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Cold-adapted enzymes produced by fungi from terrestrial and marine Antarctic environments

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ABSTRACT

Antarctica is the coldest, windiest, and driest continent on Earth. In this sense, microorganisms that inhabit Antarctica environments have to be adapted to harsh conditions. Fungal strains affiliated with Ascomycota and Basidiomycota phyla have been recovered from terrestrial and marine Antarctic samples. They have been used for the bioprospecting of molecules, such as enzymes. Many reports have shown that these microorganisms produce cold-adapted enzymes at low or mild temperatures, including hydrolases (e.g. α -amylase, cellulase, chitinase, glucosidase, invertase, lipase, pectinase, phytase, protease, subtilase, tannase, and xylanase) and oxidoreductases (laccase and superoxide dismutase). Most of these enzymes are extracellular and their production in the laboratory has been carried out mainly under submerged culture conditions. Several studies showed that the cold-adapted enzymes exhibit a wide range in optimal pH (1.0–9.0) and temperature (10.0–70.0 °C). A myriad of methods have been applied for cold-adapted enzyme purification, resulting in purification factors and yields ranging from 1.70 to 1568.00-fold and 0.60 to 86.20%, respectively. Additionally, some fungal cold-adapted enzymes have been cloned and expressed in host organisms. Considering the enzyme-producing ability of microorganisms and the properties of cold-adapted enzymes, fungi recovered from Antarctic environments could be a prolific genetic resource for biotechnological processes (industrial and environmental) carried out at low or mild temperatures.

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Introduction

Antarctica is a remote continent whose predominant characteristics include extreme low temperatures in winter (between -20.0 and -50.0 °C at the McMurdo Dry Valleys) and average temperatures below 0.0 °C, cycles of freezing and thawing, strong winds, high sublimation and evaporation rates, high radiation incidence, and long periods of darkness that limit the development of life [1]. Microorganisms inhabiting these environments may have a key role in the transport of energy, nutrient recycling and mineralization, providing the basis for the function of these terrestrial, and aquatic ecosystems [1,2].

Due to their extremophilic adaptability, fungi from Antarctic environments have been a focus of

research [3–5]. Cold-adapted enzymes are an important element of the survival strategy in Antarctic ecosystems and their properties could confer some advantages in biotechnological processes [6,7]. Some examples of cold-active enzymes from microorganisms have been reported (Cavicchioli et al. [8] and Marx et al. [9]). Although Cavicchioli et al. [8] evaluated the cold-active enzymes produced by bacteria, archaea, and fungi from cold environments; they did not obtain these microbes specifically from Antarctica. On the other hand, Marx et al. [9] contributions are related to the cold-adapted enzymes from marine Antarctic microorganisms, highlighting the comparison of these enzymes with their mesophilic counterparts. Fungi from Antarctic terrestrial samples have not been mentioned.

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Considering the ecological and biotechnological relevance of fungi from Antarctic ecosystems, this review reports the main cold-adapted enzymes produced by filamentous fungi and yeasts.

Diversity of fungi recovered from Antarctic samples

Microorganisms from Antarctic environments include representatives from all three domains, Bacteria, Archaea, and Eukarya [10]. They are classified as psychrophilic, psychrotolerant, and mesophilic-psychrotolerant. Psychrophilic microorganisms grow at maximum temperatures below 20.0 °C with optimum growth temperatures lower than, or equal to, 15.0 °C. Psychrotolerant microorganisms have the ability to grow at low temperatures, with optimum growth temperatures varying from >15.0 and ≤25.0 °C, while mesophilic-psychrotolerant microorganisms have the ability to grow at low temperatures, with optimum growth temperatures >25.0 and ≤40.0 °C [11].

Antarctic fungi were isolated from many terrestrial environments, including soils [4,7,12–16], ornithogenic soils [17,18], penguin, skua, and petrel dung and bird feathers [19], bryophytes [20,21], leaves from vascular plants [22–24], lichens and stones with lichens [18,25,26], rocks [27,28], ice-free rocks [29], and wooden structures [30,31]. On the other hand, fungi from Antarctic marine ecosystems have been isolated from sediments [18,32,33], seawater [12,33], sponges [5,18,34], and macroalgae [3,25,35,36].

Most fungal species recovered from Antarctica are common, possibly because this environment continuously receives microbial propagules from outside the Continent [2]. According to Margesin and Miteva [10], dispersal of microorganisms is possible through a combination of favorable climatic factors, such as winds and storms, in addition to dissemination by oceanic currents, dust, plant seeds, and birds.

Filamentous fungi from Antarctica belong mainly to the phylum Ascomycota. Representatives of the genera *Pseudogymnoascus* (formerly *Geomyces*) and *Mortierella* have frequently been isolated [7,36–38]. Other genera reported in the Antarctic environments include: *Acremonium*, *Alternaria*, *Antarctomyces*, *Ascobolus*, *Aspergillus*, *Beauveria*, *Cadophora*, *Capnobotryella*, *Chaetomium*, *Cladosporium*, *Dactylella*, *Davidiella*, *Embellisia*, *Entrophospora*, *Engyodontium*, *Epicoccum*, *Eurotium*, *Fusarium*, *Geomyces*, *Gyoerffyyella*, *Lecanicillium*, *Leuconeurospora*, *Microdochium*, *Mycocentrospora*, *Penicillium*, *Phaeosphaeria*, *Phoma*, *Pseudogymnoascus*, *Tetracladium*, *Thelobolus*, *Trichoderma*, and *Verticillium* [3,4,22,23,26,39–41].

Conversely, the majority of yeasts recovered from Antarctica belong to the phylum Basidiomycota. Representatives of the genera *Cryptococcus* and *Rhodotorula* have frequently been isolated from Antarctic environments [12,14,18,24]. Other common genera from the phylum Basidiomycota reported in Antarctica are: *Bensingtonia*, *Bullera*, *Cystofilobasidium*, *Dioszegia*, *Glaciozyma*, *Holtermanniella*, *Leucosporidium*, *Mrakia*, *Pseudozyma*, *Papiliotrema*, *Sporobolomyces*, and *Tausonia* (formerly *Guehomyces*). Ascomycota yeasts commonly found in Antarctica belong to the genera *Candida*, *Debaryomyces*, *Kodamaea*, *Meyerozyma*, *Metschnikowia*, *Wickerhamomyces*, and *Yamadazyma* [3,18,24–26,33,41,42].

Metschnikowia australis and *Naganishia antarctica* (formerly *Cryptococcus antarcticus*) are supposed to be autochthonous in Antarctica [2,3]. Representatives of *M. australis* were recovered from seawater [33], macroalgae [3,25,35,36], and sponge [34], whereas representatives of *Naganishia antarcticus* were isolated from soil, cold desert soil [39,43], and rhizosphere soil [33]. Considering the advances in the microbial taxonomy, some of the yeast species found in Antarctica are being reclassified [44,45]. However, a comprehensive nomenclatural revision is beyond the scope of this review; the nomenclature presented in this work is that originally presented in previous literature.

The growing number of new fungal taxa from Antarctica indicates an apparently hidden diversity in this environment. Besides, next-generation sequencing technologies and metagenomics approaches proved to be effective for the knowledge and comparison of the Antarctic microbial communities [46,47]. The following new fungal species isolated from Antarctic samples have recently been described: *Cystobasidium portillonense* sp. nov. (formerly *Rhodotorula portillonensis*) [48]; *Piskurozyma fildesensis* sp. nov. (formerly *Cryptococcus fildesensis*) [49]; *Leucosporidium escuderoi* f.a., sp. nov. [50]; *Taphrina antarctica* f.a. sp. nov. [51]; *Naganishia vaughanmartiniae* sp. nov. (formerly *Cryptococcus vaughanmartiniae*) [52]; *Cystobasidium tubakii* sp. nov. and *Cystobasidium ongulense* sp. nov. [53], and *Antarctomyces pellizariae* sp. nov. [54].

