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Microbial population changes in decaying *Ascophyllum nodosum* result in macroalgal-polysaccharide-degrading bacteria with potential applicability in enzyme-assisted extraction technologies.

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**Keywords:** *Ascophyllum nodosum*, algal cell wall degrading enzymes, enzyme-assisted extraction, iChip device



MDPI

# Legends to Supplementary figures

#### Table S1

Ascophyllum nodosum associated bacterial isolates, their closest BLAST relative and observed enzymatic activities. Bacterial strains were examined for their hydroxyethyl cellulose (HE-cellulase), lichenase and pectinase activities. Enzymatic activity is indicated by a (+) sign while a (-) sign indicates that no enzymatic activity was observed under the conditions tested

# Figure S1

Relative abundances at genus level of bacteria associated with the cultivable surface microbiota of (a) intact *Ascophyllum nodosum* and decaying *Ascophyllum nodosum* at 2, 4 and 6 weeks of decay at (a) 18 °C; 2\_18, 4\_18, 6\_18 (b) 25 °C; 2\_25, 4\_25, 6\_25 (c) 30 °C; 2\_30, 4\_30, 6\_30 which were obtained by maceration culture isolation method and (e) obtained by ichip culture isolation method. 16S rRNA gene sequences were obtained from the bacterial isolates and taxonomic analyses were performed. The relative distribution of phyla in each group is represented as a percentage

## Figure S2

Neighbor-joining phylogenetic tree representing bacterial phyla cultured from *Ascophyllum* nodosum sample before induced decay (T<sub>0</sub>). The evolutionary relationships of each phylum identified are shown with reference sequences from NCBI included. This phylogenetic analysis was performed using single representative 16S rDNA sequences from each group





identified by Fastgroup program. The number of similar sequences represented by each sequence is shown in brackets. This tree was drawn using MEGA program (version 7) and bootstrapping percentages (1000 replicates) above 50% are shown

### Figure S3

Neighbor-joining phylogenetic tree representing bacterial phyla cultured from *Ascophyllum nodosum* sample at week 2 of induced decay from ■8 °C, 25 °C and 3 °C. The evolutionary relationships of each phylum identified are shown with reference sequences from NCBI included. This phylogenetic analysis was made using single representative 16S rDNA sequences from each group identified by Avalanche NextGen Workbench version 2.30. The number of similar sequences represented by each sequence is shown in brackets. This tree was drawn using MEGA program (version 7) and bootstrapping percentages (1000 replicates) above 50% are shown

#### Figure S4

Neighbor-joining phylogenetic tree representing bacterial phyla cultured from *Ascophyllum nodosum* sample at at week 4 of induced decay from ●18 °C, ■25 °C and 30 °C. The ▲ evolutionary relationships of each phylum identified are shown with reference sequences from NCBI included. This phylogenetic analysis was made using single representative 16S rDNA sequences from each group identified by Avalanche NextGen Workbench version 2.30. The number of similar sequences represented by each sequence is shown in brackets. This tree was drawn using MEGA program (version 7) and bootstrapping percentages (1000 replicates) above 50% are shown.





# Figure S5

Neighbor-joining phylogenetic tree representing bacterial phyla cultured from *Ascophyllum nodosum* sample at the end of the decay period (week 6) from ●18 °C, ■ 25 °C and 30 °C . The evolutionary relationships of each phyla identified are shown with referendes sequences from NCBI included. This phylogenetic analysis was made using single representative 16S rDNA sequences from each group identified by Avalanche NextGen Workbench version 2.30. The number of similar sequences represented by each sequence is shown in brackets. This neighbor joining tree was drawn using MEGA program (version 7) and bootstrapping percentages (1000 replicates) above 50% are shown.

#### Figure S6

Neighbour-joining phylogenetic tree representing bacterial phyla cultured from ●18 °C, ■25 °C and 30 °C using he iChip device. The evolutionary relationships of each phylum identified are shown with reference sequences from NCBI included. This phylogenetic analysis was performed using single representative 16S rDNA sequences from each group identified by Fastgroup. The number of similar sequences represented by each sequence is shown in brackets. This neighbor joining tree was drawn using MEGA program (version 7) and bootstrapping percentages (1000 replicates) above 50% are shown





