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Research

Profiling The Growth Conditions and Persistent Organic Pollutants (POPS) Tolerance of *Phenoliferia glacialis* USM-PSY62

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ABSTRACT

Antarctica is characterized by extreme cold, isolated, and unique ecosystems. Nevertheless, Antarctica harbors diverse species of microorganisms, particularly in its ice-covered lakes and subglacial environments. These microorganisms have special adaptations to extreme cold and low-nutrient conditions. Some extremophiles, like psychrophiles can thrive in these harsh environments. *Phenoliferia glacialis* USM-PSY62, previously identified as *Rhodotorula* sp. USM-PSY62 is a psychrophilic yeast isolated from the ice brine of Antarctica. However, there is very little information on this psychrophilic yeast. This study aims to characterize the *P. glacialis* USM-PSY62 through the identification of the optimum growth parameters in different media (Yeast Peptone Dextrose, YPD & Yeast Malt, YM), temperature (4°C, 15°C, 20°C) and pH (6, 7, 8, 9) as well as their ability in carbon assimilation and extracellular enzyme production. It has an optimal growth in YPD compared to YM broth media. *P. glacialis* USM-PSY62 grows optimally at 15°C and pH 7.0. This Antarctic yeast enters the stationary phase on day six of incubation under optimum conditions. It appeared mainly as elongated-shape and oval-shaped with budding formation and was found to produce extracellular enzymes such as protease and amylase in the presence of 2% glucose concentration in YM media. *P. glacialis* USM-PSY62 also can assimilate various types of carbon sources including raffinose, arabinose, and maltose. Interestingly, the psychrophilic yeast presented growth in media supplemented with Persistent Organic Pollutants (POPs) such as dichlorophenyldichloroethylene (DDE) and polychlorinated biphenyl (PCB). These preliminary findings suggest that *P. glacialis* USM-PSY62 has tremendous potential for bioremediation application in polluted cold regions, as well as deepening our knowledge of its optimal growth conditions.

Key words: Antarctica, cold environment, pollutants, psychrotolerant, yeast

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INTRODUCTION

Antarctica, a region that is mostly covered by ice and snow harbors various unique cold-adapted species. Microorganisms living in the cold environments of Antarctica face a variety of environmental stressors such as low to freezing temperatures, limited bioavailability of nutrients, extreme pH, and salinity (Margesin & Miteva, 2011; Yusof *et al*., 2021a; Yusof *et al*., 2022). Antarctica offers a valuable opportunity to study the taxonomy and ecology of various cold-loving inhabitants including many species of bacteria, fungi, plants, and animals.

In Antarctica, yeast can be isolated from soil (Tsuji, 2018), ice, permafrost (Touchette *et al*., 2022), snow (de Menezes *et al*., 2019), water (Perini *et al*., 2019) and plant (Ferreira *et al*., 2019). The genera that have been discovered from Antarctica include *Rhodotorula* sp., *Candida* sp., *Mrakia* sp., *Cryptococcus* sp., *Leucosporidium* sp., and *Phenoliferia* sp. (Baeza *et al*., 2022). Research was done to identify the diversity of yeast in Antarctica through sampling

and amplification of internal space transcriber (ITS) regions as well as large and small subunit RNA regions. There are also other methods for identifying the species of yeast in Antarctica such as through biochemical properties, and assimilation tests using aromatic compound, carbon, and nitrogen (Wang *et al*., 2015a).

Rhodotorula sp. is recognized for its ability to break down phenol and use it as a carbon source in a broad range of temperatures, including low temperatures. As a result, the classification of the *Rhodotorula* genus is determined by the characterization of high metabolic versatility in the utilization of several phenol-related monoaromatic compounds as the primary source of carbon at 10°C and the ability to grow in the presence of high concentrations of these compounds (Margesin *et al*., 2007).

This research aims to identify the species of the Antarctic yeast obtained from the Universiti Sains Malaysia research group, which was isolated from Casey Research Station, Antarctica. Based on the previous reports on its performance in degrading phenol-related compounds, the versatility of the yeast was also tested in this study through the ability to utilize persistent organic pollutants (POPs) such as dichlorodiphenyldichloroethylene (DDE) and polychlorinated biphenyl (PCB). This is the first report on the ability of indigenous Antarctic yeast species to degrade DDE and PCB, pollutants that are also detected in the Antarctic environment (Corsolini & Sarà 2017; Terajima *et al*., 2022; Xie *et al*., 2022).

MATERIALS AND METHODS

Yeast isolation and molecular identification

P. glacialis USM-PSY62 was routinely grown in yeast peptone dextrose (YPD) agar at 15°C and kept in 20% glycerol stock at -80°C for long-term storage.

P. glacialis USM-PSY62 was cultured in YPD broth culture for seven days at 15°C. Then, the culture was observed under a compound microscope to determine the cell morphology. *P. glacialis* strain USM-PSY62 was identified based on internal transcribed spacer (ITS) and large ribosomal subunit (LSU) sequences alignment against other related sequences in the National Centre for Biotechnology Information (NCBI) Genbank.

The extraction of genomic DNA was performed using Wizard® Genomic DNA Purification Kit (USA). The internal transcribes spacer region was amplified using primer pair ITS1F (5'-GTAACAAGG TTT CCG T-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') meanwhile, the large subunit region was amplified using primer pair LROR (5'-ACCCGCTGAACTTAAGC-3') and LR6 (5'-CGCCAGTTCTGCTTACC-3'). Protocol for polymerase chain reaction was performed with DreamTaq PCR Master Mix (Thermo Scientific), according to the manufacturer's protocol. The sequence was subjected to an NCBI blast search. Sequence alignment of homologous sequences of closely related species of basidiomycetes and ascomycetes retrieved from GenBank was performed using the ClustalW option in MEGA-X software. Phylogenetic analysis based on the ITS and LSU sequences was performed using the MEGA-X program (Tamura *et al*., 2021). Phylogenetic trees were constructed using Maximum Likelihood (ML) with 1000 bootstrap replications. Evolutionary distance data were calculated using the Tamura three-parameter mode (Tamura *et al*., 2021). Further characterization of the *P. glacialis* USM-PSY62 was done through a growth optimum study, carbon assimilation, and extracellular enzyme production test.

