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Review

Cell Response to Oxidative Stress in Antarctic Filamentous Fungi

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Abstract: Antarctica, with its harsh environmental conditions, is home to a wide variety of microorganisms, including filamentous fungi. The survival of Antarctic mycoflora has led to increasing interest in their adaptation. Extreme low temperatures, low water availability, frequent freeze–thaw cycles, strong winds, ultraviolet radiation, etc., are all prerequisites for accelerated production of reactive oxygen species (ROS) and the occurrence of oxidative stress (OS). Antarctic fungi have effective mechanisms to cope with the damaging effects of ROS. While the distribution, morphology, physiology, and biotechnological potential of these fungi are well documented, the role of oxidative stress in their adaptation is poorly understood. This has been one of the main research topics of our team for a long time, and a number of papers on our results have been published. This review summarizes the existing literature on the induction of oxidative stress in Antarctic psychrophilic, psychrotolerant, and mesophilic fungi by extreme conditions. The most recent and relevant studies reporting on the relationship between cold stress and OS biomarkers and the damaging effects of ROS on key intracellular biomolecules are presented. The contribution of both enzymatic and non-enzymatic antioxidant defenses to the fungal cell response is discussed.

Keywords: Antarctica; fungi; biodiversity; ROS generation; cold stress; stress biomarkers; antioxidant response

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1. Introduction

Fungi are known for their ability to live in different ecological niches [1]. They can adapt to a wide range of extracellular stimuli such as low and high temperatures, UV radiation, low water availability, high salt concentrations, osmotic pressure, etc. In many cases, the fungi have to deal with a combination of these stimuli. Antarctica is one such habitat, characterized by extreme environmental conditions. Nevertheless, representatives of different phyla of the Kingdom Fungi have been isolated from this region [2,3]. Their survival is a complex process, and mechanisms have been identified at the morphological, physiological, biochemical, and genetic levels [1,4–6].

All the noted environmental conditions have a common mechanism of action. This is the phenomenon of oxidative stress (OS), which results from the exposure of fungal cells to biotic and non-biotic agents found in nature [7]. The term "oxidative stress" is widely used in many areas of science. It is one of the keywords of the 21st century, along with global warming and drug resistance. Oxidative stress is a complex process having negative

consequences on aerobic cells, characterized as a state in which cells generate reactive oxygen species (ROS) at a higher rate than internal antioxidant defenses [8]. ROS are characterized by the presence of free radicals such as the superoxide anion ($^{\bullet}O_2^-$), and the hydroxyl radical (•OH), along with nonradical molecules like hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), among additional species. In optimal growth environments, the synthesis of these substances occurs primarily at low levels within the organelles of fungal cells, such as mitochondria and peroxisomes [9]. However, under conditions of stress, there is a notable increase in the ROS generation rate.

A variety of environmental stresses, including temperature, drought, salinity, chilling conditions, metal toxicity, and UV-B radiation, as well as the impact of pathogens, have been demonstrated to contribute to an increased production of ROS in fungi [10]. Temperature is a critical factor influencing their growth and development. Exposure to extremely low temperatures induces oxidative stress, prompting a distinct response characterized by various stress tolerance strategies at the morphological, physiological, and molecular levels [3,6,11]. Despite the extensive research conducted on the cold stress responses of bacteria and plants, the adaptation strategies employed by fungi to cold conditions have not received adequate attention. Moreover, there is a notable lack of knowledge regarding the cellular response of Antarctic fungal isolates to low-temperature stress. Therefore, the present review was focused on the impact of low temperatures on the release of cold stress in the fungi isolated from Antarctica as well as on the cell response as a component of their adaptive strategies to survive under so harsh conditions.

2. Biodiversity of Filamentous Fungi in Antarctica

Despite the harsh conditions, Antarctica is home to a variety of microorganisms. The unique ecosystems here are dominated by fungi [12], which are the subject of growing interest in recent decades. Research on fungi in cold environments began in the late 19th century [13,14]. However, the initial research on the biodiversity of Antarctic fungi dates back to the 1960s [15]. The first evidence of the presence of fungi was obtained by studying mosses and lichens [16–20]. Microscopically viable fungi have been found on the surface and deep in permafrost sediments [21]. In the Antarctic, the most common fungal taxa to be found are *Ascomycota*, *Basidiomycota*, *Mucoromycota*, *Chytridiomycota*, and *Glomeromycota*. The reports described species belonging to the genera *Alternaria*, *Botrytis*, *Cladosporium*, *Fusarium*, *Penicillium*, *Phaeosphaeria*, and *Phoma* [22–24]. Fletcher et al. [17] reported that the major taxa identified in samples from Mac. Robertson Land and Enderby Land between December 1979 and October 1980 were *Penicillium* spp., especially *P. brevicompactum* and *P. cyclopium*, and *Cladosporium herbarum*. In this region, *Sporothrix ramosissima* was found for the first time in Antarctica. A large number of sterile forms were also isolated.

