Investigation of *Streptomyces* sp. Strain EMB24 Secondary Metabolite Profile Has Unraveled Its Extraordinary Antibacterial Potency Against Drug-Resistant Bacteria

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Received: 16 November 2021 / Accepted: 20 September 2022 / Published online: 11 October 2022
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Abstract

With the overuse and misuse of antibiotics amid COVID-19 pandemic, the antimicrobial resistance, which is already a global challenge, has accelerated its pace significantly. Finding novel and potential antibiotics seems one of the probable solutions. In this work, a novel *Streptomyces* sp. strain EMB24 was isolated and found to be an excellent source of antimicrobials as confirmed by agar-plug assay. It showed antibacterial activity against infection-causing bacteria, namely *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. In addition, *Streptomyces* sp. strain EMB24 inhibited the growth methicillin-resistant *Staphylococcus aureus* (MRSA), tetracycline-resistant *Neisseria gonorrhoeae*, and ampicillin-resistant *Neisseria gonorrhoeae*. Furthermore, to get deep insights about the genome and biosynthetic gene clusters producing antibiotics, whole genome sequencing was done. The strain EMB24 is closely related to the *Streptomyces longispororuber* as revealed by phylogenetic analysis which is a potential source of antibiotics and pigments as undecylprodigiosin and metacycloprodigiosin belonging to the class prodigiosin. Naphthyridinomycin, alkylresorcinols, desferrioxamine B and E, venezuelin, aborycin, MS-271, and siamycin are potent therapeutics that shared 100% similarity with the reference strain as revealed by the online antiSMASH tool.

Keywords *Streptomyces* · Genome-mining · Natural products · Antibiotics · Methicillin-resistant *Staphylococcus aureus* (MRSA) · *Neisseria gonorrhoeae*

Introduction

Antimicrobial-resistant (AMR) infections are expected to kill more people in the coming years than the current SARS-CoV-2 (COVID-19) pandemic, which has already resulted in multibillion-dollar investments in the discovery of new antiviral drugs, repurposing existing drugs, and developing vaccines (Miethke et al. 2021). The overuse of antibiotics as a COVID-19 (co)treatment has significantly contributed to the ongoing emergence of AMR (Goel et al. 2021a). Unfortunately, the current clinical pipeline has only 41 (60%) traditional (antibiotics) and 27 (40%) non-traditional antibiotic agents, which are insufficient to tackle the ever-growing AMR challenge (WHO 2021). This is accompanied by a significant drop in the success of traditional drug development by high-throughput screening (van Bergeijk et al. 2020).

The *Streptomyces* genus belongs to the phylum actinobacteria, which is well-recognized for its ability to create a wide range of bioactive secondary metabolites, including antibiotics (Costa et al. 2020; Goel et al. 2021b). In addition,
the *Streptomyces* genus alone represents 80% of the richest therapeutic drug-producing family in all kingdoms (Gosse et al. 2019). The rate of success of finding a new drug has significantly decreased primarily due to dereplication. However, advancement in genome sequencing has revealed > 50 biosynthetic gene clusters (BGCs) even in the well-studied *Streptomyces* species, inferring the breakthrough potentiality in producing novel antibiotics (Ward and Allenby 2018; Belknap et al. 2020).

BGCs are specific bacterial genome loci with clusters of two or more genes required to biosynthesize the natural products (Zheng et al. 2019; Chen et al. 2020). BGCs are majorly classified into three types: nonribosomal peptide synthase (NRPS), polyketide synthase (PKS), ribosomally synthesized and posttranslationally modified peptide (RiPP) based on structural differences (Chen et al. 2020). In general, NRPs and PKSs are essential targets for discovering natural products because they can synthesize an enormous number of antibiotics and essential therapeutic drugs (Zheng et al. 2019). PKSs are classified into three types based on their organization of catalytic domains: type I, type II, and type III (Wang et al. 2014). Type-I PKSs and NRPS are large multidomain consisting enzymes, each organized into modules. Each module performs a cycle of chain elongation and typically contains at least three domains. In a PKS module, the three domains are ketosynthase (KS) domain, an acyltransferase (AT) domain, and an acyl carrier protein (ACP). Whereas in NRPS module: a condensation (C) domain, an adenylation (A) domain, and a thiolation (T) domain are present (Bloudoff and Schmeing 2017; Komaki and Tamura 2020). The condensation domain in NRPS clusters is responsible for the amide bond formation during the chain elongation step, whereas in PKS, a similar reaction is carried out by KS domain. These two domains are highly conserved and are vital targets for distinguishing between various NRPS/PKS secondary metabolites pathways (Bloudoff and Schmeing 2017).

