Supplement materials

Enzymatic Verification and Comparative Analysis of Carrageenans Metabolism Pathways in Marine Bacterium *Flavobacterium algicola*

Chengcheng Jiang[a], Hong Jiang[a], Tianyu Zhang[a], Zewei Lu[a], Xiangzhao Mao[a,b] *

[a]College of Food Science and Engineering, Ocean University of China, Qingdao 266003, China

[b]Laboratory for Marine Drugs and Bioproducts of Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China,

*Corresponding author: Xiangzhao Mao

Address: College of Food Science and Engineering, Ocean University of China, Qingdao 266003, China

Tel.: +86-532-82032660

Fax: +86-532-82031789

E-mail: xzhmao@ouc.edu.cn

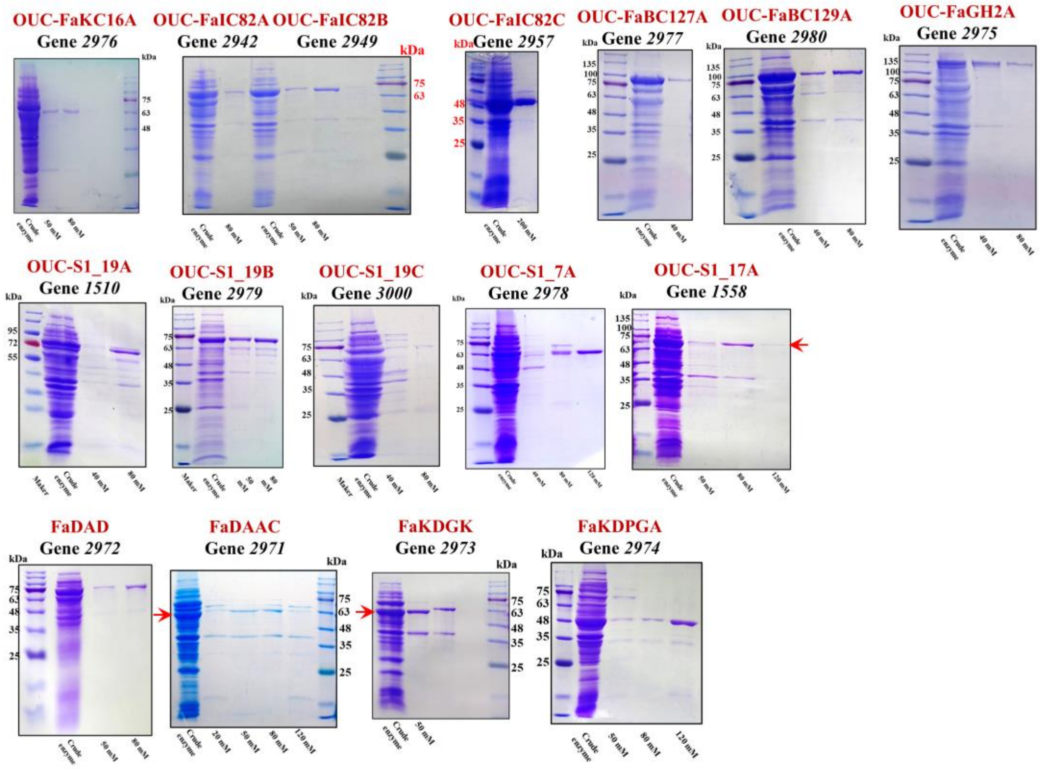

FIG S1 SDS-PAGE analyses of the purified enzymes involved the KC and IC metabolism in *F. algicola*.

