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Complete genome sequence of *Microbulbifer agarilyticus* GP101 possessing genes coding for diverse polysaccharide-degrading enzymes

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다양한 다당류를 분해하는 세균 *Microbulbifer agarilyticus* GP101의 완전한 유전체 서열

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Microbulbifer agarilyticus GP101 was isolated from the gut of a marine invertebrate *Turbo cornutus* and capable of degrading polysaccharide such as agar, alginate, and κ-carrageenan constituting algal cell wall. To obtain genomic basis of polysaccharide-degrading activity, we sequenced genome of strain GP101. The genome consists of 4,255,625 bp, 3,458 coding sequences with 55.4% G + C contents. BLASTP search revealed the presence of seven agarases, five alginate lyases, ten glucanases, four chitinases, two xylanases, one κ-carrageenase, and one laminarinase. The genomic data of strain GP101 will provide potential uses in the bioconversion process of diverse polysaccharide into bioenergy and biochemicals.

Keywords: *Microbulbifer*, agarase, carbohydrate-active enzyme, carrageenan, polysaccharide

Agar, alginate, and carrageenans are cell wall polysaccharides of various marine algae. Agar and carrageenan consist of a linear backbone of D-galactose residues linked by alternating α(1 → 3) and β(1→ 4) linkages (Michel and Czjek, 2013). In agars, the β(1,4)-linked galactose units are in the L configuration whereas they are in the D configuration in carrageenans. A further layer of complexity is added by the number and the position of the sulfate substituents per disaccharide repeat unit and the occurrence of a 3,6-anhydro bridge in the α (1,4)-linked galactose residue (Fu and Kim, 2010). Alginate is a heteropolymer of α-L-gluronate and β-D-mannuronate. Algal oligosaccharides degraded from agar, alginate, and carrageenan were reported to possess diverse physiological and biological functions and hold potential applications in food, cosmetic and medical industries (Michel and Czjek, 2013). Therefore, the search of novel microorganisms and enzymes that efficiently degrade polysaccharides is becoming increasingly crucial (Ohta *et al*., 2004; Vijayaraghavan and Rajendran, 2012; Swift *et al*., 2014; Lee and Choi, 2016; Zhu *et al*., 2016).

Genus *Microbulbifer* currently contains 22 species and mainly associated with marine environment (Parte, 2018). Up to date, three complete and twelve draft genomes of the *Microbulbifer* strains have been deposited into the NCBI genome database. To investigate gut microbiota of marine invertebrate, *Turbo cornutus* was obtained from Gapa island, Republic of Korea and its gut was aseptically dissected. A strain with agarase, alginate lyase, κ-carrageenase, and chitinase activities was isolated from homogenized gut and designated as *Microbulbifer agarilyticus* GP101 based on 16S rRNA gene

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similarity. We sequenced genome of strain GP101 and performed genomic analysis to obtain genomic basis of polysaccharide-degrading activity. Genomic DNA was isolated using Wizard Genomic DNA purification kit (Promega). Genome was sequenced by single molecule real-time sequencing method (SMRT) using PacBio RSII system from 20 kb library. The number of total read was 100,362 (319.14X coverage). *De novo* assemble was achieved using PacBio SMRT Analysis 2.3. Sequencing and assembly was performed by Chunlab Inc., which yielded a single circular chromosome (4,255,625 bp) with a G + C content of 55.4%. Genome was annotated by NCBI Prokaryotic Genome Annotation Pipeline. The genome contained 3,458 coding sequences, 34 pseudo genes, and 63 RNA-coding sequences. RNAs contained 3 copies of 5S, 16S, and 23S ribosomal RNA, 50 tRNAs, and 4 non-coding RNAs.

According to CAZy database (www.cazy.org), strain GP101 contains many carbohydrate-active enzymes such as 59 glycoside hydrolases, 36 glycosyltransferases, 7 polysaccharide lyases, and 40 carbohydrate-binding modules. BLASTP search predicted 7 agarases which cleave glycosidic bond between the repeating units alternating 3,6-anhydro-α-L-galactose and β-D-galactose to produce neoagarobiose, 3-*O*-(3,6-anhydro-L-galactosyl)-β-D-

