Biotechnology Reports 32 (2021) e00689

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

# The cold adaption profiles of *Pseudoalteromonas shioyasakiensis* D1497 from Yap trench to cope with cold

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ARTICLE INFO

*Keywords:*
Psychrophile
Cold adaption
*Pseudoalteromonas shioyasakiensis*
Low Temperature
Energy Saving

ABSTRACT

Genome sequencing of *Pseudoalteromonas shioyasakiensis* D1497, a psychrophile from the Yap trench, revealed that it contained a circle chromosome of 3,631,285 bp with 40.94% GC content and prefered to use codons with A/T in the third position. Additionally, the relative synonymous codon usage (RSCU) values indicated the codons with A and T in the third position were always the most used. Cultivation of *P. shioyasakiensis* D1497 presented lower substrate consumption rate, higher ATP pool and higher conversion rate of biomass per unit substrate consumed at low temperature (15 °C) than that of the room temperature (25 °C) culture. Comparative transcriptomic analysis revealed that the mRNA abundance of energy metabolism related genes was decreased in 15 °C culture compared with that of 25 °C culture. In addition to its codon usage biases profile, *P. shioyasakiensis* D1497 presented an energy saving metabolism strategy to cope with cold, converting more carbon source into biomass in cold environment.

## 1. Introduction

More than 80% of the biosphere on the earth is in cold environment all year round, including most of the oceans, polar regions, mountains, glaciers and stratosphere in the atmosphere. Most of the earth's biosphere is composed of cold marine environment, and 90% of the sea water in the ocean is below 5 °C throughout the year [27]. The microorganisms living in these environments become the most widely distributed and the most diverse extremophiles. It has been observed that microorganisms can survive in extreme cold condition to the extent of under −39 °C [32]. The movement of *Colwellia psychrerythraea* strain 34H was observed directly from - 1 to - 15 °C in the low temperature laboratory [19]. Microorganisms adapted to these extreme cold environments were classified into psychrophilic bacteria and psychrotolerant bacteria according to their optimal growth temperatures [29]. The optimum growth temperature of psychrophilic bacteria and psychrotolerant bacteria were less than 15 °C and 20- 35 °C, respectively [11, 29]. Since the 1970s, more and more strains of psychrophilic microorganisms have been isolated and cultured. At present, the culturable psychrophilic bacteria mainly include *Pseudomonas* [15], *Pseudoaltermonas* [35], *Vibrio* [25], *Colwellia* [17], *Acinetobacter* [36] and *Flavobacterium* [21], *et al.* With the development and wide application of in situ molecular identification technology, the biodiversity of psychrophilic microorganisms under low temperature environment would be more and more known.

Compared with mesophilic and thermophilic bacteria, psychrophilic bacteria need to face many challenges brought by extreme cold environment, such as low enzyme activity, change of cell transport system, decrease of cell membrane fluidity and cold denaturation of proteins [10]. Therefore, psychrophilic bacteria had mature survival mechanisms to cope with the adverse low temperature environment [8]. The cold adapted aminopeptidase of *Colwellia psychrerythraea* 34H maintained its activity at low temperature by improving the flexibility of global or local protein domains [16]. Under extreme environmental conditions such as low temperature and high pressure, *C. psychrerythraea* 34H could increase exopolysaccharide (EPS) production to survive as the survival rate of *C. psychrerythraea* 34H could be improved more by adding EPS than glycerol when it was stored at −80 °C [26]. In the study of psychrotrophic bacterium *Pseudoalteromonas* sp. SM9913, it was also found that the production of EPS increased with the decrease of temperature in the range of in30- 10 °C as was believed that EPS can improve the stability of cold adapted protease MCP-01 [33]. EPS of *Pseudoalteromonas* sp. SM9913 played an important role in the particle attachment life style of the strain [40]. The swimming and swarming movements mediated by

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https://doi.org/10.1016/j.btre.2021.e00689
Received 5 October 2021; Received in revised form 1 November 2021; Accepted 11 November 2021
Available online 18 November 2021

lateral and polar flagella ensured that *Pseudoalteromonas* sp. SM9913 can obtain nutrients in low temperature and oligotrophic sediments [28]. The peptide transport system of *Pseudoalteromonas* sp. SM9913 had psychrophilic properties, such as lower ligand binding temperature and higher transport efficiency, to meet the needs of substance transport at low temperature [41]. The genomic study of *C. psychrerythraea* 34H showed that it could improve the fluidity of cell membrane at low temperature by increasing the content of polyunsaturated fatty acids and Cis-isomerization of fatty acids [27]. Heat shock protein (HSP) could protect psychrophilic bacteria from heat stress at high temperature, and some HSPs could help bacteria survive and grow at low temperature [6]. In the Antarctic bacterium *Marinomonas primoryensis*, the antifreeze protein AFP could inhibit the recrystallization of extracellular ice crystals [6, 14]. Some psychrophilic bacteria, such as *C. psychhrythraea* 34H, could grow with polyhydroxyalkanoates (PHA) as carbon source which was synthesized as energy storage substances at low temperature [27].

The majority of previous research on psychrophilic bacteria focused on the survival mechanisms of psychrophilic bacteria to adapt to low temperature environment. In this study, a strain of psychrophilic bacteria *Pseudoalteromonas shioyasakiensis* D1497 was isolated from Yap trench to reveal its genomic profile and metabolism characteristic in low temperature environment to cope with cold.

## 2. Materials and methods

### 2.1. Bacterial strain and growth conditions

The Bacterial strain used in this study was isolated from a marine sediment sample at a depth of 5137.276 m in the Yap trench (8.05° N, 137.55° E) which was collected by the "Jiaolong" human occupied vehicle during cruise DY-37 of China. The sediment was gradient diluted and spread on 2216 Marine Agar plates containing (grams per liter of sea water): 1.0 yeast extract, 5.0 peptone, 1.0 beef extract, 0.01 $FePO_4 \bullet 4H_2O$ and 16.0 agar, pH 7.4- 7.6. The plates were placed in an incubator at 10 °C for 48 h. A strain D1497 with large colony was selected and identified using 16S rDNA sequence which was amplified using the forward primer 27F: 5′ -AGAGTTTGATCMTGGCTCAG-3 [M = C, A] and reverse primer 1492R: 5′ -CGGYTACCTTGTTACGACTT-3 [*Y* = *T*, C]. A phylogenetic tree was constructed using the Mega 6.0 program using Neighbour-joining method based on 16S rRNA gene. In order to investigate the optimal growth temperature, strain D1497 was cultivated in 2216 Marine Broth at 13, 15, 17, 25 and 37 °C respectively, shaking at 180 rpm for 9 days.

