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Transcriptomic response of an Antarctic yeast *Rhodotorula* sp. USM-PSY62 to temperature changes

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ABSTRACT

Aims: *Rhodotorula* sp. (USM-PSY62) is a psychrophilic yeast isolated from Antarctic sea ice that grows optimally at 15 °C. The inevitable global warming poses many challenges to the microbial community in Antarctica. Therefore, this study was conceptualized to observe how USM-PSY62 adapted to fluctuations in temperature.
Methodology and results: *Rhodotorula* sp. (USM-PSY62) was grown in YPD broth until the mid-log phase. Then, the culture was transferred to four different temperatures, specifically at 0 °C, 5 °C, 15 °C and 21 °C for 24 h. Then, the RNA was extracted, sequenced and analysed. During cold adaptation, an elevated transcription of the CorA magnesium transporter gene in USM-PSY62 indicated a higher requirement for magnesium ions to gain additional enzyme cofactors or maintain cytoplasmic fluidity. The HepA homologue coding for DNA/RNA helicase was also over-expressed with log fold change 2.89 in cold conditions possibly to reorganize secondary structures of DNA and RNA. An up-regulation of the catalase gene was also observed, reflecting an increment in the concentration of reactive oxygen species and fluctuations in the associated antioxidant system. The *YOP1* gene, which encodes a membrane protein associated with protein transport and membrane traffic, was the most down-regulated, with $\log_2$ fold change values of -6.93 lower under cold shock conditions. The genes responsible for the structural maintenance of chromosome (*SMC*) have a -8.80 in expression $\log_2$ fold change, indicating the gene was down-regulated when the temperature was shifted to 0 °C. Upon cold shock, the gene for heat shock factor protein 1 (*HSF*1) was also down-regulated with a $\log_2$ fold change value of -5.86. Hsf1 is a transcriptional regulator which regulates the heat shock responses.
Conclusion, significance and impact of study: In conclusion, the transcriptomic responses demonstrated by *Rhodotorula* sp. USM-PSY62 characterized critical physiological and biochemical compensatory mechanisms especially cellular processes and signalling, information storage and processing, and metabolism to survive at low and high temperatures. This study provides valuable data for industry, especially in the usage of molecular chaperones.

Keywords: Antarctica, cold shock, heat shock, *Rhodotorula*, transcriptome, yeast

INTRODUCTION

Antarctica is widely recognized as the coldest continent, with an average annual temperature of -55 °C and wintry conditions that can reach as low as -85 °C (Russell, 2006). It is a sanctuary with unique environmental niches consisting of different types of soil, sediments, rocks, snow and deep ice that support various microorganisms with remarkable metabolisms (Lohan and Johnston, 2005; Russell, 2006). These microorganisms are considered to be the most abundant psychrophilic or cold-adapted organisms on earth, with high species diversity and biomass (Feller and Gerday, 2003). Psychrotrophs, like psychrophiles, can also be found in cold environments, but their maximal growth temperature can be above 20 °C (Morita, 1975). Both types of microorganisms play significant roles in the biodegradation of organic compounds and the cycling of essential nutrients (Russell, 1990; Vishniac, 2006).

Studies conducted on the genomes of cold-adapted bacteria and archaea have suggested the importance of the two-component signal transduction (TCS) system for

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their growth and survival. Genome analysis of *Paenibacillus* revealed the presence of TCS genes, including thermosensor kinase (DesK-DesR), phosphate regulation, chemotaxis, sigma factors, cold shock-related genes and transporters that play crucial roles in adapting to abiotic stimuli (Dsouza *et al*., 2014). Moreover, a comparative transcriptomic study on *Rhodococcus* sp. JG3 and *Polaromonas* sp. Eur31.2.1 identified some shared strategies to respond to low-temperature environments, such as an increase in nutrient transportation and stress responses. Upregulation of fatty acids and recombination was also observed (Raymond-Bouchard *et al*., 2018).

Numerous studies have shown that yeasts exhibit a remarkable ability to adapt to cold environments (Margesin *et al*., 2003; Turkiewicz *et al*., 2003). They possess unique physiological characteristics that enable them to survive in various ecological niches (Hagler and Ahearn, 1987; Starmer and Lachance, 2010). The presence of psychrophilic and psychrotrophic yeasts in cold ecosystems has been extensively documented (Fell, 2006; Raspor, 2006; Vishniac, 2006). Basidiomycetous yeasts are the most common species in cold environments, although some ascomycetous yeasts have also been reported (Connell *et al*., 2008; Branda *et al*., 2010; de García *et al*., 2010). Examples of yeast species isolated from cold environments include *Mrakia robertii*, *Mrakia blollopis*, *Mrakia niccombsii* (Thomas-Hall *et al*., 2010), *Rhodotorula arctica* (Vishniac and Takashima 2010), *Glaciozyma antarctica* (Wong *et al*., 2019), *Dioszegia antarctica* and *Dioszegia cryoxerica* (Connell *et al*., 2010). These findings illustrate the richness and diversity of cold environments, highlighting the potential for further exploration of their diversity.

The current era of global warming presents many challenges for microbial diversity in Antarctica, making it essential to understand the associated effects of temperature increases. Therefore, it is crucial to investigate how psychrophilic microbes adapt to fluctuations in growth temperature and shed new light on their survival mechanisms (Deegenaars and Watson, 1998). Psychrophiles grow and reproduce in low-temperature environments and use several methods for survival, such as maintaining the functionality of enzymes that tend to become rigid at lower temperatures and preserving the flexibility of cytoplasmic membranes to perform signaling and biochemical reactions (Ratkowsky *et al*., 2005). Enzymes that adapt to cold conditions gain more flexibility at lower temperatures (Hoyoux *et al*., 2004) and the active sites become more accessible for enzymatic reactions (Aghajari *et al*., 2003). The evolution of cold-shock proteins is another strategy for adapting to extremely low temperatures. These proteins are regularly synthesized in various organisms during extended periods of growth in the cold (Pikuta *et al*., 2007). This study conducted transcriptomic analysis on an Antarctic yeast, *Rhodotorula* sp. USM-PSY62, to uncover more unique adaptive mechanisms to temperature fluctuations and understand how these eukaryotic extremophiles thrive in their environments. This will also help us understand how psychrophilic microbes respond to environmental changes in a fluctuating ecosystem.

