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Note (Genome Announcement)

Genome sequence of carotenoid producing *Sphingobacteriaceae* bacterium SH-48 isolated from freshwater in Korea

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카로티노이드 생산 *Sphingobacteriaceae* SH-48 균주의 유전체 염기서열 분석

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We sequenced the genome of the *Sphingobacteriaceae* bacterium SH-48 isolated from the Sohan stream in Republic of Korea by using a dilution-to-extinction culturing method. The sequences were assembled into a draft genome containing 5,650,162 bp with a G + C content of 35.4% and 4,856 protein-coding genes in 2 contigs. This strain contains the carotenoid biosynthesis genes *crtY*, *crtZ*, *crtD*, *crtI*, *crtB*, and *crtH* as gene clusters. This genomic information provides new insights into the carotenoid biosynthesis pathway.

Keywords: carotenoid biosynthesis, freshwater, genome sequencing

The carotenoids are naturally occurring groups of pigments that are widespread in bacteria, algae, and other eukaryotic organisms. These pigments have important functions in photosynthesis, nutrition, and protection against photooxidative damage (Olson and Krinsky, 1995; Shahmohammaki *et al.*, 1998). Some species of carotenoid-producing bacteria belonging to the family *Sphingobacteriaceae* have been isolated (Jagannadham *et al.*, 2000; Prasad *et al.,* 2013; Chen *et al.*, 2016; Sheu *et al.*, 2016). These pigments are ubiquitously synthesized by nonphotosynthetic bacterial lineages (Krinsky, 1978). Here, we report the draft genome sequence of *Sphingobacteriaceae* bacterium SH-48 isolated from a freshwater stream. This information will provide the basis for understanding carotenoid biosynthesis.

Strain SH-48 was isolated from a surface water sample collected from the Sohan Stream in Republic of Korea by using a high-throughput culturing based on dilution-to-extinction (HTC) (Conon and Giovannoni, 2002). Inoculation, incubation and screening of dilution cultures were performed as described by Yang *et al.* (2016). The original liquid culture of strain SH-48 was obtained after 4 weeks at 15°C and spread onto R2A agar. A representative genomic sequence of the 16S ribosomal RNA (rRNA) gene from strain SH-48 was compared with those of other members of the family *Sphingobacteriaceae* using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/). The comparative sequence analysis revealed that among the cultured isolates, this strain was most closely related to *Mucilaginibacter daejeonensis* Jip 10^T, although the level of 16S rRNA sequence similarity was low (83.8%).

The genomic DNA of strain SH-48 was extracted using the DNeasy kit (Qiagen), and the whole genome was sequenced using the PacBio sequencing platform with a 20-kb SMRTbell library, which was performed by ChunLab Inc.. The bacterial

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genome was assembled *de novo* into two contigs, with an average coverage of 114.8 × and an N50 value of 5,648,308, using the PacBio SMRT Portal (2.3.0) and the hierarchical genome assembly process (Chin *et al*., 2013). The G + C content of the genome assembly was 38.41%. Comparisons between the predicted ORFs using the SEED (Overbeek *et al*., 2005), COG (Tatusov *et al*., 2003), and Pfam (Punta *et al*., 2012) databases were conducted during the gene annotation process. Additional gene prediction analyses and functional annotations were performed with the Rapid Annotation using the Subsystem Technology (RAST) server databases (Aziz *et al*., 2008), and gene-caller GLIMMER 3.02. RNAmer 1.2 (Lagesen *et al*., 2007) and tRNAscan-SE 1.23 (Lowe and Eddy, 1997) were used to identify rRNA genes and tRNA genes, respectively. The CLgenomics software was used to visualize the genomic features. The basic genome statistics are shown in Table 1.

The draft genome size was 5,650,162 bp with a G + C content of 38.41%. As a result of gene predictions, this genome contains 4,856 CDSs, 46 tRNA, and 6 rRNA genes (Fig. 1). A total of 4,042 genes were assigned a putative function. The genes were classified into 21 COG functional categories. Sequence analyses revealed that carotenoid biosynthesis genes were localized as a cluster that included genes encoding lycopene beta-cyclase (*crtY*, MD10_00824), beta-carotene 3-hydroxylase (*crtZ,* MD10_00825), 1-hydroxycarotenoid 3,4-desaturase (*crtD*; MD10_00827, and MD10_01060), phytoene desaturase (*crtI*, MD10_00829 AND md10_01689), phytoene

Table 1. General genomic features of the strain SH-48

Features	Chromosome
Genome size (bp)	5,650,162
Contigs	2
GC content (%)	38.41
rRNA genes	6
tRNA genes	46
Protein coding genes	4,856
Genes assigned to COGs	4,042