### 2.2. Cell size determination and swimming motility

The cell morphology, size and presence of flagellum were determined by transmission electron microscopy (TEM, JEM-1230; JEOL). Strain D1497 was cultivated in 2216 Marine Broth at 15 and 25 °C respectively and then subjected to size determination on the TEM. Fifteen cells of each culture were randomly selected for length and width measurement and the cell volume was calculated. Besides, the cell volume was also measured on a BD FACSCalibur™ Flow Cytometer (BD Biosciences, United States) and polystyrene rubber beads with particle size of 1 μm was used as reference. Swimming motility was assayed in plates contained 2216 Marine Broth with 0.3% agar inoculated for 24 h at 15 °C and 25 °C, respectively [4].

Cultivation of *P. shioyasakiensis* D1497 using N-acetyl-D-glucosamine as sole carbon source at low temperature (15 °C) and room temperature (25 °C) respectively

A colony of *P. shioyasakiensis* D1497 was inoculated into a test tube containing 5 mL 2216 Marine Broth medium at 25 °C overnight. The inoculum (3% v/v) was added to 150 mL mineral salt medium in a 500-mL flask containing 2.2 mM N-acetyl-D-glucosamine as carbon source and incubated at 15 °C and 25 °C, respectively, shaking at 180 rpm for 24 h. The mineral salt medium contains (grams per liter of water): 26.0 NaCl, 5.0 $MgCl \bullet 6H_2O$, 1.4 $CaCl_2 \bullet 2H_2O$, 4.0 $Na_2SO_4$, 0.3 $NH_4Cl$, 0.1 $KH_2PO_4$, 0.5 KCl and 1 mL trace element mixture, pH 7.0. The trace element mixture contains (milligrams per liter of water): 5200.0 EDTA, 10.0 $H_3BO_3$, 5.0 $MnCl \bullet 4H_2O$, 2100.0 $Fe_2SO_4 \bullet 7H_2O$, 190.0 $CoCl \bullet 6H_2O$, 24.0 $NiCl \bullet 6H_2O$, 10.0 $CuCl \bullet 2H_2O$, 144.0 $ZnSO_4 \bullet 7H_2O$, 36.0 $Na_2MoO_4 \bullet 2H_2O$, pH 6.0. The biomass was expressed as $OD_{600}$ and determined on an ultraviolet visible spectrophotometer (Shimadzu, UV1800). The biomass and substrate concentration were measured with 4 h interval, and the conversion rate of biomass per unit substrate consumed, i.e. the ratio of the increment of $OD_{600}$ to the consumption of N-acetyl-D-glucosamine (mM), was calculated. N-acetyl-D-glucosamine was determined by High Performance Liquid Chromatography (HPLC, Agilent 1260 Infinity) equipped with an GH0525046C18AQ C18 column (5 μm, 4.6 × 250 mm, Beijing Greenherbs Science & Technology Development CO., LTD) using 100% water as mobile phase. The flow rate of the mobile phase was kept at 1.0 mL/min and the peaks were monitored under 193 nm. Standard N-acetyl-D-glucosamine was purchased from Macklin (Shanghai, China). A 1 mL cell sample was collected from 15 °C and 25 °C cultures each at 16 h, disrupted by sonication and determined their ATP concentration with an ATP detection kit (Lot number: BC0300, Solarbio, Beijing, China) according to the instruction of manufacturer. The content of ATP was indirectly reflected by measuring the content of NADPH generated by the reactions using the kit at 340 nm.

In order to eliminate the effect of different growth phases of *P. shioyasakiensis* D1497 on the conversion rate of carbon source into biomass, *P. shioyasakiensis* D1497 was first cultivated in 150 mL mineral salt medium supplemented with 1.7 mM N-acetyl D-glucosamine at 15 °C and 25 °C overnight. Two part of 75 mL cells from 15 °C and 25 °C cultures each were mixed together, centrifuged, and washed with water to remove the residual carbon source, and then re-suspended. The inoculum (100% v/v) was inoculated into 150 mL mineral salt medium with 1.7 mM N-acetyl D-glucosamine and cultivated at 15 °C and 25 °C respectively for 6 h. The biomass ($OD_{600}$) and substrate concentration were measured with 1 h interval, and the conversion rate of biomass per unit substrate was calculated.

### 2.3. Genome sequencing of P. shioyasakiensis D1497

The strain *P. shioyasakiensis* D1497 was cultured in 2216 Marine Broth medium and its genomic DNA was extracted and purified using a bacteria genomic DNA kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The genome of *P. shioyasakiensis* D1497 was sequenced on the Illumina HiSeq sequencing platform using next-generation sequencing and third-generation single-molecule sequencing technology with Pac-Bio RSII sequencer conducted at MajorBio Technologies (Shanghai, China) [13]. A total of 918,642,725 bp high-quality data were obtained and assembled into a circular chromosome using the hierarchical genome assembly process (HGAP) algorithm [5, 7]. The open reading frames were predicted by Prodigal [18], and the functions were annotated using BLAST searches of non-redundant protein sequences from the COG [37], GO [3], KEGG [20], Swiss-Prot [2], and NCBI-nr databases [12]. The tRNAs and rRNAs screening were performed using tRNAscan-SE and RNAmmer, respectively [22, 24].

### 2.4. Nucleotide content of codons and RSCU analysis

Each nucleotide at the first, second and third position of the codons (A%, T%, G% and C%) was analyzed using the Codon W. Additionally, G + C content at the first, second, and third codon positions of each sequence was calculated. The relative synonymous codon usage (RSCU) values for all amino acids in *P. shioyasakiensis* D1497 were calculated to determine the characteristics of synonymous codon usage without the influence of amino acid composition and coding sequence size following

