Complete genome sequence of *Arthrobacter* sp. PAMC25564 and comparative genome analysis for elucidating the role of CAZymes in cold adaptation

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Research Article

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Abstract

Background: The *Arthrobacter* group is a known isolate from cold areas, the species of which are highly likely to play diverse roles in low temperatures. However, their role and survival mechanisms in cold regions such as Antarctica are not yet fully understood. In this study, we compared the genomes of sixteen strains within the *Arthrobacter* group, including strain PAMC25564, to identify genomic features that adapt and survive life in the cold environment.

Results: The genome of *Arthrobacter* sp. PAMC25564 comprised 4,170,970 bp with 66.74 % GC content, a predicted genomic island, and 3,829 genes. This study provides an insight into the redundancy of CAZymes for potential cold adaptation and suggests that the isolate has glycogen, trehalose, and maltodextrin pathways associated to CAZyme genes. This strain can utilize polysaccharide or carbohydrate degradation as a source of energy. Moreover, this study provides a foundation on which to understand how the *Arthrobacter* strain produces energy in an extreme environment, and the genetic pattern analysis of CAZymes in cold-adapted bacteria can help to determine how bacteria adapt and survive in such environments.

Conclusions: We characterized the *Arthrobacter* sp. PAMC25564 complete genome and comparative analysis, provided an insight into the redundancy of CAZymes for potential cold adaptation. This provide a foundation to understand how *Arthrobacter* strain produces energy in an extreme environment, there are reports on the use of CAZymes in cold environments. Therefore, we suppose that this process has allowed *Arthrobacter* species to establish a symbiotic relationship with other bacteria in cold environments or live independently thanks to their capacity for adapting to environmental changes.

Background

The *Arthrobacter* genus is member of the family *Micrococcaceae*, which belongs to the phylum *Actinobacteria* [1, 2]. *Arthrobacter* species are often isolated from soil, where they contribute to biochemical cycles and decontamination [3]. These species have been isolated worldwide from a variety of environments, including sediments [4], human clinical specimens [5], water [6] glacier cryoconite [7], sewage [8], and glacier ice [9]. Cold environments are present in about 75% of the Earth, and their study provides information about new microorganisms and the evolution of cold environments [10]. Psychrophilic microorganisms have colonized all permanently cold environments, from the deep sea to mountains and polar regions [11]. Cold-adapted microorganisms utilize a wide range of metabolic strategies to grow in diverse environments. In general, the ability to adapt to low temperatures requires the microorganisms to sense a decrease in temperature, which induces the upregulation of cold-associated genes [12, 13]. Therefore, these kinds of enzymes have been studied for everyday life applications such as detergent additives; textile industry; food industry; and bioremediation [14].

CAZymes are enzymes associated with the biosynthesis, binding, and catabolism of carbohydrates. These enzymes are divided into several groups based on their catalytic activity: glycoside hydrolase (GH); carbohydrate esterase (CE); polysaccharide lyase (PL); glycosyltransferase (GT); and auxiliary activities (AA). CAZymes may have non-catalytic subunits. Carbohydrates are catabolized in a carbohydrate-binding module (CBM). CAZymes are well known in biotechnology, and their industrial applications are of interest to many researchers because CAZymes produce precursors for bio-based products such as food, paper, textile, animal feed, and other chemicals, including biofuels [15, 16].

Most bacteria can use glycogen as an energy storage compound, and the enzymes involved in its metabolism are well known. A recent study showed the physiological impact of glycogen metabolism in the survival of bacteria living in extreme environments [17]. Some microorganisms can adapt quickly to continuously changing environmental conditions, by accumulating energy storage compounds to cope with transient starvation periods. These strategies use glycogen-like structures such as a polysaccharides composed of α-$_D$-glycosyl units connected by α-1,4 linkages and branched by α-1,6 glycosidic linkages. These biopolymers differ in their chain length and branching occurrence. To be used as carbon and energy sources, their glucose units are released by specific enzymes [18].

Microorganisms have synergistic enzymes capable of decomposing plant cell walls to release glucose. Therefore, this phenomenon can be used as energy supply to maintain microbial growth [19]. Starch is an excellent source of carbon and energy for microbes that produce proteins responsible for the extracellular hydrolysis of starch, in-cell absorption of fructose, and further decomposition into glucose [20]. In addition, strains that metabolize glycogen show important physiological functions, including the use of energy storage compounds for glycogen metabolism. These pathways act as carbon pools that regulate carbon fluxes [21], and part of this capability are due to CAZymes. The genetic patterns of CAZymes in cold-adapted bacteria can help to understand how survival adaptation can be achieved in extreme low temperature environments.

Results And Discussion

Profile of the complete genome of Arthrobacter sp. PAMC25564

As shown in Table 1, the complete genome of *A.* sp. PAMC25564 is composed of a circular chromosome of 4,170,970 bp with a 66.74% GC content. The chromosome is predicted to include 3,829 genes, from which 3,613 protein-encoding genes were functionally assigned, whereas the remaining genes were predicted as hypothetical proteins. We annotated 147 pseudogenes, 15 rRNA genes, and 51 tRNA genes distributed through the genome. From the predicted genes, 3,449 (90.08%) were classified into 20 functional COG categories, whereas the remaining 380 (9.92%) remained un-classified. The most numerous COG categories were S genes with unknown function (705 genes), K (298 genes), E (280 genes), G (276 genes), and C (259 genes) (Fig. 1). Many of these genes are related to amino acid transport, transcription, carbohydrate transport, and energy production/conversion, which suggests that this strain utilizes CAZymes for energy storage and carbohydrate metabolism. Most bacteria rely on cell respiration to catabolize carbohydrates to obtain the energy used during photosynthesis for converting carbon dioxide into carbohydrates. The energy is stored temporarily in the form of high-energy molecules such as ATP and used in several cell processes [33, 34]. Therefore, we predicted that the PAMC25564 strain could utilize carbohydrate degradation to obtain energy.

Table 1
Genome features of *Arthrobacter* sp. PAMC25564.

