Sains Malaysiana 54(7)(2025): 1847-1857
http://doi.org/10.17576/jsm-2025-5407-17

Thermal Adaptation Response of *Glaciozyma antarctica* PI12: Statistical Analysis of Gene Expression under Temperature Conditions

(Tindak Balas Penyesuaian Terma *Glaciozyma antarctica* PI12: Analisis Statistik Pengekspresan Gen di bawah Keadaan Suhu)

NURUL SARAH IZZATI ZAHID & NORA MUDA*

Department of Mathematical Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

Received: 2 April 2024/Accepted: 7 May 2025

ABSTRACT

Glaciozyma antarctica PI12, a psychrophilic yeast isolated from Antarctic Sea ice, exhibits remarkable cold adaptation mechanisms. While its optimal growth occurs at 12 °C, its transcriptional response across broader temperature ranges remains poorly understood. We analysed the expression patterns of 7,853 genes under varying temperatures (-12 °C to 20 °C) and exposure times (6 and 24 h) to understand thermal stress responses. Our findings showed that *G. antarctica* exhibits maximum transcriptional changes (182.4% increase in differentially expressed genes) at 12 °C after 24 h exposure compared to -12 °C/6 h, suggesting an optimal temperature range for cellular adaptation. Gene expression decreased at higher temperatures (16 °C & 20 °C), indicating potential thermal stress responses. Temporal analysis showed that 24-h exposure elicited stronger transcriptional responses compared to 6-h exposure, highlighting the time-dependent nature of thermal adaptation. Statistical modelling using negative binomial regression supported these biological observations. Our findings provide new insights into *G. antarctica's* transcriptional response to thermal stress and establish a framework for analysing complex gene expression patterns in psychrophilic organisms.

Keywords: Gene expression; *Glaciozyma antarctica* PI12; negative binomial model; psychrophilic yeast; RNA sequencing; thermal adaptation

ABSTRAK

Glaciozyma antarctica PI12, yis psikrofilik yang dipencilkan daripada ais Laut Antartika menunjukkan mekanisme penyesuaian sejuk yang luar biasa. Walaupun pertumbuhan optimumnya berlaku pada 12 °C, tindak balas pentranskripsinya merentas julat suhu yang lebih luas masih kurang difahami. Kami menganalisis corak ekspresi 7,853 gen di bawah suhu berbeza (-12 °C hingga 20 °C) dan masa pendedahan (6 & 24 jam) untuk memahami tindak balas tekanan terma. Penemuan kami menunjukkan bahawa *G. antarctica* mempamerkan perubahan pentranskripsi maksimum (peningkatan 182.4% dalam pengekspresan gen berbeza) pada 12 °C selepas pendedahan 24 jam berbanding -12 °C/6 jam, mencadangkan julat suhu optimum untuk penyesuaian sel. Pengekspresan gen menurun pada suhu yang lebih tinggi (16 °C & 20 °C) menunjukkan potensi tindak balas tekanan terma. Analisis temporal menunjukkan bahawa pendedahan 24 jam menimbulkan tindak balas pentranskripsi yang lebih kuat berbanding dengan pendedahan 6 jam, menonjolkan sifat penyesuaian terma yang bergantung kepada masa. Pemodelan statistik menggunakan regresi binomial negatif menyokong pemerhatian biologi ini. Penemuan kami memberikan pandangan baharu tentang tindak balas pentranskripsi *G. antarctica* terhadap tekanan terma dan mewujudkan rangka kerja untuk menganalisis corak pengekspresan gen kompleks dalam organisma psikrofilik.

Kata kunci: *Glaciozyma antarctica* PI12; model binomial negatif; penjujukan RNA; pengekspresan gen; penyesuaian terma; yis psikrofilik

INTRODUCTION

The Antarctic continent represents one of Earth's most extreme environments, characterized by exceptionally low temperatures and nutrient levels, intense light exposure, low air humidity, and constant freeze-thaw cycles (Durán et al. 2019). Despite these harsh conditions, Antarctica hosts a diverse and substantial microbial ecosystem that surpasses those found in other continents (Garrido-Benavent et al. 2020). These microorganisms have evolved remarkable adaptations that enable them not only to survive but to thrive in these extreme environmental conditions, making them valuable models for understanding biological adaptation mechanisms.

Among the environmental challenges in Antarctica, extreme cold represents the primary factor influencing microbial survival and reproduction, with temperatures frequently dropping to -30 °C (Cvetkovska, HüNer & Smith 2017). These extreme conditions have driven the evolution of remarkable cold-adaptation mechanisms, particularly in psychrophiles, which distinguish the Antarctic ecosystem as one of Earth's most significant yet relatively unexplored biological frontiers. The successful colonization of these environments by diverse prokaryotic and eukaryotic species demonstrates the remarkable capacity of organisms to adapt to extreme cold through specialized physiological and molecular mechanisms (Kuhn 2008).

Psychrophiles, a specialized group of extremophilic microorganisms encompassing both bacteria and archaea, have evolved unique capabilities to grow and thrive at temperatures ranging from -20 °C to 20 °C, with optimal growth typically occurring around 15 °C (Singh, Fillat & Kumar 2021). Their remarkable ability to colonize frigid habitats such as polar regions and deep seas stems from sophisticated physiological adaptations, most notably the synthesis of antifreeze proteins (Firdaus-Raih et al. 2018). These proteins play a crucial role in cellular survival at sub-zero temperatures by preventing ice crystal formation and maintaining cellular integrity. Beyond their ecological significance, these cryoprotectant molecules have practical applications, particularly in food preservation technology (Singh, Fillat & Kumar 2021).