# Table S1

SAMPLE ID	TOP BLAST HIT	IDENTITY (%)	ALGAL CELL WALL POLYSACCHARIDE DEGRADING ACTIVITIES			
			HE-	Lichenase	pectinase	
			cellulase			
AN218_A2	Bacillus safensis strain Rb1S1	100	-	+	-	
AN218_H5	Bacillus sp. M101(2010) strain M101	100	+	+	-	
AN225_A5	Bacillus altitudinis strain CT10	99	-	+	-	
AN225_A11	Bacillus licheniformis strain HQB814	99	+	-	+	
AN225_B8	Bacillus licheniformis strain AG-06	100	-	-	+	
AN225_B9	Bacillus licheniformis strain ST7	99	-	-	+	
AN225_C1	Bacillus pumilus strain ASpB9	99	-	+	-	
AN225_C7	Bacillus aerius strain APBSMLB109	99	-	+	-	
AN225_C11	Bacillus sp. 11RB3	99	-	+	+	
AN225_D1	Bacillus licheniformis strain	100	+	-	+	
	APBSWPTB167					
AN225_D4	Bacillus subtilis strain HDXJ04	99	+	+	+	
AN225_D6	Bacillus pumilus strain ASpB9	100	-	+	-	
AN225_E1	Bacillus pumilus strain ASpB9	100	-	+	-	
AN225_E6	Bacillus licheniformis strain JMB003	99	-	-	+	
AN225_E7	Bacillus licheniformis strain V24	100	-	-	+	
AN225_E8	Bacillus licheniformis strain V24	100	-	-	+	
AN225_E9	Bacillus sp. strain SKS7	99	-	-	+	
AN225_E10	Bacillus licheniformis strain KB102	99	-	-	+	
AN225_E11	Bacillus pumilus strain ASpB9	100	-	+	-	
AN225_F6	Bacillus sp. strain 703	100	-	-	+	
AN225_F9	Bacillus sp. strain C60	99	-	+		
AN225_F12	Bacillus licheniformis strain V24	100	+	-	+	





AN225_G3 Bacil  AN225_G6 Bacil  AN225_G8 Bacil	lus sp. strain SKS7  llus sp. (in: Bacteria) strain VI/7  lus subtilis strain AKKVG-2-18  lus sp. (in: Bacteria) strain VI/7	100 100 100	-	-	+
AN225_G6 Bacil AN225_G8 Bacil	lus subtilis strain AKKVG-2-18			-	+
AN225_G8 Bacil		100			
	lus sp. (in: Bacteria) strain VI/7		-	+	+
AN230_A10 Bacil		100	-	-	+
	N230_A10 Bacillus pumilus strain ASpB9		-	+	-
<b>AN230_B4</b> <i>Bacil</i>	lus sp. strain SKS7	100	+	-	+
AN230_B11 Bacil	lus mycoides strain LBUM203	99	+	-	+
AN230_D9 Bacil	lus licheniformis strain V24	100	+	-	+
AN230_D11 Bacil	lus licheniformis strain V24	100	+	-	+
AN230_E3 Baci	llus sp. (in: Bacteria) strain V52	100	-	-	+
AN230_E4 Bacil	lus sp. Ph_25A	100	-	+	-
AN425_D9 Bacil	lus sp. strain CZL003	100	+	+	+
AN425_D11 Bacil	lus licheniformis strain 8B-B92	99	+	-	+
AN425_D12 Bacil	lus sp. strain SKS7	100	+	-	+
AN425_E4 Bacil	lus sp. strain BS155	100	+	+	+
<b>AN425_G7</b> <i>Bacil</i>	lus sp. strain BS155	100	+	-	+
AN618_A1 Bacil	lus pumilus strain ASpB9	100	-	+	-
AN618_A2 Bacil	lus pumilus strain ASpB9	100	-	+	-
AN618_B10 Bacil	lus pumilus strain ASpB9	100	-	+	-
	lus pumilus strain ASpB9	100	+	+	-
AN618_H4 Bacil	lus pumilus strain ASpB9	100	-	+	-
	lus pumilus strain ASpB9	100	-	+	-
	lus pumilus isolate TD22	100	-	+	-
	lus pumilus strain ASpB9	100	-	+	-
	lus hwajinpoensis strain 16E11	99	-	+	-
	lus pumilus strain ASpB9	100	-	+	-
	lus pumilus strain ASpB9	100	-	+	-
	lus pumilus strain ASpB9	100	-	+	-
	lus safensis strain Rb1S1	100	-	+	-
	o oceanisediminis strain S37	98	-	-	+
	o anguillarum strain INTA11	100	-	-	+
IC18_D7 Vibri	o anguillarum strain X0906	99	-	-	+