Adaptations to the Antarctic environments

Low temperatures cause injuries to biological systems, such as an increase in cellular viscosity, intracellular oxidative stress, the formation of ice crystals, a reduction in membrane fluidity, a decrease in solute diffusion, stabilization of inhibitory nucleic acid structures, and reductions in the molecular interactions and biochemical reaction rates [55–57].

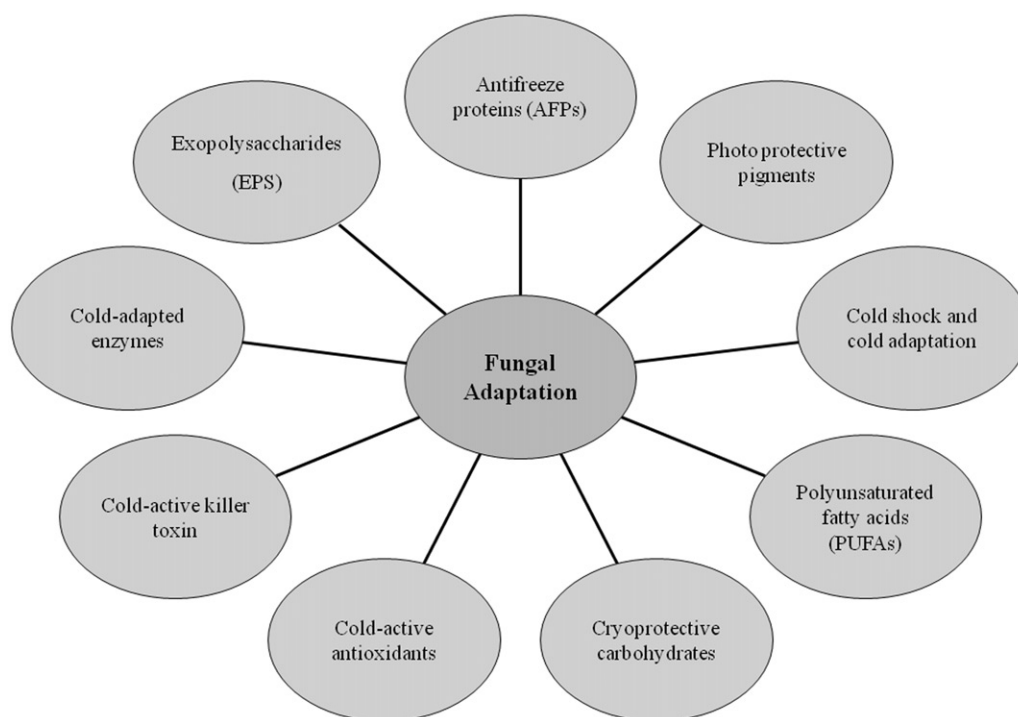


Figure 1. Main fungal adaptations to Antarctic conditions.

Survival in “extreme” environmental conditions requires a vast array of ecological and evolutionary adaptations, which are expressed at physiological, metabolic, and structural levels [27,55–57]. Survival strategies that have been observed in fungi recovered from Antarctic environments are summarized in Figure 1.

Cold-adapted enzymes

Definition and properties

Pioneering studies of psychrophilic microorganisms at the molecular level focused mainly on cold-active enzymes because this was considered as a prerequisite for environmental adaptation [58,59]. Cold-adapted enzymes are characterized by increased turnover and inherent catalytic efficiency at low temperatures. Because of their flexible structures, these enzymes show reduced activation enthalpy and negative entropy of activation compared to enzymes produced by mesophilic and thermophilic homologs [59].

The increase in flexibility has been proposed as a major molecular mechanism for the evolution of cold-adapted enzymes and relates to a combination of the following features [60–62]: (i) a decrease in core hydrophobicity, an increase in surface hydrophobicity, a lower arginine/lysine ratio, weaker inter-domain and inter-subunit interactions, and more and longer loops; (ii) a prevalence of α -helices in the secondary structure;

(iii) a decrease in the secondary structure content, less and weaker metal-binding sites, and a reduction in the number of disulfide bridges and electrostatic interactions; and (iv) a reduction in the oligomerization and an increase in the nonconformational entropy of the unfolded state.

Because of their structure, cold-adapted enzymes have properties that could be useful in many biotechnological processes. According to Feller and Gerday [58], these enzymes are up to tenfold more active at low/moderate temperatures than their mesophilic homologs, offering economic benefits by reducing energetic costs of the production processes conducted at moderate temperatures (25.0–40.0 °C); they can be inactivated by temperature before unfolding of the protein structure; and their heat-lability (mainly in psychrophilic and psychrotolerant microorganisms) can propitiate selective inactivation in complex mixtures.

Enzymes produced by fungi of Antarctic origin

Overall, there are two approaches to assess microbial resources including enzymes: based on the screening of microbial strains and based on the screening of genomic libraries. However, there is a lack of information related to the prospecting of Antarctic enzymes based on the metagenomic approach (culture-independent techniques). It is worth mentioning that metagenomics allows one to explore new environments for putative functions, but still the culturable strains are necessary

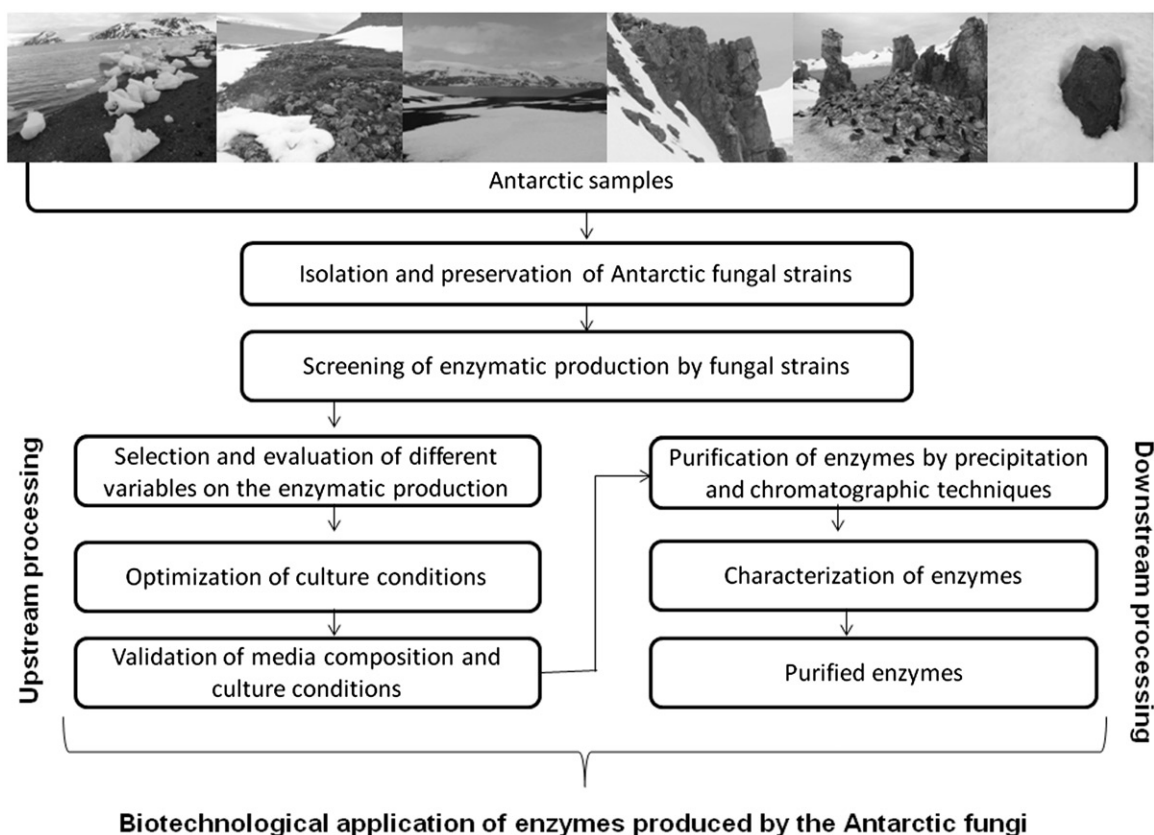


Figure 2. Prospecting of enzymes produced by fungi from Antarctica.

for applied studies. In this sense, this review only presents the data related to screening and production of enzymes using the culture-dependent approach (Figure 2).

