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MICROBIOLOGY

Antarctic environments as a source of bacterial and fungal therapeutic enzymes

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Abstract: Microbial therapeutic enzymes are the protagonists in the pharmacological treatment of different human diseases. The intrinsic enzymatic characteristics, such as high affinity and specificity to the corresponding substrate, enable effective therapies, with minimal adverse effects and complete remission. However, immunogenicity, short half-life, low enzymatic yield, and low selectivity regarding available enzyme drugs are currently the main obstacles to their development and the broad adherence to therapeutic protocols. By harboring adapted and still unexplored microbial life, environments of extreme conditions, such as Antarctica, become especially important in the prospecting and development of new enzymatic compounds that present higher yields and the possibility of genetic improvement. Antarctic microorganisms have adaptation mechanisms, such as more fluid cell membranes, production of antifreeze proteins and enzymes with more malleable structures, more robust, stable, selective catalytic sites for their respective substrates, and high antioxidant capacity. In this context, this review aims to explore enzymes synthesized by bacteria and fungi from Antarctica as potential drug producers, capable of providing therapeutic efficacy, less adverse effects, and lower production costs with highlight to L-Asparaginase, collagenase, superoxide dismutase and ribonucleases. In addition, this review highlights the unique biotechnological profile of these Antarctic extremophile microorganisms.

Key words: Antarctica, collagenase, extremophiles, L-Asparaginase, superoxide dismutase, therapeutics enzymes.

INTRODUCTION

The unexplored frozen Antarctica continent is a source of complex habitats for the development of different microbial species, including fungi, bacteria, and yeasts (Bowman 2018). In an environment with harsh and extreme characteristics, such as temperatures reaching -80 ° to -93.2 °C in winter (Parker 2014, Núñez-Montero & Barrientos 2018); strong and constant winds close to the surface (Turner et al. 2009); frequent freeze-thaw cycles forming thaw lakes with varying salinity; precipitation, low humidity, and desert regions (Bowman 2018); high incidence of ultraviolet (UV) radiation due to depletion of the ozone layer associated with reflection in the ice (Marizcurrena et al. 2017), in addition to the low nutrient availability (Yergeau et al. 2006), survival becomes a challenge for these microorganisms. Nevertheless, many microorganisms can thrive in this environment and a huge Antarctica microbial diversity are being revealed either by culture and unculture dependent methods (Duarte et al. 2016, Pulschen et al. 2017, Wentzel et al. 2019, Ogaki et al 2020).

According to Duarte et al. (2018a) the growing number of new microbial taxa from Antarctica indicates an apparently hidden diversity in this environment.

The adaptation to the Antarctic environmental conditions is characteristic of microorganisms classified as extremophiles since they are able to develop mechanisms to deal with the "extremes" imposed by the environment. Extremophilic microorganisms are a rich source of enzymes resistant to high (thermophilic) and low (psychrophilic) temperatures, salinity (halophilic), pH variations (alkalophilic and acidophilic), and UV radiation (Thomas & Dieckmann 2002). Concerning the psychrophilic microorganisms, those able to develop in environments with extreme cold, the synthesis of glycoproteins and antifreeze peptides prevents the crystallization of intracellular water, which is lethal to the cells (Morozkina et al. 2010).

The capacity to produce biomolecules with high biotechnological value explains why Antarctica has been the focus of researches related to isolation, bioprospecting, and application of microbial biomolecules in different sectors, including food, cosmetics, and pharmaceutical industries. In this sense, this review describes the Antarctic microbial enzymes with biotechnological potential for the development of new drugs and/or therapies, aiming at the reduction of adverse effects, lower production costs, and greater therapeutic efficacy.

THERAPEUTIC POTENTIAL OF THE ANTARCTIC PSYCHROPHILIC ENZYMES: STRUCTURAL AND MOLECULAR PERSPECTIVE

The application of microbial therapeutic enzymes has revolutionized the struggle against numerous human diseases. The intrinsic enzymatic characteristics, such as high affinity and specificity to the corresponding substrates, enable effective therapies, with minimal adverse effects and complete remission (Zhou et al. 2021). Although they offer too many benefits, there are still obstacles to be overcome including immunogenicity, short half-life, and low selectivity regarding the drugs currently available on the market (Bax 2020).

The bioprospecting, in turn, has become the main alternative to the search for enzymes with different catalytic profiles and that meet the pharmacological demand. For this reason, psychrophilic microorganisms, especially those living in the Antarctic regions, emerge as potential sources, due to the need for adaptive mechanisms developing to inhabit this extreme environment. These adaptations correspond to specific genetic changes, which are a consequence of long-term selection, expressed in the form of structural changes in cellular components (Sarmiento et al. 2015).

