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Genomic Report on Lycopersene Producing Streptomyces sp. VITGV38

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Abstract

Streptomyces sp. VITGV38 is an endophyte from tomato plant (*Lycopersicon esculentum*). It produces potential secondary metabolites, like coelichelin, coelibactin, hopene, undecyl prodigiosin, geosmin, albaflavenone, germicidin, ectoine, and desferrioxamin B are commercially important in human and veterinary medicine. The potentiality of this genus *Streptomyces* is the diversity of its secondary metabolites. The three days old culture in starch casein agar plates showed their purple colouration. This was transferred to broth culture to analyse the pigment. GC-MS analysis of the crude extract (ethyl acetate) revealed pigmented compounds like lycoperesene at 25.094 retention time with 1.26% of the total crude extract. Whole genome sequence analysis of *Streptomyces* sp. VITGV38 reveals the complete genome has 7.8 million base pairs, twenty-six different kinds of secondary metabolite gene clusters were found. antiSMASH was used for the identification, annotation and analysis of metabolite gene clusters. The gene clusters for terpene (62%) produce pigments such as carotenoids, β-carotenes & isorenieratene. Four nonribosomal clusters involved in the biosynthesis of peptide compounds that encodes coelichelin, coelibactin, α-lipomycin & undecylprodigiosin are reported.

Keywords: Secondary metabolite, *Streptomyces*, antimicrobial, pigment, gene cluster, lycopersene, carotene.

Introduction

Tomato has a special nutritional value that makes it one of the most essential foods for protection. The antioxidant lycopene reduces heart diseases and cancer. Like most other plants, tomato plants (*Lycopersicon esculentum*) is also colonized by a wide range of microorganisms, and such plant-microbe interactions could have a positive effect on plant growth. *Streptomyces* that inhabit plant tissues are not detrimental to the plant or the environment and have a symbiotic or mutualistic relationship with their host plant. Even to the exterior, the roots of many plants are colonized by specific *Streptomyces* which is enabling these plants get absorbable form of nutrients from the soil (1).

Streptomyces species are Gram-positive soil bacteria, that belong to the order actinobacteria. Their filamentous nature with aerial mycelium gives a phenotype similar to fungi (2). They are well-recognized for the production of novel and valuable secondary metabolites. This *Streptomyces* sp. is producing more than 10,000 bioactive compounds till now, and remain the

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largest antibiotic-producing genus (3). Various endophytic *Streptomyces* sp. are a source of agro active compounds, biocontrol agents, plant growth promoters and enhance the physiological activity of the plant and increase their yield (4,5). And some can also synthesize pigments for the plants.

Streptomyces sp. produces many specialized metabolites such as pigments, food preservatives, antibiotics, insecticides, pesticides, plant growth hormones, anticancer compounds, antidiabetic, antimalarials, and immunosuppresents. The whole genome sequencing unravels the true potential of *Streptomyces*. Analysis of various genomes of *Streptomyces* sp. revealed that there are more than one type of biosynthetic gene clusters though they were isolated based on the presence of one metabolite (6). Whole-genome sequence information can identify the known and unknown gene clusters. Lycopersene are plant-based pigmented compounds that are widely produced by both single cellular and multi cellular. Lycopersene is important with identification in maize, carrot, tomato, vetch (*Vicia sativa*) and Ascomycete fungi (*Neurospora crassa*) species, and they found less than 0.015%. As far the chemical structure of lycoperene is concerned, it is a tetraterpenoids with a polyene hydrocarbon chain yeiled from eight isoprene units. The backbone C40 is modified in many ways, to give diverse chemical structures by cyclization and desaturation. Lycopersene has wide biological applications such as antibacterial, antiproliferative, antioxidant, insecticidal, antimutagenic and anticancer activity. In this study identify a new species *Streptomyces* sp. VITGV38 (MCC4869) with a potential gene to produce lycopene pigment was analysed and confirmed by GC-MS and whole genome sequence.