The current unprecedented rate of *Streptomyces* genome mining has led to the creation and usage of bioinformatics tools aimed explicitly at discovering BGCs. Bacterial antiSMASH is a robust genome-mining tool that enables users to identify easily, annotate, and evaluate secondary metabolite BGCs (Cibichakhravarthy and Jose 2021). Based on the relevance of the *Streptomyces* genus as a hub of novel antibiotics, we have isolated *Streptomyces* sp. strain EMB24 from Pen, Maharashtra, India. The *Streptomyces* sp. strain EMB24 with a GC content of 72.40% has a genome of 7,252,958 bp. Extensive phylogenetic, genomic, secondary metabolic analyses have been carried out for genome sequencing, revealing the unique genome characteristics and diversity of the predicted BGCs. The *Streptomyces* sp. strain EMB24 was screened against various resistant bacterial strains, namely methicillin-resistant *Staphylococcus aureus* (MRSA), tetracycline-resistant *Neisseria gonorrhoeae*, and azithromycin-resistant *Neisseria gonorrhoeae* using the agar-plug assay.

**Materials and Methods**

**Bacterial Strains and Growth Conditions**

The *Streptomyces* sp. strain EMB24 (GenBank Accession number: MW582530.1) isolated from Mangrove soil collected from Pen, Maharashtra, India, was used in this study. *Escherichia coli* ATCC 25,922, *Pseudomonas aeruginosa* ATCC 27,853, and *Staphylococcus aureus* ATCC 25,923 were procured from ATCC and handled at the Indian Institute of Technology Delhi. Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, azithromycin-resistant *Neisseria gonorrhoeae* WHO P, and plasmid-mediated tetracycline-resistant *Neisseria gonorrhoeae* WHO G were revived at All India Institute of Medical Science (AIIMS), New Delhi.

**DNA Extraction and 16S rRNA Sequencing Analysis**

The *Streptomyces* sp. strain EMB24 culture was grown for five days at 30 °C in International *Streptomyces* Project-2 Medium (ISP-2, 4.0 g/L yeast extract, 10.0 g/L malt extract, 4.0/L g dextrose, pH 7.2) (Shirling and Gottlieb 1966; Hu et al. 2018) for extraction of DNA. Bacterial cell pellets were collected, and genomic DNA was isolated using the commercially available Quick-DNA Fungal/Bacterial Miniprep Kit-Zymo research as per the manufacturer’s recommended protocol. Genomic DNA was quantified using Nanodrop2000 (Thermo Scientific, USA), and quality was assessed using agarose gel electrophoresis. Sanger PCR amplification was performed with 30–50 ng of the genomic DNA as a template and 16S rRNA primers (27 forward: AGAGTTTGATCCTGGCTCAG and 1492 reverse: TACGGCTACCTTGTTACGACTT) (Lane 1991; Palkova et al. 2021). A 1.5 kb PCR product was generated, which was purified and used for Sanger sequencing to access the purity of the isolate. The sample was then sent to the Genotypic Technology company for genome sequencing.

**Illumina and Nanopore Sequencing and Assembly**

*Streptomyces* sp. strain EMB24 was sequenced using a whole-genome enzymatically fragmentation strategy by the high-throughput Illumina sequencing (Illumina, San Diego, USA) (Senol Cali et al. 2019) and the GridION X5 (Oxford Nanopore Technologies, Oxford, UK) long-read sequencing platforms: Genotypic Technology, Bangalore, India.
Firstly, the quantification of genomic DNA was assessed using a Qubit Fluorometer (Thermo Fisher Scientific, USA) and a NanoDrop2000 Spectrophotometer (Thermo Scientific, USA) and the integrity using agarose gel electrophoresis.