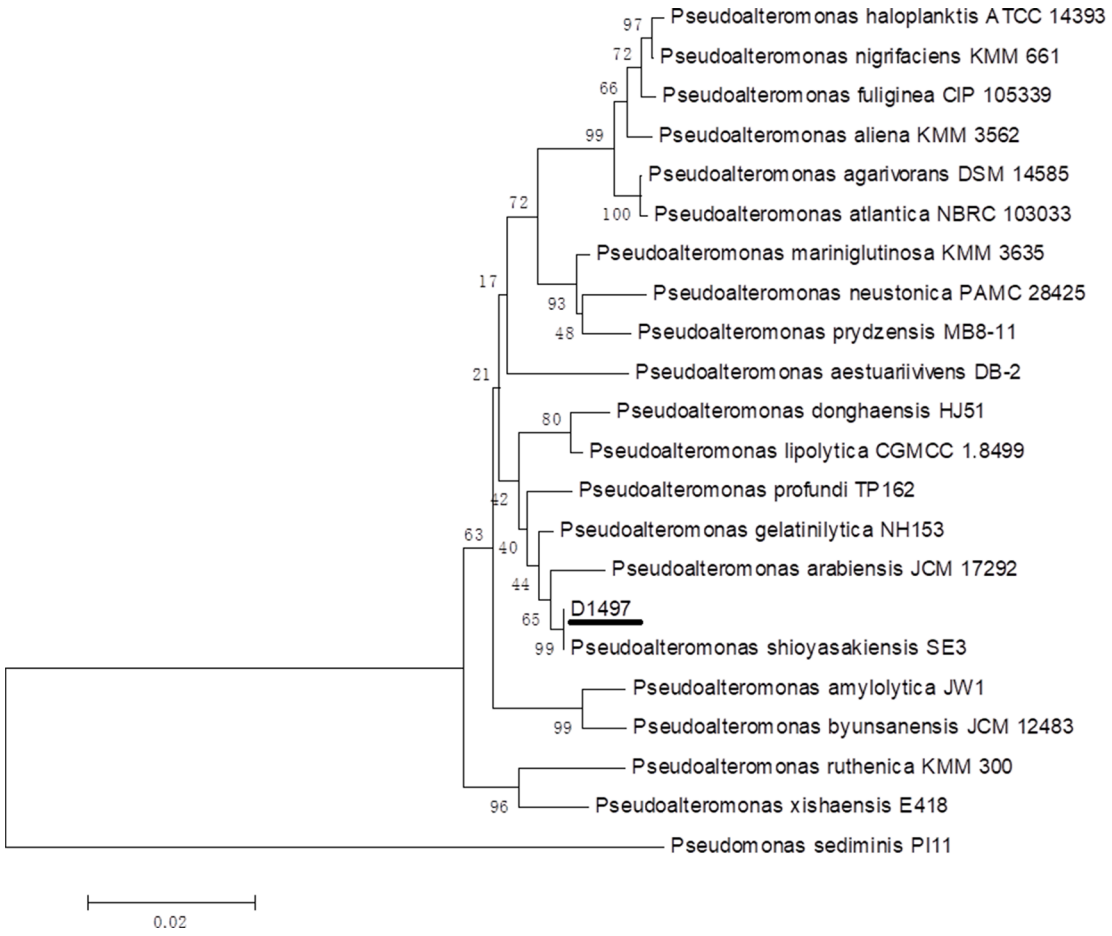


**Fig. 1.** Neighbour-joining phylogenetic dendrogram, based on 16S rRNA gene sequences, for strain D1497 and related species Bootstrap values expressed as percentages of 1000 replications are shown at branch points. Bar, 2% sequence divergence. Out group, *Pseudomonas sediminis* PI11.

a previously described method [39]. A higher RSCU value indicates that the codon is used more frequently or has a stronger codon usage bias. It is considered that there is no bias when the RSCU value is 1.0; if the RSCU value more than or less than 1.0, was considered that there is positive or negative codon usage bias, respectively.

### 2.5. RNA purification and RNA-Seq

*P. shioyasakiensis* D1497 was cultured in 2216 Marine Broth medium at 15 °C and 25 °C respectively for 16 h. Cells of 100 mL culture in the late exponential phase of growth were harvested by centrifugation at 6000 × g and 4 °C and then the centrifugation precipitates of the cultures were washed three times with ice cooled $H_2O$. Total RNA was isolated from cells using the Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Three biological replicates each were used in the preparation of RNA samples from the two different temperature cultures. Residual genomic DNA was digested with RNase-free DNase I (TaKaRa, Dalian, China). The integrity of each RNA sample was assessed by electrophoresis through a 1.2% agarose gel in 90 mM Tris-boric acid containing 2 mM EDTA (TBE). RNA concentration and purity were determined spectrophotometrically by measuring $A_{260}$ and $A_{260}/A_{280}$ ratio using Nanodrop1000 (ThermoScientific, USA) to make sure the RNA concentration higher than 100 ng/ $\mu^1$ and $OD_{260}/_{280}$ between 1.8 and 2.2. RNA-Seq and subsequent bioinformatics analysis were carried out by MajorBio Technologies (Shanghai, China). The mRNA purification and fragmentation, double-stranded cDNA synthesis, RNA-seq library preparation were carried out as described previously [34]. The Illumina HiSeq™ 2000 platform was applied for the sequencing. Sequencing quality assessment including alignment statistics, sequencing randomness assessment and distribution of reads in reference genome (*P. shioyasakiensis* D1497) were carried out as previously described [23]. All reads were mapped to reference genome using SOAP2 [23]. The RNA-Seq reads have been deposited in GenBank with accession number PRJNA643440.

### 2.6. Identification of differentially transcribed genes

Differentially transcribed genes were identified using the MajorBio platform (https://cloud.majorbio.com/). The RPKM (Reads Per Kilobase per Million mapped reads) method was used to calculate unigene transcription and directly compare gene transcription levels between samples with different cultivation temperature [30]. Genes with $\log_2$(ratio RPKM) values greater than 2.0 or less than −2.0 were considered to be increased or decreased, respectively. In addition, those genes with false discovery rate (FDR) less than 0.001 in the samples were also included.

## 3. Results

### 3.1. Strain isolation and identification

Strain D1497 was isolated from a marine sediment sample at a depth of 5137.276 m in the Yap trench (8.05° N, 137.55° E). Analysis of the 16S rRNA gene sequence showed that it belonged to the *Pseudoalteromonas* genus. A phylogenetic tree was constructed using the Mega 6.0 program which showed that the strain D1497 was more related to *Pseudoalteromonas shioyasakiensis* than other *Pseudoalteromonas* species (**Fig. 1**). Therefore, strain D1497 was designated as *Pseudoalteromonas shioyasakiensis* D1497 and deposited in Marine Culture Collection of

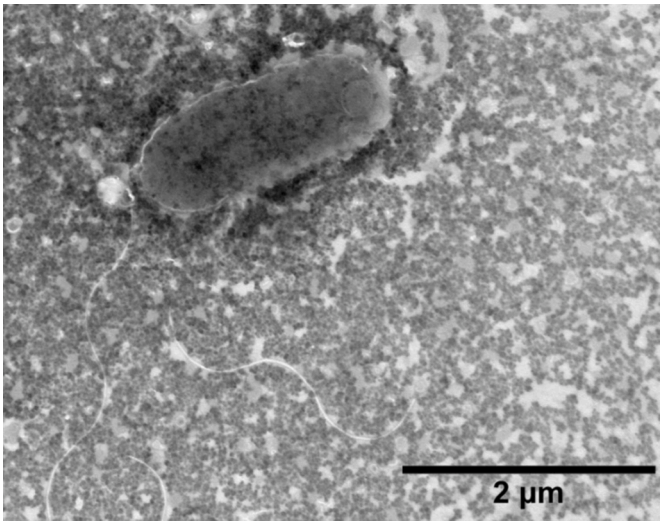


**Fig. 2.** The image of *P. shioyasakiensis* D1497 cultivated in 2216 Marine Broth detected by TEM.

China (MCCC) with deposit number of MCCC 1A17186. Cells of *P. shioyasakiensis* D1497 are Gram-negative, motile rods with a polar flagellum, 0.6 × 2.0 μm (Fig. 2). The growth curves of *P. shioyasakiensis* D1497 at different temperatures were determined in 2216 Marine Broth. The optimal growth temperature of *P. shioyasakiensis* D1497 was 15 °C indicating that it was a psychrophile (Fig. 3).

