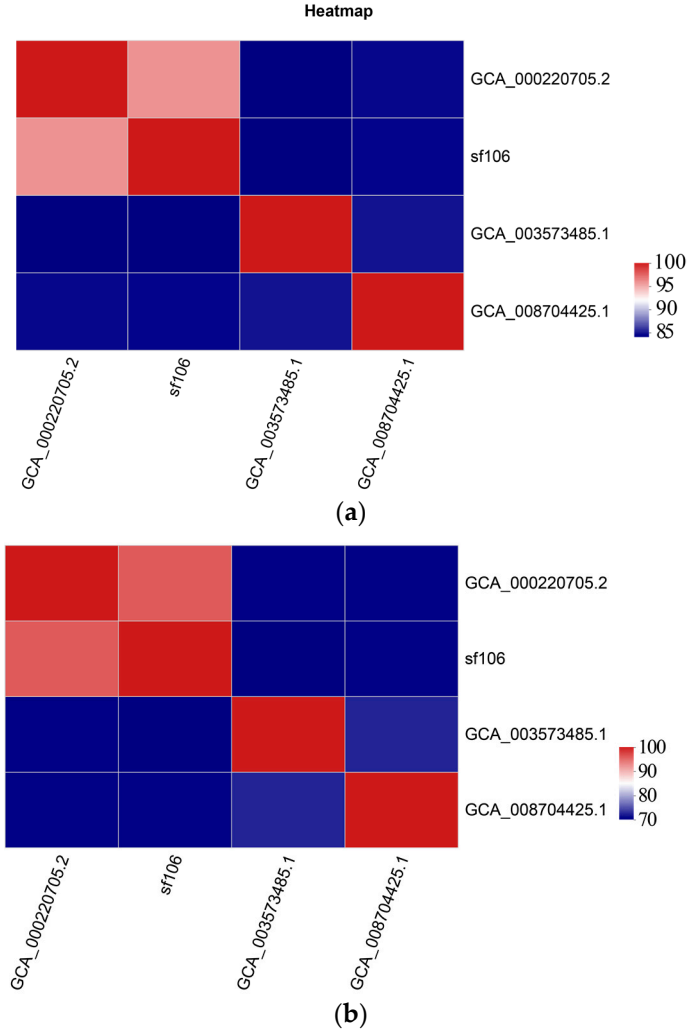


Figure 3. (**a**) Heat map of evolutionary analysis based on ANI analysis. The figure is a strain ANI matrix heat map. The lower and right sides are sample names, and different colored squares indicate nucleotide similarity between samples. (**b**) Heat map of evolutionary analysis based on AAI analysis. The figure is a strain AAI matrix heat map. The lower and right sides are sample names, and different colored squares indicate amino acid similarity between samples.

3.2. The Genomic Overview and Annotation of Streptomyces fradiae sf106

A genomic circle map can comprehensively display the characteristics of a genome, and integrating various information into a single genome circle map can provide us with a more comprehensive and intuitive understanding of the characteristics of a strain's genome. We obtained a sample genome circle map using Circos software (http://circos.ca/software/) [49].

The genomic features of *Streptomyces fradiae* sf106, including the genome size, coding DNA sequences (CDSs, total number of genes), rRNA, tRNA, GC content (guanine and cytosine content of the entire genome), and GC skew values (Figure 4), are comprehensively and visually presented using the Circos genome circle plot. The genome size of *Streptomyces fradiae* sf106 is approximately 7300 kb, with a high GC content of approximately 73% and approximately 6750 coding genes. The annotation results for these genes across various databases were summarized at the gene level to enable multi-dimensional data mining (Table 1).