Secondly, Illumina library construction was carried out using the QIASEq FX DNA Library Preparation protocol according to the manufacturer’s instructions. Briefly, 50 ng of Qubit quantified DNA was enzymatically fragmented, end-repaired, and A-tailed in a one-tube reaction using the FX Enzyme Mix provided in the QIASEq FX DNA kit. The end-repaired and adenylated fragments were subjected to adapter ligation to generate sequencing libraries, and the index incorporated Illumina adapter was ligated. These libraries were subjected to 6 cycles of Indexing-PCR to enrich the adapter-tagged fragments. Finally, the amplified libraries were purified, and libraries were paired-end sequenced on Illumina HiSeq X Ten sequencer (Illumina, San Diego, USA) as per the manufacturer’s instructions.

Thirdly, nanopore library construction was carried out, and sequencing was performed on GridION X5 (Oxford Nanopore Technologies, Oxford, UK). Finally, the raw reads generated from both Illumina and nanopore platforms were used for assembly. Hybrid assembly was carried out by Unicycler hybrid assembler using Illumina paired-end and Nanopore data (Wick et al. 2017). The assembly resulted in ~7 Mb size genome for Streptomyces sp. strain EMB24.

Genomic Prediction and Genome Annotate

The generated assembly was further used for gene prediction using the Prokka tool (Seemann 2014). The predicted proteins were searched against the UniProt protein database using the DIAMOND (Buchfink et al. 2015) BlastP program for the gene ontology and annotation. The predicted genes were subjected to pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Moriya et al. 2007). The following reference organisms were chosen for the KEGG pathway analysis: Streptomyces coelicolor (Genome ID: 100226.1), Streptomyces avermitilis (Genome ID: 227882.1), Streptomyces griseus (Genome ID: 455632.3), Streptomyces scabiei (Genome ID: 680198.5), and Thermomonospora curvata (Genome ID: 471852.6).

The pathway was generated against the gene sequences for all the samples—the KEGG ontology ID assigned for each pathway for an organism in the KEGG database. The nucleotide/protein sequence was mapped using this database against its KEGG Ontology ID to generate a pathway for each gene/protein. Ribosomal RNAs (tRNAs) and transfer RNAs (tRNAs) were predicted using RNAmmer (Version 1.2) (Lagesen et al. 2007) and tRNAscan-SE (Version 2.0) (Lowe and Chan 2016), respectively.

Phylogeny Analysis and Genome Comparison

Based on the 16S rRNA results, phylogenetic analysis was performed using MEGA 7.0 software to demonstrate the evolutionary relationship (Kumar et al. 2016), and the tree was constructed using the neighbor-joining method (Saitou and Nei 1987). In this study, 20 closest actinomycetes were selected based on the similarity search using nucleotide blast at NCBI (National Center for Biotechnology Information).

Sequences were downloaded from the NCBI database to verify the relationship further. The genome comparison analysis was performed using the BLAST Ring Image Generator (BRIG) tool, and a Circos image was generated. BRIG is a java-based tool that uses CGView for image rendering and BLAST for genome comparisons. The assembly genome was homology searched against a non-redundant database for the top organism with high query coverage and high percentage identity. The assembled genome was compared with the nearest reference strain using BRIG (Alikhan et al. 2011).

RAST Annotation and Secondary Metabolites Prediction

RAST (Rapid Annotation using Subsystem Technology) introduced in 2007 annotates fully or nearly complete bacterial and archaeal genomes and is a fully automated service. Genome annotations take around 12–24 h after submitting genome sequence either in FASTA or GenBank format. RAST (version 2.0) was used for genome annotation and comparison. SEED viewer was used to know the subsystem category distribution (Overbeek et al. 2014; Zhang et al. 2021). To predict secondary metabolites in Streptomyces sp. strain EMB24, an online software anti-SMASH (version 6.0) (Blin et al. 2021) was used (https://antismash.secondarymetabolites.org/#/start). The assembled genome of this strain was submitted to the anti-SMASH online server to search for secondary metabolite BGCs (Quemener et al. 2021). Further analysis about the gene sequence and percent identity was interpreted based upon the results.