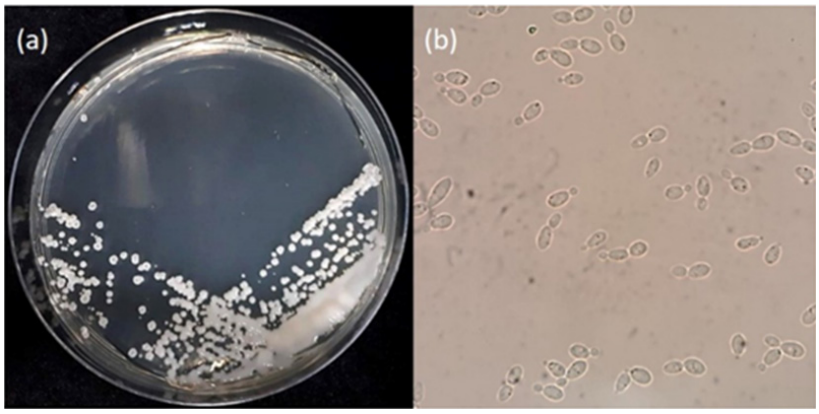
Glaciozyma antarctica PI12, isolated from Antarctic Sea ice, represents a model psychrophilic yeast system for understanding cold adaptation mechanisms (Bharudin et al. 2018). This organism has evolved a sophisticated array of physiological adaptations to cope with freezing stress, including the synthesis of antifreeze proteins and cold-active enzymes such as extracellular serine proteinase (Fujiu et al. 2021). These molecular adaptations not only enable *G. antarctica* to thrive in the Antarctic environment but also make it valuable for biotechnology applications, particularly in food technology and pharmaceutical processes requiring cryoprotection of proteins (Singh, Fillat & Kumar 2021).

Genomic analysis of *G. antarctica* PI12 has shown 7,857 protein-coding genes, providing unprecedented insight into the molecular basis of cold adaptation (Firdaus-Raih et al. 2018). The organism's complete genome sequence has uncovered unique stress response mechanisms, particularly in the expression patterns of heat shock proteins, distinguishing it from mesophilic organisms. A key feature of *G. antarctica's* cold adaptation is its specialized enzymatic machinery, characterized by high turnover numbers (kcat) and enhanced catalytic efficiency (kcat/km) at low temperatures. These cold-active enzymes maintain essential metabolic processes under conditions that would typically slow cellular activity. Unlike their thermophilic and mesophilic counterparts, these enzymes show increased thermolability, a characteristic that paradoxically enhances their functionality in cold environments (Tronelli, Maugini & Bossa 2007).

While *G. antarctica* has evolved sophisticated mechanisms to cope with cold temperatures, its ability to adapt to temperature fluctuations in its natural environment is fundamental to its survival (Yusof et al. 2016). Understanding these adaptation mechanisms at the molecular level, particularly the role of transcriptional responses to temperature changes, remains a critical challenge. Although some aspects of cold adaptation have been studied, the role of RNA-mediated responses to both extreme cold and heat stress remains poorly understood (Boo et al. 2013). Notably, while *G. antarctica's* response to cold temperatures has been documented, its transcriptional response to heat stress requires further investigation. This knowledge gap is particularly significant given the potential impacts of climate change on psychrophilic organisms.

Temperature profoundly influences *G. antarctica's* growth and morphology, with optimal development occurring at 12 °C (Koh et al. 2019). Although the organism can reproduce across a temperature range of 0 °C to 20 °C, these conditions significantly affect its cellular structure and function. While extremely low temperatures can compromise cellular integrity (Foong, Karjiban & Normi 2015), *G. antarctica* has evolved protective mechanisms. One key adaptation is the production of exopolysaccharides, visible as a mucoid, shiny layer on colony surfaces (Figure 1(a)), which protects cells from freezing damage (Bharudin et al. 2014). At the cellular level, *G. antarctica* exhibits distinctive morphological features under optimal growth conditions (Figure 1(b)). These adaptations, along with specific physiological characteristics, particularly its inability to produce lenticular bodies, have contributed to its taxonomic classification. Originally identified as *Leucosporidium antarcticum*, phylogenetic analysis of the D1/D2 region of the large-subunit (LSU) rDNA led to its reclassification as *Glaciozyma antarctica* (Turchetti et al. 2011). This reclassification reflects our evolving understanding of how morphological and molecular features contribute to cold adaptation in psychrophilic yeasts.

Cold-adapted enzymes in psychrophiles not only enable survival at low temperatures but also play essential ecological roles in nutrient cycling. Research by Brizzio, Turchettli and De Garcia (2007) showed that these enzymes serve as key indicators of metabolic adaptation, facilitating the breakdown of organic matter in cold environments. The ecological significance of these enzymatic adaptations is particularly evident in glacier environments, where melting ice provides organic carbon and nitrogen sources (Gao et al. 2020; Hood et al. 2015; Li et al. 2018). *G. antarctica*, like other psychrophilic fungi, contributes to ecosystem function by enzymatically converting complex organic compounds into simpler, bioavailable forms. This process is integrated into a broader ecological network where organic materials from dead organisms and polymers produced by algae and bacteria provide substrates for psychrophilic decomposition.



Source: Yusof, Hashim and Bharudin (2021)

FIGURE 1. Morphological adaptations of *G. antarctica* cultured on YPD agar medium at 12 °C for 10 days. (a) The mucoid, shiny colony surface represents the exopolysaccharide layer, a crucial adaptation for cryoprotection, (b) *G. antarctica* cells observed under light microscope (40× magnification)

The survival of psychrophilic organisms and their ecosystems is increasingly threatened by global climate change. Rising temperatures are accelerating glacier ice melt, fundamentally altering these cold-adapted environments. At the molecular level, temperature fluctuations trigger complex stress responses in psychrophilic organisms. Studies of *G. antarctica* PI12 have shown that sudden temperature changes induce significant modifications in gene expression patterns (Firdaus-Raih et al. 2018). Specifically, when exposed to temperature shifts between 12 °C and -12 °C, *G. antarctica* demonstrates rapid transcriptional responses, suggesting sophisticated temperature-sensing and adaptation mechanisms (Koh et al. 2019). Understanding these responses becomes increasingly crucial as climate change continues to impact polar ecosystems.

