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Whole Genome Sequence of *Streptomyces* sp. from Novel Marine Actinomycetes

Hyeon Kyeong Lee, Heung-Soon Park, Eung-Soo Kim, and Si-Sun Choi*

Department of Biological Sciences and Bioengineering, Inha University, Incheon 22212, Republic of Korea

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This paper presents the complete genome sequence of a novel marine actinomycete, *Streptomyces* sp. MMBL 11-1. The genome of *Streptomyces* sp. MMBL 11-1 was obtained through next-generation sequencing using the PacBio Sequel system and Illumina platform provided by Macrogen, Korea. The assembled genome consists of five contigs, with a total length of 8,496,900 bp and a G+C content of 71.6%. The genome harbors multiple biosynthetic gene clusters (BGCs) associated with producing microbial natural products (MNPs). The comprehensive genomic information of this type of strain will serve as a valuable resource for identifying other marine actinomycetes strains.

Keywords: *Streptomyces* sp. MMBL 11-1, marine actinomycetes, whole-genome sequencing, identification, next-generation sequencing

Marine actinomycetes are prokaryotes with significant economic value because they can produce a wide range of natural products. Several genera of marine actinomycetes have been identified, including *Actinomadura*, *Aeromicrobium*, *Gordonia*, *Marinophilus*, *Micromonospora*, *Nonomuraea*, *Rhodococcus*, *Saccharomonospora*, *Saccharopolyspora*, *Salinispora*, *Streptomyces*, *Williamsia*, and *Verrucosispora* [1]. Among these genera, *Streptomyces* is the most prevalent in natural environments, exhibiting significant morphological, physiological, and biochemical diversity [1]. Under extreme marine conditions characterized by elevated pressures, salinity, temperatures, and limited nutrient availability, actinomycetes adapt by synthesizing natural products with distinct and unique structures, which ensure their survival in such environments [2]. This paper presents the entire genome sequencing results of *Streptomyces* sp. MMBL 11-1, an aerobic Gram-positive bacterium characterized by a high G+C content and a complex secondary metabolism. *Streptomyces* sp. MMBL 11-1 was isolated from a deep-sea sponge *Spheciospongia panis* near Moon Island in Jeju, South Korea, with samples provided generously by KIOST (Korea Institute of Ocean Science & Technology). The collected sponge samples were processed into liquid samples by grinding and subsequent removal of the sponge material through filtration. An actinomycetes-specific medium (ISP4, ISP2, Marin agar) supplemented with 2% NaCl was used to selectively eliminate dominant bacteria other than actinomycetes. Furthermore, 10 μM nalidixic acid and 20 μM cycloheximide, antibiotics known to inhibit the growth of other bacteria and fungi, were added to the medium. The cultures were then incubated at 25℃ for a period ranging from one week to one month. Morphological actinomycetes-like strains were isolated from these cultures and cultivated in 500 ml Tryptic soy broth medium (BD DIFCO) for two days at 28℃.

The genomic DNA was extracted from the cultured

***Corresponding author**
Phone: +82-32-860-8825, Fax: +82-32-865-4046
E-mail: sisun95@gmail.com

strains using a Wizard® DNA Purification kit (Promega). Both draft gene sequencing and whole genome sequencing were then performed using the extracted genomic DNA. Whole genome sequencing utilized the PacBio Sequel system with PacBio Sequel Microbial Library Construction, conducted by Macrogen Inc.

The Illumina HiSeq platform with TruSeq Nano DNA (350 bp insert size) library was used for draft gene sequencing, and the sequencing process was conducted by Macrogen Inc. Subsequently, the subreads generated from the PacBio Sequel system were assembled using the Microbial assembly application of SMRTlink 10.1.0.119588, based on the Hierarchical Genome Assembly Process (HGAP) [3]. The default options were employed for the analysis within the Microbial assembly application.

Error correction was performed by quality filtering of the Illumina raw reads. Specifically, reads in which 90% of the bases had a phred score of 30 or higher were selected for the error correction step. The assembly was refined using high-quality Illumina reads using Pilon v1.21 [4].

Gene prediction and basic annotation were performed using Prokka v1.14.6 [5] with the options, --compliant, --rnammer, and --addgenes. Subsequently, the predicted protein sets underwent further annotation using InterProScan v5.30-69.0 [6] and psiblast v2.4.0 [7], using the EggNOG DB v4.5 [8]. Circular maps illustrating each contig were generated using Circos v0.69.3 [9].

Whole genome sequencing yielded five contigs, with a total genome size of 8.5 Mb. Contig 1, the largest contig, spanned 8 Mb and was analyzed using the antiSMASH program (Table 1). The analysis revealed the presence of 36 biosynthetic gene clusters (BGCs) expected to be involved in the production of microbial natural products (MNPs). In addition, contig 2 contains two BGCs. Among these BGCs, the nonribosomal peptides (NRPs), polyketides (PKSs), and NRP-PKS hybrids are particularly noteworthy and hold the potential for producing valuable natural products. The complete genome sequences of of *Streptomyces* sp. MMBL 11-1 (CP117709) and plasmids (CP117710 – CP1177013) have been deposited in the GenBank sequence database.

A phylogenetic tree was constructed based on the 16S-23S ITS region sequences of *Streptomyces* sp. MMBL 11-1, as shown in Fig. 1. The tree was generated using NCBI BLAST and MEGAX, showing that *Streptomyces* sp. MMBL 11-1 represents a novel marine-derived acti-

Table 1. Analysis of secondary metabolite 36 BGCs by antiSMASH of *Streptomyces sp*. MMBL 11-1.