The method commonly used to screen fungal enzymes is the halo/colony ratio, which is performed in solid media to quantify extracellular enzymatic activity. The culture conditions and characteristics of some enzymes produced by filamentous fungi and yeasts of Antarctic origin are described in Table 1. The majority of the enzymes are classified as hydrolases. However, some oxidoreductases, such as laccase and superoxide dismutase have also been reported. The enzyme production presented in Table 1 is mainly carried out under submerged cultures.

Some fungal species isolated from Antarctic samples show the ability to produce enzymes at 28.0, 29.0, and 37.0°C, emphasizing the potential to respond to higher growth temperatures. According to Pesciaroli et al. [11], microbes capable of growing across a wide spectrum of temperatures are more versatile in relation to environmental changes and able to colonize a wide range of ecological niches in cold environments. These microorganisms are defined as eurythermics and may be useful for industrial processes. On the other hand, the majority

of enzyme production was carried out at low and moderate temperatures (ranging from 4.0 to 26.0°C), highlighting the potential of Antarctic fungi for biotechnological applications at low/moderate temperatures. It is important to mention that the optimum temperatures for enzymatic activity of many enzymes were higher than those used for enzyme production (Table 1).

The enzymes presented in Table 1 are produced by fungi of Antarctic origin, which belong to the genera *Cystobasidium*, *Cystofilobasidium*, *Debaryomyces*, *Dioszegia*, *Glaciozyma*, *Goffeauzyma*, *Leucosporidium*, *Moesziomyces*, *Mrakia*, *Naganishia*, *Papiliotrema*, *Phenoliferia*, *Rhodotorula*, *Tausonia*, *Vanrija* (yeasts) and *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Geomyces*, *Lecanicillium*, *Penicillium*, *Phoma*, *Pseudogymnoascus*, *Trichoderma*, and *Verticillium* (filamentous fungi).

The production of polysaccharide metabolizing enzymes by Antarctic fungal strains from terrestrial samples can be explained by the availability of nutrients in these environments. On the other hand, in marine environments the presence of polysaccharides (except for chitin) is not evident, suggesting that the ocean could not be their natural reservoir. Fungal strains, recovered

Table 1. Cold-adapted enzymes produced by Antarctic fungi: growth conditions and characteristics.

Enzyme	Enzyme nomenclature (EC number)	Fungi	Source of isolate (sample)	Growth condition				Enzyme characteristics				References
				Medium	T (°C)	pH	Incubation Time (hours)	Medium quantification (Substrate)	Optimum T (°C)	Optimum pH		
Amylase (α -amylase)	3.2.1.1	<i>Moesziomyces antarcticus</i> (formerly <i>Candida antarctica</i>)	Centraal Bureau voor Schimmelcultures, Yeast Division, The Netherlands	Soluble starch 1.0%	29.0	NR	120.0	Hydrolysis of soluble starch	62.0	4.2	[63]	
		<i>Pseudogymnoascus pannorum</i> R1-2 (formerly <i>Geomyces pannorum</i>)	NR	Peptone 0.5%, yeast extract 0.2%, K_2HPO_4 0.1%, $MgSO_4$ 0.05%, supplemented with either 2% starch as the sole carbon source or 1.0% peptone 2.0%, and starch 1.0%	20.0	NR	168.0	Hydrolysis of soluble starch	70.0	6.0	[64]	
		<i>Glaciozyma antarctica</i> PI12 (formerly <i>Leucosporidium antarcticum</i>)	Seawater	Yeast extract 1.0%, peptone 2.0%, and starch 1.0%	4.0	NR	168.0–216.0	Hydrolysis of soluble starch	NR	NR	[65]	
		<i>Phenoliferia glacialis</i> (formerly <i>Rhodotorula glacialis</i>)	Soil	Yeast extract 0.3% malt extract 0.3%, and peptone 0.5% supplemented with 1.0% glucose and soluble starch	4.0–37.0	4.6, 5.4 and 6.2;	NR	Hydrolysis of soluble starch and the plates were flooded with 1.0 mL of iodine solution	10.0–22.0	5.4 and 6.2	[13]	
Amylase (Glucoamylase)	3.2.1.3	<i>Moesziomyces antarcticus</i> (formerly <i>Candida antarctica</i>)	Centraal bureau voor Schimmelcultures, Yeast Division, Delft, The Netherlands	Soluble starch 1.0%	29.0	NR	120.0	Hydrolysis of soluble starch	57.0	4.2	[63]	
Celulase	3.2.1.4	<i>Verticillium</i> sp AnsX1	Soil	Carboxymethyl cellulose sodium salt (CMC-Na) 0.5% and 2.0%	10.0	NR	96.0	Whatman No. 1 filter paper strip	38.0	5.3	[66]	
		<i>Cystoflobasidium infirmominatum</i> 071209-E8-C1-11b-lev	Marine sponge	Carboxymethyl cellulose 1.0%	15.0–23.0	5.0–7.0	168.0	NR	NR	NR	[34]	
		<i>Mrakia biolopsi</i>	Soil	Yeast extract 0.3%, malt extract 0.3%, and peptone 0.5% supplemented with 1.0% glucose and carboxymethyl cellulose	4.0–37.0	4.6, 5.4 and 6.2;	NR	Carboxymethylcellulose and the plates were flooded with 1mg/mL of Congo red solution	4.0–22.0	5.4	[13]	
Chitinase	3.2.1.14	<i>Lecanicillium muscarium</i> A3	Moss from continental Antarctica	YNB with colloidal chitin 1.0%	5.0, 15.0, and 25.0	5.5	216.0	Colloidal chitin	40.0	4.0	[67]	
		<i>Glaciozyma antarctica</i> PI12	NR	Yeast extract 0.3%, peptone 0.5%, NaCl 0.3%, and colloidal chitin 3%	4.0	NR	168.0–192.0	Colloidal chitin	15.0	4.0	[68]	
β -Galactosidase	3.2.1.23	<i>Tausonia pullulans</i> 17-1 (formerly <i>Guehomyces pullulans</i>)	Marine sediment	Lactose 3.0%, yeast extract 0.7%, polypeptone 0.3%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, and K_2HPO_4 0.3%	15.0	4.5	120.0	o-nitrophenyl- β -D-galactopyranoside	50.0	4.0	[69]	

(continued)

