Letter to the Editor

Transcriptomic Responses of *Acinetobacter harbinensis* HITLi 7^T at Low Temperatures*

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Acinetobacter harbinensis HITLi 7^T, a novel species in the genus *Acinetobacter*, was isolated from Songhua River in Harbin, China. It is a psychrotolerant heterotrophic nitrification bacterium that grows in temperatures from 2 °C to 35 °C with an optimal growth temperature of 8 °C-20 °C[1]. The strain plays an important role in ammonium removal from source water and water sanitation improvement in cold areas. Biologically enhanced activated carbon (BEAC) filters immobilized with HITLi 7^T are used to treat source water in winter, exhibiting stronger ammonium removal capability and higher microbial stability during BEAC treatment at low temperatures[2]. However, the low temperature adaptation mechanism of HITLi 7^T has not been clearly elucidated yet.

Temperature is a major environmental factor that affects bacterial growth. Low temperatures inhibit bacterial growth and alter physiological characteristics. Most research regarding bacterial adaptation to cold focuses on the cold shock response. Cold shock induces cold shock protein (Csp) production to reduce the harmful effect of temperature downshift[3] and induces changes in expressed genes associated with metabolism, regulation, and fatty acid biosynthesis[4].

In this study, we investigated the gene expression profiles of HITLi 7^T grown at a low temperature (2 °C) and optimal temperature (20 °C) by high throughput sequencing and transcriptomic analysis. We systematically assessed the change of expressed gene associated with adaptation to the low temperature conditions. Some differentially expressed genes (DEGs) were validated by RT-qPCR. This study facilitates to elucidate the molecular mechanism of HITLi 7^T adaptation to low temperatures and enrich our knowledge of the low temperature adaptation mechanism of the *Acinetobacter* genus.

HITLi 7^T was grown in liquid culture containing 0.382 g/L NH_4Cl, 2.0 g/L CH_3COONa, 0.05 g/L $MgSO_4 \cdot 7H_2O$, 0.2 g/L K_2HPO_4, and 0.12 g/L NaCl with pH 7.0 at 20 °C for 24 h, re-suspended in the flesh culture, and incubated at 2 °C and 20 °C for 4 days. The experiment was conducted in triplicate for each temperature condition. The cultures were precipitated by centrifugation at 8,000 rpm for 5 min and then washed with DEPC treated water. The cells were harvested and immediately frozen in liquid nitrogen.

Total RNA was extracted from cells using the Trizol reagent according to the manufacturer's instructions (Invitrogen). The quality and concentration of total RNA were checked by gel electrophoresis and with a spectrophotometer. Library construction and Illumina sequencing were performed at Huada Gene Technology Co., Ltd., Shenzhen, China. The library was constructed using 5 μg total RNA with TruSeq RNA Sample Prep Kit v2 from Illumina. The final library was detected by determining the average molecule length using Agilent 2100 bioanalyzer instrument (DNA 1000 Reagents) and quantifying concentration by qPCR (Taqman probe). The paired-end library was sequenced with the Illumina HiSeq 2000 system.

The raw reads were trimmed by removing low-quality reads (Q value < 20) and adapter sequences. The clean reads were assembled with Trinity assembler v2.0.6. The assembled sequences were clustered and redundancy was checked to obtain non-redundant transcripts with TGICL software v2.0.6. These transcripts were annotated to NT, NR, GO, COG, KEGG, SwissProt, and InterPro databases using Blastn, Blastx, Diamond, Blast2GO, and InterProScan5 software.

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The RSEM package was used to measure gene expression level. Differential expression analysis was performed using DEseq2 method. The RPKM values were used for deriving the log fold changes. The probability threshold *P*-value ≤ 0.05 and the absolute value of $\log_2$(Fold Change) ≥ 1 were used as criteria for judging differentially expressed genes (DEGs). A principle component analysis (PCA) was conducted using DESeq2 to determine the overall reproducibility of RNA-Seq biological replicates. The DEGs were clustered using the pheatmap function in R software. All DEGs were classified and enriched in function and biological pathways by comparison on GO and KEGG databases using the phyper function in R software.

To validate the RNA-Seq results, 16 up-regulated genes were selected to perform RT-qPCR. The primers used in RT-qPCR were given in Supplementary Table S1 (available in www.besjournal.com). RT-qPCR was performed using StepOne (ABI, America) in a final volume of 20 μL containing 1 μL of cDNA, 10 pmol of each primer, 10 μL of 2 × SuperReal Color PreMix, 2 μL of 50 × ROX together with no template as negative control. A two-step reaction procedure was adopted: pre-denaturation at 95 °C for 15 min, denaturation at 95 °C for 10 s, annealing at 60 for 30 s, 40 cycles. All procedures were performed in triplicate. The relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method with 16S rRNA as a reference gene.

The sequencing results of three parallel samples were similar; 13.73 Mb and 13.08 Mb clean reads were generated from treated and control samples, respectively. Removing partial overlapping and short sequences (< 300 bp in length) yielded 3,104 unique genes with an average size of 1,277 bp. These unique genes were annotated on the relevant databases. In total, 2,535 unique genes were annotated on 7 databases, accounting for 81.6% of total unique genes (Table 1). KEGG analysis revealed that 56.6% of unique genes participated in metabolic processes and 20.7% were assigned to genetic information processing (Supplementary Figure S1A, available in www.besjournal.com). GO classification showed that 953 unique genes were assigned to 40 GO terms. More unique genes were attributed to metabolic and cell processes (Supplementary Figure S1B). COG annotation indicated that 1,384 unique genes were clustered to 23 COG categories. The largest group is translation and ribosomal structure biogenesis, containing 258 genes (Supplementary Figure S1C).