MATERIALS AND METHODS

Yeast strain and maintenance

The Antarctic sea ice sample collected at Casey Station yielded a basidiomycete strain identified as *Rhodotorula* sp. USM-PSY62 based on 18S rRNA (Ong, 2006). USM-PSY62 was cultured and preserved in YPD medium (1% yeast extract, 2% polypeptone and 2% dextrose) at 15 °C (Vellanki, 2013) and stored for long-term use by preparing a cell suspension in YPD supplemented with 20% glycerol and frozen at -80 °C.

Exposure of USM-PSY62 to different temperatures

To initiate the experiment, a starter culture of USM-PSY62 was first grown in YPD broth for five days at its optimal temperature of 15 °C. Next, a small volume of this starter culture was used to inoculate 100 mL of fresh YPD broth in 500 mL flasks to an OD600nm of approximately 0.1. These flasks were then incubated in a shaker at 150 rpm and 15 °C until the cells reached the mid-log phase after 7 days. The cultures were then transferred to four different temperatures, namely 0 °C, 5 °C, 15 °C and 21 °C, with two replicates for each temperature, and shaken at 150 rpm for 24 h. Following the exposure period, the cells were collected by centrifugation and the supernatant was removed. The cell pellets were then immediately snap-frozen in liquid nitrogen and stored at -70 °C until RNA extraction.

Total RNA extraction and RNA-Seq

The total RNA of USM-PSY62 cells was extracted using TRIzol® Reagent (Thermo Fisher Scientific, USA) following the manufacturer's instructions after homogenization using liquid nitrogen and a grinder. TURBO DNase (Thermo Fisher Scientific, USA) was used to eliminate DNA. The extracted RNA was assessed for integrity by denaturing agarose gel electrophoresis and the RNA concentration was quantified using Nanodrop-1000 (Thermo Fisher Scientific, USA). Samples with high RIN numbers were selected for transcriptome sequencing. The Illumina HiSeq 2000 platform was used to sequence the cDNA libraries obtained from purified RNA. The transcriptome results were verified using quantitative reverse-transcription PCR (qRT-PCR).

de novo transcriptome assembly and functional annotation

The clean data generated from the transcriptome sequencing underwent a de novo assembly process. To ensure high-quality and clean data, FastQC was utilized prior to data assembly. Trinity version r2013-11-10 (Grabherr *et al*., 2011; Haas *et al*., 2013) was employed as the assembler and the option for a high expectation of

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Table 1: List of primers used in qRT-PCR.

Primers name	Sequence (5'-3')
hsf	Forward primer 5' TGATGCCGCCGAAGACTTTA 3'
	Reverse primer 5' TGGTCCCACACAAGGCAATC 3'
hepA	Forward primer 5' TCGTTGATTCCCGAGAGCG 3'
	Reverse primer 5' CGTCTTGATCGTAACGTCGC 3'
corA	Forward primer 5' TCTAACCTTCTGCGGGCAAG 3'
	Reverse primer 5' CCGGCTCAGTCCTTCGTATC 3'
smc	Forward primer 5' CTTCGACAGACCACCGTCAA 3'
	Reverse primer 5' GCTGTTGACAGCTCCTCCTT 3'
yop1	Forward primer 5' TTCCTCCTCGCCAAACTGTC 3'
	Reverse primer 5' TCCAAGAGCTCGCAAAGGAC 3'
act	Forward primer 5' GGACTGCTCTGGATCACGTT 3'
	Reverse primer 5' GAGAACTCAGTCACGGGCAA 3'

Table 2: RNA-Seq sequencing output statistics of USM-PSY62. Total clean reads were obtained after removing the adapters and low-quality sequences. An average of 25 million reads were obtained for each sample with a GC value of 61.52%.

Sample	Total raw reads	Total clean reads	Total clean nucleotides	Q20 %	GC %
0 °C_Rep 1	28,478,626	24,479,720	2,447,972,000	97.73	61.25
0 °C_Rep 2	25,860,066	24,253,342	2,425,334,200	97.68	61.43
5 °C_Rep 1	26,258,816	24,270,378	2,427,037,800	97.78	61.15
5 °C_Rep 2	25,904,280	24,214,220	2,421,422,000	97.72	61.47
15 °C_Rep 1	26,689,456	24,338,464	2,433,846,400	97.72	61.40
15 °C_Rep 2	27,375,710	23,654,056	2,365,405,600	92.23	61.68
21 °C_Rep 1	26,645,038	24,613,284	2,461,328,400	97.69	61.98
21 °C_Rep 2	28,342,708	24,505,100	2,450,510,000	92.05	61.81

paired reads with high density with UTR overlap (jaccard_clip) was used, with the minimum assembled contig length set to 100. The processed transcripts were classified using the eggNOG public database of orthology relationships, gene evolutionary histories and functional annotations using the Trinotate suite (http://trinotate.sourceforge.net/). Transcripts with more than 60% similarity to bacterial species based on BLASTN results were filtered away and the similarity of transcripts to selected organisms, including *Escherichia coli*, *Saccharomyces cerevisiae*, *Rhodotorula mucilaginosa* and *Caenorhabditis elegans*, was observed to refine the transcriptome assembly further.

Transcripts abundance estimation and differential expression analysis

The RNAseq Expectation-Maximization (RSEM) method (Li and Dewey, 2011) was utilized to perform transcript counts. The gene expression levels were normalized as fragments per kilobase of exon region per million mapped reads (FPKM). To identify the differentially expressed transcripts between the samples, the EdgeR software was applied (Robinson *et al*., 2010). TMM normalization was then performed to adjust for the differences in library sizes. The significance of the gene expression levels, normalized by TMM, was determined. The differential expression (DE) transcripts were identified as those which exhibited at least two-fold differences at a false discovery-corrected statistical significance of 0.001 or lower.

Quantitative-reverse transcription (qRT-PCR)

Validation of transcriptomic data was carried out using qRT-PCR. The genes and primers that were used for the experiment are listed in Table 1. The iTaq™ Universal SYBR® Green One-Step Kit (Bio-Rad, USA) was used to conduct qTR-PCR, with all experiments being conducted in triplicate. The *Actin* gene was used to serve as the internal control.