### 3.2. *Shioyasakiensis D1497 grew better and consumed fewer substrate at low temperature*

When 3% (v/v) inoculum was used, *P. shioyasakiensis* D1497 cultivated in 15 °C had an obvious biomass accumulation increase compared with that of 25 °C cultivation in the last 12 h (12–24 h) (Fig. 4**a**). On the contrary, the biomass accumulation of 15 °C culture was lower than that of 25 °C culture in the first 8 h of cultivation (Fig. 4**a**). Thus, the two curves of biomass accumulation cross between 8 and 12 h (Fig. 4**a**). The substrate consumption by *P. shioyasakiensis* D1497 cultivated at 25 °C was obvious faster than that of 15 °C culture in the first 12 h cultivation (Fig 4**a**). As a result, the biomass conversion rate of *P. shioyasakiensis* D1497 cultivated at 15 °C was higher than that of 25 °C in the whole cultivation process. The biomass conversion rate of *P. shioyasakiensis* D1497 cultivated at 25 °C was between 0.14 $mM^{-1}$ and 0.17 $mM^{-1}$ with the highest value of 0.17 $mM^{-1}$ at 4 h which was the late exponential phase of growth (Fig. 4**a**). When *P. shioyasakiensis* D1497 was cultivated at 15 °C, the biomass conversion rate of was 0.17– 0.27 $mM^{-1}$ with the highest value reached 0.27 $mM^{-1}$ at 12 h which was also the late exponential phase of growth (Fig. 4**a**). Compared with the highest value of biomass conversion rate of *P. shioyasakiensis* D1497 when cultivated under 15 °C and 25 °C conditions, the biomass conversion rate at low temperature had a 68.8% increase than that of the room temperature culture (Fig. 4**a**). When *P. shioyasakiensis* D1497 was cultivated at 15 °C, the $OD_{340}/OD_{600}$ (biomass) was 0.021 ± 0.005, while the value was 0.012 ± 0.001 in the 25 °C culture. This indicated the ATP pool of 15 °C cells was 1.75 fold that of the 25 °C cells.

In order to eliminate the effect of different growth phases of *P. shioyasakiensis* D1497 on the conversion of carbon source into biomass, the inoculum was raised to 100% (Fig. 4**b**). When 1.7 mM N-acetyl-D-glucosamine was used as the substrate, the biomass accumulation of *P. shioyasakiensis* D1497 was at almost the same level for both the 15 °C and 25 °C cultures in the first 3 h of cultivation (Fig. 4b). However, the biomass accumulation of the 15 °C culture had an increase compared with that of 25 °C culture from 4- 6 h (Fig. 4**b**). In the whole 6 h of cultivation, the substrate N-acetyl-D-glucosamine concentration decreased from 1.68 mM to 0.67 mM in the 15 °C culture, while from 1.68 mM to 0.36 mM in the 25 °C culture (Fig. 4**b**). Obviously, the substrate consumption rate by *P. shioyasakiensis* D1497 cultivated in 25 °C was higher than that of 15 °C. Therefore, the biomass conversion rate of *P. shioyasakiensis* D1497 was 0.11– 0.12 $mM^{-1}$ for the 25 °C culture and 0.17–0.21 $mM^{-1}$ for the 15 °C culture, respectively (Fig. 4**b**).

### 3.3. *Shioyasakiensis D1497 has larger cell volume at low temperature*

When determined with BD FACSCalibur™ Flow Cytometer, the cell volume of *P. shioyasakiensis* D1497 was 0.116 $\mu m^3$ and 0.045 $\mu m^3$ for the 15 °C and 25 °C cultures respectively (Fig. 5). This indicated the cell volume of 15 °C culture was 2.57 fold of the 25 °C culture. When observed with TEM, the cell volume of 15 °C culture was also determined to be larger than that of 25 °C culture (Fig. 5).