Feature	Value
A; Genome Statistics	
Contigs	1
Total length bp;	4,170,970
N50	4,170,970
L50	1
GC %;	66.74
B; Genome features	
Assembly level	Complete genome
Chromosome genes	3,829
Protein coding genes	3,613
Pseudogenes	147
rRNA genes	15
tRNA genes	51

16S rRNA phylogenetic analysis and ANI values

The identification of *A.* sp. PAMC25564 was verified using 16S rRNA sequence analysis (Fig. 2). This strain is phylogenetically placed among *Arthrobacter* and *Pseudarthrobacter* species. The results from phylogenetic analysis, BLAST analysis, and EzBio Cloud analysis revealed closely related strains such as *P. sulfonivirans* ALL (T) (99.09%), *P. siccitolerans* 4J27 (T) (98.48%), *A. ginsengisoli* DCY81 (T) (98.23%), and *P. phenanthrenivorans* Sphe3 (T) (98.13%). These results confirmed that isolate PAMC25564 belongs to the family *Micrococcaceae*, phylum *Actinobacteria*. Recently, several *Arthrobacter* species have been reclassified into new genera, based on 16S rRNA sequence similarities and chemotaxonomic traits such as peptidoglycan types, quinone systems, and/or polar lipid profiles [35]. Therefore, it has been proposed to reclassify within the genus *Arthrobacter* members of these five genera: *Paenarthrobacter* gen. nov., *Pseudarthrobacter* gen. nov., *Glutamicibacter* gen. nov., *Paeniglutamicibacter* gen. nov., and *Pseudoglutamicibacter* gen. nov. Among them, *Pseudarthrobacter* group would be reclassified into the genus *Arthrobacter* as: *A. chlorophenolicus*, *A. defluvii, A. equi, A. niigatensis, A. oxydans, A. phenanthrenivorans, A. polychromogenes, A. scleromae, A. siccitolerans*, and *A. sulfonivorans* [36]. Therefore, the PAMC25564 strain will probably be reclassified into the genus *Pseudarthrobacter*. As shown in Fig. 3, each ANI values ranged from 70.67 to 98.46%. So, we were confirmed that comparative genome results much lower than the common ANI values of 92–94%. In general, bacterial comparative genome analysis uses this method. The ANI analysis shows the average nucleotide identity of all bacterial orthologous genes that are shared between any two genomes and offers a robust resolution between bacterial strains of the same or closely related species (i.e., species showing 80–100% ANI) [37]. However, ANI values do not represent genome evolution, because orthologous genes can widely vary between the genomes being compared. Nevertheless, ANI closely reflects the traditional microbiological concept of DNA-DNA hybridization relatedness for defining species, so many researchers used this method, since it takes into account the fluid nature of bacterial gene pool and hence implicitly considers shared functions [38]. So, this mean the PAMC25564 strain could either belong to the species from which *Arthrobacter* diverged, or this could be a *Pseudarthrobacter* closely related new species.

CAZyme-encoding genes in Arthrobacter sp. PAMC25564

Among the 3,613 identified protein-encoding genes in PAMC25564, 108 were significantly annotated and classified into CAZyme groups: GH, GT, CE, AA, CBM, and PL using dbCAN2. The results provided an insight into the carbohydrate utilization mechanisms of PAMC25564. The signal P analysis predicted that 11 genes contained signal peptides. We found that proteins were distributed as follows: 33 GHs, 45 GTs, 23 CEs, 5 AAs, and 2 CBMs. However, no protein was assigned to the PL group. Most annotation results of GH genes revealed that the PAMC25564 genome has genes involved in glycogen and trehalose metabolism pathways such as β-glucosidase (GH1), glycogen debranching proteins (CBM48 and GH13_11), (1→4)-α-D-glucan 1-α-D-glucosylmutase (GH13_26), α-glucosyltransferase (GH13), α-trehalose phosphorylase (GH65), and 4-α-glucanotransferase (GH77) (Table 2). Previous studies showed the complex interplay of glycogen metabolism in colony development of *Streptomycetes* (in *Actinomycetes* species was only reported), showing that spore germination is followed by an increase in glycogen metabolism [39]. The underlying genetic and physiological mechanisms of spore germination remain unknown, but some mechanisms associated with the accumulation of nutrients as biomass and storage materials in the substrate mycelium during morphological phases of development have been reported [40]. Recently, *Shigella* sp. PAMC 28760, of pathogens isolated from Antarctica, was also reported to be able to adapt and survive in cold environments through glycogen metabolism [64]. Nonetheless, glycogen metabolism in bacteria remains unknown, even though it has been well-studied in eukaryotes [41]. However, we could predict the specificity of PAMC25564 strain genes involved in glycogen and trehalose metabolism.

Table 2
List of CAZyme GH enzymes from *Arthrobacter* sp. PAMC25564.

CAZyme group	Enzyme activity	Gene position	EC number	Number
GH1	β-Glucosidase	1206507_1208054	EC 3.2.1.21	2
		1548357_1546930		
GH2	β-Glucuronidase	230633_228825	EC 3.2.1.31	1
GH3	β-Glycosyl hydrolase	226049_223737	-	2
		1615559_1617091		
GH4	6-Phospho-β-glucosidase	1608453_1606978	EC 3.2.1.86	1
GH13	Malto-oligosyltrehalose/ trehalohydrolaseGH13_10;	1599543_1601333	EC 3.2.1.141	8
	Limit dextrin α-1,6-maltotetraose-hydrolase GH13_11;	4158486_4156372	EC 3.2.1.196	
	Trehalose synthase α-Amylase GH13_16;	4150667_4148871	EC 5.4.99.16 EC 3.2.1.1	
	Malto-oligosyltrehalose synthase GH13_26;	1597186_1599498	EC 5.4.99.15	
	Glucanase glge GH13_3;	4152718_4150673	EC 3.2.1.-	
	α-Glucosidase GH13_30;	1490553_1492259	EC 3.2.1.20	
	Limit dextrin α-1,6-maltotetraose-hydrolase CBM48 + GH13_11;	1594748_1597189	EC 3.2.1.196	
	1,4-α-Glucan glycogen; branching enzyme CBM48 + GH13_9;	4148869_4145174	EC 2.4.1.18	
GH15	Glucoamylase	2725735_2726796	EC 3.2.1.3	4
		1210063_1211832		
		2262201_2264033		
	Trehalose-6-phosphate phosphatase	943180_945807	EC 3.1.3.12	
GH23	Peptidoglycan-binding lysm	3355397_3356797	-	2
	Membrane-bound lytic murein transglycosylase	3043759_3043151	EC 4.2.2.-	
GH25	1,4-β-N-Acetylmuramidase	1863081_1865612	EC 3.2.1.92 -	1
GH30	Endo-1,6-β-galactosidase	731722_733167	EC 3.2.1.164	1
GH32	Sucrose-6-phosphate hydrolase	3442058_3440550	EC 3.2.1.26	2
	β-Fructosidase	1383596_1384843	EC 3.2.1.26	
GH33	Sialidase	613011_614603	EC 3.2.1.18	1
GH38	α-Mannosidase	4082745_4079713	EC 3.2.1.24	1
GH53	Galactosidase	2955779_2956930	-	1
GH65	Maltose phosphorylase/ Trehalose phosphorylase	392560_390227	EC 2.4.1.8 EC 2.4.1.64	2
	Trehalose-6-phosphate phosphatase	342376_339176	EC 3.1.3.12	
GH76	Fructose-bisphosphate aldolase	137645_138862	EC 4.1.2.13	1
GH77	4-α-Glucanotransferase amylomaltase;	2730522_2728363	EC 2.4.1.25	1
GH109	Gluconokinase	787401_788513	EC 2.7.1.12	2
		236269_235103		

