Complete Genome Sequence of *Streptomyces* Sp. HSG2 From Rhizosphere Soil of Mangrove in Qingmei Gang, Sanya

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Research Article

Keywords: Secondary metabolites, Biosynthesis, Degradation, Natural products, Streptobactin, Nystatin A1

Posted Date: March 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-298540/v1>

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Abstract

Streptomyces sp. HSG2, was isolated from rhizosphere soil of mangrove forest sampled at Qingmei Gang, Sanya. The complete genome sequence of the strain HSG2 was obtained using PacBio Sequel HGAP.4 and comprised of 5,282,528 base pairs with a 71.9 mol% G + C content, 4504 protein-coding genes, 71 RNAs. In silico analysis confirmed the genes associated with polysaccharide hydrolyzation, hydrocarbon degradation and aerobic denitrification. 24 natural product biosynthesis gene clusters for secondary metabolites were identified as well, including those for Streptobactin and Nystatin A1. The complete genome sequence indicated that *Streptomyces* sp. HSG2 will provide insight into the biosynthesis and regulatory mechanisms for the secondary metabolites, and propose potential use in biotechnological and novel bioactive natural product biosynthesis applications.

Introduction

The genus *Streptomyces* was proposed by Waksman & Henrici (Waksman and Henrici 1943) to accommodate aerobic, spore-forming actinomycetes, which having almost 700 taxonomically validated species (www.bacterio.net/streptomyces.html) and being the largest genus in the phylum *Actinobacteria*. Members of the phylum *Actinobacteria* have been one of the major origins of bioactive natural products, owing to their capability to produce plentiful secondary metabolites comprising diverse chemical structures and biological activities (Lee et al. 2014). In particular, the genus *Streptomyces* has been recognized for their ability to produce a wide range of bioactive compounds with antiviral, antifungal, antiparasitic, antitumoral and immunosuppressant activity (Jones and Elliot 2017). Because of the beneficial properties of *Streptomyces*, people are sticking in exploring these living beings in different habitats such as marine, terrestrial, desert, plant tissues and extreme environments (Cheng et al. 2015). In recent years, people are increasing interest in the isolate of new *Streptomyces* strains from under-explored zones such as the mangrove environment and hope extract new and beneficial compounds from these novel species. For example, *Streptomyces malaysiense* MUSC 136^T with antioxidative activity and cytotoxic potential against human cancer cell lines (Ser et al. 2016a). A novel mangrove soil actinobacterium *Streptomyces antioxidans* MUSC 164^T (Ser et al. 2016b) with antioxidative and neuroprotective potential, has been isolated from a mangrove soil. Indeed, it is known that mangrove forest is mostly located in the intertidal area of tropical and subtropical coastal regions (Ser et al. 2015) and nurtures a variety of terrestrial, freshwater, and marine organisms. Therefore, the unique environment of mangroves leads to vastly rich in nutrient and organic matter resulting from countless microbial enzymatic and metabolic activities (Mangamuri et al. 2012).

In this study, we report the complete genome sequence of *Streptomyces* sp. HSG2, which was isolated from rhizosphere soil of mangrove forest sampled at Qingmei Gang, Sanya. Using the genome sequencing, 24 putative secondary metabolite biosynthetic gene clusters required for the biosynthesis of several natural products were predicted in the genome of HSG2. Besides, we founded some genes associated with aerobic denitrification, hydrocarbon degradation and polysaccharide hydrolyzation, as well as several other genes involved in the degradation of xenobiotic substances. To the best of our

knowledge, this is the first report on the bioactive properties of *Streptomyces* from mangrove environments in Qingmei Gang, Sanya. Thus, the complete genome sequence of HSG2 will as an intriguing case to provide insight into the biosynthesis and regulatory mechanisms for the secondary metabolites, and propose potential use in biotechnological and natural product biosynthesis applications.