In total, 393 DEGs were identified, of which 339 and 54 were up- and down-regulated, respectively, at 2 °C, accounting for 86.3% and 13.7% of total DEGs, respectively (Figure 1). The results indicated that the larger number of DEGs induced up-regulation to adapt to low temperature conditions. GO function annotation showed that 289 DEGs were enriched in 28 GO terms (Supplementary Figure S2A, available in www.besjournal.com). Most DEGs were associated with metabolic, cellular, regulatory, and stimuli response processes. KEGG analysis indicated that 80 DEGs were enriched in 30 pathways. Most DEGs participated in metabolism pathways (Supplementary Figure S2B).

Of down-regulated genes, 39 were predicted to encode a hypothetical protein with unknown function and 19 were putatively involved in biological processes. The majority of hypothetical protein genes were down-regulated more than five times. Thus, further work is necessary to evaluate the possible role of these genes in low temperature adaptation. The gene Unigene1254_All encoding ClpV1 family T6SS ATPase and the gene Unigene1274_All encoding type VI secretion protein EvpB were putatively related to type VI secretion system (T6SS) and down-regulated (3.0-fold and 3.3-fold) in low temperature conditions, implying reduced T6SS-mediated intoxication toward cell growth at low temperatures. Some down-regulated genes were listed in Supplementary Table S2 available in www.besjournal.com.

The majority of up-regulated genes were involved in metabolic, regulatory, substance transport, translation, stimuli response, and cell division processes (Supplementary Table S3, available in www.besjournal.com), indicating that the adaptation of HITLi 7^T to low temperatures involves the extensive regulation of biological processes. A significant effect of low temperature on metabolism was observed. A large number of genes associated

Table 1. Results of Functional Annotation of Unique Genes

Values	Total	Nt	Nr	Swiss Prot	KEGG	COG	Inter pro	GO	Inter Section	Overall
Number	3,104	948	1,720	1,575	1,742	1,384	2,115	953	577	2,535
Percentage (%)	100.0	30.5	55.4	50.7	56.1	44.5	68.1	30.7	18.5	81.6

with carbohydrate and amino acid metabolism appeared to be up-regulated, implying the increase of the basal metabolic activity of HITLi 7^T at low temperatures and supply of sufficient substrate and energy for rapid growth at low temperature.

Carbohydrate metabolism provides energy and the carbon skeleton for cell growth; 39 genes related to carbohydrate metabolism appeared to be up-regulated. Seven genes involved in glycolysis and eight genes involved in citric acid cycle (TCA) were up-regulated, suggesting the enhancement of glycolysis and TCA at low temperatures. Additionally, several genes involved in pentose phosphate, propanoate, pyruvate, glycolate, carboxylic, and sugar metabolism were also up-regulated. Thus, we speculate that the increase of carbohydrate metabolism was one of the main mechanisms of HITLi 7^T low temperature adaptation.

Of genes related to amino acid metabolism and transporters, 42 were up-regulated, indicating the activation of amino acid metabolism at low temperatures. Some genes involved in lysine, leucine, arginine, histidine, glutamite, glutamate, and tryptophan synthesis were up-regulated, indicating that HITLi 7^T strived to accumulate and utilize these amino acids at low temperatures. Additionally, several genes related to lysine and leucine degradation and transport also appeared to be up-regulated, demonstrating the remolding of lysine and leucine metabolism, as well as uptake and utilization enhancement of lysine and leucine at low temperatures. Leucine is critical for production of branched-chain fatty acid[5]. The up-regulation of genes (Unigene1324_All and Unigene536_All) encoding leucine synthesis and branched-chain amino acid transport may promote branched-chain fatty acid synthesis at low temperatures; the expression of these genes was validated by RT-qPCR (Figure 2).

Transcription regulation of HITLi 7^T in low temperature conditions was controlled by many global regulators, including RNA polymerase sigma factors, transcription regulator activators, temperature sensors, and AraC, Fur, IclR, Crp, LysR, ArsR, TetR, ArgP family transcription regulators. Sigma factor, as a bacterial transcription initiation factor, is responsible for regulating transcription initiation. Two RNA polymerase sigma factors encoding genes, Unigene1683_All (σ^{70}) and Unigene1454_All (σ^{32}), were up-regulated in low temperature conditions, implying the activation of transcription initiation. Additionally, σ^{32} is likely to positively regulate the expression of heat shock genes in HITLi 7^T at low temperatures due to the up-regulation of several heat shock genes (hscA, dnaJ, dnaK, htpG, clpB). Two transcription regulator activators, integration host factor subunit alpha (Unigene1333_All) and RNA polymerase-associated protein RapA (Unigene109_All), were 9.3-fold and 4.0-fold up-regulated in low temperature conditions, respectively, hinting the activation of transcription rate, frequency, and extent at low temperature. Additionally, a large number of transcription regulator encoding genes, such as AraC, Fur, IclR, Crp, LysR, ArsR, TetR, and ArgP family transcription regulators, were also up-regulated. These transcription

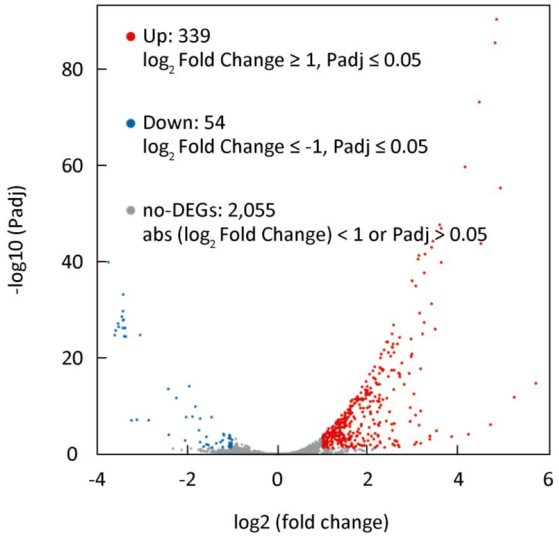

Figure 1. Volcano plot of differentially expressed genes.