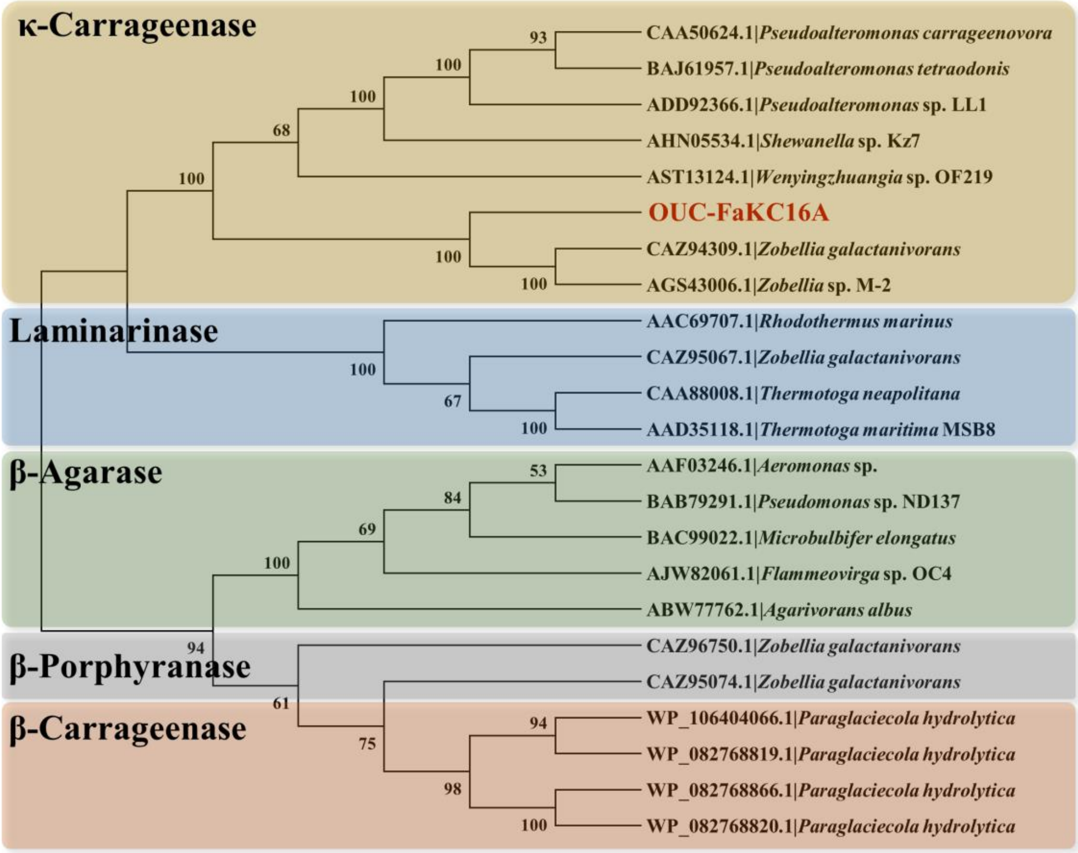

FIG S2 Sequence-based relationship of gene *2976* encoded κ-carrageenase OUC-FaKC16A from *F. algicola* with other characterized GH16 hydrolases. Accession number and taxonomy is indicated for each protein. The β-agarases, β-porphyrases, κ-carrageenases, and laminarinases represent characterized enzymes according to the CAZy database.