Since the beginning of the 21st century, research on the abundance and diversity of fungi in the Antarctic regions has made a great deal of progress (Table 1) [25–27]. The number of habitats under investigation is increasing, and the list of fungi isolated and identified is rapidly growing. Antarctic soils are characterized by great fungal diversity because of their exceptional capacity for cold adaptation. Spores in the dormant state make up a greater portion of fungal biomass [21,28]. In general, Antarctic soils contain mainly *Ascomycota* filamentous fungi and *Basidiomycota* yeasts [29], and *Zygomycota* and *Chytridiomycota* have been very rarely isolated [30,31]. Dos Santos et al. [32] presented information on 309 strains isolated from soils of Collins Glacier. Most of them belong to the phylum *Ascomycota* (more than 90%), followed by phylum *Mortierellomycota*, and phylum *Basidiomycota*. The strains were assigned to the genera *Pseudogymnoacus*, *Pseudeurotium*, *Mortierella*, *Passalora*, *Pholiota*, *Russulales*, *Acremonium*, *Schizophyllum*, *Thelebolus*, and *Xylaria*. Similar data for the phylum *Ascomycota* were reported by Frisvad [33], who mentioned

9 orders (*Eurotiales*, *Hypocreales*, *Leotiales*, *Microascales*, *Onygenales*, *Pezizales*, *Saccharomycetales*, *Sordariales*, and *Trichosphaeriales*) with 12 families. For phylum *Basidiomycota*, the classes *Tremellomycetes* and *Cystobasidiomycetes* were reported [34], and for *Mucoromycota*—the family *Mortierellaceae* [35]. Marfenina et al. [36] isolated 38 species from Antarctic soils belonging to the phylum *Ascomycota* (the genera *Aspergillus*, *Penicillium*, *Trichoderma*, *Alternaria*, *Aureobasidium*, *Cladosporium*, *Doratomyces*, *Trichurus*, and *Emericella*) and 1 species belonging to the phylum *Zygomycota*. A similar ratio between phyla was found in the fungal community identified in the soil of Syowa Station, Antarctica (11 genera of *Ascomycota*, 7 genera of *Basidiomycota*, and 2 genera of *Zygomycota*) [37]. A total of 145 fungal strains have been isolated from soil samples collected at Casey Station, Terra Nova Bay, and South Georgia [38]. They belong to the phyla *Deuteromycota*, *Ascomycota*, and *Zygomycota* and the genera *Penicillium*, *Aspergillus*, *Mucor*, *Cladosporium*, *Alternaria*, *Verticillium*, *Botrytis*, etc.

Table 1. Fungal strains isolated from Antarctica.

№	Strain	Antarctic Samples	Origin	References
1.	*Acremonium berkeleyanum*	Soil	Russian Research Station	[39]
2.	*Alternaria alternata*	Lichens	Livingston Island	[40]
3.	*A. arborescens*	Seals nasal swab	Primavera Cape/ Cierva Cove	[41]
4.	*A. alternata*	Soil	McMurdo Dry Valleys	[42]
5.	*A. maritima*	Soil	Livingston Island	[43]
6.	*Angustimassarina populi*	Soil	Livingston Island	[43]
7.	*Antarctomyces pelizarie*	Soil	Penguin Island and Robert Island	[44]
8.	*Antarctomyces psychrotrophicus*	Water	Lakes in Admiralty Bay (King George Island)	[45]
9.	*Arthrobotrys ferox*	Soil	Victoria Land	[19]
10.	*A. ferox*	Moss	Edmonson Point	[46]
11.	*Aspergillus flavus*	Soil	Russian Research Station	[39]
12.	*A. fumigatus* 1-9	Soil	Livingston Island	[40]
13.	*A. glaucus*	Lichens	Livingston Island	[47]
14.	*A. sydowii*	Soil	Livingston Island	[40]
15.	*A. tenneseensis*	Seals nasal swab	Primavera Cape/ Cierva Cove	[41]
16.	*A. versicolor* (Vuill.)	Soil under moss	Lamplugh Island	[46]
17.	*Aureobasidium pullulans var. pullulans*	Antarctic permafrost	McMurdo Dry Valleys	[42]
18.	*Cadophora malorum*	Water	Lakes in Admiralty Bay (King George Island)	[45]
19.	*Cladosporium oxysporum*	Lichens	Livingston Island	[43]
20.	*C. cladosporioides*	Antarctic permafrost	McMurdo Dry Valleys	[42]
21.	*C. herbarum*	Antarctic permafrost	McMurdo Dry Valleys	[42]
22.	*Coelomycetes Incertaesedis*	Permafrost sediments	Banger Oasi	[2]
23.	*Cordyceps farinose*	Seals nasal swab	Primavera Cape/ Cierva Cove	[41]