Antimicrobial Activity Against Resistant Strains

Agar-plug diffusion assay was performed to check the antimicrobial activity of isolated actinobacteria against P. aeruginosa ATCC 27853, E. coli ATCC 25922, S. aureus ATCC 25923, MRSA, azithromycin-resistant N. gonorrhoeae, and tetracycline-resistant N. gonorrhoeae (Quemener et al. 2021). The assay is based on the antagonism between the microorganisms. The actinobacteria were tightly streaked onto ISP2 agar media and incubated at 30 °C for 6 days. After sporulation, the wells were cut and placed onto the nutrient agar plates previously swabbed with the test microorganisms. After 24 h incubation at
respective temperatures, plates were checked for the zone of inhibition (Balouiri et al. 2016).

Results and Discussion

Sequencing, Assembly, Phylogenetic Analysis, and the Genomic Characteristics

The Illumina-compatible sequencing library for the sample showed an average fragment size of 343 bp, and 7,524,682 reads were generated. Nanopore read statistics were as follows: 136,162 reads were generated with an average read length of 5097 bp. The maximum read length was 58,086 bp, and the minimum was 94 bp. The genome size was linear of 7,252,958 bp size with a GC content of 72.40% and N50 length 7,252,958, contigs 1. The genome consists of 6433 protein-coding sequences with 3150 pseudogenes; in total, 77 tRNA and 18 rRNA were found. The statistical results are summarized in Table S1. Phylogenetic analysis revealed that Streptomyces sp. strain EMB24 is closely related to the Streptomyces longispororuber (MT760611.1) (Fig. S1), which is a potential source of antibiotics and pigments as underelyprodigiosin and metacycloprodigiosin belonging to the class Prodigiosin (Harir et al. 2018). In addition, genomic comparison analysis unraveled the maximum similarity with Streptomyces sp. ETH9427 (CP029624.1), which is 7,745,357 bp genome (Fig. S2) and has 6864 protein-coding sequences.

Genomic Prediction and Genome Annotation

The gene prediction from the assembled genome was carried out using the PROKKA tool (Seemann 2014). Out of the 6433 total number of predicted proteins, 6365 (98.94%) were annotated. The gene ontology (GO) of 6365 proteins is divided into three classes: biological process (10 branches), molecular function (10 branches), and cellular component (8 branches). Gene ontology annotation resulted in the top 5 abundant protein functions: DNA-binding protein, transcriptional regulator, transposase, TetR family transcriptional regulator, and oxidoreductase. The complete gene annotation summary and protein predictions are shown in Fig. S3. The predicted genes were subjected to pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Fig. S4). All the genes are mainly concerned around 23 pathway functions of which the maximum number of genes is involved in protein families: genetic information processing (419) and signaling and cellular processes (383). This is followed majorly by carbohydrate metabolism (306), amino acid metabolism (264), and protein families: metabolism (214). There are 28 genes associated with the pathways of metabolism of terpenoids and polyketides responsible for antibiotic synthesis, vancomycin (KO01055), enediyne (KO01059), tetracycline (KO00253), type I PKS (KO01052), along with other potential antibiotics. In addition, 38 genes belong to the pathways of biosynthesis of secondary metabolites: prodigiosin biosynthesis (KO00333) consistent with the 16S RNA phylogenetic relation with the Streptomyces longispororuber responsible for prodigiosin pigment production; streptomycin biosynthesis (KO00521); penicillin and cephalosporin biosynthesis (KO00311); neomycin, kanamycin, and gentamicin biosynthesis (KO00524); and novobiocin biosynthesis (KO00401) (Table S2).