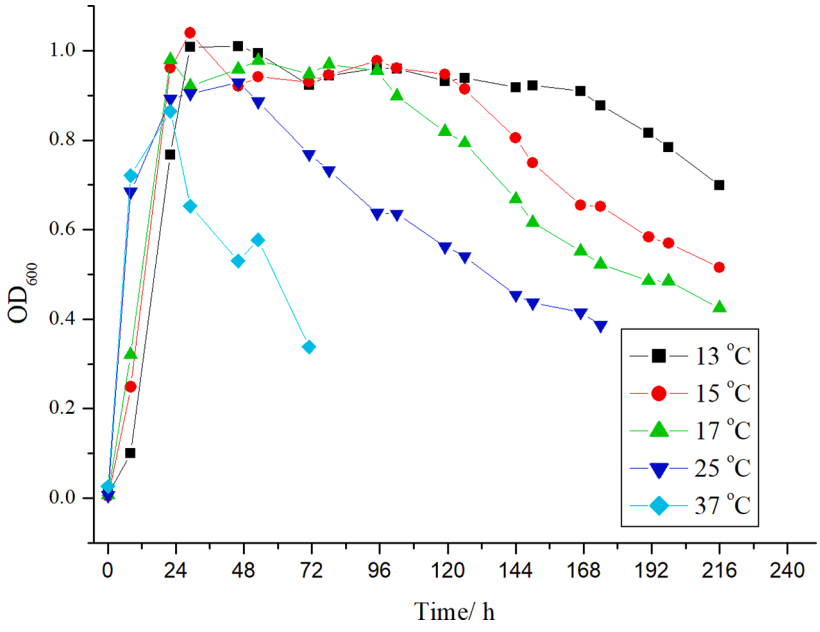


**Fig. 3.** Growth curves of *P. shioyasakiensis* D1497 at different temperatures.

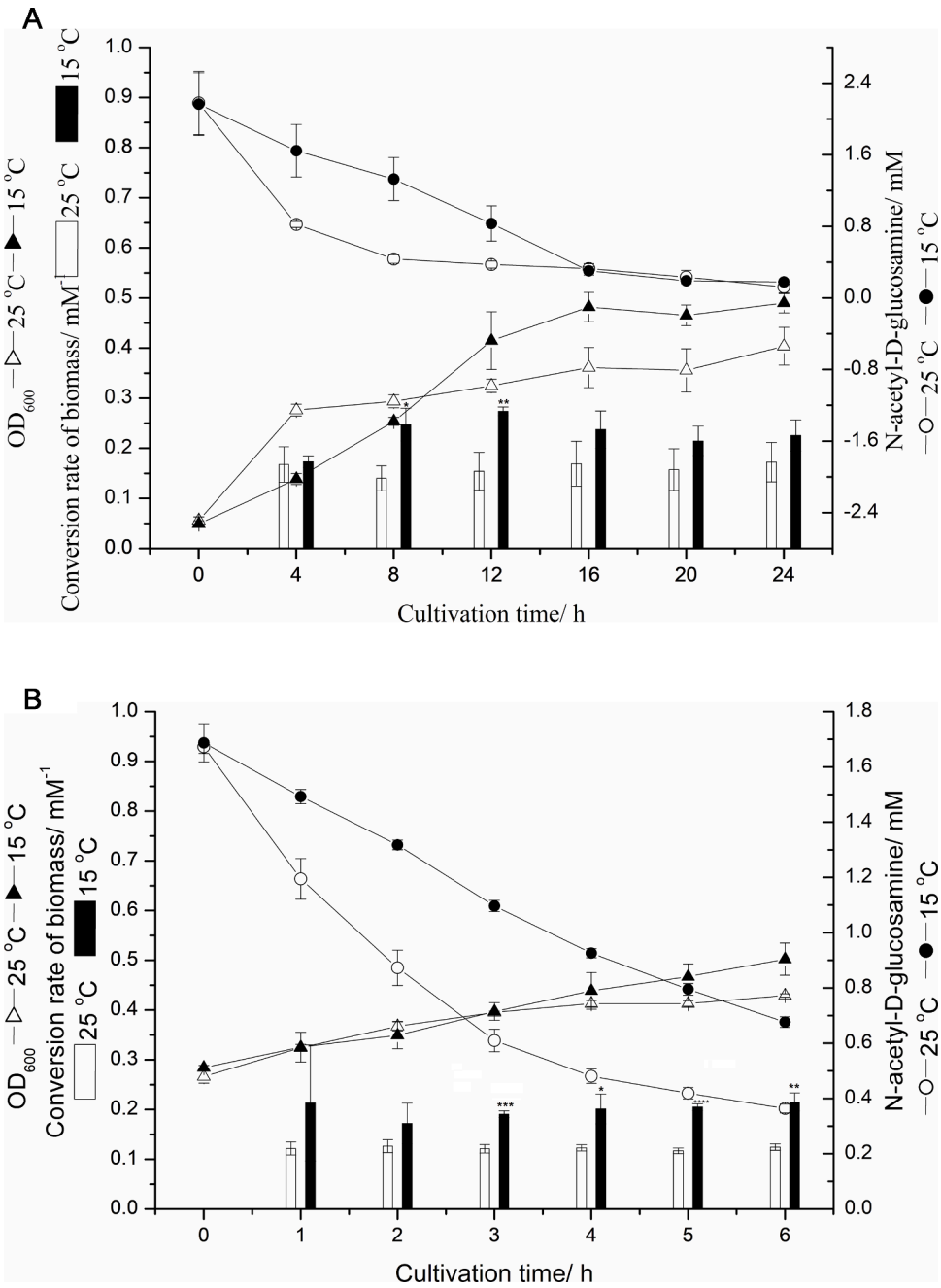


**Fig. 4.** Biomass accumulation, N-acetyl-D-glucosamine concentration and biomass conversion rate of *P. shioyasakiensis* D1497 at 15 °C and 25 °C respectively with 3% (a) or 100% (b) inoculum.

### *3.4. Complete genome sequencing of P. shioyasakiensis D1497*

The complete genome of *P. shioyasakiensis* D1497 was made up of a circle chromosome of 3631,285 bp with 40.94% GC content and no plasmid (**Table S1**). The graphical map of the chromosome of *P. shioyasakiensis* D1497 was illustrated in **Fig. S1**. The genome of *P. shioyasakiensis* D1497 encodes 3113 proteins, 25 rRNAs and 106 tRNAs (**Table S1**). The complete genome sequence of *P. shioyasakiensis* D1497 had been submitted to GenBank with the accession number of CP058414.1 and would facilitate improving the general understanding of how it adapts to the cold environment.

### *3.5. Shioyasakiensis D1497 preferred to use codons with A/T in the third position*

The total numbers of codons (except stop codon) in *P. shioyasakiensis* D1497 were 1074,422. $A + T$ content in the third position of codons was 65.76% which was higher than that of the first (46.82%), second (63.01%) positions and the average value (58.53%) of the three positions (Table 1). Among protein coding genes, Asn (8.87%) and Cys (0.97%) were the most and the least used amino acids (Fig. 6**a**). The relative synonymous codon usage (RSCU) values of *P. shioyasakiensis* D1497 were summarized in Fig. 6**b**. The RSCU indicated the five most used codons CGT (Arg), TTA (Leu), GGT (Gly), ATT (Ile) and CCA (Pro) were all with A/T in the third position (Fig. 6**b**). Additionally, compared with other synonymous codon, the codons with A and T in the third position were always the most used (Fig. 6**b**).

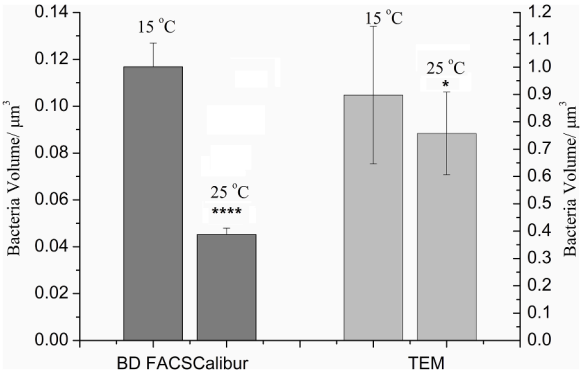


**Fig. 5.** The cell volume of *P. shioyasakiensis* D1497 at 15 °C and 25 °C determined by BD FACSCalibur™ flow cytometer and TEM respectively.

**Table 1**
The content of four DNA base in three positions of codons of *P. shioyasakiensis* D1497.

| Condon | A% | C% | G% | T% | A + T% | C + G% |
|---|---|---|---|---|---|---|
| First site | 27.94 | 19.23 | 33.93 | 18.88 | 46.82 | 53.17 |
| Second site | 32.7 | 21.28 | 15.69 | 30.31 | 63.01 | 36.98 |
| Third site | 29.9 | 17.91 | 16.31 | 35.85 | 65.76 | 34.23 |
| Average | 30.18 | 19.48 | 21.98 | 28.35 | 58.53 | 41.46 |

### *3.6. General features of the transcriptional profiles of P. shioyasakiensis D1497 generated by RNA-Seq*

Comparative analysis of the transcribed gene profiles had provided extensive biological information about the response of *P. shioyasakiensis* D1497 grown at low temperature (15 °C) and room temperature (25 °C) on a genomic scale. Using statistical criteria described previously, a 2.0 $\log_2$(ratio RPKM) of median cutoff was considered as differential gene transcription under the two growth conditions [30]. A total of 1200 genes showed a differential transcription profile, of which 536 were increased (by up to 14.4-fold) and 664 were decreased (by up to 13.5-fold) at low temperature (15 °C) compared to room temperature (25 °C) **(Fig. S2)**. Genes exhibiting differential transcription were annotated by Gene Ontology and KEGG and were found to be mostly associated with pathways of metabolism including amino acid metabolism, biosynthesis of other secondary metabolites, carbohydrate metabolism, energy metabolism, glycan biosynthesis and metabolism, lipid metabolism, metabolism of cofactors and vitamins, metabolism of other amino acids, metabolism of terpenoids and polyketides, nucleotide metabolism and xenobiotics biodegradation and metabolism (**Fig. 7**). Genes and operons involved in the energy metabolism pathways were analysed in further detail according to their differential mRNA abundance levels (**Table 2**).