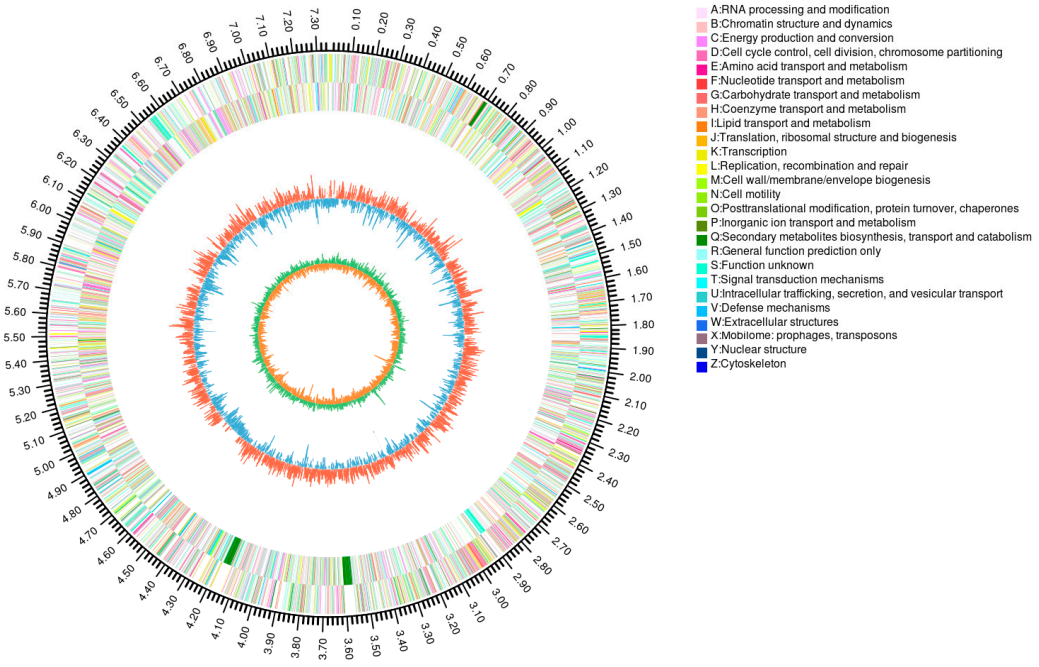


Figure 4. Circos genome map of *Streptomyces fradiae* sf106. The outermost circle of the Circos diagram represents the size of the genome. The second and third circles represent the coding sequences (CDS) on the positive and negative strands, with different colors indicating different functional classifications based on COG. The fourth circle represents rRNA and tRNA. The fifth circle represents the GC content, with the outward red region indicating higher GC content compared with the average GC content of the entire genome. The higher the peak, the greater the difference in GC content from the average value. The inward blue region indicates lower GC content compared with the average GC content of the entire genome. The higher the peak, the greater the difference in GC content from the average value. The innermost circle represents the GC skew value, which is calculated as $(G - C)/(G + C)$. It can help determine the leading and lagging strands, with a positive GC skew indicating the leading strand and a negative GC skew indicating the lagging strand. It can also assist in identifying the replication origin (minimum cumulative offset) and terminus (maximum cumulative offset).

Table 1. Summary of genomic overview and gene annotation.

Sample	Genome Size (kb)	GC Content (%)	CDS No.	tRNA No	rRNA No.	KEGG	GO	COG	NR	Pfam	Swiss-Prot
Streptomyces fradiae sf106	7351	73	6750	60	20	2300	3500	4950	6100	4860	3920

Note: (1) Sample Name: name of the sample; (2) genome size: size of the genome; (3) GC content (%): GC content of the chromosome or plasmid; (4) CDS No.: number of predicted coding genes; (5) tRNA: number of predicted tRNAs; (6) rRNA: number of predicted rRNAs; (7–12) number of genes annotated with information obtained from different databases and reference genomes in the sample.

3.3. Analysis of Mobile Genetic Elements

Horizontal gene transfer can allow for the exchange of genetic material between different biological entities or within individual cellular organelles. Common mobile genetic elements include genomic islands, CRISPR/Cas systems, and insertion sequences, among others. Strain sf106 has a total of 12 genomic islands, 5 insertion sequences, 13 CRISPR/Cas systems, and 1 prophage. The analysis of mobile genetic elements indicated a high degree of horizontal gene transfer in this strain (Table 2).

Table 2. Predicted table of mobile genetic elements.

Sample Name	GI No.	Is No.	Prophage No.	CRISPR-Cas No.
Streptomyces fradiae sf106	12	5	1	13

3.4. Prediction of Antibiotic Resistance Genes

The Comprehensive Antibiotic Resistance Database (CARD) hosts an extensive range of reference genes associated with antibiotics across various organisms, genomes, and plasmids. This database serves as a valuable resource for investigating antibiotic resistance mechanisms in environmental, human, and animal microbial communities. The annotation analysis using CARD suggested that *Streptomyces fradiae* sf106 harbors multiple classes of resistance genes (Figure 5a). Specifically, the strain contains 88 macrolide, 69 tetracycline, and 50 penam resistance genes, along with additional resistance genes against other antibiotics, including 36 against fluoroquinolone, 27 against aminocoumarin, 26 against aminoglycoside, 26 against cephalosporin, and other types of resistance genes.