Table 1. *Cont.*

№	Strain	Antarctic Samples	Origin	References
24.	*C. bassiana*	Antarctic permafrost	McMurdo Dry Valleys	[42]
25.	*Cryptococcus victoriae*	Soil	Rip Point, Nelson Island	[27]
26.	*Dendryphiella salina*	Moss and soil under moss	Lake Carezza	[46]
27.	*Dioszegia hungarica*	Snow samples	Trinity Peninsula	[44]
28.	*Epicoccum nigrum*	Lichens	Livingston Island	[40]
29.	*E. nigrum*	Lichens	Livingston Island	[43]
30.	*Lecanicillium muscarium*	Moss	Victoria Land	[48]
31.	*L. muscarium*	Permafrost sediments	Bellingshausen Station	[2]
32.	*L. muscarium*	Soil	Livingston Island	[43]
33.	*Microdochium phragmitis*	Water	Lakes in Admiralty Bay (King George Island)	[45]
34.	*Monodictys austriana*	Soil	Livingston Island	[43]
35.	*Mortierella elongate*	Soil	Progress station	[49]
36.	*Mrakia blollopsi*	Soil	Syowa station	[50]
37.	*Mucor hiemalis*	Soil	Livingston Island	[40]
38.	*Neomicrosphaeropsis italica*	Snow samples	King George Island	[44]
39.	*Penicillium aurantiogriseum*	Soil	Livingston Island	[43]
40.	*P. brevicompactum*	Permafrost sediments	Bellingshausen Station	[2]
41.	*P. chrysogenum*	Mosses	Livingston Island.	[40]
42.	*P. chrysogenum*	Antarctic permafrost	McMurdo Dry Valleys	[42]
43.	*P. citrinum*	Soil	Livingston Island	[43]
44.	*P. commune*	Soil	Livingston Island	[40]
45.	*P. commune* 161	Soil	Livingston Island	[47]
46.	*P. dierckxii*	Soil	Livingston Island	[43]
47.	*P. expansum*	Permafrost sediments	Beacon Valley	[2]
48.	*P. expansum*	Antarctic permafrost	McMurdo Dry Valleys	[42]
49.	*P. fimorum*	Lichens	Livingston Island	[43]
50.	*P. glandicola*	Snow sample	Trinity Peninsula	[44]
51.	*P. griseofulvum*	Soil	Terra Nova Bay	[51]
52.	*P. olsonii* p14	Soil	Casey Station	[52]
53.	*P. palitans*	Antarctic permafrost	McMurdo Dry Valleys	[42]
54.	*P. rubens* III11-2	Soil	Livingston Island	[53]
55.	*P. rubens*	Soil	Livingston Island	[40]
56.	*P. solitum*	Marine sediments	King George Island	[54]
57.	*P. tardochrysogenum*	Snow samples	King George Island	[44]
58.	*P. waksmanii* m12	Soil	Terra Nova Bay	[52]
59.	*Phenoliferia glacialis*	Snow samples	Trinity Peninsula	[44]

Table 1. *Cont.*

№	Strain	Antarctic Samples	Origin	References
60.	*Phenoliferia psychrophenolica*	Snow samples	King George Island	[44]
61.	*Phoma herbarum*	Water	Lakes in Admiralty Bay (King George Island)	[45]
62.	*Pseudogymnoascus pannorum*	Soil	Livingston Island	[40]
63.	*Pseudogymnoascus* spp.	Lichen thalli and soils	South Shetland Islands	[55]
64.	*Talaromyces pinophilus*	Soil	Livingston Island	[40]
65.	*Thelebolus microspores*	Penguin sample	Potter Peninsula/Cove	[41]
66.	*T. microsporus*	Water	Lakes in Admiralty Bay (King George Island)	[45]
67.	*Torulopsis psychrophila*	Soil	Schirmacher Oasis	[56]
68.	*Trichoderma harzianum*	Permafrost sediments	Bellingshausen Station	[2]

From the lakes in the Antarctic Peninsula have been isolated species such as *Geomyces pannorum*, *Thelebolus* sp., *Mortierella* sp., *Cadophora cf. luteo-olivacea*, *Cadophora malorum*, *Davidiella tassiana*, *G. pannorum*, *Mortierella cf. alpina*, *Thelebolus cf. microspores*, etc. [45]. DNA metabarcoding indicates that the dominant phyla in lakes on the South Shetland Islands and James Ross Island are *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota*, and *Chytridiomycota*. Species belonging to the phyla *Aphelidiomycota*, *Basidiobolomycota*, *Blastocladiomycota*, *Monoblepharomycota*, *Rozellomycota*, *Zoopagomycota*, and unknown fungal taxa were also detected [57].

Microbiological culture approaches or different molecular techniques have been used to demonstrate the presence of fungi in Antarctica [26,58–62]. Culturable soil fungi isolated from King George Island, Deception Island, and Robert Island were identified to 19 genera, namely, *Penicillium*, *Pseudogymnoascus*, *Lambertella*, *Cadophora*, *Candida*, *Mortierella*, *Oxygenales*, *Geomyces*, *Vishniacozyma*, *Talaromyces*, *Rhizopus*, *Antarctomyces*, *Cosmospora*, *Tetracladium*, *Leptosphaeria*, *Lecanicillium*, *Thelebolus*, *Bjerkandera* [63]. The culture-dependent method has been used successfully to assess fungal diversity in a number of Antarctic regions [28,41,64,65]. In addition, various methods such as direct or luminescence microscopy, scanning electron microscopy, and microarray analysis have been used to study Antarctic mycobiota [49,66–68].

On the other hand, molecular approaches allowed for the expansion of the number of identified fungi from different niches in Antarctica [26,57,69–71]. The simultaneous use of both methods increases the chances of obtaining more accurate information about fungal diversity [72]. The miscellany of black fungi in the Antarctic cryptoendolithic habitat was described by combining high-throughput sequencing and culture-dependent methods (genera *Cyphellophora*, *Cryomyces*, *Catenelustroma*, *Coniospora*, *Elasticomyces*, *Friedmanniomyces*, *Knufia*, *Meristemomyces*, *Rachicladosporium*, *Cladophialophora*, *Exophiala*, *Extremus*, *Hortaea*, *Neodevriesia*, *Oleoguttula*) [59]. Varrella et al. [60] reviewed the use of culture-dependent and molecular methods for the identification of fungi in the Antarctic marine environment. In coastal and offshore Antarctic waters, genera belonging to the orders *Eurotiales*, *Hypocreales*, *Chaetothyriales*, *Kriegeriales*, *Penicillium*, *Metschnikowia*, *Rhodotorula*, and *Glaciozyma* were found. In addition, the genera *Pseudocercosporella*, *Toxicocladosporium*, *Trichoderma*, *Humicola*, *Paraconiothyrium*, *Phaeoacremonium*, and *Phenoliferia* have been identified in Antarctic marine sediments. Molecular tools have allowed the identification of the orders *Saccharomycetales*

and *Eurotiales*, the genera *Wickerhamomyces*, *Geotrichum*, *Letendraea*, and *Bullera*, and the families *Saccharomycetaceae* and *Aspergillaceae*.

