Korean Journal of Microbiology (2022) Vol. 58, No. 2, pp. 96-98
DOI https://doi.org/10.7845/kjm.2022.2022

pISSN 0440-2413
eISSN 2383-9902

Whole genome sequence of a *Pseudoalteromonas* sp. strain SiA1 isolated from a *Pterocirrus* species in the Antarctic Ocean

Kyuwon Jeon[1], Sia Kang[1#], Young Jun Lee[1], Dockyu Kim[2], and Eungbin Kim[1*]

[1]*Department of Systems Biology, Yonsei University, Seoul 03722, Republic of Korea*
[2]*Division of Polar Life Sciences, Korea Polar Research Institute, Incheon 21990, Republic of Korea*
[#]*Present address: Molecular Diagnosis Development Team1, SD Biosensor, Suwon 16690, Republic of Korea*

남극해 서식 불가사리(*Pterocirrus* sp.)에서 분리한 *Pseudoalteromonas* sp. strain SiA1의 유전체 염기서열

전규원[1] · 강시아[1#] · 이영준[1] · 김덕규[2] · 김응빈[1*]

[1]연세대학교 시스템생물학과, [2]극지연구소 극지생명과학연구부, [#]현재 소속: SD 바이오센서 분자진단개발본부

(Received April 1, 2022; Revised June 17, 2022; Accepted June 17, 2022)

In this study, whole-genome sequencing analysis was conducted on *Pseudoalteromonas* sp. strain SiA1 isolated from the intestines of the Antarctic Ocean-dwelling starfish (*Pterocirrus* sp.). Its complete genome sequence consists of two chromosomes, which have 3,355,071 bp and 715,567 bp, respectively. Also, their G + C contents are 40.44% and 39.79%, respectively. A computer-aided analysis detected a total of 3,597 open reading frames, of which at least 98 (~2.7%) are suspected to encode different kinds of proteolytic enzymes.

Keywords: Antarctic Ocean, extracellar protease, starfish, symbiotic

The Antarctic Ocean is the world's southernmost ocean. The Ross Sea is a deep bay in Antarctica, located between Victoria Land and Marie Byrd Land at approximately 75°S, 175°W. It has unique geological features. Its continental shelf is extensive and its accelerated current causes substantial vertical and horizontal exchanges of nutrient-rich water making it a diverse habitat (Smith *et al*., 2007). The Ross Sea is one of the most productive regions of the Antarctic Ocean and is home to a wide array of organisms (Fabiano and Pusceddu, 1998). The benthos such as starfish, oysters, and sea cucumbers live at the bottom of seas and lakes . Some microorganisms are known to live on surfaces or inside of benthos and produce proteolytic enzymes, which help their hosts digest food (Burnett *et al*., 1997; Russell, 1998; Ravenschlag *et al*., 2001; Li *et al*., 2009; Cristóbal *et al*., 2011; Lee *et al*., 2014). This study was initiated to gain insights into such symbiotic bacteria.

The intestines of starfish (a member of genus *Pterocirrus*) caught from the Terra Nova Bay of the Ross Sea were homogenized using a tissue homogenizer and spreaded on Marine Broth (MB) agar medium containing 1% skim milk to differentiate bacteria with extracellular proteolytic activities. Initially isolated as protease positive, strain SiA1 produced a clear zone of 37 mm in diameter surrounding a 1.0-mm diameter colony in 24 h at 25°C.

In order to taxonomically identify strain SiA1, its 16S rRNA gene was amplified via colony PCR 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primers and the PCR product was directly sequenced with same primer pairs. Analysis of the nucleotide sequence on BLAST showed that SiA1 16S rRNA sequence has high levels of identity (ca. 99%) with those from several different

***For correspondence.** E-mail: eungbin@yonsei.ac.kr;
Tel.: +82-2-2123-2651; Fax: +82-2-312-5657

Pseudoalteromonas species. SiA1 was thus positively identified as a *Pseudoalteromonas* sp.

In an effort to better understand the proteolytic potential of SiA1, its genome was sequenced using PacBio RSII and Illumina HiSeq (Macrogen, Inc.). The resulting sequences were de novo assembled using HGAP v.3.0 and Pilon v.1.21. There were a total of 839,170,695 subread bases and the genome coverage was 206x. Then, the genome was annotated using the NCBI prokaryotic genome annotation pipeline. Annotation method and software revision were GenMarks-2+ and v.5.2, respectively.

The features of SiA1's complete genome are summarized in Table 1. SiA1's whole genome sequence was composed of 4,070,638 bp across two chromosomes. Chromosomes 1 and 2 were determined to contain 3,355,071 bp and 715,567 bp, respectively. A total of 3,597 coding sequences (CDSs) were identified (3,001 in chromosome 1 and 596 in chromosome 2). G + C content of each chromosome was calculated to be 40.44% and 39.79%, respectively. Also, average nucleotide identity (ANI) analysis revealed that SiA1 was most closely related to *Pseudoalteromonas tetraodonis* (Fig. 1).