Comparison of Arthrobacter sp. PAMC25564 genome characteristics with those from closely related species

We compared CAZyme genes from Arthrobacter species to speculate about their bacterial lifestyles and identified relevant CAZymes for potential applications in biotechnology. Considering the accessibility of available genome data, the complete genomes of 26 strains were chosen for the comparative analysis of CAZymes: 19 genomes of Arthrobacter spp., 1 genome of A. *crystallopoietes*, 3 genomes of A. *alpinus*, and 3 genomes of *Pseudarthrobacter* spp. (Table 3). Our results showed that the number of genes encoding glycogen and trehalose metabolism-associated CAZymes ranged from a minimum of 56 (A. sp. YC-RL1) to a maximum of 177 (A. sp. YN). We predicted that genes such as CE14, CE9, GH23, GH65, GT2, GT20, GT28, GT39, GT4, and GT51 were in each of the 26 genomes. In addition, GH13, GH65, GH77, GT5, and GT20 (glycogen and trehalose-related genes) are involved in energy storage. These genes are involved in glycogen degradation and trehalose pathways and were found in strains PAMC25564, 24S4-2, FB24, Hiyo8, KBS0702, MN05-02, PGP41, QXT-31, U41, UKPF54-2, A6, Ar51, and sphe3. These genes code for proteins with a strong ability to store and release energy. We found that strain PAMC25564 had the largest number of CAZyme genes. In general, CAZymes are large group of protein and this is mainly responsible for the degradation and biosynthesis/modification of polysaccharide but not all the members of this group are secreted proteins. This confirmed the little difference through results (Fig. 4).

Table 3
Genome information and comparative data of CAZymes from 26 strains including *Arthrobacter* sp. PAMC25564.

Species	Strain	Size Mb;	GC %;	Replicons	Plasmid	Gene	Protein	tRNAs	rRNAs	References
Arthrobacter sp.	PAMC25564	4.17097	66.70	NZ_CP039290.1	0	3,829	3,613	51	15	**This study**
	24S4-2	5.56375	65.10	NZ_CP040018.1	0	5,152	4,522	50	15	Unpublished
	YN	5.06355	62.70	NZ_CP022436.1	0	4,673	4,387	55	18	Unpublished
	QXT-31	5.04157	66.00	NZ_CP019304.1	0	4,593	4,379	54	18	Unpublished
	Rue61a	5.08104	62.23	NC_018531.1/CP003203.1	2	4,693	4,568	53	18	[55]
	FB24	5.07048	65.42	NC_008541.1/CP000454.1	3	4,623	4,486	51	15	[56]
	PAMC25486	4.59358	62.80	NZ_CP007595.1	0	4,154	3,995	53	18	Unpublished
	ZXY-2	5.05871	63.35	NZ_CP017421.1	5	4,700	4,505	54	18	Unpublished
	U41	4.79263	66.38	NZ_CP015732.1	3	4,407	4,134	51	15	Unpublished
	DCT-5	4.53075	66.22	NZ_CP029642.1	1	4,040	3,816	50	15	Unpublished
	PGP41	4.27024	65.40	NZ_CP026514.1	0	3,917	3,760	49	12	Unpublished
	ERGS1:01	4.93669	65.41	NZ_CP012479.1	2	4,481	4,232	41	6	[57]
	YC-RL1	4.01864	64.04	NZ_CP013297.1	2	3,754	3,606	66	19	[58]
	Hiyo4	3.77925	65.00	AP014718.1	0	5,182	5,120	50	12	[59]
	KBS0702	3.64955	67.90	NZ_CP042172.1	0	3,373	3,243	51	15	Unpublished
	UKPF54-2	3.51782	68.50	NZ_CP040174.1	0	3,238	3,110	50	15	Unpublished
	MN05-02	3.64342	68.81	AP018697.1	1	3,608	3,543	52	12	Unpublished
	Hiyo8	5.02672	63.76	AP014719.1	2	7,108	7,038	53	15	[59]
	ATCC21022	4.43490	63.40	NZ_CP014196.1	0	4,078	3,910	53	12	[60]
Arthrobacter crystallopoietes	DSM 20117	5.03270	64.36	NZ_CP018863.1	2	4,634	4,425	48	15	Unpublished
Arthrobacter alpinus	R3.8	4.04645	62.20	NZ_CP012677.1	0	3,732	3,523	51	18	Unpublished
	ERGS4:06	4.33365	60.59	NZ_CP013200.1	1	3,850	3,581	53	25	[61]
	A3	4.45829	60.64	NZ_CP013745.1	1	4,033	3,902	52	19	Unpublished
Pseudarthrobacter phenanthrenivorans	Sphe3	4.53532	65.38	NC_015145.1/CP002379.1	2	4,278	4,052	50	12	[62]
Pseudarthrobacter chlorophenolicus	A6	4.98087	65.98	NC_011886.1/CP001341.1	2	4,685	4,505	49	15	[63]
Pseudarthrobacter sulfonivorans	Ar51	5.04376	64.70	NZ_CP013747.1	1	4,640	4,408	50	12	Unpublished