Soil samples collected from the permanent Bulgarian Antarctic base "St. Kliment Ohridski" (62°38′29″ S, 60°21′53″ W) on Livingston Island have demonstrated the presence of 109 filamentous fungi [43,47]. Among them, the strains belonging to the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Geomyces*, *Lecanicillium*, *Monodictys*, *Talaromyces*, *Epicoccum*, etc.

3. Growing Temperature Requirements of Antarctic Fungi

The Antarctic mycoflora includes endemic and indigenous fungi that are able to grow and reproduce at low temperatures. They are well adapted, with the majority of them being psychrotolerant or rapid sporulating forms that can complete their lifecycles in an extremely short period. In addition, mesophilic cosmopolitan fungi may be found, transported as propagules from nearby areas, which are unable to grow in extreme conditions [24,25,73]. In most reports, the terminology used to characterize the temperature type of Antarctic fungi is that of Morita [6,34,74–77]. Fungi isolated from Antarctica can be classified as psychrophilic, psychrotolerant (psychrotrophic), and mesophilic–psychrotolerant [25,78]. Psychrophilic fungi can grow below 0 °C with optimum growth temperatures (OGT) around 15 °C or lower and maximum growth temperatures (MGT) around 20 °C, while psychrotolerant (psychrotrophic) fungi are able to grow at 5 °C or lower and have MGT between 20 and 25 °C [79]. Mesophilic–psychrotolerant fungi (mesophilic behavior and psychrotolerant adaptation) have been classified as strains that do not grow at 5 °C, showing optimal growth at 35 °C, and can grow at 45 °C [54,80–82]. In addition to the above-noted classification of microorganisms isolated from cold environments, Cavicchioli and Siddiqui [83] proposed the terms 'eurypsychrophile' (instead of 'psychrotolerant' or 'psychrotroph') and 'stenopsychrophile' (instead of 'true psychrophile'). In the present review, we follow the definition of Morita [74].

The published data indicate a correlation between the temperature class of Antarctic fungi and their provenance [81]. In most cases, the Antarctic psychrophilic fungi are endemic species that can grow and reproduce in these harsh conditions [25,55,84,85]. From the soils of Schirmacher Oasis, East Antarctica, isolated 16 psychrophilic fungal and one yeast strains belonging to genera *Acremonium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Trichoderma*, and *Torulopsis* [56]. Endemic psychrophilic strains were classified as species *Metschnikowia australis*, *Antarctomyces psychrotrophicus*, *Antarctomyces pellizariae*, *Cryomyces antarcticus*, *Friedmanniomyces simplex*, *Friedmanniomyces endolithicus*, *Mortierella antarctica*, *Penicillium antarcticum*, *Penicillium tardochrysogenum*, *Thelebolus globosus*, *Thelebolus ellipsoideus*, *Thelebolus balaustiformis*, and *Thelebolus spongiae* [84]. Two endemic psychrophilic species of the genus *Antarctomyces* (*A. psychrotrophicus* and *A. pellizariae*) have been identified in the samples from the seasonal snow of the Antarctic Peninsula [44]. However, the Antarctic mycoflora consists mainly of cosmopolitan psychrotolerant taxa. For example, several cold-tolerant species belonging to the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, and *Rhodotorula* have been recognized as cosmopolitan taxa, adapted to the extreme low temperatures of Antarctica [84]. Out of the 51 fungal isolates from King George Island, 76% have been identified as psychrotolerant and 26% as psychrophilic [86]. The prevalence of psychrotolerant fungi in surface soils of cold environments has been documented in several studies. According to Robinson [6], this could be attributed to solar radiation causing seasonal and diurnal increases in soil temperature, which favors the development of cold-adapted microorganisms over strict psychrophiles. Furthermore, the Antarctic mycoflora includes species displaying mesophilic–psychrotolerant behavior as a cold-adaptive strategy [24]. Such mesophilic–psychrotolerant fungi have been isolated

from soils and marine sediment samples collected from King George Island and Whalers Bay [79]. The authors found that two strains of *Penicillium* cf. *oxalicum* (LAMAI 2400 and LAMAI 2402) could grow at temperatures between 15 and 30 °C. Similar results have been reported for Antarctic isolates belonging to the genus *Penicillium* [38,54,87], as well as for *Friedmanniomyces* sp. CCFEE 507 [75], *Aspergillus glaucus* [88], *Chaetomium thermophilum* [89], and so forth.

