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Comparative Genomic Study of Polar Lichen-Associated *Hymenobacter* sp. PAMC 26554 and *Hymenobacter* sp. PAMC 26628 Reveals the Presence of Polysaccharide-Degrading Ability Based on Habitat

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Abstract

The genus *Hymenobacter* is classified in the family Hymenobacteraceae under the phylum Bacteroidetes. They have been isolated from diverse environments, such as air, soil, and lichen, along with extreme polar environments, including the Arctic and Antarctic regions. The polar regions have attracted intense research interest for the discovery of novel microorganisms and their functions. Analysis of the polysaccharide utilization-related carbohydrate-active enzyme among the two lichen-associated polar organisms *Hymenobacter* sp. PAMC 26554 and *Hymenobacter* sp. PAMC 26628 was performed, along with its comparison with the complete genome of the same genus available in the NCBI database. The study was conducted relying on the AZCL screening data for the two polar lichen-associated species. While comparing with eight other complete genomes, differences in polysaccharide preferences based on the isolation environment and biosample source were discovered. All the species showed almost similar percentage of cellulose synthesis and degradation genes. However, the polar lichen-associated microorganism was found to have a high percentage of hemicellulose degradation genes, and less starch and laminarin degradation. The *Hymenobacter* species with higher number of hemicellulose degradation genes was found to have a lower number of starch and laminarin degradation genes and vice versa, highlighting the differences in polysaccharide utilization among the species.

Introduction

Up to 80% of the Earth's surface is covered by a cold ecosystem [1]. Recently, polar regions have been enthusiastically investigated to explore new possibilities for microbiomes [2]. Cold-active microorganisms, designated as psychrophilic microorganism, have colonized environments such as high mountains, polar regions, and deep sea. Although these extreme habitats are very challenging for the organism, they have developed cold adaptation strategies for their survival. The features of the microorganisms that allow them to adjust to such cryoenvironments are of significant interest to researchers. Likewise, various cold-adaptive polysaccharide-degrading enzymes with biotechnological importance have been characterized by polar microorganisms; these enzyme include xylanase, β-galactosidase, glucanases, amylase, and cellulase [3–5]. Several studies have also reported a cluster of polysaccharide utilization loci in psychrophilic organisms responsible for hydrolysis, especially from the family Bacteroidetes [6–8]. They have been found to incorporate various cellulose and hemicellulose synthesis genes, including algal polysaccharides, e.g., laminarin, lichenan, and carrageenan.

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However, there are still more possibilities for exploration in such a cryoenvironment with diverse creatures.

Polysaccharides are composed of a large number of monosaccharides arranged together by glycosidic linkages. The most abundant polysaccharides in the environment include cellulose and hemicellulose, especially xylan [9]. Similarly, starch is also one of the major storage polysaccharides among various plants [10]. The complex arrangement of these polysaccharides occurring in nature carries the utmost potential as a utilizable carbon in the biosphere [11]. They can be renewable sources to combat greenhouse gas emission by obtaining bioenergy through waste plant products, agricultural wastes, and other energy crops, such as Switchgrass, Eucalyptus, and Giant reed [12]. Lichens are one of the major habitats of the polar microorganism. Generally, lichen is a symbiotic association formed between algae and fungi; these associations are also observed in the polar region. Lichens and bryophytes significantly cover terrestrial vegetation in the Antarctic regions [13, 14]. The Arctic regions are also highly colonized by lichens; they are involved in the cyclization of minerals among the Arctic habitats [15]. Several studies have revealed the polysaccharide constituents of lichen: galactomannans, β-glucans, and α-glucans [16].

Carbohydrate-active enzyme (CAZyme) is involved in carbohydrate metabolism, including the complexly arranged polysaccharides [17]. In the CAZy database, CAZymes are classified into different families based on amino acid sequences with a resemblance to at least one biochemically characterized member [18]. These includes glycosyl hydrolase (GH), carbohydrate esterase (CE), polysaccharide lyase (PL), and auxiliary activities (AA) for catabolism, while glycosyltransferase (GT) function for anabolism. Meanwhile, the family also consists of a carbohydrate-binding domain (CBM) that plays a key role in the binding of sugar moieties to CAZyme in the reaction process (www.cazy.org). Among these families, GH has been studied widely, due to its roles in plant biomass decomposition, and it consists of the most abundant number of enzymes. CAZyme from extremophiles has attracted the attention of researchers. CAZyme study has been carried out from psychrophilic, hyper (thermophilic), acidophilic, and other extremities too. Psychrophilic enzymes are found to have high catalytic efficiency and high molecular flexibility [19], and thus offer good potential as commercial enzymes.

The genus *Hymenobacter* is classified in the family Hymenobacteraceae under the phylum Bacteroidetes [20]. They are Gram-negative, rod-shaped, and aerobic organisms, usually with red to pinkish brown colonies. They have been isolated from diverse environments in air, soil, and lichen, along with extreme environments, such as the Arctic and Antarctic regions [20–23]. Species belonging to the phylum Bacteroidetes have a great tendency to degrade polysaccharide and utilize carbon sources for energy [24–26]. However, the polysaccharide utilization ability of the genus *Hymenobacter* has not previously been highlighted. Therefore, this study presents an analysis of the polysaccharide-degrading ability of lichen-associated *H.* sp. PAMC 26554 and *H.* sp. PAMC 26628 obtained from the Antarctic and Arctic regions, respectively; they are compared among eight other complete strains from the same genus based on their different habitats. Likewise, the study focuses on major enzymes degrading polysaccharides, like cellulose, hemicellulose, starch, and a specific polysaccharide laminarin (β-1,3 glucan) usually found in lichen, highlighting its isolation source.