It is known that, at low temperatures, the kinetic energy available is insufficient to achieve the energy needed for rearrangements in the conformational factors related to catalysis in mesophilic organisms (Martorell et al. 2019). Furthermore, proteins often tend to undergo denaturation processes, as the availability of water molecules greatly decreases since they become more orderly and less associated with proteins (Sarmiento et al. 2015). Cold adapted enzymes, however, overcome these energy

barriers through very diverse structural and molecular solutions (Mangiagalli et al. 2021).

Active sites of psychrophilic enzymes are robust and have greater accessibility to the substrate, resulting in low energy costs during protein-ligand conjugation (Ashok et al. 2019). Regarding the energetic perspective of the system, a decrease in enthalpy is observed, caused by the reduction in the number of interactions that must be broken during the transition from the basic enzyme-substrate complex to the transition state (activated complex) of the reaction (Santiago et al. 2016). Thus, the activation energy of the reactions is considerably lower, when compared to that of the mesophilic and thermophilic counterparts. On the other hand, to compensate for the lower enthalpy, there is an increase in entropy due to the change in the stability, as well as the increase in structural protein flexibility (Santiago et al. 2016, Bruno et al. 2019). Together, these characteristics contribute to the main adaptative attribute inherent to cold enzymes, the high catalytic rate at low temperatures (Yang et al. 2021). In pharmacological application, the high enzymatic activity translates into smaller amounts of the drug to obtain the same results as its counterparts, under ideal conditions, and, therefore, the patient will be preserved from receiving high doses.

Several specific compositional adaptations and secondary, tertiary, and/or quaternary structural properties are identified in these polypeptides (Figure 1) aiming to achieve sufficient conformational flexibility (Siddiqui et al. 2013). They show a decreased central hydrophobicity, however, with an increased surface hydrophobicity and, thus, a greater presence of nonpolar residues in the external protein structure (Martorell et al. 2019). The content of larger α-helices at the expense of β-sheets is a fundamental and determinant characteristic of malleability (Jin et al. 2019). The percentage of proline residues is higher in α-helices, but in loops, they tend to be reduced (Siddiqui et al. 2013). It is also worth noting the predilection for small amino acids in the enzymatic constitution, such as Ala (alanine),

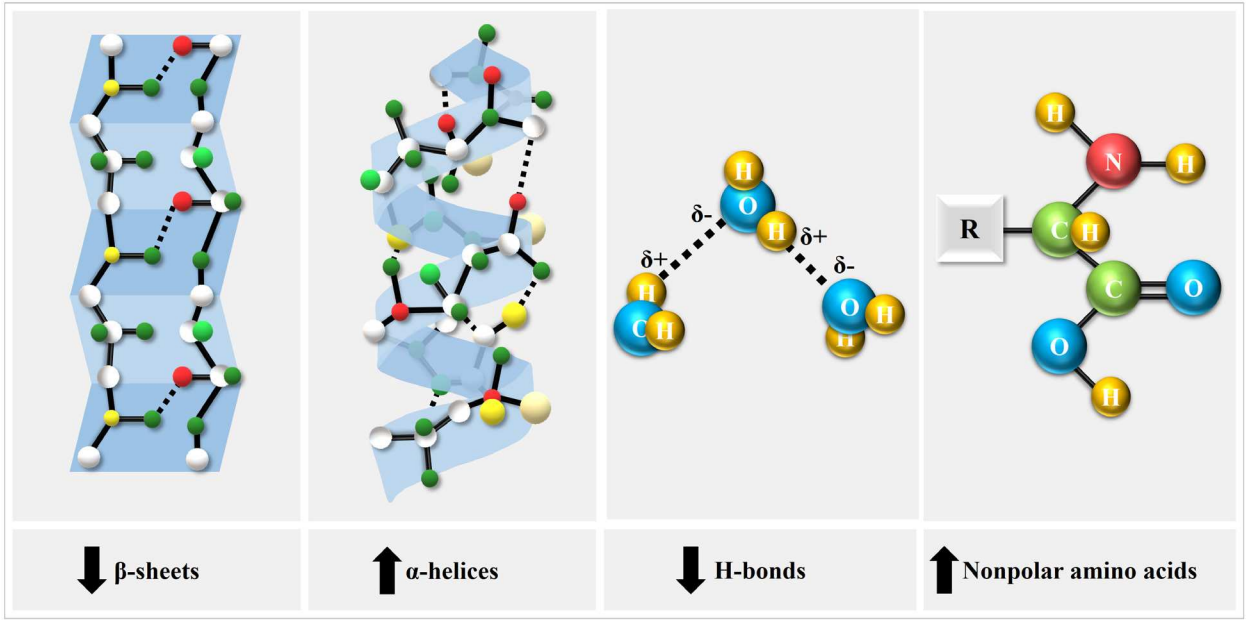

Figure 1. Structural and molecular adaptations of the enzymes produced by cold-adapted microorganisms.