### *3.7. mRNA abundance of genes involved in energy metabolism decreased at low temperature (15 °C)*

There are two distinct ATP-producing mechanisms, oxidative phosphorylation and substrate-level phosphorylation. Oxidative phosphorylation is typically associated with respiration, where the reduction of terminal electron acceptors such as $O_2$ is coupled to proton motive force (PMF) generation, and the PMF subsequently contributes to ATP synthesis via ATP synthase (ATPase). It can be clearly seen from **Table 2** that the mRNA abundance of genes of the ATP synthase operon (HYD28_02260- HYD28_02300) of *P. shioyasakiensis* D1497 was decreased by as much as 2.13-fold in low temperature (15 °C) growth condition. Substrate-level phosphorylation is associated with the production of ATP through direct transfer of a phosphoryl group to ADP via the action of enzymes like phosphate acetyltransferase (Pta), acetate kinase (AckA) and pyruvate kinase (Pyk). In *P. shioyasakiensis* D1497, the mRNA abuandance of *pta* (HYD28_09315) encoding phosphate acetyltransferase, *ackA* (HYD28_09320) encoding acetate kinase and *pyk* (HYD28_10,235) encoding pyruvate kinase was also decreased by up to 0.79-fold in low temperature (15 °C) growth condition. The phage shock protein (Psp) system was first identified as a response to phage infection in *Escherichia coli*. Recently, it was suggested that dissipation of the PMF was probably an inducing signal for the Psp response. In *E. coli*, PspA was thought to be important in the maintenance of the PMF under inducing condition [9]. When *P. shioyasakiensis* D1497 was cultivated at low temperature (15 °C), the transcriptional level of genes involved in oxidative phosphorylation and substrate-level phosphorylation as mentioned above was decreased. Additionally, a higher level of ATP pool was observed when *P. shioyasakiensis* D1497 was cultivated at low temperature (15 °C) compared with that of room temperature (25 °C). As a result, PMF of 15 °C cells of *P. shioyasakiensis* D1497 would be maintained in a higher level than that of 25 °C cell culture. In this case, the mRNA abundance of *pspA* (HYD28_13,720) was decreased by 2.39-fold at low temperature (15 °C) compared with that of room temperature (25 °C) (**Table 2**).

## 4. Discussion

Psychrophile *P. shioyasakiensis* D1497 was isolated from the Yap trench at a depth of 5137.276 m. *P. shioyasakiensis* D1497 was revealed to possessed a set of energy saving strategies to cope with the slowing down of energy metabolism and production caused by low temperature environment in the deep sea.

First, *P. shioyasakiensis* D1497 contained a circle chromosome of 3631,285 bp with GC content as low as 40.94%. It was presumed that low GC content of genome might facilitate *P. shioyasakiensis* D1497 to carry out life activities such as DNA replication, mRNA transcription and protein translation in cold environment. It was known that less energy was needed to open AT base pair than GC base pair.

Additionally, *P. shioyasakiensis* D1497 prefered to use codons with A/T in the third position. When all the codons were counted, *P. shioyasakiensis* D1497 had the highest $A + T$ content in the third position of codons (**Table 1**). Besides, the RSCU values of *P. shioyasakiensis* D1497 indicated the codons with A and T in the third position were always the most used (**Fig. 6b**). This phenomenon had also been reported in the sea cucumber *Benthodytes marianensis* collected from the Mariana trench [31]. AT base pair in the third position of codons would make the translation process more energy saving.

Besides, when it was cultured at low temperature, the metabolic process would be rearranged, slowing down the energy production pathways (**Table 2**). The cultivation experiments confirmed that the biomass conversion rate of *P. shioyasakiensis* D1497 cultivated in 15 °C was higher than that of 25 °C. *P. shioyasakiensis* D1497 grew better at low temperature, although the substrate consumption was slower (**Fig. 4**). These results indicated more substrate was converted into biomass under low temperature condition. Therefore, the conversion rate of biomass per unit substrate consumed at low temperature was improved compared with room temperature (**Fig. 4**). In the deep sea environment with low temperature, the primary task of *P. shioyasakiensis* D1497 was to save energy and substrates to reproduce itself as the substrates transportation and energy production processes were all slowed down. It was speculated that some metabolic activities unrelated with its survival and reproduction of itself such as swimming and synthesis of secondary metabolite would be slowed down or shut down at low temperature. Swimming assays confirmed that less energy was spent by *P. shioyasakiensis* D1497 at low temperature (15 °C) as its swimming distance was obviously shorter than that of room temperature (25 °C) **(Fig. S3)**. At low temperature (15 °C), the ATP pool of *P. shioyasakiensis* D1497 was maintained at a higher level compared with that of the room temperature (25 °C) condition. This phenomenon was