Optimum growth study

The three conditions for yeast growth were tested. For media, YPD and yeast malt (YM) agar were used to grow *P. glacialis* USM-PSY62 at 4°C. Then, the optimum temperature was tested at 4°C, 15°C, and 20°C at 180 rpm. Eventually, the optimum pH was tested from pH 6 to pH 9. Triplicates of each type of condition were prepared. *P. glacialis* USM-PSY62 was grown in 50 mL YPD broth supplemented with 50 mg/mL kanamycin and 50 mg/mL ampicillin for 8 to 9 days. The optical density of each culture at the 600 nm absorbance was measured every 24 hr. A growth curve was plotted based on the average of the OD_{600} reading of the replicates for every day.

Extracellular enzyme activity assay

Extracellular enzyme activities were performed using the yeast malt agar (YMA) media containing 2% of glucose (Carrasco *et al*., 2012). Each of these media was supplemented with different carbon sources, 2.5% hydrolysate chitin, 0.5% carboxymethyl cellulose (CMC), 0.2% soluble starch, and 2% casein. 1 µL of yeast cell suspension grown to mid-log phase was inoculated onto the agar medium and incubated at 4°C for 14 days. The colonies were washed off from the agar medium with distilled water. The identification of enzyme activities followed the method by Hankin and Anagnostakis (1975). To identify cellulase activity, the medium was stained with 1 mg/mL of congo red solution for 15 min and de-

stained with 1 M sodium chloride (NaCl) for 15 min. A halo zone on the agar medium indicated that the substrate had been degraded (Carrasco *et al*., 2012). For soluble starch, the plates were flooded with 1 mL of iodine solution and the appearance of a clear halo around the colony indicated positive activity of amylase. Protease activity was defined by the white precipitate around the fungal colony in media supplemented with casein (Hankin & Anagnostakis, 1975). The activity of chitinase was presented directly by the presence of a clear halo region around the colony, indicating the utilization of chitin.

Determination of carbon source assimilation

Assimilation of different carbon sources was carried out according to protocols in "Preparation of Yeast Media" (2003). In yeast, nitrogen base (YNB) media, 2% of raffinose, maltose, arabinose, glucose, and galactose were added. From a single colony, *P glacialis* USM-PSY62 was streaked on the media and after 10 days of incubation, the growth of *P. glacialis* USM-PSY62 was observed.

Growth of *P. glacialis* in the presence of POPs

P. glacialis USM-PSY62 was cultured in YPD media supplemented with 1 ppm, 5 ppm, 10 ppm, 20 ppm, and 30 ppm DDE. PCB, at a concentration of 0.01 µg/mL was also being supplemented in the media and was conducted in duplicate. YPD with no addition of POPs served as control. The incubation time was seven days at 15°C.

RESULTS

Identification of *P. glacialis*

The yeast previously called *Rhodotorula* sp. USM-PSY62, was subjected to sequencing and BLAST search. However, the top BLAST search showed that *Phenoliferia* sp.has the highest percent identity (>90%) to the subjected sequence with a low e-value of 0 for the ITS sequence. The ITS and LSU sequences of other psychrophilic *Rhodotorula* sp. were used for multiple sequence alignment before generating a phylogenetic tree to support the claim of the BLAST result. In Figure 1, the phylogenetic trees were built to observe the phylogenetic relation between *Rhodotorula* sp. and *Phenoliferia* sp. The Antarctic yeast used in the study is located in the same clade as other *Phenoliferia* sp while the rest of *Rhodotorula* sp. accumulate in different clades for both phylogenetic trees. A paper in 2007 mentioned that *Phenoliferia* sp. was initially known as *Rhodotorula*, specifically for *Rhodotorula glacialis*, *Rhodotorula psychrophila,* and *Rhodotorula psychrophenolica* (Margesin *et al*., 2007). Regarding that, an article published in 2015, reported phylogenetic analysis revision including genera within Pucciniomycotina which include both *Rhodotorula* (the revised genera) and *Phenoliferia* (the proposed genera) (Wang *et al*., 2015b). Table 1 summarises the relation between *Rhodotorula* sp. and *Phenoliferia* sp. It is very important to be updated with the common name used by the species as well as its latest name as this can influence the database search engines. Furthermore, *Phenolifera* was given to describe the metabolic performance of utilize several phenol-related monoaromatic compounds as the primary source of carbon (Margesin *et al*., 2007).

Growth performance in different temperatures, media, and pH

Phenoliferia glacialis shows the appearance of creamy-white colonies on the YPD agar. Figure 2 shows the morphology of *P. glacialis* on agar culture. Colonies are round and there was no hyphae or pseudomycelium formation. The cell morphology under microscope showed the cells can occur in singly or in small chains and the budding is polar (Margesin *et al*., 2007). Figure 3 shows the cell shape. of *P. glacialis* USM-PSY62.

The growth curve of nine days revealed that *P. glacialis* USM-PSY62 grew optimally in YPD and YM agar media. Nevertheless, the YPD medium supported better cell growth than YM given that the doubling time in YPD media (15.7 hr) is shorter compared to in YM media (17.9 hours). *P. glacialis* USM-PSY62 has a shorter doubling time at 15°C (9.4 hr) compared with at 4°C (14.5 hr) and 20°C (18.5 hr). Figure 4, 5 and 6 show the growth curve of *P. glacialis* USM-PSY62 in different media, temperature, and pH respectively.