Materials and Methods:

Isolation

The strain VITGV38 was isolated from the traditional variety (*Arka Rakshak*) of tomato plants in the southern districts of Tamil Nadu, IndiaThe plant samples were subjected to a surface sterilization process, which involved treating them with a series of solutions including 70% ethanol for 1 min, 90% ethanol for 1 min, sodium hypochlorite (0.9%) for 4 min, 70% ethanol for 30 seconds, 10% NaHCO3 for 5 min, and finally, rinsing them in sterile distilled water for 3 minutes. (7). The plant parts, such as upper and lower stems, were then cut into small pieces, these cut segments were streaked in ISP2 (International Streptomyces Project - 2) agar plates for actinomycetes growth. After the microbial plates were inoculated, they were incubated at 30ºC for a period of seven days. Following this, individual colonies were separated and transferred into starch casein broth for further analysis (8, 9).

Characterization

Normal growth of *Streptomyces* sp. VITGV38 was observed only in the Starch casein agar. Morphology of VITGV38 was observed in a phase-contrast microscope (Model - MT5210/ MEIJI, Japan) and Scanning Electron Microscope (SEM; Carl Zeiss – Evo 18, Japan).

Biomass production

Pure *Streptomyces* sp. VITGV38 strain, which is an endophyte in tomato plants, was cultured in Starch Casein broth; when it reaches lag phase, it is used to seed, a separate 1000 ml Erlenmeyer flasks containing 250 ml of ISP2 medium, placed on a rotary shaker at 150 rpm. This is allowed to grow for seven days at 30°C.

For the metabolite extraction, cells were removed by centrifugation (2,000 rpm; 5 minutes; 4°C) and subsequently passed through a Whatman filter paper to get cell-free culture filtrate. Ethyl acetate was used as solvents at 1:1 ratio to extract the secondary metabolite. This is left in the shaker for overnight at 150 rpm. The extracts were recovered, and the organic solvent was evaporated. This process was repeated thrice, using a rotary evaporator (Eyela N-1000, Japan).

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GC-MS analysis of metabolite

The ethyl acette extract of *Streptomyces* ***sp. VITGV38 was*** analysed in GC-MS (Thermo Scientific, Waltham, MA). The temperature of the transfer line was 280°C. The temperature program as: an initial 50°C for 2 min, 50–150°C for 7°C per min, then 150–270°C for 5°C per min, and a final temperature of 270–310°C increasingly at 3.5°C per min. The peaks were compared with NIST library.

Whole-genome sequencing

The genomic DNA of the strain VITGV38 was isolated from a seven-day-old broth culture(10). The TruSeq Nano library preparation kit was used to prepare libraries, which were then sequenced using the Illumina platform on a NextSeq 500 machine by Macrogen Eurofins Genomics India Pvt. Ltd. Paired-end reads of 150 bp were produced with a minimum Phred quality score of Q30. To remove low-quality reads, a sliding window trimming of 10 bp was performed with a threshold of 20. The resulting reads were de novo assembled into scaffolds using the SPAdes assembler (v-3.13.0) method. A homology-based approach was used to identify the most closely related organism(s) by analyzing the assembled scaffolds of both models. Protein coding and RNA genes were identified in the final assembled draft genome(s) of VITGV38, and Prokka was used for gene prediction. KEGG annotations were performed for the proteins predicted by Prokka. (version 1.12). To functionally annotate the predicted genes of the VITGV156 genome sequences, a BlastX search was performed against the NCBI non-redundant protein database (nr) using the Basic Local Alignment Search Tool with an E-value cutoff of 1e-05 through the Diamond tool. Genomic map of the VITGV156 genome as a reference sequence blasted using CGViewer Server V1.0. (11). Thus, the circular genome map of VITGV156 was constructed.

Pigment gene cluster

antiSMASH v6.0 revealed the presence of biosynthetic gene clusters, its identification, annotation, and analysis of pigment-producing genes (12).

Result and Discussion

Tomato is the second most highly consumed plant-based food worldwide, with a broad range of clinically valuable secondary bioactive metabolites and the plant harbours a wide range of microbes (13). The surface sterilization process is the primary step to isolate and purify the endophytes. As the culture plates were without any contaminations, thus the sterilization protocol was confirmed to be perfect. Based on the morphological observation, 240 endophytic actinomycetes were categorized. The most abundant genus was found to be *Streptomyces* sp., as a similar finding was reported in the tomato plant by Goudjal *et al.* (14).

