Genes for degradation and utilization of uronic acid-containing polysaccharides of a marine bacterium *Catenovulum* sp. CCB-QB4

Go Furusawa [Corresp., 1] , **Nor Azura Azami** [1] , **Aik-Hong Teh** [1]

[1] Centre for Chemical Biology, Universiti Sains Malaysia, Bayan Lepas, Penang, Malaysia

Corresponding Author: Go Furusawa
Email address: furusawa@usm.my

Background

Oligosaccharides from polysaccharides containing uronic acids are known to have many useful bioactivities. Thus, polysaccharide lyases (PLs) and glycoside hydrolases (GHs) involved in producing the oligosaccharides have attracted interest in both medical and industrial settings. The numerous polysaccharide lyases and glycoside hydrolases involved in producing the oligosaccharides were isolated from soil and marine microorganisms. Our previous report demonstrated that an agar-degrading bacterium, *Catenovulum* sp. CCB-QB4, isolated from a coastal area of Penang, Malaysia, possessed 183 glycoside hydrolases and 43 polysaccharide lyases in the genome. We expected that the strain might be degraded and use uronic acid-containing polysaccharides as a carbon source, indicating that the strain has a potential for a source of novel genes for degrading the polysaccharides.

Methods

To confirm the expectation, the QB4 cells were cultured in artificial seawater media with uronic acid-containing polysaccharides, namely alginate, pectin (and saturated galacturonate), ulvan, and gellan gum, and the growth was observed. The genes involved in degradation and utilization of uronic acid-containing polysaccharides were explored in the QB4 genome using CAZy analysis and BlastP analysis.

Results

The QB4 cells were capable of using these polysaccharides as a carbon source, and especially, the cells exhibited a robust growth in the presence of alginate. 28 PLs and 22 GHs related to degradation of these polysaccharides were found in the QB4 genome based on the CAZy database. 11 polysaccharide lyases and 16 glycoside hydrolases contained lipobox motif, indicating that these enzymes play an important role in degrading the polysaccharides. 14 of 28 polysaccharide lyases were classified into ulvan lyase, and the QB4 genome possessed the most abundant ulvan lyase genes in the CAZy database. Besides, genes involved in uronic acid metabolisms were also present in the genome. These results were consistent with cell growth. In the pectin metabolic pathway, the strain had genes for three different pathways. However, the growth experiment using saturated galacturonate exhibited that the strain can only use the pathway related to unsaturated galacturonate.

1   **Genes for degradation and utilization of uronic acid-containing polysaccharides of a**

2   **marine bacterium Catenovulum sp. CCB-QB4**

4   **Go Furusawa* Nor Azula Binti Azami, and Aik-Hong Teh**

6   Author's institutional affiliation:

7   Centre for Chemical Biology, Universiti Sains Malaysia, 10 Persiaran Bukit Jambul, 11900

8   Bayan Lepas, Penang, Malaysia

10   Contact information;

11   Go Furusawa

12   Centre for Chemical Biology, Universiti Sains Malaysia, 10 Persiaran Bukit Jambul, 11900

13   Bayan Lepas, Penang, Malaysia

14   E-mail: furusawa@usm.edu.my

32   **Abstract**

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36   producing the oligosaccharides have attracted interest in both medical and industrial settings.

37   The numerous polysaccharide lyases and glycoside hydrolases involved in producing the

38   oligosaccharides were isolated from soil and marine microorganisms. Our previous report

39   demonstrated that an agar-degrading bacterium, *Catenovulum* sp. CCB-QB4, isolated from a

40   coastal area of Penang, Malaysia, possessed 183 glycoside hydrolases and 43 polysaccharide

41   lyases in the genome. We expected that the strain might be degraded and use uronic acid-

42   containing polysaccharides as a carbon source, indicating that the strain has a potential for a

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47   and gellan gum, and the growth was observed. The genes involved in degradation and utilization

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51   The QB4 cells were capable of using these polysaccharides as a carbon source, and especially,

52   the cells exhibited a robust growth in the presence of alginate. 28 PLs and 22 GHs related to

53   degradation of these polysaccharides were found in the QB4 genome based on the CAZy

54   database. 11 polysaccharide lyases and 16 glycoside hydrolases contained lipobox motif,

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56   polysaccharide lyases were classified into ulvan lyase, and the QB4 genome possessed the most

57   abundant ulvan lyase genes in the CAZy database. Besides, genes involved in uronic acid

58   metabolisms were also present in the genome. These results were consistent with cell growth. In

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60   growth experiment using saturated galacturonate exhibited that the strain can only use the

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62

63 **Introduction**

64 Uronic acids are a class of sugar acids oxidized the hydroxyl group on C6 of aldoses. Uronic acids

65 including D-glucuronic acid, D-galacturonic acid, D-mannuronic acid, L-guluronic acid, and L-

66 iduronic acid are components of polysaccharides produced by animals (heparin), terrestrial plants

67 (pectin), seaweed (alginate and ulvan), and bacteria (gellan gum) (de Lederkremer and Marino,

68 2003). In general, these polysaccharides such as pectin, alginate, ulvan, and gellan gum, have broad

69 potential in many applications due to their excellent properties of biocompatibility, non-toxic,

70 immunogenicity, availability, and relatively low cost (Morelli. A. and Chiellini, 2010; Venkatesan

71 et al., 2015; Rahman et al., 2017).

72   Pectin is a polymer with a linear structure characterized by a backbone consisting of a few

73 hundred to thousand D-galacturonic acid units linked together by α-(1→4)-glycosidic linkages. It

74 found in the cell walls of the plant and intracellular layer of plant cells, mainly fruits, such as

75 apples, oranges, and lemons (Mudgil, 2017). Pectin contains a significant amount of neutral sugar,

76 typically L-rhamnose, L-arabinose, D-galactose, D-xylose, and D-glucose which linked to the

77 hydroxyl groups on the number 2 and 3 carbons of the main chain. Pectin attracted attention due

78 to its gelling capabilities (Penhasi and Meidan, 2015). Pectin is widely used as a thickener and

79 stabilizing agent in the food industry (Munarin et al., 2012).

80   Alginate, also known as alginic acid is an unbranched polymer composed of β-D-

81 mannuronic acid (M) and α-L-guluronic acid (G), which are covalently (1-4)-liked. The residues

82 are randomly arranged into MM-, GG- and MG-blocks (Meng and Liu, 2013). Alginate is

83 distributed widely in the cell wall of marine brown agar and has long been used in the industry

84 such as medical field, fabric, food, and beverage industries as thickening, gel-forming, and

85 colloidal stabilizing agents (Liakos et al., 2013; Martău et al., 2019).

86   Ulvan is water-soluble polysaccharides found in the cell wall of green algae

87 (*Ulva* and *Enteromorpha*) composed mainly of 3‑sulfated rhamnose (Rha3S), glucuronic acid

88 (GlcA), iduronic acid (IdoA), and xylose (Xyl) (Kim et al., 2011). Ulvan has attracted

89 pharmaceutical and medical applications for its anti-viral, anti-coagulant, and anti-proliferative

90 activities towards cancer cells and its immune-stimulating properties (Alves, Sousa & Reis, 2013).

91 In addition, ulvan also showed to be an activator of plant defense and an inducer of plant resistance

92 (Alves, Sousa & Reis, 2013). Therefore, it is a good potential for agricultural applications.

93   Gellan gum is an exopolysaccharide produced from non-pathogenic, Gram-negative

94   bacterium, *Sphingomonas elodea* (earlier *Pseudomonas elodea*) using aerobic fermentation

95   (Vendrusculo et al., 1994). Gellan gum consisting of repeating tetrasaccharide units of glucose,

96   glucuronic acid, and rhamnose residues in a 2:1:1 ratio: [→3)-β-D-glucose-(1→4)-β-D-glucuronic

97   acid-(1→4)-β-D-glucose-(1→4)-α-L-rhamnose-(1→] (Jansson et al., 1983). Gellan gum has been

98   used in medicine, pharmaceutical formulations, cosmetics, or tissue engineering. As a

99   biocompatibility polysaccharide, gellan gum is used in contact with or inside the body. Besides,

100   gellan gum also useful in the food and biotechnology industry as immobilization of enzymes and

101   yeast cells (Iurciuc (Tincu) et al., 2015).