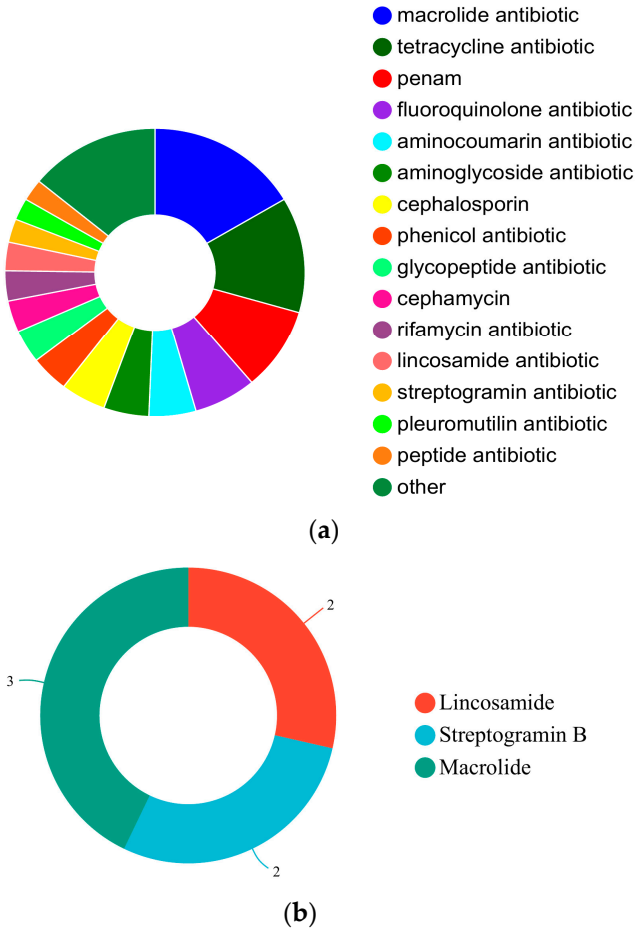


Figure 5. (**a**) Categorized statistics of predicted resistance genes. Different colors represent different drug classes, and their areas indicate the relative proportion of genes in that class. (**b**) Predictive statistical analysis of drug resistance genes based on ResFinder. Different colors represent different drug classes, and their areas indicate the relative proportion of genes in that class. The numbers in the figure represent the number of genes.

To corroborate these findings, the ResFinder database, comprising experimentally validated antibiotic resistance genes, was employed. Utilizing ResFinder with a threshold of identity $\geq$ 80% and a coverage $\geq$ 60%, three major classes of resistance genes were identified: lincosamide (2), streptogramin B (2), and macrolide (3). The elucidation of these

resistance profiles is crucial for understanding the mechanisms underlying drug-resistant mutations and the development of new antibiotics.

3.5. Metabolic System Analysis

3.5.1. Carbohydrate-Active Enzyme Annotation

The Carbohydrate-Active Enzymes Database (CAZy, http://www.cazy.org/ (accessed on10 January 2021)) is a specialized database for enzymes involved in the synthesis or degradation of complex carbohydrates and glycoconjugates. These enzymes are categorized into six major protein families according to amino acid sequence similarities in their domains: glycoside hydrolases (GHs), glycosyl transferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), carbohydrate-binding modules (CBMs), and auxiliary activities (AAs). Figure 6 reveals that the strain sf106 primarily contains genes related to the glycoside hydrolases category, followed by glycosyl transferases.

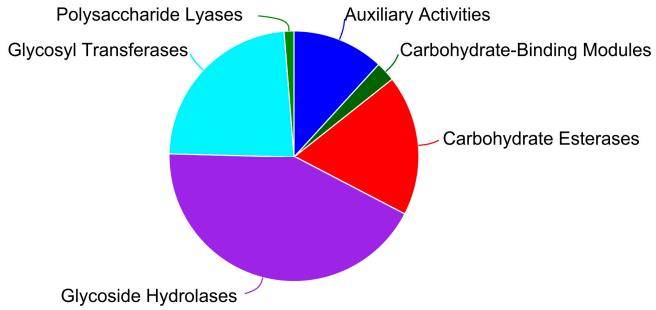


Figure 6. Statistics of carbohydrate-active enzyme annotation. The different colors in the pie chart represent different categories, and the area represents the relative proportion of genes in that category.

3.5.2. Secondary Metabolite Analysis

Microorganisms synthesize secondary metabolites, compounds not essential for their basic metabolic processes, when they reach a certain growth stage. These metabolites are often controlled by gene clusters called secondary metabolite biosynthetic gene clusters. The complete genome of sf106 was analyzed using the antiSMASH database (https://antismash.secondarymetabolites.org/#!/start (accessed on 6 October 2021)) with relaxed detection. This analysis predicted a total of 22 secondary metabolite biosynthetic gene clusters (Table 3), encompassing a diverse range of compounds, such as terpenes, NRPS (non-ribosomal peptide synthetase), and PKS (polyketide synthase), among others. NRPS and PKS are two crucial biosynthetic enzymes used for the synthesis of complex natural products. They play a key role in drug development and biotechnology.

We used the MIBiG (Minimum Information about a Biosynthetic Gene Cluster) database (https://mibig.secondarymetabolites.org/ (accessed on 6 October 2021)) to compare each of the predicted clusters against known biosynthetic gene clusters. The comparison analysis was performed using the protocluster-to-region format, resulting in the identification of the 22 known compounds most similar to the predicted gene clusters (Table 3). This provided a basis for the development of similar metabolites in *Streptomyces fradiae* sf106, and it is worth noting that actinobacteria are still the major source of these metabolites. It was found that the genes in the ninth gene cluster (Figure 7) were highly similar to the synthesis of the antibiotic tylosin, and they were highly similar to the known compound tylactone (Table 4), which is closest to the predicted gene cluster. This comparison identified the closest known compounds to the predicted clusters, providing insight into the metabolic potential of sf106. Importantly, one cluster was found to be highly similar to genes involved in the synthesis of the antibiotic tylosin. This suggests that sf106 has the capacity for tylosin synthesis. This has great value for the excavation and application of tylosin in *Streptomyces fradiae* sf106.

Table 3. Prediction of secondary metabolites in *Streptomyces fradiae* sf106.