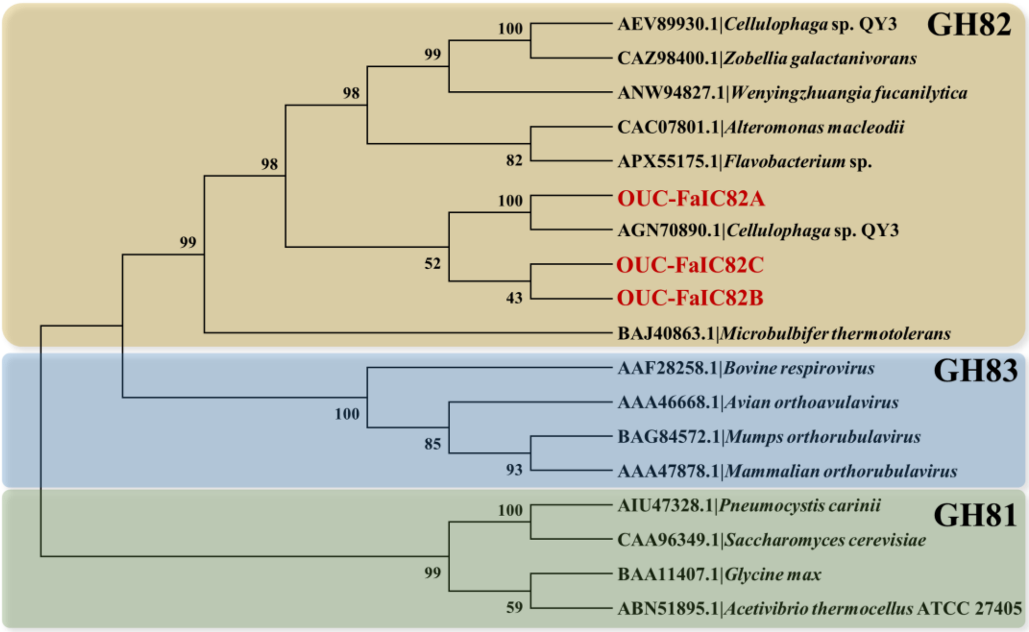

FIG S3 Sequence-based relationship of gene *2942*, *2949*, and *2957* encoded ι-carrageenases OUC-FaIC82A, OUC-FaIC82B, and OUC-FaIC82C, respectively, from *F. algicola* with other characterized GH81, GH82, and GH83 hydrolases. Accession number and taxonomy is indicated for each protein. The sequences of characterized enzymes were obtained from CAZy database.

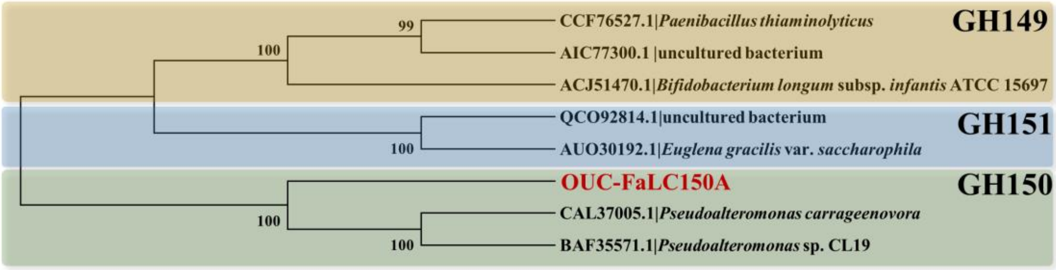

FIG S4 Sequence-based relationship of gene *2941* encoded λ-carrageenase OUC-FaLC150A from *F. algicola* with other characterized GH149, GH150, and GH151 hydrolases. Accession number and taxonomy is indicated for each protein. The sequences of characterized enzymes were obtained from CAZy database.