102   These polysaccharides could also be alternative sustainable sources for fermentative

103   biofuel production (John et al., 2011). The PLs and GHs play an important role in the

104   saccharification of the polysaccharides in the process of biofuel production (Edwards et al., 2011;

105   Takeda et al., 2011; Li et al., 2015). For example, endo- and exolytic alginate lyases from

106   *Saccharophagus degradans* A1 were co-displayed on the yeast cell surface, and the co-displaying

107   yeasts were able to effectively produce monosaccharides (Takagi et al., 2016b). On the other hand,

108   oligosaccharides from uronic acids generated by PLs are known to have multiple biological

109   activities. For instance, alginate oligosaccharides stimulate the growth of human endothelial

110   (Kawada et al., 1997) and keratinocytes cells (Kawada et al., 1999). In addition, the

111   oligosaccharides also promote the growth and root elongation of rice and barley (Tomoda et al.,

112   1994; Hien et al., 2000). Mandalari and colleagues reported that pectin oligosaccharides (POS)

113   have probiotic effects through the improvement of bifidobacteria and lactobacillus (Mandalari et

114   al., 2007). Besides, POS inhibited inflammation, fibrosis formation, as well as cancer progression,

115   transformation, and metastasis (Bonnin Garnier & Ralet, 2014). Thus, PLs and GHs have attracted

116   considerable interest in both academic and commercial spheres.

117   Many bacterial species, such as the genera *Agrobacterium, Bacillus, Cellulophaga,*

118   *Clostridium, Erwinia, Flammeovirga, Flavobacterium, Microbulbifer, Psuedoalteromonas,*

119   *Pseudomonas, Saccharophagus, Sphingomonas, Vibrio, Xanthomonas,* and *Zobellia*, are known

120   as bacteria that are capable of degrading uronic acid-containing polysaccharides, mainly alginate

121   and pectin (Liu et al., 2019; Dubey et al., 2016; Takagi et al., 2016). The genus *Catenovulum*

122   consisted of three species, *C. agarivorans, C. maritimum*, and *C. sediminis*, was also known to

123   degrade agar (all three strains) and alginate (*C. maritimum*, and *C. sediminis*). Besides that,

124   *Catenovulum* sp. LP was able to produce an ulvan lyase (Li et al., 2015; Qiao et al., 2020).

125   However, the degradation and utilization pathway of uronic acid-containing polysaccharides

126   containing alginate, gellan gum, pectin, and ulvan by the genus *Catenovulum* are poorly

127   understood. Our group isolated *Catenovulum* sp. CCB-QB4 (referred to hereafter as QB4) from

128   Queens Bay of Penang, Malaysia, and the complete genome sequence was reported (Lau et al.,

129   2019). From the study, it was reported that the QB4 genome contained 183 GHs and 43 PLs. Based

130   on the information, this bacterium is predicted to have the ability to utilize many polysaccharides.

131   To confirm the expectation, in this study, the cell growth of QB4 in the presence of polysaccharides

132   containing uronic acids, namely, alginate, pectin, ulvan, and gellan gum, was confirmed using a

133   shake flask fermentation method. In addition, genes involved in degrading and utilizing these

134   polysaccharides were explored in the QB4 genome. This study is the first report to describe the

135   degradation and probable metabolic pathways for utilization of four different uronic acid-

136   containing polysaccharides in the genus Catenovulum.

137

138   **Materials and Methods**

139

140   **Strain and chemicals**

141   The QB4 cells (Lau et al., 2019) was cultured using high nutrient artificial seawater medium (H-

142   ASWM) condition [0.5% tryptone, 2.4% (w/v) artificial sea salt mix (Marine Enterprises

143   International), 10mM HEPES, pH 7.6], as reported by Furusawa and co-workers, 2015 (Furusawa

144   et al., 2015). 0.1 % of polygalacturonic acid [pectic acid] (Nacalai Tesque), sodium alginate

145   (Sigma-Aldrich), Gelzan™ CM (gellan gum) (Sigma-Aldrich), and ulvan were used as carbon

146   source throughout the research.

147

148   **Purification of ulvan**

149   The ulvan was purified according to the method described by Tabarsa et al. (2012) with slight

150   modification. 5 g of the dry powder of *Ulva pertusa* was dissolved and stirred in 100 mL of water

151   and stirred at 65 ºC for 3 h. The mixture was cooled and centrifuged at 10,000 g for 20 min at 15

152   ºC using Sorvall™ RC 6 Plus Centrifuge (ThermoFisher Scientific). 4 volume of cold isopropanol

153   was added into the supernatant to precipitate crude polysaccharide. The solution was left at 4 ºC

154   for overnight. The crude ulvan was harvested by filtration, washed with 70 % isopropanol several

155   times, and dried overnight at 60 ºC.

156

157   **Determination of bacterial growth**

158   The QB4 cells were inoculated in 10 mL of H-ASWM broth and cultured overnight at 30 ºC. 0.1

159   mL of the cell suspension was inoculated into 100 mL of H-ASWM medium with 0.2% of each

160   uronic acid as the carbon sources. Media with and without 0.2% glucose (Fisher Scientific) were

161   used as positive and negative controls, respectively.

162   Each flask was inoculated with 0.1 mL of pre-cultured bacterial cell suspension and incubated at

163   30 ºC with an agitation speed of 200 rpm on the orbital shaker. To monitor the bacterial growth,

164   the colony-forming unit (CFU) counts were conducted by culturing the cells on H-ASWM agar

165   plates because the optical density measurement was not suitable for the sample with gelzan in

166   which the broth was solidified by calcium ion contained in H-ASWM medium. 100 μl of the cell

167   suspension from each sample was collected every 3 hours. The suspension was diluted into 900 μl

168   of H-ASWM medium. After that, sequential 10-fold serial dilutions were made, and 100μl of

169   aliquots of each dilution were plated on H-ASWM agar plates. Colonies were scored after

170   incubation for 48 h at 30 ºC, and the growth curve was constructed. The experiment was performed

171   in triplicates. The generation time (G) was estimated by the method described by Aparna et al.

172   based on the growth curve (Aparna et al., 2020). The number of generation (n) was determined by

173   the method of n = (logb – logB)/log2, where B and b are the number of bacteria at the beginning

174   of a time interval and at the end of the time interval, respectively. The generation time (G) was

175   determined by the method of G = t/n, where t is the time interval (min).

176   To confirm the utilization of saturated galacturonate, the QB4 cells were culture in an H-ASWM

177   medium with 0.2% saturated galacturonate. Media with and without 0.2% glucose was used as

178   positive and negative controls, respectively. To monitor the bacterial growth, the optical density

179   (OD_{600nm}) was measured at 3 h intervals for 30 h using UV spectrophotometer UV-1800

180   (Shimadzu). The experiment was performed in triplicates.

181

182   **Genomic analysis of *Catenovulum* sp. CCB-QB4**

183   The complete genome of QB4 deposited at GenBank under the accession number CP026604-

184   CP026605 was determined by Lau et al. (Lau et al., 2019). Genes involved in the carbohydrate-

185 active enzyme (CAZymes) in QB4 were predicted using dbCAN pipelines (Yin et al., 2012).

186 Several enzymes related to uronic acid metabolism in QB4 were predicted by Blastp at the National

187 Center for Biotechnology Information (NCBI) server (Bethesda, MD, U.S.A.) and were found

188 using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Kanehisa and

189 Goto, 2000). Finally, the amino acid sequence similarity of all enzymes was confirmed by using

190 Blastp analysis with Protein Data Base (PDB). Signal peptide prediction was conducted by LipoP

191 1.0 (Juncker et al., 2003) and SignalP 5.0 (Armenteros, et al., 2017). For sequence alignment of

192 DEH reductase, the amino acid sequence of A1-R and A1-R' from *Sphingomonas* sp. A1 and DEH

193 reductase from *Saccharophagus degradans* 2-40 were obtained from GenBank

194 (https://www.ncbi.nlm.nih.gov/genbank/). The sequence alignment with 4 sequences was

195 conducted by ClustalW (Thompson et al., 1994) at PRABI Lyon-Gerland (https://npsa-

196 [prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html](https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html)).