Materials and Methods

Bacterial Isolation and Complete Genome Sequencing

Two *Hymenobacter* species namely, PAMC 26554 and PAMC 26628 isolated from lichens from Antarctic and Arctic regions, respectively, were obtained from the Korean Polar Research Institute (KOPRI, Incheon, Korea). The strain PAMC 26554 was isolated from the lichen *Usnea* sp. that was collected from Barton Peninsula, King George Island, Antarctica (62°13′ S, 58°47′ W). Similarly, the strain PAMC 26628 was isolated from lichen *Stereocaulon* sp. from Ny-Ålesund, Svalbard, Arctic (78° 55′ N, 11° 56′ E). They were isolated in the R2A medium (MB Cell Ltd., Seoul, Korea), and grown at 4 to 37 °C. The authors have already reported their complete genome information elsewhere [23]. The genomic information of both the species is available in the NCBI database with GenBank accession numbers NZ_CP014771.1 and NZ_CP014304.1, respectively. This result is a follow-up study of information of their genome already reported. However, the previous study did not highlight their habitat and their polysaccharide utilization ability, which are discussed here.

AZCL Screening for Diverse Polysaccharide-Degrading Ability

Azurine-crosslinked (AZCL) activity was performed, using a commercial kit available from Megazyme© (Bray, Ireland). The screening method was conducted using a medium containing 1% agarose and 23 mM phosphoric buffer to polysaccharides substrate powder. The medium was sterilized and cooled to (65–70) °C, poured into Petri dishes, and allowed to solidify. Eleven AZCL substrates were used to test enzyme activity: AZCL-amylose, AZCL-barley β-glucan, AZCL-HE-cellulose, AZCL-xylan (Beechwood), AZCL-arabinoxylan (Wheat), AZCL-xylan (Birchwood),

AZCL-xyloglucan (Tamarind), AZCL-chitosan, AZCL-curdlan, AZCL-galactomannan, and AZCL-debranched arabinan. A positive enzyme activity reaction leads to the release of dyed water-soluble fragments into the 1% agarose plate, and the blue zone is thus a quantitative measure for an enzyme activity that can be compared between strains or protein [27, 28].

Comparative Genomics Analyses

For the comparison of polysaccharide-degrading ability, all the genomic information of *Hymenobacter* species under comparative study was obtained from the NCBI genome database. The gene bank accession numbers are NZ_CP012623.1 (*H.* sp. DG25A), NZ_CP032317.1 (*H.* sp. sh-6), NZ_CP007145.1 (*H. swuensis* DY53), NZ_CP029145.1 (*H. nivis* strain NBRC 111535), NZ_CP013909.1 (*H. sedentarius* strain DG5B), NZ_CP037922.1 (*H.* sp. 17J36-26), NZ_CP006587.1 (*H.* sp. APR13), and NZ_CP010054.1 (*H.* sp. DG25B). Only selected complete genomes available in the database during the time of this study were selected.

Phylogenetic Analysis

The phylogenetic tree was constructed using MEGA-X based on the 16S rRNA gene sequence of the *Hymenobacter* strains using a maximum likelihood tree. For the reliability of the interior branch length, a bootstrap test was performed with 1,000 replications. All of the species of *Hymenobacter* and *Flavobacterium* as an outgroup were selected to build the tree.

CAZyme Annotation

For CAZyme annotation, all the complete genome of the species were subjected to the dbCAN2 meta server. Each genome was annotated using DIAMOND, HMMER, and Hotpep via CAZy, dbCAN, and PPR database, as previously described [29]. Analysis of the putative function took place with the subjection of each sequence to the NCBI protein blast (BLASTP). For domain analysis, the sequences were also subjected to the Conserved Domain Database (CDD) server within NCBI. All the gathered information was used to compare the polysaccharide utilization ability of *Hymenobacter* species.

Pathway Mapping and Cluster Analysis

Pathway mapping was performed manually using the Kyoto encyclopedia of genes and genomics (KEGG) for the *H.* sp. PAMC 26554 and *H.* sp. PAMC 26628. The genome of the microorganism was inserted into the database, and the outcome was analyzed for the availability of enzymes involved in the degradation pathway. On the other hand, the cluster analysis for laminarin degradation was performed using the Polysaccharide Utilization Loci DataBase (pulDB).

Results

General Features of *Hymenobacter* Strains

The two *Hymenobacter* species, PAMC 26554 and PAMC 26628 isolated from two different parts of the polar lichen showed a gene size of (5.45125 and 5.36698) Mb, respectively. Table 1 lists all the genomic information of *Hymenobacter* species taken from NCBI database. This includes genomic information, along with sampling and isolation sources. The species were isolated from diverse environments and sources, with most of them taken from the soil, along with feces and snow. One more species, *H. nivis* strain NBRC 111535, was isolated from the Antarctic snow.

Table 1 General genomic features and isolation information of selected *Hymenobacter* sp. for comparative study

Name of species	Size (Mb)	Gene content	Protein content	Isolation source	Isolation country
H. sp. PAMC 26554	5.45125	4735	4577	Lichen host	Antarctica
H. sp. PAMC 26628	5.36698	4636	4457	Lichen *Stereocaulon* sp. host	Svalbard
H. swuensis DY53	5.25207	4163	4073	Mountain soil	South Korea
H. nivis NBRC 111535	5.0276	4451	4252	Red snow	Antarctica: Yatude Valley
H. sedentarius DG5B	4.86885	3989	3882	Soil	South Korea: Seoul
H. sp. APR13	5.01059	4236	4112	Silkworm feces	China: Jiangsu
H. sp. DG25B	4.36003	3755	3634	Soil	South Korea: Seoul
. sp. DG25A	3.77714	3260	3181	Soil	South Korea
H. sp. Sh-6	4.18881	3618	3520	Nm	China
H. sp. 17J36-26	4.91897	4285	3843	Soil	South Korea: Seoul

Nm not mentioned

Figure 1 shows that in the phylogenetic analysis, the two *Hymenobacter* from the Antarctic region clustered in the same clade, while the Arctic species showed divergence.

Specialized Screening by AZCL Activity

AZCL activity from the strains PAMC26554 and PAMC 26628 was checked. If the bacteria produce related enzyme, the enzymes degrade the large polysaccharides insoluble molecules, which have been blue dyed with AZCL. The small hydrolyzed compounds are dyed blue, and diffuse in the plate, developing blue circle zones around the colonies, so positive enzyme activity reaction can be easily found. Both strains showed activity against barley β-glucan, galactomannan, and debranched arabinan. Similarly, strain PAMC 26554 could show activity in all the substrates except AZCL-chitosan, but strains PAMC 26628 could not show activity, except for three substrates, as shown in Table 2.