Materials And Methods

Bacterial strain and genomic DNA extraction

The strain of HSG2 was isolated from rhizosphere soil of mangrove forest sampled at Qingmei Gang, Sanya (18.23° S, 109.63° E) and spread on isolation medium (10 g glucose, 5 g peptone, 5 g yeast extract, 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10 g NaHCO_3 , 27 g $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$, 20 g agar; 1 litre natural seawater, pH 10) and incubated at 28°C for three weeks. The individual bacterial colony was picked and continuously streaked on plates with marine agar 2216 (MA; Becton Dickinson) to procure pure cultures. They were then stored at -80°C as glycerol suspension [15% (v/v) with 0.5% (w/v) trehalose]. Genomic DNA was extracted using an EasyPure Bacteria Genomic DNA Kit (TransGen Biotech, Beijing, China) according to the manufacturer's instructions.

Genome sequencing and annotation

The genome of strain HSG2 was then sequenced by Tianjin Biochip Corporation (Tianjin, China) using the PacBio Sequel system (Pacific Biosciences, Menlo Park, CA, USA). The mean depth of genome coverage was 639-fold. *De novo* genome assembly was carried out following a hierarchical genome assembly process (HGAP) (Chin et al. 2013) using HGAP.4 (SMRT Link V6.0.0.47841; Pacific Biosciences), to form a single contig with no gaps. The bacterial proteome was predicted using a protein Basic Alignment Search Tool-based comparison against the Clusters of Orthologous Groups database. Analysis and comparison of the functional annotation were performed using the Kyoto Encyclopedia of Genes and Genomes automatic annotation server. RNAmmer (Lagesen et al. 2007) and tRNAScanSE (Lowe and Chan 2016) were used to identify ribosomal RNA (rRNA) genes and transfer RNA (tRNA) genes, respectively. Clustered regularly interspaced short palindromic repeats (CRISPR) elements were identified by CRISPRCasFinde (Couvin et al. 2018). Genomic islands were determined with the web tool IslandViewer using two independent methods Islander and IslandPath-DIMOB (Bertelli et al. 2017). The presence of secondary metabolite gene clusters was examined by antiSMASH 5.0 platform (Blin et al. 2019).

Phylogenetic analysis

The near-complete 16S rRNA gene sequences were obtained with two universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') for Sanger sequencing. The sequences of the 16S rRNA genes were compiled with Contig Express software and uploaded to EzBioCloud (www.ezbiocloud.net/) to achieve the gene sequence similarity values with the reference species which have valid published names. For 16S rRNA gene phylogenetic analysis, the type strains of

closely related species were included in the alignments. All sequence alignments were carried out using MEGA 7 software (Kumar et al. 2016). Phylogenetic trees were reconstructed with the Neighbor-Joining (Saitou and Nei 1987) and bootstrap values based on 1000 replications. To further investigate the taxonomic rank of strain HSG2, ninety-two bacterial core genes based on the up-to-date bacterial core gene (UBCG) tool (<https://www.ezbiocloud.net/tools/ubcg>) were used for phylogenomic tree reconstruction using default parameters (Na et al. 2018). The selection of the representative genes was based on 1429 complete genome sequences, covering 28 phyla and providing a set of genes present in the majority of the genomes or highly conserved single copy genes.

Nucleotide sequence accession number

The complete genome sequence of strain HSG2 has been deposited in the GenBank database under the accession number CP066801. The strain is available from the Marine Culture Collection of China under the accession number MCCC 1A06678.

Results And Discussion

The complete 16S rRNA gene sequence confirmed that strain HSG2 belonged to the genus *Streptomyces*, with 98.0% nucleotide sequence identity to the closest type strain, *Streptomyces hyderabadensis* OU-40^T (Reddy et al. 2011). Neighbor-joining tree (Fig. S1) based on 16S rRNA gene sequences and phylogenomic tree (Fig. 1) based on the 92 bacterial highly conserved single-copy genes using the UBCG tool obviously indicated strain HSG2 constituted a separated branch in the clade of the genus *Streptomyces*. Therefore, we propose that strain HSG2 is a novel member of the genus *Streptomyces*.