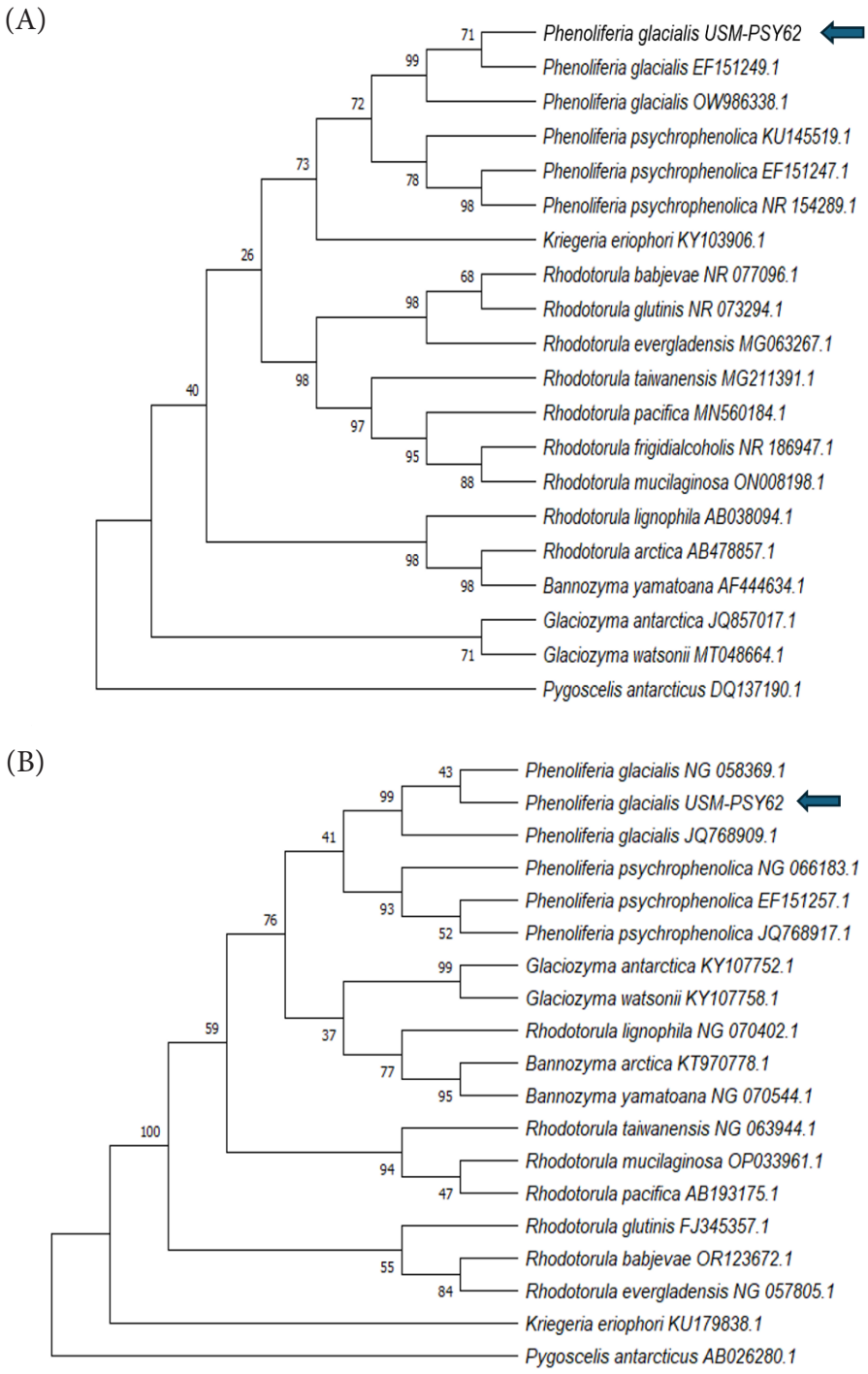


Fig. 1. (A)The phylogenetic tree showing the relationship between ITS region from *Phenoliferia glacialis* USM-PSY62, *Phenoliferia* sp, *Rhodotorula* sp, and other psychrophilic and psychrotolerant basidiomycetes. (B) The phylogenetic tree shows the relationship between LSU regions of *Phenoliferia glacialis* USM-PSY62 and other basidiomycetes. The species used in this study is indicated with a dark arrow. *Pygoscelis antarctic* was included as the outlier.

Table 1. The difference of order between *Rhodotorula* sp. and *Phenoliferia* sp. (Wang, *et al*., 2015a)

Taxonomic rank	*Rhodotorula* sp.	*Phenoliferia* sp.
Subkingdom	Dikarya	
Phylum	Basidiomycota	
Subphylum	Pucciniomycotina	
Class	Microbotryomycetes	
Order	Sporidiobolales	Kriegeriales
Family	Sporidiobolaceae	Kriegeriaceae
Genus	*Rhodotorula*	*Phenoliferia*

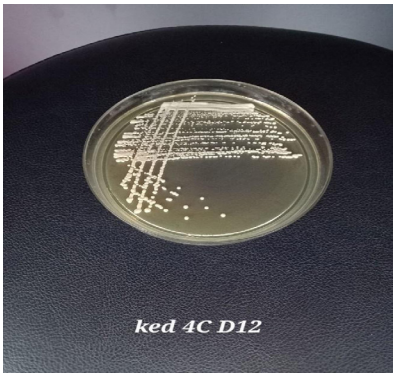


Fig. 2. Growth of *Phenoliferia glacialis* USM-PSY62 on YPD agar medium at 15°C after seven days.