Asp (aspartate), Gly (glycine), Ser (serine), and Thr (threonine); lower arginine/lysine ratio and higher glutamate + aspartate/arginine + lysine ratio compared to mesophilic counterparts (Bruno et al. 2019, Yang et al. 2021).

From a therapeutic perspective, the different amino acid composition of these enzymes gives a wider range of epitopes and, consequently, produces different immunogenicity profiles of the marketed enzymatic drugs. It is emphasized that these characteristics point to a promising scenario, with more effective treatments and few adverse effects, especially in immunological bias, by reducing hypersensitivity reactions (Ghosh et al. 2019).

Notably, cold-active proteins have weaker molecular interactions, such as interdomain and intersubunit interactions, fewer disulfide bridges, and reduced electrostatic interactions (e.g. H-bonds, salt bridges, cation-pi interactions, aromatic-aromatic interactions) (Siddiqui et al. 2013). In contrast to the decrease in secondary structures and oligomerization, there is an increase in the number and size of the loops. In addition to contributing to increased flexibility, these particularities are usually related to the attenuation of stability (Bruno et al. 2019). Nevertheless, studies have shown that thermolability, although frequent in these polypeptides, is not universal. Antarctic bacteria *Pseudoalteromonas haloplanktis* TAC125, for example, produced GroEL and thioredoxin with thermostability similar to the *Escherichia coli* (Parrilli & Tutino 2017). Therefore, the plurality of attributes inherent to the Antarctic extremozymes stands out, and they can even natively display the combination of the various profiles desired by the pharmaceutical industry in just one protein.

Different enzymes (L-asparaginase, collagenase, superoxide dismutase, and ribonucleases) produced by microorganisms of Antarctica are described below. In this review, the attention was given to enzymes that presented a good biotechnological profile and had their activity and enzymatic profile evaluated, in addition to characteristics that made them potential targets for biotechnological development and application in innovative therapies in the health area. Data related to the enzymes and the producer microorganisms are shown in Tables I and II.

L-ASPARAGINASE

L-asparaginase (L-asparagine amidohydrolase, L-ASNase, E.C.3.5.1.1) is a chemotherapeutic drug listed as an essential drug by the World Health Organization (WHO) (Beckett & Gervais 2019). The incorporation of this enzyme in the treatment of Acute Lymphocytic Leukemia (ALL) increased the complete remission of the disease from 20 to 80-90%, configuring, therefore, as an indispensable medicine to face the respective neoplasia (Costa-Silva et al. 2020). Besides, it is emphasized the role of L-ASNase in therapeutic protocols for acute myelocytic leukemia, lymphosarcoma, acute myelomonocytic leukemia, reticulosarcoma, and melanosarcoma (Golbabaie et al. 2020).

The antineoplastic mechanism of L-ASNase comes from the inherent ability to catalyze the conversion of the amino acid L-asparagine (L-Asn) to L-aspartate (L-Asp) and ammonia (Golbabaie et al. 2020). In contrast to healthy cells, the expression of asparagine synthase, an enzyme responsible for producing L-Asn in the intracellular medium, is low in neoplastic cells. Thus, the maintenance of tumor metabolism depends on extracellular sources of the respective amino acid. The biopharmaceutical, by decreasing the patient's serum L-asparagine levels, causes a nutritional restriction in the

Table I. Characteristics of therapeutic enzymes produced by Antarctic bacteria and fungi.

