The Draft Genome Sequence of The Endophytic Streptomyces Strain VITGV156

Veilumuthu P

VIT University

Nagarajan T

Madurai Kamaraj University

Sasikumar S

Madurai Kamaraj University

Siva R

Vellore Institute of Technology: VIT University

J Godwin Christopher (✉ godwinj@vit.ac.in)

VIT University <https://orcid.org/0000-0002-9328-983X>

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Abstract

Streptomyces species is one among the dominant group of bacteria in the family Actinobacteria with a rich repertoire of secondary metabolites. Secondary metabolites with antimicrobial activity and plant growth promotor have been isolated from various *Streptomyces* sp. Here in this investigation, we present the draft genome of a new species, *Streptomyces* sp. VITGV156 isolated from healthy tomato plant (*Lycopersicon esculentum*) which has some rare antimicrobial secondary metabolites, like coelichelin, fluostatin, vicanistatin, nystatin, sipanmycin, and informatipeptin. The genome is 8.18 Mb in size with 6,259 protein coding genes. The average GC content of the genome is 72.61 %. Preliminary analysis with antiSMASH 6.0 revealed the presence of 29 biosynthetic gene clusters for the synthesis of potential secondary metabolites. These includes 4 NRPS (non – ribosomal peptide synthetase), 7 PKS (Polyketide Synthases), 2 RiPP (Ribosomally synthesized and post-translationally modified peptides) clusters. When we look into genes associated with secondary metabolites, 406 genes are present which includes 184 genes for cofactor and vitamins, 72 genes for terpenoids and polyketides, 70 genes for xenobiotics and 80 genes for other metabolites are present. Comparative genome analysis of VITGV156 with its closest neighbor *Streptomyces luteus* strain TRM45540 revealed ANI 91.22% and dDDH value 44.00%.

Introduction

Tomatos are usually protective foods because of its special nutritive value. The limited caloric supply, relatively provision of minerals, vitamins and phenols such as flavonoids make the tomato an excellent functional food providing additional physiological benefits as well as meeting basic nutritional requirements. This plant has a diverse microbial association throughout its life. It also has may actinomycetes as endophytes, specially *Streptomyces* Members of the genus *Streptomyces* are Gram-positive bacteria that resemble filamentous fungi. *Streptomyces* sp. typically inhabit soil, and also interact with plants, animals and lichens (Klykleung et al. 2020). They are renowned for producing a wide variety of biologically active secondary metabolites, and therefore, efforts were made to exploit them as Plant Growth Promoters (PGP) or Biological Control Agents (BCA) in agriculture. Hence there is a surge in interest towards using *Streptomyces* sp. to manage plant diseases and enhance the plant growth promotion (Kunova et al. 2021). Around 23,000 bioactive secondary metabolites from microorganisms have been reported so far, and nearly half of them (~ 10,000) are from actinomycetes (Kalyani et al. 2019) and *Streptomyces* sp. being the major contributors (Dewi et al. 2017). Many of these secondary metabolites have antimicrobial potential which made *Streptomyces* the primary bioactive metabolite producing organisms exploited by the agricultural and pharmaceutical industry. Also, among the endophytic actinobacteria recovered from medicinal plants, *Streptomyces* sp. accounts for the most abundant genus followed by *Micromonopora*, *Actinopolyspora*, *Nocardia*, *Saccharopolyspora*, *Streptosporangium*, *Promicromonospora* and *Rhodococcus*plants (Passari et al. 2017).

Endophytic *Streptomyces* sp. suppressed the growth of pathogen and promote the growth of tomato plants (Vurukonda et al. 2018; Suárez-Moreno et al. 2019). In addition to their ability for secondary metabolite production, *Streptomyces* are also considered as key players in the production of vitamins.