CAZyme Analysis

Based on the AZCL screening result, genome analysis for CAZyme was performed for the two *Hymenobacter* species from polar lichens under study. Meanwhile, other *Hymenobacter* species obtained from the NCBI genome database were also analyzed for the CAZyme comparison. The analysis revealed the presence of a high number of glycosyl hydrolase (GH), followed by some carbohydrate esterase

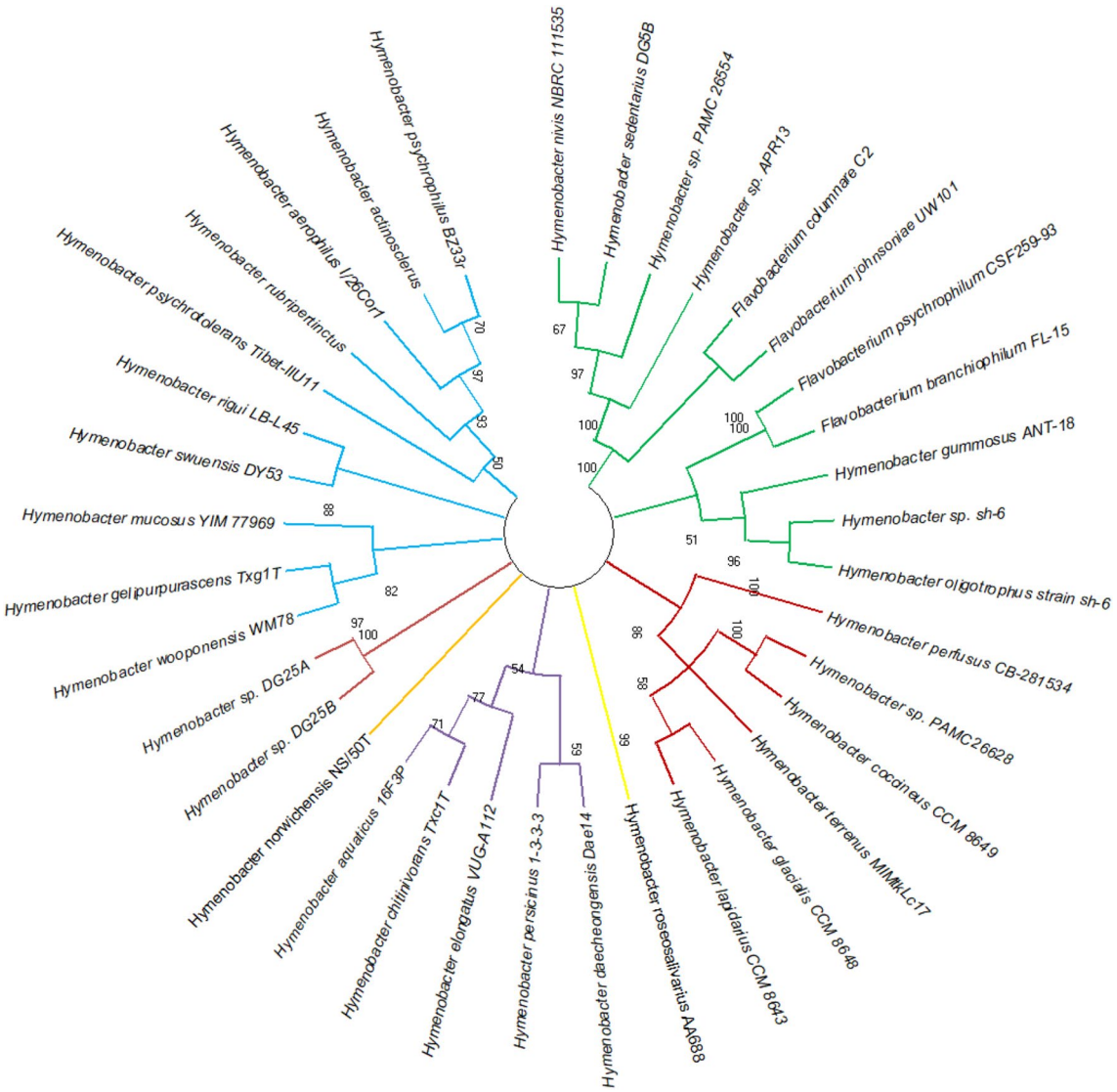

Fig. 1 The phylogenetic placement of the *Hymenobacter* and *Flavobacterium* species under study was constructed using the maximum likelihood tree using MEGAX software. 16 s rRNA sequences were used for tree construction. The bootstrap number represents the confidentiality of the generated branches. The color code symbolizes the separation of the clade between the species (Color figure online)

Table 2 Result of the AZCL screening for the polysaccharide ability of *Hymenobacter* sp. PAMC 26554

Carbohydrates	AZCL screening for carbohydrate abilities	
	Hymenobacter sp. PAMC 26554	*Hymenobacter* sp. PAMC 26628
AZCL-amylose	+	−
AZCL-barley β-glucan	+	+
AZCL-HE-cellulose	+	−
AZCL-xylan (Beechwood)	+	−
AZCL-arabinoxylan (Wheat)	+	−
AZCL-xylan (Birchwood)	+	−
AZCL-xyloglucan (Tamarind)	+	−
AZCL-chitosan	−	−
AZCL-curdlan	+	−
AZCL-galactomannan	+	+
AZCL-debranched arabinan	+	+

The screening involves the analysis of cellulose, hemicellulose, starch, and chitin degradation abilities