Enzyme	Microorganism	Site and source	Optimum temperature (°C)	Optimum pH	Enzymatic activity	Specific activity ($U.mg^{-1}$)	Reference
L-asparaginase	*Leucosporidium muscorum* CRM 1648	Marine sediments (Admiralty Bay – King George Island)	NC	NC	NC	NC	Freire et al. (2020)
	Leucosporidium scottii L115	Marine sediments (Antarctic Peninsula)	NC	NC	NC	NC	Moguel et al. (2020)
	Leucosporidium scottii L120	Seafloor sediments (Antarctic environment)	NC	NC	1.1×10^{-3} $U.mg^{-1}$	NC	Correa et al. (2020)
	Cryptococcus victoriae L92	Seafloor sediments (Antarctic environment)	NC	NC	0.9×10^{-3} $U.mg^{-1}$	NC	Correa et al. (2020)
	Trichosporon asahii IBBLA1	Schirmacher Hills (Dronning Maud Land)	NC	NC	20.57 $U.mL^{-1}$	NC	Ashok et al. (2019)
	Coprinopsis cinerea IBBLA4	Schirmacher Hills (Dronning Maud Land)	NC	NC	16.23 $U.mL^{-1}$	NC	Ashok et al. (2019)
	Cosmospora sp. OB4B	Whale bones (King George Island)	NC	NC	> 0.4 $U.mL^{-1}$	NC	Duarte et al. (2018)
	Cosmospora sp. OB1B	Whale bones (King George Island)	NC	NC	> 0.2 $U.mL^{-1}$	NC	Duarte et al. (2018)
	Cosmospora sp. OB2	Whale bones (King George Island)	NC	NC	> 0.2 $U.mL^{-1}$	NC	Duarte et al. (2018)
	Penicillium sp. C2	Sea star (King George Island)	NC	NC	> 1.3 $U.mL^{-1}$	NC	Duarte et al. (2018)
	Penicillium sp. E2B	Sea star (King George Island)	NC	NC	> 0.4 $U.mL^{-1}$	NC	(Duarte et al. 2018)
	Pseudogymnoascus sp. S2B	*Salpa* sp. (King George Island)	NC	NC	> 0.2 $U.mL^{-1}$	NC	Duarte et al. (2018)
	Fungal sp. OB7A	Whale bones (King George Island)	NC	NC	> 0.5 $U.mL^{-1}$	NC	Duarte et al. (2018)
	Fungal sp. L2-16A	Lichen (King George Island)	NC	NC	> 0.1 $U.mL^{-1}$	NC	Duarte et al. (2018)

Table I. Continuation.

Collagenase	*Arthrobotrys tortor*	Antarctica	NC	NC	NC	NC	Tosi et al. (2002)
Superoxide Dismutase	*Pseudoalteronomas haloplanktis*	Marine sediments (Antarctica)	25	6.9	NC	14.10^3	Castellano et al. (2006)
	Marinomonas sp. NJ522	Sea ice in Antarctic	40	9.0	NC	148.0	Zheng et al. (2015)
	Aspergillus glaucus 363	Livingston Island (Antarctica)	25	7.0	NC	39.61	Tosi et al. (2010)
	Penicillium sp. P14	Antarctic soils	10	7.8	NC	NC	Gocheva et al. (2006)
	Penicillium sp. M12	Antarctic soils	10	7.8	NC	NC	Gocheva et al. (2006)
Ribonuclease	*Pseudomonas fluorescens* 10CW	Schirmacher Oases (Antarctica)	40	7.4	NC	120.0	Reddy et al. (1994)

tumor and, consequently, activates apoptotic mechanisms (Chand et al. 2020, Borges et al. 2020).

Despite the enormous therapeutic importance, too many adverse effects prevent its prolonged use and, even, the administration in a significant portion of the population. Pancreatitis, hyperglycemia, bronchospasm and coagulation disorders, leading to intracranial thrombosis or hemorrhage, are recurrent side effects, which are related to the catalytic activity adjacent to the amino acid L-glutamine present in the current commercialized L-asparaginases (Cecconello et al. 2020). However, the main limitation to therapy is the high immunogenicity, causing hypersensitivity reactions between 30 and 75% of individuals submitted to the native enzymatic form of *E. coli*, and approximately 70% develop anti-EcAII antibodies (González-Torres et al. 2020).

According to Lopes et al. (2017) due to its importance in the treatment of several types of cancers, in particular leukemia, the searcher for new microbial sources of L-ASNase can drive the increase in its availability and in the reduction of side effects. In this sense, extremophile isolates, whose adaptive mechanisms produce amidohydrolases with different profiles, have become an extremely promising alternative. Antarctica is emerging in this area, as it is characterized by being a cradle of extremophilic microbial biodiversity.

The yeast *Leucosporidium muscorum* CRM 1648, isolated from marine sediments from Admiralty Bay, King George Island, Antarctica, produced an L-ASNase devoid of glutaminase activity. The respective strain was able to produce 490.41 $U.L^{-1}$ of L-ASNase, resulting in a volumetric productivity of 5.12 $U.L^{-1}.h^{-1}$ (Freire et al. 2020). The L-asparaginases available on the market for chemotherapy use come from two Gram-negative bacteria, *E. coli* and *Erwinia chrysanthemi*. Nonetheless, yeast and fungal enzymes are expected to have less immunogenicity, since, being eukaryotic, they are evolutionarily closer to humans compared to bacteria (Chand et al. 2020). Moreover, yeast ASNase demonstrated better serum stability and optimal pH close to physiological conditions (Moguel et al. 2020).

Table II. Characteristics of the Antarctic therapeutic enzymes produced by heterologous expression.