Previously vitamin B12 production was reported in *Streptomyces Olivaceus* and *Streptomyces fulvissimus* (Hall et al. 1953; Atta 2007). Removing xenobiotics pollutants from soil, water or sediments through complete transformation or mineralization into harmless end products like CO₂ and H₂O is an urgent need of bioremediation strategy. The most important organisms in biodegradation are fungi, bacteria and algae. Recently some researchers have reported Xenobiotic degrading *Streptomyces* strains such as *Streptomyces* sp. M7 and *Streptomyces aureus* strain HP-S-01 from the natural environment (Chen et al. 2011; Mishra et al. 2021)

Therefore, in this investigation, we isolate an endophytic *Streptomyces* sp. with a rich repertoire of antimicrobial secondary metabolites, its genome was sequenced. Whole genome sequencing (WGS) offers the heterologous expression of valuable secondary metabolite for future use. Exploring the source of secondary metabolite along with unlocking the bioengineering potential will certainly facilitates identification of novel antibiotics and helps to combat infections better. The draft genome sequences were analysed and biosynthetic gene clusters responsible for the metabolism of secondary metabolite were identified and the results are discussed.

Materials And Methods

In this study, the strain *Streptomyces* sp. VITGV156 was isolated from healthy tomato plants (*Lycopersicon esculentum*) from southern region of the state Tamil Nadu in India (9.9420° N, 77.9724° E). The collected host samples were surface sterilized. The samples were then dissected and *Streptomyces* strains were isolated in ISP2 agar plates by streaking method (Singh and Dubey 2020; Promnuan et al. 2020). The plates were incubated for up to 15 days at 30°C. The isolated samples were further segregated and individual colonies were isolated

The genomic DNA was extracted from a 7-days old culture grown in ISP2 medium (Law et al. 2017) using cetyl-trimethylammonium bromide (CTAB) based method (Kieser et al, 2000). The quality of the isolated DNA was assessed in 0.8% agarose gel electrophoresis. The draft genome of *Streptomyces* sp. VITGV156 was analysed using Illumina Nextseq500 platform. The sequenced raw data of the samples were processed using Trimmomatic v0.38 (Bolger et al. 2014) to obtain high quality clean reads. The filtered high quality Paired-End (PE) reads of the samples were assembled into scaffolds using SPAdes assembler (v-3.13.0) (Bankevich et al. 2012). Annotation of protein coding and RNA genes from the genome were identified in the samples using the final assembled draft genome. The process of gene prediction was performed by Prokka (version 1.12) (Seemann 2014). The pathway annotation of predicted genes was carried out against the curated KEGG GENES database using KAAS (KEGG Automatic Annotation Server (<http://www.genome.jp/kegg/ko.html>)). The genome is uploaded to NCBI as *Streptomyces ferrovit* under BioProject ID: SAMN20499087 Accession ID: 20499087 and SRA: SRS9645416.

The circular genome was constructed using the CGViewer server (Grant and Stothard 2008). A circular genome map showing, ORF, GC skew+, GC skew- and GC content was created using default parameters.

Phylogenetic analysis was performed using the 16s ribosomal RNA sequences (collected from NCBI) using MegaX (Kumar et al. 2018). The biosynthetic clusters involved in the synthesis of secondary metabolites were analyzed using antiSMASH 6.0 (Blin et al. 2021) and BAGEL 4 (Heel et al. 2018).

Finally, comparative genome analysis for *Streptomyces* sp. VITGV156 with its closest neighbour in the phylogenetic tree, *Streptomyces luteus* strain TRM45540 (Accession ID – PRJNA248298; Taxonomic ID – 67332) was performed by means of the comparative genomic tool Ezbiocloud. To determine the relatedness between the *Streptomyces* sp. VITGV156 and *Streptomyces luteus* strain TRM45540, an Average Nucleotide Identity (ANI) analysis and digital DNA – DNA Hybridization (dDDH) estimation were performed as described by Meier-Kolthoff et al. (2014). Phylogenetic analysis performed using 16s ribosomal RNA sequences revealed the uniqueness of this strain. The 16s rRNA gene sequence of *Streptomyces* sp. VITGV156 has been deposited in NCBI GenBank (Accession ID – MZ750937). In phylogenetic tree, *Streptomyces* sp. VITGV156 falls on a separate branch which shows its distinct nature.