This study investigates the transcriptional response of *G. antarctica* to thermal stress by analyzing the expression patterns of its 7,853 genes across different temperatures and exposure times. Our primary goal is to understand how this psychrophilic yeast adapts to temperature variations, particularly focusing on the temporal dynamics of gene expression under both cold and heat stress conditions. Through examining *G. antarctica's* transcriptional responses to temperatures ranging from -12 °C to 20 °C over different time periods (6 & 24 h), we seek to characterize the molecular basis of thermal adaptation in this psychrophilic yeast, identify key temperature-responsive genes, and understand the temporal progression of stress responses.

This investigation provides insights into how psychrophilic organisms respond to temperature fluctuations, contributing to our understanding of cold adaptation mechanisms and potential responses to climate change. Furthermore, understanding *G. antarctica's* heat stress response adds crucial knowledge about the thermal adaptation limits of psychrophilic organisms, particularly relevant in the context of global warming and its impact on polar ecosystems.

DATA AND MATERIALS

Data Sources and Description

The current study analyzed RNA-seq data from the psychrophilic yeast *Glaciozyma antarctica* PI12 strain, originally obtained from the Antarctic Microbial Culture Collection, School of Biological Sciences, Universiti Sains Malaysia. The dataset comprises gene expression measurements across five temperature conditions (-12 °C, 0 °C, 12 °C, 16 °C, and 20 °C) and two exposure times (6 & 24 h), with three independent biological replicates per condition. This experimental design was implemented to investigate thermal adaptation responses in this psychrophilic yeast.

Dataset Characteristics

The analyzed dataset includes expression values for 7,853 genes previously identified in the *Glaciozyma antarctica* PI12 genome (Firdaus-Raih et al. 2018). The RNA-seq data was originally generated using the Illumina GA platform. In total, the comprehensive dataset contains 78,529 gene count data points spanning all experimental conditions and replicates.

Statistical Analysis Methodology

Gene expression values in the dataset were normalized using RPKM (Reads Per Kilobase of transcript per Million mapped reads) to account for variations in gene length and sequencing depth. For the current study, data integration and analytical procedures were performed using R version

4.0.2 (R Core Team 2022), employing the tidyverse, dplyr, plyr, and readr packages for data processing. The edgeR package from Bioconductor was specifically used for normalizing gene count data. Raw and normalized expression data, along with complete R scripts for all analyses conducted in this study, are available in the supplementary materials.

METHODOLOGY

GENE EXPRESSION ANALYSIS

Glaciozyma antarctica PI12 transcriptional responses to thermal stress were analyzed using RNA sequencing data. Expression levels were quantified with reads mapped to the *G. antarctica* PI12 genome using CLC Genomic Workbench. The experimental design examined five temperature conditions (-12 °C, 0 °C, 12 °C, 16 °C & 20 °C) across two exposure periods (6 & 24 h), with -12 °C at 6 h serving as the reference condition. Three independent biological replicates were maintained for each condition. Gene-level and sample-level expression data were integrated using the *G. antarctica* PI12 yeast interactive dashboard (Safinah Sharuddin et al. 2020).

STATISTICAL ANALYSIS

Statistical analysis of the gene expression count data from 7,853 genes was conducted using R statistical software (version 4.0.2). Initial testing showed significant overdispersion in the data (dispersion ratio = 2184.61), violating the Poisson regression assumption that variance equals the mean. Therefore, we evaluated multiple count-based regression models: Poisson regression, Negative Binomial regression, Zero-Inflated Poisson regression, and Zero-Inflated Negative Binomial regression.

The Negative Binomial regression model with temperature-time interaction effects was formulated as:

$$\log(\mu) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 (X_1 X_2)$$

where μ represents the expected gene count; X_1 represents temperature conditions; X_2 represents exposure time; and X_1X_2 represents their interaction. The probability density function of the model is:

$$P_b\{y;\mu,\phi\} = \frac{\Gamma(y+\phi)}{\Gamma(\phi)\Gamma(y+1)}\left(\frac{\phi}{\mu+\phi}\right)^{\phi}\left(1-\frac{\phi}{\mu+\phi}\right)^{y}, \quad y = 0,1,2,\ldots,k$$

with mean $E(Y) = \mu$ and variance $Var(Y) = \mu + \mu^2/\phi$, where ϕ is the dispersion parameter.

We focus on this model as it emerged as the most appropriate based on comprehensive model selection criteria including Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and likelihood ratio tests. The Negative Binomial model demonstrated superior performance with a significantly improved dispersion ratio, effectively accommodating the inherent variability in the RNA-seq data while avoiding the complexity of zero-inflated models. The significance level was set at $\alpha = 0.001$ for all statistical inferences. Analysis was performed using R packages including *tidyverse*, MASS, and *pscl*. All raw and normalized expression data, along with the complete R scripts for data analysis, are available in the supplementary materials.

RESULTS AND DISCUSSION

RESULTS

The transcriptional response of *Glaciozyma antarctica* PI12 to thermal stress was comprehensively analyzed using negative binomial regression with interaction analysis. The statistical modeling showed intricate patterns of gene expression across different temperature and time conditions (Table 1). Table 1 details the statistical parameters of the negative binomial regression model, including estimations, standard errors, *Z*-values, and significance levels for each temperature and time interaction.

