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# Draft genome sequences of *Vibrio splendidus* KCTC 11899BP, which produces hyaluronate lyase in the presence of hyaluronic acid

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# 히알우론산 유도하에 히알우로네이트 라이아제를 생산하는 *Vibrio splendidus* KCTC 11899BP균주의 유전체 서열 분석

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We, for the first time, isolated and identified a *Vibrio splendidus* KCTC 11899BP producing hyaluronate lyase from seawater. This enzyme is produced only when hyaluronic acid (HA) is added to the basal medium. Hyaluronate lyases are produced by microorganisms, which degrade the β-(1, 4) bond of HA to produce disaccharide. The genome of KCTC 11899BP, which consist of two circular contigs that are 3,522 kb (contig 1) long and 1,986 kb (contig 2) long respectively, as like other *Vibrio* sp. that contained 2 chromosomes. The genome included 4,700 predicted open reading frames, G + C content 44.12%, 137 tRNA genes, and 46 rRNA genes.

**Keywords:** *Vibrio splendidus* KCTC 11899BP, genome sequence, hyaluronate lyase, hyaluronic acid

Hyaluronic acid (HA) is a biomaterial present in the skin and joints of animals. It is a macromolecule with a molecular weight of hundreds of thousands to several million daltons (Fraser *et al*., 1997). HA is widely used as a moisturizing agent in cosmetics, and as an articular lubricant (Toole, 2000). It is also used as a filler to spread wrinkles on the face (Chen and Abatangelo, 1999). However, HA is not easily absorbed into the body for use in cosmetic applications because of its high molecular weight (Fakhari and Berkland, 2013). Therefore, technologies for the lowering molecular weight have been developed, for example, treatment by acid, alkali, heat or enzymes (Shimada and Matsumura, 1980; Kubo *et al*., 1993; Tawada *et al*., 2002). Among these methods, we favor enzymatic treatment for cost and environmental reasons. Thus we tried to screen hyaluronate lyase producing bacteria, and isolated *Vibrio splendidus* KCTC 11899BP and identified the hyaluronate lyase coding gene from it. In enzymology, a hyaluronate lyase (EC 4.2.2.1) is an enzyme that catalyzes the chemical reaction cleaving hyaluronate chains at a β-D-N-acetyl galactosamine-(1→4)-β-D-glucuronic acid. The hyaluronate lyase coding gene, *hylB*, was initially reported in Group B Streptococci which is a major causative agent for perinatal infection in human. Sequence analysis of positive clones identified an open reading frame capable of coding for a 984 amino acid (111 kDa) (Bo *et al*., 1994). Genetic diversity of *hyl* have been studied by restriction fragment length polymorphism (RFLP) profiles and

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sequence analysis and the results indicated that a pattern of diversity shared with many surface proteins having a variable 5' region by mutations (Samantha *et al*., 2004).

For screening of novel marine microorganisms capable of degrading HA, various possible candidates from seawater were incubated on minimal nutrients (0.1% peptone, 3% NaCl) adding 0.3% HA. The selected strain was identified according to Bergey's manual (Krieg and Holt, 1984) and sugar utilization tests (accession no. *Vibrio splendidus* KCTC 11899BP). The enzyme activity of this strain was confirmed. The initial molecular weight ≥ 1.5 MDa of HA was degraded to 50 kDa by hyaluronate lyase from this strain for 105 min treatment.

To obtain whole genome sequences, purified genomic DNA was analyzed by commissioning to Macrogen Inc. The genomic DNA of KCTC 11899BP was sequenced using PacBio RSII sequencing method. After assembly, the resulting 2 contigs were polished by Pilon (version: 1.21) with the Paired-end reads from Illumina HiSeq 2500 (https://github.com/broadinstitute/pilon/wiki). Genome annotation was conducted by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

Genome statistics are summarized in Table 1. The genome of strain KCTC 11899BP was composed of 5,508,387-bp long with 4,700 coding regions. To identify the hyaluronate lyase coding gene sequences, the 15 residues amino acid sequences of hyaluronate lyase from KCTC11899BP was blasted with the genome sequence of KCTC11899BP by using BLASTp. Hyaluronate lyase coding gene was confirmed that it consisted of 2,409 base pairs long starting from the 1612 base pairs and ending at the 4021 base pairs of contig 1 with an open reading frame capable of coding for 803 amino acid (about 90 kDa).

**Table 1. Genome statistics for *Vibrio splendidus* strain KCTC 11899BP**

| Attribute | Value |
|---|---|
| No. of Contigs | 2 |
| Genome size (bp) | 5,508,387 |
| G + C contents (%) | 44.12 |
| Coding sequences | 4,700 |
| tRNA | 137 |
| rRNA | 46 |
| Sequencing depth | 180 |
| Circular | Yes |

The *hyl* gene of the Gram-positive strain *Strptoccoci* encoded 984 amino acids (111 kDa), while that of the Gram-negative strain *Vibrio splendidus* KCTC 11899BP encoded 803 amino acids (90 kDa). The identity of the amino acid sequence was confirmed by BLASTp, which showed 96% identity in *Allivibrio wondanis* (WP_061029046.1) and 92% in *Allivibrio fischeri*. It showed also a 50% identity with the most virulent *Vibrio parahaemolyticus* (ODZ65005.1) in *Vibrio* sp. with E-value 0. Hyaluronate lyase coding genes have been conserved in other *Vibrio* sp. such as *V. campbellii*, *V. harveyi*, *V. fluvialis*, *V. vulnificus*, *V. ponticus*, and *Photobacterium* sp. such as *P. jeanii*, *P. leiognathi*, *P. aquimaris*, *P. damselae*, *P. kishitanii*, *P. phosphoreum*, *P. sanctipauli*, as well. This enzyme plays important roles in invasion and spread into the host tissue via degradation of the extracellular matrix of aquatic host.

In conclusion, the draft genome sequences of *Vibrio splendidus* KCTC 11899BP was presented and the hyaluronate lyase coding gene was confirmed in this study.

The genome sequence of *Vibrio splendidus* KCTC11899BP has been deposited in NCBI GenBank under accession number CP031055-CP031056.

## 적 요

우리는 처음으로 바닷물에서 히알우론산 분해효소를 생산하는 균주인 *Vibrio splendidus* KCTC 11899BP를 분리하고 동정했다. KCTC 11899BP는 히알우론산(HA)이 기초 배지에 첨가 될 때에만 Hyaluronate lyase를 생산하며, 이 효소는 HA의 β-(1, 4) 결합을 분해하여 이당(disaccharide)을 생성시키는 효소로서 미생물에 의해 생산된다. 게놈 염기서열분석을 통해, KCTC 11899BP의 게놈은 2개의 염색체를 보유하는 다른 *Vibrio* sp.와 유사하게 각각 3,522 kb (contig 1)와 1,986 kb (contig 2)인 두 개의 원형 contig로 구성되어 있다는 것을 확인하였다. 또한 4,700개의 예측 오픈 리딩 프레임, G + C 함량 44.12%, 137개의 tRNA 유전자 및 46개의 rRNA 유전자를 포함하고 있다는 것을 확인했다.

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