Results And Discussion

Tomato is the second mostly consumed plant produce worldwide with wide range of pharmaceutically valuable secondary metabolites (Quinet et al. 2019). Sequencing of this *Streptomyces* sp. genome results in 7,670,883 reads representing 8.18 Mb genome with an average GC content of 72.61 %. The genome was assembled from 32 scaffolds (containing 7,207,566 bp) with N50s of 592,385 bp. The details of the genome were tabulated in Table 1. Similar kind of genome results recorded in *Streptomyces avermitilis* contains 9,025,608 bases average GC content, 70.7% (Ikeda et al. 2003). Whole genome sequencing (WGS) is a complete requirement for the modern natural product discovery for the agricultural, biomedical and pharmaceutical products.

Table 1
Genome details of the *Streptomyces* sp. VITGV156

Features	<i>Streptomyces</i> sp. VITGV156
Genome size (bp)	8.18 Mb
Total number of bases sequenced	7,207,566 bp
GC content	72.61
Total genes	6,386
ANI value <i>Streptomyces luteus</i> strain TRM45540 (%)	91.22
dDDH value <i>Streptomyces luteus</i> strain TRM45540 (%)	44.00
Genes with no blast hit	151
Protein coding genes	6,259
rRNA operons	5
tRNA operons	81

A representative genome map was drawn using CGViewer server V1.0. Schematic circular representation of genome sequences for strain *Streptomyces* sp. VITGV156 (Fig. 1). Genome map of *Streptomyces* sp. VITGV156, open reading frames with GC skew and GC content was highlighted in the map. The outer circle from first to third circle shows the positions of the ORF genes in clockwise and from fourth to six counter-clockwise directions, respectively. The seventh circle shows GC skew (higher than average GC content in green, lower than average in purple) and the second innermost circle shows the GC content of the entire genome sequence. The innermost circle shows the size of the genome *Streptomyces* sp. VITGV156. Chen et al. (2019) report shows similar circular representation of the ORF, GC skew, GC content and genome size in the strain *Streptomyces* sp. S063 complete genome. Phylogenetic analysis performed using 16s ribosomal RNA sequences revealed the uniqueness of this strain. The 16s rRNA gene sequence of *Streptomyces* sp. VITGV156 has been deposited in NCBI GenBank (Accession ID – MZ750937). In phylogenetic tree, *Streptomyces* sp. VITGV156 falls on a separate branch which shows its distinct nature. BLAST analysis shows similarity with *Streptomyces luteus* (99%), *Streptomyces mutabilis* (99%), *Streptomyces enissocaesilis* (99%), *Streptomyces minutiscleroticus* (99%), *Streptomyces rochei* (99%), and *Streptomyces tuius* (99%). This result is in accordance with previous analytical report of Kim and Goodfellow (2002) in *Streptomyces avermitilis* sp. nov.

The results of the comparative genome analysis with neighbour *Streptomyces luteus* strain TRM45540 revealed an ANI value of 91.22%, and dDDH value of 44.00% were calculated for *Streptomyces* sp. VITGV156 with *Streptomyces luteus* strain TRM45540 (Accession ID – PRJNA248298; Taxonomic ID – 67332) as shown in Table 1. This reveals that VITGV156 is a distinctively new species. High ANI value (above 95%) and high DDH estimations (Formula 2 above 70%) were usually observed for bacterial species differentiation previously (Yoon et al. 2017). Therefore, both strains are closely related, but both

are not belonging to the same species. The results show as *Streptomyces* sp. VITGV156 could be a new species of the genus *Streptomyces*.

A total of 6,259 protein coding genes were identified (Table 1) and 151 genes out of them showed no resemblance to the existing genes which could be due to the presence of new metabolic pathways. Similar report previously recorded in *Streptomyces* sp. RLA2-12 showed 157 genes showed potential property in involved in the metabolic pathway by Nicault et al. (2020). Previous reports from the endophytic *Streptomyces* sp. obtained from tomato plant showed a significant antibacterial (Shah et al. 2017), antifungal, activity, enhance disease resistance (Abbasi et al. 2019) and plant growth promotion (Zarandi et al. 2021).