Enzyme	Microorganism	Isolation source	Optimum temperature (°C)	Optimum pH	Enzymatic activity	Specific activity	Reference
Superoxide Dismutase (GMO)	*Pseudoalteromonas* sp. ANT506	Antarctic sea-ice	30	8.0	NC	587.4 $U.mg^{-1}$	Wang et al. (2015)
	Rhodotorula mucilaginosa AN5	Antarctic sea-ice	20	3.0	NC	92.11 $U.mg^{-1}$	Kan et al. (2017)
Ribonuclease (GMO)	*Psychrobacter* sp. ANT206	Antarctic sea-ice	30	6.0	NC	115.60 $\mu mol^{-1}.min^{-1}.mg^{-1}$	Wang et al. (2019)

Moguel et al. (2020) reported the synthesis of this amidohydrolase by a psychrotolerant microorganism isolated from marine sediments, Antarctic Peninsula. The yeast *Leucosporidium scottii* L115, under suitable process conditions, reached an enzymatic productivity of 35.11 $U.L^{-1}.h^{-1}$, a value high enough to develop scale-up studies. Correa et al. (2020) also described an L-ASNase from the eukaryote *L. scotti*. The L120 strain, collected from seafloor sediments of the Antarctic environment, showed an enzyme with catalytic activity equal to 1.1 $U.g^{-1}$. The same study reported the Antarctic strain of *Cryptococcus victoriae* L92 as a producer of an L-ASNase with an activity of 0.9 $U.g^{-1}$.

Ashok et al. (2019) identified 30 filamentous fungi isolated from Schirmacher Hills, in Antarctica, as producers of L-asparaginases. Among them, *Trichosporon asahii* IBBLA1 showed the highest enzymatic activity, followed by *Coprinopsis cinerea* IBBLA4, with maximum activities of 20.57 $U.mL^{-1}$ and 16.23 $U.mL^{-1}$, respectively. The parameters used to achieve the optimal enzymatic activity were temperature of 30 °C and neutral pH (7.0). These values are auspicious, pointing to potential development in the pharmacological area. In addition, it is necessary to highlight that the L-ASNases produced by the aforementioned fungi did not present activities for the nitrogen sources glutamine and urea (Ashok et al. 2019).

These characteristics are extremely relevant, especially in the current therapeutic scenario, whose main adverse effects of the enzyme drug are caused by the low selectivity for the acting substrate, L-asparagine, being, therefore, capable of hydrolyzing other nitrogen compounds (Beckett & Gervais 2019). Thereby, the high biotechnological value of Antarctic L-ASNases is corroborated, with the potential to masterfully replace current preparations, offering greater efficacy in treatment, fewer side effects, and, possibly, lower productive and commercial costs.

Furthermore, studies prove the wide therapeutic capacity of L-ASNase from filamentous fungi to act in different conditions. The fungal species *Purpureocillium lilacinum*, for example, demonstrated antimicrobial activity against a range of bacteria: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Listeria monocytogenes*. It should be noted that this enzyme obtained a minimum

inhibitory concentration (MIC) against *P. vulgaris* equal to 21.06 mg.mL^{-1} (Vimal & Kumar 2020). Such results are important since the acquisition of new drugs to treat infections is highly desired, especially in the advent of the era of antibiotic resistance.

A total of eight filamentous fungi isolated from samples collected on King George Island exhibited L-ASNase activity in liquid medium. Three of them were identified as *Cosmospora* sp. (isolated from whale bones: OB4B, OB1B, and OB2), two as *Penicillium* sp. (recovered from sea star: C2 and E2B), and one *Pseudogymnoascus* taxa (recovered from *Salpa* sp.: S2B). Isolates OB7A (recovered from whale bones) and L2-16A (recovered from lichen) could not be identified. It is noteworthy that *Penicillium* sp. C2 and the unidentified fungus OB7A showed the highest enzymatic activities. The first presented L-ASNase activity > 1.3 U.mL^{-1} and the second > 0.5 U.mL^{-1} (Duarte et al. 2018b).

COLLAGENASE

Collagen is a protein composed of a triple helix structure. However, its rigidity makes it undegradable to most proteases, reacting only to collagenases (Microbial collagenase, E.C. 3.4.24.3) (De Albuquerque Wanderley et al. 2020). The applications of collagenolytic enzymes are vast, especially in the medical and pharmaceutical segments. The enzyme produced by *Clostridium histolyticum* has been approved by the Food and Drug Administration (FDA) for use as a drug in the treatment of Dupuytren's disease, characterized by abnormal tissue thickening due to exacerbated collagen deposition (Cooper et al. 2020). This enzyme is also used in the wound healing and burns (Alipour et al. 2016).