The genome characteristics of strain HSG2 are presented in Table 1. The complete genome of HSG2 contains a circular chromosome of 5,282,528 bp with an average G + C content of 71.9 mol%, which is consisted of 4504 protein-coding genes, 63 tRNA genes, 18 rRNA operons. Seven CRISPRs and thirty genomic islands are identified in the genome (Table S1 and Table S2). A circular map of the genome was generated in GCView (Stothard and Wishart 2005) (Fig. 2).

Table 1
General genome features of *Streptomyces* sp.
HSG2

Feature	Value
Genome size (bp)	5,282,528
N50 Contig Length	5,277,571
GC content (%)	71.9
Total genes	4585
Protein coding genes	4504
CDS region ratio (%)	85.9
CRISPRs	7
Genomic islands	30
tRNA genes	63
rRNA genes	18
BioProject	PRJNA685641
BioSample	SAMN17088907
GenBank accession no.	CP066801

The genome of strain HSG2 contains genes encoding multiple enzymes that could be applied in biodegradation processes, including amylase, protease, lipase, phospholipase and glycosyl hydrolase (Table S3). In addition, the genome of strain HSG2 contains genes encoding a partial denitrification pathway (*nirABDJK*). Genes encoding four hydrocarbon degradation-associated proteins and other xenobiotic degradation-associated proteins were also recognized. Therefore, the genome mining analysed in this study proposes that strain HGS2 has functional potential in biodegradation applications and provides a frame to better learn biodegradation mechanisms and processes.

The presence of secondary metabolite gene clusters was examined by antiSMASH 5.0 platform (Blin et al. 2019). We found 24 putative biosynthetic gene clusters, which were predicted to be related to the biosynthesis of different types of secondary metabolites, including four non-ribosomal peptides, four terpenes, three polyketides, three ectoines, one indole, one ladderane, one oligosaccharide, one bacteriocin, one blactam, one TfuA-related, one linaridin, one thiopeptide, one lantipeptide and one siderophore (Table S4). Among the 24 putative biosynthetic gene clusters, five putative gene clusters show 100% similarity (100% of genes showing similarity) to the reported gene clusters staurosporine, isorenieratene, geosmin, venezuelin and ectoine. Five putative gene clusters show high similarity (72%-84%) to the reported nystatin A1 (72%), spore pigment (75%), hopene (76%), streptobactin (76%) and triacsins (84%) gene clusters, respectively. The remaining ten putative gene clusters show low

similarity ($\leq 30\%$) to the most similar clusters and four gene clusters are no similar to any reported gene clusters, which may be new ones. The existence of plenty secondary metabolite gene clusters indicates that strain HSG2 could be a producer for novel bioactive natural products.

Nystatin A1 is a polyene macrolide antibiotic, which has 37 membered lactone rings containing 6 conjugated double bonds (Fig. 3b). The special structural feature most likely make a contribution to the type of action of these compounds, decide to be an interaction with sterols present in the fungal membranes (Bolard 1987). These interactions induce production of polyene–sterol complexes, which made themselves into membrane channel making the membrane penetrable to ions and water, which lead to cell death. Therefore, the polyene antibiotics not only being antifungal and antibacterial agents, but also have antiviral and immunostimulant activities (Abu-Salah 1996; Ciftci et al. 1984). All core biosynthetic genes (*nysABCDEFGHIJK*) and transport-related genes (*nysGH*) (Fig. 3a, Table S5) implicated in production nystatin A1 were found in the genome of strain HSG2. Functional assignments for the proteins encoded within the cluster open possibilities for genetic manipulations with the aim of producing novel nystatin derivatives.