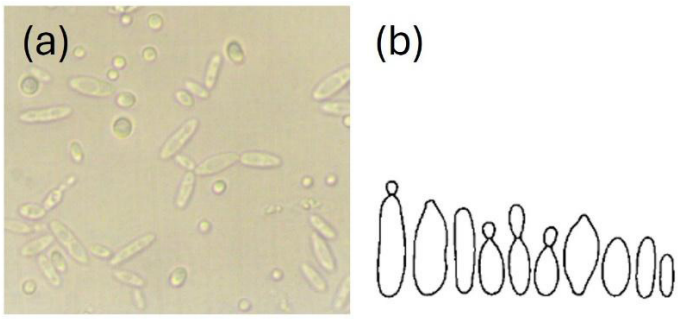


Fig. 3. Cell shape of *P. glacialis* USM-PSY62 (a) The morphology of *P. glacialis* under 400x magnification after seven days incubation at 15°C, (b) Line drawing of *Rhodotorula glacialis* described by Margesin *et al*. (2007).

Extracellular enzyme activity assay

Phenoliferia glacialis USM-PSY62 exhibited amylase and protease activities by the formation of halos in starch-containing media and the formation of white precipitate around the colony in casein-containing media respectively (Table 2). However, cellulase and chitinase activity was not detected in the CMC-containing media and chitin-containing media, respectively.

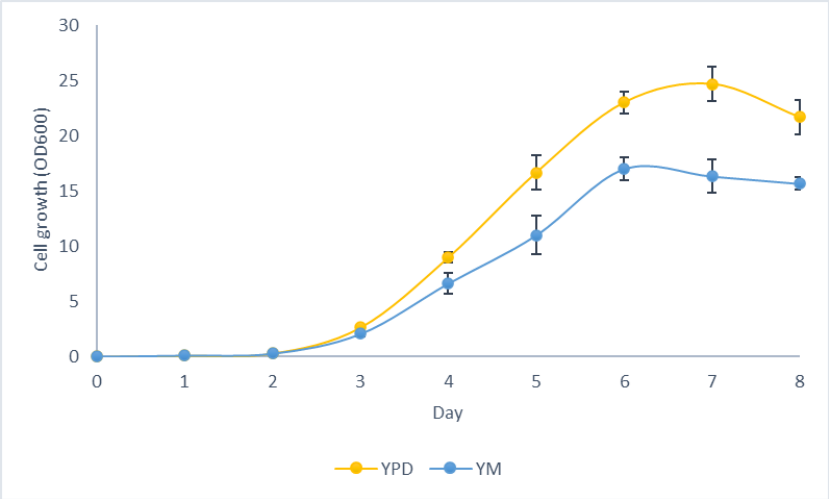


Fig. 4. The growth curve of media optimization in YPD and YM liquid media.

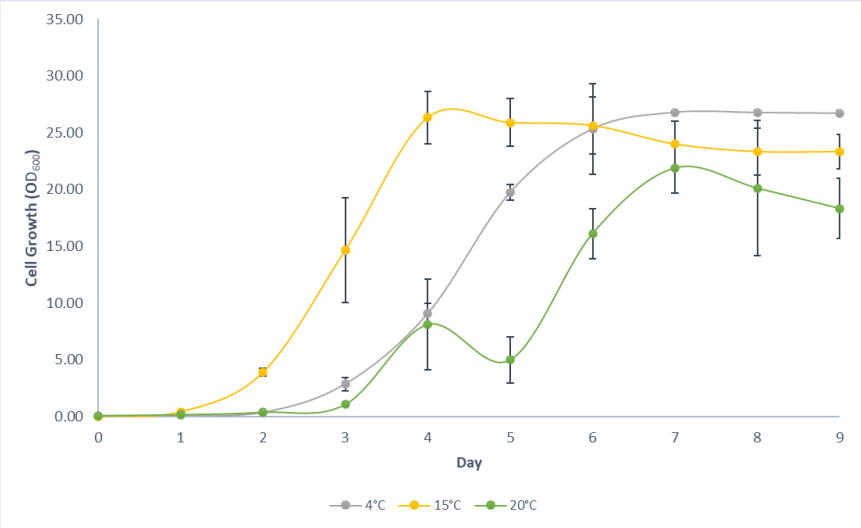


Fig. 5. The growth curve of temperature optimization at 4°C, 15°C and 20°C.

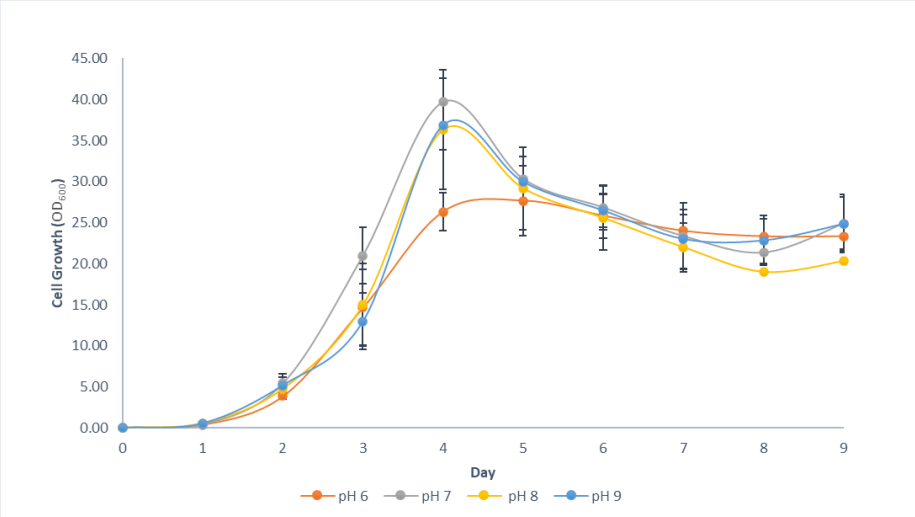
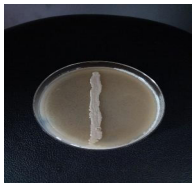
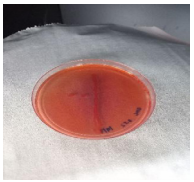
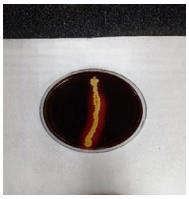
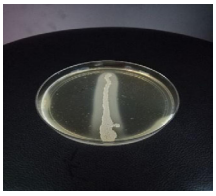


Fig. 6. The growth curve of pH optimization at pH 6, 7, 8 and 9.