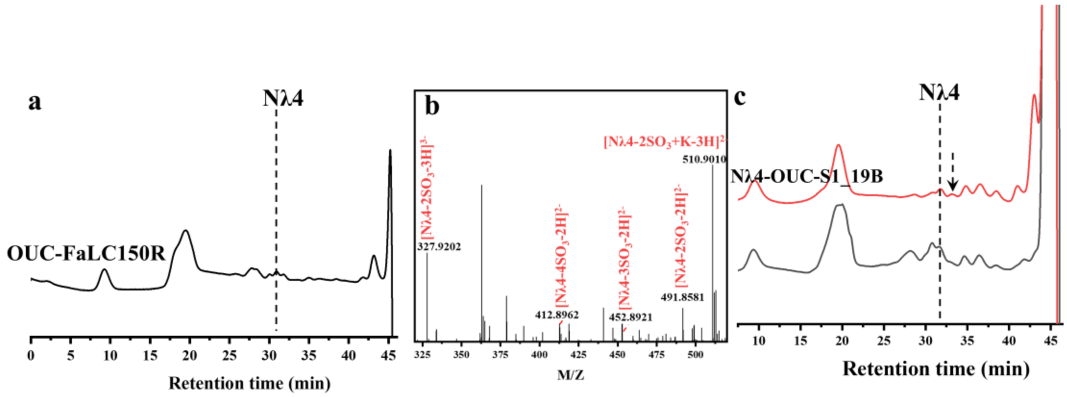

FIG S5 (c) HPLC for analyzing the main products of OUC-FaLC150A from hydrolyzing LC. (d) MS for analyzing the end products of OUC-FaLC150A from hydrolyzing IC for 24 h. (c) HPLC for analyzing the ability of OUC-S1_19B to remove the G4S group from Nλ4. LC: λ-carrageenan; Nλ4: λ-neocarratetrose; G4S: C4 hydroxyl sulfated D-galactose residue

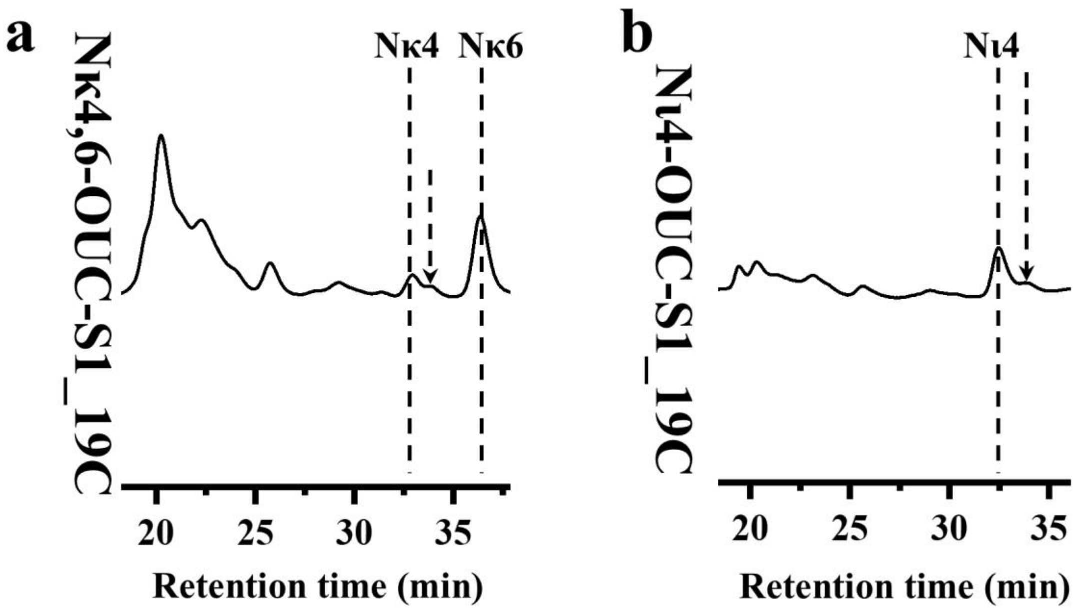

FIG S6 (a) HPLC analysis of the activities of OUC-S1_19A (encoded by gene *3000*) for removing the G4S group from the mixture of Nκ4 and Nκ6. (b) HPLC analysis of the activity of OUC-S1_19A for removing the G4S group from Nι4. G4S: C4 hydroxyl sulfated D-galactose residue; Nκ4: κ-neocarratetrose; Nκ6: κ-neocarrahexaose; Nι4: ι-neocarratetrose