Additionally, the use of collagenase from *Clostridium histolyticum* (CCH) as a first-line treatment in Peyronie's disease (PD) has increased substantially (Gabbrielson et al. 2017). PD manifests itself as a penile abnormality, expressed by fibrosis of the tunica albuginea, causing penile curvature and deformity, penile pain, and sexual dysfunction (Hatfield et al. 2020). Although surgical treatment is the most common, there is a gradual inversion of this scenario, with the rise of injection therapies, aiming, above all, to minimize the following adverse effects of application: shortening of the penis, change in penile sensation, erectile dysfunction, and palpable sutures (Sukumar et al. 2020).

Given the therapeutic importance, the search for even more effective collagenases is undoubtedly encouraged. Nevertheless, the present study recovered only one scientific manuscript referring to the collagenolytic protease produced constitutively by an Antarctic-derived microorganism, the fungus *Arthrobotrys tortor* (Tosi et al. 2002). Therefore, the need for investments in the field of bioprospecting of this enzyme is emphasized, in order to develop collagenases with different profiles and increase the scope of its action and pharmacological application.

SUPEROXIDE DISMUTASE (SOD)

Reactive oxygen species (ROS) are a set of oxygen free radicals that include superoxide ($^{\cdot}O_2^-$), hydroxyl ($OH^{\cdot}$), peroxyl ($RO_2^{\cdot}$), and hydroperoxyl ($HO_2^{\cdot}$) radicals, and certain nonradical oxidizing agents, such as hydrogen peroxide (H_2O_2) (Bayir 2005). ROS are important for several physiological mechanisms such as the synthesis of molecular structures and participation in body's defense system against pathogens (Pizzino et al. 2017). When there is an imbalance between the production of ROS and

that of enzymes that metabolize them, oxidative stress occurs. These ROS attack other molecules such as carbohydrates, proteins, lipids, and the DNA itself, causing changes in their molecular structure and function, being studied as factors that cause cancer, degenerative diseases, cardiac disorders and is related to the aging process (Dalton et al. 1999, Rowe et al. 2008). In response to this scenario of oxidative stress, superoxide dismutase (Superoxide dismutase, SOD, E.C.1.15.1.1) appear to play a fundamental role in reducing these reactive oxygen species.

SODs are enzymes that incorporate a metal as a cofactor in its structure (metalloenzymes) responsible for the degradation of superoxide radicals ($\cdot O_2$-) in hydrogen peroxide (H_2O_2), which is also a ROS, later degraded by catalase (Zheng et al. 2006). They are classified into 4 types according to the metal associated with the enzyme: Cu/Zn-SOD (copper and zinc), Mn-SOD (manganese), Fe-SOD (iron) and Ni-SOD (nickel). Cu/Zn-SOD is found in several organisms, being isolated from the periplasm of bacterial species. Studies demonstrate its role in the virulence of bacterial strains, being the main line of defense against erythrocyte SOR (Kim et al. 2010). MN-SOD is found in prokaryotes and in eukaryotic mitochondria, where they are responsible for eliminating ROS, especially the superoxide formed during the respiratory chain (Candas & Li 2014). Fe-SOD, found mainly in prokaryotes and chloroplasts, has shown interesting studies in its role in joint therapy against cancer, eliminating the ROS of cancer cells (Bafana et al. 2011a). Little reported in the literature, in a 1996 study, Ni-SOD was isolated from strains *Streptomyces* sp. IMSNU-1 and *Streptomyces coelicolor* ATCC 10147 (Youn et al. 1996).

Enzymatic antioxidants are widely used to combat ROS due to various factors such as bioavailability, specificity, and non-consumption during the antioxidant reaction, with SOD and catalase being among the most potent antioxidants found in nature (Ratnam et al. 2006). Studies point out the importance of using and increasing the expression of SODs in cancer treatments (Hu et al. 2005), urinary bladder cystitis by pelvic irradiation (Kanai et al. 2002), rheumatoid arthritis (Goebel & Storck1983), progressive systemic sclerosis (Morita et al. 1996), skin inflammation (Kwon et al. 2012), vitiligo (Jain et al. 2011), among other potential applications.

Psychrophilic or psychrotolerant microorganisms have small rates of metabolism, with less use of ATP and consequent accumulation of electrons in their production process, with an increase in the production of Reactive Oxygen Species (ROS), such as superoxide radicals (·O2) (Gocheva et al. 2006). In addition to this mechanism, exposure of oxygen to UV radiation or chemical reducers such as oxidases, peroxidases, and mono- and dihydrooxygenases also increase the production of ROS (Xie et al. 2009). In order to combat this excess of ROS, these microorganisms develop differentiated physiological processes, with consequent production of SODs of high biotechnological value (Brenchley 1996).