RESULTS

Raw data and sequence assembly

The RNA-Seq post-filtering and assembly generated relatively consistent raw data, with an average of around 25 million reads per sample and an average GC content of 61.52%. Table 2 provides a summary of the raw data. The de novo assembly of the raw data produced a total of 34170 contig sequences ranging from 101 bp to 9227 bp in length. The median and mean lengths of the contigs were 309 bp and 673 bp, respectively, with an N50 value of 1400 bp. The overall assembly statistics generated by Trinity are shown in Table 3. Figure 1 illustrates that the most abundant contig lengths in the assembly were 101 bp to 500 bp, while the least abundant was 2501 bp to 3000 bp. Alignment of the transcripts to selected species revealed that 2.99% mapped to the *E. coli* genome, 0.17% to *S. cerevisiae*, 8.92% to *R. mucilaginosa* and 0.57% to *C. elegans*. The raw data are available in NCBI

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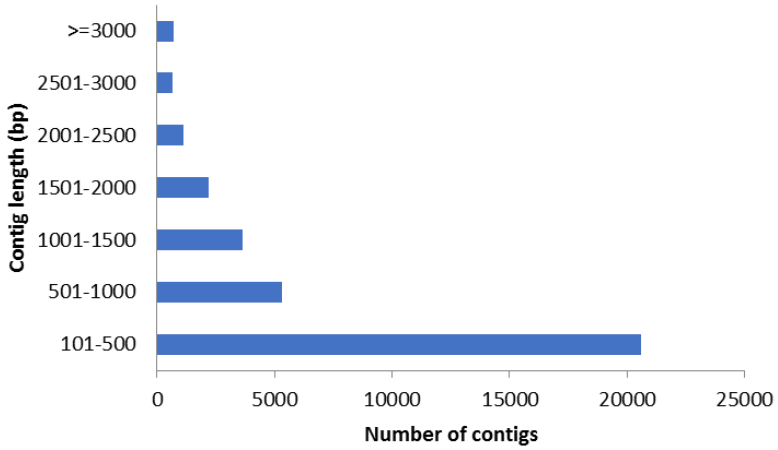

Figure 1: Graph showing the length distribution of Trinity-assembled contigs. A total of 34170 contigs were assembled. The most abundant contig lengths in the assembly were in the range of 101 bp to 500 bp, while the least abundant were in the range of 2501 bp to 3000 bp.

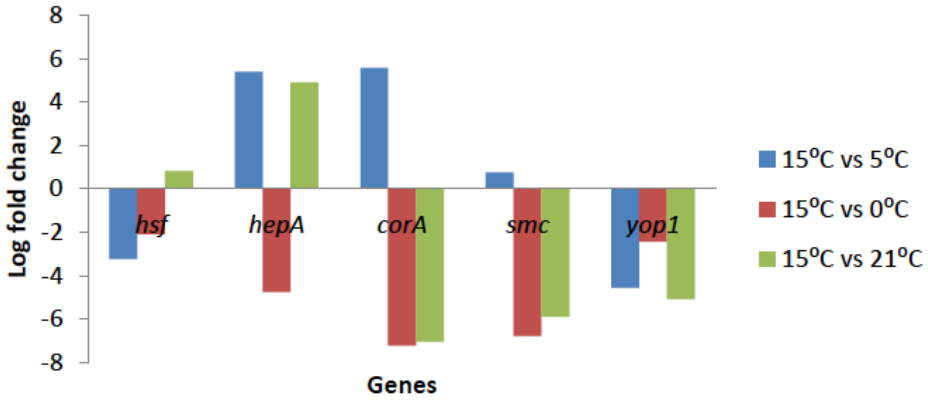

Figure 2: Log fold-change of *hsf*, *hepA*, *corA*, *smc* and *yop1* genes. The validation of transcriptome analysis was performed using qRT-PCR. The relative expression of each gene was normalized using the actin gene and analyzed using Livak's ($2^{-\Delta\Delta Cq}$). The results corresponded with transcriptome analysis.

Table 3: Trinity assembly statistics. From the assembly, the total number of transcripts generated were 34170 with a minimum contig length of 101 bp and a maximum of 9227 bp. The average contig length was 673 bp with a GC content of 62.47%.

Description	Value
Total Trinity transcripts	34170
Total bases	22,966,978
Minimum contig length	101 bp
Maximum contig length	9227 bp
Average contig length	673 bp
Median contig length	309 bp
Contig N50	1400 bp
(A + T)s	37.53%
(G + C)s	62.47%
Ns	0.00%

under BioProject ID PRJNA522043.

Differentially expressed transcripts and functional annotation

Three pairwise comparisons were performed on the transcriptomes, namely 15 °C vs 5 °C, 5 °C vs 0 °C and 15 °C vs 21 °C. A total of 133 transcripts were identified to be differentially expressed at least two-fold, with some overlaps observed among the three pairwise comparisons (refer to Tables 4-6). The significance threshold was set at a false discovery-corrected statistical significance of 0.001 based on the transcripts' normalized expression values, which were measured in FPKM (fragments per feature kilobase per million reads mapped). However, before generating the FPKM values, the RNA-Seq count data underwent normalization using the TMM method.

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Table 4: Brief description based on functional groups of 45 transcripts represented in Figure 2, where *Rhodotorula* sp. USM-PSY62 was exposed to a temperature shift from 15 °C to 5 °C.

No.	Transcript ID	Log fold change	Expression level	Accession number
Cellular processes and signalling				
1	comp2729_c0_seq1	-2.643854	down	COG0330
2	comp2184_c0_seq1	-1.263762	down	COG0702
3	comp6513_c0_seq1	-1.493231	down	COG0071
4	comp15275_c0_seq1	-1.695049	down	COG5074
5	comp12847_c0_seq1	9.705356	up	COG4238
6	comp6405_c0_seq1	-6.931226	down	COG5052
7	comp8473_c0_seq9	-2.430342	down	COG0642
Information storage and processing				
8	comp7487_c0_seq2	8.018526	up	COG1670
9	comp8916_c0_seq6	-6.37717	down	COG0507
10	comp8916_c0_seq10	1.746675	up	COG0507
11	comp8916_c0_seq3	3.015206	up	COG0507
12	comp8916_c0_seq1	-8.012335	down	COG0507
13	comp8936_c0_seq12	9.042724	up	COG0553
14	comp8936_c0_seq4	2.409688	up	COG0553
15	comp8191_c0_seq3	2.876829	up	COG0553
16	comp8936_c0_seq8	-1.634288	down	COG0553
17	comp8739_c1_seq3	1.688598	up	COG5179
18	comp5490_c0_seq1	-1.228738	down	COG5084
19	comp2135_c0_seq2	1.620837	up	COG5147
20	comp5468_c0_seq1	-5.856946	down	COG5169
21	comp8426_c1_seq6	2.20702	up	COG5256
Metabolism				
22	comp6898_c1_seq2	-1.841753	down	COG0477
23	comp4572_c0_seq1	-2.040901	down	COG0477
24	comp11718_c0_seq1	-1.4509	down	COG1028
25	comp6796_c0_seq1	-1.300862	down	COG1902
26	comp7209_c0_seq2	-2.670481	down	COG2273
27	comp7230_c0_seq2	-8.091322	down	COG1893
28	comp13825_c0_seq1	-1.331793	down	COG1028
29	comp8089_c0_seq4	-1.615364	down	COG0604
30	comp7629_c0_seq3	-5.659021	down	COG0604
31	comp14257_c0_seq1	-1.210936	down	COG0807
32	comp8113_c0_seq6	1.471048	up	COG1785
33	comp6337_c0_seq1	-2.091992	down	COG2124
34	comp3495_c0_seq2	-1.842732	down	COG3250
35	comp8089_c0_seq3	-2.837434	down	COG0604
36	comp3523_c0_seq1	1.597053	up	COG0534
37	comp8278_c0_seq4	6.999321	up	COG0598
38	comp7430_c0_seq2	-3.390306	down	COG0604
39	comp8366_c0_seq2	-4.041479	down	COG1472
40	comp5954_c0_seq2	-2.249075	down	COG0408
41	comp8366_c0_seq4	-3.486855	down	COG1472
42	comp6452_c0_seq1	-1.474342	down	COG0807
43	comp6069_c1_seq1	-1.380557	down	COG2072
44	comp8844_c0_seq2	2.812792	up	COG2937
45	comp2593_c0	2.6337254	up	COG27164