The equation model used to analyze the negative binomial regression model with interaction can written as follows:

$$\log(\hat{\mu}) = \hat{\beta}_0 + \hat{\beta}_1 X_1 + \hat{\beta}_2 X_2 + \hat{\beta}_3 X_3 + \hat{\beta}_4 X_4 + \hat{\beta}_5 X_5 + \hat{\beta}_6 X_6 + \hat{\beta}_7 X_7 + \hat{\beta}_8 X_8 + \hat{\beta}_9 X_9$$

$$\hat{\mu} = \exp(\hat{\beta}_0 + \hat{\beta}_1 X_1 + \hat{\beta}_2 X_2 + \hat{\beta}_3 X_3 + \hat{\beta}_4 X_4 + \hat{\beta}_5 X_5 + \hat{\beta}_6 X_6 + \hat{\beta}_7 X_7 + \hat{\beta}_8 X_8 + \hat{\beta}_9 X_9)$$

which

$$\hat{\mu} = \exp(6.53 + 0.045X_1 - 0.307X_2 + 0.404X_3 + 0.476X_4 - 0.249X_5 + 0.126X_6 + 1.038X_7 - 0.183X_8 + 0.290X_9)$$

The negative binomial regression model in Table 1 indicates strong temperature-dependent and time-dependent effects on gene expression in *Glaciozyma antarctica*, with significant interactions between these factors. Our analysis of main effects showed distinct temperature-dependent and time-dependent gene expression patterns in *G. antarctica*. Compared to the baseline temperature (-12 °C), expression at 12 °C showed significant downregulation ($p < 0.001$), while expression at higher temperatures demonstrated significant upregulation (16 °C & 20 °C, both $p < 0.001$), with only a slight non-significant change observed at 0 °C ($p = 0.078$). Additionally, extended exposure time (24 h) resulted in a significant decrease in gene expression ($p < 0.001$) compared to the shorter exposure time (6 h) at the baseline temperature, suggesting that both temperature magnitude and exposure duration are critical factors in *G. antarctica's* transcriptional response to thermal stress.

TABLE 1. Analysis of Negative Binomial Regression Models with interactions

Parameter	Estimation	Standard Error	*Z*- value	*p*- Value	Significance
Intercept β_0	6.530	0.018	357.462	< 2e- 16	***
Temp 0 °C, β_1	0.045	0.026	1.758	0.078823	.
Temp 12 °C, β_2	-0.307	0.026	-11.872	< 2e- 16	***
Temp 16 °C, β_3	0.404	0.026	15.629	< 2e- 16	***
Temp 20 °C, β_4	0.476	0.026	18.407	< 2e- 16	***
Time 24 h, β_5	-0.249	0.026	-9.633	< 2e- 16	***
Temp 0 °C : Time 24 h, β_6	0.126	0.037	3.441	0.000579	***
Temp 12 °C : Time 24 h, β_7	1.038	0.037	28.401	< 2e- 16	***
Temp 16 °C : Time 24 h, β_8	-0.183	0.037	-5.013	5.35e -07	***
Temp 20 °C : Time 24 h, β_9	0.290	0.037	7.939	2.04e -15	***
Residual deviance	101347				
Degree of freedom	78520				
Dispersion ratio	1.291				
Theta	0.382				

Note: Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ALTERNATIVE MODEL EVALUATION

Multiple statistical models were explored to characterize the complex gene expression patterns of *Glaciozyma antarctica*. Initial Poisson regression analysis as shown in Table 2 showed significant overdispersion, necessitating alternative modeling approaches. To address the overdispersion issue identified in the Poisson model, we implemented a negative binomial regression model (Table 1), which accommodates greater variance in the count data than the Poisson distribution allows. This model provided a substantially improved fit to the gene expression data, with a more reasonable dispersion parameter of 1.29 much closer to the ideal value of 1.0. The negative binomial analysis confirmed the significant effects of both temperature and exposure time on gene expression patterns, while properly accounting for the inherent biological variability across the 7,853 genes under investigation.

The Negative Binomial regression model with interaction effect emerged as the most appropriate analytical framework. Table 3 demonstrates the initial model without interaction, characterized by a dispersion ratio of 1.29. The 24-h time variable exhibited limited statistical significance ($p = 0.935$), indicating the need for a more nuanced approach.

The Zero-Inflated Negative Binomial model (Table 4) demonstrated non-significant coefficients, further supporting the superiority of the interaction-based Negative Binomial approach.

Model selection criteria provided additional validation. Table 5 presents the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) analysis, demonstrating the interaction model's statistical efficiency. Comprehensive model comparison in Table 6 confirmed the Negative Binomial Regression Model with interaction as the optimal analytical framework, exhibiting the lowest AIC (1,134,402) and BIC (1,134,597) values.

Table 7 presents the likelihood ratio test results, which provides a critical statistical assessment of model performance. The analysis showed a log-likelihood difference of 1296.8 with 4 degrees of freedom, demonstrating statistical significance ($p < 2.2e\text{-}16$). This test confirmed the superiority of the Negative Binomial model with interaction effect over the model without interaction.

This rigorous model selection process ensured a robust statistical approach to understanding the transcriptional dynamics of *G. antarctica* under thermal stress.

INCIDENCE RATE ANALYSIS

Table 8 presents the incidence rate ratios (IRR) for each parameter, demonstrating the estimated changes in gene expression across different temperature and time conditions. The exponent estimates, confidence intervals, and significance levels provide a comprehensive view of *G. antarctica's* transcriptional responses.

From Table 8, we can clearly see that the temperature significantly influenced gene expression patterns. For the main temperature effects (compared to -12 °C baseline), 0 °C shows a slight 4.6% increase in gene expression (IRR=1.046, CI: 0.99-1.10), which is not statistically significant as the confidence interval includes 1.0; 12 °C demonstrates a significant 26.4% decrease (IRR=0.736, CI: 0.70-0.77); 16 °C exhibits a significant 49.8% increase (IRR=1.498, CI: 1.42-1.58); and 20 °C shows the largest

main effect with a significant 60.9% increase (IRR=1.609, CI: 1.53-1.69). For exposure time, 24-h exposure causes a significant 22.1% decrease in expression (IRR=0.779, CI: 0.74-0.82) compared to 6-h exposure at baseline temperature.