AZCL Azurine crosslinked

(CE) and polysaccharide lyase (PL). However, the genes coding for auxiliary activities (AA) were not detected in any species (Table 3). Therefore, highlighting the role of glycosyl hydrolase (EC 3.2.1.x) family was considered, further having regard to its abundance. Figure 2 shows the glycosyl hydrolase families present among all the species and their distribution. In total, 53 glycosyl hydrolase families distributed among the *Hymenobacter* species were identified. Among them, GH136 was unique to PAMC 26554, while GH128 was unique to PAMC 26628. On the other hand, GH26 and GH78 were unique for three *Hymenobacter* isolated from the polar region, adding NBRC 111535 along with the other two. Glycosyl hydrolase families, such as GH1, GH2, GH3, GH13, GH31, GH65, and GH97, were found among all the species. The number of genes coding for GH43 was significantly high among all the species except APR13, which does not involve any gene for GH43. On finding the putative functions of these glycosyl hydrolases, the abundance of genes coding for cellulose, hemicellulose, and starch degradation genes among all the species was determined. Figure 2 shows that GH13, GH43, and GH3 families were most abundant, representing starch, hemicellulose, and cellulose degradation enzymes, respectively. Figure 3 shows the estimated pathway for the utilization of cellulose, hemicellulose, and starch as obtained from the KEGG database when the *Hymenobacter* species from the polar lichen were subjected for analysis. The pathway shows that both the *Hymenobacter* species have enzymes for utilizing the aforementioned polysaccharides leading to either of the fates like glycolysis, pentose phosphate pathway, and amino acid or nucleotide sugar metabolism. The products of cellulose, galactan, and starch show their fate towards glycolysis. Meanwhile, the products of xylan and arabinan have fates towards pentose phosphate pathway. On the other hand, mannan shows its fate towards amino acid and nucleotide sugar metabolism along with galactan that also shows possible fate towards glycolysis, as revealed in Fig. 3. However, the database shows the lack of enzymes for laminarin degradation.

Cellulose Decomposition

Cellulose is one of the abundant polymers synthesized by plants, mostly as a part of their cell wall, with approximately (20–50) % coverage. It consists of β-1,4 linked subunits

Table 3 Number of annotated CAZyme in the genome of *Hymenobacter* species

Name of species	CAZyme classification					
	GH	GT	PL	CE	CBM	AA
H. sp. PAMC 26554	70	29	1	13	9	-
H. sp. PAMC 26628	70	33	-	7	12	-
H. swuensis DY53	84	33	3	12	21	-
H. nivis NBRC 111535	68	30		6	10	-
H. sedentarius DG5B	75	32	4	12	8	-
H. sp. APR13	39	32	-	6	6	-
H. sp. DG25B	36	31	-	3	10	-
H. sp. DG25A	37	31	-	4	11	-
H. sp. sh-6	18	26	-	3	-	-
H. sp. 17J36-26	89	28	3	9	10	-

This includes all the classification of CAZyme, and the common result provided by HMMER, DIAMOND, and Hotpep databases

GH glycosyl hydrolase, *GT* glycosyltransferase, *PL* polysaccharide lyase, *CE* carbohydrate esterase, *CBM* carbohydrate-binding module, *AA* auxiliary activities