Table 1. Continued

Enzyme	Enzyme nomenclature (EC number)	Fungi	Source of isolate (sample)	Growth condition				Enzyme characteristics			
				Medium	T (°C)	pH	Incubation Time (hours)	Medium quantification (Substrate)	Optimum T (°C)	Optimum pH	References
Invertase	3.2.1.26	Glaciomyces antarctica 17 (formerly Leucosporidium antarcticum)	Seawater	Bactopeptone 0.5%, yeast extract 0.3%, marine salt 3.5% and sucrose 1.0% (in certain cultures replaced by glucose or maltose 1.0%)	6.0	6.50	384.0	Sucrose and raffinose	30.0	4.55 (sucrose) and 4.75 (raffinose)	[70]
Laccase	1.10.3.2	<i>Dioszegia hungarica</i> (formerly <i>Bullera armeniaca</i>) <i>Goffeauzyma gilvescens</i> (formerly <i>Cryptococcus gilvescens</i>) <i>Dioszegia</i> sp. <i>Debaryomyces hansenii</i> <i>Leucosporidium creatinivorum</i> (formerly <i>Leucosporidiella creatinivora</i>) <i>Rhodotorula</i> sp. <i>Rhodotorula mucilaginosa</i> <i>Cystobasidium laryngis</i> (formerly <i>Rhodotorula laryngis</i>)	Marine macroalgae, rocks and soils	Basal Medium with 0.02% guaiacol	15.0	NR	240.0	NR	NR	NR	[71]
Lipase	3.1.1.3	<i>Geomyces</i> sp. P7	Soil from King George Island	Corn steep liquor 0.3% and olive oil 0.5%	10.0	NR	504.0	Emulsion containing synthetic triacylglycerols (triacetin, tributyrate, tricaprylate, tripalmitate or tristearin)	35.0	8.0	[72]
		<i>Leucosporidium scottii</i> L117	Marine sediment	Solid corn steep liquor 0.06% and olive oil 4.0%	20.0	8.0	120.0	p-nitrophenyl-palmitate	40.0	5.0	[73]
		<i>Mrakia blolopsis</i> SK-4	Algal mat in sediment	KH ₂ PO ₄ 0.2%, Na ₂ PO ₄ 0.29%, NH ₄ Cl 0.02%, CaCl ₂ 0.04%, FeCl ₃ 0.001%, yeast extract 0.5%, and Tween 80 1.0%	10.0	NR	324.0	p-nitrophenyl-palmitate	60.0–65.0	8.0–9.0	[74]
		<i>Penicillium allii</i> cloned in fungal hots <i>Trichoderma reesei</i>	NR	KH ₂ PO ₄ 0.75 g (NH ₄) ₂ SO ₄ 0.25 g, Avicel 1 g, soy hydrolysate 0.75 g, 20% lactose (2.5 mL), 1 M MgSO ₄ (121 µL), and 1 M CaCl ₂ (204 µL)	28.0	NR	144.0	p-nitrophenyl caprate (C10)	25.0	7.9	[75]
		<i>Penicillium expansum</i> SM3	NR	Avicel cellulose 2.5%, soy hydrolysate 1.5%, Tween 80 0.02%, lactose 1%, MgSO ₄ 0.0024M, and CaCl ₂ 0.0054M	25.0	6.0	168.0	p-nitrophenyl laurate (C12), p-nitrophenyl myristate (C14), p-nitrophenyl palmitate (C16) and p-nitrophenyl stearate (C18)	10.0–30.0	8.0	[76]

(continued)

Table 1. Continued

Enzyme	Enzyme nomenclature (EC number)	Fungi	Source of isolate (sample)	Growth condition				Enzyme characteristics			
				Medium	T (°C)	pH	Incubation Time (hours)	Medium quantification (Substrate)	Optimum T (°C)	Optimum pH	References
β -mannanase	3.2.1.78	<i>Alternaria alternata</i> <i>Phoma</i> sp.	Ornithogenic soils	Minimal medium containing locust bean gum 0.5%	25.0	NR	168.0	Locust bean gum	NR	NR	[77]
Phytase	3.1.3.26	<i>Papiliotrema laurentii</i> (formerly <i>Cryptococcus laurentii</i> AL27)	NR	Different carbon sources (glucose, galactose, fructose, sucrose, and lactose) and sodium phytate as phosphorous sources.	20.0–26.0	2.43–6.9	NR	NR	40.0	4.8	[78]
		<i>Rhodotorula mucilaginosa</i> JMUY14	Deep-sea sediment	Calcium phytate 0.5%, NH_4NO_3 0.5%, KCl 0.05%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05%, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.03%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.03%, and glucose 3%	15.0	5.7	160.0	Sodium phytate	50.0	5.0	[79]
Protease	3.4.	<i>Vanniija humicola</i> (formerly <i>Candida humicola</i>)	Soil	YNB without aminoacids or ammonium sulfate but containing glucose and casein	22.0	4.0	48.0	Casein, hemoglobin, BSA	37.0	1.0 (hemoglobin, BSA) 1.0, 5.0–7.0 (casein)	[80]
		<i>Rhodotorula mucilaginosa</i> L7	Marine Macroalgae	Saboraud Dextrose Broth	25.0	5.6	48.0	Azocasein	50.0	5.0	[81]
		<i>Acremonium</i> sp. L1-4B	Lichen	Medium containing cactus pear being used as the main carbon source.	15.0–25.0	4.0–8.0	96.0	Azocasein	50.0	8.0	[82]
Protease (Subtilase)	3.4.21	<i>Glaciozyma antarctica</i> 171 (formerly <i>Leucosporidium antarcticum</i>)	Sub-glacial waters (depth of 200 m)	Bactopeptone 0.5%, yeast extract 0.3%, saccharose 1%, and marine salt 3.5%	6.0	NR	288.0	Elastine protein	25.0	6.5–6.8 hemoglobin hemolysis	[83]
Superoxide dismutase	1.15.1.1	<i>Aspergillus glaucus</i> 363	Soil	Casein 3.0 g/L, soybean flour 4.0 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g/L, CuSO_4 0.0011 g/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.0029 g/L, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.0043, MnSO_4 0.0013 g/L	25.0	NR	72.0	Nitro-blue tetrazolium (NBT)	NR	NR	[84]
Tannase TAH I	3.1.1.20	<i>Verticillium</i> sp. P9.	Soil	Czapek–Dox medium with 5.0% extract of black tea	16.0	5.8	336.0	Tannic acid or methyl gallate	20.0	5.0	[85]
Tannase TAH II		<i>Verticillium</i> sp. P9.	Soil	Czapek–Dox medium with 5.0% extract of black tea	16.0	5.8	336.0	Tannic acid or methyl gallate	25.0	5.0	[85]
Xylanase	3.2.1.8	<i>Cladosporium</i> sp	Marine sponges	Czapek medium with 1.0% beechwood xylan	23.0	NR	168.0	Xylose	50.0	6.0	[86]
		<i>Penicillium hirsutum</i> <i>Phoma</i> sp.	Ornithogenic soils	Birchwood xylan 0.5%	10.0–37.0	NR	168.0	Birchwood xylan	NR	NR	[77]
		<i>Trichoderma reesei</i> <i>Naganishia adeliensis</i> (formerly <i>Cryptococcus adeliensis</i>)	NR	$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 2.14 g/L; KH_2PO_4 1.01 g/L; NH_4NO_3 2.2 g/L; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g/L; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1 g/L, 1 mL trace element solution, and xylose or xylan 10.0 g/L	10.0	7.5	72.0	Birchwood xylan	40.0–45.0	5.0–5.5	[87]

from tissues of marine sponges, are present as spores and/or propagules derived from the water but do not necessarily form an ecological association with the invertebrate [88].

Hydrolytic enzymes

Cellulases

The production of cellulases has been reported by Antarctic fungi isolated from marine sponges [34], soil [13,66], ornithogenic soils [7,77], and from wood and other organic materials [89–91]. The introduction of wood and other organic materials for human survival by early explorers may have introduced exogenous fungi and new nutrient sources for the indigenous fungi. Wood degradation in Antarctic sites appears to occur via “soft rot” by asexual ascomycetes, rather than by “white rot” or “brown rot” basidiomycete species [30–31,89–93].

For the screening of cellulases from fungi of Antarctic origin, media supplemented with carboxymethylcellulose as a carbon source have been used [34,66,77]. Filamentous fungi recovered from Antarctic samples that belong to the genera *Cadophora*, *Cladosporium*, *Geomyces*, *Penicillium*, *Pseudeurotium*, *Pseudogymnoascus*, *Verticillium*, and *Wardomyces* have shown the ability to produce cellulases [7,66,89,90]. The production of these enzymes was also observed in Antarctic yeasts belonging to the genera *Cryptococcus* and *Mrakia* [7,13,33,94]. Additionally, *Penicillium roqueforti*, *Cadophora malorum*, *Geomyces* sp., and *Mrakia blollopis* can grow and use cellulose substrates in temperatures ranging from 4.0 to 22.0 °C [13,89,90].