The interaction effects show complex temperature-time relationships: at 12 °C with 24-h exposure, there is a dramatic 182.4% increase in expression (IRR=2.824, CI: 2.63-3.03), representing the strongest interaction effect; 20 °C with 24-h exposure shows a significant 33.6% increase (IRR=1.336, CI: 1.24-1.44); 0 °C with 24-h exposure demonstrates a 13.4% increase (IRR=1.134, CI: 1.06-1.22); and 16 °C with 24-h exposure shows a 16.7% decrease (IRR=0.833, CI: 0.78-0.89). These results highlight that 12 °C with extended exposure represents a critical condition that triggers substantial changes in gene expression patterns, potentially indicating an important thermal adaptation threshold for this psychrophilic yeast.

Based on the explanation of each coefficient's rate ratio, it is evident that the rate ratio for temperature 12 °C and time 24 h has a more significant effect on the number of significantly expressed *G. antarctica* PI12 yeast genes.

PREDICTIVE MODELLING

The predictive modelling, illustrated in Figure 2, demonstrated the negative binomial regression model's capacity to estimate gene expression patterns. The prediction was run using the R software's *magrittr* package function with version 2.0.3. The comparative analysis

TABLE 2. Overdispersion Poisson Regression Model analysis

Parameter	Estimation	Standard Error	*Z*- value	*p*- Value	Significance
Intercept β_0	6.407	0.0003	18479.5	< 2e- 16	***
Temp 0 °C, β_1	0.102	0.0004	230.0	< 2e- 16	***
Temp 12 °C, β_2	0.280	0.0004	654.9	< 2e- 16	***
Temp 16 °C, β_3	0.328	0.0004	773.2	< 2e- 16	***
Temp 20 °C, β_4	0.613	0.0004	1528.7	< 2e- 16	***
Time 24 h, β_5	0.014	0.0003	56.8	< 2e- 16	***
Residual Deviance	171543948				
Degree of Freedom	78524				
Dispersion Ratio	2184.61				

Note: Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

TABLE 3. Analysis of Negative Binomial Regression Model without interaction

Parameter	Estimation	Standard Error	*Z*- value	*p*- Value	Significance
Intercept β_0	6.414	0.014	450.631	< 2e- 16	***
Temp 0 °C, β_1	0.102	0.018	5.577	2.45e- 08	***
Temp 12 °C, β_2	0.285	0.018	15.265	< 2e- 16	***
Temp 16 °C, β_3	0.328	0.018	17.827	< 2e- 16	***
Temp 20 °C, β_4	0.613	0.018	33.368	< 2e- 16	***
Time 24 h, β_5	-0.0009	0.012	-0.082	0.935	
Residual Deviance	101504				
Degree of Freedom	78524				
Dispersion Ratio	1.29				
Theta	0.377				

Note: Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

TABLE 4. Analysis of Zero-Inflated Negative Binomial Regression Models with interactions

Parameter	Estimation	Standard Error	*Z*- value	*p*- Value	Significance
Intercept β_0	-22.645	2248.516	-0.010	0.992	
Temp 0 °C, β_1	7.614	2248.938	0.003	0.997	
Temp 12 °C, β_2	18.464	2248.516	0.008	0.993	
Temp 16 °C, β_3	6.238	2251.558	0.003	0.998	
Temp 20 °C, β_4	5.309	2251.299	0.002	0.998	
Time 24 h, β_5	5.157	2253.457	0.002	0.998	
Temp 0 °C : Time 24 h, β_6	-9.609	2293.672	-0.004	0.997	
Temp 12 °C : Time 24 h, β_7	-4.258	2253.457	-0.002	0.998	
Temp 16 °C : Time 24 h, β_8	-7.988	2277.118	-0.004	0.997	
Temp 20 °C : Time 24 h, β_9	-8.194	2307.373	-0.004	0.997	

Note: Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

TABLE 5. AIC and BIC Analysis of Negative Binomial Regression Models

Test/Criteria	Model 1	Model 2
Likelihood Ratio	19.675	
AIC	1135852	1134563
BIC	1135917	1134665
Model 1	Negative Binomial Regression Model without interaction	
Model 2	Negative Binomial Regression Model with interaction	

TABLE 6. Analyses of the AIC and BIC for five models

Test/Criteria	Model 1	Model 2	Model 3	Model 4	Model 5
AIC	1134563	172078731	169450575	163477496	1134402
BIC	1134665	172078786	169450668	163477681	1134597
Model 1	Negative Binomial Regression Model with interaction				
Model 2	Poisson Regression Model without interaction				
Model 3	Poisson Regression Model with interaction				
Model 4	Zero-Inflated Poisson Regression Model with interaction				
Model 5	Zero-Inflated Negative Binomial Regression Model with interaction				

TABLE 7. Likelihood Ratio Test

Model	*df*	LogLik		X^2	*p*-value
Negative Binomial Model	7	-567919			
Negative Binomial Model with Interaction	11	-567271	4	1296.8	< 2.2e -16

TABLE 8. Incidence Rates Ratio (IRR) on average, with confidence intervals

Parameter	Estimate	Exponent (estimate)	Incidence rate percentage	2.5% Confidence Interval	97.5% Confidence Interval
Intercept β_0	6.530	685.39	685.39 -1 = 684.39 × 100 = 68439%	661.81	710.95
Temp 0 °C, β_1	0.045	1.046	1.046-1= 0.046 × 100 = 4.6%	0.99	1.10
Temp 12 °C, β_2	-0.307	0.736	0.736-1= -0.264 × 100 = -26.4%	0.70	0.77
Temp 16 °C,β_3	0.404	1.498	1.498-1=0.498 × 100 = 49.8%	1.42	1.58
Temp 20 °C, β_4	0.476	1.609	1.609-1=0.609 × 100 = 60.9%	1.53	1.69
Time 24 h, β_5	-0.249	0.779	0.779-1= -0.221 × 100 = -22.1%	0.74	0.82
Temp 0 °C : Time 24 h, β_6	0.126	1.134	1.134-1= 0.134 × 100 = 13.4%	1.06	1.22
Temp 12 °C : Time 24 h, β_7	1.038	2.824	2.824-1 = 1.824 × 100 = 182.4%	2.63	3.03
Temp 16 °C : Time 24 h, β_8	-0.183	0.833	0.833-1 = -0.167 × 100 = -16.7%	0.78	0.89
Temp 20 °C : Time 24 h, β_9	0.290	1.336	1.336-1= 0.336 × 100 = 33.6%	1.24	1.44

between observed and predicted data showed notable characteristics of *Glaciozyma antarctica's* transcriptional response.