Region	Type	From	To	Most Similar Known Cluster		Similarity
Region 1	redox-cofactor	11,912	34,657	Alanylclavam/2-hydroxymethylclavam/2-fomyloxymethyclavam/clavam-2-carboxylate	Other: Non-NRP beta- lactam	12%
Region 2	T3PKS, terpene	367,053	406,828	endophenazine A/endophenazine B	Other: Phenazine	38%
Region 3	NRPS	670,555	724,534	scabichelin	NRP	20%
Region 4	NRPS-like, PKS-like, betalactone, T2PKS, RRE- containing	1,232,101	1,317,375	LL-D49194α1 (LLD)	Polyketide	39%
Region 5	lanthipeptide-class-iii	1,980,221	2,002,953	SapB	RiPP: Lanthipeptide	100%
Region 6	siderophore	2,354,686	2,365,381			
Region 7	ectoine	2,484,580	2,494,984	ectoine	Other	100%
Region 8	T3PKS	3,482,933	3,524,030	flaviolin	Other	50%
Region 9	T1PKS	3,545,272	3,626,176	carrimycin	Polyketide	55%
Region 10	NRPS-like, lassopeptide	3,657,076	3,699,170	citrulassin B	RiPP	100%
Region 11	NRPS	4,128,379	4,201,561	isocomplestatin	NRP	93%
Region 12	terpene	4,360,731	4,379,029			
Region 13	RiPP-like, NRPS-like	4,422,841	4,466,244	nunapeptin/nunamycin	NRP	14%
Region 14	lanthipeptide-class-i	4,599,449	4,622,053			
Region 15	terpene	4,728,801	4,752,252		Terpene	53%
Region 16	terpene	4,832,715	4,849,274			
Region 17	RRE-containing, LAP, thiopeptide	4,936,167	4,971,075			
Region 18	siderophore	5,095,314	5,106,980	desferrioxamine	Other	83%
Region 19	RiPP-like	5,186,566	5,198,233			
Region 20	siderophore	5,473,885	5,488,679	ficellomycin	NRP	3%
Region 21	lanthipeptide- class-i	5,836,957	5,862,133	tetrocarcin A	Polyketide	4%
Region 22	terpene	7,023,507	7,049,988	isorenieratene	Terpene	87%

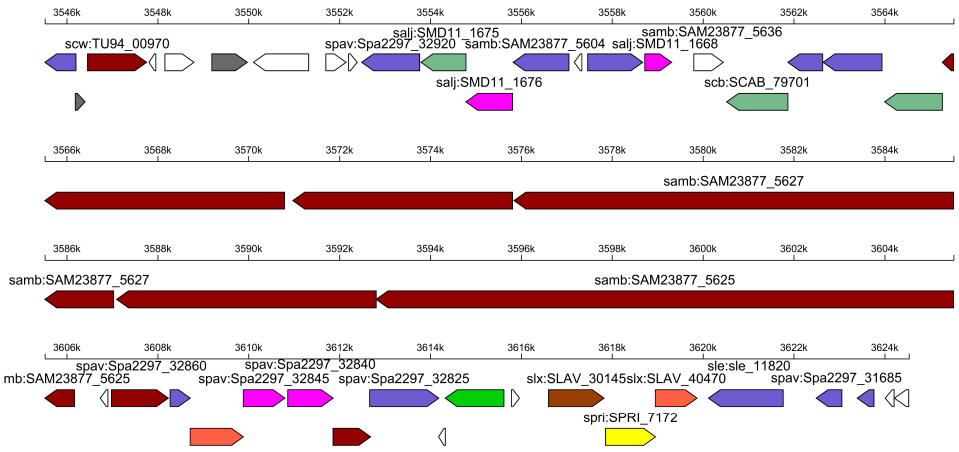


Figure 7. Linear map of secondary metabolite biosynthetic gene cluster (cluster 9). The map shows all genes in the predicted gene cluster, and the colors of different annotated genes are displayed according to the color of the gene in the COG classification. The functions represented by different colors are detailed in the COG analysis interface, and the gray represents the gene not annotated in COG.

Table 4. Comparison of similar gene clusters from MIBiG comparison.

	Reference	Similarity Score	Type	Compound(s)	Organism
Cluster1	BGC0001156.1	0.15	Terpene	platencin	*Streptomyces platensis*
Cluster2	BGC0000674.1	0.71	Terpene	(-)-δ-cadinene	*Streptomyces clavuligerus* ATCC 27064
Cluster3	BGC0000426.1	0.42	NRP	sevadicin	*Paenibacillus larvae*