Morphology

The morphology of the endophytic *Streptomyces* sp. isolated from the tomato plants in Starch casein agar plate is shown in Fig. 1A. Its cultural characteristics, and other colony morphology were similar to other *Streptomyces* (Fig. 1A). The hyphae appeared as a continuous aerial structure and were found to form small, smooth-surfaced chains of spores. Each spore was observed to possess a cavity on one side at its center (Fig. 1B).

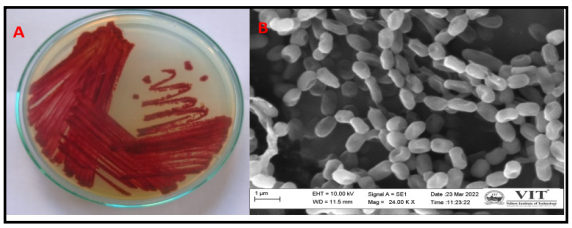

Fig. 1: Images of *Streptomyces* sp. VITGV38. (A) Petri plate with seven days old culture in starch casein agar with thick purple colour. (B) SEM image showing spore chain**.**

Biomass production

The liquid culture of VITGV38 started to develop small clumps of globules from the seventh day. The flask developed a few cell masses

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at the end of the 15th day. *Streptomyces* sp. VIT-GV38 grown in liquid culture media produced secondary metabolites. These dark purple pigments (Fig. 2) in the culture media are formed by the strain *Streptomyces* sp. VITGV38, which grows inwards, and secondary mycelium which grows outwards. A similar clump formation was reported in *Streptomyces olidensis* (15).

Fig. 2: Lycopersene production is shown *Streptomyces* sp. VITGV156 in ISP2 broth

A- Control broth. B – 15 days old nutrient broth for lycopersene production.

GC-MS analysis

The condensed ethyl acetate extract of *Streptomyces* sp. VITGV38 showed 30 peaks in the GC-MS chromatogram. Among them, lycopersene was identified at a retention time of 25.094 min (Fig. 3). Further analysis of GC-MS data and its retrieval of reference data with the NIST mass spectrophotometry database confirmed the presence of squalene in the extract. Lycopersene was reported in the squalene-hopene cyclase pathway of *Streptomyces globisporus* 1912 by Holembiovs'ka and Matselukh (16)

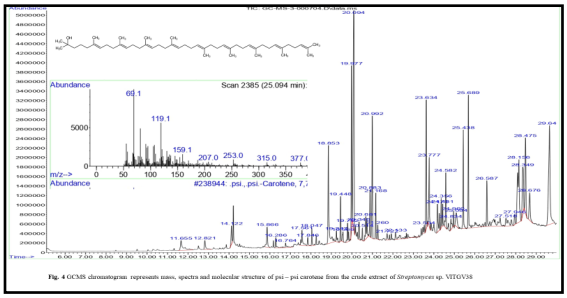

Fig 3: GCMS chromatogram represents mass, spectra and molecular structure of lycopersene from the crude extract of *Streptomyces sp*. VIT-GV38

Whole-genome sequencing

The draft genome details of *Streptomyces* sp. VITGV38 are summarized in Table 1. There were 7,715,899 reads representing 8.27 Mb and the *N*50 value of the assembly was 421,805 bp, with a G+C composition of the DNA of 72.28% and a genome size of 7,863,511 bp. A similar genome result was recorded for *Streptomyces* sp. FH025 contained 8,381,474 bp and an average G+C content of DNA, 72.51% (17). The number of genes not associated with blast hits was 151, and the total number of tRNA and rRNA operons was 81 and 5, respectively. Our previous study on *Streptomyces* sp. VITGV100 [18] also had 190 blast hit genes for tRNA and rRNA operons were 85 and 5, respectively. An overview of the genome features, G+C skew, G+C content, and genome size were identified using CG Viewer server V1.0 and Fig. 4 represents the circular genomic view of *Streptomyces* sp. VITGV156. In the circular genomic map generated for *Streptomyces* sp. VITGV38. Ring 1 of the circular view represents the open reading frame (ORF) of the forward strand (ORF genes). Rings 2, 3, and 4 indicate the start and stop codons of the forward strand, whereas Rings 5, 6, and 7 indicate the start and stop codons of the reverse strand of VITGV38. Rings 7 and 8 show the GC content and GC skew of the circular genomic view of VITGV38, with higher than average GC content in green and lower than average in purple. The first inner circle of

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the circular view shows the predicted size of the entire genome of Streptomyces sp. VITGV38. Interestingly, a similar representation of ORF genes, forward and reverse strands, GC content, GC skew, and circular genome size was also observed in the strain *Streptomyces mexicanus* Q0842 (19).