Table 2. Summary of the chitinase, cellulase, amylase, and protease activity of *P. glacialis*

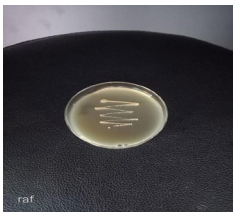
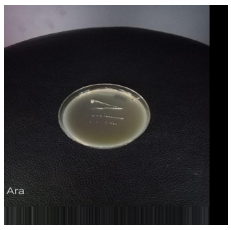
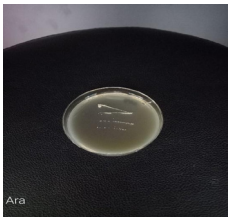
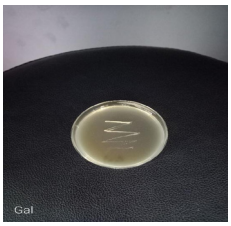
Polysaccharides	Chitin	CMC	Starch	Casein
Enzyme activity				
	-	-	+	+

#+, with activity; -, no activity

Determination of carbon source assimilation

According to Wang *et al*. (2015b), the proposed genera (*Phenoliferia*) shows the inability to grow on maltose but not on raffinose. Conversely, the *P. glacialis* USM-PSY62 in this study was able to grow in the presence of maltose as well as other carbon sources tested including raffinose, galactose, and arabinose. Table 3 describes the findings of the carbon assimilation test. A study reported by Margesin *et al*. (2007) also mentioned the inability of *R. glacialis* to grow on maltose, galactose, and arabinose. *Phenoliferia glacialis* USM-PSY62 in this study might have adapted new characteristics to be able to use various types of carbon sources. Table 4 summarises the findings on *P. glacialis* characterization while comparing with previously reported data on *P. glacialis* or *R. glacialis*.

Table 3. Summary of the assimilation of different carbon sources of *P. glacialis*

Carbon source	Raffinose	Maltose	Arabinose	Galactose
Growth				
	+	+	+	+

#+, with activity; -, no activity

Growth of *P. glacialis* USM-PSY62 in the presence of DDE and PCB

Phenoliferia glacialis USM-PSY62 was cultured in YPD media supplemented with specific concentrations of DDE and PCB. They were incubated for seven days at 15°C. Interestingly, *P. glacialis* USM-PSY62 demonstrated growth, indicating its resilience and adaptive capacity in environments contaminated with POPs such as DDE and PCB. Figure 7 shows the growth of *P. glacialis* USM-PSY62 in the screening of POPs.

DISCUSSION

The optimum growth condition for microbes can be determined through medium, pH, and temperature optimization. *P. glacialis* USM-PSY62 has an optimum growth temperature of 15°C and can grow at a maximum temperature of 20°C (Halim *et al*., 2023). In the current study, *P. glacialis* USM-PSY62 has optimum growth temperature at 15°C while slow growth at 20°C can be observed. The yeast has the least doubling time at 15°C compared to growth at 4°C and 20°C. YPD, PDA, and YM medium are examples of suitable media for the growth of yeast (Kurtzman *et al*., 2011). *P. glacialis* USM-PSY62 in this study favored YPD over YM media while both contained yeast extract and bacto-peptone that are richer in nutrient contents when compared to potato dextrose (PDA) medium ingredients (Peng *et al*., 2020). Cold-adapted yeast or fungi tend to survive a wide range of pH despite some being unable to grow at lower pH such as at pH 4 and below (Hankin & Anagnostakis, 1975; Berry & Foegeding, 1997). In this study, *P. glacialis* USM-PSY62 showed growth across all pH tests. Overall, YPD and 15°C are the best growth conditions for *P. glacialis* USM-PSY62. This condition was used in subsequent identification experiments such as the optimum condition to grow *P. glacialis* USM-PSY62.

Table 4. Comparison of phenotypic characteristics of *P. glacialis* in this study with previously reported *P. glacialis*

Characteristics	*P. glacialis* in this study	*Rh. glacialis* (Margesin *et al*., 2007)	*P. glacialis* (Carrasco *et al*., 2012)	*P. glacialis* (Martorell *et al*., 2017)
Temperature				
4°C	+	N/A	N/A	+
15°C	+	+	N/A	+
20°C	+	+	N/A	+
25°C	-	-	N/A	-
30°C	-	N/A	N/A	-
Extracellular Enzyme				
Chitinase	-	N/A	-	N/A
Cellulase	-	N/A	-	-
Amylase	+	N/A		+
Protease	+	N/A	-	+
Carbon assimilation				
Raffinose	+	+	N/A	N/A
Maltose	+	-	N/A	N/A
Galactose	+	-	N/A	N/A
Arabinose	+	-	N/A	N/A
pH				
6	+	N/A	N/A	N/A
7	+	N/A	N/A	N/A
8	+	N/A	N/A	N/A
9	+	N/A	N/A	N/A
Salinity				
5%	+	N/A	N/A	N/A
10%	-	-	N/A	N/A
15%	-	N/A	N/A	N/A

