Short Communication

Whole genome sequence of a freshwater agar-degrading bacterium Cellvibrio sp. KY-GH-1

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A B S T R A C T

We investigated the whole genome sequence of a freshwater agar-degrading bacterium Cellvibrio sp. KY-GH-1 (KCTC13629BP) to explore genetic information encoding agarases which hydrolyze agar into its monomers. The complete genome of KY-GH-1 comprised 5,762,391 base pairs (bp) with 47.9% GC content, and contained 5080 protein-encoding sequences, including nine β-agarase genes and two α-neoagarobiose hydrolase (α-NABH) genes in an agarase gene cluster spanning approximately 77 kb. Based on these genetic information, the degradation of agar into monomers (D-galactose and 3,6-anhydro-L-galactose) by KY-GH-1 was predicted to be initiated by endolytic GH16 β-agarases and endolytic GH86 β-agarases, further processed by exolytic GH50 β-agarases, and then terminated by exolytic GH17 α-NABHs. This study reveals the diversity and abundance of agarase genes, and provides insight into their roles in the agar-degrading enzyme machinery of Cellvibrio sp. KY-GH-1.

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Agar is a complex polysaccharide that is abundant in the cell walls and intracellular spaces of marine red algae such as Gelidium and Gracilaria. It comprises a mixture of neutral agarose and charged agarpectin. Agarose contains alternating residues of α-1,3-linked 3,6-anhydro-L-galactose (AHG) and β-1,4-linked D-galactose; agarpectin has the same repeating units with some replacement of AHG residues with L-galactose sulfate and partial replacement of the D-galactose residues with pyruvic acid acetal 4,6-O-(1-carboxyethylidene)-D-galactose [1].

Agar forms a stable gel at low concentrations and is generally resistant to microbial degradation.

Therefore, it is widely used as a gelling agent in food industries, microbial culture media, and several other biotechnological techniques. In addition, agar-oligosaccharides and neoagaro-oligosaccharides, which are agar-degrading products, have been shown to exert antioxidant activity [2], antitumor activity [3,4], prebiotic effect [5], and moisturizing effect [6], anti-inflammatory activity [7], and anti-diabetic and anti-obesity effects [8]. Since an agar-degrading bacterium Bacillus gelaticus was first isolated from seawater by Gran in 1902, several genera of agar-degrading bacteria including Acinetobacter, Agarivorans, Alteromonas, Cellulophaga, Cytophaga, Flammeoaviga, Gayadomonas, Microbulbifer, Pseudoalteromonas, Pseudomonas, Saccharophagus, Thalassomonas, Vibrio, and Zobellia have been identified, mainly in marine environments [9–11]. Few genera of non-marine agar-degrading bacteria have also been isolated from freshwater environments or terrestrial soil environments, including Alteromonas, Bacillus, Cellvibrio, Cytophaga, Paenibacillus, Streptomyces, and Thermoanaerobacter [11–13].

Agar-degrading bacteria produce agarases to degrade agar into D-galactose and AHG, which act as carbon and energy sources. Agarases are divided into two types depending on their mode of cleavage: α-agarase (EC 3.2.1.158) cleaves the α-(1,3) glycosidic linkage, whereas the β-agarase (EC 3.2.1.81) cleaves the β-(1,4) glycosidic linkage [9]. Although numerous researchers have studied the biochemical and enzymatic properties of bacterial agarases, they have examined either a single agarase purified from agar-degrading bacteria or a recombinant agarase produced in a bacterial expression system from its gene. The reported agarases were mostly agar-liquefying endo-type β-agarases which cleave the β-1,4-linkage of agar to produce neoagaro-oligosaccharides [9,10]. There have been fewer reports on α-agarases which hydrolyze the α-1,3-linkage of agar to produce agaro-oligosaccharides. Analyses of the amino acid sequence similarities of bacterial agarases based on the Carbohydrate-Active Enzymes (CAZyme) database have revealed that β-agarases belong to four glycoside hydrolase (GH) families, i.e., GH16, GH50, GH86, and GH118 [14–16], whereas α-agarases belong to two families, i.e., GH96 and GH117 [17,18]. The GH16, GH86, and GH118 β-agarases are
endolytic and mainly produce neoagarotetraose (NA4)/neoagarohexaose (NA6), NA6/neoagaroctaose (NA8), and NA8/neoagaroodecaose (NA10), respectively. The GH50 β-agarases are exolytic and mainly produce neoagarobiose (NA2) [9,19]. The GH96 α-agarases mainly produce agarobiose from agarose; the GH117 enzyme α-neoagarobiose hydrolase (α-NABH) catalyzes the hydrolytic degradation of NA2 to D-galactose and AHG [18].