197

198

199 **Results**

200 **Growth confirmation of QB4 using uronic acids.**

201 To confirm the growth of QB4 in the presence of uronic acids, the cells of QB4 were cultured in

202 H-ASWM with alginate, pectin, ulvan, and gelzan. As shown in figure 1, the cells of QB4

203 showed good growth in the presence of the uronic acid-containing polysaccharides and glucose

204 except for negative control. The cells of QB4 reached the early stationary phase at 9 h in H-

205 ASWM with alginate and gelzan. The generation time of alginate and gelzan was 76.76 and

206 74.07 min. However, the cell number of the sample with gelzan was 5.7 times lower than that of

207 the sample with alginate. On the other hand, the cells cultured by pectin and glucose reached the

208 early stationary phase at 18 h. Besides that, in the case of using ulvan, the cells reached

209 stationary phase at 24 h, and the sample exhibited the highest cell number at 30 h in the

210 experiment. The generation time of the samples with glucose, pectin, and ulvan was 91.37,

211 95.74, and 85.10 min, which were longer than that of the samples with alginate and gelzan. Thus,

212 QB4 cells exhibited a robust growth in the presence of uronic acids. Subsequently, we focused

213 on genes for degradation and utilization of uronic acids in the genome of QB4.

214

215 **Genes for alginate degradation and utilization**

216   Alginate is a major polysaccharide found in the cell wall of brown algae and consisted of

217   guluronate and mannuronate arranged as 1,4-linked polysaccharides. First, alginate is degraded

218   into oligomeric or monomeric units by alginate lyases. Eight alginate lyase genes were found in

219   the QB4 genome (Table 1). Ad1-PL6, Ad2-PL6 and Ad3-PL6, Ad4-PL7, Ad5-PL7, Ad6-PL7,

220   Ad7-PL7, and Ad8-PL17 were classified into family PL6, PL7, and PL17 based on the CAZy

221   database, respectively. As a result of Blastp search with Protein Data Bank (PDB), Ad proteins

222   were similar to Alygc from *Paraglaciecola chathamensis* (Ad1-PL6 and Ad3-PL6), AlyQ from

223   *Persicobacter* sp. CCB-QB2 (Ad2-PL6, Ad4-PL7, and Ad5-PL7), alginate lyase of *Klebsiella*

224   *pneumoniae* (Ad6-PL7 and Ad7-PL7) and *Saccharophagus degradans* 2-40 (Ad8-PL17) (Table

225   S1). LipoP 1.0 (Juncker et al., 2003) was used to predict the signal peptides and their type of

226   each enzyme. Table 1 demonstrated that Ad1-PL6, Ad2-PL6, Ad4-PL7and Ad5-PL7 possessed

227   type I signal peptide, which was cleaved by signal peptidase I, and Ad6-PL7, Ad7-PL7 and Ad8-

228   PL17 have type II lipoprotein signal peptide which was cleaved by signal peptidase II. The

229   lipoprotein signal peptide referred to as "lipobox" plays an important role in anchoring the

230   protein on the outer surface of the cell membrane after secretion and modification of N-terminal

231   cysteine residue (Pugsley et al., 1986; Hutcheson et al., 2011). This suggested that Ad6-PL7,

232   Ad7-PL7, and Ad8-PL17 may localize on the cell surface. In contrast, Ad1-PL6, Ad2-PL6, Ad4-

233   PL7, and Ad5-PL7 may be released into the culture medium. On the other hand, Ad3-PL6, which

234   does not possess any signal peptides, may locate at the cytoplasm.

235   Takase et al. (Takase et al., 2010) described that an exotype oligoalginate lyase, A1-IV, from

236   *Sphingomonas* sp. A1 degraded oligoalginates into monosaccharides, which are then

237   nonenzymatically converted to 4-deoxy-L-erythro-5-hexoseulose uronic acid (DEH). Takagi and

238   colleagues also reported that an alginate lyase, Alg7K, from *Saccharophagus degradans* showed

239   exolytic activity and produced monosaccharides from oligoalginates (Takagi et al., 2016a). The

240   amino acid sequence of the alginate lyase, Ad6-PL7, showed high similarity (77.3 %) to that of

241   Alg7K, suggesting that Ad6-PL7 might mediate hydrolysis of oligoalginate to produce

242   monomers. The DEH is converted to 2-keto-3-deoxy-D-gluconate (KDG) by NADH or NADPH-

243   dependent DEH reductase (Takase et al., 2010; Takase et al., 2014; Kim et al., 2016). A result of

244   the BLASTp search demonstrated that the SDR family oxidoreductase, Au1, found in the QB4

245   genome (Table 1) showed high similarity to NADH (A1-R', 60.9 %) and NADPH (A1-R, 50.8

246   %)-dependent DFH reductase. In addition, the gene aliments of DEH reductases showed that the

247 TGXXXGX motif and catalytic triad (Ser, Tyr, and Lys) are highly conserved in SDR family

248 enzymes (Takase et al., 2014) were conserved in the SDR family oxidoreductase (Fig. S1). These

249 results indicated that Au1 might function as DEH reductase. KDG kinase catalyzes the

250 conversion of KDG to 2-keto-3-deoxy-phosphogluconate (KDPG), and then KDGP is converted

251 into D-glyceraldehyde-3-phosphate and pyruvate by 2-dehydro-3-deoxy-phosphogluconate

252 aldolase via Entner -Doudoroff pathway. Two of the genes encoding the KDG kinase, Au2 and

253 Au3, and the aldolase, Au4, and Au5, were found in the genome of QB4 (Table 1). Finally,

254 pyruvate produced by the pathway goes into further metabolic pathways for generating energy.

255 The five Au proteins also demonstrated a high degree of amino acid sequence similarity (>50 %)

256 to DEH reductase A1-R' from *Sphingomonas* sp. A1 (Au1), KDG kinase *Shigella flexneri* (Au2

257 and 3), 2-keto-3-deoxy-6-phosphogluconate aldolase from *Thermotoga maritima* (Au4), and

258 Kdpg aldolase from *Escherichia coli* (Au5) (Table S1). As mentioned above, QB4 possessed

259 many alginate lyases and enzymes responsible for alginate utilization. This is consistent with

260 robust growth of QB4 in the presence of alginate, as shown in Fig. 1.

261

262 **Genes for pectin degradation and utilization**

263 As a first step, pectin is depolymerized by pectin lyases or polygalacturonases. Pectin and pectate

264 lyases are classified into five families of PLs. Although pectin lyases attack highly methyl-

265 esterified pectin, pectate lyases specifically attack non-methylated polygalacturonate or

266 methylated pectin with a very low degree (Hugouvieux-Cotte-Pattat et al., 2014). These enzymes

267 degrade pectin to unsaturated pectic-oligosaccharides and disaccharides.

268 In the CAZy database, seven pectate lyases belonging to family PL1 (Pd1_PL1, Pd2_PL1,

269 Pd3_PL1, Pd4_PL1, and Pd5_PL1), PL3 (Pd6_PL3), and PL10 (Pd7_PL10) family pectate

270 lyases were found in the genome of QB4. The result of Blastp search using PDB database on Pd

271 proteins also demonstrated that these Pd proteins were similar (>45 %) to pectate lyase

272 (Pd1_PL1, Pd2_PL1, Pd3_PL1, Pd5_PL1, Pd6_PL3, and Pd7_PL10) and pectinesterase

273 (Pd4_PL1) from other bacterial species (Table S2). CBM13 found in Pd1_PL1 and Pd6_PL3

274 and CBM35 found in Pd2_PL1 and Pd3_PL1 were capable of binding to multi-ligands, such as

275 arabinan, arabinoxylan, and pectin (Fujimoto, 2013; Dhillon et al., 2018). However, CBM77

276 contained in Pd2_PL1 recognized homogalacturonan (Fujimoto 2013). Pectin methylesterase

277 (PME) domain from family 8 Carbohydrate Esterase (CE8) was found in Pd4_PL1 and

278 Pd5_PL1. PMEs produce de-esterified homogalacturonan by catalyzing the de-esterification of

279 the methoxyl group of the pectin; as a result, the products are effectively degraded by pectate

280 lyases (Kashyap et al., 2001). Besides, although Pd1_PL1, Pd3_PL1, Pd4_PL1, and Pd7_PL10

281 possessed type I signal peptide, Pd5_PL1 and Pd6_PL3 had lipobox, indicating that both two

282 lyases localize on the cell surface. Hence, these results suggested that Pd5_PL1 plays an

283 important role in the pectin degradation of QB4 due to its de-esterification and depolymerization

284 activities and its localization.