RAST Annotation and Secondary Metabolites Prediction

Fig. S5 shows that 72 genes are involved in stress response, and 46 genes are involved in virulence, diseases, and defense, which may be associated with the upregulation of secondary metabolites to protect nutrients available while switching life from substrate-to-substrate aerial mycelium. In total, 16 secondary BGCs were identified using the online antiSMASH tool predicted for NRPS, Lanthipeptide-class-i, ii, iv, PKS-like, T1PKS, T2PKS, T3PKS, terpene, ectoine, siderophore, RiPP-like, and lasso peptide (Table 1). Region 1 has 100% similarity with naphthyridinomycin and antimycin and 93% with splenocin. Naphthyridinomycin, isolated from Streptomyces lupinus, belongs to the tetrahydroisoquinoline (THIQ) alkaloids that exhibit strong antitumor and antibacterial activity against MRSA (Zhang et al. 2018; Pu et al. 2013). Splenocins are first reported from Streptomyces sp. CNQ43 shares structural dilactone scaffold identity with antimycins and has potent anti-inflammatory activity (Strangman et al. 2009; Chang et al. 2015). Many of the therapeutic drugs are believed to be produced by Streptomyces sp. strain EMB24 as predicted by the online antiSMASH tool such as alkylresorcinols (T3PKS), curamyacin (T2PKS), ectoine, desferrioxamine B and desferrioxamine E (siderophore), albaflavenone and geosmin (terpene), venezuelin (lanthipeptide class iv), and aborycin, MS-271, and sianmycin (lasso peptide), each of them shares 100% similarity with the reference (Table 1). In addition, ectoines are one kind of compatible solutes produced by microorganisms to cope with the varied habitats due to the continuous fluctuations in the surrounding environmental osmolarity. Ectoines protect DNA from damage by ionizing radiation (Schröter et al. 2017), help stabilize the lipid bilayers (Harishchandra et al. 2010), protect the functionality of proteins caused by various challenges (Kolp et al. 2006), and also confer protection to the bacterial cell at extreme temperature conditions (Salvador et al. 2018; Richter et al. 2019). While iron is an essential trace element used by virtually all microorganisms to carry out critical redox reactions, the bioavailability of this element is low (Emerson et al. 2012). To cope up
| Region | Type | From | To | Most similar known cluster | Reference strain | Similarity (%) | Accession number |
|--------|------|------|----|----------------------------|------------------|----------------|-----------------|
| 1      | NRPS, lanthipeptide class I, lanthipeptide class II, PKS-like, T1PKS | 374 | 151,659 | Naphthyridomycin | Streptomyces lusitanus | 100 | JQ996389.1 |
|        |      |      |    | Antimycin | Streptomyces argillaceus | 100 | LT989885.1 |
|        |      |      |    | Splenocin | Streptomyces sp. CNQ431 | 93 | KP719128.1 |
| 2      | T3PKS | 325,391 | 366,464 | Alkylresorcinol | Streptomyces griseus subsp. griseus NBRC 13350 | 100 | AP009493.1 |
| 3      | Terpene | 547,951 | 570,453 | Isorenieratene | Streptomyces griseus subsp. griseus NBRC 13350 | 85 | AP009493.1 |
|        |      |      |    | Carotenoid | Streptomyces griseus subsp. griseus NBRC 13350 | 75 | AF272737.1 |
| 4      | T2PKS | 751,882 | 824,391 | Curamycin | Streptomyces cyaneus | 100 | X62518.1 |
|        | Spore Pigment | | | Spore Pigment | Streptomyces collinus | 85 | AF299354.1 |
|        | Spore Pigment | | | Streptomyces avermitilis | | 83 | AB070937.1 |
| 5      | Ectoine | 1,659,866 | 1,670,264 | Ectoine | Streptomyces anulatus | 100 | AY52544.1 |
| 6      | Siderophore | 2,695,825 | 2,706,436 | Desferrioxamine B | Streptomyces griseus subsp. griseus NBRC 13350 | 100 | AP009493.1 |
|        | Desferrioxamine E | | | Streptomyces sp. ID38640 | | 100 | MG59167.1 |
| 7      | Lanthipeptide-class-iv | 3,186,753 | 3,209,425 | Venezuelin | Streptomyces venezuelae ATCC 10712 | 100 | HQ328852.1 |
| 8      | Lasso peptide | 3,984,509 | 4,007,002 | Aborycin | Streptomyces sp. ZS0098 | 100 | NZ_MKCP01000039.1 |
|        | MS-271 | | | Streptomyces sp. | | 100 | LC381634.1 |
|        | Siamycin | | | Streptomyces nodosus | | 100 | CP009313.1 |
| 9      | Terpene | 4,904,610 | 4,925,378 | Albaflavenone | Streptomyces coelicolor A3(2) | 100 | AL645882.2 |
| 10     | Lanthipeptide-class-i | 5,700,585 | 5,725,904 | Kanamycin | Streptomyces kanamyceticus | 1 | AB254080.1 |
| 11     | RiPP-like | 5,855,764 | 5,866,761 | | | | |
| 12     | Terpene | 5,891,595 | 5,912,178 | Geosmin | Streptomyces coelicolor A3(2) | 100 | AL645882.2 |
| 13     | Siderophore | 6,045,930 | 6,059,169 | Grincamycin | Streptomyces lusitanus | 8 | KC962511.1 |
| 14     | Terpene | 6,525,062 | 6,551,758 | Hopene | Streptomyces coelicolor A3(2) | 92 | AL645882.2 |
| 15     | PKS-like, T1PKS, hglE-KS | 6,764,420 | 6,833,902 | Caboxamycin | Streptomyces coelicolor A3(2) | 80 | AL645882.2 |
|        | Nataxazole | | | Streptomyces sp. Tu 6176 | | 62 | KK106988.1 |
| 16     | RiPP-like | 6,951,136 | 6,961,351 | Informatipeptin | Streptomyces viridochromogenes DSM 40736 | 57 | GG657757.1 |