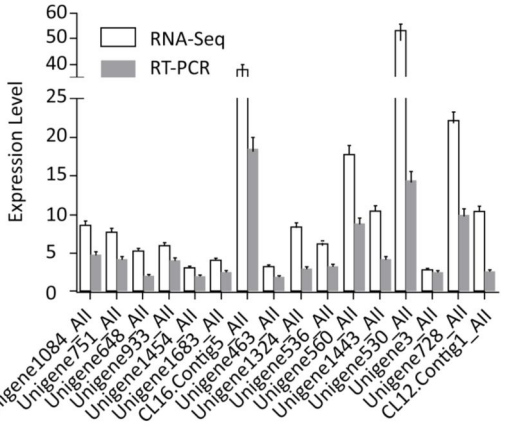

Figure 2. Validation of differently expressed genes by RT-qPCR.

regulators regulated multiple metabolic processes. Their up-regulation enhanced metabolic ability of the cell in low temperature conditions. The RT-qPCR results of the genes *AraC* (Unigene933_All), *rpoH* (Unigene1454_All), and *RpoD* (Unigene1683_All) were consistent with transcriptome analysis (Figure 2).

Of genes related to temperature and oxidative stress response, 15 were up-regulated. The Csps encoding gene (CL12.Contig1_All) exhibited an enhanced expression level of over 10-fold. Six hsps genes exhibited the enhanced expression (3.6-fold to 17.7-fold). Csps facilitate efficient translation at low temperatures by hampering the formation of the stable secondary mRNA structure acting as transcriptional activators or RNA chaperones. Hsps facilitates protein fold under stress by acting as molecular chaperones. Generally, hsps genes are up-regulated in increased temperatures[6-7]. However, the opposite result was observed in our study. This is likely due to differences in molecular responses of different bacteria at low temperatures. The up-regulation of Csp (CL12.Contig1_All) and hsp (Unigene560_All and Unigene1443_All) were verified by RT-qPCR (Figure 2). Previous proteome analysis of HITLi 7^T also revealed the overexpression of two chaperone proteins in low temperature conditions [8]. These findings revealed that Csps and hsps played an important role in low temperature adaptation in HITLi 7^T by improving DNA binding and protein folding. The oxidative stress response genes, including catalase (Unigene902_All), superoxide dismutase (Unigene1524_All), flavoprotein (Unigene740_All), and alkyl hydroperoxide reductase subunit F (CL47.Contig2_All), were observed to be up-regulated, suggested that low temperature adaptation of HITLi 7^T also involved the oxidative stress response.

Low temperatures inhibit translation due to decreased ribosomal structural integrity and tRNA synthesis. Of genes associated with translation, 24 were up-regulated at low temperatures, including ribosome-associated genes, translation initiation factor IF2, IF1, translation elongation factor p, and tRNA synthesis- and transport related-genes. The up-regulation of these genes activated the translation of HITLi 7^T in low temperature conditions. Thus, we speculated that the translation activity of HITLi 7^T at a low temperature was enhanced by increasing ribosomal activity, mRNA longevity, translation initiation, and elongation as well as tRNA synthesis and transport. The RT-qPCR results of three genes (Unigene1084_All, Unigene751_All, and Unigene648_All) were consistent with transcriptome analysis (Figure 2) further revealed that translation level modulation may be an important process involved in cellular response to low temperatures.

To maintain nucleic acid structure and improve DNA replication and transcription at low temperatures, some genes related to DNA replication and recombination and RNA and DNA repair appeared to be up-regulated. Six DEAD-box RNA helicase encoding genes were significantly up-regulated (5.2-fold 37.8-fold), suggested that DEAD/DEAH box helicase played an important role in HITLi 7^T low temperature adaptation. DEAD-box proteins participate in transcription and translation as RNA helicase and chaperone and play a key role in cold tolerance in a multitude of organisms[9-10]. One gene (CL16.Contig5_All) was validated by RT-qPCR (Figure 2). Several DNA gyrase and topoisomerase, exo- and endonucleases, DNA polymerase, DNA binding protein, DNA methyltransferase, DNA mismatch repair protein, and DNA recombination/repair protein genes involved in DNA replication and repair were induced in low temperature conditions. These findings indicated that the genes responsible for maintaining the activity and integrity of DNA and RNA are activated at low temperatures.

Of genes associated with protein, lipid, amino acid, organic, and ion transport and those associated with cell division proteins, 44 and 4, respectively, were up-regulated in low temperature conditions, indicating the improvement of substance transport and cell division at low temperatures. In addition, 66 hypothetical protein genes with uncharacterized function appeared to be up-regulated in low temperature conditions. Thus, more experiments are required to illuminate the function of these genes.

In conclusion, this study provides new insights into the low temperature adaptation of HITLi 7^T. The low-temperature adaptation of HITLi 7^T was elaborate and involved nearly every cellular process, mainly relying on basal metabolism regulation.