Antarctic fungi are not always tolerant of the temperature of the cold environment from which they have been isolated. For example, psychrophilic fungi can grow at optimum and maximum temperatures much higher than the temperature of the original habitat [90]. It is worth mentioning that the isolation of Antarctic fungi belonging to different temperature classes depends on the isolation temperature, which can affect the isolation frequency [6]. The fungal strains obtained from forest soils in Rhode Island at an isolation temperature of 0 °C were mainly psychrotolerant, and some psychrophiles [91]. Isolations conducted at 25 °C yielded predominantly mesophilic organisms, exhibiting optimal growth between 5 and 10 °C and reaching a maximum at temperatures above 25 °C. Similar trends in the ratio between psychrophilic, psychrotolerant, and mesophilic isolates have been described for samples from King George Island [63]. Furthermore, the mitochondrial genomes of psychrophilic and psychrotolerant fungi demonstrated a higher fraction of protein-coding regions and a lower GC content compared to those of mesophilic fungi [82]. Tosi et al. [43] reported that among 109 fungal isolates, 16 were collected from agar plates incubated at 4 °C, 61 from those at 10 °C, and 32 from plates at 25 °C. The results revealed that the preferred growth temperature for most of the isolated strains was between 15 and 25 °C.

4. ROS and Oxidative Stress

4.1. ROS: Origin, Sources, Damages

The designation ROS denotes a collective term of oxygen-containing reactive species that are formed during the process of incomplete oxygen reduction. They include superoxide radicals ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•), singlet oxygen ($^1O_2$), peroxyl radical (LOO•), alkoxyl radical (LO•), lipid hydroperoxide (LOOH), peroxynitrite (ONOO−), hypochlorous acid (HOCl), and ozone (O_3), among others [92]. Some of ROS contain one or more unpaired electrons, which are called oxygen radicals or oxygen free radicals, for example $^{\bullet}O_2^-$, OH•, LOO•, and LO•. The ROS that do not contain any unpaired electrons, such as H_2O_2, HOCl, and O_3, are not free radicals [93,94]. ROS are inevitable by-products of the normal aerobic metabolism of cells. The mitochondrial respiratory chain is the major source of ROS. Under normal oxy conditions, only 1–2% of total oxygen is converted into free radicals [95]. The primary species $^{\bullet}O_2^-$, H_2O_2, and HO• are the result of successive processes of monovalent O_2 reduction [Figure 1].

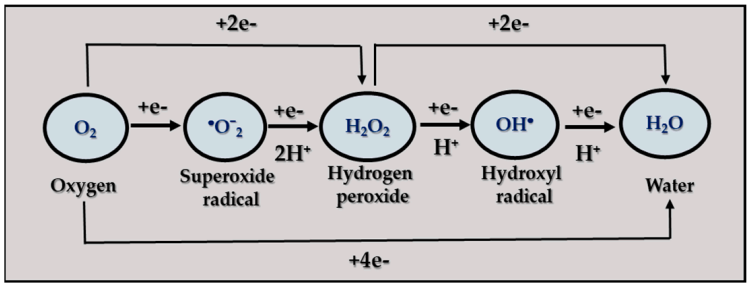


Figure 1. Mechanisms of ROS generation.

The initial monovalent reduction results in the formation of $^{\bullet}O_2^-$, which is subsequently followed by the reduction of a second electron, leading to the release of H_2O_2. $^{\bullet}O_2^-$ also reacts with H_2O_2 to generate HO• (Haber–Weiss reaction) [96]. The superoxide

radical is most commonly produced in mitochondria. It has a very short lifetime and relatively low reactivity. $^{\bullet}O_2^-$ does not react with most biological molecules. Its chemical activity depends on where it is produced in the cell, its ability to be protonated to a more powerful oxidant (peroxyl radical), and its collision with a suitable substrate [97].

Hydrogen peroxide is an unstable and slowly decaying molecule containing an oxygen–oxygen bond. The main source of H_2O_2 is the superoxide radical dismutation reaction or the two-electron reduction of oxygen in reactions catalyzed by xanthine oxidase, glucose oxidase, and others. The H_2O_2 molecule is uncharged and can cross biological membranes. It is a powerful oxidant, but the kinetics of its reaction with biomolecules are quite slow, leading to its accumulation in cells at relatively high concentrations [98].

The hydroxyl radical is one of the most reactive radicals present in biological systems and has a relatively short half-life (10^{-8} to 10^{-9} s) [99]. It exhibits a direct effect near its point of origin, without making long transitions into the living cell, causing damage to the first molecule it encounters.

Singlet oxygen is a non-radical form of oxygen. It is an oxygen molecule in which one of the two free electrons is in an excited state. Thus, $^1O_2$ can transfer energy to other molecules and act as a catalyst for the formation of free radicals.

ROS can originate from both external sources and intracellular processes. Their formation occurs across a variety of biochemical and physiological functions [94]. Besides the mitochondrial electron transport chain, various cellular organelles, including peroxisomes, lysosomes, the endoplasmic reticulum, etc., which depend on oxygen for their activities, also contribute to the intracellular reservoir of ROS. A range of enzymes such as NADPH oxidases, xanthine oxidase, cyclooxygenase, lipoxygenase, and other free ions (such as iron and copper) has been associated with the generation of ROS. The group of exogenous sources includes ultraviolet radiation, polycyclic aromatic hydrocarbons (PAHs), γ-irradiation, and environmental pollution. Exposure to different drugs like cisplatin, doxorubicin, paclitaxel, metformin, atorvastatin, and others can also cause an increase in the ROS level.