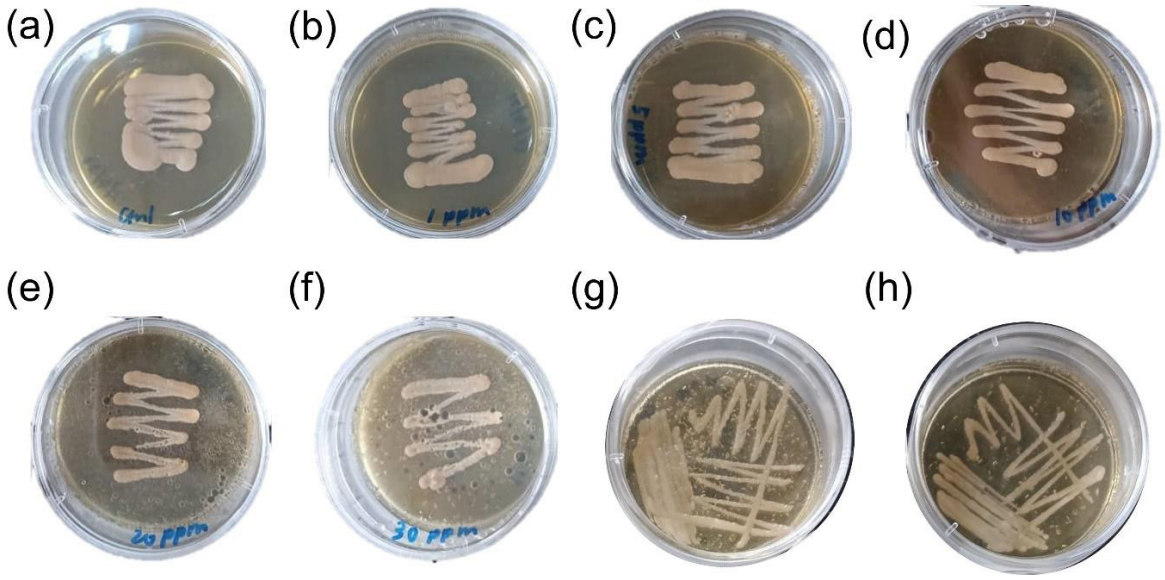


Fig. 7. The growth of *P.glacialis* USM-PSY62 with the presence of DDE and PCB. (a) Control with no addition of DDE and PCB, (b) 1 ppm DDE, (c) 5 ppm DDE, (d) 10 ppm DDE, (e) 20 ppm DDE, (f) 30 ppm DDE, (g) 0.01 µg/L PCB, (h) 0.01 µg/L PCB.

Phenoliferia glacialis USM-PSY62 enters the log phase after two days of incubation at its optimum temperature and stays in the log phase for two days before entering the stationary phase. Psychrophiles take a longer time to grow compared to mesophiles. By growing slowly, psychrophiles can compensate for the limited nutrient availability, prevent the rapid exhaustion that causes starvation, and eventually lead to maximized cellular fitness (Bharudin *et al*., 2018; Gonzalez & Aranda, 2023). Besides affecting growth, temperature also influences microbial activities such as in producing cold-adapted enzymes which is valuable in the field of biotechnology using psychrophiles for the production of cold-active compounds (Margesin, 2009; Yusof *et al*., 2021b; Kamaruddin *et al*., 2022).

Phenoliferia glacialis USM-PSY62 is isolated from Antarctica, an environment with limited nutrient and carbon sources. *Phenoliferia glacialis* USM-PSY62 presented the ability to produce several hydrolytic enzymes that enable the cells to utilize several complicated substrate types other than glucose as the major source of carbon for its growth and energy. Consequently, an organism with the capacity to utilize a variety of carbon sources will have an advantage to survive in a nutrient-poor environment. Besides, the results from the characterization study were compared with other reported characterizations of *P. glacialis* USM-PSY62 (Table 3). There were newly discovered characteristics such as the growth of *P. glacialis* observed in the presence of maltose, arabinose, and galactose while previously it was reported that the yeast presented no growth when those carbon sources were added into the media. Additionally, there are differences between several published studies in terms of the extracellular enzymes produced. These differences in observation could be influenced by the location and the type of samples of isolation.

Margesin *et al*. (2007) reported remarkable phenol degradation activities by *Phenoliferia* sp. at low temperatures. In this study, surprisingly, *P. glacialis* can survive up to 30 ppm of DDE and 0.01 µg/mL supplemented in the growth media. There is still scarce reported degradation performance of DDE and PCB by cold-adapted yeast especially the indigenous species of Antarctica. However, there was a study reported that while some Antarctic isolates presented no growth when phenol and heavy metals salts were added into the rich culture media, several Antarctic isolates presented abundant growth with the presence of those compounds. (Fernández *et al*., 2017). The resistance towards organic pollutants and heavy metals allows the surviving strain to be a promising environment treatment in extremely cold regions. A study regarding the ability of cold-adapted yeast to degrade compounds such as lignin monomers and polycyclic aromatic hydrocarbon (PAH) (Martínez-Ávila *et al*., 2021; Margesin *et al*., 2022).

Therefore, based on the ability of *P. glacialis* USM-PSY62 to grow in the presence of PCB and DDE, *P. glacialis* USM-PSY62 might as well have the potential to degrade the pollutants. The microbial response towards xenobiotic compounds like PCB and DDE was reported to induce the degradation and detoxification response by the microbial cells (Murínová *et al*., 2014; Martínez-Ávila *et al*., 2021). While the microbial response is highly dependent on the species and the type of xenobiotics compound, it will be fortunate if the response of *P. glacialis* USM-PSY62 to both PCB and DDE can be revealed in future studies. This preliminary study will pave the way for deeper insight into studying cold-adapted yeast as one of the bioremediation strategies, not only in temperate regions but also in polluted extreme polar regions.

CONCLUSION

In this study, previously reported *Rhodotorula* sp. was identified as *Phenoliferia glacialis* USM-PSY62. This yeast shared some of the traits of other reported *P. glacialis* while also having some distinctive traits of its own such as the ability to assimilate wider types of carbon source. These characteristics may explain the success in adapting in various low-nutrient settings, including Antarctica. Currently, there is no information concerning DDE and PCB degradation by *P. glacialis* USM-PSY62 was detected, however, several *Phenoliferia* species have been reported to degrade phenol. Thus, this is the first report on *P. glacialis* ability to potentially be utilized in the bioremediation of POPs especially towards DDE and PCB.