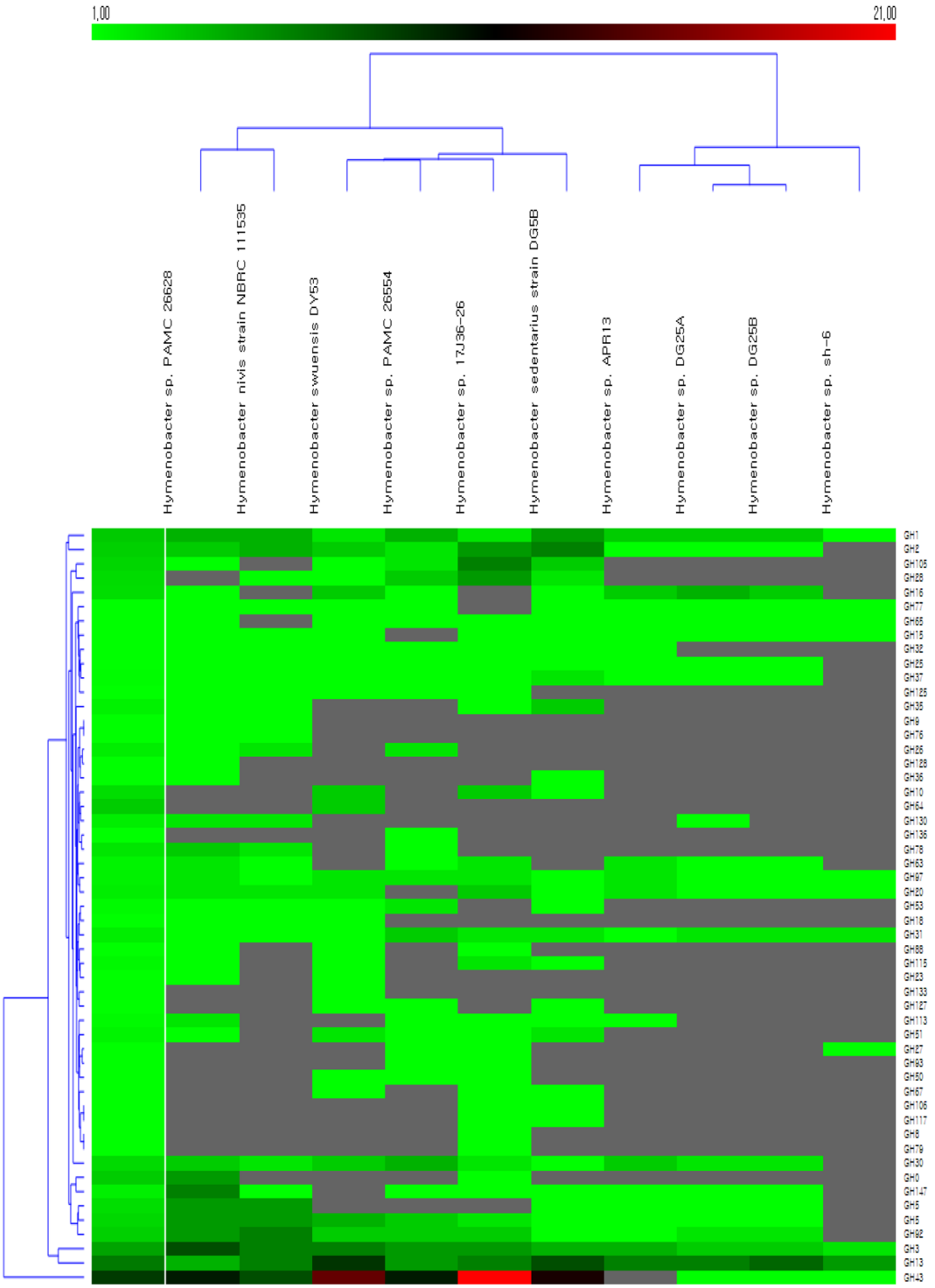

Fig. 2 GH family in the proteome of *Hymenobacter* species. The abundance of each family is represented in color code increasing from green to red. Gray color indicates the absence of GH in a particular organism (Color figure online)

of glucose arranged together in crystalline form [30]. Its decomposition has attracted great attention in bioenergy production, along with its importance in the textile, food, agriculture, brewery, and paper industries. The characterized cellulases are mainly under the glycosyl hydrolase family GH1, GH3, GH5, GH6, GH8, GH9, GH12, GH45, GH48, GH51, and GH74 present in the CAZy database [31]. The *Hymenobacter* from polar lichen incorporated (9 and 12)

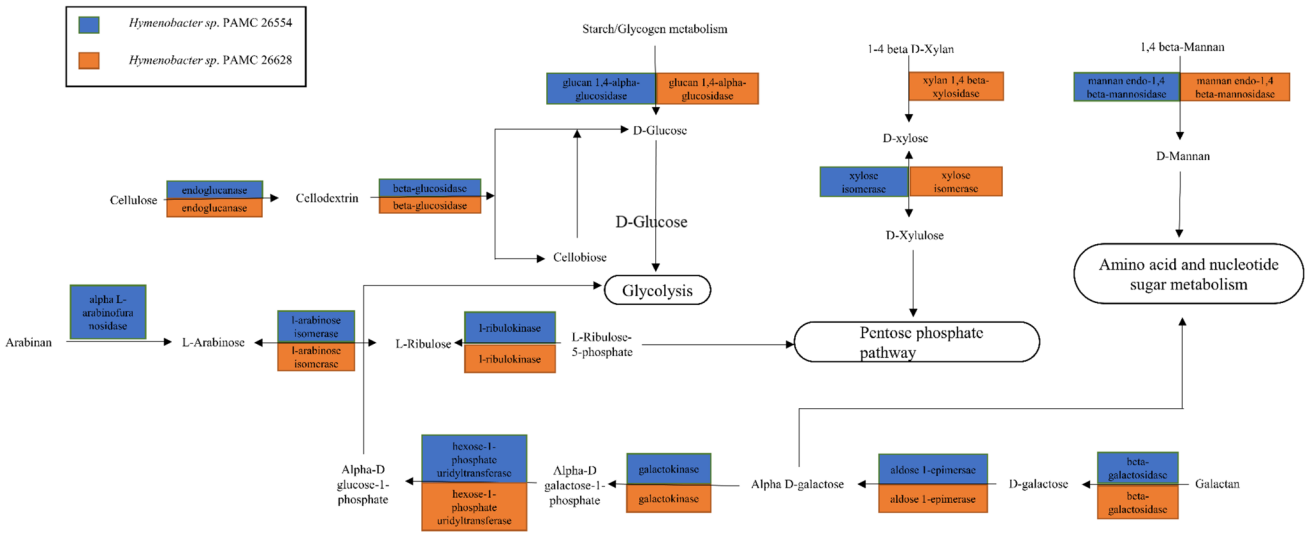

Fig. 3 The estimated pathway for cellulose, hemicellulose, and starch degradation analyzed from the KEGG database. The blue color symbolizes the enzymes present in *H.* sp. PAMC 26554, while orange symbolizes *H.* sp. PAMC 26628. The possible pathway for polysaccharide degradation is obtained from the KEGG pathway map for carbohydrate metabolism. However, the pathway for laminarin degradation in the KEGG pathway was not available (Color figure online)

% genes involved in cellulose degradation in PAMC 26554 and PAMC 26628, respectively; which is approximately the same amount for other species. Figure 4 shows the distribution of cellulose genes among all the species ranging (7–12) %, without any distinct variable in the percentage of any species. The most abundant number of cellulose genes was present in PAMC 26628 and NBRC 111535 isolated from the polar region. Overall, the *Hymenobacter* species contained the cellulose degradation genes from families like GH1, GH3, GH5, GH9, GH147, and GH115 (Table 4).