Table 4. *Cont.*

	Reference	Similarity Score	Type	Compound(s)	Organism
Cluster4	BGC0000248.1	1.65	Polyketide	naphtocyclinone	*Streptomyces arenae*
Cluster5	BGC0000551.1	0.47	RiPP	SapB	*Streptomyces coelicolor* A3(2)
Cluster6	BGC0001572.1	0.34	Other	desferrioxamine E	*Pantoea agglomerans*
Cluster7	BGC0000853.1	0.64	Other	ectoine	*Streptomyces anulatus*
Cluster8	BGC0001310.1	0.46	Terpene	naringenin	*Streptomyces clavuligerus* ATCC 27064
Cluster9	BGC0000166.1	0.78	Polyketide	tylactone	*Streptomyces fradiae*
Cluster10	BGC0001552.1	1.16	RiPP	citrulassin F	*Streptomyces avermitilis* MA-4680 = NBRC 14893
Cluster11	BGC0000326.1	0.41	NRP	isocomplestatin	*Streptomyces lavendulae*
Cluster12	BGC0001910.1	0.28	Terpene	cyslabdan	*Streptomyces cyslabdanicus*
Cluster13	BGC0000500.1	0.41	RiPP	carnolysin A1, carnolysin A2	*Carnobacterium maltaromaticum*
Cluster14	BGC0000544.1	0.19	RiPP	planosporicin	*Planomonospora alba*
Cluster15	BGC0000648.1	0.34	Terpene	carotenoid	*Myxococcus xanthus*
Cluster16	BGC0001910.1	0.34	Terpene	cyslabdan	*Streptomyces cyslabdanicus*
Cluster17	BGC0001146.1	1.02	RiPP	cyclothiazomycin C	*Streptomyces* sp. NRRL WC-3908
Cluster18	BGC0001478.1	0.58	Other	desferrioxamine E	*Streptomyces* sp. ID38640
Cluster19	No matches found				
Cluster 20	BGC0001531.1	0.26	Other	bisucaberin B	*Tenacibaculum mesophilum*
Cluster21	BGC0001727.1	0.23	RiPP	paenilan	*Paenibacillus polymyxa* E681
Cluster22	BGC0000664.1	0.42	Terpene	isorenieratene	*Streptomyces griseus* subsp. griseus NBRC 13350

3.6. Preprocessing of Metabolite Expression Data

The preprocessing parameters for metabolomic data include several key steps: missing value filtering of raw data (applied when the proportion of missing values in each group exceeds 20%), imputation of missing values using the minimum value method, recoding of missing values through simulation, data conversion (log10 transformation was performed to obtain the final data matrix), and data normalization for subsequent analysis, among other steps. Data preprocessing is a commonly used method in metabolomics analysis.

Preprocessing identified a total of 1855 metabolites (Table 5). Of these, 970 metabolites were identified in positive ion mode, and 885 metabolites were identified in negative ion mode. Subsequent annotation was carried out using public databases, including the Human Metabolome Database (HMDB) and Lipidmaps (https://www.lipidmaps.org/ (accessed on 25 August 2022)).

Table 5. Total ion count and identification statistics.

Ion Mode	All Peaks	Identified Metabolites	Metabolites in Library	Metabolites in KEGG
pos	6480	970	697	229
neg	8049	885	790	238

Note: (1) Ion mode: the ion mode of the substance detected by the mass spectrometer, mainly: pos (positive ion mode) and neg (negative ion mode); (2) all peaks: the number of peaks extracted by the software; (3) identified metabolites: the number of metabolites finally identified by the first and second levels of mass spectrometry data, searching libraries (self-built library, Metlin, HMDB) through the primary and secondary mass spectrometry data; (4) metabolites in library: the number of metabolites annotated to public databases, such as HMDB and Lipidmaps; (5) metabolites in the KEGG: the number of metabolites annotated to the KEGG database.

3.7. Metabolites KEGG Compounds Classification

The classification of metabolites in strain sf106 based on the KEGG compound database yielded the following four main categories, as shown in Figure 8. The first category was Compounds with Biological Roles (111); this category is primarily composed of Amino Acids, Phospholipids, Neurotransmitters, and Carboxylic Acids, among others. The second category was Lipids (82); the dominant types of lipids are FA01 Fatty Acids and Conjugates,

PR01 Isoprenoids, GP03 Glycerophosphoserines, and ST02 Steroids, among others. The third category was Phytochemical Compounds (65); this category is primarily composed of triterpenoids (C30), diterpenoids (C20), monolignols, and flavonoids, among others. The fourth category was Pesticides (4), i.e., the dominant types of plant growth regulators and insecticides, among others.