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ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Baeza, M., Zúñiga, S., Peragallo, V., Gutierrez, F., Barahona, S., Alcaino, J. & Cifuentes, V. 2022. Response to cold A comparative transcriptomic analysis in eight cold-adapted yeasts. Frontiers in

Microbiology, 13(February): 828536. https://doi.org/10.3389/fmicb.2022.828536
Berry, E.D. & Foegeding, P.M. 1997. Cold temperature adaptation and growth of microorganisms. Journal of Food Protection, 60(12): 1583-1594. https://doi.org/10.4315/0362-028X-60.12.1583
Bharudin, I., Abu Bakar, M.F., Hashim, N.H.F., Mat Isa, M.N., Alias, H., Firdaus-Raih, M., Md Illias, R., Najimudin, N., Mahadi, N.M., Abu Bakar, F.D. & Abdul Murad, A.M. 2018. Unravelling the adaptation strategies employed by Glaciozyma antarctica PI12 on Antarctic sea ice. Marine Environmental Research, 137: 169-176. https://doi.org/10.1016/j.marenvres.2018.03.007
Carrasco, M., Rozas, J. M., Barahona, S., Alcaíno, J., Cifuentes, V. & Baeza, M. 2012. Diversity and extracellular enzymatic activities of yeasts isolated from King George Island , The Sub- Antarctic Region. BMC Microbiology, 12: 251. https://doi.org/10.1186/1471-2180-12-251
Corsolini, S. & Sarà, G. 2017. The trophic transfer of persistent pollutants (HCB, DDTs, PCBs) within polar marine food webs. Chemosphere, 177: 189-199. https://doi.org/10.1016/j.chemosphere.2017.02.116
de Menezes, G. C. A., Amorim, S. S., Gonçalves, V. N., Godinho, V. M., Simões, J. C., Rosa, C. A. & Rosa, L. H. 2019. Diversity, distribution, and ecology of fungi in the seasonal snow of Antarctica. Microorganisms, 7(10): 1-16. https://doi.org/10.3390/microorganisms7100445
Fernández, P.M., Martorell, M.M., Blaser, M.G., Ruberto, L.A.M., de Figueroa, L.I.C. & Mac Cormack, W.P. 2017. Phenol degradation and heavy metal tolerance of antarctic yeasts. Extremophiles, 21(3): 445-457. https://doi.org/10.1007/s00792-017-0915-5
Ferreira, E.M.S., de Sousa, F.M.P., Rosa, L.H. & Pimenta, R.S. 2019. Taxonomy and richness of yeasts associated with angiosperms, bryophytes, and meltwater biofilms collected in the Antarctic Peninsula Eskálath. Extremophiles, 23(1): 151-159. https://doi.org/10.1007/s00792-018-1069-9
Gonzalez, J.M. & Aranda, B. 2023. Microbial growth under limiting conditions-future perspectives. Microorganisms, 11(7): 1641. https://doi.org/10.3390/microorganisms11071641
Halim, M.A., Chai, C.N., Yam, H.C., Rosli, N., Azlan, A., Mohamad, F., Azzam, G. & Najimudin, N. 2023. Transcriptomic response of an Antarctic yeast *Rhodotorula* sp. USM-PSY62 to temperature changes. Malaysian Journal of Microbiology, 19(5): 458-471. https://doi.org/10.1017/CBO9781107415324.004
Hankin, L. & Anagnostakis, S.L. 1975. The use of solid media for detection of enzyme production by fungi. Mycologia, 67(3): 597. https://doi.org/10.2307/3758395
Kamaruddin, S., Redzuan, R.A., Minor, N., Seman, W.M.K.W., Md Tab, M., Jaafar, N.R., Ahmad Rodzli, N., Jonet, M.A., Bharudin, I., Yusof, N.A., Xia, D.Q.H., Mahadi, N.M., Murad, A.M.A. & Bakar, F.D.A. 2022. Biochemical characterisation and structure determination of a novel cold-active proline iminopeptidase from the psychrophilic yeast, *Glaciozyma antarctica* PI12. Catalysts, 12(7): 722. https://doi.org/10.3390/catal12070722
Kurtzman, C.P., Fell, J.W., Boekhout, T. & Robert, V. 2011. Methods for isolation, phenotypic characterization and maintenance of yeasts. In: The Yeasts. C.P. Kurtzman, J.W. Fell and T. Boekhout (Eds.). Elsevier B.V. pp. 87-110. https://doi.org/10.1016/B978-0-444-52149-1.00007-0
Margesin, R. & Miteva, V. 2011. Diversity and ecology of psychrophilic microorganisms. Research in Microbiology, 162(3): 346-361. https://doi.org/10.1016/j.resmic.2010.12.004
Margesin, R. 2009. Effect of temperature on growth parameters of psychrophilic bacteria and yeasts. Extremophiles, 13: 257-262. https://doi.org/10.1007/s00792-008-0213-3
Margesin, R., Fonteyne, P., Schinner, F. & Sampaio, P. 2007. *Rhodotorula psychrophila* sp. nov., *Rhodotorula psychrophenolica* sp. nov. and *Rhodotorula glacialis* sp. nov., novel psychrophilic basidiomycetous yeast species isolated from Alpine environments. International Journal of Systematic and Evolutionary Microbiology, 57(9): 2179-2184. https://doi.org/10.1099/ijs.0.65111-0
Margesin, R., Ludwikowski, T.M., Kutzner, A. & Wagner, A.O. 2022. Low-temperature biodegradation of lignin-derived aromatic model monomers by the cold-adapted yeast Rhodosporidiobolus colostri isolated from Alpine forest soil. Microorganisms, 10(3): 515. https://doi.org/10.3390/microorganisms10030515
Martínez-Ávila, L., Peidro-Guzmán, H., Pérez-Llano, Y., Moreno-Perlín, T., Sánchez-Reyes, A., Aranda, E., Ángeles de Paz, G., Fernández-Silva, A., Folch-Mallol, J.L., Cabana, H., Gunde-Cimerman, N. & Batista-García, R.A. 2021. Tracking gene expression, metabolic profiles, and biochemical analysis in the halotolerant basidiomycetous yeast *Rhodotorula mucilaginosa* EXF-1630 during benzo[a] pyrene and phenanthrene biodegradation under hypersaline conditions. Environmental Pollution, 271: 116358. https://doi.org/10.1016/j.envpol.2020.116358
Martorell, M.M., Ruberto, L.A.M., Fernández, P.M., Castellanos de Figueroa, L.I. & Mac Cormack, W.P. 2017. Bioprospection of cold-adapted yeasts with biotechnological potential from Antarctica. Journal of Basic Microbiology, 57(6): 504-516. https://doi.org/10.1002/jobm.201700021
Murínová, S., Dercová, K. & Dudášová, H. 2014. Degradation of polychlorinated biphenyls (PCBs)