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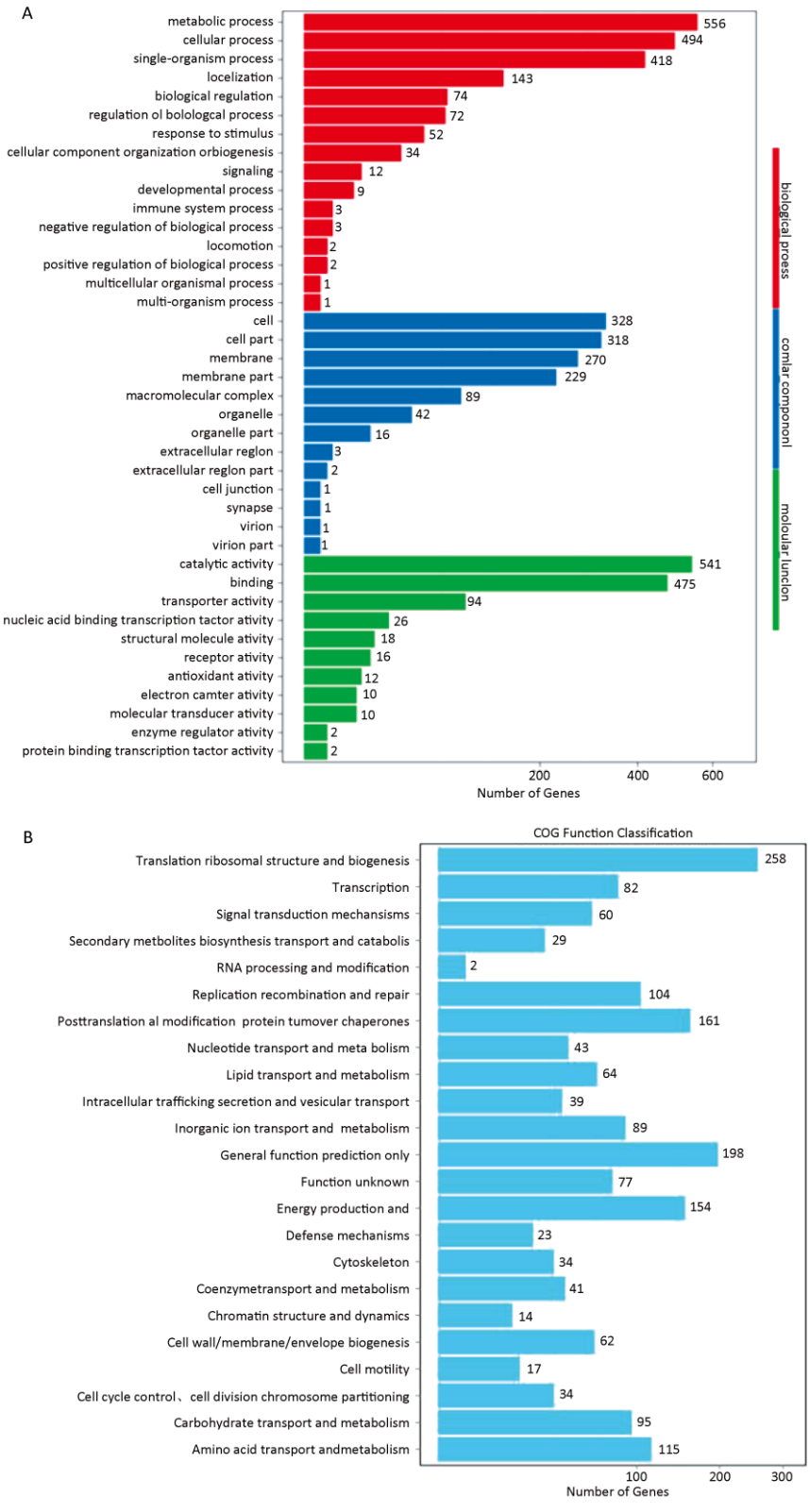
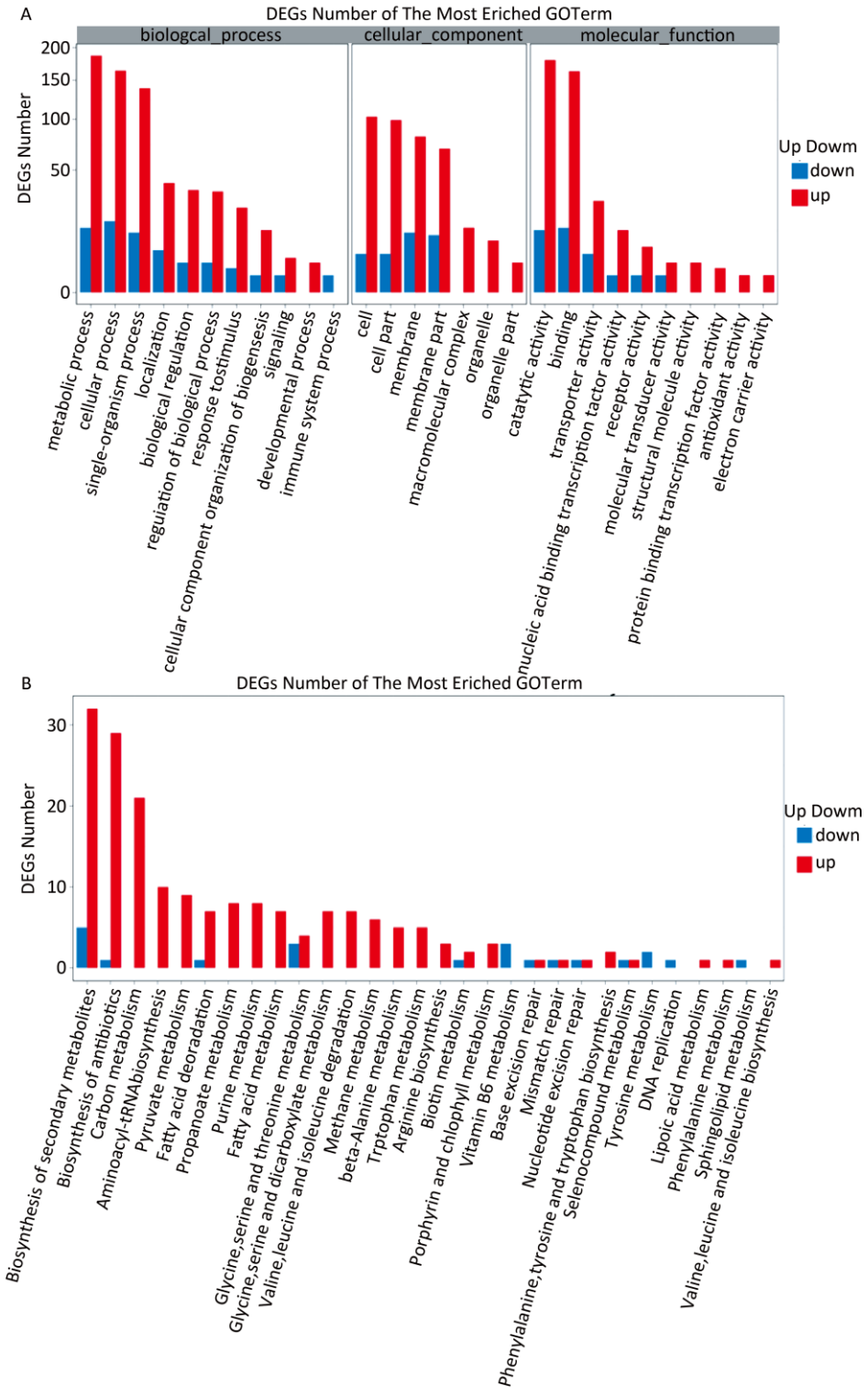
Supplementary Table S1. Primers Used for RT-qPCR