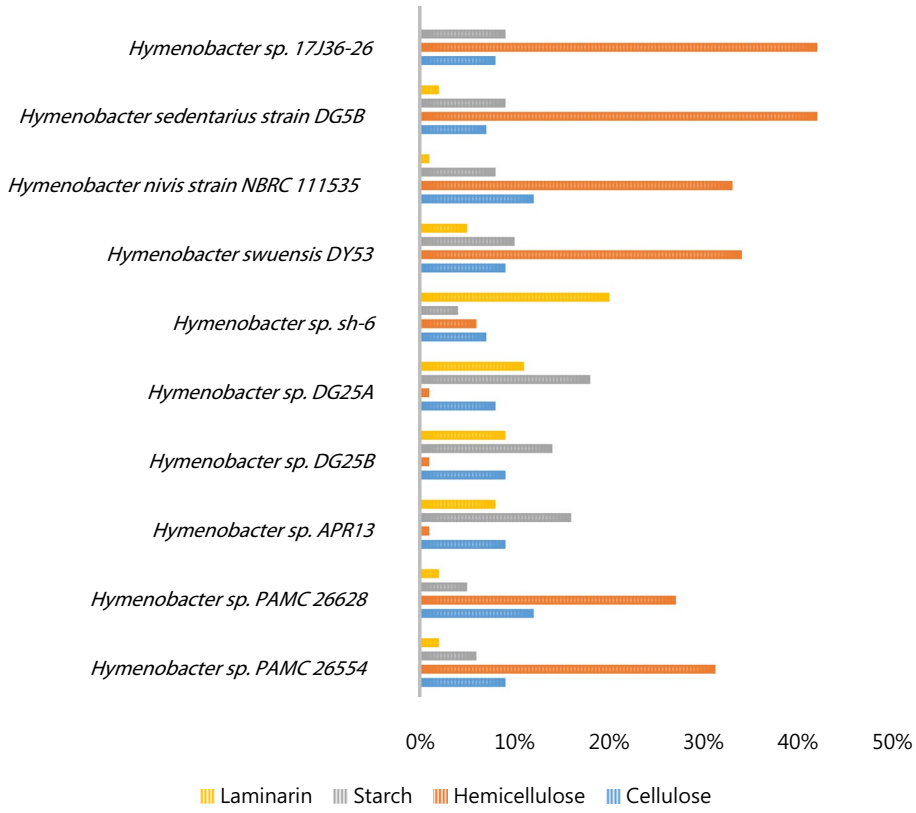

Fig. 4 Bar graph of the total number of cellulose, hemicellulose, starch, and laminarin-degrading genes among *Hymenobacter* species. All of the species include genes involved in the degradation of all four polysaccharides except 17j36-26, which did not include the gene for laminarin degradation (Color figure online)

Table 4 CAZyme with the total number of genes of GH family involved in cellulose, starch, and laminarin degradation

Name of species	Cellulose decomposition (No.[a])		Starch decomposition (No.[a])				Laminarin (β-1,3 glucan) decomposition (No.[a])	
	β-Glucosidase/cellulase		α-Amylase		α-Glucosidase		Laminarinase	
H. sp. PAMC 26554	GH1, GH3, GH5	(10)	GH13	(4)	GH13, GH31	(2)	GH16	(1)
H. sp. PAMC 26628	GH1, GH3, GH5	(14)	GH13	(4)	GH15, GH31	(2)	GH16	(1)
H. swuensis DY53	GH1, GH3, GH5	(12)	GH13	(9)	GH15, GH31, GH63	(3)	GH16, GH64	(4)
H. nivis NBRC 111535	GH1,GH3, GH5, GH9	(13)	GH13	(5)	GH31, GH15	(2)		
H. sedentarius DG5B	GH1, GH3, GH5	(8)	GH13	(8)	GH15, GH31	(2)	GH16	(1)
H. sp. APR13	GH1, GH3, GH5	(7)	GH13	(8)	GH31, GH63	(3)	GH16, GH64	(3)
H. sp. DG25B	GH1, GH3, GH5	(6)	GH13	(6)	GH15, GH31, GH63	(4)	GH16	(3)
H. sp. DG25A	GH1, GH3, GH5	(6)	GH13	(7)	GH15, GH31, GH63	(4)	GH16	(4)
H. sp. sh-6	GH1, GH3	(3)	GH13	(5)	GH15, GH31	(3)		
H. sp. 17J36-26	GH1, GH3, GH5, GH147, GH115	(10)	GH13	(6)	GH15, GH31, GH63	(5)		

[a]Organizing GH family and number of contained genes

Hemicellulose Decomposition

Hemicellulose consists of the heterogeneously distributed polysaccharides that primarily consist of xylan, arabinoxylan, glucuronoxylan, xyloglucan, and glucomannan with different side chains. This covers (15–35) % of the cell wall of plants [32]. However, the decomposition of hemicellulose requires the action of a set of enzymes, such as xylanase, xylosidase, galactosidases, glucuronidases, arabinosidases, and glucosidases, due to the complexity of their chain arrangement [33]. In the CAZy database, hemicellulolytic enzymes fall under GH2, GH10, GH11, GH16, GH26, GH30, GH31, GH39, GH42, GH43, and GH53 families. Along with cellulose, hemicellulose also carries good potential for bioenergy and biotechnological application. The CAZyme analysis for hemicellulose utilizing genes in this paper showed a visible difference in the distribution among the *Hymenobacter* species. High percentage of hemicellulose genes was found in polar lichen-associated PAMC 26554 and PAMC 26628 with (27 and 31.25) %, respectively. Similarly, DY53 and NBRC 111535 showed percentage of (34 and 33) %, respectively. However, the highest percentage was observed in strain DG5B and 17J36-26, i.e., 42%; both isolated from soil. On the other hand, significant decrement of (1, 1, 1, and 6) % was observed in species APR13, DG25B, DG25A, and sh-6, respectively. The hemicellulose genes in the case of *Hymenobacter* lay in the families GH1, GH2, GH3, GH5, GH10, GH26, GH27, GH30, GH31, GH35, GH36, GH43, GH51, GH53, GH67, GH76, GH88, GH92, GH105, GH115, GH117, GH125, GH127, GH130, and GH147 (Table 5).

Starch Decomposition

Starch is one of the vital sources of energy for microorganisms, and is a stored food source of plants. It consists of α (1→4) glyosidic linkage, i.e., either amylose or α (1→6) glycosidic linkage, i.e., amylopectin. Amylase is the enzyme most involved in the degradation of starch, which falls under the glycoside hydrolase family GH13 in the CAZy database [34]. Amylase has great importance in industry, due to its applicability to the fermentation, pharmaceuticals, paper, textile, and food industries [35]. The least percentage of starch genes was found in polar lichen-associated PAMC 26554 and PAMC 26628 with (6 and 5) %, respectively, along with sh-6 having 4%; whose isolation source has been mentioned as R2A medium in the NCBI database, biosample. Among other *Hymenobacter* species subjected for analysis of starch genes, DG25A and APR13 showed maximum percentage of (18 and 16) % isolated from feces and soil, respectively. The remaining showed distribution in the range (8–14) %. The genes were found to belong to hydrolase families, such as GH13, GH15, GH31, GH63, and GH133 (Table 5).