The graph in Figure 2 compares predicted values across different temperature and time conditions, highlighting the model's capacity to estimate transcriptional variability. This graph shows the distribution of forecasted gene expression counts at different temperature conditions (0 °C, 12 °C, 16 °C & 20 °C) with 24-h exposure time in *G. antarctica*. The *x*-axis represents predicted gene expression counts, while the *y*-axis shows the frequency of genes exhibiting those counts. These histograms illustrate the pronounced differences in gene expression distributions across temperature conditions.

At 0 °C and 16 °C, the distributions show higher frequencies of genes with low expression values and a gradual decrease in frequency as expression increases. In contrast, the 12 °C condition demonstrates a uniquely different pattern with a wider range of expression values (extending to 61 counts) and more genes showing moderate to high expression levels, consistent with the strong positive interaction effect (182.4% increase) observed in the negative binomial regression model. The 20 °C condition shows an intermediate pattern with greater expression diversity than 0 °C and 16 °C but less extreme than 12 °C. These distribution patterns highlight the non-linear temperature response of *G. antarctica's* transcriptome, with 12 °C and 24-h exposure representing a potential critical threshold in the yeast's thermal adaptation mechanisms.

DISCUSSION

The transcriptional responses of *Glaciozyma antarctica* provide critical insights into molecular mechanisms of thermal adaptation in psychrophilic organisms. The observed gene expression patterns suggest a sophisticated cellular response system enabling survival in extreme environmental conditions.

Our analysis shows that *G. antarctica* employs distinct transcriptional strategies across different temperature conditions, with 12 °C emerging as a critical threshold in its thermal adaptation profile. The significant 26.4% decrease in gene expression at 12 °C with short exposure contrasts dramatically with the 182.4% increase observed after prolonged exposure at the same temperature. This non-linear response suggests the activation of specialized molecular pathways when the yeast experiences extended exposure to temperatures approaching its upper growth limit. In contrast, the consistent upregulation observed at higher temperatures (16 °C & 20 °C) likely reflects the activation of canonical heat shock response pathways and cellular protection mechanisms that shield essential cellular components from thermal damage.

The complex interaction between temperature and exposure time showed by our negative binomial regression model highlights the sophisticated nature of *G. antarctica's* transcriptional regulation. The model effectively captured the overdispersion inherent in gene expression data, providing a more accurate representation of the probabilistic nature of transcriptional responses to thermal stress. The discrepancies between observed and predicted gene expression distributions underscore the limitations of simple linear models in capturing the nuanced adaptive strategies employed by psychrophilic organisms. This finding aligns with previous research on extremophiles, which has consistently demonstrated that adaptation to extreme environments involves complex, non-linear regulatory processes.

The temporal dynamics of gene expression further illuminate the adaptive strategies of *G. antarctica*. The significant differences between 6-h and 24-h exposures

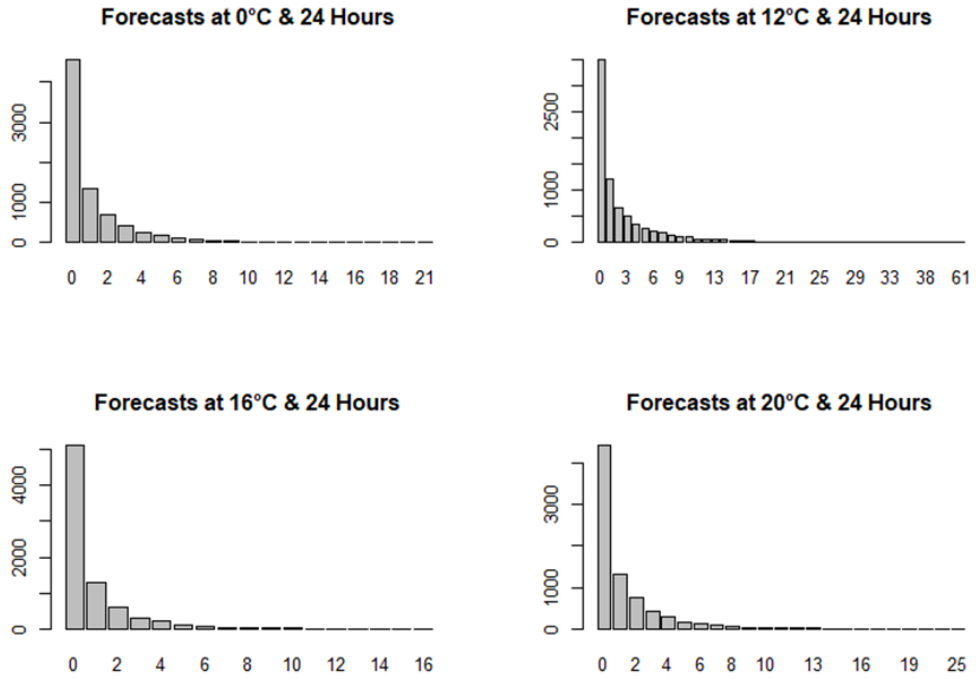


FIGURE 2. Distribution of forecasted gene expression counts in *Glaciozyma antarctica* at different temperatures (0 °C, 12 °C, 16 °C & 20 °C) with 24-h exposure

suggest that this psychrophilic yeast employs temporally regulated molecular mechanisms to maintain cellular homeostasis under thermal stress. This temporal adaptability aligns with studies by Margesin and Miteva (2011), and Morgan-Kiss et al. (2006) who demonstrated that psychrophilic microorganisms from polar environments typically exhibit time-dependent transcriptional responses that enable adjustment to diurnal temperature fluctuations. Additionally, Blanc et al. (2012) observed comparable temporal regulation in cold-adapted algae, where extended exposure to temperature shifts elicited more comprehensive transcriptional reorganization than acute exposure.