Although the previous studies on the individual agarases have improved our understanding of the diversity and redundancy of bacterial agarases, it remains unclear what combination of agarases is required for efficient degradation of agar into its monomers in agar-degrading bacteria. Recently, we have isolated an agar-degrading bacterial strain KY-GH-1 from freshwater sediments from Shinchun River, Daegu city, Republic of Korea. The bacterial strain KY-GH-1 was capable of exerting shallow depression around the colonies on the basal medium plate (0.2% NaNO₃, 0.05% polypeptide, 0.05% K₂HPO₄, 0.05% MgSO₄·7H₂O, 0.01% CaCl₂·2H₂O, 0.01% NaCl, 0.002% FeSO₄·7H₂O, 0.02% MnSO₄, and 1.8% agar), and was curved rod form (Fig. 1A and B). For the taxonomic identification of the strain KY-GH-1, the 16S rRNA gene (1.5 kb) was selectively amplified from the chromosomal DNA by polymerase chain reaction and the nucleotide sequence was determined as previously described [11]. When the 16S rDNA sequence was aligned with those available in the GenBank nucleotide sequence database by using the BLASTN search program, it exhibited maximum homology with Cellvibrio species and appeared to be 99.1% identical to Cellvibrio fibrivorans strain R-4079 (Fig. 2).

To elucidate the agar-degrading enzyme machinery in Cellvibrio sp. KY-GH-1, we analyzed the entire genome using a PacBio RS II Sequencer [20], and conducted the genome assembly using SMRT Analysis software (v2.3.0 HCAP2). We found that the complete circular genome, which consisted of one contig, comprised 5,762,391 bp with 47.9% GC contents (Table 1). The read coverage was 210×. The tRNAscan-SE programs predicted that there were 48 tRNA genes and 9 rRNA genes. Protein-coding sequences (CDS), which were predicted using Glimmer 3.0 and were annotated by comparisons with NCBI-nr, UniRef90, COG, and KEGG databases using BLAST version 2.2.26, numbered 5080. Among the 5080 CDS, we detected four GH16 β-agarase genes (β-CvAga16A, β-CvAga16B, β-CvAga16C, β-CvAga16D), three GH50 β-agarase genes (β-CvAga50A, β-CvAga50B, β-CvAga50C), and two GH86 β-agarase genes (β-CvAga86A, β-CvAga86B), as well as two GH117 α-NABH genes (α-CvNabh11A, α-CvNabh11B) in an agarase gene cluster spanning approximately 77 kb (Fig. 3 and Table 2). The agarase gene cluster appeared to be located between +3313 kb and +3390 kb based on the translation start site (+1) of the DNA replication initiation protein gene, DnaA, on the genome sequence. In contrast, neither the GH117 β-agarase gene nor the GH96 α-agarase gene was detected in the CDS.

![Fig. 1](image1.png) **Fig. 1.** An agar plate with an agar-degrading KY-GH-1 colonies (A), and photomicrograph of the agarolytic strain KY-GH-1 taken by light microscopy with ×1000 magnification (B).

![Fig. 2](image2.png) **Fig. 2.** Phylogenetic relationships of KY-GH-1 and related Cellvibrio species based on 16S rDNA gene sequence similarities. The tree was constructed by the neighbor-joining method [21], and approximately 1497 nucleotides were used for comparison. The 16S rRNA gene (DQ084459) of Pseudomonas fluorescens FLM05-1 was used as the outgroup. Numbers at nodes are levels of bootstrap support; only values above 50% are given. Scale bar represents one nucleotide substitution per 100 nucleotides.

| Table 1 | Features of the Cellvibrio sp. KY-GH-1 genome. |
|---------|-----------------------------------------------|
| Features | Value                                          |
| Total reading base pairs (bp) | 1,219,212,882 |
| Contig number | 1 |
| Total size, N₅₀ (bp) | 5,762,391 |
| Read coverage | 210 |
| GC content (%) | 47.9 |
| tRNAs | 48 |
| rRNAs | 9 |
| Protein-coding sequences | 5080 |
If *Cellvibrio* sp. KY-GH-1 produces endolytic GH16 β-agarases, endolytic GH86 β-agarases, exolytic GH50 β-agarases, and exolytic GH117 α-NABHs as components of its agar-degrading enzyme machinery, the degradation of agar into monomers (D-galactose and AHG) will be initiated by endolytic GH16 β-agarases and endolytic GH86 β-agarases, further processed by exolytic GH50 β-agarases, and then terminated by exolytic GH117 α-NABHs (Fig. 4).

### Nucleotide sequence accession numbers

The complete genome sequence of *Cellvibrio* sp. KY-GH-1 has been deposited in the GenBank database under accession number CP031728. The strain is available from the Korean Collection for Type Cultures (Jeongeup, Republic of Korea) under the accession number KCTC13629BP.

### Conflict of interest

All the authors report no conflicts of interest.

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Fig. 4. Schematic diagram of the process by which Cellvibrio sp. KY-GH-1 degrades agarose.

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