Gene ID	Sequence 5'to 3'	Size (bp)	Reference
Unigene1084_All	GCGTAAAGGCCAGCGTATTG AGAGTGTGTCATGCGTCCAG	180	This study
Unigene751_All	ATGCCAGTGCTGATGTCGAA CGCATCACGTTCACGAATCC	119	This study
Unigene648_All	CCGGTAACCGAAGTGTGTGA AAGGGTAAGGGCTTGATGGC	105	This study
Unigene933_All	TATGTGTCCGTTCAGCGAGG TGAACAGCCAGTCGCAGAAT	116	This study
Unigene1454_All	GGTTTCAGGAGAGCTGGCAT ATGGCGTCTGCCATGAGAAA	102	This study
Unigene1683_All	GAATGCCGACGTCTTGAAGC CATCTCCCAGCCTCGATCAC	73	This study
CL16.Contig5_All	ACGCTCAGTACGTGGTTTGT GATCGTCCTGCTACTCCACG	141	This study
Unigene463_All	GGCGGTGACTGGTTAAGTGA CATCGGCGTTACGGTCAAAC	112	This study
Unigene1324_All	AATCGACTGTCGTGCCACTT AGCCTTACAATGGTCGGTGG	118	This study
Unigene536_All	TTCAGAAATAGGCGCTGCGA CACTGGCCGTACTGGGTATC	92	This study
Unigene560_All	ACGGTGATGACTGCACCATT AATGCCCGCTTTTGAAACCC	129	This study
Unigene1443_All	AGGGTGAATAATGGCGGCAA AAGAAAAGCGTGAGCAAGCG	83	This study
Unigene530_All	CTAAACACGCTGAAGCGGC TCTGCTTTGGCTTTGGTTGC	70	This study
Unigene3_All	GCGTGCAAGCACCTTTTGTA GGAAGCTTTTATGGCGCTCG	117	This study
Unigene728_All	TCGATGGTTTTCCCTGGTCG AGAGATGCGTATTGGCCGAG	102	This study
CL12.Contig1_All	CAGCAGGGCGTATGTTC TCGCTAACTCTGGCTTC	185	This study
16s RNA	CTCCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	162	This study

Note. 16 up-regulated genes at low temperature involved in metabolism, regulation, translation, stimulus response and DNA repair are randomly selected for confirmation by RT-qPCR. The specificity of these primers was verified by conventional PCR amplification and gene sequencing.