Under normal conditions, the level of radicals is adequate to the level of the cell's defense system, and they manage to maintain an oxidation–reduction equilibrium; i.e., a balance is observed between the formation and elimination of ROS. However, the overproduction of ROS can exceed the protective mechanisms of cells, leading to detrimental impacts on cellular elements such as lipids, proteins, and nucleic acids [97] [Figure 2]. Carbohydrates, albeit to a lesser degree, are also affected by ROS [100].

Although DNA is a stable, well-protected molecule, ROS can interact with it and cause various types of damage. In the human body, such processes have been shown to occur at a rate of 104 lesions per cell per day. ROS damages the DNA molecule by removing an electron from it and turning it into an unstable molecule. Mitochondrial and chloroplast DNA are more susceptible to oxidative damage than nuclear DNA due to the lack of protective proteins and histones and their proximity to ROS-producing systems. ROS has been demonstrated to induce a series of molecular events, including binding to DNA bases or deoxyribose, DNA chain breakage, removal of nucleotides, modifications in nucleotides, and subsequent mutations. Additionally, covalent binding to a protein molecule has been observed as a result of ROS action (Figure 3) [97,101,102].

Proteins are also susceptible to biological oxidation due to their prevalence in cells and their high rate of constant interaction with numerous types of free radicals. Consequently, protein damage is a significant consequence of free radical formation within and outside the cell. ROS can cause hydrogen removal, electron transfer (oxidation or reduction), addition, fragmentation and rearrangement reactions, dimerization, disproportionation, and substitution in amino acids, peptides, and proteins, etc. (Figure 4) [100,103,104].

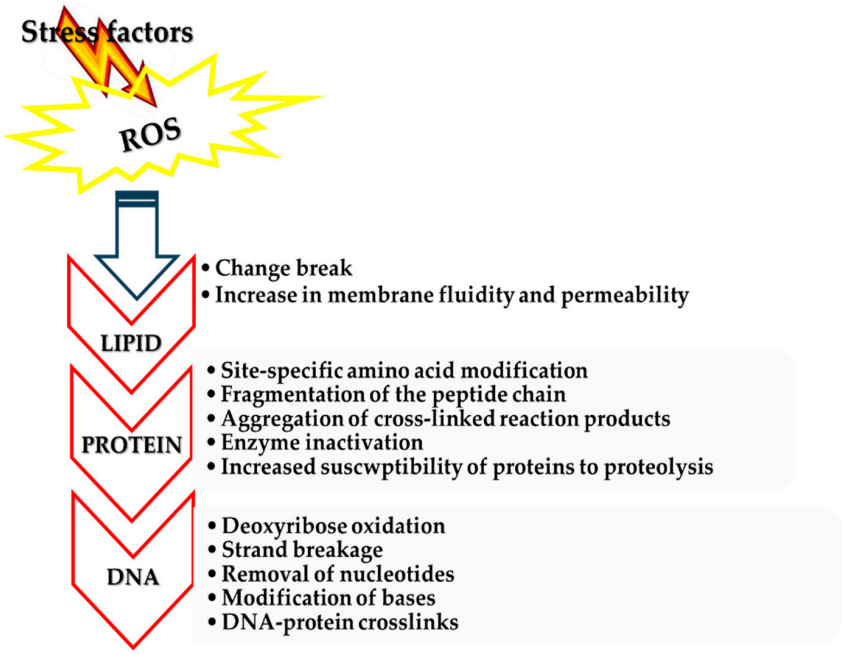


Figure 2. Oxidative damage to intracellular molecules.

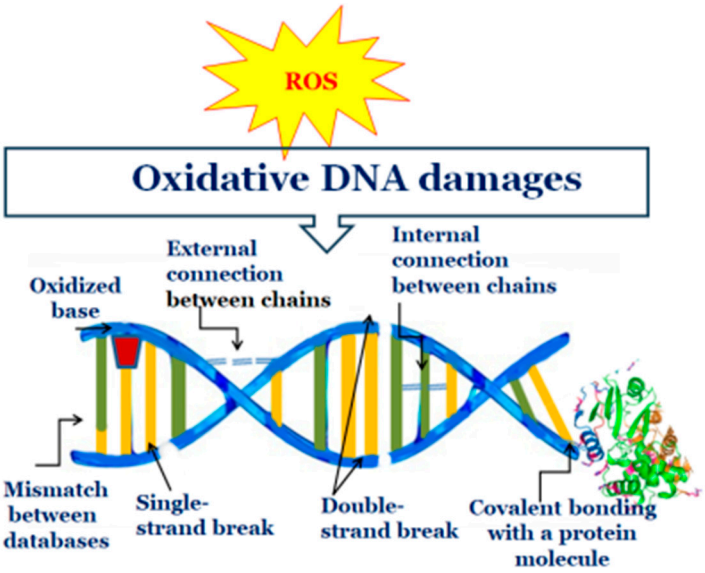


Figure 3. Changes in DNA as a result of oxidative damage.

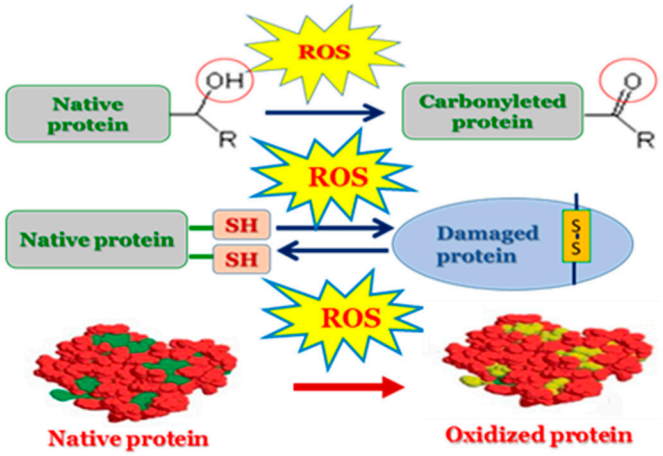


Figure 4. Oxidative-induced damage of proteins.