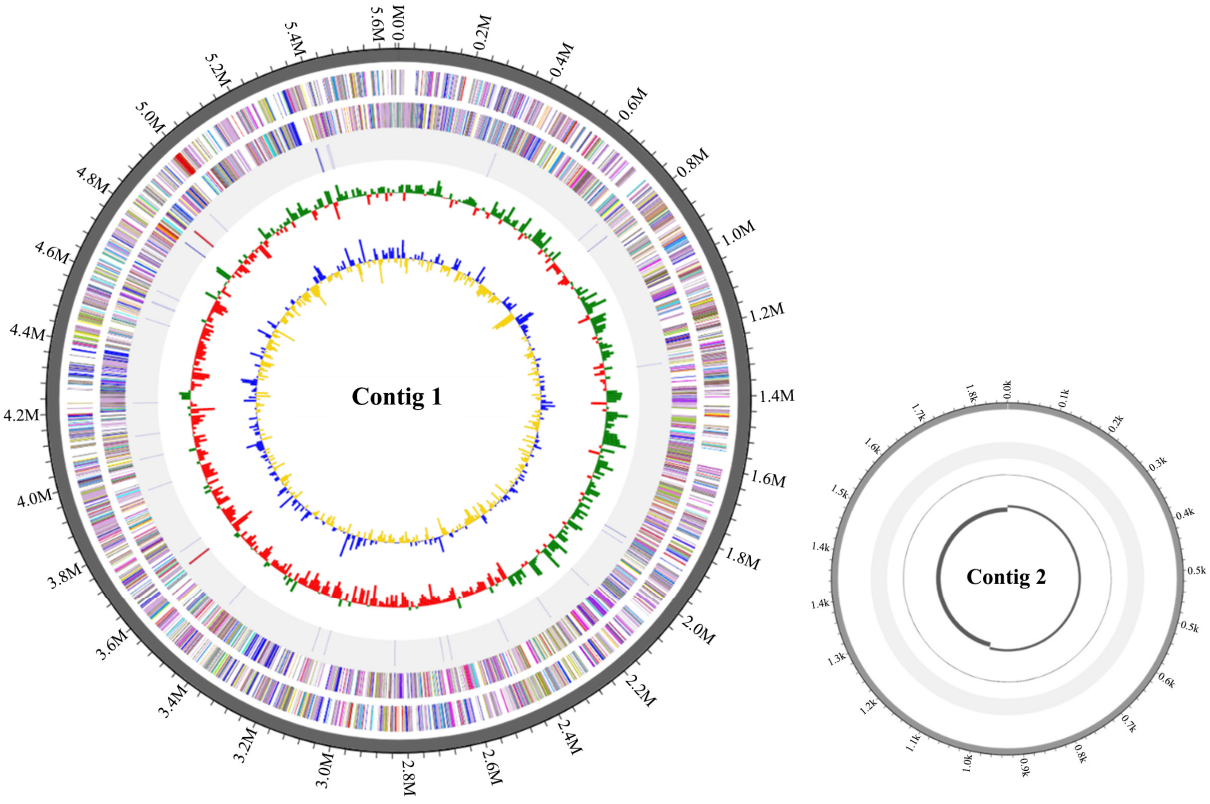

Fig. 1. Graphical circular map of strain SH-48. From bottom to top: genes on the forward strand (colored by COG categories), genes on the reverse strand (colored by COG categories), RNA genes (tRNA-green, rRNA-red, other RNAs-black), GC content, and GC skew (purple/olive).

Table 2. Carotenoid biosynthesis genes in strain SH-48

Gene	Description	Length (AA)	Top BLAST hit	Species	*E*-value	AA identity
crtY	lycopene cyclase	388	WP_071506339.1	*Arsenicibacter rosenii*	2e-96	41%
crtZ	beta-carotene hydroxylase	159	WP_050855217.1	*Flavobacterium frigoris*	2e-79	73%
crtD	phytoene desaturase	476	WP_073090694.1	*Cyclobacterium lianum*	1e-175	53%
crtD	C-3',4' desaturase	522	WP_066033154.1	*Flavobacterium johnsoniae*	1e-180	52%
crtI	phytoene desaturase	492	WP_090559445.1	*Pedobacter hartonius*	1e-133	59%
crtI	phytoene desaturase	462	WP_092738084.1	*Hymenobacter psychrophilus*	5e-137	44%
crtB	phytoene synthase	278	WP_090559444.1	*Pedobacter hartonius*	2e-128	66%
crtH	Phytoene isomerization	557	APR83373.1	*Minicystis rosea*	1e-143	41%

synthase (*crtB*; MD10_00830), prolycopene isomerase (*crtH*, MD10_00956). Notably, the genome contains *crt* genes coding for proteins with homology to enzymes that performed the core steps of carotenoid biosynthesis. However, the *crt* genes of SH-48 showed low similarity to existing bacterial genes (Table 2). This genome and its comparison with the genomes of other carotenoid-producing bacteria may provide a better understanding of the mechanisms of carotenoid synthesis.

Nucleotide sequence accession numbers

Sequence and annotation data of strain SH-48 were deposited in DDBJ/EMBL/GenBank under the accession number NVQB00000000. The version described in this paper is version NVQB00000000.1. This study was based on the first version of this genome.

적 요

그람 음성이며 막대모양의 *Sphingobacteriaceae* bacterium SH-48은 삼척 소한천에서 분리하였다. SH-48에 대한 유전체 분석을 실시하였으며, G + C 비율이 38.4%인 5,650,162 bp 크기의 염기서열을 얻었다. 유전체 특징은 카로티노이드 생합성 유전자인 *crt* 유전자 클러스터를 보유하고 있어 균주의 잠재적 중요성을 보여준다. 이러한 유전체 정보는 카로티노이드 생합성 경로에 대한 새로운 정보를 제공한다.

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