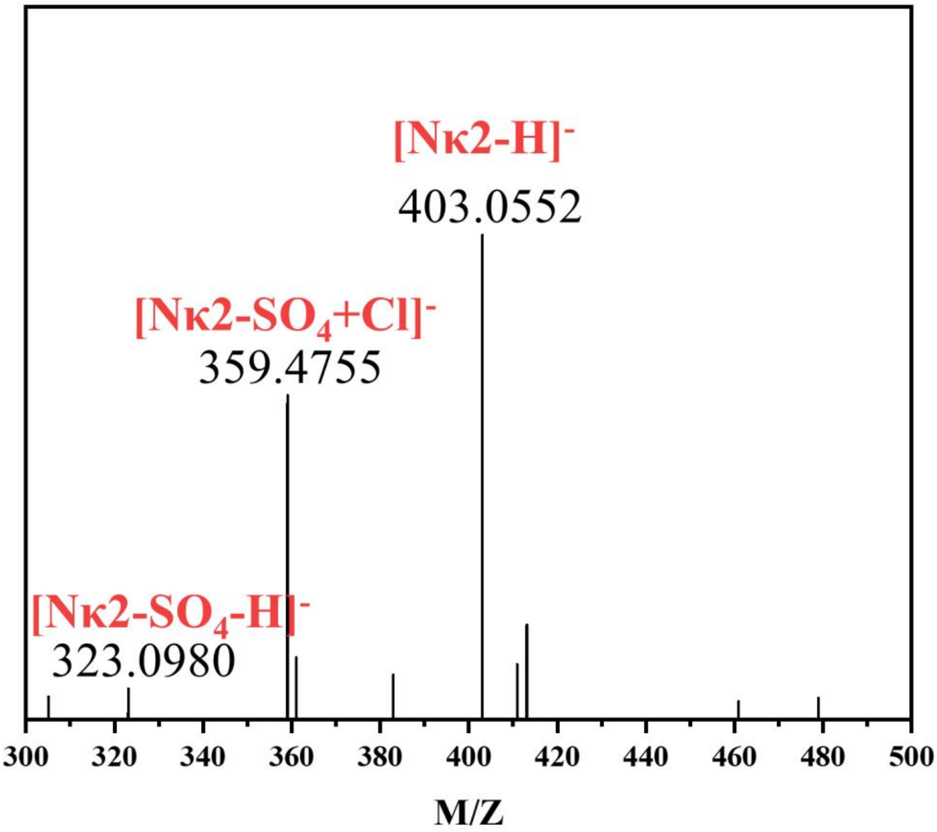

FIG S7 HPLC analysis of the activities of d OUC-S1_19B for removing the G4S group from the mixture of Nκ2. G4S: C4 hydroxyl sulfated D-galactose residue; Nκ2: κ-neocarrabiose