Cold-adapted cellulases can be used in the textile and paper industries, in cotton processing, paper recycling, detergent production, juice extraction, as animal feed additives, and for biofuel production [13,66,95,96]. Cold-adapted cellulolytic enzymes tolerant to organic solvents can be used for manufacturing volatile and heat-sensitive compounds such as flavors, fragrances, and perfumes [97].

Xylanases

Fungi able to produce xylanases have been isolated from Antarctic samples including ornithogenic soil [77], soil [12], and marine invertebrates [18,34,86]. Screening of xylanases produced by Antarctic fungi was performed using substrates such as birchwood xylan, beechwood xylan, and wheat bran. Representatives of fungi from the genera *Alternaria*, *Candida*, *Cryptococcus*, *Dioszegia*, *Naganishia*, *Penicillium*, *Tausonia*,

Trichoderma, and *Phoma* were reported as xylanase producers [12,18,77,87,98].

In the study performed by Del-Cid et al. [87], the fungus *Cladosporium* sp., recovered from Antarctic marine sponge, showed high xylanolytic activity at low temperatures using pure xylyans from hardwoods (birchwood and beechwood). Duarte et al. [18] reported the production of xylanases by almost all of the Basidiomycota yeasts (80.56%) recovered from Antarctic samples. In the study carried out by Gomes et al. [87], the definition of culture medium composition using statistical design increased the enzymatic activity of *Naganishia adeliensis* (formerly *Cryptococcus adeliensis*) 4.3-fold at 4.0 °C. According to Petrescu et al. [98], the tridimensional model of xylanase produced by *Naganishia adeliensis* shared 84% identity with the xylanase produced by its mesophilic counterpart *Naganishia albida* (formerly *Cryptococcus albidus*). However, the xylanase from *Naganishia adeliensis* was less thermostable than the one produced by *Naganishia albida*. Additionally, in the temperature range of 0.0–20.0 °C, the cold-adapted xylanase produced by *Naganishia adeliensis* displayed lower activation energy and higher catalytic efficiency, suggesting a less compact, and more flexible molecular structure. Cold-adapted xylanases can be applied in the production of flour and biofuel, in the field of bioremediation, and for the generation of chemicals from lignocellulose [87].

Pectinases

The majority of fungal strains with pectinase activity were recovered from the marine environment in Antarctica. In the study reported by Fenice et al. [99], more than 50% of Antarctic fungal strains produced extracellular polygalacturonase and/or pectin lyase. Representatives of Antarctic fungi from the genera *Arthrotrichum*, *Aureobasidium*, *Cladosporium*, *Leucosporidium* showed the ability to produce pectinases [33,99]. Cold-adapted pectinases can be used in the food processing industry in fruit juice clarification, juice wastewater treatment, and to improve the quality of wines [100].

Chitinases

Antarctic fungal strains able to produce chitinases have been isolated from soil samples [12] and sandstone rocks [28]. Representatives of the genera *Phoma*, *Lecanicillium*, *Leuconeurospora*, *Dioszegia*, *Mrakia*, *Metschnikowia*, *Sporidiobolus*, and *Glaciozyma* were reported as chitinase producers [12,28,67,68,99,101,102]. According to Ramli et al. [68],

chitin comprises approximately 20–58% of the dry weight of marine environment organisms, such as shrimp, crabs, squid, oysters, and krill, the latter being considered the main component of the Antarctic food chain.

In the study reported by Fenice et al. [102], the yield of chitinase production in a bioreactor by *Lecanicillium muscarium* CCFEE-5003 was higher from shrimp shells than from crab shells. This strain produced a complex enzymatic system comprising five proteins that act on chitin [103]. These chitinolytic enzymes have MWs and isoelectric points ranging from ca. 20.0 to 75.0 kDa and pH 4.5 to 5.0, respectively [104]. Two additional chitinolytic enzymes (CHI1 and CHI2) presented differences in chitin hydrolysis and were defined as “chitobiase” and “exo-chitinase”, respectively [102–104].

The structure of psychrophilic chitinase (CHI II) from *Glaciozyma antarctica* P112 was elucidated by Ramli et al. [61]. The authors found that the enzyme has low sequence identity with other chitinase sequences and observed that, similar to other psychrophilic enzymes, the substitutions of certain amino acids in the surface and loop regions conferred an increase in the flexibility of the enzyme, as a result of adaptation to cold temperatures.

Chitinases with activity at low temperatures have significant advantages in industrial applications, such as the treatment of residues and control of microbial contamination in refrigerated foods and increasing shelf life [68,101]. Additionally, cold-adapted chitinases can be used in the treatment of chitin-rich waste at low temperatures, biocontrol of microbial spoilage, and phytopathogens [68].

Proteases

Antarctic fungi able to produce proteases were recovered from soil [7,12,80,99], marine invertebrates and macroalgae [18,34], melt water [12], and lichens [82]. Protease screening has been measured in solid media with skim milk, defatted milk, or casein. In liquid media, the proteolytic activity has been usually determined in the presence of azocasein as substrate.

Antarctic fungi reported as protease producers belong to the genera: *Acremonium*, *Candida*, *Cryptococcus*, *Chrysosporium*, *Embellisia*, *Exophiala*, *Geomyces*, *Glaciozyma*, *Glomerella*, *Leuconeurospora*, *Leucosporidium*, *Mrakia*, *Phoma*, *Pseudogymnoascus*, *Rhodotorula*, *Trichoderma*, *Vanrija*, and *Wickerhamomyces* [12,18,33,34,80–83,99,105]. In most cases, the secretion of proteases was dependent on the medium composition, temperature, and the phase of growth [80–82]. In the study performed by Duarte et al.

[18], 14% of the Antarctic yeasts were able to produce proteases in solid and liquid media. *Rhodotorula mucilaginosa* L7 produced the greatest amounts of enzyme [106]. The extracellular acid protease from the *R. mucilaginosa* L7 was active at temperatures ranging from 15.0 to 60.0 °C [81].

According to Ray et al. [80], the protease of *Vanrija humicola* (formerly *Candida humicola*) was active at temperatures ranging from 0.0 to 45.0 °C and was resistant to freeze-thaw cycles. In the study reported by Turkiewicz et al. [107], the serine protease secreted by *Glaciozyma antarctica* 107 (formerly *Leucosporidium antarcticum*) was most active at 25.0 °C and exhibited activity even at –10.0 °C. On the other hand, the serine protease purified from *Acremonium* sp. L1–4B was active at temperatures ranging from 10.0 to 40.0 °C [82]. In another study, the protease produced by *Pseudogymnoascus pannorum* (formerly *Geomyces pannorum*) exhibited the greatest activity at 4.0 °C [7].

Cold-adapted proteases can be used in the food industry to accelerate cheese ripening, softening frozen/refrigerated meat products, preserving the quality of heat-sensitive nutrients, and can also be effective against haze-producing proteins in winemaking and brewing. In the textile industry, these proteases can be used in detergents and soap powder for dirt removal for “cold washing” processes and washing textiles at room temperature [57,105,107,108].

Lipases

The production of esterases and lipases by Antarctic fungi may be associated with the mechanisms of cold tolerance since the maintenance of cell membrane fluidity is essential to survival and may be achieved by increasing the degree of unsaturation of fatty acids [109,110].

Representatives of Antarctic fungi belonging to the genera *Beauveria*, *Candida*, *Cryptococcus*, *Geomyces*, *Leucosporidium*, *Moesziomyces*, *Mrakia*, *Pseudozyma*, *Penicillium*, *Phoma*, *Pseudogymnoascus*, *Verticillium*, and *Trichosporon* were reported to be lipase producers [18,33,72–74,76,99,111–113]. Substrates used for lipase production by Antarctic fungal strains include vegetable and fish oils, animal fats, synthetic triacylglycerides, and Tween 20, 40, 60, and 80. Lipases can be screened using carbon sources, such as olive oil plus rhodamine B [18,77].

The yeast *Moesziomyces antarcticus* (formerly *Candida antarctica*) is the best-known fungus of Antarctic origin with industrial applications [42]. This yeast produces two lipases, A and B, the latter being sold as Novozym 435 by Novozymes (Bagsvaerd,

Denmark) [114]. Lipase B is involved in many organic synthesis applications related to food and feed processing, pharmaceuticals, and cosmetics [113].