Table 1 Overview of 17 secondary metabolic biosynthesis gene clusters of *Streptomyces* sp. strain EMB24 detected by antiSMASH 2.0 server.
with the shortage, microorganisms have evolved in many ways to take this essential trace element; one of the ways is the secretion of siderophores. Siderophores are secondary metabolites released into the environment that scavenge iron and make soluble Fe\(^{3+}\) complex, further taken up by the microorganisms via specific receptors (Kramer et al. 2020). *Streptomyces* sp. EMB24 strain predicted to produce the two potent siderophores: desferrioxamine B and desferrioxamine E, with 100% similarity with the references *Streptomyces griseus* subsp. *griseus* and *Streptomyces* sp. ID38640 respectively. Desferrioxamin B has been used to treat iron load in humans and sold under the brand name Desferal (Jia et al. 2017).

In the present study, naphthyridinomycin BGCs were shown aligned with the *Streptomyces lusitanus* and 85 open reading frames exhibiting various roles in regulating the production of the naphthyridinomycin, 3 of which encode modular NRPS (Fig. 1A). Similarly, curamycin (T2PKS)

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**A.**

Most similar known structure: **Naphthyridinomycin**

Cluster Type: **NRP**

Naphthyridinomycin biosynthetic gene cluster from *Streptomyces* sp. EMB24

![Naphthyridinomycin biosynthetic gene cluster from Streptomyces lusitanus](JQ996398.1)

| Transcriptional regulator (94%) | UV-Repair Protein (98%) |
|--------------------------------|------------------------|
| Phosphopantetheinyl transferase (98%) | Membrane Protein (99%) |
| Short chain dehydrogenase (99%) | Monoxygenase (100%) |
| Methyl transferase (98%) | Thiamin diphosphate binding domain of transketolase (99%) |
| Oxidoreductase (99%) | |
| Transmembrane-transport protein (98%) | |
| SAM dependent methyltransferase (99%) | |

Non-ribosomal peptide synthetase

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**B.**

Most similar known structure: **Curamycin**

Cluster Type: **T2PKS**

Curamycin biosynthetic gene cluster from *Streptomyces* sp. EMB24

![Curamycin biosynthetic gene cluster from Streptomyces cyaneus](X62518.1)

| Beta-ketoacyl synthase (86%) | Cyclase (75%) |
|------------------------------|---------------|
| Acyl Carrier Protein (60%) | |

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**C.**

Most similar known structure: **Alkylresorcinol**

Cluster Type: **T3PKS**

Alkylresorcinol biosynthetic gene cluster from *Streptomyces* sp. EMB24

![Alkylresorcinol biosynthetic gene cluster from Streptomyces griseus subsp. griseus NBRC 13350](AP009493.1)

| Monooxygenase (60%) | Methyltransferase (70%) |
|---------------------|------------------------|
| (62%)               | T3 Polyketides (T3PKS) |