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Table 5: Brief description based on functional groups of 47 transcripts represented in Figure 3(A-C), where *Rhodotorula* sp. USM-PSY62 was exposed to a temperature shift from 5 °C to 0 °C.

No.	Transcript ID	Log fold change	Expression level	Accession number
Cellular processes and signalling				
1	comp6513_c0_seq1	1.129581	up	COG0071
2	comp3254_c1_seq1	-1.018463	down	COG0326
3	comp6918_c0_seq1	-8.803772	down	COG1196
4	comp6405_c0_seq1	-1.387866	down	COG5052
5	comp4593_c0_seq2	1.176855	up	COG1215
6	comp7922_c4_seq2	7.138811	up	COG5391
7	comp8473_c0_seq9	1.93212	up	COG0642
8	comp7948_c0_seq3	1.251941	up	COG0642
Information storage and processing				
9	comp7487_c0_seq2	-5.560906	down	COG1670
10	comp8916_c0_seq6	-1.868045	down	COG0507
11	comp8916_c0_seq10	6.261352	up	COG0507
12	comp8916_c0_seq1	1.590589	up	COG0507
13	comp8936_c0_seq12	-7.436077	down	COG0553
14	comp7744_c0_seq3	7.384942	up	COG0553
15	comp6218_c0_seq1	-2.007918	down	COG5169
16	comp9006_c1_seq2	2.325696	up	COG5211
17	comp8936_c0_seq4	-2.195524	down	COG0553
18	comp8191_c0_seq3	6.03144	up	COG0553
19	comp8936_c0_seq8	10.16288	up	COG0553
20	comp8739_c1_seq3	5.647731	up	COG5179
21	comp5490_c0_seq1	-1.547657	down	COG5084
22	comp5468_c0_seq1	-2.241933	down	COG5169
Metabolism				
23	comp2909_c1_seq1	3.184432	up	COG0477
24	comp8176_c0_seq2	1.43372	up	COG0477
25	comp6898_c1_seq2	1.137336	up	COG0477
26	comp4572_c0_seq1	1.063855	up	COG0477
27	comp8732_c0_seq1	1.720333	up	COG0477
28	comp11718_c0_seq1	1.589077	up	COG1028
29	comp7209_c0_seq2	3.95132	up	COG2273
30	comp7230_c0_seq2	-1.822073	down	COG1893
31	comp13825_c0_seq1	1.839644	up	COG1028
32	comp8089_c0_seq4	2.621868	up	COG0604
33	comp14257_c0_seq1	-1.192267	down	COG0807
34	comp8113_c0_seq6	-2.119545	down	COG1785
35	comp9040_c0_seq32	7.775199	up	COG2303
36	comp3495_c0_seq2	2.738382	up	COG3250
37	comp8089_c0_seq3	2.604183	up	COG0604
38	comp3523_c0_seq1	6.589149	up	COG0534
39	comp8278_c0_seq4	-8.863971	down	COG0598
40	comp7430_c0_seq2	1.253994	up	COG0604
41	comp8089_c0_seq2	3.820099	up	COG0604
42	comp8366_c0_seq2	8.047347	up	COG1472
43	comp8592_c0_seq12	5.558765	up	COG0531
44	comp8366_c0_seq4	5.263325	up	COG1472
45	comp6452_c0_seq1	1.099371	up	COG0807
46	comp6069_c1_seq1	-1.044898	down	COG2072
47	comp2593_c0	-2.956503	down	COG27164

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Table 6: List of 41 transcripts that were differentially expressed at least two-fold under conditions from 15 °C to 21 °C, sorted according to eggNOG predictions.

No.	Transcript ID	Log fold change	Expression level	Accession number
Cellular processes and signalling				
1	comp7774_c1_seq2	8.011089	up	COG5074
2	comp6513_c0_seq1	1.904158	up	COG0071
3	comp3272_c0_seq2	1.736735	up	COG0386
4	comp6918_c0_seq1	-8.71795	down	COG1196
5	comp6405_c0_seq1	-6.848557	down	COG5052
6	comp7922_c4_seq2	-1.471228	down	COG5391
7	comp8473_c0_seq9	5.981473	up	COG0642
8	comp7948_c0_seq3	2.782955	up	COG0642
Information storage and processing				
9	comp8916_c0_seq6	-8.292012	down	COG0507
10	comp8916_c0_seq10	4.544521	up	COG0507
11	comp8916_c0_seq3	3.208995	up	COG0507
12	comp8916_c0_seq1	-6.989533	down	COG0507
13	comp7744_c0_seq3	7.813475	up	COG0553
14	comp8936_c0_seq4	10.47262	up	COG0553
15	comp8191_c0_seq3	9.073612	up	COG0553
16	comp8936_c0_seq8	-1.474423	down	COG0553
17	comp2135_c0_seq2	7.809846	up	COG5147
18	comp8426_c1_seq6	1.16017	up	COG5256
Metabolism				
19	comp8176_c0_seq2	1.348369	up	COG0477
20	comp6898_c1_seq2	1.026357	up	COG0477
21	comp4572_c0_seq1	1.813883	up	COG0477
22	comp8732_c0_seq1	1.643989	up	COG0477
23	comp9014_c0_seq4	2.028149	up	COG0277
24	comp9040_c0_seq8	-8.432328	down	COG2303
25	comp6796_c0_seq1	1.319276	up	COG1902
26	comp7230_c0_seq2	-8.361887	down	COG1893
27	comp13825_c0_seq1	2.271468	up	COG1028
28	comp8089_c0_seq4	2.619352	up	COG0604
29	comp8113_c0_seq6	-1.531392	down	COG1785
30	comp7773_c0_seq2	1.408593	up	COG0281
31	comp8089_c0_seq3	2.037236	up	COG0604
32	comp8592_c0_seq1	-8.392003	down	COG0531
33	comp3523_c0_seq1	-1.252989	down	COG0534
34	comp8089_c0_seq2	3.721356	up	COG0604
35	comp8113_c0_seq4	7.728536	up	COG1785
36	comp5954_c0_seq2	2.103894	up	COG0408
37	comp8366_c0_seq4	-1.07985	down	COG1472
38	comp8366_c0_seq3	7.650236	up	COG1472
39	comp4669_c0_seq1	2.011104	up	COG0340
40	comp2862_c0_seq1	1.062615	up	COG3000
41	comp5671_c0_seq2	1.329442	up	COG3000

