Biotechnology Reports 23 (2019) e00346

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Short Communication

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

Gi Hyun Kwon, Mi Jung Kwon, Ju Eun Park, Young Ho Kim*

Laboratory of Immunobiology, School of Life Science and Biotechnology, College of Natural Sciences, Kyungpook National University, Daegu, 41566, Republic of Korea

ARTICLE INFO

Article history:
Received 14 January 2019
Received in revised form 24 April 2019
Accepted 14 May 2019

Keywords:
Agar-degrading bacteria
Cellvibrio
Genome sequence
β-Agarase genes
α-NABH genes
Agarase gene cluster

ABSTRACT

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 GH117 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.

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 agaropectin. Agarose contains alternating residues of α-1,3-linked 3,6-anhydro-L-galactose (AHG) and β-1,4-linked D-galactose; agaropectin 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, agaro-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*, *Flammeovirga*, *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

* Corresponding author.
E-mail address: ykim@knu.ac.kr (Y.H. Kim).

https://doi.org/10.1016/j.btre.2019.e00346

endolytic and mainly produce neoagarotetraose (NA4)/neoagarohexaose (NA6), NA6/neoagarooctaose (NA8), and NA8/neoagarodecaose (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% $NaNO_3$, 0.05% polypeptone, 0.05% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.01% $CaCl_2 \cdot 2H_2O$, 0.01% NaCl, 0.002% $FeSO_4 \cdot 7H_2O$, 0.02% $MnSO_4$, 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 HGAP.2). 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 RNAmmer and 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-CvNabh117A, α-CvNabh117B) 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 GH118 β-agarase gene nor the GH96 α-agarase gene was detected in the CDS.

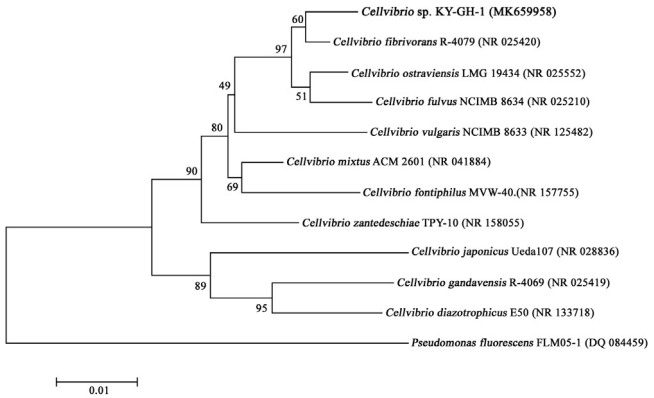

Fig. 2. Phylogenetic relationships of KY-GH-1 and related *Cellvibrio* species based on 16S rDNA gene sequence similarities. The tree was constructed by the neighbor-joining method [21], and approximately 1497 nucleotides were used for comparison. The 16S rRNA gene.
(DQ084459) of *Pseudomonas fluorescence* 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, N_{50} (bp)	5,762,391
Read coverage	210
GC content (%)	47.9
tRNAs	48
rRNAs	9
Protein-coding sequences	5080

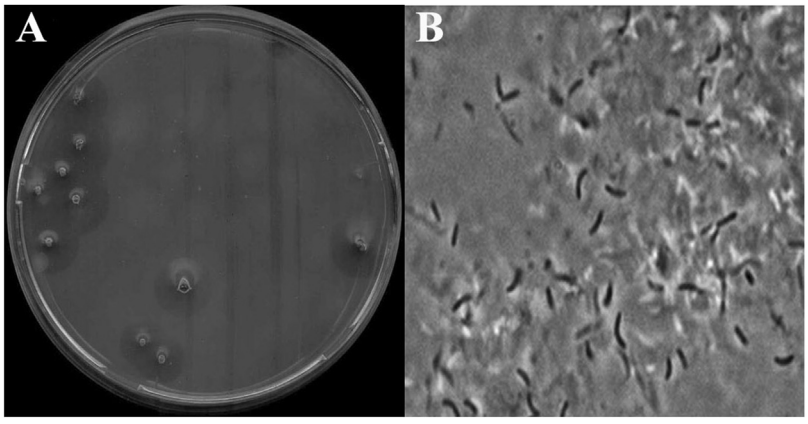

Fig. 1. An agar plate with an agar-degrading KY-GH-1 colonies (A), and photomicrograph of the agarolytic strain KY-GH-1 taken by light microscopy with ×1000 magnification (B).