Bacterial glycogen metabolism in a cold environment

Glycogen is an energy source for plants, animals, and bacteria and is one of the most common carbohydrates. Glycogen consists of D-glucose residues joined by α (1→4) links; and it is a structural part of cellulose and dextran [42]. Glycogen is a polymer with approximately 95% of α-1, 4 linkages, and 5% of α-1, 6 branching linkages. In bacteria, glycogen metabolism includes five essential enzymes: ADP-glucose pyrophosphorylase (GlgC), glycogen synthase (GlgA), glycogen branching enzyme (GlgB), glycogen phosphorylase (GlgP), and glycogen debranching enzyme (GlgX) [43]. To adapt and survive in a cold environment, organisms need well-developed functional energy storage systems, one of which is glycogen synthesis. Bacteria have a passive energy saving strategy to adapt to cold environmental conditions such as nutrient deprivation, by using a slow glycogen degradation. Glycogen has the hypothesis of durability energy reserves, which have been reported as a Durable Energy Storage Mechanism (DESM) to account for the long-term survival of some bacteria in cold environments [44]. Metabolism of maltodextrin has been linked with osmoregulation and sensitivity of bacterial endogenous induction to hyperosmolarity, which is related to glycogen metabolism. Glycogen-generated maltotetraose is dynamically metabolized by maltodextrin phosphorylase (MalP) and maltodextrin glucosidase (MalZ), while 4-α-glucanotransferase (MalQ) is responsible for maltose recycling to maltodextrins [45]. Maltotetraose is produced using GlgB, MalZ, MalQ, and glucokinase (Glk), which act on maltodextrin and glucose. On the other hand, glucose-1-phosphate can be formed by MalP for glycogen synthesis or glycolysis [46]. Thus, glycogen degradation can play an essential role in bacterial adaptation to the environment. Additionally, maltose may form capsular α-glucan, which plays a role in environmental adaptation through the (TreS)-Pep2-GlgE-GlgB pathway [47, 48]. Previous studies indicate that trehalose is involved in bacterial adaptation to temperature fluctuation, hyperosmolarity, and desiccation resistance. Recently, the accumulation of trehalose and glycogen under cold conditions in *Propionibacterium freudenreichii* has been reported [49, 50]. Therefore, the role of glycogen in bacterial energy metabolism is closely linked to several metabolic pathways associated to bacterial persistence under environmental stresses such as starvation, drying, temperature fluctuations, and hyperosmolarity. Maltodextrin and trehalose pathways are examples of the relationship between glycogen and other metabolic pathways, as shown in Fig. 5. However, further exploration is needed to elucidate the relationship of glycogen with other compounds, and the mechanisms involved in bacterial persistence strategy [45]. The comparative analysis of predicted pathways for glycogen metabolism in *Arthrobacter* isolates (Additional file 1: Table S1), showed that in PAMC25564 the trehalose biosynthesis follows three metabolic pathways (OtsAB, TreYZ, and TreS) as in *Mycobacterium* [51]. The trehalose biosynthesis is well-known in numerous bacteria, for example, a defense strategy involving the accumulation of trehalose and three metabolic pathways to regulate osmotic stress is been reported in *Corynebacterium glutamicum* [52]. These three metabolic pathways are used for producing trehalose in *C. glutamicum*, where the gene *galU*/ *otsAB* allows the increase of trehalose levels up to six times [53, 54]. This pathway was found in *A.* sp. PAMC25564, and it was predicted that such isolate could produce energy in cold environments.

Glycogen metabolism and trehalose pathway in Arthrobacter species

We investigated the glycogen metabolic pathways in each Arthrobacter strains (Fig. 6). To determine the 3 pathways of glycogen metabolism and trehalose pathway in Arthrobacter species, the level of dissimilarity was analyzed based on the composition of GH, GT, and other major enzymes from the 16 genomes. The analysis showed that only QXT-31, U41, and PGP41 shared with our strain the same genes and pathway, but other strains have a little different pattern. Therefore, we assumed that the PAMC25564 strain uses different pathways to obtain energy or degrade polysaccharides. Based on the above mentioned pathway-related genes, we confirmed that strains YN, Rue61, PAMC25486, ZXY-2, ERGS1:01, YC-RL1, ATCC21022, R.3.8, and A3 lack the malQ gene, which is responsible for maltose recycling to maltodextrins. Therefore, the energy supply may be compromised in such isolates. These Arthrobacter species showed a low number of genes for the three main pathways of trehalose. Therefore, we assumed that the PAMC25564 strain uses different pathways to obtain energy or degrade polysaccharides. Although most strains showed GalU/OtsAB genes, strains Hyo8 and ERGS1:01 lack the otsB gene (Fig. 6; Additional file 1: Table S1). So, we predicted that these strains would produce a significantly lower amount of trehalose, compared with the isolates having the OtsB gene. Additionally, we investigated the phosphotransferase system-related genes in strains R.3.8 and A3. These enzymes constitute another method used by bacteria for sugar uptake when the source of energy is phosphoenolpyruvate. As a result, these two strains were expected to produce polysaccharides by themselves or from an external source using phosphoenolpyruvate rather than consuming energy. Most of the compared strains were isolated from low temperature environments (-18 to 15 ℃), and most of them are known to adapt quickly to environmental changes. We expect that our results on glycogen metabolism, trehalose, and maltodextrin pathways will have a significant impact in industrial applications. The predicted trehalose metabolism in bacteria is important since they could be used for bioremediation. Additionally, these isolates would be an alternative for a cost-effective production of trehalose. We also predicted that trehalose metabolism varies among bacteria, depending on their metabolism and environmental conditions.

Conclusions

In this study, we elucidated the complete genome sequence of *Arthrobacter* sp. PAMC25564 and conducted a comparative genome analysis with other species for studying CAZyme patterns. We isolated bacteria from cryoconite under laboratory conditions and confirmed that the isolate is an *Arthrobacter* species, based on the analysis of 16S rRNA sequences. Although it has been reported the isolation of this species from extreme or contaminated environments, there are no reports on the use of CAZymes in cold environments. Therefore, we suppose that this process has allowed *Arthrobacter* species to establish a symbiotic relationship with other bacteria in cold environments, or live independently thanks to their capacity for adapting to environmental changes. We predicted that *Arthrobacter* sp. PAMC25564 could produce energy by its own as fast as it can adapt to the environment. In summary, the PAMC25564 strain genome is 4.17 Mb in size with a GC content of 66.74%. The analysis of its complete genome suggested that the isolate has glycogen, trehalose, and maltodextrin pathways associated to CAZyme genes. We confirmed that PAMC25564 has 108 active CAZyme genes from the following groups, 5 AA, 2 CBM, 23 CE, 33 GH, and 45 GT. In addition, a comparative genome analyses of *Arthrobacter* species revealed that they adapt quickly to the environment. In conclusion, we expect the genome sequence analysis provides valuable information regarding novel functional enzymes, especially CAZymes, which are active at low temperatures and can be used for biotechnological applications and fundamental research purposes. Additionally, this study provides a foundation to understand how the PAMC25564 strain produces energy in an extreme environment.

Methods

Isolation of Arthrobacter sp. PAMC25564 and genomic DNA preparation

Arthrobacter sp. PAMC25564 strain was isolated from the Cryoconite of Wurmkogel, Ötztaler Alps, Austria (47°04' N, 12°41' E, 2820 m to a maximum elevation) using 0.1 X R2A agar (MB cell Ltd., Seoul, Korea). The strain was isolated at an environmental temperature of 20 ℃. The bacterial sample for DNA analysis was isolated at 15 ℃ by using a pure R2A agar. DNA from *A.* sp. PAMC25564 was extracted using a QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA). The quantity and purity of genomic DNA were determined using a spectrophotometer (Biochrome, Libra S35PC, UK). The extracted DNA was checked by agarose gel electrophoresis to evaluate its quality. DNA was stored at -20 ℃ until use.