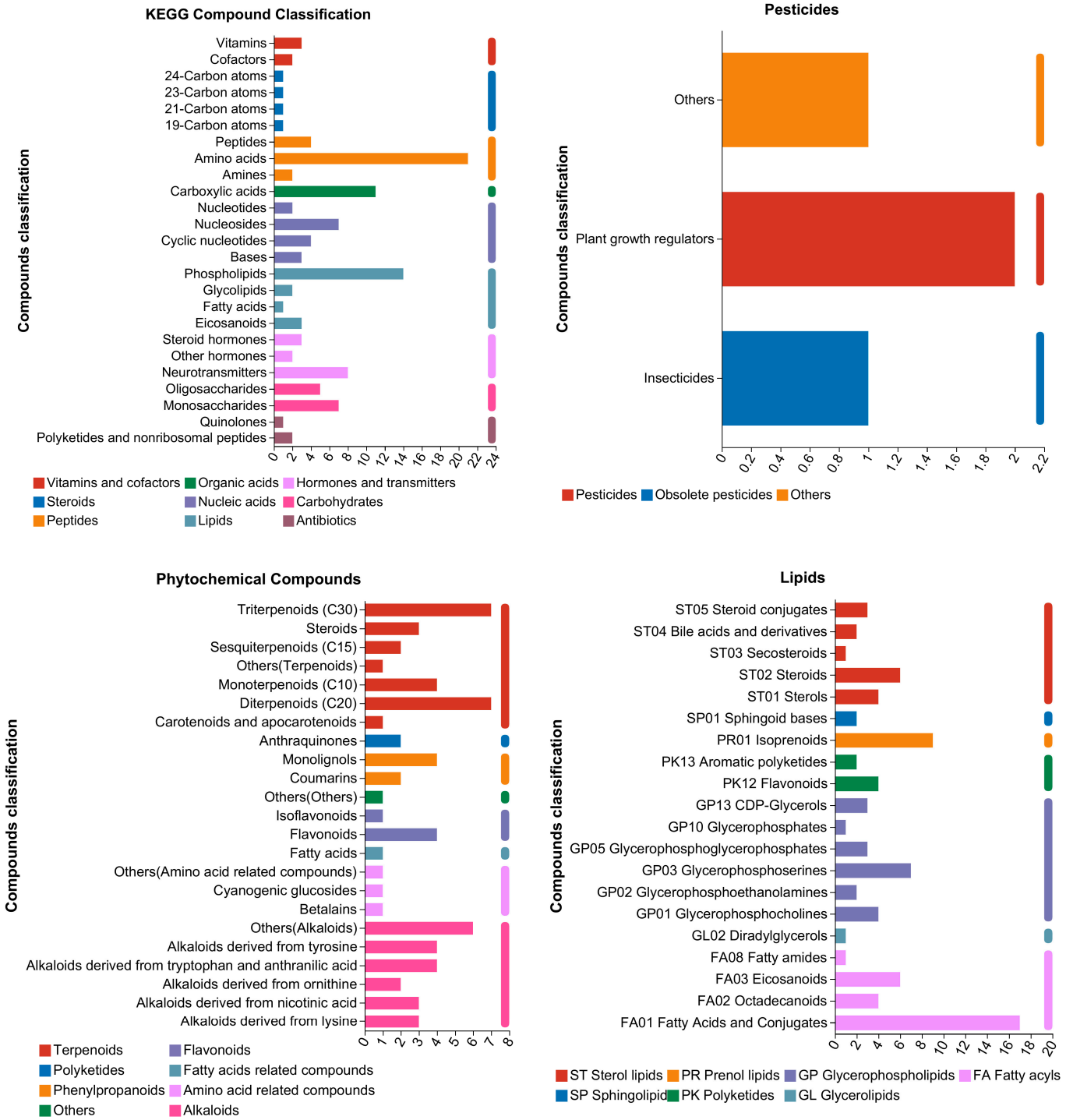


Figure 8. KEGG compound classification. The vertical axis represents the classification of KEGG compounds, and the horizontal axis represents the number of compounds annotated to that type. The color of the bar indicates that it belongs to the first-level classification category of compounds.

3.8. Differential Metabolite Analysis

For the 1855 identified metabolites, a multi-group ANOVA was conducted using the Kruskal–Wallis rank sum test (Kruskal–Wallis H test). The analysis focused on the top 20 metabolites in terms of abundance that could be matched to known names (Figure 9). The results showed highly significant differences in mean relative abundance between the groups (A, B, C, and D) studied for the majority of these top metabolites.