285   The unsaturated disaccharides generated by the pectate lyases are converted to 5-keto-4-

286 deoxyuronate (DKI) by two distinct processes. One is that the disaccharides are degraded by

287 oligogalacturonate lyase belonging to family PL22, and then the product, Δ-4,5-unsaturated

288 galacturonate, is linearized into DKI by KdgF (Hobbs et al., 2016). The other is that unsaturated

289 galacturonyl hydrolases belonging to GH105 degrades the disaccharides and directly releases

290 DKI (Hobbs et al., 2019). In the QB4 genome, no PL22 enzymes were found in the genome.

291 However, three GH105 proteins, Pd8_GH105, Pd9_GH105, and Pd10_GH105, were present in

292 the genome (Table 2). These enzymes showed a high degree of similarity (49 ~ 67 %) on

293 unsaturated rhamnogalacturonyl hydrolases (YteR) based on Blastp search with PDB database.

294 These results indicated that oligogalacturonate might directly convert to DKI by GH105 proteins

295 in QB4. DKI is converted to 2-keto-3-deoxygluconate (KDG) by two enzymes, a DKI isomerase

296 (KduI) and a 2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase (KduD) (Hobbs et al., 2019). The

297 pathway analysis based on KEGG showed that four of KduI (Pu1, Pu2, Pu3, and Pu4) and KduD

298 (Pu5, Pu6, Pu7, and Pu8) were present in the genome of QB4. Blastp search with PDB database

299 also exhibited that these enzymes showed a high degree of similarity (63 ~ 71 %) on KduD and

300 KduI from *Enterococcus faecalis*, *Escherichia coli*, and a pectolytic bacterium, *Pectobacterium*

301 *carotovorum* (Table S2). These results indicated that QB4 use pectin as a carbon source for cell

302 growth and is consistent with the result shown in Fig. 1.

303 On the other hand, the pathway analysis also showed the other pathway using saturated

304 galacturonate (monosaccharide) produced by polygalacturonases. As mentioned above, although

305 QB4 possesses seven pectate lyases, only two polygalacturonases (GH28), Pd11_GH28 and

306 Pd12_GH28, which were highly similar (>66 %) to polygalacturonase from pectolytic bacteria,

307 such as *Erwinia carotovora* and *Thermotoga marítima* (Table S2), were found in the genome.

308 Based on SignalP 5.0 analysis, it was found that Pd11_GH28 and Pd12_GH28 contained Tat and

309 type I signal peptide, respectively. Saturated galacturonate is converted to D-tagaturonate by

310 glucuronate isomerase. Three glucuronate isomerases, Pu9, Pu10, and Pu11, which were highly

311 similar (> %) to glucuronate isomerase of *Salmonella enterica* subsp. *enterica* serovar

312 Typhimurium and uronate isomerase of *Caulobacter vibrioides* CB15 (Table S2), were found in

313 the QB4 genome. The conversion of D-tagaturonate to KDG occurs through two distinctly

314 different pathways. One is that the process consists of two steps involving tagaturonate reductase

315 and altronate hydrolase and one intermediate, D-altronate (Richard and Hilditch, 2009). The

316 other is that tagaturonate is converted via three steps involving tagaturonate epimerase,

317 fructuronate reductase, and mannonate dehydratase and two intermediates, D-fructuronate and D-

318 mannonate (Valk et al., 2020). As shown in Table 2, QB4 possessed all genes involved in the

319 two different pathways, namely tagaturonate reductase (Pu12), altronate hydrolase (Pu13),

320 tagaturonate epimerase (Pu14), fructuronate reductase (Pu15), and mannoate dehydratase (Pu16).

321 Homologous enzymes of these proteins were also found in the PDB database (Table S2). This

322 result suggested that QB4 is also capable of using saturated galacturonate as a carbon source. To

323 confirm which pathway is functioning when QB4 cells grew in H-ASWM broth with

324 polygalacturonic acid, the QB4 cells were cultured in H-ASWM broth with polygalacturonic

325 acid or saturated galacturonate. As shown in Figure 2, although the cells with glucose

326 demonstrated robust growth, the cells with were unable to grow in the broth with saturated

327 galacturonate as well as the negative control in the incubation period. This result indicated that

328 the unsaturated galacturonate utilization pathway is the main pathway to utilize polygalacturonic

329 acid of QB4. Interestingly, genes for all pectate lyases, polygalacturonases, and one set of KduI

330 (Pu4), KduD (Pu8), glucuronate isomerase (Pu11), and tegaturonate reductase were located in a

331 plasmid (https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP026605.1) found in QB4 (Table 2).

332

333 **Genes for ulvan degradation and utilization**

334 Ulvan lyases classified into 5 families, PL24, PL25, PL28, PL 37, and PL40 in the CAZy

335 database (Li et al., 2020) were isolated from several marine bacteria, such as genera,

336 *Alteromonas*, *Pseudoalteromonas, Formosa*, and *Nonlabens* (Kopel et al., 2016; Qin et al., 2018;

337 Ulaganathan et al., 2018a; Ulaganathan et al., 2018b; Reisky et al., 2019). Ulvan lyases cleave

338 between L-rhamnose 3-sulfate (Rha3S) and D-glucuronic acid (GlcA) or L-iduronic acid (IdoA).

339 Fourteen ulvan lyases in QB4 were classified into three PLs families, PL24 (Ud1_PL24,

340   Ud2_PL24, Ud3_PL24, Ud4_PL24, Ud5_PL24, Ud6_PL24, Ud7_PL24, Ud8_PL24, and

341   Ud9_PL24), PL25 (Ud10_PL25, Ud11_PL25, Ud12_PL25, and Ud13_PL25) and PL40

342   (Ud14_PL40). Ud proteins belonging to the PL24 family were highly similar (>62 %) to short

343   ulvan lyase of *Alteromonas* sp. LOR, and Ud proteins belonging to the PL25 family were highly

344   similar (>57 %) to ulvan lyase-PL25 of *Pseudoalteromonas* sp. PLSV (Table S3). The number of

345   the ulvan lyases was obviously higher than that of *F. agariphila* (three PL40 proteins) (Reisky et

346   al., 2019) and *Alteromonas* sp. LOR (one PL24 and one nonclassified protein) (Foran et al.,

347   2017). Ud6_PL24 and Ud9_PL24 and the enzymes belonging to PL25 possessed lipobox.

348   Although Ud2_PL24, Ud3_PL24, Ud4_PL24, Ud5_PL24, and Ud8_PL24 contained type I signal

349   peptide, the remaining enzymes did not have any signal peptides. Although only one pectin lyase

350   had a lipobox, many ulvan lyases possessed lipobox as well as alginate lyases, indicating that

351   alginate and ulvan degradation activity of QB4 may be more effective than pectin degradation of

352   the strain.

353   The unsaturated uronyl residue at the non-reducing end of the oligosaccharides produced by the

354   ulvan lyases may be released by unsaturated glucuronyl hydrolases (GH105), because five

355   putative unsaturated glucuronyl hydrolases, Ud15_GH105, Ud16_GH105, Ud17_GH105,

356   Ud18_GH105, and Ud19_GH105 were found in the QB4 genome and showed similarity on

357   unsaturated 3s-rhamnoglycuronyl hydrolase of *Nonlabens ulvanivorans,* which is known as a

358   ulvan-degrading bacterium, based on BLASTp search (Table 3; Table S3) (Collén et al., 2014).