Genome sequencing and assembly of the whole genome of Arthrobacter sp. PAMC25564

Genome sequencing was performed using PacBio sequel single-molecule real-time (SMRT) sequencing technology (Pacific Biosciences, Menlo Park, CA, USA). SMRTbell library inserts (20 kb) were sequenced using SMRT cells. Raw sequence data were generated from 77,075 reads and 821,081,934 bp that were assembled de novo by using the hierarchical genome-assembly process (HGAP) protocol [22] and HGAP4 assembly using SMRT analysis software (ver. 2.3; Pacific Biosciences, https://github.com/PacificBiosciences/SMRT-Analysis). The complete genome sequence was deposited in the GenBank database under the GenBank accession number NZ_CP039290.1.

Genome annotation of Arthrobacter sp. PAMC25564

The PAMC25564 genome was annotated using the rapid annotation subsystem technology (RAST) server [23]. The predicted gene sequences were translated and searched in the National Center for Biotechnology Information (NCBI) non-redundant database, the Clusters of Orthologous Groups (COG) from the eggnog v.4.5.1 database [24], and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. A circular map of the PAMC25564 genome was prepared using the CGView comparison tool [25]. CAZyme gene analyses were carried out by running dbCAN tool [26] scans using hidden Markov model (HMM) profile downloaded from dbCAN2 HMMdb (version 7.0). The e-value cutoff was 1e-15 and the coverage cutoff was > 0.35. In addition, we used DIAMOND [27] (e-value < 1e102) and Hotpep [28] (frequency > 2.6, hits > 6) to improve the prediction accuracy.

Phylogenetic analysis

Strain PAMC25564 was compared with other *Arthrobacter* species on the base of 16S rRNA phylogenetic analysis. Alignments were performed using Basic Local Alignment Search Tool (BLAST) from the NCBI database and analyzed using EzBio Cloud (www.ezbiocloud.com). 16S rRNA sequences were aligned using MUSCLE [29, 30] and MEGA X [31] to reconstruct a neighbor-joining tree and maximum likelihood tree with 1000 bootstrap replications.

Comparative genomics of Arthrobacter sp

We used all complete genome sequences of *Arthrobacter* species available in GenBank (https://www.ncbi.nlm.nih.gov). Firstly, we determined the relationship of PAMC25564 with other strains from the same species using complete genome sequences and checked their similarity by comparing values of average nucleotide identity (ANI), calculated using a OrthoANI [32]. The genome information of several *Arthrobacter* species is available in GenBank, and we compared the CAZymes from registered species referenced in CAZy (http://www.cazy.org). Based on complete genome sequence, we found 25 strains from a same species. All those sequences were downloaded from the database and all CAZymes were reannotated using the dbCAN2 server.

Abbreviations

GH: Glycoside Hydrolase; CE: Carbohydrate Esterase; PL: Polysaccharide Lyase; GT: Glycosyltransferase; AA: Auxiliary Activities; CBM: Carbohydrate-Binding Module; ANI: Average Nucleotide Identity; NCBI: National Center for Biotechnology Information.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

H. Park and T.-J. Oh designed and supervised the project. S.-R. Han and B. Kim performed the experiments; S.-R. Han, B. Kim, J.H. Jang, H. Park, and T.-J. Oh wrote the manuscript. All authors discussed the results, commented on the manuscript, and approved the manuscript.

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Availability of data and materials

The datasets analyzed during the current study are available in the NCBI repository, accession numbers: NZ_CP039290.1for *Arthrobacter* sp. strain PAMC25564, complete genome; NZ_CP040018.1 for *Arthrobacter* sp. strain 24S4-2, complete genome; NZ_CP022436.1 for *Arthrobacter* sp. strain YN, complete genome; NZ_CP019304.1 for *Arthrobacter* sp. strain QXT-31, complete genome; NZ_CP003203.1 for *Arthrobacter* sp. strain Rue61a; NZ_CP000454.1 for *Arthrobacter* sp. strain FB24, complete genome; NZ_CP007595.1 for Arthrobacter sp. strain PAMC25486, complete genome; NZ_CP017421.1 for *Arthrobacter* sp. strain ZXY-2, complete genome; NZ_CP015732.1 for *Arthrobacter* sp. strain U41, complete genome; NZ_CP029642.1 for *Arthrobacter* sp. strain DCT-5, complete genome; NZ_CP026514.1 for *Arthrobacter* sp. strain PGP41, complete genome; NZ_CP012479.1 for *Arthrobacter* sp. strain ERGS1:01, complete genome; NZ_CP013297.1 for *Arthrobacter* sp. strain YC-RL1 , complete genome; AP014718.1 for *Arthrobacter* sp. strain Hiyo4, complete genome; NZ_CP042172.1 for *Arthrobacter* sp. strain KBS0702, complete genome; NZ_CP040174.1 for *Arthrobacter* sp. strain UKPF54-2, complete

genome; AP018697.1 for *Arthrobacter* sp. strain MN05-02, complete genome; AP014719.1 for *Arthrobacter* sp. strain Hiyo8, complete genome; NZ_CP014196.1 for *Arthrobacter* sp. strain ATCC21022, complete genome. NZ_CP018863.1 for *Arthrobacter crystallopoietes* DSM 20117, complete genome; NZ_CP012677.1 for *Arthrobacter alpinus* strain R3.8, complete genome; NZ_CP013200.1 for *Arthrobacter alpinus* strain ERGS4:06, complete genome; NZ_CP013745.1 for *Arthrobacter alpinus* strain A3, complete genome; CP002379.1 for *Pseudarthrobacter phenanthrenivorans* strain Sphe3, complete genome; CP001341.1 for *Pseudarthrobacter chlorophenolicus* strain A6, complete genome; NZ_CP013747.1 for *Pseudarthrobacter sulfonivorans* strain Ar51, complete genome.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors have declared that no conflict of interest exists.