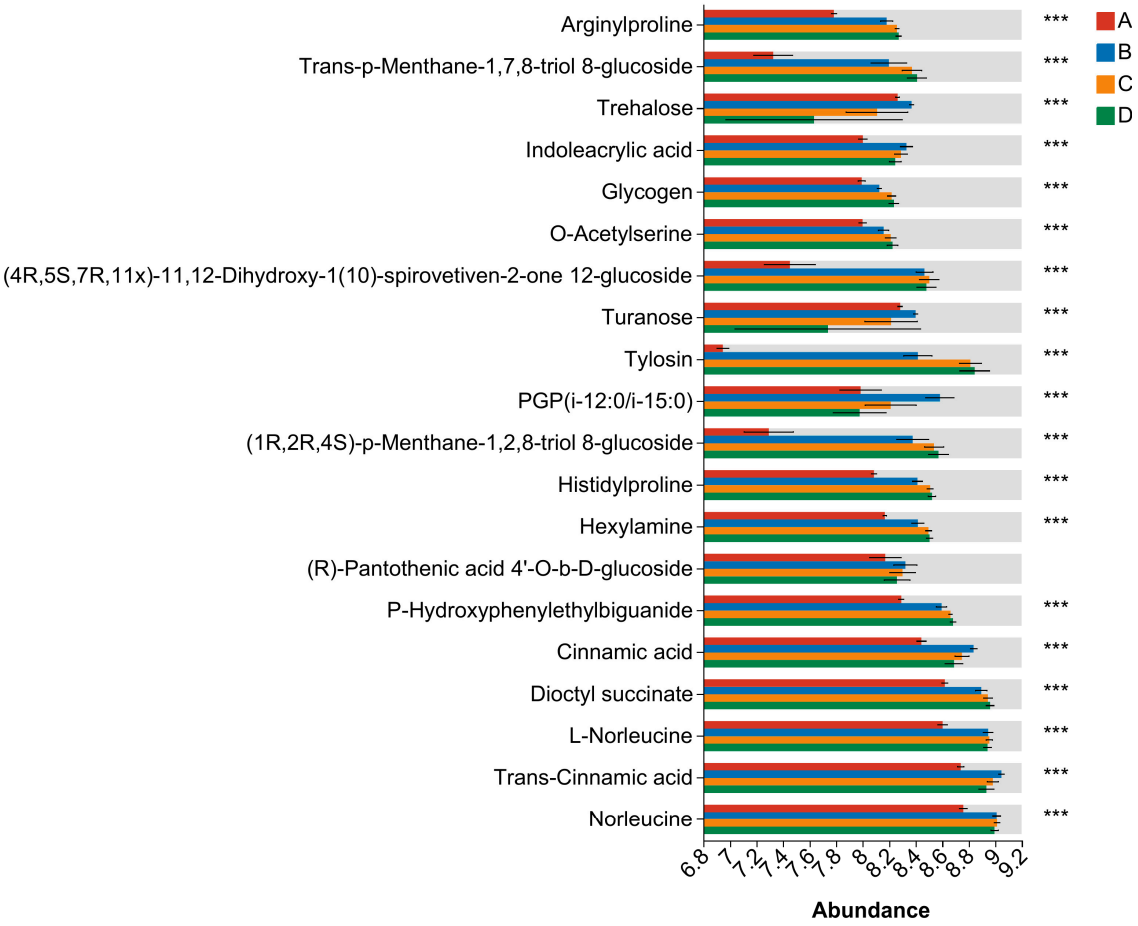


Figure 9. Top 20 metabolites in abundance and matched to names. Y-axis indicates metabolite name, *X*-axis indicates the average relative abundance of metabolites in different subgroups, and different colored columns indicate different subgroups; *p*-values are on the far right: ***, $p \leq 0.001$. A is the logarithmic pre-growth phase, B is the logarithmic mid-growth phase, C is the logarithmic late-growth phase, and D is the stationary phase.

Further analysis centered on the relative mean abundance of tylosin across the different growth phases (Figure 10). The highest mean abundance of tylosin was observed during the stationary phase. Interestingly, the average relative abundance of tylosin in the late logarithmic growth phase was not significantly different from that in the stationary phase. This suggests that the strain may reach the maximum tylosin biosynthesis toward the end of the logarithmic growth phase and continue to accumulate it during the stationary phase. These insights are valuable for understanding the optimal time for tylosin biosynthesis, which has implications for industrial production.

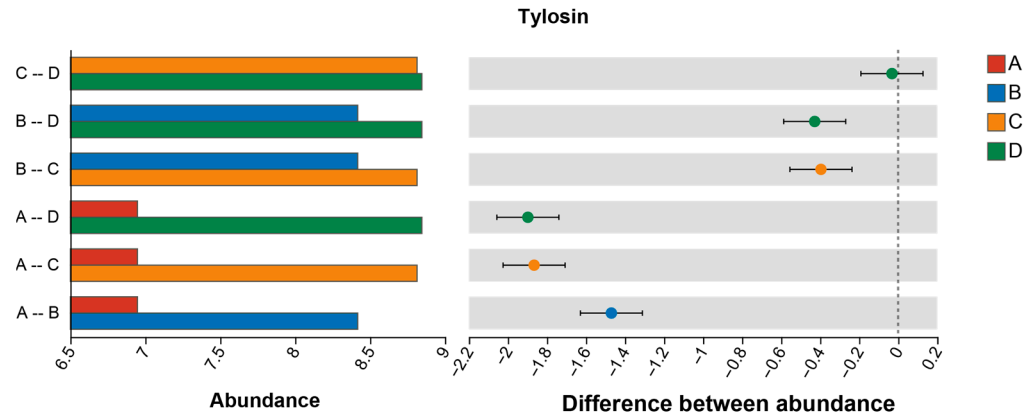


Figure 10. Differences in mean relative abundance of tylosin between groups. The X-axis of the bar chart on the left side indicates the average relative abundance of a metabolite in different subgroups, the vertical coordinate indicates the subgroup category of the two-by-two comparison, and the different colors indicate different subgroups. The middle area is the confidence interval set, and the values corresponding to the dots indicate the difference in the average relative abundance of the metabolite in the two subgroups. The color of the dots shows the color of subgroups whose metabolite abundance accounts for the larger proportion, and the I-type intervals on the dots are the upper and lower limits of the difference.

4. Discussion

The widespread use of antibiotics, such as tetracycline, cephalosporins, and erythromycin, has revolutionized medical treatment and agricultural practices. This underscores the critical role of antibiotics and natural products, making the discovery of new secondary metabolites a research priority [50–52]. Despite advances, many metabolites remain undiscovered or underutilized. Leveraging genomic information is now a focal point in natural product research [53,54]. Whole-genome sequencing allows the prediction of potential secondary metabolic gene clusters, significantly aiding in the discovery of novel natural products. [55–57]. *Streptomyces* spp. microorganisms have become important sources for discovering novel lead compounds. Determining the synthesis genes (clusters) of their secondary metabolites and discovering the metabolites of other metabolic pathways has become a mainstream research direction. With the development of compound isolation, structure identification, and fermentation technology, new natural products continue to be discovered and applied. Secondary metabolites are active substances of great value and play a significant role in regulating life activities [58,59]. In our study, the identified bacteria belong to the *Streptomyces fradiae* species, sharing high nucleotide and amino acid identity with *Streptomyces xinghaiensis* S187.