The differentially expressed transcripts are depicted in Tables 4-6.

The qRT-PCR validation graph is given in Figure 2. Based on the transcriptomic analysis, five transcripts with significant up- or down-regulation in expression were chosen for qRT-PCR validation. The relative expression of each transcript was normalized against the expression of a control gene, actin, and analyzed using Livak's ($2^{-\Delta\Delta Cq}$) method. To validate the transcriptomic analysis, the qRT-PCR result was compared against it. As an example, the down-regulated expression of HSF1 (comp5468_c0_seq1) by a $\log_2$ fold change value of -5.86 (15 °C to 5 °C) was similarly illustrated by qRT-PCR technique, which showed a $\log_2$ fold change value of approximately -3.00 (Figure 2).

The differentially expressed transcripts were classified into three main groups based on the eggNOG annotations: 'cellular processing and signaling', 'information storage and processing' and finally, 'metabolism'.

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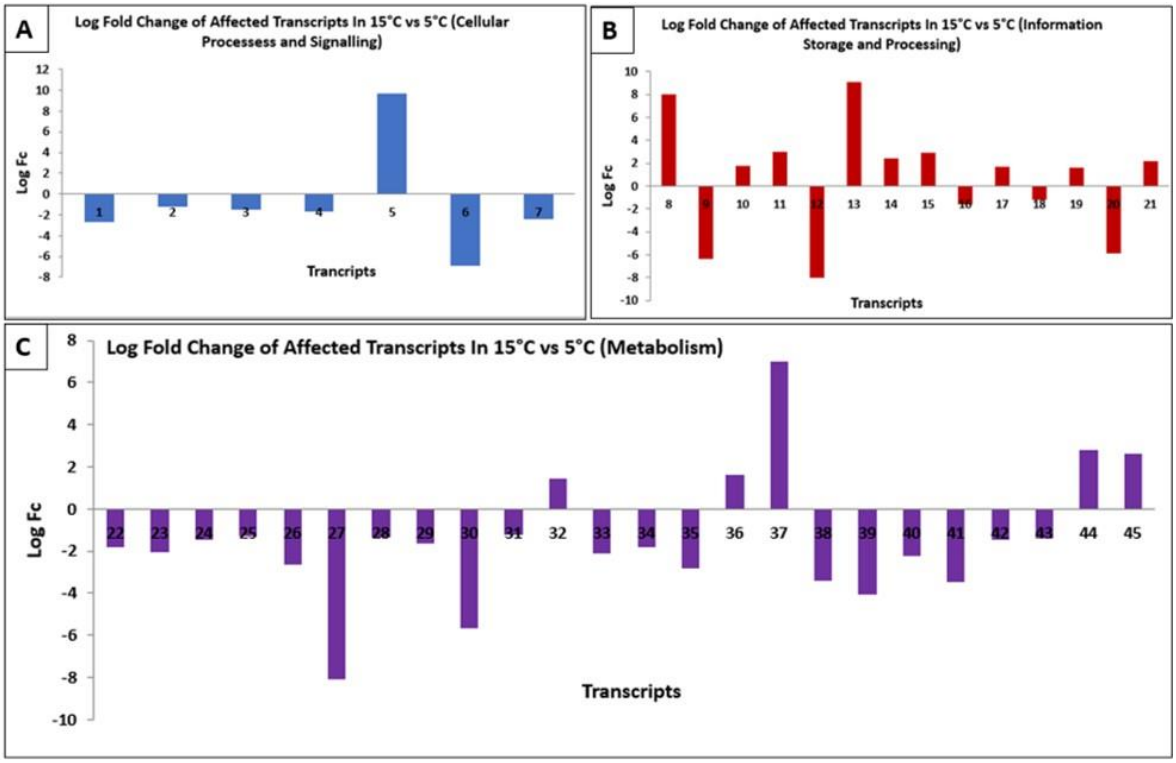

Figure 3: Graphs showing log fold change of transcripts expression when temperature shifted from 15 °C to 5 °C. (A) shows affected transcripts under the "Cellular Processes and Signalling" category, (B) shows affected transcripts under "Information Storage and Processing" and (C) shows affected transcripts under "Metabolism".

Bar graphs representing the detailed response of every differentially expressed transcript between cold adaptation responses (15 °C to 5 °C and 5 °C to 0 °C) and heat adaptation responses (15 °C to 21 °C) are shown in Figure 3, Figure 4 and Figure 5, respectively.

DISCUSSION

Organisms face the challenge of acclimatizing to stressful environmental conditions in order to survive. One common source of stress in ecosystems is changes in ambient temperature, which disrupts the metabolism of the inhabitants. The populations must adapt in order to thrive in their new environment (Hoffmann and Hercus, 2000). These changes primarily affect the chemical and enzyme-based reactions within the organisms and the structural composition of their cellular components. Temperature shifts also trigger intricate cellular responses involving genes, with transcriptional reactions engaging numerous genes that can be classified based on the external variations that were imposed.