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Figures

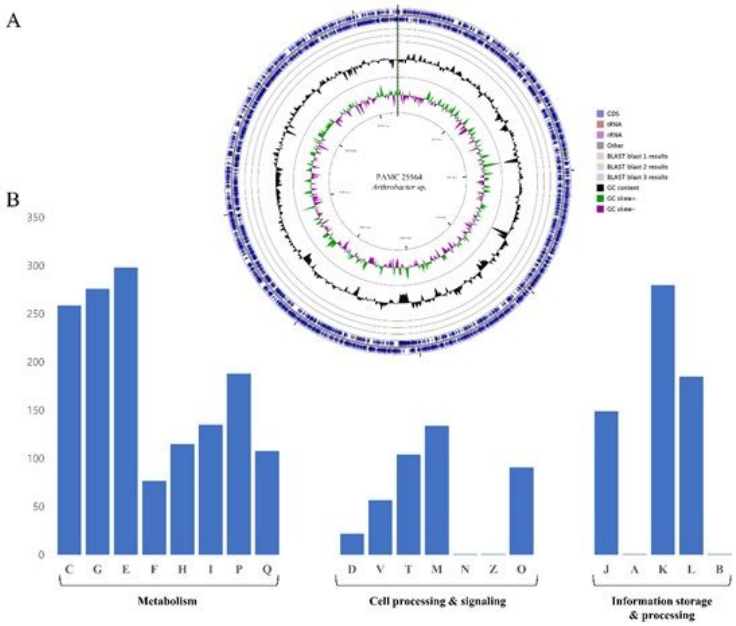
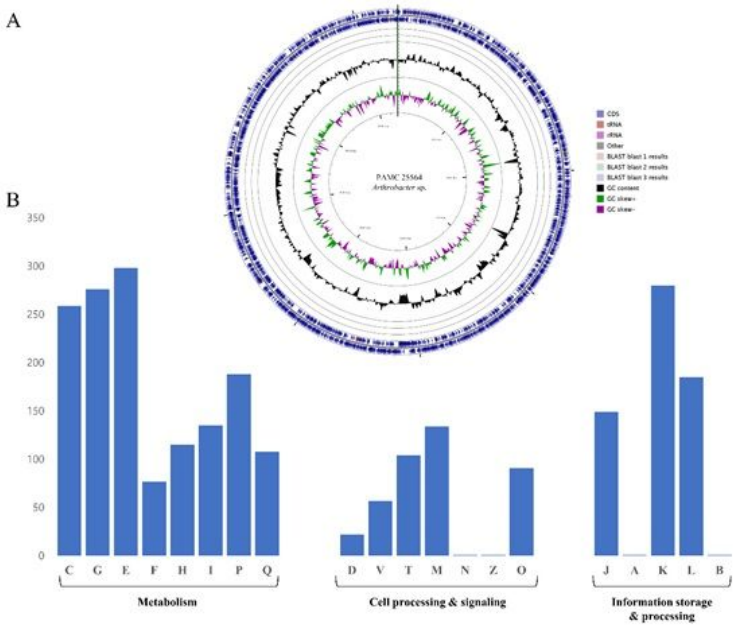


Figure 1

A; Circular map of the genome of Arthrobacter sp. PAMC25564, B; COG functional categories for forward coding sequences. Metabolism: C, energy production and conversion; G, carbohydrate transport and metabolism; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; H, coenzyme transport and metabolism; I, lipid transport and metabolism; P, inorganic ion transport and metabolism; and Q, secondary metabolites biosynthesis, transport, and catabolism. Cell processing and signaling: D, cell cycle control, cell division, and chromosome partitioning; V, defense mechanisms; T, signal transduction mechanisms; M, cell wall/membrane/envelope biogenesis; N, cell motility; Z, mobilome, prophages, and transposons; and O, posttranslational modification, protein turnover, and chaperones. Information storage and processing: J, translation, ribosomal structure, and biogenesis; A, RNA processing and modification; K, transcription; L, replication, recombination and repair; and B, chromatin structure and dynamics.



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A; Circular map of the genome of Arthrobacter sp. PAMC25564, B; COG functional categories for forward coding sequences. Metabolism: C, energy production and conversion; G, carbohydrate transport and metabolism; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; H, coenzyme transport and metabolism; I, lipid transport and metabolism; P, inorganic ion transport and metabolism; and Q, secondary metabolites biosynthesis, transport, and catabolism. Cell processing and signaling: D, cell cycle control, cell division, and chromosome partitioning; V, defense

mechanisms; T, signal transduction mechanisms; M, cell wall/membrane/envelope biogenesis; N, cell motility; Z, mobilome, prophages, and transposons; and O, posttranslational modification, protein turnover, and chaperones. Information storage and processing: J, translation, ribosomal structure, and biogenesis; A, RNA processing and modification; K, transcription; L, replication, recombination and repair; and B, chromatin structure and dynamics.

Figure 2

Phylogenetic tree of Arthrobacter sp. PAMC25564. The phylogenetic was generated using the neighbor-joining method and MEGA X, based on 16S rRNA sequences. The tree shows the relationship between three Arthrobacter strains and six Pseudarthrobacter strains, and their phylogenetic position.

Figure 2

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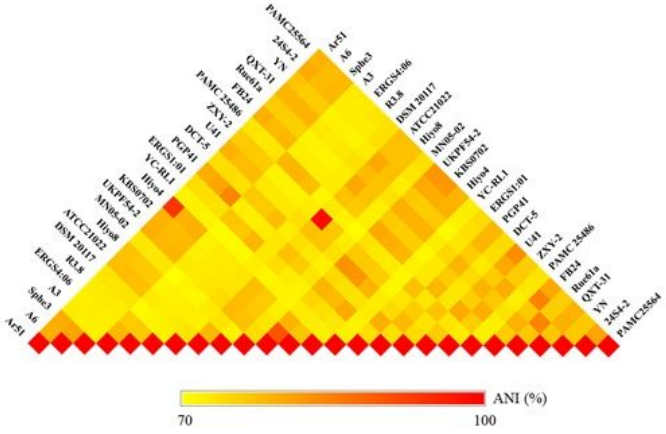
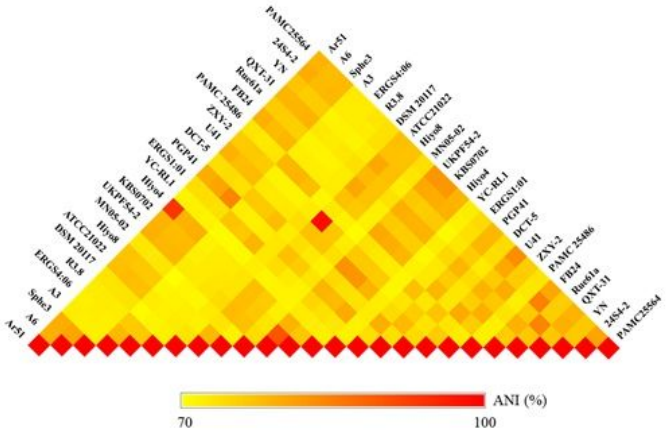


Figure 3

Average Nucleotide Identity ANI; values %; among Arthrobacter sp. PAMC25564 genome and 25 other genomes calculated using OrthoANI. ANI results are colored yellow to red according to their value mean 70-100 %. Strains belonging to the same species are marked with strong color. Arthrobacter sp.: PAMC25564, 24S4-2, YN, QXT-31, Rue61a, FB24, PAMC25486, ZXY-2, U41, DCT-5, PGP41, ERGS1:01, YC-RL1, Hiyo4, KBS0702, UKPF54-2, MN05-02, Hiyo8, and ATCC21022; Arthrobacter crystallopoietes: DSM 20117; Arthrobacter alpinus: R3.8, ERGS4:06, and A3; Pseudarthrobacter phenanthrenivorans: Sphe3; Pseudarthrobacter chlorophenolicus: A6; and Pseudarthrobacter sulfonivorans: Ar51.