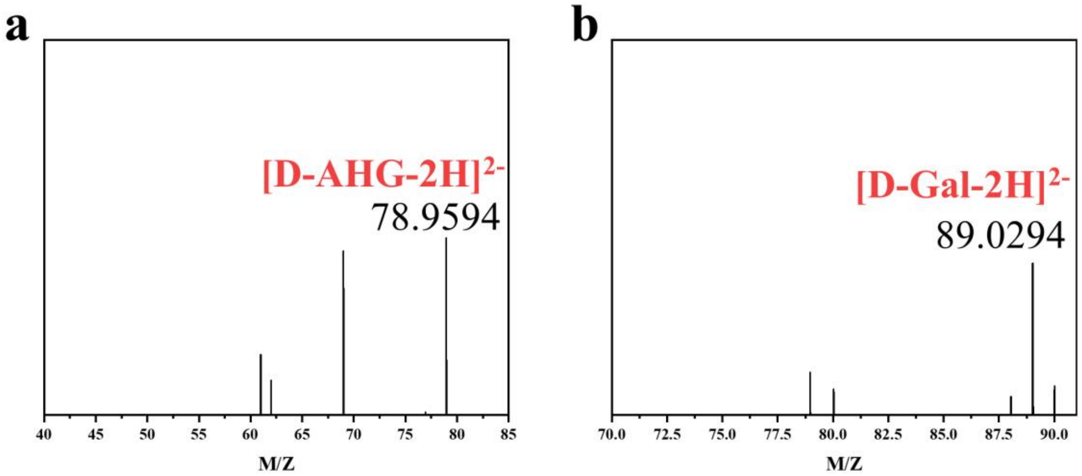

FIG S8 (a) MS analysis of the D-AHG production from OUC-FaBC127A and OUC-FaBC129A hydrolysis of the OUC-S1_19B-treated Nκ4. (b) MS analysis of the D-Gal production from OUC-FaGH2A hydrolysis of the COS with DP3 (G-DA-G4S). ADAG: α-3,6-anhydro-D-galactosidase; BG: β-galactosidase; Nκ4: κ-neocarratetrose; D-AHG: 3,6-anhydro-D-galactose; D-Gal: D-galactose

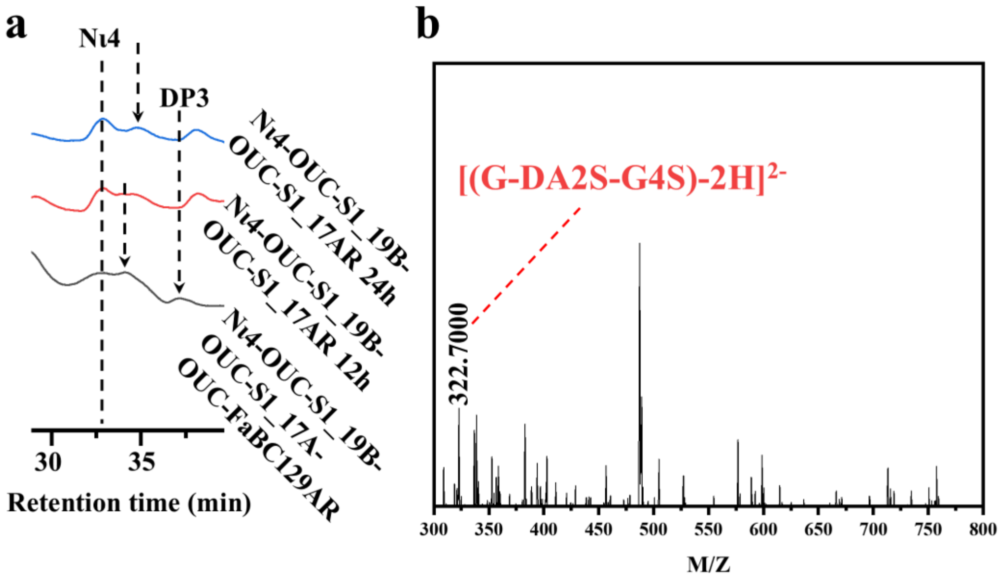

FIG S9 The HPLC (a) and MS (b) analyses of the DP3 (G-DA2S-G4S) products from hydrolyzing Nι4 which treated successively with OUC-S1_19B and OUC-S1_17A by OUC-FaBC129A