While our statistical analysis provides robust insights into the transcriptional responses of *G. antarctica*, several limitations must be acknowledged. The current study focused primarily on gene count patterns rather than individual gene identities, representing a critical area for future investigation. Identifying the specific genes and pathways that drive these distinctive expression patterns would provide deeper insights into the molecular basis of psychrophilic adaptation. Additionally, further research is needed to determine whether the observed transcriptional changes translate to corresponding alterations in protein levels and cellular function. Despite these limitations, our findings contribute significantly to understanding thermal adaptation in psychrophilic yeasts, demonstrating the remarkable transcriptional flexibility that enables survival in extreme environments.

CONCLUSION

This study provides a comprehensive investigation of the transcriptional responses of *Glaciozyma antarctica* PI12 to thermal stress, showing the sophisticated molecular adaptation mechanisms of this psychrophilic yeast. The research demonstrates the remarkable transcriptional flexibility that enables survival in extreme Antarctic environments. The negative binomial regression model with interaction effects uncovered complex gene expression patterns across different temperatures and exposure times. The most significant finding was the exceptional gene expression response at 12 °C with 24-h exposure, suggesting a critical temperature point in the yeast's thermal adaptation strategy. The temperature-dependent transcriptional responses ranged from minimal changes at 0 °C to substantial modulations at 16 °C and 20 °C, showing complex interactions between temperature and exposure time. These findings provide evidence of sophisticated molecular mechanisms for maintaining cellular homeostasis under thermal stress.

Methodologically, the study highlighted the importance of advanced statistical approaches in analyzing complex biological data. The negative binomial regression model with interaction provided superior insights compared to traditional analytical methods, demonstrating the value of sophisticated statistical techniques in molecular biology research. The current study's limitations include the focus on gene count rather than individual gene identity. Future

research should prioritize identifying specific genes driving thermal stress responses, investigating molecular pathways underlying these transcriptional changes, and exploring the functional significance of observed expression patterns.

The findings contribute significantly to our understanding of psychrophilic organism adaptation, offering insights into the molecular strategies that enable survival in extreme environmental conditions. This research provides a foundation for future investigations into thermal adaptation mechanisms in microorganisms. Ultimately, the study shows the remarkable complexity of *G. antarctica's* transcriptional response, highlighting the sophisticated molecular mechanisms that enable survival in one of the world's most challenging environments. By elucidating the intricate gene expression patterns, this research advances our comprehension of how microorganisms adapt to extreme thermal conditions, opening new avenues for understanding cellular resilience and evolutionary adaptation.

ACKNOWLEDGEMENTS

The authors acknowledge the School of Biological Sciences USM Malaysia' Microbial Culture Collection for providing the data. We are greatly grateful for the in-depth discussion by the anonymous reviewers that have improved the quality of the manuscript.

REFERENCES

Bharudin, I., Zolkefli, R., Abu Bakar, M.F., Kamaruddin, S., Md Illias, R., Najimudin, N., Mahadi, N.M., Abu Bakar, F.D. & Abdul Murad, A.M. 2018. Pengenalpastian dan profil pengekspresan gen biosintesis asid amino yis psikrofil, *Glaciozyma antarctica*. *Sains Malaysiana* 47(8): 1675-1684. doi:10.17576/jsm-2018-4708-06

Bharudin, I., Zaki, N.Z., Abu Bakar, F.D., Mahadi, N.M., Najimudin, N., Illias, R.M. & Murad, A.M.A. 2014. Comparison of RNA extraction methods for transcript analysis from the psychrophilic yeast, *Glaciozyma antarctica. Malays. Appl. Biol.* 43: 71-79.

Blanc, G., Agarkova, I., Grimwood, J., Kuo, A., Brueggeman, A., Dunigan, D.D., Gurnon, J., Ladunga, I., Lindquist, E., Lucas, S., Pangilinan, J., Pröschold, T., Salamov, A., Schmutz, J., Weeks, D., Yamada, T., Lomsadze, A., Borodovsky, M., Claverie, J-M., Grigoriev, I.V. & Van Etten, J.L. 2012. The genome of the polar eukaryotic microalga *Coccomyxa subellipsoidea* reveals traits of cold adaptation. *Genome Biology* 13: R39.

Boo, S.Y., Wong, C.M.V.L., Rodrigues, K.F., Najimudin, N., Murad, A.M.A. & Mahadi, N.M. 2013. Thermal stress responses in Antarctic yeast, *Glaciozyma antarctica* PI12, characterized by real-time quantitative PCR. *Polar Biology* 36(3): 381-389. doi:10.1007/S00300-012-1268-2

Brizzio, S., Turchettli, B. & De Garcia, V. 2007. Extracellular enzymatic activities of basidiomycetous yeasts isolated from glacial and subglacial waters of northwest Patagonia (Argentina). *Canadian Journal of Microbiology* 53: 519-525.

Cvetkovska, M., HüNer, N.P.A. & Smith, D.R. 2017. Chilling out: The evolution and diversification of psychrophilic algae with a focus on *Chlamydomonadales. Polar Biology* 40: 1169-1184. doi: 10.1007/s00300-016-2045-4

Durán, P., Barra, P.J., Jorquera, M.A., Viscardi, S., Fernandez, C., Paz, C., de la Luz Mora, M. & Bol, R. 2019. Occurrence of soil fungi in pristine Antarctic environments. *Frontiers in Bioengineering and Biotechnology.* https://doi.org/10.3389/fbioe.2019.00028

Firdaus-Raih, M., Hashim, N.H.F., Bharudin, I., Abu Bakar, M.F., Huang, K.K., Alias, H., Lee, B.K.B., Mat Isa, M.N., Mat-Sharani, S., Sulaiman, S., Tay, L.J., Zolkefli, R., Muhammad Noor, Y., Law, D.S.N., Abdul Rahman, S.H., Md-Illias, R., Abu Bakar, F.D., Najimudin, N., Abdul Murad, A.M. & Mahadi, N.M. 2018. The *Glaciozyma antarctica* genome reveals an array of systems that provide sustained responses toward temperature variations in a persistently cold habitat. *PLoS ONE* 13(1): e0189947. doi:10.1371/JOURNAL.PONE.0189947

Foong, P., Karjiban, R. & Normi, Y. 2015. Bioinformatics survey of the metal usage by psychrophilic yeast *Glaciozyma antarctica* PI12. *Metallomics* 7: 156-164.