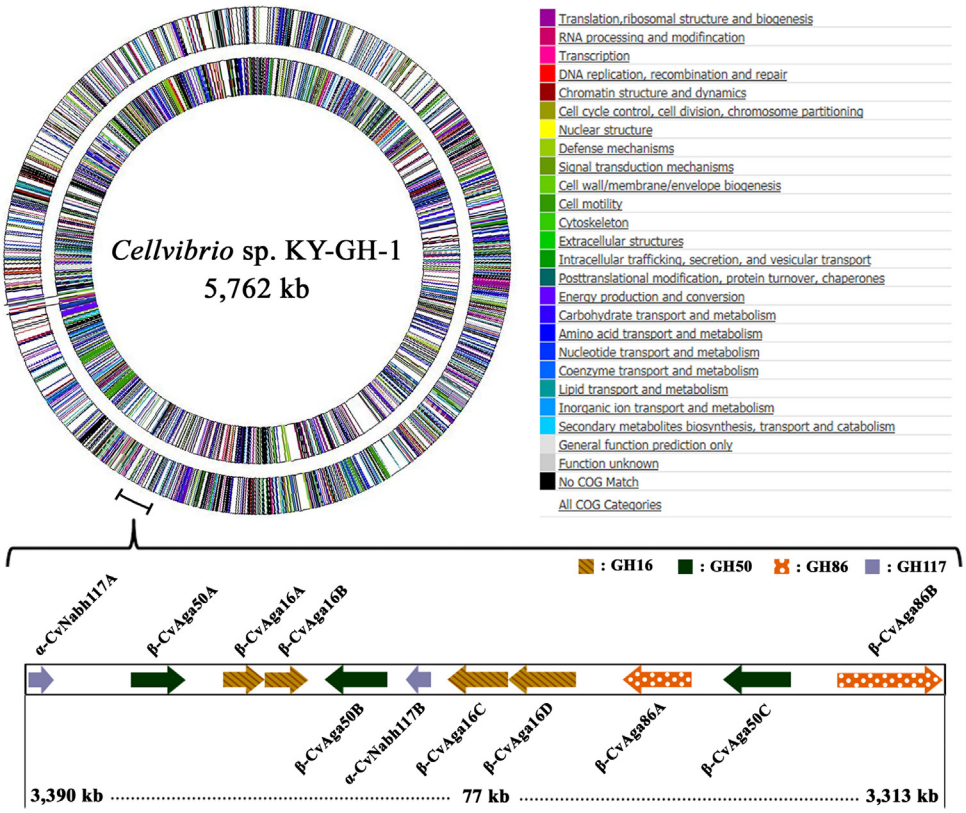

Fig. 3. Circular representation of *Cellvibrio* sp. KY-GH-1 genome showing genomic features, and structure of the agarase gene cluster (77 kb) encoding nine β-agarases and two α-neoagarobiose hydrolases (α-NABHs).

Table 2
Characteristics of predicted agarase genes in the *Cellvibrio* sp. KY-GH-1 genome.

Gene name	Mode of activity	Nucleotide length (bp)	Protein size (kDa)	Major products
β-CvAga16A	endolytic-β-agarase	1,434	52.5	NA4, NA6
β-CvAga16B	endolytic-β-agarase	1,794	65.7	NA4, NA6
β-CvAga16C	endolytic-β-agarase	1,773	64.9	NA4, NA6
β-CvAga16D	endolytic-β-agarase	2,997	109.8	NA4, NA6
β-CvAga50A	exolytic-β-agarase	2,397	87.8	NA2
β-CvAga50B	exolytic-β-agarase	2,319	84.9	NA2
β-CvAga50C	exolytic-β-agarase	2,313	84.7	NA2
β-CvAga86A	endolytic-β-agarase	2,136	78.2	NA6, NA8
β-CvAga86B	endolytic-β-agarase	4,515	165.4	NA6, NA8
α-CvNabh117A	exolytic-α-NABH	1,095	40.0	D-galactose, AHG
α-CvNabh117B	exolytic-α-NABH	1,179	43.1	D-galactose, AHG

Symbols: AHG, 3,6-anhydro-L-galactose; NA2, neoagarobiose; NA4, neoagarotetraose; NA6, neoagarohexaose; NA8, neoagarooctaose; NA10, neoagarodecaose.

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.

Acknowledgment

This work was supported by a grant from Kyungpook National University, Daegu 41566, Republic of Korea, 2018.