359   After forming DKI by the enzymes, the following process may be the same as pectin utilization.

360   In the ulvan-degrading process of *F. agariphila*, Rha3S-Xyl-Rha3S was also the main product by

361   ulvan lyases (Reisky et al., 2019). First, Rha3S at the non-reducing end is desulfated by

362   sulfatases. The amino acid sequences of two sulfatases, Ulu1 and Ulu2, in the QB4 genome

363   showed high similarity (77.3 and 86.2 %) to the sulfatases, WP.032096151.1 and

364   WP.632096147.1, located into ulvan utilization loci of *Alteromonas* sp. LOR (Foran et al., 2017),

365   indicating that the two sulfatases may involve desulfation of the Rha3S. The Rha3 will remove

366   the Rha3-Xyl-Rha3S by α-L-rhamnosidase. Five α-L-rhamnosidases classified into GH78 family,

367   Ud20_GH78, Ud21_GH78, Ud22_GH78, Ud23_GH78, and Ud24_GH78, were found in the

368   QB4 genome and showed similarity on rhamnosidase from *Bacillus* sp. GL1, *Dictyoglomus*

369   *thermophilum, Streptomyces avermitilis,* and *Bacteroides thetaiotaomicron* (Table S3).

370   Ud21_GH78, Ud22_GH78, Ud23_GH78, and Ud24_GH78 contained lipobox, suggesting that

371 these enzymes are located on the cell surface. In addition, Ud20_GH78 and Ud22_GH78

372 possessed the CBM67 domain, which binds L-rhamnose in a calcium-dependent manner

373 (Fujimoto et al., 2013), suggesting that the two α-L-rhamnosidases are the main components to

374 generate Rha3.

375 Rha3 metabolic pathway was described by Reisky et al., 2019. α-L-rhamnose is converted to β-

376 L-rhamnose by L-rhamnose mutarotase. Next, isomerization of the β-L-rhamnose to L-

377 rhamnulose is catalyzed by rhamnose isomerase. The product is converted to L-rhamnulose-1-

378 phosphate by pentulose/hexulose kinase (rhamnulokinase), and subsequently, the L-rhamnulose-

379 1-phosphate is cleaved into L-lactaldehyde and dihydroxyacetone phosphate by rhamnulose

380 aldolase. Finally, the L-lactaldehyde is converted to pyruvate by aldehyde dehydrogenase and

381 lactate dehydrogenase. Table 3 displayed that the corresponding genes (Ulu3, Ulu4, Ulu5, Ulu6,

382 Ulu7, and Ulu8) were found in the QB4 genome, and these proteins showed similarity on

383 corresponding proteins in the PDB database (Table S3). This result indicated that Rha3 is

384 metabolized by QB4 cells. The genes involving the metabolic pathway downstream of the

385 mutarotation in *F. agariphila* form a gene cluster (Reisky et al., 2019). However, the genes in

386 QB4 were randomly distributed in the genome.

387 Xyl will be released by β-xylosidase classified into GH3 and GH43 families. Based on the CAZy

388 database, seven GH43 (Ud25_GH43, Ud26_GH43, Ud27_GH43, Ud28_GH43, Ud29_GH43,

389 Ud30_GH43, and Ud31_GH43) proteins were found in the QB1 genome while *F. agariphila*

390 possessed two each of GH3 and GH43 proteins (Reisky et al., 2019). Ud25_GH43, Ud29_GH43,

391 and Ud31_GH43 were similar to glycoside hydrolases from *Zobellia galactanivorans* (50.00 %)

392 and *Halothermothrix orenii* H 168 (45.75 and 39.10 %) (Table S3). The D-xylose is converted to

393 D-xylulose-5-phosphate via xylose isomerase and xylulose kinase, and then the product is passed

394 to the pentose phosphate pathway. Two genes encoding xylose isomerase (Ulu9) and xylulose

395 kinase (Ulu10) were found in the QB4 genome based on RAST server annotation and formed a

396 gene cluster with transcription repressor, *xylR* (Ulu11) (Table 3). These results suggested that

397 QB4 cells may use not only glucuronic acid but also L-rhamnose and D-xylose as carbon sources.

398 In addition, the presence of numerous genes encoding ulvan lyase may promote strong ulvan

399 degradation and its utilization. Actually, the QB4 cells exhibited a robust cell growth in the

400 presence of ulvan as well as that in the presence of alginate, as shown in Figure 1.

401

402 **Genes for gellan gum degradation and utilization**

403 Gellan gum is depolymerized gellan lyases classified into PL33 family proteins. Gellan lyases

404 are found in several bacterial species, such as *Bacillus* sp. GL1, *Geobacillus* stearothermophilus

405 98, and *Opitutaceae bacterium* TAV5 (Hashimoto et al., 1997; Derekova et al., 2006; Helbert et

406 al., 2019). Gellan gum is degraded to tetrasaccharides composed of [→3)-β-D-glucose-(1→4)-β-

407 D-glucuronic acid-(1→4)-β-D-glucose-(1→4)-α-L-rhamnose-(1→] by gellan lyases. The

408 tetrasaccharides are completely degraded to monosaccharides by β-D-glucosidase (GH1, GH2,

409 GH3, GH5, GH9, GH16, GH30, GH39, and GH116), unsaturated glucuronyl hydrolase (GH88

410 and GH105), and α-L-rhamnosidase (GH78) (Hashimoto et al., 2003).

411 Figure 1 demonstrated that QB4 might also use gellan gum as a carbon source for its growth.

412 However, gellan lyases were not found in the genome. As shown in Table 3, QB4 possessed five

413 unsaturated glucuronyl hydrolases and five α-L-rhamnosidases (Table 3). The hydrolysates,

414 DEH, and α-L-rhamnose, may be metabolized by the pathway described above. In addition, one

415 β-D-glucosidase classified into the GH1 family (Gd1-GH1) was also found in the genome, and

416 Gd1-GH1 showed high similarity (63.23 %) on β-glucosidase A of *Hungateiclostridium*

417 *thermocellum* in the PDB database (Table 4; Table S3). β-D-glucose produced by the β-D-

418 glucosidases is converted to β-D-glucose-6-phosphate by glucokinase, and then the product is

419 converted to β-D-fructose-6-phosphate, which is an intermediate of glycolysis, by glucose-6-

420 phosphate isomerase. One of these genes, Gu1 and Gu2, were found in the QB4 genome (Table

421 4) and were similar on glucokinase and glucose-6-phosphate Isomerase of *E. coli* in PDB

422 database, respectively (Table S4), indicating that β-D-glucose is converted to β-D-fructose-6-

423 phosphate and is metabolized by glycolysis.

424

425 **Discussion**

426 Polysaccharides from plants, seaweed, and bacteria, especially their oligosaccharides, have

427 attracted considerable attention due to various biological activities. Thus, polysaccharide lyases

428 and glycosyl hydrolases were isolated from microorganisms, including fungi and bacteria.

429 The genus *Catenovulum* have been known as bacteria that can degrade agar and polysaccharide

430 containing uronic acids, such as alginate, pectin, and ulvan (Cui et al., 2014; Li et al., 2015; Lee

431 et al., 2019; Liu et al., 2019). Our previous report demonstrated that many genes encoding

432 polysaccharide degrading enzymes were present in the QB4 genome (Lau et al., 2019. In this

433   study, expectedly, QB4 cells were able to degrade and used the polysaccharides as a carbon

434   source for their growth (Fig. 1). Especially, the QB4 cells exhibited a robust growth in the

435   presence of alginate and ulvan, suggesting that polysaccharides from marine algae are effectively

436   used by QB4 cells rather than that of plants and bacteria. The growth profile of QB4 was

437   different from *S. degradans* of which the cells reached the early stationary phase at 9 h with

438   alginate, glucose, and pectin (Takagi et al., 2016a).