by four bacterial isolates obtained from the PCB-contaminated soil and PCB-contaminated sediment. International Biodeterioration and Biodegradation, 91: 52-59. https://doi.org/10.1016/j.ibiod.2014.03.011

Peng, T.C., Peng, K.S. & Ling, C.M.W.V. 2020. Characterisation of an Antarctic yeast, Glaciozyma antarctica PI12. Borneo International Journal of Biotechnology (BIJB), 1(December): 89-102. https://doi.org/10.51200/bijb.vi.2154

Perini, L., Gostinčar, C. & Gunde-Cimerman, N. 2019. Fungal and bacterial diversity of Svalbard subglacial ice. Scientific Reports, 9: 20230. https://doi.org/10.1038/s41598-019-56290-5

Singh, V.P., Singh, P. & Haritashya, U.K. 2011. Encyclopedia of Snow, Ice and Glaciers. Springer Dordrecht. https://doi.org/10.1007/978-90-481-2642-2

Tamura, K., Stecher, G. & Kumar, S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution, 38(7): 3022-3027. https://doi.org/10.1093/molbev/msab120

Terajima, T., Shibahara, A., Nakano, Y., Kobayashi, S., Godwin, J.R., Nagaoka, K., Watanabe, G., Takada, H. & Mizukawa, K. 2022. Age-related accumulation of persistent organic chemicals in captive king penguins (*Aptenodytes patagonicus*). Journal of Veterinary Medical Science, 84(11): 1551-1555. https://doi.org/10.1292/jvms.22-0245

Touchette, D., Altshuler, I., Gostinčar, C., Zalar, P., Raymond-Bouchard, I., Zajc, J., McKay, C. P., Gunde-Cimerman, N. & Whyte, L. G. 2022. Novel Antarctic yeast adapts to cold by switching energy metabolism and increasing small RNA synthesis. ISME Journal, 16(1): 221-232. https://doi.org/10.1038/s41396-021-01030-9

Treco, D.A. & Lundblad, V. 1993. Preparation of yeast media. Current Protocols in Molecular Biology, 23(1): 13.1.1-13.1.7). https://doi.org/10.1002/0471142727.mb1301s23

Tsuji, M. 2018. Genetic diversity of yeasts from East Ongul Island, East Antarctica and their extracellular enzymes secretion. Polar Biology, 41(2): 249-258. https://doi.org/10.1007/s00300-017-2185-1

Wang, Q.M., Groenewald, M., Takashima, M., Theelen, B., Han, P.J., Liu, X.Z., Boekhout, T. & Bai, F.Y. 2015a. Phylogeny of yeasts and related filamentous fungi within Pucciniomycotina determined from multigene sequence analyses. Studies in Mycology, 81: 27-53. https://doi.org/10.1016/j.simyco.2015.08.002

Wang, Q.M., Yurkov, A.M., Göker, M., Lumbsch, H.T., Leavitt, S.D., Groenewald, M., Theelen, B., Liu, X.Z., Boekhout, T. & Bai, F.Y. 2015b. Phylogenetic classification of yeasts and related taxa within Pucciniomycotina. Studies in Mycology, 81(January 2016): 149-189. https://doi.org/10.1016/j.simyco.2015.12.002

Xie, J., Tao, L., Wu, Q., Bian, Z., Wang, M., Li, Y., Zhu, G. & Lin, T. 2022. Bioaccumulation of organochlorine pesticides in Antarctic krill (*Euphausia superba*): Profile, influencing factors, and mechanisms. Journal of Hazardous Materials, 426: 128115. https://doi.org/10.1016/j.jhazmat.2021.128115

Yusof, N.A., Charles, J., Wan Mahadi, W.N.S., Murad, A.M.A. & Mahadi, N.M. 2021a. Characterization of Inducible HSP70 Genes in an Antarctic Yeast, *Glaciozyma antarctica* PI12, in Response to Thermal Stress. Microorganisms, 9: 2069. https://doi.org/10.3390/microorganisms9102069

Yusof, N.A., Hashim, N.H.F. & Bharudin, I. 2021b. Cold adaptation strategies and the potential of psychrophilic enzymes from the Antarctic yeast, *Glaciozyma antarctica* PI12. Journal of Fungi, 7(528): 1-16. https://doi.org/10.3390/jof7070528

Yusof, N.A., Masnoddin, M., Charles, J., Thien, Y.Q., Nasib, F.N., Wong, C.M.V.L., Abdul Murad, A.M., Mahadi, N.M. & Bharudin, I. 2022. Can Heat Shock Protein 70 (HSP70) serve as biomarkers in antarctica for future ocean acidification, warming and salinity stress? Polar Biology, 45(3): 371-394. https://doi.org/10.1007/s00300-022-03006-7