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**Fig. 1** A Proposed biosynthetic gene cluster of naphthyridinomycin compound in *Streptomyces* sp. strain EMB24. The most similar gene cluster from *Streptomyces lusitanus* is shown. B Proposed biosynthetic gene cluster of curamycin compound in *Streptomyces* sp. strain EMB24. The most similar gene cluster from *Streptomyces cyaneus* is shown. C Proposed biosynthetic gene cluster of alkylresorcinol compound in *Streptomyces* sp. strain EMB24. The most similar gene cluster from *Streptomyces griseus* subsp. *griseus* NBRC 13350 is shown with related genes and percentage depicted to highlight intracluster gene rearrangements
and alkylresorcinol (T3PKS) BGCs are shown aligned with the *Streptomyces cyaneus* and *Streptomyces griseus* subsp. *griseus*, respectively (Fig. 1B and C).

**Antimicrobial Activity Against Resistant Strains**

The isolated *Streptomyces* sp. EMB24 strain were screened for antibacterial activity against *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, MRSA ATCC 43300, azithromycin-resistant *N. gonorrhoeae* (WHO P), and tetracycline-resistant *N. gonorrhoeae* (WHO G). The zone of inhibition measured is as follows: *P. aeruginosa* (10 mm), *E. coli* (12 mm), *S. aureus* (20 mm), MRSA (22 mm), azithromycin-resistant *N. gonorrhoeae* (18 mm), and tetracycline-resistant *N. gonorrhoeae* (20 mm) as shown encircled in Fig. S6. As mentioned earlier, naphthyridinomycin exhibits strong antibacterial activity against MRSA, confirmed by the agar-plug assay. These results show that the *Streptomyces* sp. EMB24 strain is a potent source of antibiotics, as predicted by the online antiSMASH tool and the agar-plug assays. The strain is in further evaluation to isolate and purify the compounds.

**Conclusion**

In this study, a novel *Streptomyces* sp. EMB24 strain was isolated from salt pan, Maharashtra, India. The strain exhibits potent antimicrobial activity against various drug-resistant bacteria growth, including methicillin-resistant *Staphylococcus aureus* (MRSA), tetracycline-resistant *Neisseria gonorrhoeae*, and azithromycin-resistant *Neisseria gonorrhoeae*. Furthermore, genome analysis revealed various therapeutics BGCs producing drugs such as naphthyridinomycin, anti-nycin, splenocin, alkylresorcinals, curamycin, enteoin, desferrioxamin B, desferrioxamine E, albaflavenone, geosmin, venezuelin, aborycin, MS-271, and siamycin, each of them sharing 100% similarity with the reference strain. Isolation, characterization, and purification of the compounds continue to evaluate the potentiality of this EMB24 strain.

**Data Availability**

The whole-genome of *Streptomyces* sp. EMB24 has been deposited at the NCBI genome database under the accession number CP075343. The assembly reported in the paper is associated with NCBI BioProject: PRJNA729206 and BioSample: SAMN19113948. The authors confirm that all the data needed to support the study is represented within the article and supplementary files.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10126-022-10168-2.

**Acknowledgements** The authors gratefully acknowledge the financial grant provided by the Indian Institute of Technology Delhi (IITD) for carrying out the present work. NG also appreciates the financial assistance provided by the Ministry of Human Resource Development (MHRD), Govt. of India, and IIT Delhi. NG sincerely acknowledges the help from Dr. Seema Sood and her group for providing her laboratory facilities to check the antibacterial activity against resistant strains. We thank the genome sequencing services provided by Genomic Technology Pvt. Ltd. Bangalore, India, for providing the sequencing data used in the study.

**Author Contribution** Nikky Goel: conceptualization, methodology, formal analysis, writing original draft; Rajendra Singh: visualization, review MS; Seema Sood: supervision, review MS; Sunil K. Khare: supervision, review MS.

**Declarations**

**Competing Interest** The authors declare no competing interests.

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