The whole-genome sequence was further analyzed for the presence of a secondary metabolite gene cluster using antiSMASH 6.0. In total, twenty-six biosynthetic gene clusters were identified in the strain VITGV38. Among these, eight gene clusters showed similarity to other BGCs with known functions. Eight biosynthetic gene clusters exactly match the existing BGCs (100%) (Table 2 and Fig. 5). Previously, *Streptomyces* sp. Babs14 showed 29 biosynthetic secondary metabolite gene clusters predicted using antiSMASH, among which eight gene clusters showed 100% genes from the known cluster (20). *Streptomyces iranensis, Streptomyces himalayensis and Streptomyces griseus* are showed important therapeutic potentioal (21).

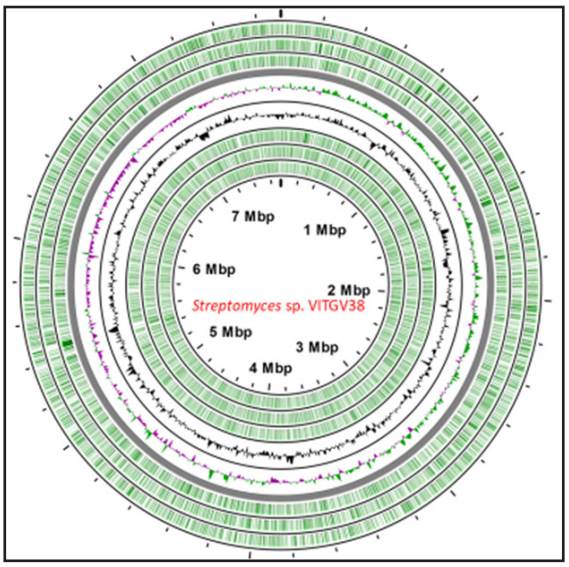

Fig 4: Genome map of *Streptomyces* sp. VTVGV38 generated using CG Viewer. Open reading frames with GC skew and GC content was highlighted in the map.

Table 1: General features of *Streptomyces* sp. VITGV38

S.no	Features	*Streptomyces* sp. VITGV38
1	Genome size (bp)	8.27 Mb
2	Total number of bases sequenced	7,715,899
3	GC content	72.28
4	Total genes	6,938
5	**orthoANIu value** *Streptomyces coelicoflavus* Strain NBRC15399	92.50
6	**DDH value** *Streptomyces coelicoflavus* Strain NBRC15399 &	48.40

Table 2: Potential BGCs of secondary metabolites in *Streptomyces* sp. VITGV38, predicted using antiSMASH 6.0

S. No	Region	Type	Most similar known cluster	Similarity
1	Region 2.1	NRPS	Coelichelin	100%
2	Region 2.3	NRPS	Coelibactin	100%
3	Region 2.4	Terpene	Hopene	100%
4	Region 2.5	Lanthipeptide class III	SapB	100%
5	Region 4.2	Terpene	Geosmin	100%
6	Region 6.2	Terpene	Abaflavenone	100%
7	Region 24.1	Ectoine	Ectoine	100%
8	Region 26.2	T3PKS	Germicidin	100%

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Fig. 5: Region of gene cluster predicted using antiSMAS

Conclusion

This study showed that *Streptomyces* sp. VITGV38 is a unique species producing pigments lycopersene. antiSMASH analysis proves this by decoding the genes in terpenoid cluster located in 26.3 core hits. To explore the pigmentation pattern and isolation for commercialization, further studies are required.

Availability of data and materials

The draft genome of *Streptomyces* sp. VITGV38 was submitted to in NCBI SRA portal under BioProject accession number PRJNA750621, BioSample accession number SAMN20475137, and SRA accession number SRR15293244.

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