Supplementary Figure S1. Function annotation statistics of unique genes.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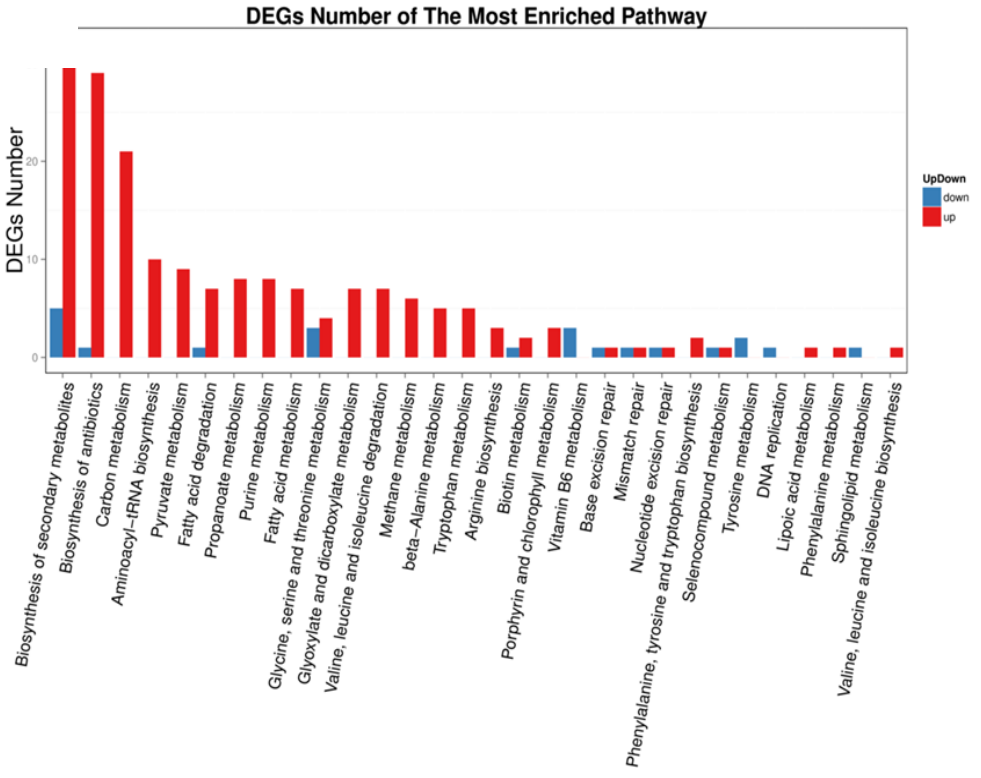
Pyruvate metabolism
Fatty acid deoradation
Propanoate metabolism
Purine metabolism
Fatty acid metabolism
Glycine,serine and threonine metabolism
Glycine,serine and dicarboxylate metabolism
Valine,leucine and isoleucine metabolism
Methane metabolism
beta-Alanine metabolism
Trptophan metabolism
Arginine biosynthesis
Biotin metabolism
Porphyrin and chlophyll metabolism
Vitamin B6 metabolism
Base excision repair
Mismatch repair
Nucleotide excision repair
Phenylalanine,tyrosine and tryptophan biosynthesis
Selenocompound metabolism
Tyrosine metabolism
DNA replication
Lipoic acid metabolism
Phenylalanine metabolism
Sphingolipid metabolism
Valine,leucine and isoleucine biosynthesis



Supplementary Figure S2. GO and KEGG function annotation of differently expressed genes.

Supplementary Table S2. Down-regulated DEGs at Low Temperature

Gene ID	Description	Fold Change
CL6.Contig1_All	hypothetical protein	13.5
Unigene630_All	hypothetical protein	12.2
Unigene473_All	IclR family transcriptional regulator	3.4
Unigene399_All	biotin synthase BioB	4.1
Unigene1254_All	ClpV1 family T6SS ATPase	3.0
Unigene1274_All	type VI secretion protein EvpB	3.3
CL55.contig1_All	MFS transporter	5.4
Unigene1085_All	proline/sodium symporter	2.8
Unigene1160_All	cation/acetate symporter	3.9
Unigene919_All	fumarylacetoacetase	3.5
Unigene999_All	alcohol dehydrogenase	3.7
CL6.Contig4_All	hypothetical protein	12.0
Unigene183_All	hypothetical protein	10.5
Unigene1621_All	hypothetical protein	9.4

Supplementary Table S3. Up-regulated DEGs at Low Temperature

Functional Group	Gene ID	Description	Fold Hange
TCA cycle	Unigene581_All	fumarate hydratase	4.2
	Unigene226_All	2-oxoglutarate dehydrogenase	3.1
	Unigene788_All	isocitrate dehydrogenase	2.4
	Unigene569_All	dihydrolipoyl dehydrogenase	2.5
	Unigene230_All	phosphoenolpyruvate carboxylase	2.9
	Unigene1260_All	aconitate hydratase B	2.7
	Unigene463_All	succinate dehydrogenase flavoprotein subunit	3.1
	Unigene103_All	malate synthase G	3.0
Pentose phosphate pathway	Unigene1253_All	fructose-bisphosphatase	2.1
	Unigene585_All	transketolase	2.8
Pronanoate mechanism	Unigene1166_All	acyl-CoA dehydrogenase	2.9
	Unigene569_All	dihydrolipoyl dehydrogenase	2.5
	Unigene899_All	acetyl-CoA synthetase	2.6
	Unigene115_All	acyl-CoA dehydrogenase	4.6
	Unigene945_All	aminoglycoside phosphotransferas	3.3
	Unigene652_All	acyl-CoA dehydrogenase	2.8
	Unigene1172_All	acetyl-CoA acetyltransferase	5.6
	Unigene758_All	acyl-CoA dehydrogenase	2.8
Glycolysis	Unigene1068_All	phosphoglycerate mutase	3.3
	Unigene650_All	glucose-6-phosphate 1-epimerase	2.8
	Unigene195_All	phosphoenolpyruvate synthase	2.5
	Unigene899_All	acetyl-CoA synthetase	2.6
	Unigene569_All	dihydrolipoyl dehydrogenase	2.5
	Unigene1253_All	fructose-bisphosphatase	2.1
	Unigene54_All	phosphoenolpyruvate carboxykinase	5.8