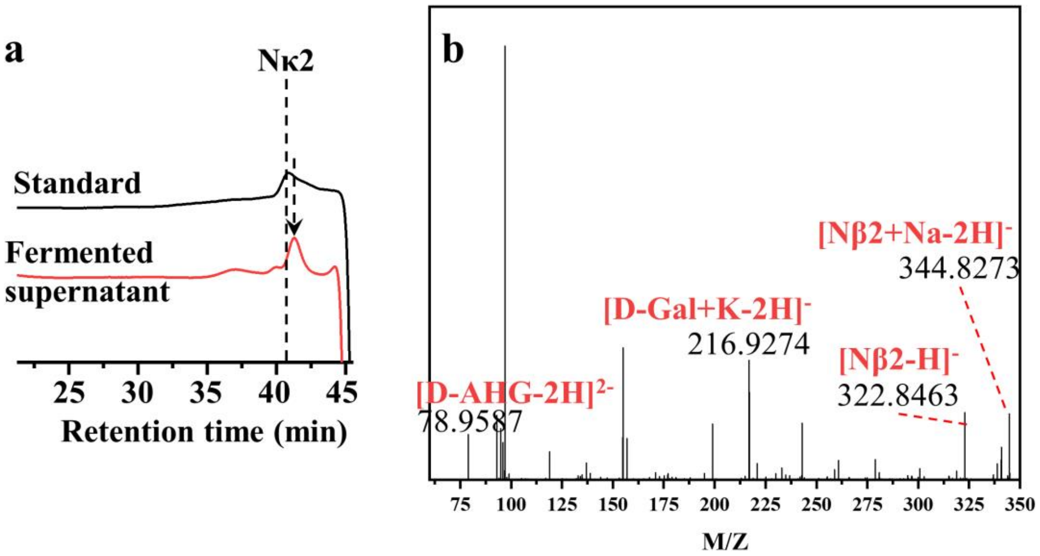

FIG S10 The HPLC (a) and MS (b) analyses of fermented supernatant of *F. algicola* collected from M9 medium with KC as the sole carbon source. Nκ2: κ-neocarrabiose; D-AHG: 3,6-anhydro-D-galactose; D-Gal: D-galactose

Table S1 The BLAST results of carrageenases of *F. algicola* with the closest characterized enzymes from NR databases

Gene	Protein	CAZy family	Accession no. of closest characterized protein	Percent Identity	Query Coverage
2976	OUC-FaKC16A	GH16, κ-carrageenase	CAZ94309.1, κ-carrageenase	60%	68%
2942	OUC-FaIC82A	GH82, ι-carrageenase	AGN70890.1, ι-carrageenase	56%	88%
2949	OUC-FaIC82B	GH82, ι-carrageenase	APX55175.1, ι-carrageenase	28%	98%
2957	OUC-FaIC82C	GH82, ι-carrageenase	APX55175.1, ι-carrageenase	37%	96%
2941	OUC-FaLC150A	GH150, λ-carrageenase	BAF35571.1, λ-carrageenase	45%	99%
			CAL37005.1, λ-carrageenase	45%	98%

Table S2 The BLAST results of sulfatases, oligosaccharide glycosidases, and D-AHG utilization related enzymes of *F. algicola* with the reported enzymes involved the carrageenan metabolism of the *Zobellia galactanivorans* Dsij^{T} and *Pseudoalteromonas* species

Gene	Protein	Accession no. of closest characterized protein	Percent Identity	Query Coverage
1510	OUC-S1_19A	CAZ97290.1, exo-acting G4S NκCOS sulfatase S1_19B	37%	79%
1558	OUC-S1_17A	KAA1157105.1, S1_17 DA2S-sulfatase ZGAL_3151	56%	88%
2064	OUC-S1_17B	CAZ97290.1, S1_17 DA2S-sulfatase ZGAL_3151	36%	81%
2978	OUC-S1_7A	CAZ97285.1, S1_7 KC G4S-sulfatase	55%	98%
2979	OUC-S1_19B	CAZ97284.1, S1_19 NιCOS G4S-sulfatase	65%	94%
		KAA1157105.1, KC/IC G4S-sulfatase	53%	99%
3000	OUC-S1_19C	CAZ97284.1, S1_19 NιCOS G4S-sulfatase KAA1157105.1,	59%	95%
		KC/IC G4S-sulfatase	56%	97%
2977	OUC-FaBC127A	CAZ97289.1, GH127 ADAG	67%	93%
2980	OUC-FaBC129A	CAZ97291.1, GH129-like ADAG	64%	99%
2971	FaDAAC	CAZ97295.1, 3,6-anhydro-D-galactonate cycloisomerase	56%	97%
2972	FaDAD	CAZ97294.1, 3,6-anhydro-D-galactose dehydrogenase	73%	98%
2973	FaKDGK	CAZ97293.1, KDGal kinase	48%	98%
2974	FaKDPGA	CAZ97292.1, KDPGal aldolase	64%	97%