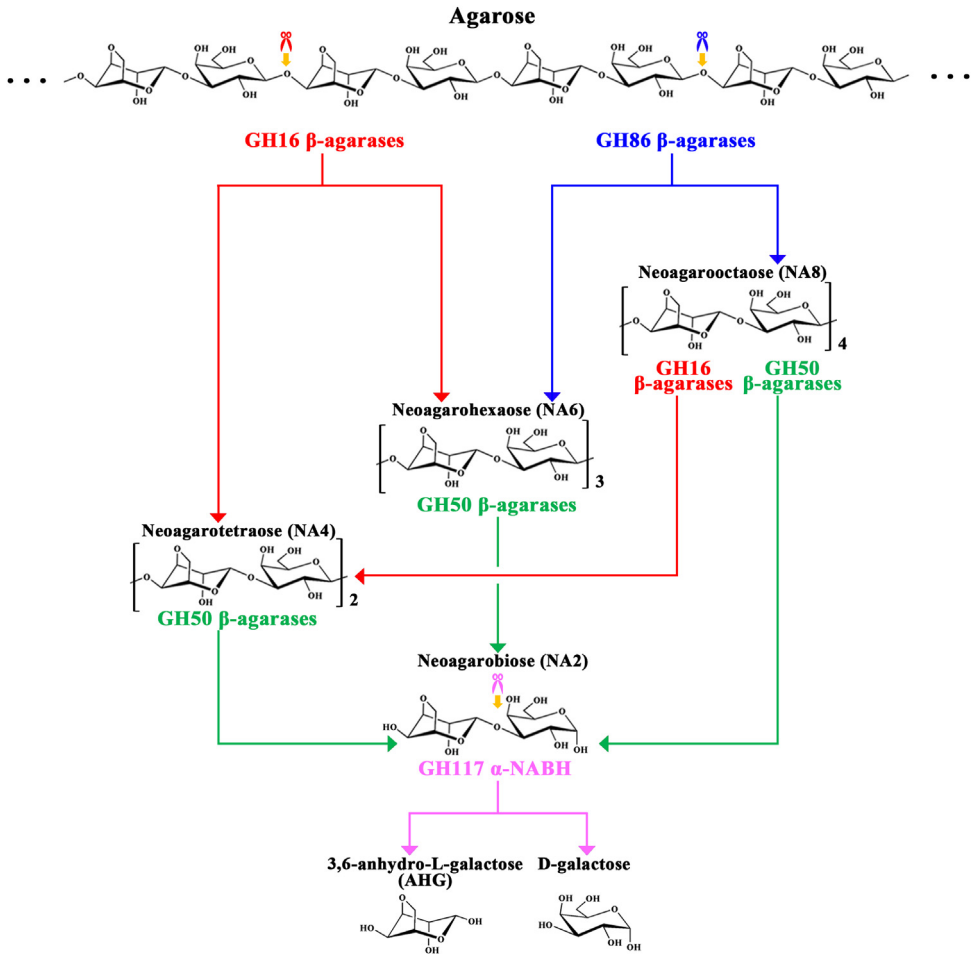

Fig. 4. Schematic diagram of the process by which *Cellvibrio* sp. KY-GH-1 degrades agarose.

References

[1] M. Duckworth, W. Yaphe, The structure of agar. Part I. Fractionation of a complex mixture of polysaccharides, Carbohydr. Res. 16 (1971) 189–197.

[2] H.M. Chen, X.J. Yan, Antioxidant activities of agaro-oligosaccharides with different degrees of polymerization in cell-based system, Biochim. Biophys. Acta 1722 (2005) 103–111, doi:http://dx.doi.org/10.1016/j.bbagen.2004.11.016.

[3] T. Enoki, T. Tominaga, F. Takashima, H. Ohnogi, H. Sagawa, I. Kato, Anti-tumor-promoting activities of agaro-oligosaccharides on two-stage mouse skin carcinogenesis, Biol. Pharm. Bull. 35 (2012) 1145–1149, doi:http://dx.doi.org/10.1248/bpb.b12-00188.

[4] M.H. Lee, J.H. Jang, G.Y. Yoon, S.J. Lee, M.G. Lee, T.H. Kang, Y.M. Park, Neoagarohexaose-mediated activation of dendritic cells via Toll-like receptor 4 leads to stimulation of natural killer cells and enhancement of antitumor immunity, BMB Rep. 50 (2017) 263–268, doi:http://dx.doi.org/10.5483/BMBRep.2017.50.5.014.

[5] B. Hu, Q. Gong, Y. Wang, Y. Ma, J. Li, W. Yu, Prebiotic effects of neoagaro-oligosaccharides prepared by enzymatic hydrolysis of agarose, Anaerobe 12 (2006) 260–266, doi:http://dx.doi.org/10.1016/j.anaerobe.2006.07.005.

[6] R. Kobayashi, M. Takisada, T. Suzuki, K. Kirimura, S. Usami, Neoagarobiose as a novel moisturizer with whitening effect, Biosci. Biotechnol. Biochem. 61 (1997) 162–163, doi:http://dx.doi.org/10.1271/bbb.61.162.