The yeast *Leucosporidium scottii* L117 isolated from an Antarctic marine sediment can produce lipase at 20.0 °C in medium containing olive oil and corn steep liquor as carbon and nitrogen sources, with optimal activity levels at 40.0 °C [73]. In the study performed by Tsuji et al. [74], lipase from *Mrakia blollopis* SK-4 remained stable in a wide range of temperatures. Additionally, the lipase produced by the Antarctic fungus *Geomyces* sp. P7 has an optimum temperature between 8.0 and 35.0 °C (with 15% of maximum activity at 0.0 °C) and performs enantioselective transesterification of 1-phenylethanol with vinyl acetate [72].

Lipases from psychrophilic/psychrotolerant fungi have drawn attention because of their potential for biotechnological applications, including in the food and beverage, chemical, and pharmaceutical industries [115]. These enzymes can also be used in the composition of cleaning products, in the conversion of vegetable oils to biodiesel, for the decomposition of milk at low temperatures, in wastewater treatment, especially in temperate regions, and the cleaning of oil spills [15,60,74,109,116,117].

α -Amylases and glucoamylases

Antarctic fungal strains representative of the genera *Cryptococcus*, *Dioszegia*, *Glaciozyma*, *Holtermanniella*, *Leuconeuropa*, *Mrakia*, *Moesziomyces*, *Penicillium*, *Pseudogymnoascus*, *Rhodotorula*, *Phenoliferia*, and *Thelebolus* were reported to be amylase producers [12,13,32,62–65,99,118]. Amylase activity has been assayed by growing the selected strains on media supplemented with starch as a carbon source.

In the study reported by De Mot and Verachtert [63], α -amylase and glucoamylase from *Moesziomyces antarcticus* CBS 6678 (*Candida antarctica*) were purified; both act preferentially on high-molecular-mass substrates, with α -amylase being active on cyclodextrins. Ramli et al. [62] elucidated the structure of a novel cold-active α -amylase produced by *Glaciozyma antarctica* PI12. This enzyme showed the presence of binding sites for a conserved calcium ion (CaI), non-conserved calcium ions (CaII and CaIII), and sodium ion (Na). The authors suggest that the low stability of this enzyme is related to its ability to act at different temperatures.

Amylases can be used in a wide range of industries, including baking, paper, textiles, brewing, and detergents [13,118]. Cold-adapted amylases that are inactivated by heat are important in the food industry

because they can prevent modification of heat-sensitive substrates and products [119].

Other hydrolytic enzymes

According to the literature, fungi of Antarctic origin can produce other hydrolytic enzymes, including β -galactosidases, tannases, phytases, β -mannanases, and invertases (Table 1). However, data related to these cold-adapted enzymes remains scarce.

β -galactosidases can be applied in the food and pharmaceutical industries, including for sugar reduction in different foods, development of additives for animal and human diets, and bioconversion of milk whey to reduce industrial effluents [69]. Zhang et al. [120], reported that the marine Antarctic yeast *Tausonia pullulans* 17-1 (formerly *Guehomyces pullulans*) showed highest extracellular β -galactosidase activity in a medium containing only lactose as the carbon source.

Tannases are utilized in a number of industrial applications, including the manufacture of instant tea, beer, fruit juices, some wines, and gallic acid production [121]. In the study performed by Kasiaczka-Burnecka et al. [85], two Antarctic cold-adapted extracellular tannases (TAH I and TAH II) produced by *Verticillium* sp. P9 were purified and presented optimum activities at 25.0 and 20.0 °C, respectively.

Cold-adapted phytases might have considerable advantages in direct inclusion in feed for monogastric animals and use in aquaculture [79]. Pavlova et al. [78] reported that *Papiliotrema laurentii* AL27 (formerly *Cryptococcus laurentii*) showed highest intracellular phytase activity at an optimal temperature of 40.0 °C. In another study, Yu et al. [79] reported the production of extracellular phytase by *Rhodotorula mucilaginoso* JMUY14, which showed resistance to both pepsin and trypsin degradation after purification.

β -Mannanases are used in the production of animal feed and laundry detergents, also being applied in the pulp, paper, food, pharmaceutical, and energy industries [122]. According to Bradner et al. [77], fungi from the genera *Penicillium* and *Phoma* recovered from ornithogenic soils in Antarctica presented high levels of mannanase activity at 0.0 °C. Parvizpour et al. [122] elucidated a novel psychrophilic β -mannanase from *Glaciozyma antarctica* PI12. The enzyme exhibited adaptations related to molecular flexibility and enzymatic efficiency at low temperatures.

Fungal invertases are used mainly in the production of invert sugars to be employed in the food and beverage industries [70]. Turkiewicz et al. [70] reported the production of an intracellular fructofuranosidase by the Antarctic yeast *Glaciozyma antarctica* 171 (formerly

Leucosporidium antarcticum), with an optimum temperature of 30.0 °C.

Oxidoreductases

Laccases

Despite the relevance of these enzymes for industrial and environmental applications, studies related to their production by Antarctic fungi are scarce. Rovati et al. [71] reported the ability of yeasts recovered from different Antarctic samples to produce laccase at 15.0 °C in a medium supplemented with guaiacol, useful for textile dyes and lignin degradation. Guaiacol has been widely employed as a substrate for ligninolytic enzymes, such as laccase, peroxidases, and lignin peroxidase because an intense reddish-brown color is produced in the medium around laccase-producing organisms in the presence of this compound [123].

Ligninolytic enzymes produced at low/moderate temperatures can be applied for pollutant degradation in cold environments or processes [57]. In this context, some studies have reported the ability of fungi of Antarctic origin to grow and/or degrade environmental pollutants. However, the involvement of laccases in these processes was not mentioned. Litova et al. [124] showed that three fungal strains recovered from Antarctic soil (*Penicillium commune* AL2, *Aspergillus fumigatus* AL3, and *Penicillium rugulosum* AL7) were able to utilize phenol as their sole carbon source at 5.0 °C. Gerginova et al. [125] reported that Antarctic fungi were able to tolerate and/or degrade polycyclic aromatic hydrocarbons, including phenanthrene (*Alternaria maritima* AL10), anthracene (*Penicillium rugulosum* AL7 and *Penicillium waksmanii* AL14), and naphthalene (*Aspergillus fumigatus* AL9, *Penicillium chrysogenum* AL12, and *Mucor* sp. AL13).

Superoxide dismutases and catalases

High concentrations of superoxide dismutase were produced by the Antarctic fungus *Aspergillus glaucus* 363 following cold stress at 10.0 °C using a nitro-blue tetrazolium substrate [84]. Tosi et al. [126] reported catalase production by fungal strains recovered from soil samples belonging to the genera *Aspergillus*, *Cladosporium*, and *Penicillium*.

Cold-adapted antioxidant enzymes, such as superoxide dismutases and catalases can be very important for the prevention of skin injury when used in cosmetic formulations. These enzymes can also be used in medicine, as a pulp-capping agent, as an inhibitor of

pulmonary vascular oxidative stress, and in the treatment of septic shock [84,126].

Heterologous expression

Some of the cold-adapted enzymes, produced by Antarctic fungi, have been cloned and expressed in different hosts. Data related to host organisms, vectors, production and amino acid, and nucleotide sequences are presented in Table 2.