439   Three pathways of pectin metabolisms were known in bacteria. One is the polygalacturonate

440   pathway metabolized unsaturated disaccharides. The pathway was found in bacteria, such as

441   *Escherichia coli* and phytopathogenic enterobacterium, *Erwinia chrysanthemi* (Chatterjee et al.,

442   1985; Richard and Hilditch 2009). The other two pathways metabolize saturated galacturonate

443   through uronate isomerase (isomerase pathway) (Richard and Hilditch, 2009) or tagaturonate

444   epimerase (epimerase pathway) (Rodionova et al., 2012). Table 2 displayed that the QB4

445   possessed genes involved in the three pathways. However, QB4 cells did not grow in the

446   presence of saturated galacturonate (Fig. 2). Although a pectin-degrading marine bacterium,

447   *Pseudoalteromonas* sp. PS47, also has genes for the epimerase pathway, the strain was unable to

448   grow on saturated galacturonate (Hobbs et al., 2019). Hobbs and colleagues proposed that

449   GH28s of the strain are periplasmic proteins and, thus, that saturated galacturonates would be

450   produced in the periplasm (Hobbs et al., 2019). It was suggested that the strain might be unable

451   to transport extracellular saturated galacturonate. On the other hand, although Pd11_GH28

452   containing Tat signal peptide would be exported into periplasm through Tat (for twin-arginine

453   translocation) system (Stanley et al., 2000), Pd12_GH28 would be secreted to the outside of the

454   cells via Sec system, suggesting that saturated galacturonate might be generated in the culture

455   broth in contrast to the strain PS47. However, the QB4 did not grow using saturated

456   galacturonate. Thus, it seems like the BQ4 cells are also unable to transport extracellular

457   saturated galacturonate as well as the strain PS47. In other cases, some investigators reported that

458   the isomerase pathway is not crucial for utilizing pectin in *Dickeya dadantii* (formerly *E.*

459   *chrysanthemi*) (Hugouvieux-Cotte-Pattat et al., 1996; Pédron et al., 2018). It was known that *D.*

460   *dadantii* belonging to the class Gammaproteobacteria, which is a plant pathogen and pectinolytic

461   bacterium, also possessed the polygalacturonate pathway and the isomerase pathway

462   (Hugouvieux-Cotte-Pattat et al., 1996). Even though the enzyme production of the isomerase

463   pathway was not impaired by a mutation in genes of the polygalacturonate pathway (*kduD and*

464   *kduI*), these mutants did not exhibit their growth on polygalacturonate (Hugouvieux-Cotte-Pattat

465   et al., 1996). In addition, the transcriptomic analysis of *D. dadantii* during the early colonization

466   on the plant leaf demonstrated that genes involved in the polygalacturonate pathway were

467   upregulated in the condition (Pédron et al., 2018). These results suggested that the isomerase

468   pathway was not the main pathway for utilizing pectin in the bacterium. The third pathway,

469   epimerase pathway, involving the conversion of D-tagatose to D-fructuronate by tagatose

470   epimerase (UxaE), was found in the hyperthermophilic bacterium, *Thermotoga maritima*,

471   belonging to the phylum Thermotoga. However, the pathway was not found in other bacteria,

472   such as *Escherichia* and *Bacillus* (Kuivanen et al., 2019). *uxaE* and other epimerase pathway

473   genes, such as hexuronate catabolism regulator (*uxaR*), fructuronate reductase (*uxaD*), predicted

474   D-mannonate utilization enzyme (*gntE)* constituted a regulon regulated by the GntR-like

475   transcription factor UxaR in the genus *Thermotoga* (Rodionova et al., 2012). A regulon is a gene

476   cluster or operon that is regulated by the same regulatory protein. However, Table 2

477   demonstrated that genes encoding Pu14, Pu15, and Pu16 were scattered in the chromosome and

478   plasmid, suggesting that Pu14, Pu15, and Pu16 might not constitute a regulon. In addition, figure

479   2 demonstrated that the saturated galacturonate, which is a substrate of the epimerase pathway,

480   was not used by QB4 cells. These results suggested that the epimerase pathway might not

481   function in QB4.

482   Figure 1 demonstrated that the QB4 cells exhibited a robust growth using ulvan as a carbon

483   source. Qiao *et al*. (2020) fermented *Catenovulum* sp. LP using the shaking-flask method

484   containing 1.2 % purified ulvan, of which the concentration was 6 times-higher than that of the

485   QB4. The bacterial culture reached the stationary phase at 36 h incubation period (Qiao et al.,

486   2020) that was quite slower than that of the QB4. Table S5 showed that the QB4 genome

487   contained 14 ulvan lyase genes, which is the largest number compared to *Siansivirga*

488   *zeaxanthinifaciens* CC-SAMT-1 (8 genes) *Tamlana* sp. UJ94 (7 genes), *Polaribacter* sp. BM10

489   (7 genes), and *Wenyingzhunangia fucanilytica* CZ1127 (7 genes) in the CAZy database (Table

490   S5). The numerous genes in the QB4 genome may be crucial to efficiently degrade ulvan and to

491   stimulate robust growth.

492   Figure 1 demonstrated that the QB4 cells were capable of using gelzan as a carbon source,

493   however, no gallan lyases found in the QB4 genome. It was known that *Bacillus* sp. GL1 and *G.*

494   *stearothermophilus* 98, which possessed all genes for gellan gum degradation and utilization,

495   were able to use gellan gum as a sole carbon source (Hashimoto et al., 1998; Derekova et al.,

496   2006). On the other hand, although *Paludisphaera borealis* PX4 belonging to the order

497   *Planctomycetales* does not have any gellan lyases, the bacterium was capable of degrading gellan

498   gum (Ivanova et al., 2017). The authors suggested that an unsaturated glucuronyl hydrolase and

499   two α-L-rhamnosidases of the bacterium are involving degrading gellan gum. Tables 3 and 4

500   showed that QB4 cells possess five unsaturated glucuronyl hydrolases, five α-L-rhamnosidases,

501   and one β-D-glucosidase. We expected that these hydrolases play a critical role in degrading

502   gellan gum in QB4 cells. On the other hand, the lack of gellan lyases may cause lower

503   degradation efficiency. Thus, the cell growth with gellan gum was not robust compared to those

504   with other uronic acids.

505

506   **Conclusion**

507   The agar-degrading bacterium, *Catenovulum* sp. CCB-QB4, used uronic acids, including

508   alginate, pectin ulvan, and gellan gum as carbon sources for its growth. Especially, QB4 cells

509   exhibited a robust cell growth in the presence of alginate and ulvan from seaweed. In gene

510   analysis based on the CAZy database, a large number of polysaccharide lyases and hydrolases

511   involved in degrading these uronic acids were found in the QB4 genome. Many alginate lyases,

512   ulvan lyases, unsaturated glucuronyl hydrolases, α-L-rhamnosidase, and β-xylosidase contained

513   lipobox, indicating that QB4 cells can effectively degrade alginate and ulvan and uptake the

514   oligosaccharides. Of course, genes for metabolizing the uronic acids were also present in the

515   QB4. These results were suggested that QB4 will become a source of novel uronic acid

516   degradation enzymes.

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520   **Conflict of interest**

521   The authors do not have any conflict of interest in relation to the work described.

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758 Figure legends

759 Figure 1. Growth confirmation of QB4 cultured with four different uronic acid-containing

760 polysaccharides, alginate (Alg), pectin (Pct), Ulvan (Ulv), and Gelzan (Gel). The growth was

761 measured by cfu/mL. Media with and without 0.2% glucose was used as positive (Glu) and

762 negative controls (NC), respectively. All data shown are mean values from three replicate

763 experiments. Error bars denote the standard deviation of triplicate samples.

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765 Figure 2. Growth confirmation of QB4 cultured with saturated galacturonate (Sat. Gal). The

766 growth was measured as optical density ($OD600_{nm}$). Media with and without 0.2% glucose was

767 used as positive (Glu) and negative controls (NC), respectively. All data shown are mean values

768 from three replicate experiments. Error bars denote the standard deviation of triplicate samples.

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Figure 1

Growth confirmation of QB4 cultured with four different uronic acid-containing polysaccharides, alginate (Alg), pectin (Pct), Ulvan (Ulv), and Gelzan (Gel).