Laminarin Decomposition

Since two *Hymenobacter* species under study from lichen were isolated, their lichen-associated polysaccharide utilization ability was analyzed. Laminarin is a β-1,3 linked chain of glucan mostly found in red algae [36]. Laminarinase (EC 3.2.1.6) has been characterized in families GH16, GH17, and GH64, and this enzyme is part of multiple glycosyl hydrolase families in the CAZy database. This enzyme has great

Table 5 CAZyme with the total number of genes of GH family involved in hemicellulose degradation

Name of species	Hemicellulose decomposition (No.[a])									
	Arabinosidase		Galactosidase		Mannosidase		Glucuronidase		Xylosidase	
H. sp. PAMC 26554	GH43, GH53	(9)	GH1, GH2, GH35, GH53	(9)	GH5, GH26, GH92, GH125, GH130	(8)	GH2	(4)	GH43	(5)
H. sp. PAMC 26628	GH43, GH51	(9)	GH1, GH2, GH36	(7)	GH2, GH92, GH125, GH130, GH147	(10)	GH2, GH88, GH115	(3)	GH43	(2)
H. swuensis DY53	GH43, GH51, GH53, GH127	(16)	GH1, GH2, GH53	(7)	GH92	(3)	GH2, GH67, GH88, GH115	(6)	GH3, GH43	(12)
H. nivis NBRC 111535	GH43	(2)	GH2, GH3, GH35	(8)	GH5, GH26, GH92, GH130, GH147	(13)	GH2	(3)	GH31, GH43	(9)
H. sedentarius DG5B	GH43, GH51, GH53, GH127	(14)	GH1, GH2, GH35, GH53	(15)	GH5, GH92	(2)	GH2, GH10, GH67, GH115	(8)	GH43	(11)
H. sp. APR13			GH1	(3)	GH92, GH147	(2)				
H. sp. DG25B	GH43	(1)	GH1, GH2	(3)	GH30, GH92	(3)	GH2	(1)	GH43	(1)
H. sp. DG25A	GH43	(3)	GH1, GH2	(3)	GH92, GH130	(3)	GH2	(1)	GH43	(1)
H. sp. sh-6	GH43	(1)	GH1, GH2	(2)			GH2	(1)	GH43	(1)
H. sp. 17J36-26	GH43, GH51, GH117	(12)	GH1, GH2, GH3, GH27, GH35	(9)	GH5, GH92, GH125	(5)	GH2, GH3, GH67, GH88, GH115	(11)	GH43	(18)

[a]Organizing GH family and number of contained genes

importance for third generation biofuel production from algal polysaccharides [37]. All the *Hymenobacter* species, except 17J36-26, showed laminarin activity that involved GH16 family only. However, the amount of genes was comparatively lower, as in the case of cellulose, hemicellulose, and starch degradation. Unexpectedly, the two *Hymenobacter* species isolated from lichen had the least number of enzymes for this algal polysaccharide. The maximum percentage of the gene was found in sh-6; which showed the least percentage of gene for starch and cellulose degradation, and comparatively less percentage in the case of hemicellulose as well.

Figure 5 shows that the polysaccharide utilization loci were also analyzed for further confirmation of the laminarin degradation gene clusters, as per the authors' hypotheses. The polysaccharide utilization loci contain the cluster of enzymes that are responsible for the utilization of certain polysaccharides [38]. The cluster showing laminarin utilization loci in *Hymenobacter* was screened for. For that, the laminarin utilization loci of *Gillisia* sp. and *Formosa* sp. were kept as a reference. Eight out of ten of the studied *Hymenobacter* species were found to have a cluster for laminarin. Both *Hymenobacter* species from polar lichen had SusC and SusD transporter located in similar orientation. However, PAMC 26628 showed more similarity with the well-known references, as there are more than one glycosyl hydrolase family involved in the cluster. Meanwhile, PAMC 26554 had only GH16, which showed similarity with only APR13. All the other clusters of *Hymenobacter* species were also found to have SusC and SusD transporter. Besides showing potential genes for laminarinase activity, sh-6 was not found to have a cluster for laminarin degradation according to pulDB. Meanwhile, the other species 17j36-26 was not submitted to pulDB. Overall, the presence of laminarin utilization loci somehow confirms that the two *Hymenobacter* from polar lichen have activity towards it, besides having only one gene for coding laminarinase in the CAZyme annotation result.

Discussion

In this study, the genomic comparison was conducted to analyze the polysaccharide-degrading ability of the polar lichen-associated *H.* sp. PAMC 26554 and *H.* sp. PAMC 26628 with other species from the same genus, considering their isolation environment, and biosample source. The genus *Hymenobacter* was selected for the study, as it falls under the phylum Bacteroidetes, which several studies have reported to depolymerize polysaccharide [39–41]. Therefore, the lack of study of polysaccharide decomposition from *Hymenobacter* allowed this paper to explore a new insight into the enzymes