From the group of SOD-producing bacteria isolated from the Antarctic environment, Zheng et al. (2006) worked with the species *Marinomonas* sp. NJ522 isolated from the icy Antarctic sea between 2001 and 2002. Characterized as belonging to the Fe-SOD group, this enzyme showed a specific activity of 148 $U.mg^{-1}$ protein and a purification of approximately 39-fold with a yield of 9%. Despite being produced by a psychrophilic bacterium, the optimal temperature of this SOD was 40 °C, which leaves it in a range of activity similar to that of the human organism, a characteristic that may indicate a biotechnological potential

for the development of new antioxidant drugs (Zheng et al. 2006).

In another study, Castellano et al. (2006) worked with *Pseudoalteromonas haloplanktis* TAK125 isolated in 1992 from marine sediments collected near the French Antarctic Station Dumont d'Urville, which was able to produce a psychrophilic superoxide dismutase denominated PhSOD (Birolo et al. 2000). PhSOD showed a specific activity of 14.000 $U.mg^{-1}.mol\ Fe^{-1}$ mol subunit with a purification of 50-fold. An interesting feature of this enzyme in the Fe-SOD group was that its specific activity practically did not vary over a wide range of temperature (5-25 °C) and pH (7.8-10.85), although new techniques are needed to confirmation of this profile. Although it is a psychrophilic enzyme, it showed a good resistance to thermal denaturation, reaching a denaturation/inactivation temperature of 30 °C higher than the maximum growth temperature of *P. haloplanktis* TAK125 (Castellano et al. 2006). Considering the difficulties related to recombinant sources of SODs application in therapeutics due to problems related to availability and pharmacokinetics, enzymes with a more stable profile may emerge as perspectives for the future of the development of new antioxidant therapies (Bafana et al. 2011b).

Pseudoalteromonas sp. ANT506, a bacterium also isolated from the Antarctic sea-ice presented the production of a SOD (PsSOD) from the group of Fe-SODs with an interesting biotechnological profile. After the identification of the PsSOD encoding gene, it was inserted into *E. coli* and an rPsSOD enzyme was produced, which showed a specific activity of 587.4 $U.mg^{-1}$ protein and a 12.6-fold purification, and 22.9% yield. Despite not having great thermal stability, characteristic of cold-adapted enzymes, this enzyme demonstrated stability in pH (7.0-9.0), and an important resistance to salinity, maintaining 62.4% of its optimal activity even in 2.5 M of chloride sodium (NaCl) (Wang et al. 2016).

Kan et al. (2017) studied *Rhodotorula mucilaginosa* AN5, a cold-adapted yeast isolated from the Antarctic environment that produces Fe-SOD. The gene encoding this SOD was cloned and expressed in *E. coli* giving rise to the recombinant RmFeSOD. This enzyme demonstrated a specific activity of 92.11 $U.mg^{-1}$ protein and a yield of 0.735 mg SOD protein per gram of fresh weight. An interesting feature of RmFeSOD, was that it showed the highest stability in different pH ranges, when compared with other Fe-SOD, maintaining about 70% of its maximum activity in a pH range between 1.0 and 9.0 (Kan et al. 2017). This characteristic makes the RmFeSOD of *R. mucilaginosa* AN5 a potential enzyme for application in a wide range of functions, from pharmacological to food industry.

Filamentous fungi also stand out in the production of SODs with high biotechnological potential. Representatives of the psychrophilic and mesophilic *Penicilium* from the Antarctic soil, *Penicillium* sp. p14, and *Penicillium* sp. m12, respectively, were compared with another mesophilic strain isolated from a temperate region of Bulgaria (*Penicillium* sp. T35) regarding several aspects, among them, the production of SOD in different temperature ranges (Gocheva et al. 2006). Strains of Antarctic origin produced SODs with greater specific activity at lower temperatures, indicating the adaptation to oxidative stress, which is common in regions with extreme environmental characteristics such as Antarctica.

In another study, Tosi et al. (2010) evaluated the antioxidant activity of enzymes produced by filamentous fungus *Penicillium* sp. 161, *Penicillium* sp. p31, *Cladosporium cladosporioides* B611, *Cladosporium oxysporum*

212, *Epicoccum nigrum*, and *Aspergillus glaucus* 363 isolated from soil collected near the permanent Bulgarian Antarctic base "St. Kliment Ohridski" on Livingston Island. Among them, *A. glaucus* 363 showed the highest antioxidant activity (specific activity of 39.61 $U.mg^{-1}$ protein) due to the production of SOD and catalase (Tosi et al. 2010). The SOD produced by *A. glaucus* 363 was characterized as a Cu/Zn-SOD, an enzyme with a fundamental role in the defense system of aerobic organisms against oxidative stress.