Out of the total genes, a total of 406 genes were involved in metabolism of cofactors, vitamins, terpenoids, polyketides and other secondary metabolites (Table 2). Moreover, the previous study by Zhang et al. (2019) reports the similar scenario in *Streptomyces* sp. CC0208 contains 439 genes which involved in the metabolism of cofactors, vitamins, terpenoids, glycan, polyketides and other secondary metabolites.

Table 2

Genes associated with secondary metabolites

Pathways	<i>Streptomyces</i> sp. VITGV156
Metabolism of cofactors and vitamins	184
Metabolism of terpenoids and polyketides	72
Xenobiotics biodegradation and metabolism	70
Biosynthesis of other secondary metabolites	80

The genome was further analysed for the presence of BGCs using antiSMASH 6.0 (Medema et al. 2011). A total of 29 putative secondary metabolites were predicted by antiSMASH 6.0 (Table 3). Sivakala et al. (2021) reported 36 well-defined BGCs from *Streptomyces* sp. SAJ using antiSMASH.

Table 3

Secondary metabolite Biosynthetic Gene Cluster of *Streptomyces* sp. VITGV156 using antiSMASH 6.0

Region	Type	Most similar known cluster	Similarity
Region 2.1	Indole	5-isoprenylindole-3-carboxylate β -D-Glycosyl ester	23%
Region 2.2	Terpene	Isorenieratene	63%
Region 3.1	NRPS-like	Streptothricin	95%
Region 3.2	T3PKS	Herboxidiene	8%
Region 7.1	Ectoine	Ectoine	100%
Region 7.2	Melanin	Melanin	60%
Region 7.3	Siderophore	Desferrioxamin B / Desferrioxamine E	83%
Region 10.1	NRPS-like	-	-
Region 10.2	PKS-like, furan, Lanthipeptide-class-V	Methylenomycin A	9%
Region 11.1	Lanthipeptide-class-III	Catenulipeptin	60%
Region 12.1	Terpene	Albaflavenone	100%
Region 12.2	T2PKS	Spore Pigment	66%
Region 13.1	T2PKS, PKS-like	Fluostatins M-Q	67%
Region 15.1	Butyrolactone	Prejadomycin / Rabelomycin / Gaudimycin C / Gaudimycin D / Uwm6 / Gaudimycin A	6%
Region 16.1	Siderophore	-	-
Region 17.1	RiPP-like	-	-
Region 17.2	Terpene	Geosmin	100%
Region 17.3	Siderophore	Paulomycin	9%
Region 18.1	NRPS	CDA1b / CDA2a / CDA2b / CDA3a / CDA3b / CDA4a / CDA4b	72%
NRPS - non – ribosomal peptide synthetase			
T1PKS – Type 1 Polyketide Synthases			
T2PKS – Type 2 Polyketide Synthases			
T3PKS – Type 3 Polyketide Synthases			
RiPP - Ribosomally synthesized and post-translationally modified peptides			

Region	Type	Most similar known cluster	Similarity
Region 18.2	Terpene	Hopene	100%
Region 18.3	T1PKS	Vicenistatin	60%
Region 19.1	T1PKS	Sipanmycin	13%
Region 20.1	T1PKS	Nystatin A1	27%
Region 21.1	T1PKS	Streptovaricin	29%
Region 21.2	Terpene	Versipelostatin	5%
Region 21.3	RiPP-like	Informatipeptin	42%
Region 21.4	NRPS	Coelichelin	100%
Region 21.5	NRPS, lanthipeptide-class-i	Coelibactin	90%
Region 21.6	Lanthipeptide-class-iii	SapB	100%
NRPS - non – ribosomal peptide synthetase			
T1PKS – Type 1 Polyketide Synthases			
T2PKS – Type 2 Polyketide Synthases			
T3PKS – Type 3 Polyketide Synthases			
RiPP - Ribosomally synthesized and post-translationally modified peptides			