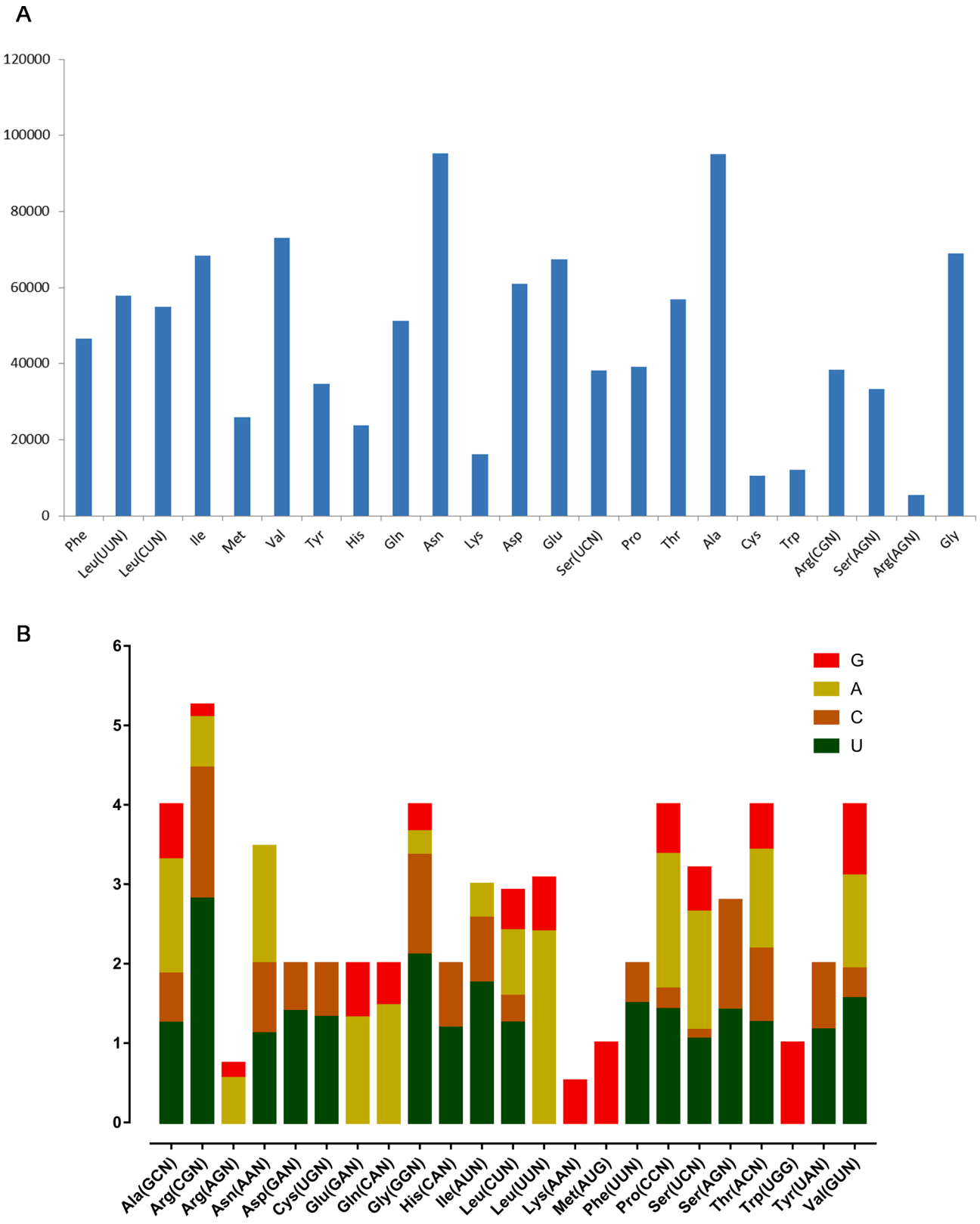


**Fig. 6.** Codon usages (a) and the relative synonymous codon usage (RSCU) (b) of *P. shioyasakiensis* D1497 genome Numbers to the left refer to the total numbers of codons (a) and the RSCU value (b). Codon families are provided on the X-axis.

in accordance with that found in *Psychrobacter cryohalolentis* [1]. Cell volume measurement proved that the cell volume of *P. shioyasakiensis* D1497 cultivated at 15 °C was larger than that of the 25 °C culture. This phenomenon was in accordance with the higher substrate consumption at 25 °C as smaller cell volume represented larger relative cell membrane area to improve substrate transportation efficiency.

Comparative analysis of the transcribed gene profiles of *P. shioyasakiensis* D1497 grown at low temperature (15 °C) and room