The growth was measured by cfu/mL. Media with and without 0.2% glucose was used as positive (Glu) and negative controls (NC), respectively. All data shown are mean values from three replicate experiments. Error bars denote the standard deviation of triplicate samples.

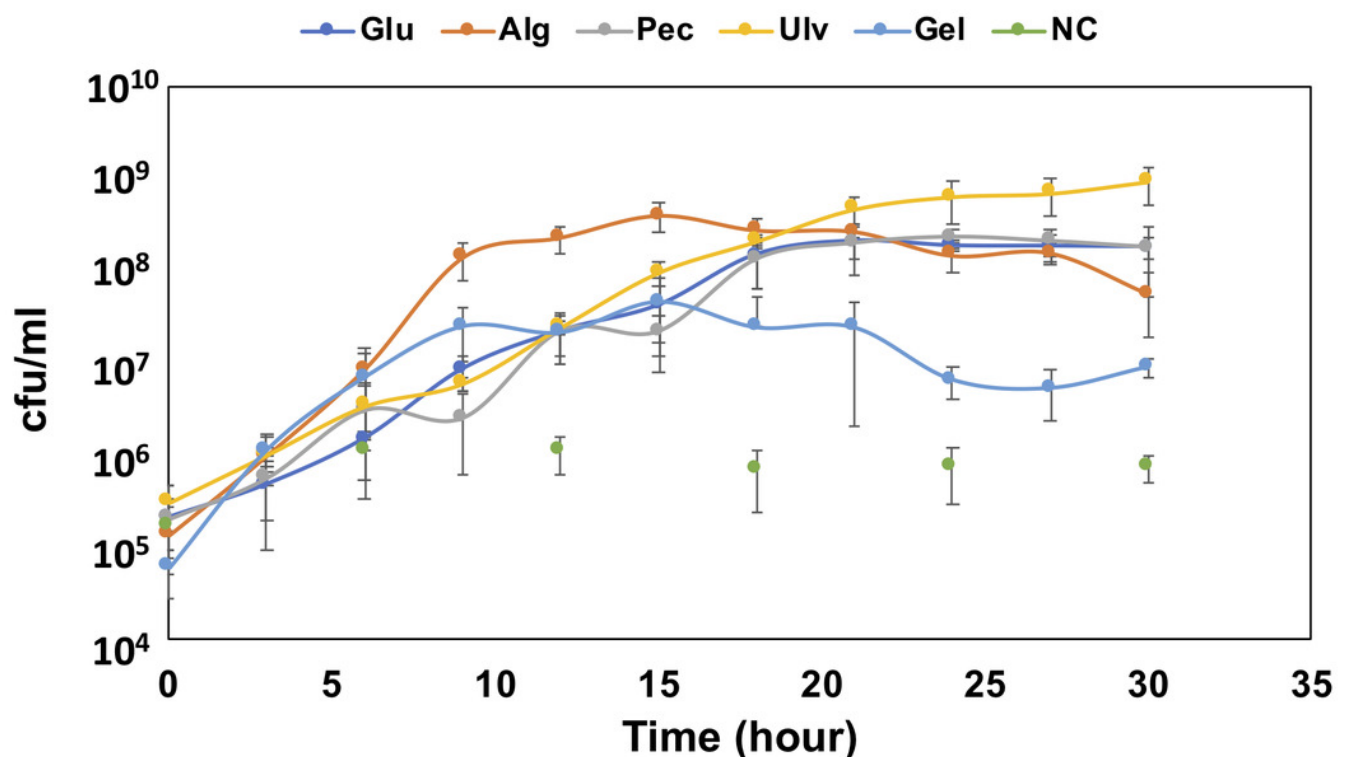
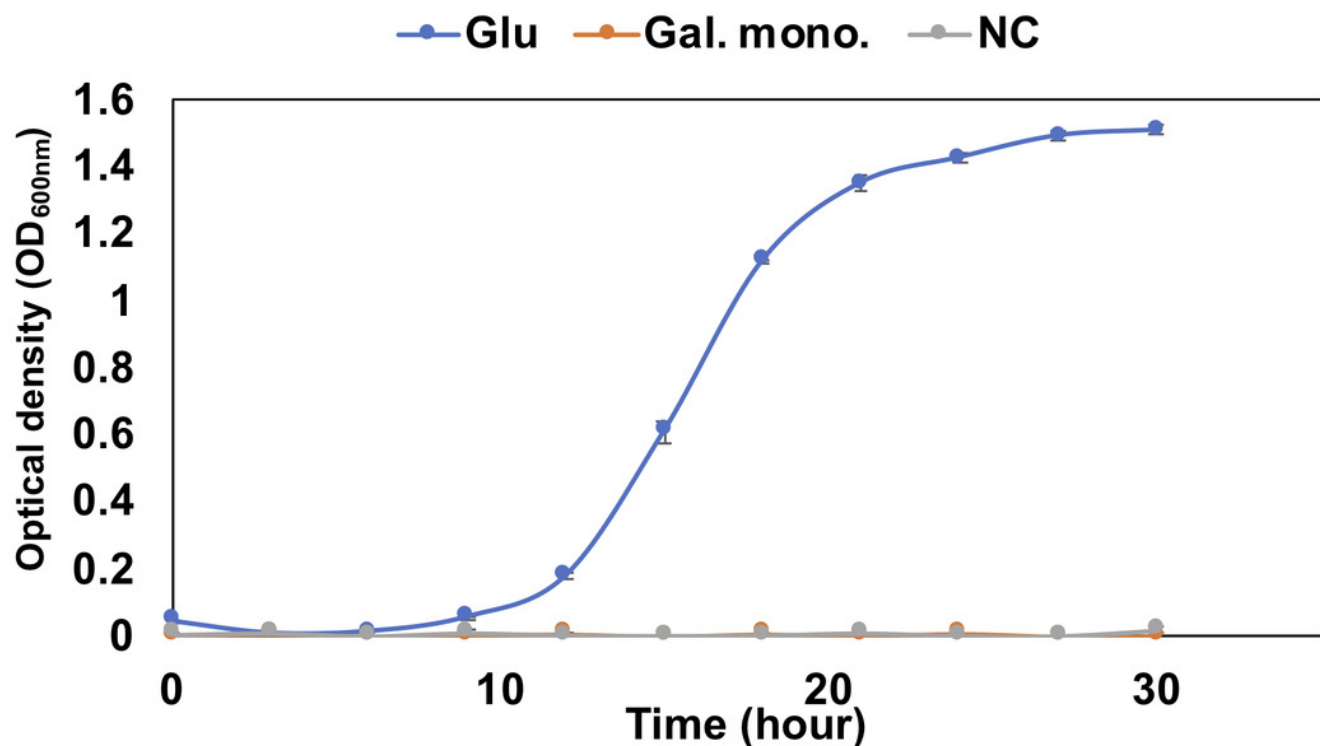



Figure 2

Growth confirmation of QB4 cultured with saturated galacturonate (Sat. Gal).

The growth was measured as optical density ($OD600_{nm}$). Media with and without 0.2% glucose was used as positive (Glu) and negative controls (NC), respectively. All data shown are mean values from three replicate experiments. Error bars denote the standard deviation of triplicate samples.

Genes involving in alginate metabolism.

1   Table 1. Genes involving in alginate metabolism.

2   Alginate-degrading enzymes

Abbreviation	Function	CAZy	Sp.	GenBank
Ad1_PL6	Alginate lyase	PL6, CBM32, CBM32	Type I	WP_108601791.1
Ad3_PL6	Alginate lyase	PL6, CBM16	-	WP_108605000.1
Ad4_PL7	Alginate lyase	PL7, CBM32	Type I	WP_159084278.1
Ad5_PL7	Alginate lyase	PL7, CBM32, CBM32	Type I	WP_108602212.1
Ad6_PL7	Alginate lyase	PL7	Lipobox	WP_108602720.1
Ad7_PL7	Alginate lyase	PL7	Lipobox	WP_108603319.1
Ad8_PL17	Alginate lyase	PL17	Lipobox	WP_108601502.1

11   Alginate-utilization proteins

Abbreviation	Function	GenBank
Au1	DEH reductase	WP_108601028.1
Au2	KDG kinase	WP_108601027.1
Au3	KDG kinase	WP_108603513.1
Au4	2-dehydro-3-deoxyphosphogluconate aldolase	WP_108602314.1
Au5	2-dehydro-3-deoxyphosphogluconate aldolase	WP_108603883.1

18   Sp. indicates signal peptide.

Genes involving in pectin metabolism.