RIBONUCLEASES

Ribonucleases (Ribonucleases, RNases, EC 3.1.26.-) have several roles in the metabolism of RNA molecules. In addition to participating in the degradation of RNA molecules that are no longer useful, or with some type of damage, RNases actively participate in the maturation of RNA molecules and their transformation into alternative forms (Li & Deutscher 2004). They are classified into 2 main groups, according to their RNA cleavage mechanism. Endoribonucleases are responsible for cleaving the phosphodiester bonds that bind ribonucleotides internally releasing fragments of RNA of various sizes. Among the endoribonucleases isolated in *E. coli* are RNases I, II, P, E, G, HI, and HII (Li & Deutscher 2004). Exoribonucleases cleave RNA molecules generally from the 3' or 5' terminal position promoting the release of ribonucleotides that will participate in the formation of new RNA molecules (Nicholson 1999). In the group of exoribonucleases characterized in *E. coli* are RNases II, R, D, T, PH, BN, polynucleotide phosphorylase (PNPase), and oligoribonuclease (ORNase) (Li & Deutscher 2004).

RNases have a key role in the regulation and maturation of DNA, being studied for their role in combating several diseases, in several species of organisms (Shruti et al. 2016). The bovine seminal vesicle RNase (BovineBS-RNase – EC.3.1.27.5) isolated from the *Bos taurus* species showed the potential to promote apoptosis of human thyroid cancer cells, becoming an alternative for the development of new antineoplastic drugs (Spalletti-Cernia et al. 2003). Enzymes from a species of the frog *Rana pipiens* from the RNase A family, Onconase and Amphinase, have shown great cytotoxic potential against several cancer cell lines (Ardelt et al. 2003, 2009, Singh et al. 2007). Bacteria are also an important source of isolation of RNases with anti-cancer activity. RNases from representatives of *Bacillus intermedius* presented apoptosis induction in ovarian cancer cells (Garipov et al. 2014). *Bacillus amyloliquefaciens* is a producer of barnase, an RNase with high cytotoxic power (Prior et al. 1996). *Streptomyces aureofaciens* is also a producer of cytotoxic RNase (Makarov et al. 2008). The search for microbial species that produce RNAs in environments with extreme characteristics such as Antarctica, generates the possibility of isolating RNAses with therapeutic activity, but which have a different profile of stability and potential for the development of new drugs

In the search for cold-adapted RNases, Reddy et al. (1994) worked with 13 species of psychrotrophic bacteria isolated from the soil of the region around Zub Lake, Schirmacher Oasis Antarctica (Shivaji et al. 1988). The bacterial extracts were exposed to a temperature of 65 °C for 30 minutes and immediately tested for enzymatic activity. Among the bacteria that showed thermolabile characteristics, the species *Pseudomonas fluorescens* showed a specific activity of 125 $U.mg^{-1}$ protein, with a purification of 36.8-fold, and a yield of 14.7%. Despite *P. fluorescens* demonstrating the ability to completely hydrolyze RNA from *E. coli*, when compared to bovine pancreatic RNase A, it

demonstrated total inactivation at 65 °C (Reddy et al. 1994).

RNase R was cloned from a *Psychrobacter* sp. ANT206 isolated from the Antarctic sea-ice and tested for its structure and catalytic properties (Wang et al. 2019). The PsRNR enzyme showed a specific activity of 115.60 μmol.min^{-1}.mg^{-1} with purification of 5.58-fold, yield of 48.85%, and maximum activity at pH 6.0 and temperature of 30 °C. This enzyme was characterized as cold-adapted and presented a salinity tolerance profile (Wang et al. 2019). Due to the characteristics of RNases R, degeneration of mRNAs and stable defective RNAs, and their importance for the expression of *Shigella* virulence genes and *E. coli* enteroinvasive strains (Cairrão et al. 2003), PsRNR appears as a promising alternative to antimicrobial treatments.

CONCLUSIONS

Enzymes produced by fungi and bacteria isolated from Antarctica have potential biotechnological value in therapeutics. The catalytic profile of these enzymes differs from the mesophilic counterparts, presenting greater specificity to their respective substrates, such as the L-asparaginases, which showed a lack of adjacent enzymatic activity. In addition to presenting peculiar molecular characteristics, psychrophilic polypeptides demonstrate potential compatibility with humans, whether due to temperature and pH similar to that of the human body or low immunogenicity. The studies that seek the isolation of superoxide dismutases and Antarctic L-asparaginases are growing. Considering the rich Antarctic ecosystem, it is possible to infer that there is a vast unexplored field in the search for microbial enzymes with biotechnological potential. Thus, it is concluded that the bioprospecting of Antarctic microorganisms is a promising alternative to the development of new enzymatic drugs, especially when looking at a market with different demands.

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