The expression of enzymes from eukaryotic organisms in bacteria can be unsatisfactory, mainly because of their lack of ability to secrete extracellular proteins; additionally, eukaryotic post-translational modifications do not occur in prokaryotic organisms. Therefore, the expression must be performed in other eukaryotic organisms; *P. pastoris* appears to be a good host for fungal cold-adapted enzyme expression. According to Gao et al. [64], the α -amylase from *Pseudogymnoascus pannorum* R2-1 (formerly *Geomyces pannorum*) cloned in *P. pastoris* presented low homology to other α -amylases, with thermophilic characteristics (optimal activity at 70.0 °C). In another study, Ramli et al. [68] reported that the chitinase from *G. antarctica* PI12 expressed in *P. pastoris* was most active at 15.0 °C, being stable in the range of 5.0–25.0 °C. According to Feller and Gerday [58], a great contribution in this field could be associated with the use of a psychrophilic microorganism as a host for the expression of genes at low temperatures, preventing the formation of inclusion bodies and protecting heat-sensitive gene products.

Purification of cold-adapted enzymes from Antarctic fungi

Hydrolases and oxidoreductases produced by fungi of Antarctic origin have been purified and characterized (Table 3). The techniques used were diverse and yielded purification factors from 1.70- to 1568.00-fold and yield ranging from 0.65 to 86.20%. Enzymes secreted into the medium by Antarctic fungi are first concentrated by techniques, such as ultrafiltration [68,74,80,81,98,130], ammonium sulfate precipitation [63,64], ammonium sulfate and dialysis [79,85], or acetone precipitation [107]. After concentrating, the protein, purification can be carried out by a single or multiple steps of chromatography. In studies carried out by Lario et al. [81], Petrescu et al. [98], and Ray et al. [80], ion exchange chromatography was performed. On the other hand, in the studies reported by Abrashev et al. [84], De Mot and Verachtert [63], Florczak et al. [72], Gao et al. [64], Turkiewicz et al. [107], and Yu et al. [79] size exclusion with ion exchange and/or affinity chromatography were combined.

Table 2. Antarctic fungi and host organisms used in heterologous expression of cold-adapted enzymes.

Enzyme	Antarctic fungi	Host organisms	Induction enzyme production	Vector	Nucleotide sequence (pb)	Aminoacid sequence (aa)	Reference
α -amylase	<i>Pseudogymnoascus pannorum</i> (formerly <i>Geomyces pannorum</i>)	<i>Aspergillus oryzae</i>	Medium containing (100 mL): NaNO ₃ 0.6 g, KCl 0.05 g, KH ₂ PO ₄ 0.08 g, K ₂ HPO ₄ 0.104 g, Dextrose 1 g, MgSO ₄ 0.052 g, Trace element 100 μ L, sucrose 20.54 g, agar 1.25 g Medium supplement with 0.5% methanol	Plasmid pBC12FNH	1497	494	[127]
	<i>Pseudogymnoascus pannorum</i> R2-1 (formerly <i>Geomyces pannorum</i>)	<i>Pichia pastoris</i>	Medium supplement with 0.01–0.05 mM isopropyl-thiogalactopyranosid (IPTG)	Plasmid pPIC9K	1761	586	[64]
	<i>Glaciozyma antarctica</i> P112	<i>Escherichia coli</i>	Medium supplement with 1% methanol	Plasmid pET-22b (+)	1590	530	[65]
Chitinase	<i>Glaciozyma antarctica</i> P112	<i>Pichia pastoris</i>	Medium supplement with 1 mM IPTG	Plasmid pPICZ α -A	1215	404	[68]
Lipase B – CalB	<i>Moesziomyces antarcticus</i> (formerly <i>Candida antarctica</i>)	<i>Escherichia coli</i>	Medium supplement with 1 mM L-arabinose and IPTG	Plasmid pET-22b (+)	–	–	[128]
Lipase	<i>Penicillium expansum</i> SM3	<i>Escherichia coli</i>	Medium supplement with 1 mM IPTG	Plasmid pMAL-c5E	1142	285	[76]
	<i>Penicillium dillii</i> DAR74613	<i>Trichoderma reesei</i> VTT-D-79125	Medium containing (50 mL): KH ₂ PO ₄ 0.75 g (NH ₄) ₂ SO ₄ 0.25 g, Avicel 1.0 g, soy hydrolysate 0.75 g, 20% lactose 2.5 mL, MgSO ₄ 1M and CaCl ₂ 1M	Plasmid pHEN54	NR	308	[75]
Protease	<i>Glaciozyma antarctica</i> P112	<i>Pichia pastoris</i>	Medium supplement with up 1% methanol	Plasmid pPIC9	2892	963	[129]

Purification of extracellular enzymes involves environmental concerns because of the use of large quantities of salts and the high costs associated with chromatographic steps. Because of this, the search for other separation methods is important. Liquid–liquid extraction has been used for cold-adapted enzyme purification and has proven to be efficient for lipase from *Leucosporidium scottii* L117 [73] and protease from the *Rhodotorula mucilaginosa* L7 [131].

Patents

To find patents (or patent requests) related to enzymes from Antarctic fungi, a survey of 14 databases from around the world was performed. A total of eight keywords were used to conduct the search and the results are listed in Table 4. Despite the high number of keywords used, the survey did not retrieve many relevant results, indicating that this environment has a great unexplored biotechnological potential.

Records of patents or requests are associated mostly with bacteria, such as the lipase from *Bacillus pumilus* from Antarctica (10–1596435-0000), the cold-adapted protease HSPA-2 from a marine bacterium (WO2013177834), and anticancer and antimicrobial compounds from Lakes Schirmacher Oasis' bacteria, in East Antarctica (US20110301216). Most patents of cold-adapted enzymes recovered from Antarctic fungal strains are related to the lipase produced by *Candida antarctica* (Table 5).

An attempt to survey patents in a database known as BioProspector maintained by the Institute of Advanced Studies (United Nations University) was performed, but unfortunately this database no longer exists. It used to contain information about patents and genetic resources from both Arctic and Antarctic environments. Now that it is inaccessible, there is one less resource for the promotion of the research regarding on extreme environments.

Conclusions and future perspectives

Fungi from Antarctic environments demonstrate a remarkable ability to produce enzymes (hydrolases and oxidoreductases), suggesting that the fungal community plays an important role in decomposition processes within Antarctic ecosystems. The search for cold-adapted enzymes produced by Antarctic fungi has been performed mainly using culture-dependent methods, which allowed for the structuring of fungal culture collections of Antarctic origin. Although several studies in this field are being conducted, knowledge about microorganisms and their adapted biomolecules

Table 3. Strategies used for purification and data related to cold-adapted enzymes produced by Antarctic fungi.