Streptobactin is a catechol-type siderophore, which possesses a catechol bonded arginine-substituted siderophore framed on a cyclic triester backbone of threonine (Fig. 3c). As we known, siderophore facilitates the microbial acquisition of iron in the ocean environment and due to its efficient siderophore-mediated iron acquisition mechanisms, siderophores may be developed as novel antimicrobials by covalently attaching clinical antibiotics to marine siderophores or novel iron chelator desferals (Hamilton et al. 2014; Page 2013; Souto et al. 2012; Waksman and Henrici 1943). In genome of strain HSG2, the genes (*dhbACEBG*) coding for converting chorismate to 2,3- dihydroxybenzoate and non-ribosomal peptide biosynthesis, as well as genes (*griA-H*) involved in the import, uptake, and utilization of siderophores were all existed (Fig. 3d, Table S6), suggesting that it may produce new natural products with great potential in medicine.

Declarations

Funding

This work was supported by grants from the Scientific Research Foundation of Third Institute of Oceanography, MNR, No. 2019011 and China Ocean Mineral Resources R&D Association (COMRA) Program (No. DY135-B2-01).

Conflicts of interest

The authors have no financial conflicts of interest to declare.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

References

1. Abu-Salah KM (1996) Amphotericin B: an update. Br J Biomed Sci 53:122-133. [https://doi.org/10.1016/S0009-8981\(00\)89024-5](https://doi.org/10.1016/S0009-8981(00)89024-5)
2. Bertelli C et al. (2017) IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. Nucleic Acids Res 45:W30-W35. 10.1093/nar/gkx343
3. Blin K et al. (2019) antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:W81-W87. <https://doi.org/10.1093/nar/gkz310>
4. Bolard J (1987) How do the polyene macrolide antibiotics affect the cellular membrane properties? Biochimica et Biophysica Acta 864:257-304. [https://doi.org/10.1016/0304-4157\(86\)90002-X](https://doi.org/10.1016/0304-4157(86)90002-X)
5. Cheng K, Rong X, Pinto-Tomas AA, Fernandez-Villalobos M, Murillo-Cruz C, Huang Y (2015) Population genetic analysis of *Streptomyces albidoflavus* reveals habitat barriers to homologous recombination in the diversification of streptomycetes. Appl Environ Microbiol 81:966-975. <https://doi.org/10.1128/AEM.02925-14>
6. Chin CS et al. (2013) Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563-569. <https://doi.org/10.1038/nmeth.2474>
7. Ciftci T, Borkman TA, McDaniel LE, Schaffner CP (1984) Comparative analysis of hexaene antibiotics. J Antibiot (Tokyo) 37:876-884. <https://doi.org/10.7164/antibiotics.37.876>
8. Couvin D et al. (2018) CRISPRCasFinder, an update of CRISPRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. Nucleic Acids Res 46:W246-W251. <https://doi.org/10.1093/nar/gky425>
9. Hamilton JL, Hatef A, Imran ul-Haq M, Nair N, Unniappan S, Kizhakkedathu JN (2014) Clinically approved iron chelators influence zebrafish mortality, hatching morphology and cardiac function. PLoS One 9:e109880. <https://doi.org/10.1371/journal.pone.0109880>