Oxidative effects on lipids play a particularly important role in cell biology, human health, and the food industry. In cells, a process called lipid peroxidation takes place in which free radicals attack lipids (particularly polyunsaturated fatty acids, PUFAs), removing hydrogen while incorporating oxygen. Responsible for this process are hydroxyl radicals (OH•), which are mainly formed by the Fenton [Reaction 1] and Haber–Weiss [Reaction 2] reactions [10]. Theoretically, the Haber–Weiss reaction cannot play a major role in vivo because its rate constant is lower than that of the dismutation reaction. However, the Fenton reaction, which exploits the ability of iron ions to participate in redox–cyclic interactions and increases the rate of the process, is much more feasible.

$$Fe^{3+} + {}^{\bullet}O^{-}_{2} \longleftrightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \quad \text{[Reaction 1]}$$

Fenton reaction

$${}^{\bullet}O^{-}_{2} + H_2O_2 \longleftrightarrow O_2 + HO^{\bullet} + OH^{-} \quad \text{[Reaction 2]}$$

Haber-Weiss reaction

Lipid peroxidation is considered to be a major molecular mechanism involved in oxidative damage to all structures that can lead to cell death [105,106]. Cell membranes and membranes of subcellular organelles composed of polyunsaturated fatty acids are the first targets of radical attack. These include mitochondrial membranes, microsomes, peroxisomes, and cytoplasmic membranes. Glycolipids, phospholipids, and cholesterol are also damaged or fatally modified by lipid peroxidation (Figure 5).

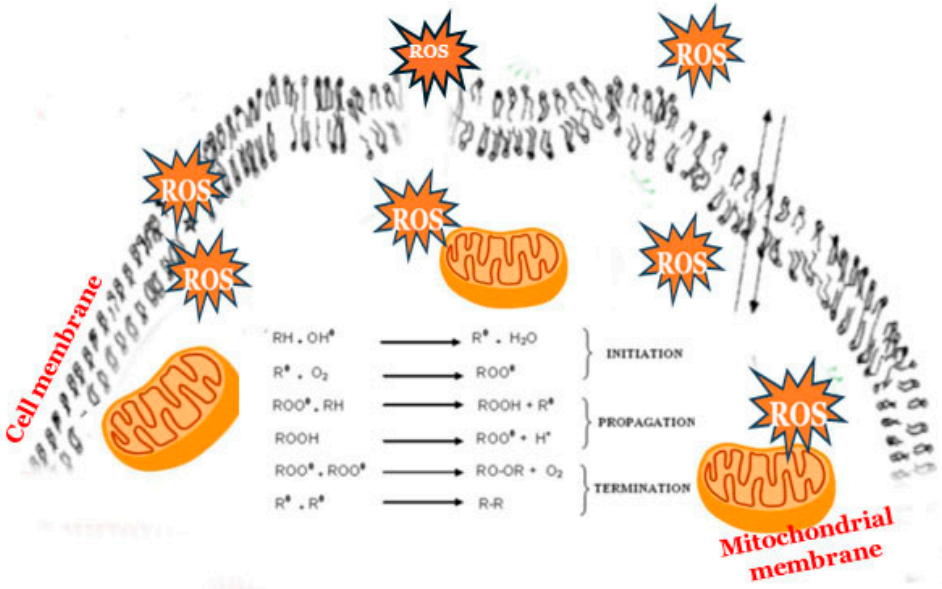


Figure 5. Lipid peroxidation in cell membranes.

4.2. The "Beneficial" Role of ROS

Is it possible that nature has involved ROS in beneficial activities? There is no doubt that the "helpful" effect of these radicals is very limited. From the data currently available, it is clear that ROS are important for the modeling of secondary signaling molecules, differentiation of cells, metabolism of xenobiotics, stimulation of the acrosome reaction in cell fertilization, etc. [107,108]. In addition, maintaining an optimum ROS level plays a vital role in molecular signaling in fungal growth, development, adaptation, and response to various abiotic and biotic stresses, as well as for the virulence of pathogenic fungi [109,110]. The beneficial role of ROS for fungi has been demonstrated in their resistance to antifungal compounds [111] or in the degradation of lignocellulose [112].

5. Oxidative Stress and Antioxidant Defence

5.1. Oxidative Stress and Its Physiological Role

A delicate balance between the rate of their formation and the rate of their removal controls the toxicity of ROS by the cell's defense mechanisms. In cells with normal aerobic respiration, ROS formation proceeds at a relatively low rate. The antioxidant system present in normoxia is able to protect the organism against the action of radicals. However, under certain conditions, the concentration of toxic metabolites exceeds the physiological capacity of the cell, leading to an imbalance between pro- and antioxidants with a predominance of the former [96,113,114]. Excessive levels of pro-oxidants can cause OS. The concept of oxidative stress was introduced in greater detail for the first time in 1985 [115]. This concept caused extensive research on the relationship between oxidants and antioxidants, including their origins and metabolic features [116–118]. It is known that the OS plays a significant role in different physiological processes, in particular, cell signaling, differentiation, proliferation, tissue repair, aging, immune response, etc. [1,110,118]. OS is involved in the regulation of cell growth, differentiation, and apoptosis [119]. The detoxification of various compounds like drugs and xenobiotics can also be realized due to the OS [120].