Organisms' ability to adapt to changes in their environment is crucial for their survival and this is particularly true for psychrophilic yeasts like *Rhodotorula* sp. USM-PSY62. In order to understand how this yeast adapts to changing conditions, we investigated its physiological response to cold and heat shocks. Cold-shock was induced by reducing the temperature from its optimal 15 °C to 5 °C and 0 °C, while heat-shock was induced by increasing the temperature from 15 °C to 21 °C. Psychrophilic microorganisms typically adjust the expression of several genes in response to sudden temperature changes, either up or down-regulating them (De Maayer *et al.*, 2014). These physiological responses are initiated by the recognition of the temperature stress, which then triggers a cascade of adaptive responses within the cellular machinery (Xiong *et al.*, 2002).

Under cold shock conditions, the *YOP1* gene in USM-PSY62 showed the most significant down-regulation in expression, with log2 fold change values of -6.93 (comp6405_c0_seq1; Table 3; Figure 3A; 15 °C to 5 °C) and -1.39 (comp6405_c0_seq1; Table 4; Figure 4A; 5 °C to 0 °C), respectively. Yop1 is a membrane protein that works with other proteins to create the protein transport machinery in eukaryotic cells. Specifically, Yop1 binds to Yip1p, which is crucial in mediating membrane traffic (Calero *et al.*, 2001). These proteins are involved in many aspects of membrane traffic, including vesicle formation, movement along actin and tubulin networks, and membrane fusion, and undergo a cycle of binding and hydrolysis during protein transportation (Calero *et al.*, 2001). The decrease in *YOP1* expression could be due to the reduction in cell membrane fluidity and membrane-associated functions caused by cold stress. In general, psychrophilic microorganisms detect rigidification of the membrane as the primary signal when exposed to low

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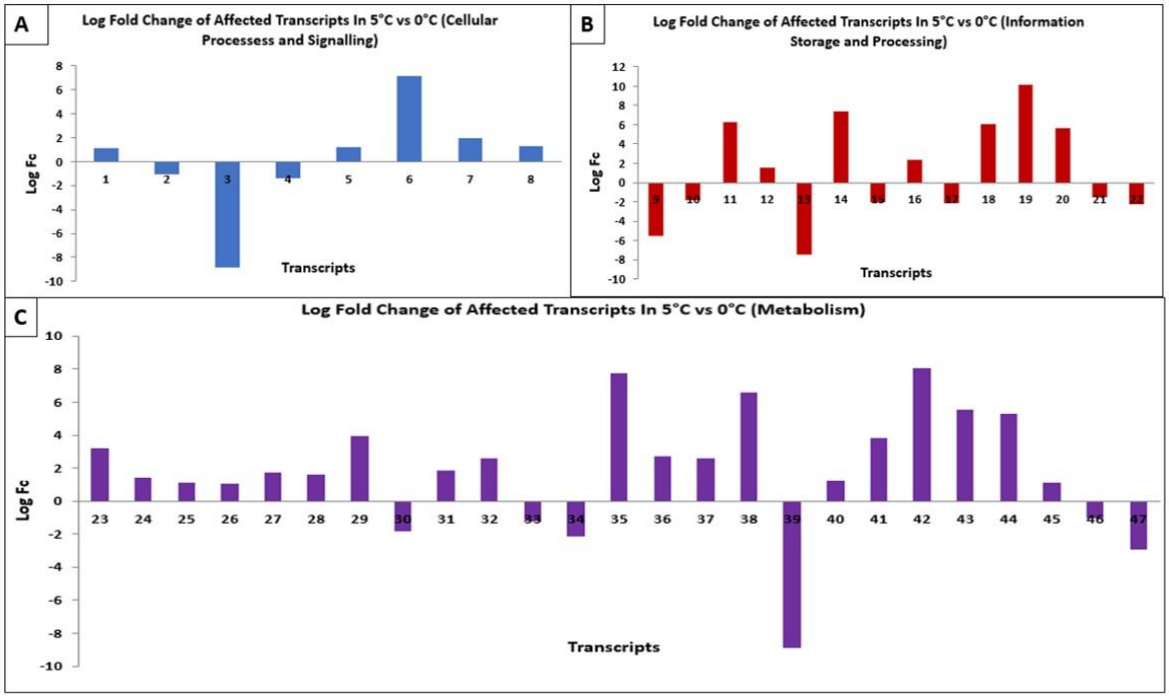

Figure 4: Graphs showing log fold change of transcripts expression when temperature shifted from 5 °C to 0 °C. (A) shows affected transcripts under the "Cellular Processes and Signalling" category; (B) shows affected transcripts under "Information Storage and Processing;" and (C) shows affected transcripts under "Metabolism".