10. Jones SE, Elliot MA (2017) *Streptomyces* exploration: competition, volatile communication and new bacterial behaviours. Trends Microbiol 25:522-531. <https://doi.org/10.1016/j.tim.2017.02.001>
11. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33:1870-1874. 10.1093/molbev/msw054
12. Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100-3108. <https://doi.org/10.1093/nar/gkm160>
13. Lee LH et al. (2014) Diversity and antimicrobial activities of actinobacteria isolated from tropical mangrove sediments in Malaysia. Sci World J 2014:698178. <https://doi.org/10.1155/2014/698178>
14. Lowe TM, Chan PP (2016) tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44:54-57. <https://doi.org/10.1093/nar/gkw413>
15. Mangamuri et al. (2012) Isolation, Identification and Molecular Characterization of Rare Actinomycetes from Mangrove Ecosystem of Nizampatnam. Malaysian J of Microbiol
16. Na S-I, Kim YO, Yoon S-H, Ha S-m, Baek I, Chun J (2018) UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. J Microbiol 56:280-285. <https://doi.org/10.1007/s12275-018-8014-6>
17. Page MGP (2013) Siderophore conjugates. Ann Ny Acad Sci 1277:115-126. <https://doi.org/10.1111/nyas.12024>
18. Reddy TVK, Mahmood S, Paris L, Reddy YHK, Wellington EMH, Idris MM (2011) *Streptomyces hyderabadensis* sp. nov., an actinomycete isolated from soil. Int J Syst Evol Microbiol 61:76-80. <https://doi.org/10.1099/ijs.0.020446-0>
19. Saitou NM, Nei M (1987) The Neighbor-Joining Method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 24:189-204. 10.1093/oxfordjournals.molbev.a040454
20. Ser HL, Palanisamy UD, Yin WF, Chan KG, Goh BH, Lee LH (2016a) *Streptomyces malaysiense* sp. nov.: A novel Malaysian mangrove soil actinobacterium with antioxidative activity and cytotoxic potential against human cancer cell lines. Sci Rep 6:24247. <https://doi.org/10.1038/srep24247>
21. Ser HL et al. (2016b) *Streptomyces antioxidantans* sp. nov., a novel mangrove soil actinobacterium with antioxidative and neuroprotective potentials. Front Microbiol 7:899. <https://doi.org/10.3389/fmicb.2016.00899>
22. Ser HL, Zainal N, Palanisamy UD, Goh BH, Yin WF, Chan KG, Lee LH (2015) *Streptomyces gilvigriseus* sp. nov., a novel actinobacterium isolated from mangrove forest soil. Antonie Van Leeuwenhoek 107:1369-1378. <https://doi.org/10.1007/s10482-015-0431-5>
23. Souto A, Montaos M, Balado M, Osorio C, Rodriguez J, Lemos M, Jiménez C (2012) Synthesis and antibacterial activity of conjugates between norfloxacin and analogues of the siderophore vanchrobactin. Bioorgan Med Chem 21 <https://doi.org/10.1016/j.bmc.2012.10.028>
24. Stothard P, Wishart DS (2005) Circular genome visualization and exploration using CGView. Bioinformatics 21:537-539. <https://doi.org/10.1093/bioinformatics/bti054>

Figures

Figure 1

Phylogenomic tree showing the relationship between strain HGS2 and members of the genus *Streptomyces*. The phylogenetic relationship of the related genomes was determined using UBCG pipeline3 tool with GTR + CAT model based on concatenated alignment of 92 core genes. Genome sequences for each of the type strains are available from the NCBI databases, with the GenBank accession numbers shown in parentheses. Bootstrap values (expressed as percentages of 1000 replications) over 50 % are shown at branching nodes. Bar, 0.02 substitutions per nucleotide position

Figure 2

Complete genome map of *Streptomyces* sp. HSG2. Rings from the outside to the center indicate: 1) scale marks for genome size (Mb); 2,3) protein-coding genes on the forward/reverse strand; 4,5) tRNA (black) and rRNA (orange) genes on the forward/reverse strand; 6) Genomic islands, prediction methods: Integrated (red) and IslandPath-DIMOB (blue); 7) GC content. Protein-coding genes are color coded according to their COG categories.

Figure 3

a Overview of the Nystatin A1 biosynthetic pathway. b Chemical structure of the Nystatin A1. c Chemical structure of the Streptobactin. d Overview of the Streptobactin biosynthetic pathway. The transcriptional orientation of the genes is indicated by arrows and different colors show the genetic organization of the gene cluster.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [HSG2supplement.pdf](#)