1 Table 2. Genes involving in pectin metabolism.

2 Pectin-degrading enzymes

	Abbreviation	Function	CAZy	Sp.	GenBank
3					
4	Pd1_PL1	Pectate Lyase (Plasmid)	PL1, CBM13	Type I	WP_108605188.1
5	Pd2_PL1	Pectate Lyase (Plasmid)	PL1, CBM35, CBM77	-	WP_108605291.1
6	Pd3_PL1	Pectate Lyase (Plasmid)	PL1, CBM35	Type I	AWB69198
7	Pd4_PL1	Pectate Lyase (Plasmid)	PL1, CE8	Type I	WP_159084287.1
8	Pd5_PL1	Pectate Lyase (Plasmid)	PL1, CE8	Lipobox	WP_108605272.1
9	Pd6_PL3	Pectate Lyase (Plasmid)	PL3, CBM13	Lipobox	WP_108605187.1
10	Pd7_PL10	Pectate Lyase (Plasmid)	PL10	Type I	WP_108605295.1
11	Pd8_GH105	Unsaturated galacturonyl hydrolases (Plasmid)	GH105	Lipobox	WP_108605228.1
12	Pd9_GH105	Unsaturated galacturonyl hydrolases (Plasmid)	GH105	Type I	WP_108605230.1
13	Pd10_GH105	Unsaturated galacturonyl hydrolases (Plasmid)	GH105	Lipobox	WP_108605292.1
14	Pd11_GH28	Polygalacturonases (Plasmid)	GH28	Tat	WP_108605238.1
15	Pd12_GH28	Polygalacturonases (Plasmid)	GH28	Type I	WP_108605277.1

16 Pectin-utilization proteins

	Abbreviation	Function	GenBank
17			
18	Pu1	5-dehydro-4-deoxy-D-glucuronate isomerase (KduI)	WP_108601704.1
19	Pu2	5-dehydro-4-deoxy-D-glucuronate isomerase (KduI)	WP_108601929.1
20	Pu3	5-dehydro-4-deoxy-D-glucuronate isomerase (KduI)	WP_108602166.1
21	Pu4	5-dehydro-4-deoxy-D-glucuronate isomerase (KduI) (Plasmid)	WP_108605242.1
22	Pu5	2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase (KduD)	WP_108601158.1
23	Pu6	2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase (KduD)	WP_108601705.1
24	Pu7	2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase (KduD)	WP_108602165.1
25	Pu8	2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase (KduD) (Plasmid)	WP_108605243.1
26	Pu9	Glucuronate isomerase	WP_108601474.1
27	Pu10	Glucuronate isomerase	WP_108604964.1
28	Pu11	Glucuronate isomerase (Plasmid)	WP_108605248.1
29	Pu12	Tagaturonate reductase	WP_108601943.1
30	Pu13	Altronate hydrolase	WP_108601944.1
31	Pu14	Tagaturonate epimerase (Plasmid)	WP_108605252.1
32	Pu15	Fructuronate reductase	WP_108602164.1
33	Pu16	Mannoate dehydratese	WP_108601469.1

34   Plasmid indicated that the gene is present in plasmid, not the genome.

Genes involving in ulvan metabolism.

1 Table 3. Genes involving in ulvan metabolism.

Ulvan-degrading enzymes				
Abbreviation	Function	CAZy	Sp.	GenBank
Ud1_PL24	Ulvan lyase	PL24		WP_108601530.1
Ud2_PL24	Ulvan lyase	PL24	Type I	WP_108601531.1
Ud3_PL24	Ulvan lyase	PL24	Type I	WP_108604943.1
Ud4_PL24	Ulvan lyase	PL24	Type I	WP_108601549.1
Ud5_PL24	Ulvan lyase	PL24	Type I	WP_108601554.1
Ud6_PL24	Ulvan lyase	PL24	Lipobox	WP_108602230.1
Ud7_PL24	Ulvan lyase	PL24, CBM32		WP_108604992.1
Ud8_PL24	Ulvan lyase	PL24	Type I	WP_108602276.1
Ud9_PL24	Ulvan lyase	PL24	Lipobox	WP_108602290.1
Ud10_PL25	Ulvan lyase	PL25	Lipobox	WP_108601547.1
Ud11_PL25	Ulvan lyase	PL25	Lipobox	WP_108601636.1
Ud12_PL25	Ulvan lyase	PL25	Lipobox	WP_108601685.1
Ud13_PL25	Ulvan lyase	PL25	Lipobox	WP_108602265.1
Ud14_PL40	Ulvan lyase	PL40		WP_108602237.1
Ud15_GH105	Unsaturated glucuronyl hydrolase	GH105	Lipobox	WP_108601620.1
Ud16_GH105	Unsaturated glucuronyl hydrolase	GH105	Lipobox	WP_108601696.1
Ud17_GH105	Unsaturated glucuronyl hydrolase	GH105	Lipobox	WP_108601928.1
Ud18_GH105	Unsaturated glucuronyl hydrolase	GH105	Lipobox	WP_108602225.1
Ud19_GH105	Unsaturated glucuronyl hydrolase	GH105	Lipobox	WP_108602277.1
Ud20_GH78	α-L-rhamnosidase	GH78, CBM67	Type I	WP_108601540.1
Ud21_GH78	α-L-rhamnosidase	GH78	Lipobox	WP_108604942.1
Ud22_GH78	α-L-rhamnosidase	GH78, CBM67	Lipobox	WP_108601635.1
Ud23_GH78	α-L-rhamnosidase	GH78	Lipobox	WP_108602268.1
Ud24_GH78	α-L-rhamnosidase	GH78	Lipobox	WP_108602275.1
Ud25_GH43	Putative β-xylosidase	GH43	Type I	WP_108601365.1
Ud26_GH43	Putative β-xylosidase	GH43	Lipobox	WP_108601561.1
Ud27_GH43	β-xylosidase	GH43	Type I	WP_108601631.1
Ud28_GH43	β-xylosidase	GH43	Lipobox	WP_108602133.1
Ud29_GH43	β-xylosidase	GH43	Lipobox	WP_159084088.1
Ud30_GH43	β-xylosidase	GH43	Lipobox	WP_108602377.1

34	Ud31_GH43	β-xylosidase		GH43	Lipobox	WP_108602397.1
35	Ulvan-utilization proteins					
36	Abbreviation	Function				GenBank
37	Ulu1	Sulfatase				WP_108601682.1
38	Ulu2	Sulfatase				WP_108601697.1
39	Ulu3	L-rhamnose mutarotase				WP_108601622.1
40	Ulu4	Rhamnose isomerase				WP_108601699.1
41	Ulu5	Pentulose/hexulose kinase (rhamnulokinase)				WP_108602148.1
42	Ulu6	Rhamnulose aldolase				WP_108604932.1
43	Ulu7	Aldehyde dehydrogenase				WP_108601159.1
44	Ulu8	Lactate dehydrogenase				WP_108602249.1
45	Ulu9	Xylose isomerase				WP_108604489.1
46	Ulu10	Xylulose kinase				WP_108604490.1
47	Ulu11	Transcriptional regulatory protein XylR				WP_10860449.1

Genes involving in ulvan metabolism.

1 Table 3. Genes involving in ulvan metabolism.

Gellan-degrading enzymes

Abbreviation	Function	CAZy	Sp.	GenBank
Gd1_GH1	β-D-glucosidase	GH1		WP_159084202.1

Gellan-utilization proteins

Abbreviation	Function			GenBank

Gu1 Glucokinase WP_108603884.1
Gu2 Glucose-6-phosphate isomerase WP_108604264.1