Horizontal gene transfer via mobile genetic elements significantly influences bacterial survival [60]. In *Streptomyces fradiae* sf106, 12 genomic islands, 1 prophage, and 13 CRISPR/Cas systems were identified. These genomic fragments break the boundaries of phylogenetic relationships, increasing microbial diversity and playing a vital role in bacterial adaptation and evolution. Genomic islands are the most important form of horizontal transfer elements that carry genes that can provide selective advantages to bacteria. They contain genes related to various biological functions and can be classified into pathogenicity islands, resistance islands, metabolic islands, and symbiotic islands according to the types of genes they contain. Studying the resistance genes found in genomic islands can further analyze the mechanisms of organism resistance and provide a basis for the development of new antibiotics and resistance targets. Genomic islands are an important source of new functional characteristics of strains. By studying the functional characteristics of genomic islands and their related genes, the mechanism of bacterial pathogenicity and resistance can be greatly promoted, providing us with new ideas for exploring metabolites. Horizontal transfer of mobile genetic elements (MGEs) promotes the functionality and genomic diversity of microbiomes. Some genes encoded by MGEs may help bacteria defend themselves

or enhance their ability to compete for resources. These elements contribute to bacterial adaptability and could be crucial in understanding resistance mechanisms.

Carbohydrate-active enzymes (CAZymes) are pivotal in biological functions such as glycosidic bond formation and breakdown. Our study identified six protein families involved in carbohydrate activities in *Streptomyces fradiae* sf106. Glycoside hydrolases accounted for the largest proportion, followed by glycosyl transferases. Carbohydrate-active enzymes are important enzymes involved in the degradation, modification, and generation of glycosidic bonds. In-depth research on CAZymes is of great significance for revealing the metabolic mechanisms of microbial carbohydrates. According to the prediction results of secondary metabolites, *Streptomyces fradiae* sf106 has 22 secondary metabolite gene clusters [61], indicating a great potential for producing terpenes and clustered natural products. Moreover, the MIBiG comparison showed that *Streptomyces* spp. is still one of the major sources of natural products. Exploring these secondary metabolite gene clusters can help us identify and characterize more important natural compounds from unexplored microorganisms [62], providing an important method for the targeted exploration or discovery of novel natural products and greatly reducing the possibility of discovering duplicate compounds. In addition, our research group has long focused on tylosin, an antibiotic used in animal husbandry. We found multiple genes capable of producing tylosin in the ninth gene cluster predicted in sf106, which can guide us to further determine the tylosin content of this strain. Although there is increasing controversy regarding the use of antibiotics as feed additives, tylosin is widely used due to its high efficiency, low residual, and specific application to livestock and poultry. With the development of animal husbandry, there will be broader possibilities for its use. Therefore, it is necessary to further improve the production capacity of this strain. With the continuous maturity of gene editing technology, targeted genetic modification of certain metabolites through genetic editing has made it possible to discover various natural products, making it possible to explore and improve the production of secondary metabolites and enhance the activities of active components.

Non-targeted metabolome sequencing identified a total of 1855 metabolites that could be matched to names, of which 972 metabolites were identified in positive ion mode and 888 metabolites in negative ion mode; moreover, the large number of metabolites that were not matched to names also indicate that this strain has the ability to produce unknown natural products. The categorization of metabolites with consistent expression trends will help us further search for metabolites with similar expression trends or similar functions, which can greatly facilitate the discovery of unknown natural compounds and their derivatives. In addition, differential metabolite analysis showed that the average relative abundance of most metabolites differed significantly among groups, for example, the differential metabolites were largest in pre-logarithmic (A) and mid-logarithmic (B) growth and then decreased in the late stage of strain growth, which implies that the synthesis patterns of different metabolites in different periods can be found. This further clarifies the metabolic trends of the strains and biosynthesis patterns of the target metabolites and has a significant influence on the further clarification of the metabolic trends of the strain and the biosynthesis pattern of target metabolites.

The potential for tylosin production was identified in sf106, which is noteworthy given tylosin's widespread use in animal husbandry. The need for optimizing this strain for higher tylosin yields is evident. Differential metabolite analysis revealed significant differences in metabolite abundance between growth phases, providing insights into metabolic trends and biosynthesis patterns. These data contribute to uncovering the origin and evolution of life and provide sufficient research foundations for more in-depth studies on the biosynthesis of tylosin and other secondary metabolites in *Streptomyces fradiae* sf106. These findings lay the groundwork for further genetic manipulation at the genomic level of *Streptomyces fradiae* sf106.

5. Conclusions

Whole-genome sequencing confirmed the high rates of similarity between *Streptomyces fradiae* sf106 and *Streptomyces xinghaiensis* S187, establishing sf106 as a metabolically versatile strain. This has positive implications for industrial production. Guided by whole-genome and metabolome information, this confirmed the ability of the strain to produce tylosin. Additionally, the strain harbors abundant exogenous functional genes, such as resistance genes. Analysis of mobile genetic elements enhances our understanding of the genetic diversity of this strain and highlights the importance of further research on them. This study serves as a foundational step for future research aimed at enhancing natural product yields, understanding resistance mechanisms, and exploring microbial diversity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9100866/s1. Table S1: DNA sample detection results of Streptomyces fradiae sf106. Figure S1: Growth of Streptomyces fradiae sf106 on different media.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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