Enzyme type	Antarctic fungi	Source	Concentration method	Column matrices	Purification factor (fold)	Yield (%)	Km (mM)	Vmax	Weight (kDa)	Reference
α -amylase	<i>Moesziomyces antarcticus</i> CBS 6678 (formerly <i>Candida antarctica</i>)	Centraal Bureau voor Schimmelcultures, Yeast Division, The Netherlands	Protamine sulfate precipitation	Sephadex G-75	15.6	63.7	4.4 g/L for soluble starch	NR	50.0	[63]
			Ammonium sulfate precipitation	Ultrogel ACA 54 Anion exchange chromatography Hydroxyapatite	22.8 35.0 38.6	76.1 53.2 34.4				
Chitinase	<i>Pseudogymnoascus pannorum</i> RT-2 (formerly <i>Geomyces pannorus</i>) <i>Glaciozyma antarctica</i> P112 cloned in <i>Pichia pastoris</i>	–	Ammonium sulfate precipitation	DEAE-Sephacel Sephadex G-100	5.72 17.2	33.2 11.2	2.07 mg/mL toward soluble starch	3.659 μ mol/(mL.min) toward soluble starch	54.0	[64]
		Nucleotide sequence obtained from a GSS survey of the <i>G. antarctica</i> P112 genome was identified to encode the consensus domain of the glycosyl hydrolase family 18 using NCBI databases.	Ultrafiltration	Affinity chromatography (His-Tag)	NR	NR	NR	27981 (mg/mL) for colloidal chitin	3559 μ mol/(\mu g.h) for colloidal chitin	39.0 (non glycosylated) 50.0 (glycosylated)
	<i>Lecanicillium muscarium</i> A3	Moss from continental	Ammonium sulfate precipitation and preparative isoelectric focusing (Rotorf)	Q-Sepharose Chromatography	218.0	14.0	–	–	45.0	[67]
Galactosidase	<i>Naganishia albid</i> AL3 (formerly <i>Cryptococcus albidus</i>)	Marine sediment	Ultrafiltration	DEAE-Sephadex A-50 Sephadex G-200	5.4 45.1	49.8 15.4	0.64 mM	140 U/mg p-nitrophenil- β -D-glucopyranoside	100.0	[130]
		Marine sediment	Ultrafiltration	Sephadex-G200 CM-Sepharose Fast Flow	2.2 2.4	19.3 16.1	o-nitrophenil-phenyl- β -D-galactopyranoside	9.2 μ mol/min	335	[69]
Glucoamylase	<i>Moesziomyces antarcticus</i> CBS 6678 (formerly <i>Candida antarctica</i>)	Centraal bureau voor Schimmelcultures, Yeast Division, Delft, The Netherlands	Protamine sulfate precipitation	Sephadex G-75	2.6	86.2	0.97 g/L for soluble starch	NR	48.5	[63]
			Ammonium sulfate precipitation	Ultrogel ACA 54 Anion exchange chromatography Affinity chromatography	3.4 5.3 6.0	72.8 47.8 29.5				
Lipase	<i>Geomyces</i> sp. P7	Soil from King George Island	–	HiTrapQ FF Chromatography HiLoad Superdex 200 Chromatography HiTrapQ FF	1.7 39.0 41.6	76.0 76.0 61.0	8.5 mM	NR	65.0	[72]
		Algal mat in sediment	Ultrafiltration	Chromatography Toyopearl-butyl 650 M (Tosho, Tokyo, Japan) hydrophobic interaction chromatography	20.1	9.4	1 mM p-nitrophenyl ester	–	60.0	[74]

(continued)

Table 3. Continued

Enzyme type	Antarctic fungi	Source	Concentration method	Column matrices	Purification factor (fold)	Yield (%)	Km (mM)	Vmax	Weight (kDa)	Reference
Phytase	<i>Rhodotorula muclaginos</i> JMUY14	Deep-sea sediment	Ammonium sulfate precipitation and dialysis	DEAE SFF anion exchange SP SFF cation exchange Sephadex G-100 gel filtration	3.9 9.9 15.1	48.0 30.0 5.0	0.247 mM (phytate)	1326 Mmol/mg/min	63.0	[81]
Protease	<i>Varijia humicola</i> (formerly <i>Candida humicola</i>) <i>Glaciozyma antarctica</i> 171 (formerly <i>Leucosporium antarcticum</i>) <i>Rhodotorula muclaginos</i> L7 <i>Aspergillus glaucus</i> 363	Soil from Schirmacher Oasis Sub-glacial waters at a depth of 200 m in Admiralty Bay Antarctic marine macroalgae Soil	Ultrafiltration Acetone precipitation Ultrafiltration Ultrafiltration	Anion exchange chromatography Sephadex G75 Anion exchange chromatography Sephacryl S-100 Cation exchange chromatography Superdex 100 column chromatography Phenyl-Sepharose column chromatography Q-Sepharose I column chromatography Q-Sepharose II column chromatography	NR 46.0 440.0 1,568.0 15.6 11.8 28.8 153.4 23.5	NR 66.0 27.9 22.7 29.7 35.0 8.1 9.7 0.6	NR 0.51 mM for N-SucAAPFPNA NR NR NR	NR 40.4	36.0 35.0 35.0 38.1 (SODI) 15.8 (SODII)	[80] [107] [81] [84]
Superoxide dismutase										
Xylanase	<i>Naganishia adeliensis</i> (formerly <i>Cryptococcus adeliae</i>)	Decayed algae in the icepack at the Antarctic station Dumont d'Urville Antarctic soil	Ultrafiltration	Anion exchange chromatography	NR	48.0	NR	NR	41.4	[98]
Tannase TAH1	<i>Verticillium</i> sp. P9	Antarctic soil	Ammonium sulfate precipitation and dialysis	DEAE-cellulose Chromatography	7.9	1.6	2.20 in Tannic acid at 5.0°C	11.6 in tannic acid at 5.0°C	40.0	[85]
Tannase TAH11	<i>Verticillium</i> sp. P9	Antarctic soil	Ammonium sulfate precipitation and dialysis	DEAE-cellulose Chromatography	10.5	0.9	2.12 in Tannic acid at 5.0°C	22.2 in tannic acid at 5.0°C	46.0	[85]

Table 4. Databases and keywords used to find patents (or requests) related to enzymes from Antarctic microorganisms.

Database	Keywords							
	"Cold adapted enzymes"	"Antarctic Microorganisms"	"Antarctic cold adapted enzymes"	"Psychrophilic enzymes"	"Enzyme fungus Antarctica"	"Cold adapted lipase"	"Cold adapted protease"	"Cold adapted xylanase"
European Patent Office	6	3	1	3	0	0	0	0
World intellectual property organization (WIPO)	13	4	3	26	0	0	0	0
Korean intellectual property rights information service (KIPRIS)	1	0	2	3	0	3	0	0
AusPat (Australia)	0	0	0	0	0	0	0	0
Canadian intellectual property office	0	0	0	1	0	0	0	0
Taiwan Patent Search System	0	0	0	0	0	0	0	0
Swissreg IGE/IPI	0	0	0	0	0	0	0	0
Swedish patent database	0	0	0	0	0	0	0	0
Norwegian industrial property office	0	0	0	0	0	0	0	0
BPP – Benelux patent platform	0	0	0	0	0	0	0	1
Israel patent office	0	0	0	0	0	0	0	0
DPMA/Germany	0	0	0	0	0	0	1	0
LATIPAT	0	0	0	0	0	0	0	0
INPI (Brazil)	0	0	0	0	0	0	0	0

Table 5. Patents related to cold-adapted enzymes from Antarctic fungi.

Patent number	Fungi source	Enzyme	Company/ Organization	Research field
US20080020088, EP1723229, WO/2005/087916 NZ333445 US5928933	<i>Moesziomyces antarcticus</i> (formerly <i>Candida antarctica</i>)	Lipase	AstraZeneca/ Sweden, UK	Chemical processing
US6020180 (2000), EP0652945A1, US6074863, WO1994001541A1 US5407828 (1995)	<i>Moesziomyces antarcticus</i> (formerly <i>Candida antarctica</i>)	Lipase	Du Pont/ USA Novo Nordisk	Chemical processing Molecular biology/biotech
KR20040066373	<i>Moesziomyces antarcticus</i> (formerly <i>Candida antarctica</i>)	Lipase	DSM NV/ Netherlands Korea Ocean Res Dev Inst/ Republic of Korea Pais	Chemistry Chemical processing
KR20010092006	<i>Moesziomyces antarcticus</i> (formerly <i>Candida antarctica</i>)	Lipase	Shin Dong Bang Corp/ Republic of Korea	Chemical processing
JP2002262859	KA-616 (mold)		Nagata Sangyo/ Japan	Chemical processing

remains limited. In this sense, the use of a culture-independent approach, for both structural and functional analyses, could increase our knowledge related to the diversity and ecology of these microorganisms as well as expand the prospection of biomolecules, including those produced by non-cultivable microorganisms.

Considering the complexity and costs related to Antarctic expeditions, researchers involved in the prospecting steps (particularly the ones responsible for microbial isolation and preservation) should consider depositing these strains in an appropriate culture collection to ensure the maintenance of these valuable genetic resources. There are plenty of renowned culture collections all over the world, and many of them operate on the basis of international rules related to Access and Benefit Sharing – ABS (Nagoya Protocol).

Studies related to the prospecting, production, and characterization of Antarctic fungal enzymes should be encouraged since they could result in the isolation of

new enzymes with different properties that can be used in environments and/or processes with low/mild temperatures. In this sense, national and international programs should be established to promote biotechnology advances in this area.


Disclosure statement

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