Fujiu, S., Ito, M., Kobayashi, E., Hanada, Y., Yoshida, M., Kudoh, S. & Hoshino, T. 2021. Basidiomycetous yeast, *Glaciozyma antarctica*, forming frost-columnar colonies on frozen medium. *Microorganisms* 9(8): 1679. doi:10.3390/MICROORGANISMS9081679/S1

Gao, T., Kang, S., Zhang, Y., Sprenger, M., Wang, F., Du, W., Wang, X. & Wang, X. 2020. Characterization, sources and transport of dissolved organic carbon and nitrogen from a glacier in the Central Asia. *Science of The Total Environment* 725: 138346. https://doi.org/10.1016/j.scitotenv.2020.138346

Garrido-Benavent, I., Pérez-Ortega, S., Durán, J., Ascaso, C., Pointing, S.B., Rodríguez-Cielos, R., Navarro, F. & de Los Ríos, A. 2020. Differential colonization and succession of microbial communities in rock and soil substrates on a maritime Antarctic glacier forefield. *Frontiers in Microbiology* 11: 126. doi:10.3389/FMICB.2020.00126/BIBTEX

Hossain, M.Z. 1998. AIC and BIC - The two competitive information criteria for model selection in economics and statistics. *The Jahangirnagar Review, Part II*: Social Science. XIX-XXII: 133-140. https://www.researchgate.net/publication/282731304_AIC_and_BIC_-_The_two_competitive_information_criteria_for_model_selection_in_economics_and_statistics

Koh, J.S.P., Wong, C.M.V.L., Najimudin, N. & Mahadi, N.M. 2019. Gene expression patterns of *Glaciozyma antarctica* in response to temperature shifts. *Polar Science* 4: 45-54. doi:https://doi.org/10.1016/j.polar.2018.11.007

Kuhn, M. 2008. The climate of snow and ice as boundary condition for microbial life. In *Psychrophiles: From Biodiversity to Biotechnology*, edited by Margesin, R., Schinner, F., Marx, J.C. & Gerday, C. Berlin, Heidelberg: Springer https://doi.org/10.1007/978-3-540-74335-4_1

Li, X., Ding, Y., Xu, J., He, X., Han, T., Kang, S., Wu, Q., Mika, S., Yu, Z. & Li, Q. 2018. Importance of mountain glaciers as a source of dissolved organic carbon. *Journal of Geophysical Research: Earth Surface* 123(9): 2123-2134. https://doi.org/10.1029/2017JF004333

Margesin, R. & Miteva, V. 2011. Diversity and ecology of psychrophilic microorganisms. *Research in Microbiology* 162(3): 346-361.

Morgan-Kiss, R.M., Priscu, J.C., Pocock, T., Gudynaite-Savitch, L. & Huner, N.P. 2006. Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiology and Molecular Biology Reviews* 70(1): 222-252.

R Core Team. 2022. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Safinah Sharuddin, Nora Muda, Nazalan Najimudin & Abdul Rahman Othman. 2020. An interactive dashboard for visualization of RNAseq data of yeast Glaciozyma antarctica PI12. *International Journal of Engineering Trends and Technology (IJETT)- Editor's Issues*. doi: 10.14445/22315381/CATI1P220. 110-113.

Safinah Sharuddin, Nora Muda, Nazalan Najimudin & Abdul Rahman Othman. 2020. An interactive dashboard for visualization of RNAseq data of yeast Glaciozyma antarctica PI12. *International Journal of Engineering Trends and Technology (IJETT)- Editor's Issues*: 110-113. doi: 10.14445/22315381/CATI1P220.

Singh, P., Fillat, M. & Kumar, A. 2021. *Cyanobacterial Lifestyle and Its Applications in Biotechnology*. Massachusetts: Academic Press. https://doi.org/10.1016/B978-0-323-90634-0.00032-9

Tronelli, D., Maugini, E. & Bossa, F. 2007. Structural adaptation to low temperatures - Analysis of the subunit interface of oligomeric psychrophilic enzymes. *The FEBS Journal* 274: 4595-4608.

Turchetti, B., Thomas Hall, S.R., Connell, L.B., Branda, E., Buzzini, P., Theelen, B., Müller, W.H. & Boekhout, T. 2011. Psychrophilic yeasts from Antarctica and European glaciers: Description of *Glaciozyma* gen. nov., *Glaciozyma martinii* sp. nov. and *Glaciozyma watsonii* sp. nov. *Extremophiles* 15: 573.

Yusof, N.A., Hashim, N.H.F., Beddoe, T., Mahadi, N.M., Illias, R.M., Bakar, F.D.A. & Murad, A.M.A. 2016. Thermotolerance and molecular chaperone function of an SGT1-like protein from the psychrophilic yeast, *Glaciozyma antarctica. Cell Stress & Chaperones* 21(4): 707-715. doi:10.1007/S12192-016-0696-2

Yusof, N.A., Hashim, N.H.F. & Bharudin, I. 2021. Cold adaptation strategies and the potential of psychrophilic enzymes from the antarctic yeast, *Glaciozyma antarctica* PI12. *Journal of Fungi* 7(7): 528. doi:10.3390/JOF7070528

*Corresponding author; email: noramuda@ukm.edu.my