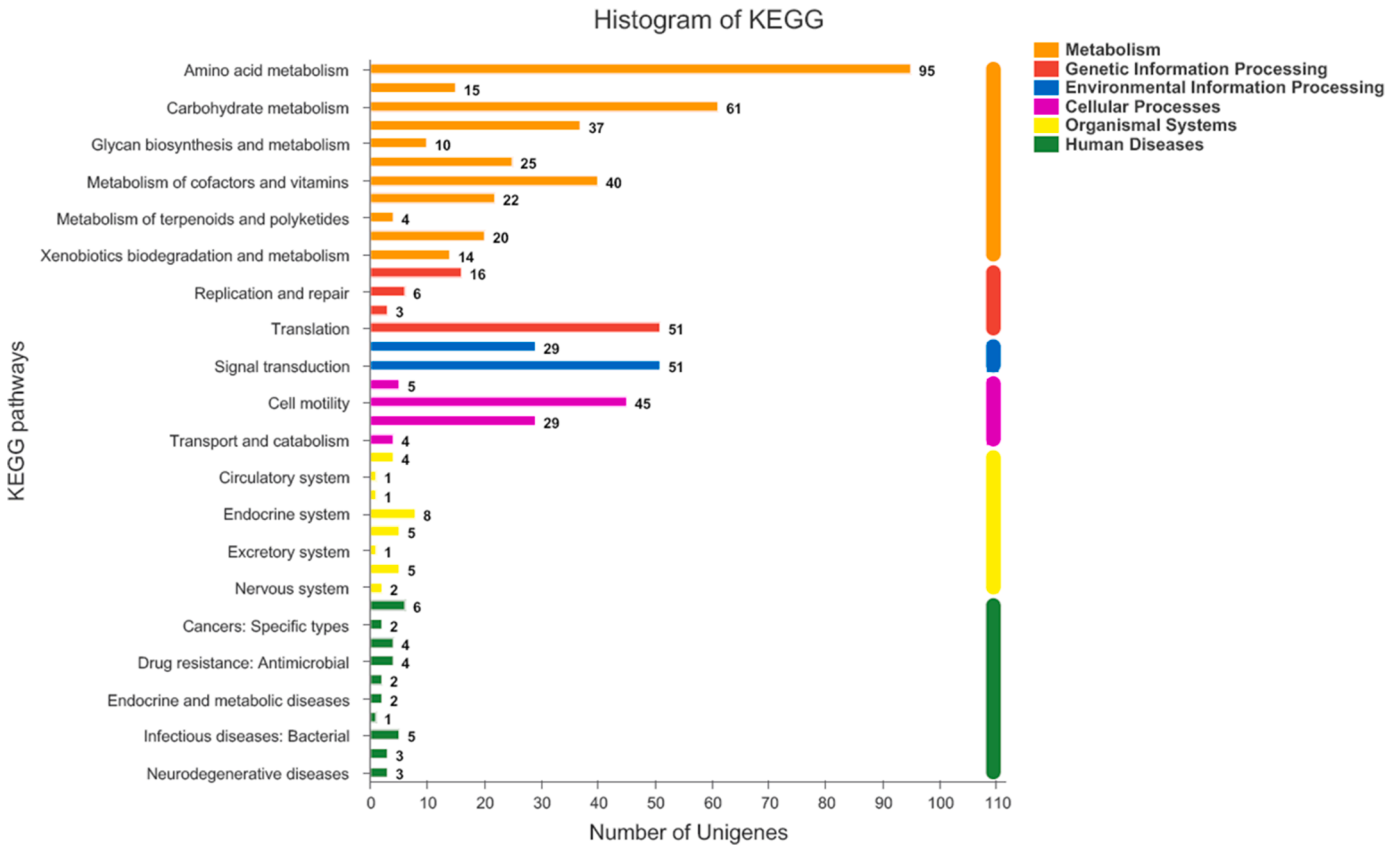


**Fig. 7.** Enrichment of KEGG metabolic pathways for genes with different transcribed levels under 15 °C and 25 °C culture conditions of *P. shioyasakiensis* D1497 Y-axis, KEGG metabolic pathways of 6 classes: Metabolism, Genetic Information Processing, Environmental Information Processing, Cellular Processes, Organismal Systems, Human Diseases; X-axis, the number of unigenes enrichment of KEGG metabolic pathways; The subclasses of metabolism with the number of unigenes enrichment are amino acid metabolism (95), biosynthesis of other secondary metabolites (15), carbohydrate metabolism (61), energy metabolism (37), glycan biosynthesis and metabolism (10), lipid metabolism (25), metabolism of cofactors and vitamins (40), metabolism of other amino acids (22), metabolism of terpenoids and polyketides (4), nucleotide metabolism (20) and xenobiotics biodegradation and metabolism (14).

**Table 2**
Genes involved in the energy metabolism of *P. shioyasakiensis* D1497.

| Sequence ID | Gene | Function | R_RPKM[a] | L_ RPKM[b] | logFC(L/R)[c] | FDR[d] |
|---|---|---|---|---|---|---|
| **ATP synthase operon** | | | | | | |
| HYD28_02260 | *atpC* | ATP synthase F0F1 subunit epsilon | 1313.6 | 299.2 | −2.13 | $2.79 \times 10^{-13}$ |
| HYD28_02265 | *atpD* | ATP synthase F0F1 subunit beta | 1748.5 | 617.4 | −1.5 | $1.85 \times 10^{-14}$ |
| HYD28_02270 | *atpG* | ATP synthase F0F1 subunit gamma | 1445.3 | 510.5 | −1.5 | $2.71 \times 10^{-15}$ |
| HYD28_02275 | *atpA* | ATP synthase F0F1 subunit alpha | 1592.8 | 808.6 | −0.98 | $8.73 \times 10^{-11}$ |
| HYD28_02280 | *atpH* | ATP synthase F0F1 subunit delta | 1374.3 | 631.1 | −1.12 | $1.31 \times 10^{-13}$ |
| HYD28_02285 | *atpF* | ATP synthase F0F1 subunit B | 2741.8 | 1161 | −1.24 | $1.14 \times 10^{-07}$ |
| HYD28_02290 | *atpE* | ATP synthase F0F1 subunit C | 365.00 | 239.7 | −0.61 | $3.34 \times 10^{-06}$ |
| HYD28_02295 | *atpB* | ATP synthase F0F1 subunit A | 611.03 | 400.6 | −0.61 | $1.21 \times 10^{-06}$ |
| HYD28_02300 | *atpI* | ATP synthase F0F1 subunit I | 127.52 | 99.63 | −0.36 | $1.12 \times 10^{-02}$ |
| **Substrate-level phosphorylation** | | | | | | |
| HYD28_09315 | *pta* | phosphate acetyltransferase | 323.06 | 187.2 | −0.79 | $9.63 \times 10^{-08}$ |
| HYD28_09320 | *ackA* | acetate kinase | 318.67 | 235.6 | −0.44 | $2.50 \times 10^{-06}$ |
| HYD28_10,235 | *pyk* | pyruvate kinase | 504.76 | 427.6 | −0.24 | $2.87 \times 10^{-03}$ |
| **phage shock operon** | | | | | | |
| HYD28_13,710 | *pspC* | phage shock protein C | 1232.6 | 377.0 | −1.71 | $5.25 \times 10^{-14}$ |
| HYD28_13,715 | *pspB* | phage shock protein B | 1108.9 | 345.5 | −1.68 | $9.65 \times 10^{-28}$ |
| HYD28_13,720 | *pspA* | negative regulatory gene for the psp opreon, phage shock protein A | 619.31 | 117.7 | −2.39 | $2.98 \times 10^{-18}$ |
| HYD28_13,725 | *pspF* | transcriptional activator of phage shock proteins | 73.28 | 119.0 | 0.7 | $1.71 \times 10^{-01}$ |

[a] RPKM values of three biological replicates of genes from *P. shioyasakiensis* D1497 cultured in room temperature (25 °C). RPKM, reads per kb per million reads.
[b] RPKM values of three biological replicates of genes from *P. shioyasakiensis* D1497 cultured in low temperature (15 °C).
[c] The $\log_2$ ratio of L_RPKM/R_RPKM.
[d] False discovery rate.

temperature (25 °C) revealed that genes exhibiting differential transcription were found to be mostly associated with pathways of metabolism including amino acid metabolism (Fig. 7). Metabolic changes in amino acid metabolism and carbohydrate metabolism induced by cold stress in psychrotolerant *Microbacterium sediminis* YLB-01 also had been reported [38]. Further, it was found that the mRNA abundance of energy

metabolism genes was decreased in low temperature (15 °C) growth condition (Table 2). These evidences provided an insight into the gene transcription profile of *P. shioyasakiensis* D1497 for slowing the processes of substrate metabolism and energy production at low temperature. These results were in accordance with the low substrate consumption rate of *P. shioyasakiensis* D1497 at low temperature (15 °C) (Fig. 4). At the same time, a higher biomass accumulation of *P. shioyasakiensis* D1497 cultivated at low temperature indicated it carried out an energy saving metabolism lifestyle in cold environment. From the energy saving aspect, *P. shioyasakiensis* D1497 could be used as an excellent microbial cell chassis in synthetic biology for production of chemical compounds in the future.

## Availability of data and materials

The genome sequence of *P. shioyasakiensis* D1497 was deposited in GenBank with accession number of CP058414.1. The RNA-Seq reads have been deposited in GenBank with accession number of PRJNA643440.

## Compliance with ethical standards

This study did not involve any research involving human participants or animals.

## Author contribution statement

WG conceived and designed research. JD conducted experiments, analyzed data and wrote the manuscript. All authors read and approved the manuscript.

## Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

## Acknowledgements

The study was financially supported by grants from the Natural Science Foundation of Fujian Province, China (grant number: 2019J05019), the Scientific Research Foundation of Third Institute of Oceanography, Ministry of Natural Resources (NO. 2019031), the Natural Science Foundation of Fujian Province, China (grant number: 2020J01101), Xiamen Youth Innovation Foundation Project (3502Z20206100), and the China Ocean Mineral Resources R & D Association (COMRA) program (grant number: DY135-B2–01).

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.btre.2021.e00689.

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