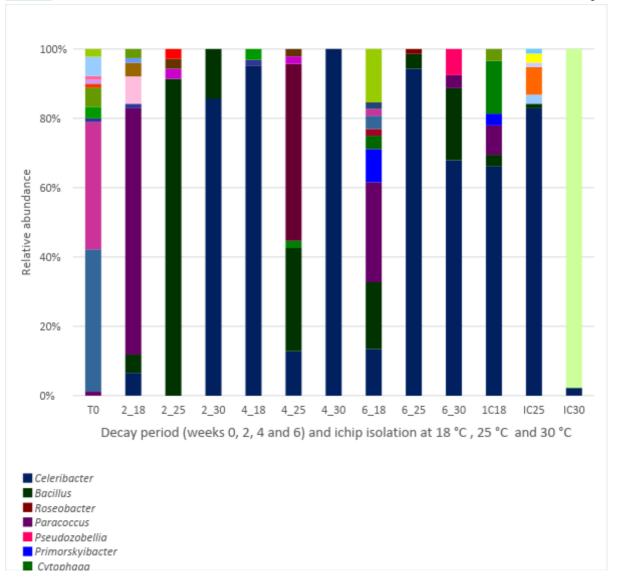


					-
IC18_D8	Vibrio oceanisediminis strain S37	99	-	-	+
IC18_D9	Vibrio anguillarum strain X0906	99	-	-	+
IC18_E2	Vibrio oceanisediminis strain S37	98	-	-	+
IC18_E6	Vibrio anguillarum strain KAP1	100	-	-	+
IC18_E7	Vibrio oceanisediminis strain S37	99	-	-	+
IC18_E8	Vibrio anguillarum strain INTA11	100	-	-	+
IC25_C11	Micrococcus yunnanensis	100	-	-	+
IC25_F10	Micrococcus yunnanensis	100	-	-	+

Figure S1













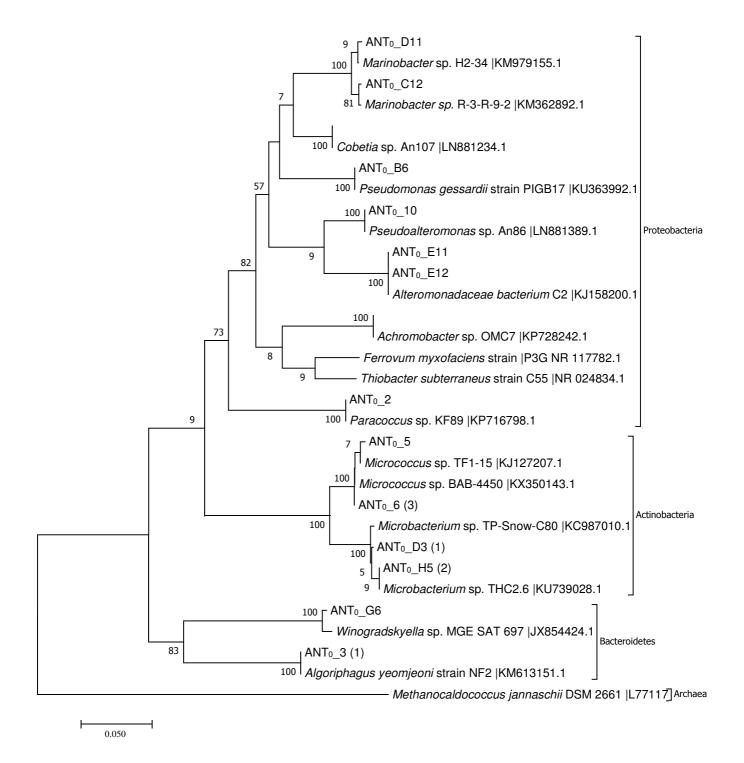


Figure S3











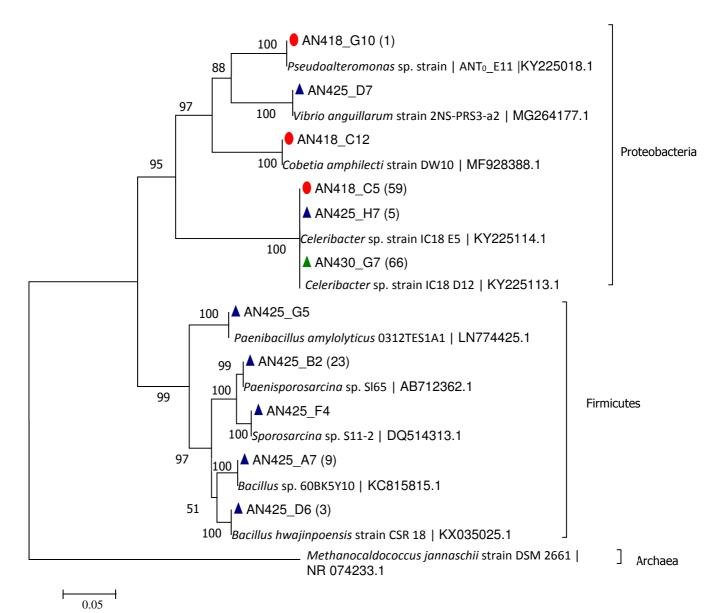


Figure S5







Figure S6