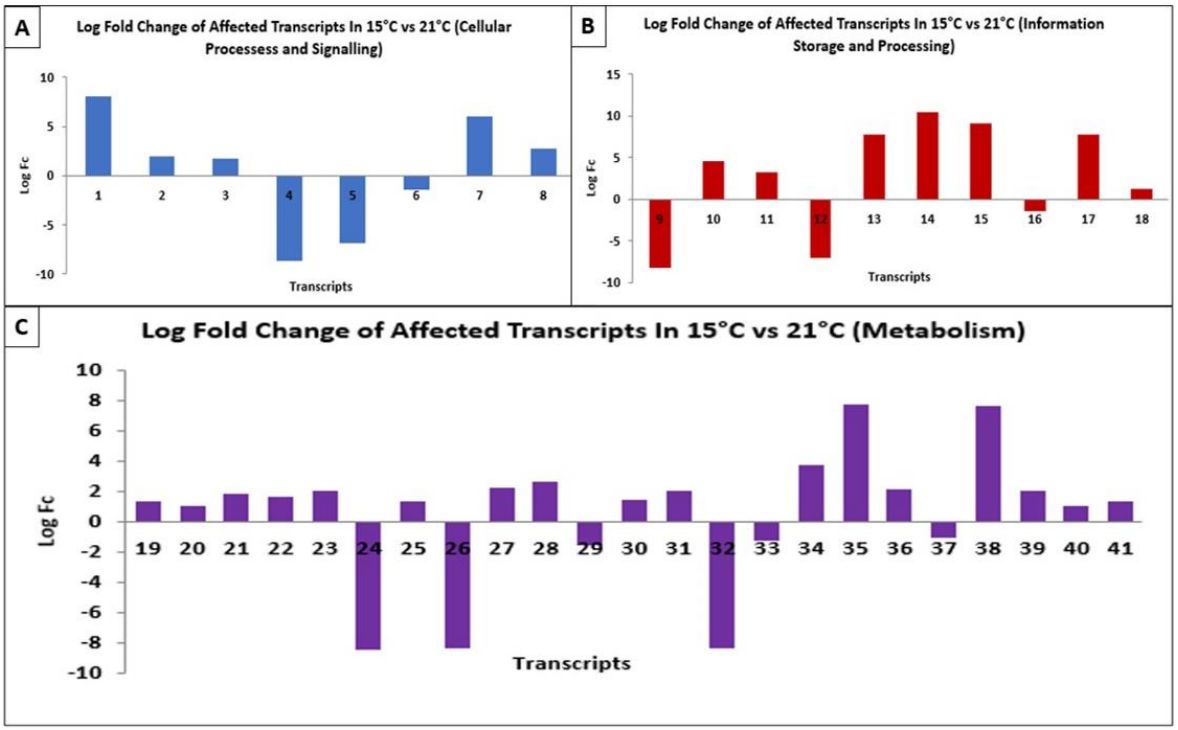

Figure 5: Graphs showing log fold change of transcripts expression when temperature shifted from 15°C to 21°C. (A) shows affected transcripts under the "Cellular Processes and Signalling" category; (B) shows affected transcripts under "Information Storage and Processing;" and (C) shows affected transcripts under "Metabolism."

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temperature, which triggers a series of cold adaptation mechanisms (Shivaji and Prakash, 2010). USM-PSY62 might down-regulate YOP1 to prevent cell death, as over-expression could inhibit growth and enlarge cellular size, possibly due to the accumulation of internal membrane structures. Moreover, over-expression of *YOP1* could interfere with the transport and trafficking of vesicles from the endoplasmic reticulum to the Golgi apparatus (Calero *et al.*, 2001).

Under the cold-shock condition of 15 °C to 5 °C, the gene for murein lipoprotein (comp12847_c0_seq1; Table 4; Figure 3A) exhibited an up-regulation with log2 fold change values of 9.71, which may have been in response to restore the fluidity of the cell membrane. The murein lipoprotein is a component involved in the biogenesis of the outer cell membrane envelope. Previous reports have shown that it is also involved in cell envelope assembly (Braun and Bosch, 1972), cell division (Torti and Park, 1976; Weigand *et al.*, 1976) and the passive transport of small molecules through the outer membrane (Inouye, 1974). Additionally, the up-regulation of the murein lipoprotein gene may have been a compensatory response for the reduced expression of *YOP1*, which led to decreased cell membrane fluidity and membrane-associated functions.

The gene responsible for the structural maintenance of chromosome (*SMC*) exhibited a down-regulation with a log2 fold change value of -8.80 (comp6918_c0_seq1; Table 5; Figure 4A) when the temperature was lowered from 5 °C to 0 °C. SMC proteins are crucial in ensuring the proper segregation of chromosomes during mitosis and a wide range of chromosomal events (Losada and Hirano, 2005; Nasmyth and Haering, 2005). These proteins are present in almost all organisms, from bacteria to humans, in large numbers (Hirano, 2006). They play a vital role in chromosomal condensation, segregation, cohesion, and recombinational DNA repair in eukaryotes, as well as introducing positive supercoiling into DNA *in vitro* (Graumann, 2001). The down-regulation of the SMC protein genes in USM-PSY62 indicated a change in chromatin structure in response to cold shock. This observation is consistent with a previous study that showed a change in DNA curvature toward negative supercoiling at low temperatures (Barria *et al.*, 2013). The down-regulation of SMC proteins could potentially result in negative supercoiling of nucleic acids as a response to cold temperatures. The increased DNA-negative supercoiling may, in turn, elicit more cold adaptive responses (Barria *et al.*, 2013).

Low temperatures lead to decreased transcriptional and translational activity of cells due to the stabilization of DNA and RNA molecules (Satyanarayana *et al.*, 2005). In response, cells overproduce DNA and RNA helicases to maintain protein expression by reorganizing the secondary structures of DNA and RNA molecules (Barria *et al.*, 2013). This adaptive response was observed in USM-PSY62, where a putative homologue of DNA/RNA helicase, HepA, was overexpressed under cold conditions. The *hepA* homologues showed significant up-regulation with log2 fold change values of 2.89 and 6.03 (comp8191_c0_seq3; Table 4 and 6, respectively) from 15 °C to 5 °C and 5 °C to 0 °C, as well as 9.04 (comp8936_c0_seq12; Table 4) and 10.2 (comp8936_c0_seq8; Table 5) from 15 °C to 5 °C and 5 °C to 0 °C, respectively. This finding is consistent with previous studies on psychrophiles (De Maayer *et al.*, 2014). The *hepA* homologue was among the many genes that were strongly up-regulated in response to cold exposure. Cold-induced RNA helicases can destabilize the secondary structures of DNA and RNA molecules and prevent interference in transcription (De Maayer *et al.*, 2014).

The metabolism of cells plays a crucial role in providing the necessary energy and components to sustain cellular functions. At low temperatures, Antarctic basidiomycetous yeast cells were observed to prioritize survival-related metabolisms rather than growth (Amato *et al.*, 2009). Under cold-shock conditions, the expression of the homologue coding for CorA was up-regulated with a log2 fold change value of 7.0 (comp8278_c0_seq4; Table 4; Figure 3C; 15 °C to 5 °C) in USM-PSY62. The CorA protein family is essential for transporting magnesium ions in both prokaryotic and eukaryotic cells, facilitating the uptake and distribution of these ions to subcellular compartments (Moomaw and Maguire, 2008). The increased transcription of CorA homologues in USM-PSY62 suggests a higher requirement for magnesium ions as cofactors for enzymes and proteins induced during cold-shock conditions. Apart from transporting magnesium ions, CorA proteins may also transport other metal ions. In prokaryotes, CorA proteins have been observed to uptake cobalt ions (Hmiel *et al.*, 1986). The presence of CorA proteins could also increase the ionic concentration in the cell cytoplasm, which decreases the freezing point of the cytoplasmic fluid and prevents ice crystal damage, which can be detrimental to cells (Sherwood *et al.*, 2012). To maintain cell fluidity, USM-PSY62 may have increased its ionic intake. Other Antarctic yeast species, such as *Mrakia psychrophila* and *M. blollopis*, have up-regulated glycerol 3-phosphate dehydrogenase genes to prevent osmotic shrinkage during cold stress acclimation (Wolfe and Bryant, 1999; Su *et al.*, 2016; Tsuji, 2018). Additionally, the sea ice psychrophilic basidiomycete *Glaciozyma antarctica* PI12 was found to possess nine antifreeze proteins to adapt to sub-zero freezing conditions (Firdaus-Raih *et al.*, 2018).