Continued

Functional Group	Gene ID	Description	Fold Hange
Pyruvate mechanism	Unigene195_All	phosphoenolpyruvate synthase	2.5
	Unigene103_All	malate synthase G	3.0
	Unigene1324_All	2-isopropylmalate synthase	8.3
	Unigene1447_All	histidine kinase	2.3
	Unigene820_All	malate dehydrogenase	2.0
	Unigene569_All	dihydrolipoyl dehydrogenase	2.5
	Unigene899_All	acetyl-CoA synthetase	2.6
	Unigene1172_All	acetyl-CoA acetyltransferase	5.6
	Unigene581_All	fumarate hydratase class II	4.2
	Unigene300_All	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	2.7
	Unigene1068_All	phosphoglycerate mutase	3.3
Amino acid metabolism	Unigene340_All	amidase	3.2
	Unigene1102_All	glutamate synthase	10.8
	Unigene1429_All	imidazole glycerol phosphate synthase	6.4
	Unigene1528_All	glutathione synthase	2.5
	Unigene1419_All	argininosuccinate lyase	3.4
	Unigene582_All	glutamine--fructose-6-phosphate aminotransferase	3.4
	Unigene2_All	glutamine synthetase	2.1
	Unigene1324_All	2-isopropylmalate synthase	8.3
	Unigene226_All	2-oxoglutarate dehydrogenase	3.1
	Unigene402_All	glutamate-1-semialdehyde aminotransferase	5.9
	Unigene1172_All	acetyl-CoA acetyltransferase	5.6
	Unigene1166_All	acyl-CoA dehydrogenas	2.9
	Unigene115_All	acyl-CoA dehydrogenase	4.6
	Unigene945_All	aminoglycoside phosphotransferase	3.3
	Unigene652_All	acyl-CoA dehydrogenase	2.8
	Unigene758_All	acyl-CoA dehydrogenase	2.8
	CL5.Contig1_All	glycerate dehydrogenase	2.1
	CL5.Contig2_All	glycerate dehydrogenase	2.6
	Unigene1599_All	L-glutamate gamma-semialdehyde dehydrogenase	7.7
	Unigene1090_All	diaminopimelate decarboxylase	3.8
	Unigene988_All	3-phosphoshikimate 1-carboxyvinyltransferase	6.3
	Unigene50_All	N-acetylglutamate synthase	2.3
	Unigene729_All	lipid-A-disaccharide synthase	2.8
	Unigene1165_All	anthranilate phosphoribosyltransferas	3.1
	Unigene788_All	isocitrate dehydrogenase	2.4
	Unigene585_All	transketolase	2.8
	Unigene1324_All	2-isopropylmalate synthase	8.3
	Unigene1165_All	anthranilate phosphoribosyltransferase	3.1
Protein transport	Unigene1163_All	outer membrane lipoprotein carrier protein LolA	2.7
	Unigene99_All	signal recognition particle protein	3.7

Continued

Functional Group	Gene ID	Description	Fold Hange
	Unigene390_All	permease	3.6
Lipid transport	Unigene1076_All	lipid A export permease	3.2
	CL13.Contig1_All	LPS biosynthesis protein	2.8
	CL13.Contig2_All	LPS biosynthesis protein	2.1
Amino acid transport	Unigene1071_All	lysine transporter	2.2
	Unigene1122_All	threonine transporter RhtB	5.9
	Unigene1415_All	D-alanine/D-serine/glycine permease	2.1
	Unigene375_All	amino acid transporter	10.6
	Unigene536_All	branched-chain amino acid transport system II carrier protein	6.0
Ion transport	Unigene551_All	macrolide transporter	3.4
	Unigene107_All	ferrous iron transporter B	12.3
	Unigene1595_All	MFS transporter	3.4
	Unigene1416_All	SNF family Na^+-dependent transporter	4.6
	Unigene1_All	MATE family efflux transporter	3.6
	Unigene1261_All	MFS transporter	3.3
	Unigene29_All	uracil transporter	2.4
	Unigene1177_All	NAD(P)($^+$) transhydrogenase	5.9
	Unigene9_All	ATPase	4.1
	Unigene11_All	sulfate transporter	3.7
	Unigene1240_All	sodium:proton antiporter	3.1
	Unigene1642_All	sodium:proton antiporter	2.8
	Unigene984_All	zinc ABC transporter	2.2
	Unigene1348_All	potassium-transporting ATPase subunit B	9.0
	Unigene120_All	cation transporter	2.0
	CL10.Contig3_All	copper ABC transporter	3.0
	Unigene605_All	magnesium transporter	2.8
	Unigene1086_All	betaine/carnitine/choline family transporter	3.0
	Unigene1261_All	MFS transporter	3.3
	Unigene583_All	TonB-dependent receptor	3.7
	Unigene1693_All	TonB-dependent receptor	2.0
	Unigene243_All	iron-sulfur cluster insertion protein ErpA	7.0
Transcription	Unigene1333_All	integration host factor subunit alpha	9.3
regulator	Unigene1454_All	RNA polymerase factor sigma-32 RpoH	2.4
	Unigene1683_All	RNA polymerase sigma factor RpoD	3.9
	Unigene109_All	RNA polymerase-associated protein RapA	4.0
	Unigene738_All	hypothetical protein	2.4
	Unigene933_All	AraC family transcriptional regulator	5.9
	Unigene1500_All	Fur family transcriptional regulator	2.7
	Unigene392_All	transcriptional regulator Crp	2.3
	Unigene578_All	DNA-binding response regulator	2.1