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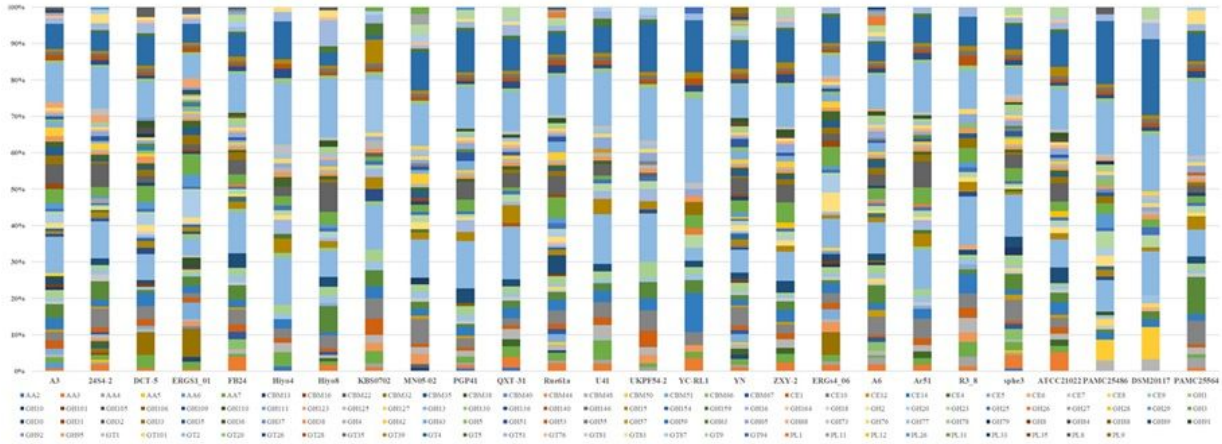
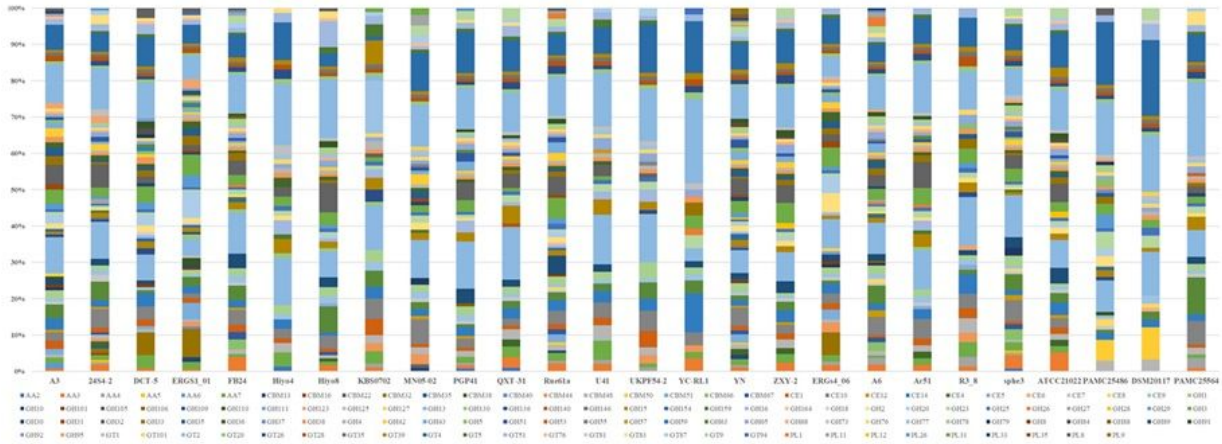


Figure 4

Comparative CAZyme-encoding genes found in the genome of Arthrobacter species. GT, glycosyl transferase; GH, glycoside hydrolase; CE, carbohydrate esterase; CBM, carbohydrate binding; and AA, auxiliary activities. CAZyme-encoding genes are colored as indicated below the figure. Arthrobacter sp.: PAMC25564, 24S4-2, YN, QXT-31, Rue61a, FB24, PAMC25486, ZXY-2, U41, DCT-5, PGP41, ERGS1:01, YC-RL1, Hiyo4, KBS0702, UKPF54-2, MN05-02, Hiyo8, and ATCC21022; Arthrobacter crystallopoietes: DSM 20117; Arthrobacter alpinus: R3.8, ERGS4:06, and A3; Pseudarthrobacter phenanthrenivorans: Sphe3; Pseudarthrobacter chlorophenolicus: A6; and Pseudarthrobacter sulfonivorans: Ar51.