The annotation results revealed the presence of a total of 98 CDSs related to proteolytic activities. Among the 98 CDSs, eighty three are present on chromosome 1 while chromosome 2 harbors the remaining fifteen. Subsequently, all the 98 CDSs were carefully examined for the presence of signal peptide sequences to select extracellular enzyme candidates. Twenty five CDSs from chromosome 1 and eleven ORFs from chromosome 2 were found to have signal peptide sequences, respectively. The whole genome sequences were deposited in GenBank under accession numbers CP076084 (chromosome 1) and CP076085 (chromosome 2).

Table 1. Genome features of *Pseudoalteromonas* sp. strain SiA1

	Chromosome 1	Chromosome 2
Genome size (bp)	3,355,071	715,567
Number of contigs	1	1
G + C contents (%)	40.44	39.79
CDS	3,001	596
tRNA	100	0
rRNA	25	0

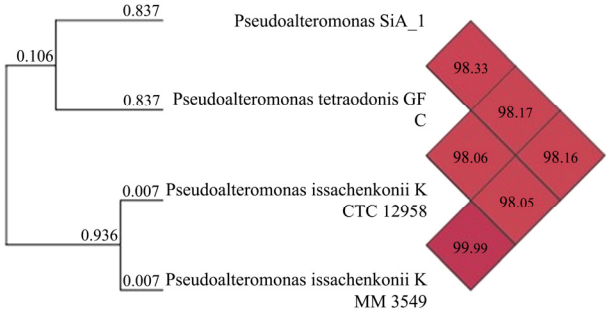

Fig. 1. Dendrogram and heatmap of ANI values of genomes from four closely related *Pseudoalteromonas* spp. ANI value was calculated using OrthoANI (Lee *et al*., 2016).

적 요

남극해에 서식하는 불가사리의 내장에서 분리한 *Pseudoalteromonas* sp. strain SiA1은 총2개의 염색체(염색체 1, 염색체 2)를 가지고 있는 것으로 밝혀졌다. 그 크기는 각각 3,355,071 bp와 715,567 bp이며, 유전체의 G + C 비율은 염색체 1에서 40.44%, 염색체 2에서 39.79%로 나타났다. 컴퓨터 분석 결과 3,597개의 코딩서열이 확인되었는데, 이 가운데 최소 98개(약 2.7%)가 세포 외 단백질 분해 효소 단백질을 암호화 하는 것으로 추정된다. 이는 분리 과정에서 확인된 SiA1의 탁월한 단백질 분해 능력을 유전자 차원에서 입증하는 결과라고 추측된다.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF-2019S1A5C2A04083293).

Conflict of Interest

The authors have no conflict of interest to report.

References

Burnett WJ and McKenzie JD. 1997. Subcuticular bacteria from the brittle star *Ophiactis balli* (Echinodermata: Ophiuroidea) represent a new lineage of extracellular marine symbionts in the a subdivision of the class *Proteobacteria. Appl. Environ. Microbiol.* **63**, 1721–1724.

Cristóbal HA, López MA, Kothe E, and Abate CM. 2011. Diversity of protease-producing marine bacteria from sub-antarctic environments. *J. Basic Microbiol.* **51**, 590–600.

Fabiano M and Pusceddu A. 1998. Total and hydrolizable particulate organic matter (carbohydrates, proteins and lipids) at a coastal station in Terra Nova Bay (Ross Sea, Antarctica). *Polar Biol*. **19**, 125–132.

Lee I, Kim YO, Park SC, and Chun J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int. J. Syst. Evol. Microbiol*. **66**, 1100–1103.

Lee YM, Jung YJ, Hong SG, Kim JH, and Lee HK. 2014. Diversity and physiological characteristics of culturable bacteria from marine sediments of Ross Sea, Antarctica. *Korean J. Microbiol*. **50**, 119–127.

Li H, Yu Y, Luo W, Zeng Y, and Chen B. 2009. Bacterial diversity in surface sediments from the Pacific Arctic Ocean. *Extremophiles* **13**, 233–246.

Ravenschlag K, Sahm K, and Amann R. 2001. Quantitative molecular analysis of the microbial community in marine Arctic sediments (Svalbard). *Appl. Environ. Microbiol*. **67**, 387–395.

Russell NJ. 1998. Molecular adaptations in psychrophilic bacteria: potential for biotechnological applications, pp. 1–21. *In* Antranikian G. (ed.), Biotechnology of Extremophiles, vol. 61. Springer, Berlin, Heidelberg, Germany.

Smith WO, Ainley DG, and Cattaneo-Vietti R. 2007. Trophic interactions within the Ross Sea Continental shelf ecosystem. *Phil. Trans. R. Soc. B* **362**, 95–111.