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Functional Group	Gene ID	Description	Fold Hange
	Unigene1508_All	LysR family transcriptional regulator	4.0
	Unigene113_All	transcriptional regulator	2.6
	Unigene1519_All	AraC family transcriptional regulator	2.6
	Unigene1290_All	AraC family transcriptional regulator	2.7
	Unigene1675_All	ArsR family transcriptional regulator	5.8
	Unigene1599_All	bifunctional proline dehydrogenase (RHH-type transcriptional regulator)	7.7
	Unigene1288_All	TetR family transcriptional regulator	3.5
	CL12.Contig1_All	cold-shock protein	10.3
	Unigene1079_All	hypothetical protein	2.6
	Unigene806_All	DNA-binding protein	8.1
	Unigene1296_All	ribosomal RNA small subunit methyltransferase B	6.5
	Unigene1431_All	transcription-repair coupling factor	2.2
Nucleic acid replication and repair	Unigene1069_All	DNA gyrase subunit B	5.5
	Unigene1583_All	DNA topoisomerase IV subunit B	4.9
	Unigene747_All	DNA topoisomerase I subunit omega	3.5
	Unigene15_All	DNA topoisomerase IV	2.5
	CL16.Contig5_All	DEAD/DEAH box helicase	37.8
	CL16.Contig4_All	DEAD/DEAH box helicase	30.7
	CL16.Contig2_All	DEAD/DEAH box helicase	28.8
	CL16.Contig1_All	DEAD/DEAH box helicase	28.2
	Unigene561_All	DEAD/DEAH box helicase	6.7
	CL16.Contig3_All	DEAD/DEAH box helicase	8.4
	Unigene382_All	ATP-dependent DNA helicase PcrA	4.8
	Unigene1417_All	replicative DNA helicase	2.4
	Unigene1094_All	DNA replication protein	2.4
	CL33.Contig2_All	DNA primase	2.6
	CL44.Contig1_All	primosomal protein N'	2.0
	Unigene1080_All	DNA mismatch repair protein mutS	2.6
	Unigene1679_All	DNA methyltransferase	4.4
	Unigene1611_All	DNA recombination/repair protein RecA	5.8
	Unigene545_All	single-stranded-DNA-specific exonuclease RecJ	4.4
	Unigene1520_All	excinuclease ABC subunit A	3.7
	Unigene543_All	DNA polymerase III subunit alpha	2.0
Translation	Unigene825_All	30S ribosomal protein S15	2.0
	Unigene1084_All	30S ribosomal protein S1	8.6
	Unigene1296_All	ribosomal RNA small subunit methyltransferase B	6.5
	Unigene559_All	ribosome biogenesis GTPase	2.8
	Unigene648_All	multifunctional CCA protein	5.3
	Unigene644_All	ribonuclease R	5.0

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Functional Group	Gene ID	Description	Fold Hange
	Unigene92_All	ribonuclease E	5.3
	Unigene792_All	SsrA-binding protein	4.7
	Unigene1622_All	peptidyl-prolyl cis-trans isomerase	2.0
	Unigene7_All	aspartyl/glutamyl-tRNA amidotransferase subunit B	3.1
	Unigene192_All	valine--tRNA ligase	2.0
	Unigene197_All	methionine--tRNA ligase	7.6
	Unigene369_All	cysteine--tRNA ligase	3.2
	Unigene290_All	aspartate--tRNA ligase	2.8
	Unigene201_All	glutamate--tRNA ligase	3.8
	Unigene189_All	threonine--tRNA ligase	4.2
	Unigene464_All	glutamine--tRNA ligase	3.9
	Unigene1081_All	tyrosine--tRNA ligase	4.3
	CL20.Contig1_All	phenylalanine--tRNA ligase subunit beta	4.5
	Unigene751_All	hypothetical protein/translation initiation factor IF-1	7.8
	CL53.Contig1_All	translation initiation factor IF-2	2.5
	Unigene1064_All	elongation factor P	3.6
	Unigene981_All	elongation factor P--(R)-beta-lysine ligase	2.8
	Unigene1285_All	peptide deformylase	4.2
Temperature response	Unigene560_All	molecular chaperone HscA/hsp70	17.8
	Unigene1443_All	molecular chaperone DnaJ/hsp70	10.3
	Unigene837_All	molecular chaperone DnaK/hsp70	7.9
	Unigene1424_All	molecular chaperone HtpG/hsp90	4.9
	Unigene1000_All	ATP-dependent chaperone ClpB	3.7
	CL12.Contig1_All	cold-shock protein	10.3
	Unigene1615_All	DNA-binding protein /Cold shock protein domain	2.5
	Unigene1345_All	ATP-dependent Clp protease ATP-binding subunit	9.6
Oxidative stress response	CL47.Contig2_All	alkyl hydroperoxide reductase subunit F	4.8
	Unigene902_All	Catalase	4.3
	Unigene1524_All	Superoxide dismutase	2.9
	Unigene446_All	thiol reductase thioredoxin	3.0
	Unigene1177_All	NAD(P)(+) transhydrogenase	5.9
	Unigene734_All	thiol:disulfide interchange protein	4.1
	Unigene740_All	flavoprotein	4.8
Cell division	Unigene328_All	Cell division protein ZipA	5.2
	Unigene1241_All	cell division protein FtsX	2.2
	Unigene940_All	cell division protein FstK	2.1
	Unigene1589_All	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso -diaminopimelate ligase	3.1