[7] W. Wang, P. Liu, C. Hao, L. Wu, W. Wan, X. Mao, Neoagaro-oligosaccharide monomers inhibit inflammation in LPS-stimulated macrophages through suppression of MAPK and NF-κB pathways, Sci. Rep. 7 (2017) 44252, doi:http://dx.doi.org/10.1038/srep44252.

[8] S.J. Hong, J.H. Lee, E.J. Kim, H.J. Yang, J.S. Park, S.K. Hong, Anti-obesity and anti-diabetic effect of neoagarooligosaccharides on high-fat diet-Induced obesity in mice, Mar. Drugs 15 (2017) 90, doi:http://dx.doi.org/10.3390/md15040090.

[9] X.T. Fu, S.M. Kim, Agarase: review of major sources, categories, purification method, enzyme characteristics and applications, Mar. Drugs 8 (2010) 200–218, doi:http://dx.doi.org/10.3390/md8010200.

[10] S.T. Jahromi, N. Barzkar, Future direction in marine bacterial agarases for industrial applications, Appl. Microbiol. Biotechnol. 102 (2018) 6847–6863, doi:http://dx.doi.org/10.1007/s00253-018-9156-5.

[11] Y.J. Rhee, C.R. Han, W.C. Kim, D.Y. Jun, I.K. Rhee, Y.H. Kim, Isolation of a novel freshwater agarolytic *Cellvibrio* sp. KY-YJ-3 and characterization of its extracellular β-agarase, J. Microbiol. Biotechnol. 20 (2010) 1378–1385, doi:http://dx.doi.org/10.4014/jmb.1007.07010.

[12] J.A.C. Agbo, M.O. Moss, The Isolation and characterization of agarolytic bacteria from a lowland river, J. Gen. Microbiol. 115 (1979) 355–368.

[13] G.E. Bannikova, S.A. Lopatin, V.P. Varlamov, B.B. Kuznetsov, I.V. Kozina, M.L. Miroshnichenko, N.A. Chernykh, T.P. Turova, E.A. Bonch-Osmolovskaia, The thermophilic bacteria hydrolyzing agar: characterization of thermostable agarase, Appl. Biochem. Microbiol. 45 (2008) 366–371, doi:http://dx.doi.org/10.1134/S0003683808040054.

[14] B.L. Cantarel, P.M. Coutinho, C. Rancurel, T. Bernard, V. Lombard, B. Henrissat, The carbohydrate-active EnZyme database (CAZy): an expert resource for glycogenomics, Nucleic Acids Res. 37 (2009) D233–238, doi:http://dx.doi.org/10.1093/nar/gkn663.

[15] W.J. Chi, Y.K. Chang, S.K. Hong, Agar degradation by microorganisms and agar-degrading Enzymes, Appl. Microbiol. Biotechnol. 94 (2012) 917–930.

[16] G. Michel, P. Nyval-Collen, T. Barbeyron, M. Czjzek, W. Helbert, Bioconversion of red seaweed galactans. A focus on bacterial agarases and carrageenases, Appl. Microbiol. Biotechnol. 71 (2006) 23–33, doi:http://dx.doi.org/10.1007/s00253-012-4023-2.

[17] D. Flament, T. Barbeyron, M. Jam, P. Potin, M. Czjzek, B. Kloareg, G. Michel, α-Agarases define a new family of glycoside hydrolases, distinct from β-agarase families, Appl. Environ. Microbiol. 73 (2007) 4691–4694, doi:http://dx.doi.org/10.1128/AEM.00496-07.

[18] S.C. Ha, S. Lee, J. Lee, H.T. Kim, H.J. Ko, K.H. Kim, I.G. Choi, Crystal structure of a key enzyme in the agarolytic pathway, α-neoagarobiose hydrolase from *Saccharophagus degradans* 2-40, Biochem. Biophys. Res. Commun. 412 (2011) 238–244, doi:http://dx.doi.org/10.1016/j.bbrc.2011.07.073.

[19] J.H. Hehemann, G. Correc, F. Thomas, T. Bernard, T. Barbeyron, M. Jam, W. Helbert, G. Michel, M. Czjzek, Biochemical and structural characterization of the complex agarolytic enzyme system from the marine bacterium *Zobellia galactanivorans*, J. Biol. Chem. 287 (2012) 30571–30584, doi:http://dx.doi.org/10.1074/jbc.M112.377184.

[20] A. Rhoads, K.F. Au, PacBio sequencing and its applications, Genom. Proteomics Bioinf. 13 (2015) 278–289, doi:http://dx.doi.org/10.1016/j.gpb.2015.08.002.

[21] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406–425, doi:http://dx.doi.org/10.1093/oxfordjournals.molbev.a040454.