Table 1. Predicted polysaccharide-degrading enzymes of *M. agarilyticus* GP101

Predicted function	Locus tag*	CAZy family	Accession number of closest characterized protein	Percent identity
Alginate lyase	00390	PL7	BAV10560.1 (*Falsirhodobacter* sp. alg1)	47.08
	00420	PL17	AHW45238.1 (*Shewanella* sp. Kz7)	61.09
	00425	PL6	AHC69713.1 (*Shewanella* sp. Kz7)	63.31
	07840	PL7	WP_053404615.1 (*Persicobacter* sp. CCB-QB2)	72.84
	17615	PL7	ASA33933.1 (*Vibrio* sp.)	34.59
Agarase	02005	GH50	AGT98631.1 (*Thalassotalea agarivorans*)	45.71
	05875	GH86	BAG48880.1 (*Cellvibrio* sp. OA-2007)	28.78
	05880	GH86	BAG48880.1 (*Cellvibrio* sp. OA-2007)	51.68
	05925	GH16	WP_066965750.1 (*Microbulbifer* sp. Q7)	84.73
	05935	GH50	AGT98631.1 (*Thalassotalea agarivorans*)	51.92
	06020	GH50	AGT98631.1 (*Thalassotalea agarivorans*)	54.14
	06025	GH50	BAA04744.1 (*Vibrio* sp.)	49.13
κ-Carrageenase	05680	GH16	AAW20552 (*Pseudoalteromonas carrageenovora*)	40.60
Glucanase	00780	GH1	ASU50143.1 (*Cellulomonas biazotea*)	44.68
	04970	GH5	AAK39540.1 (*Bacillus subtilis*)	46.80
	04975	GH30	AAP04424.1 (*Pseudoalteromonas* sp. DY3)	54.26
	04990	GH5	AAK39540.1 (*Bacillus subtilis*)	35.35
	05015	GH3	ABG59531.1 (*Cytophaga hutchinsonii*)	32.38
	05395	GH3	ABG59531.1 (*Cytophaga hutchinsonii*)	30.72
	05405	GH3	ABG59531.1 (*Cytophaga hutchinsonii*)	33.59
	05415	GH5	ABC30636.1 (*Hahella chejuensis* KCTC 2396)	28.81
	15220	GH9	ABN51651.1 (*Ruminiclostridium thermocellum* ATCC 27405)	30.77
	15460	GH3	ABG59531.1 (*Cytophaga hutchinsonii*)	32.62
Chitinase	05070	GH18	WP_051089467.1 (*Microbulbifer variabilis*)	68.19
	06600	GH18	DAA01334.1 (*Saccharophagus degradans* 2-40)	34.55
	07130	GH18	BAP19085.1 (*Stenotrophomonas maltophilia* N4)	36.88
	13195	GH18	ACI24006.1 (*Bacillus licheniformis* MS-3)	54.17
Laminarinase	15465	GH16	CAZ96583 (*Zobellia galactanivorans* Dsij)	31.50
Xylanase	00765	GH26	AIX87981.1 (*Pseudomonas vesicularis* MA103)	32.24
	03600	GH10	ABD81893.1 (*Saccharophagus degradans* 2-40)	51.58

* Prefix for locus tag of strain GP101 is "Mag101_".

galactopyranose or agarobiose, 4-*O*-(β-D-galactopyranosyl)-3,6-anhydro-L-galactose. Anhydrogalactose-degrading pathway was also predicted (Lee *et al.*, 2014, 2016). In addition, BLASTP search identified many genes related to polysaccharide degradation; five alginate lyases, ten glucanase, four chitinases, two xylanases, one κ-carrageenase, and one laminarinase (Table 1).

Genes coding for polysaccharide-degrading enzymes could be utilized as biological parts for engineering metabolic pathway to produce oligosaccharide and biochemical. Therefore, the genome data of *M. agarilyticus* GP101 could provide abundant novel enzymes for biodegrading various polysaccharides and enhancing bioconversion efficiency of polysaccharide biomass.

Strain and nucleotide sequence accession numbers

M. agarilyticus GP101 was deposited to Korean Collection for Type Cultures (KCTC) under accession KCTC 52777. The complete genome sequence of GP101 has been deposited at DDBJ/EMBL/GenBank under the accession CP019650.

적 요

Microbulbifer agarilyticus GP101은 소라(*Turbo cornutus*)의 내장에서 분리되었으며 해조류 유래 다당류인 한천, 알긴산, κ-카라기난을 분해하는 특징이 있다. GP101 균주의 유전체는 4,255,625 bp 크기로 3,458개의 코딩 서열을 포함하며 55.4%의 GC 함량을 가진다. BLASTP 분석 결과 7개의 agarase, 5개의 alginate lyase, 10개의 glucanase, 4개의 chitinase, 2개의 xylanases, 1개의 κ-carrageenase, 1개의 laminarinase의 존재를 확인하였다. *M. agarilyticus* GP101의 유전체 정보는 다당류의 생물전환 공정에 이용할 수 있는 유전 정보를 제공할 수 있을 것이다.

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