The Table 3 also shows some rare antibiotics such as coelichelin, fluostatins, vicenistatin, nystatin, sipanmycin, and informatipeptin. These antibiotics were previously reported in several strains such as coelichelin – *Streptomyces* sp. ICC1 (Gosse et al. 2019), fluostatins – *Streptomyces* sp. PKU-MA00045 (Jin et al. 2018), vicenistatin - *Streptomyces* sp. SD85 (Low et al. 2018), nystatin – *Streptomyces youssoufiensis* OUC6819 (Yao et al. 2018), sipanmycin – *Streptomyces* sp. strain CS149 (Malmierca et al. 2018), and informatipeptin – *Streptomyces* sp. VN1 (Nguyen et al. 2020).

Table 3 show how this species is unique with many biosynthetic gene clusters, having more possibility for novel compounds belonging to different clusters such as terpene (versipelostatin 5%), butyrolactone (prejadomycin 6%), T3PKS herboxidiene 8%), Siderophore (paulomycin 9%), PKS Lanthipeptide-class-V (methylenomycin A 9%) and T1PKS (sipanmycin 13%). As the compounds are having very low similarity (5 to 13%) they could be new derivatives of existing compounds.

BGCs are including an unidentified non ribosomally synthetases, and a ribosomally synthesized and post-translationally modified peptides and a siderophore compound. Also, three compounds show relatively less similarity (less than 20%) to the known natural products suggesting them to be unidentified

compounds. Cruz-Morales et al. (2017) reported these secondary metabolites like siderophore in *Streptomyces* sp. CC77. Of the total 29 BGCs responsible for production of secondary metabolites, 34% (10 BGCs) corresponds to the biosynthesis of peptide compounds, 24 % (7 BGCs) corresponds to the biosynthesis of polyketides, 13% (4 BGCs) corresponds to the synthesis of terpenes and 10% (3 BGCs) corresponds to the synthesis of siderophores. Similar report was published in *Streptomyces* sp. which consists by Park et al. (2021) 32 biosynthetic gene cluster, Non-ribosomal peptide synthetases, siderophores, and terpenes were also present in genomes.

An indole compounds less similar to 5-isoprenylindole-3-carboxylate β -D-Glycosyl ester was produced by the strain. Indole compounds are well known for their growth promoting activity. Similarly, Goudjal et al. (2015), suggested that Siderophores associated with *Streptomyces* sp.TL7 is involved in promoting plant growth. Analysis of bacteriocins by BAGEL revealed the presence of antimicrobial peptide zoocin A and lanthipeptides belong to class I and IV. This same Lanthipeptides are ribosomally synthesized and post-translationally modified microbial secondary metabolites by Iftime et al. (2015) in the strain *Streptomyces collinus* Tü 365. These results indicate that the strain *Streptomyces* sp. VITGV156 is indeed a novel strain producing a rich repertoire of secondary metabolites with promising applications.

Declarations

Author contributions

Authors PV, TN and JGC designed the work, executed it and compiled the results. PV, TN SS, KS and JGC discussed and wrote the manuscript

Availability of data and materials

The draft genome of *Streptomyces* sp. VITGV156 was submitted to in NCBI SRA portal under BioProject accession number PRJNA750872, BioSample accession number SAMN20499087, SRA accession number SRS9645416 and Accession ID 20499087

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Compliance with ethical standards

Conflict 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.

Ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

Figure 1

Genome map of *Streptomyces sp. VITGV156* generated using CG Viewer Genome map of *Streptomyces sp. VITGV156*, open reading frames with GC skew and GC content was highlighted in the map. The outer circles from first to third circle shows the positions of the ORF genes in clockwise and from fourth to six counter-clockwise directions, respectively. The seventh circle shows GC skew (higher than average GC content in green, lower than average in purple) and the second innermost circle shows the GC content of

the entire genome sequence. The innermost circle shows the size of the genome *Streptomyces* sp. VITGV156.