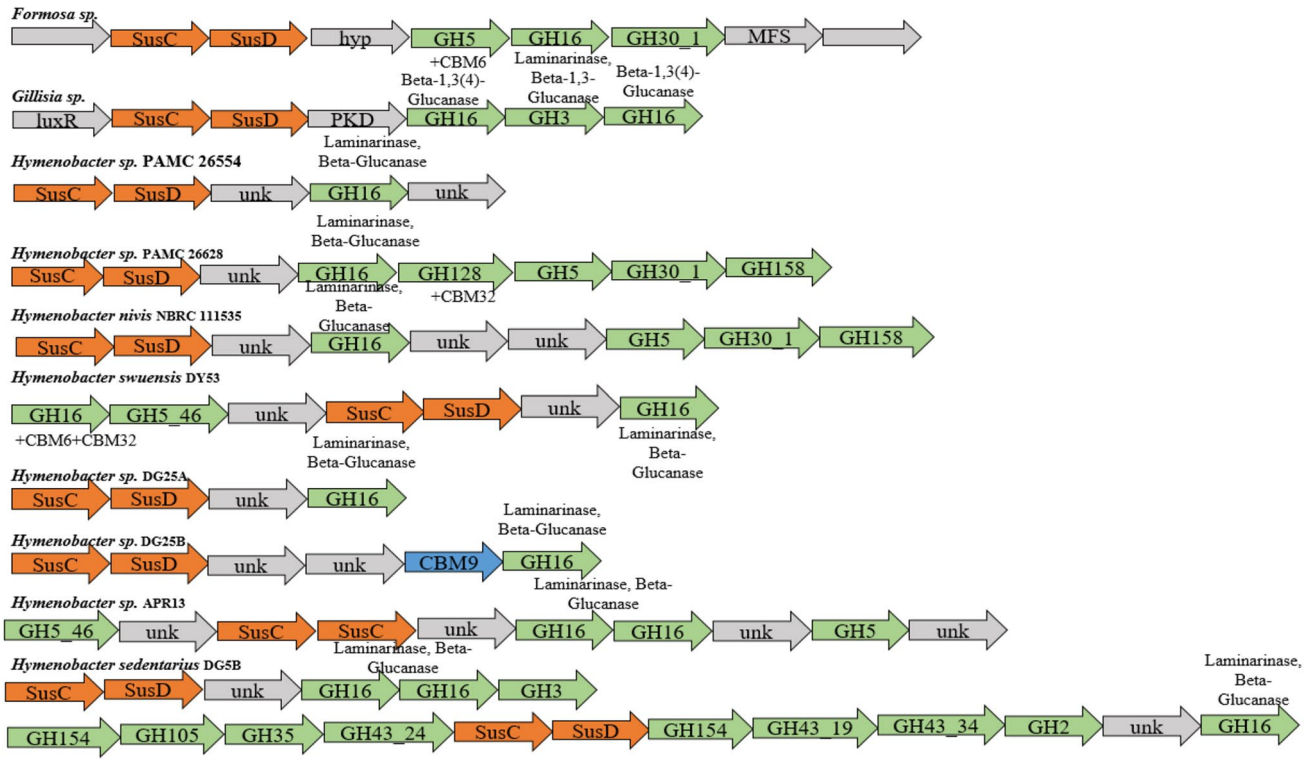

Fig. 5 Laminarin utilization cluster as observed in pulDB of *Hymenobacter* species. The color coding represents different genes involved in the cluster as shown above: green, GH; orange, Transporter; blue, Carbohydrate-binding domain; and gray, Unknown (Color figure online)

involved in polysaccharide degradation, focusing on glycosyl hydrolases. Also, while there are several reports regarding polysaccharide-decomposing enzymes from cold-associated microorganism, soil, and feces as well [1, 16, 37, 42], to the best of the authors' knowledge, little study has previously been conducted into the polysaccharide decomposition from polar lichen-associated bacteria.

It was found that, along with two lichen-associated polar organisms, PAMC 26554 and PAMC 26628, other *Hymenobacter* species were isolated from diverse environments, such as Antarctica and different parts of Asia, with biosample source, soil, feces, and snow. In the phylogenetic tree, the species belonging to Antarctic and Arctic regions showed separation in the clade. The *Hymenobacter* species, PAMC 26554 and NBRC 111535, isolated from Antarctic region were placed in the same clade, whereas PAMC 26628 from Arctic region was placed in a different clade. The separation in phylogenetic placement between Antarctic and Arctic organisms was also observed by Gawor et al. [43]. On the other hand, bacterial species failed to separate in the clade based on their bio sample source.

All the complete strains of *Hymenobacter* species were analyzed for the polysaccharide utilization gene, focusing on hydrolysis, based on their isolation environment and source. The analysis revealed a visible difference in the number of hemicellulose, starch, and laminarin decomposition genes, while cellulose decomposition genes showed uniformity among all the species. The organisms PAMC 26554, PAMC 26628, NBRC 111535, and *H. swuensis* DY53 from cold isolation environment were found to have abundant hemicellulose genes, which showed similarity with the study by Ma et al. as well [1]. On the other hand, APR13, DG25A, and DG25B isolated from silkworm feces and soil from normal environment, respectively, showed the highest percentage of starch degradation gene and low hemicellulose degradation gene. Likewise, sh-6, whose isolation source is not mentioned, showed maximum percentage of laminarin degradation gene, with much very less hemicellulose and starch genes. This result might symbolize the primary preference of polysaccharides among the species based on the environment, isolation source, or some other factor, as the ones that showed the least percentage of hemicellulose genes were found to have a greater percentage of starch, as well as laminarin genes, and vice versa.

The lichen-associated species were found to have a high percentage of hemicellulose degradation genes. The species PAMC 26554 and PAMC 26628 had both the highest hemicellulose degradation gene, and less starch and laminarin genes in their genome. The very much lower percentage of starch-degrading genes in lichen-associated bacteria also somehow clarifies the abundance of polysaccharide-degrading genes according to the adapted habitat. This finding resembles the plant ecology of polar regions, which mostly consists of lichen, bryophytes abundant in hemicellulose cellulose, and β-1,3 glucan, instead of vascular plants whose major storage compound is starch [44]. But, the lower percentage of laminarinase in lichen-associated bacteria was the major concern, and devoid of the expected outcome in this study. However, the polysaccharide utilization cluster shows the insertion of GH16 with laminarinase activity. This supports the presence of laminarinase activity in the organism, which is further supported by AZCL screening result too for lichen-associated PAMC 26554 and PAMC 26628.

The pattern of genomic analysis for CAZyme as shown by PAMC 26554 and PAMC 26628, of polar lichen-associated organism, was both similar and contradictory to the result obtained from AZCL screening. This screening method has been utilized by several studies, such as Brunecky et al. and Wilkens et al. [45, 46], which to a degree clarifies its reliability. However, the result from PAMC 26554 showed a concomitant relationship with AZCL screening, while the lack of cellulose and amylose utilization activity during the screening was not similar to the genomic data of PAMC 26628. In this paper, genes involved in cellulose, hemicellulose, starch, and glucan decomposition were found in PAMC 26554 and PAMC26628. Similarly, neither of them showed activity towards chitin utilization, as observed in the AZCL screening. To the best of the authors' knowledge, such differences between experimental and genomic data have not been justified for polysaccharide utilization by other studies.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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