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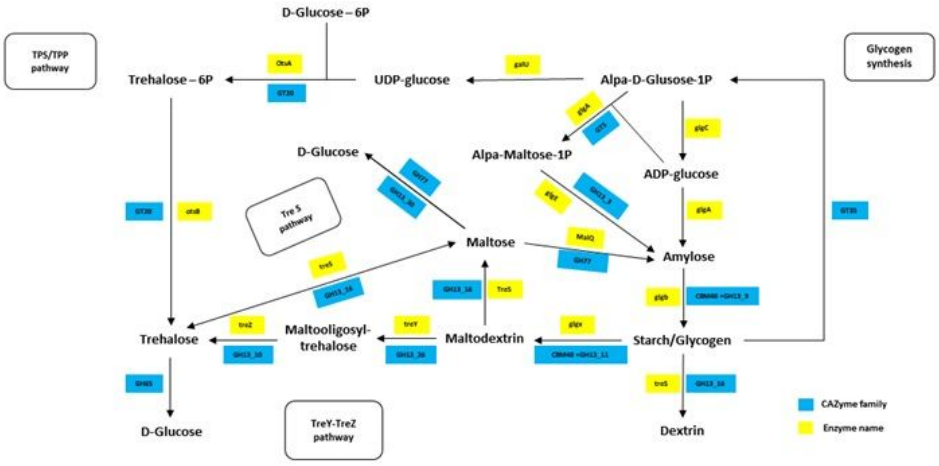
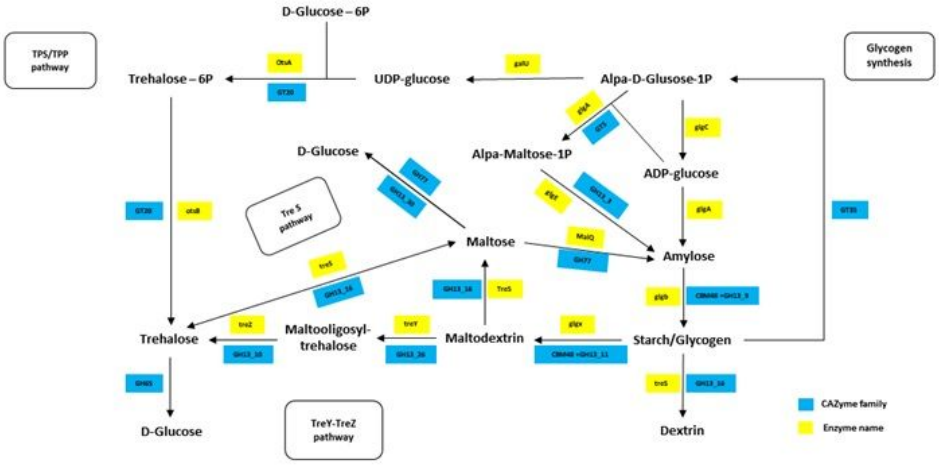


Figure 5

Predicted pathways for glycogen and trehalose metabolism in Arthrobacter sp. PAMC25564 as a response to cold adaptation. Kyoto Encyclopedia of Genes and Genomes KEGG;-predicted enzyme pathways yellow square;; and dbCAN2-predicted CAZyme family blue square;.



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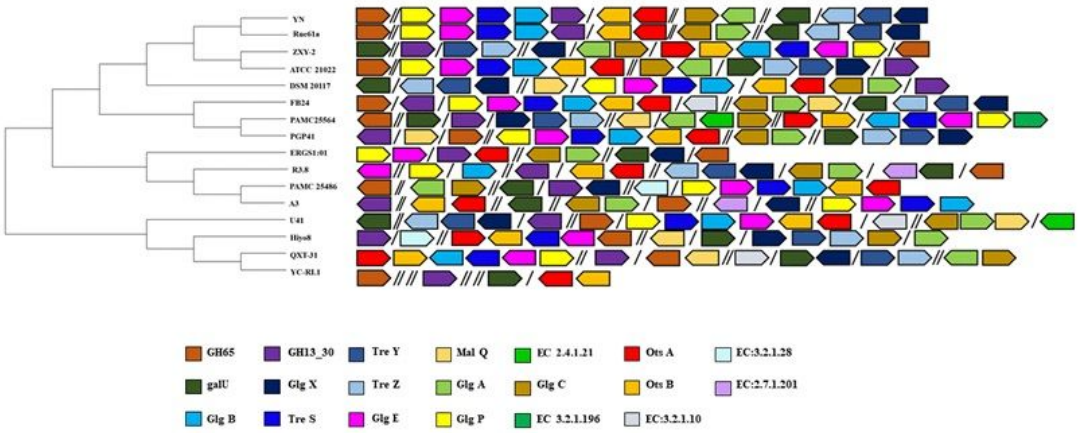
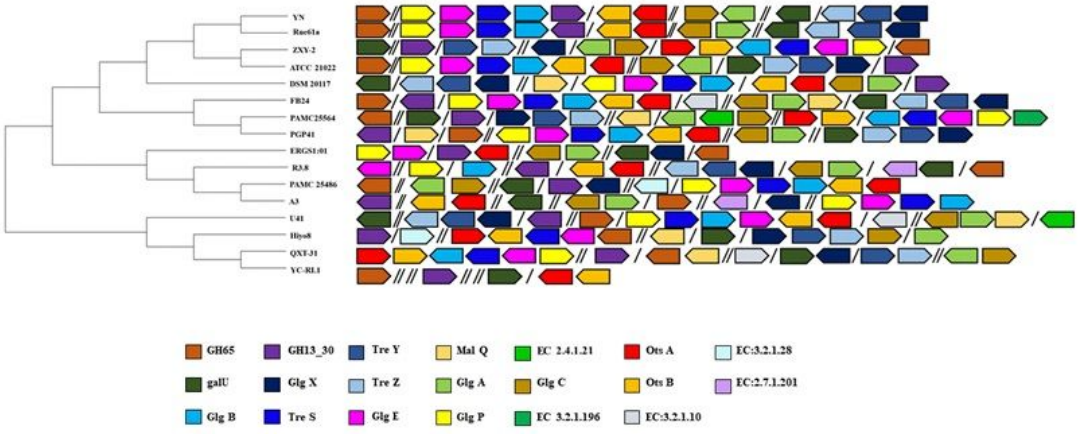


Figure 6

Comparative anlysis of predicted glycogen and trehalose metabolic pathways in Arthrobacter species. Glycogen and trehalose metabolism-associated gene are colored as indicated below the figure. The figure shows the differences in direction, presence, and location of genes among the strains. GH65: α,α-trehalose phosphorylase; GH13_30: α-1,4-glucan-maltose-1-phosphate maltosyltransferase; TreY: 1→4;-α-D-glucan 1-α-D-glucosylmutase; MalQ: 4-α-glucanotransferase; 2.4.1.21: glycogen synthase, and ADP-glucose transglucosylase; OtsA: trehalose 6-phosphate synthase; 3.2.1.28: α-trehalase; GalU: UTP-glucose-1-phosphate uridylyltransferase; GlgX: glycogen debranching protein; TreZ: maltooligosyltrehalose trehalohydrolase, α-glucosidase, and α-trehalase; GlgA: glycogen synthase, and ADP-glucose transglucosylase; GlgC: glucose-1-phosphate adenylyltransferase; OtsB: trehalose 6-phosphate phosphatase; 2.7.1.201: PTS system, and sugar-specific IIA component; GlgB: 1,4-α-glucan branching enzyme; TreS: maltose α-D-glucosyltransferase/α-amylase; GlgE: α-1,4-glucan-maltose-1-phosphate maltosyltransferase, PTS system, and sugar-specific IIA component; GlgP: glycogen phosphorylase; and 3.2.1.10: oligo-1,6-glucosidase.



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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx
- TableS1.docx