Organisms commonly respond to environmental stresses by increasing the expression of heat shock proteins (HSPs). These genes are activated in response to high-temperature conditions, which can cause severe damage to cellular proteins and membranes, leading to protein malfunction (Richter *et al.*, 2010). As molecular chaperones, HSPs play a protective role by alleviating the harmful effects of protein misfolding and degradation. While the response of HSPs to heat stress has been widely studied, their response to cold stress has been less explored. HSPs are associated with both extremely cold and heat shock conditions, as they are rapidly up-regulated in response to stress until favourable conditions prevail again (Rinehart, 2007).

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Although the heat shock response of mesophilic and thermophilic microorganisms has been well documented, there is still limited knowledge of this phenomenon in psychrophilic microorganisms. Psychrophilic yeasts have been shown to produce heat shock proteins (HSPs) as a defence mechanism (Buzzini *et al.*, 2012). In this study, the gene for heat shock factor protein 1 (HSF1) was down-regulated in response to a shift in temperature (comp6218_c0_seq1; Table 5; Figure 4B; 15 °C to 5 °C, comp5468_c0_seq1; Table 5; Figure 4B; 5 °C to 0 °C and comp5468_c0_seq1; Table 4; Figure 3B; 15 °C to 5 °C). Hsf1 is a transcriptional regulator that belongs to a group of proteins called heat shock factors (HSFs), which are involved in regulating the heat shock response. Under normal conditions, Hsf1 exists in a complex with Hsp40/70 or Hsp90 as an inactive monomer (Branda *et al.*, 2010). During thermal stress, Hsf1 is released from the chaperone complex and undergoes trimerization. Hsf1 is then transported to the nucleus, where it becomes hyperphosphorylated and binds to DNA containing heat shock elements, which consist of repeating five-nucleotide units of NGAAN (Shamovsky and Nudler 2008). Hsf1 has a helix-turn-helix DNA-binding domain that enables it to bind to DNA (Bonner *et al.*, 1992). Interestingly, overexpression of HSF1 halts the vegetative growth of haploid yeast cells, while deletion or null mutants are lethal. Temperature-sensitive mutants have been found to be defective in several processes, including preservation of cell wall integrity, duplication of spindle pole body, and transportation of proteins (Boeke *et al.*, 1984; Oettinger and Struhl, 1985; Sadler *et al.*, 1989; Mosser *et al.*, 1990).

One of the responses exhibited by USM-PSY62 during the stepped down-shifts of temperature was the up-regulation of the gene for catalase (CAT, EC 1.11.1.6). Catalase, an antioxidant enzyme present in all aerobic organisms, decomposes hydrogen peroxide (H_2O_2) into the water and molecular oxygen and oxidizes low molecular alcohols, nitrites and formate that are generated during biological oxidation when H_2O_2 concentrations are low (Kurutas, 2016). The expression of CAT in USM-PSY62 was up-regulated with a log2 fold change value of 2.6 (comp2593_c0; Table 4; Figure 3C; 15 °C to 5 °C) when the temperature shifted from 15 °C to 5 °C. A similar observation was made in *S. cerevisiae*, in which a downshift in the growth temperature from 30 °C to 10 °C resulted in increased transcript levels of genes encoding catalase T (CTT1), CuZn superoxide dismutase (SOD1) and γ-glutamylcysteine synthetase (GSH1), which are involved in the antioxidant response, suggesting that downshifting temperature could lead to oxidative stress (Zhang *et al.*, 2003). In *Arabidopsis thaliana* and wheat, low-temperature stress prompts the sub-cellular antioxidant systems to safeguard photosynthetic electron transport (Caverzan *et al.*, 2016; Juszczak *et al.*, 2016).

In addition to the heat shock response, metabolic strategies are also crucial for cold adaptation. *G. antarctica* PI12 and *M. blollopis*, for instance, have been found to differentially express genes involved in carbon metabolism and energy production under low-temperature conditions (Firdaus-Raih *et al.*, 2018; Tsuji, 2018). Similarly, USM-PSY62 also employs these strategies to cope with the effects of low temperatures. For example, the genes encoding homologues of ProP (permeases of the major facilitator superfamily; comp2909_c1_seq1; Table 5; Figure 4C), BglX (beta-glucosidase-related glycosidases; comp8366_c0_seq2 & comp8366_c0_seq4; Table 5; Figure 4C), and LacZ (beta-galactosidase; comp3495_c0_seq2; Table 5; Figure 4C), which are involved in carbohydrate transport metabolism, were significantly up-regulated at lower temperatures. Genes involved in amino acid transport and metabolism, such as those encoding PotE (amino acid transporter; comp8592_c0_seq12; Table 5; Figure 4C) and BetA (choline dehydrogenase and related flavoproteins; comp9040_c0_seq32; Table 5; Figure 4C), were also up-regulated. Furthermore, a gene involved in energy production and conversion, namely *qor*, which encodes a NADPH:quinone reductase and related Zn-dependent oxidoreductase (comp8089_c0_seq4, comp8089_c0_seq3, comp8089_c0_seq2; Table 5; Figure 4C), was transcriptionally enhanced.

In conclusion, the transcriptomic responses demonstrated by USM-PSY62 characterized critical physiological and biochemical compensatory mechanisms to survive at low as well as high temperatures. The responses have relevance to its survival and are essential for the endurance of eukaryotic microbes in the freezing Antarctic environment. Bioinformatics studies on species other than model organisms are crucial to uncovering new molecular pathways or new genes responsible for adaptation to thermal stresses.

CONCLUSION

In conclusion, this study examined how the psychrophilic yeast *Rhodotorula* sp. (USM-PSY62) adapted to temperature fluctuations, which is an important consideration given the potential impact of global warming on the microbial community in Antarctica. The transcriptomic analysis of USM-PSY62 showed that the yeast exhibited critical physiological and biochemical compensatory mechanisms, including cellular processes and signalling, information storage and processing, and metabolism, to survive at low and high temperatures. The up-regulation of genes such as CorA magnesium transporter, HepA homologue coding for DNA/RNA helicase, and catalase, as well as the down-regulation of genes such as YOP1 and HSF1, provided valuable insights into the molecular mechanisms underlying the yeast's adaptation to temperature changes. These findings could have significant implications for industry, particularly in the usage of molecular chaperones. Overall, this study adds to our understanding of how microorganisms adapt to changing environmental conditions and highlights the importance of studying their responses to such changes.

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