BGC	Type	Length (kb)
#1	butyrolactone	11
#2	terpene	22
#3	terpene	24
#4	NRP-metallophore, NRPS	55
#5	T3PKS	40
#6	NRPS, NRPS-like	48
#7	hgIE-KS	53
#8	NRPS-like	28
#9	NRPS-like, betalactone, terpene	61
#10	ectoine	10
#11	NRPS	73
#12	NI-siderophore	10
#13	lanthipeptide-class-ii, lanthipeptide-class-iii	31
#14	NI-siderophore	10
#15	lanthipeptide-class-i	26
#16	lanthipeptide-class-v	42
#17	NRPS, T1PKS	53
#18	lanthipeptide-class-iii	21
#19	NRPS-like, T1PKS	51
#20	thiopeptide, LAP	27
#21	melanin	8
#22	lanthipeptide-class-iii	23
#23	terpene	18
#24	NI-siderophore	13
#25	T2PKS, PKS-like, betalactone, RiPP-like, arylpolyene, ladderane, NRPS-like	92
#26	NRPS	42
#27	NRPS-like, NRPS, T3PKS, ectoine, phenazine	133
#28	terpene	27
#29	NRPS, T1PKS	49
#30	RiPP-like	10
#31	NRPS-like, T1PKS, NI-siderophore	46
#32	betalactone	31
#33	RiPP-like	9
#34	melanin	10
#35	T3PKS	41
#36	LAP, thiopeptide	19

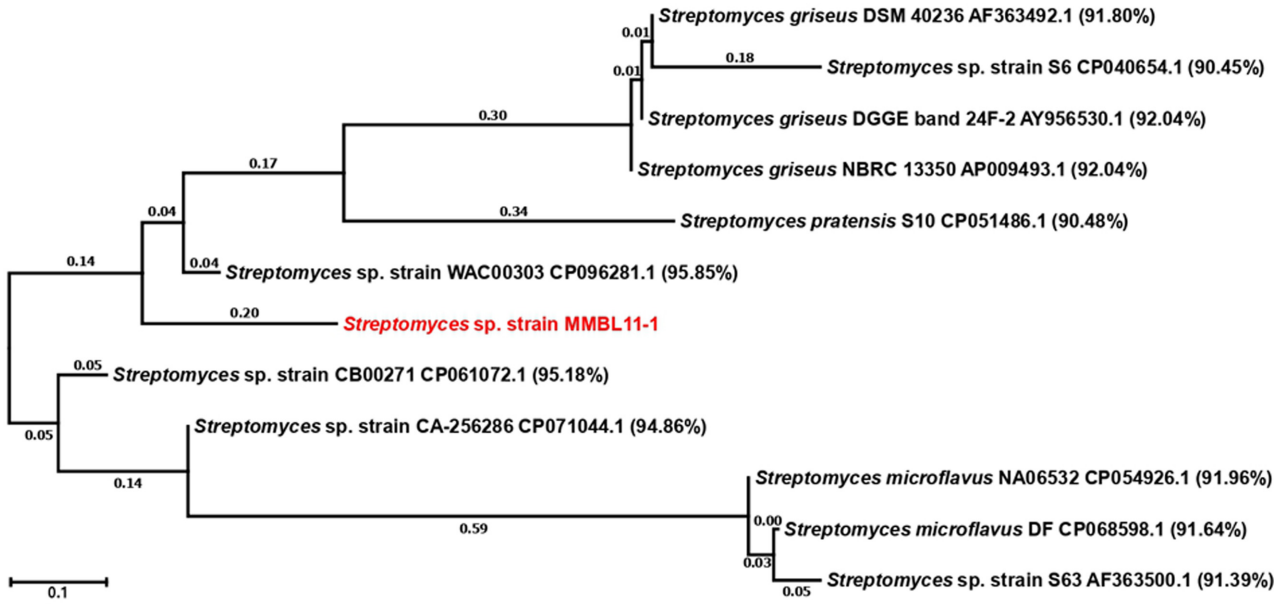

Fig. 1. Unrooted phylogenetic tree constructed to illustrate the relationship between *Streptomyces* sp. MMBL 11-1 and other related taxa within the genus *Streptomyces*. The Neighbor-Joining method [10] was used to infer the evolutionary history, and the analyses were performed using MEGAX.

nomycete. This study lays the groundwork for exploring marine actinomycetes and discovering novel microbial natural products (MNPs). Furthermore, these MNPs have the potential to act as promising lead compounds with drug-like properties, thus providing invaluable assistance in pharmaceutical research and development.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Dharmaraj S. 2010. Marine *Streptomyces* as a novel source of bioactive substances. *Springer* **26**: 2123-2125.
2. Li X, Qin L. 2005. Metagenomics-based drug discovery and marine microbial diversity. *Trends Biotechnol.* **23**: 539-543.
3. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, *et al.* 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat. Methods* **10**: 563-569.
4. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, *et al.* 2014. Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* **9**: e112963.
5. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**: 2068-2069.
6. Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, *et al.* 2014. InterProScan 5: genome-scale protein function classification. *Bioinformatics* **30**: 1236-1240.
7. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, *et al.* 2009. BLAST+: architecture and applications. *BMC Bioinformatics*. **10**: 421.
8. Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, *et al.* 2016. eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Res.* **44**: D286-293.
9. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, *et al.* 2009. Circos: An information aesthetic for comparative genomics. *Genome Res.* **19**: 1639-1645.
10. Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406-425.