5.2. Antioxidants and Antioxidant Defence

According to Halliwell [121], antioxidants are "any substance that delays prevents or removes oxidative damage to a target molecule". They can also be defined as sufficiently stable molecules capable of accepting or donating an electron to any free radical and eliminating its unpaired state (Figure 6) [122]. Antioxidants cover a wide range of substances produced within cells or supplied by food substrates. Their function includes reducing the potential of ROS to cause damage, or interacting with ROS and preventing the chain reaction of their generation before damage occurs to molecules vital to the cell.

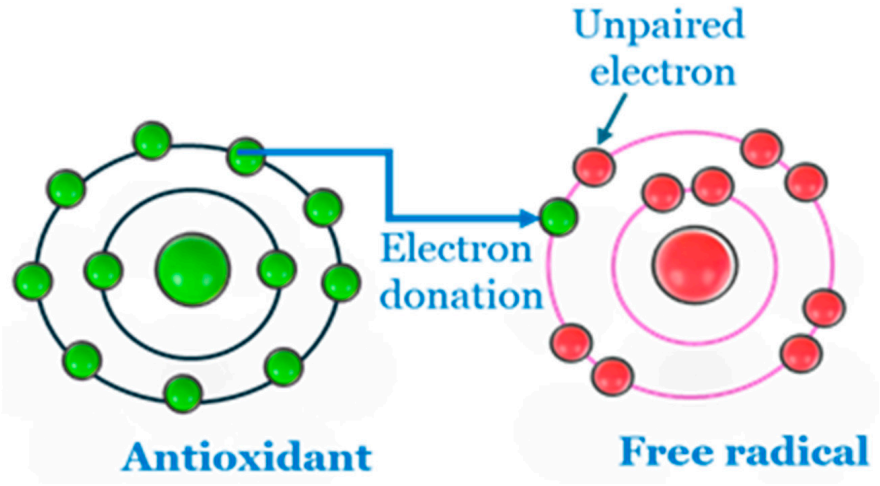


Figure 6. Antioxidant action against free radicals.

Antioxidant activity can be found in the cellular defense system at different levels, depending on the strategy used: prevention, scavenging and destroying radicals, repairing damage, and adaptation. They can also be classified as natural and synthetic, enzymatic and non-enzymatic, low molecular and high molecular, intracellular and extracellular, water-soluble and lipid-soluble (Figure 7) [96,123].

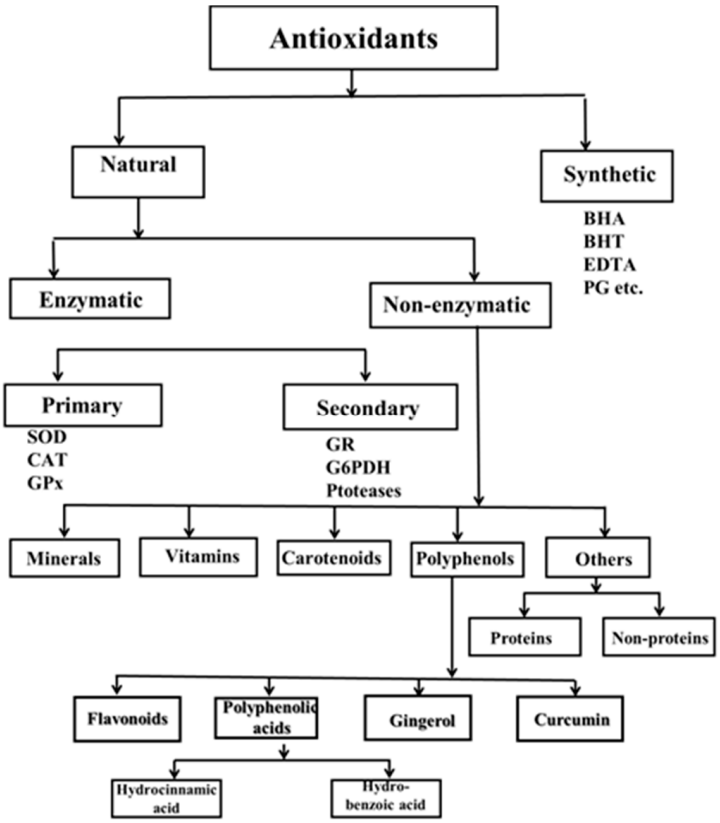


Figure 7. Classification of antioxidants.

Enzymatic antioxidants are the first line of defense and are the most effective antioxidants in aerobic cells. They are classified as primary and secondary. Primary enzymes include superoxide dismutase (SOD) and catalase (CAT). Depending on the developmental stage of the organism, they are joined by glutathione peroxidase (GPx). SOD is responsible for the dismutation reaction in which $^{\bullet}O_2^-$ is converted to H_2O_2. In subsequent reactions catalyzed by CAT or GPx, H_2O_2 is converted to water and oxygen. Glutathione reductase and glucose-6-phosphate dehydrogenase are part of the secondary enzymatic defense. Glutathione reductase converts glutathione from its oxidized to its reduced state to recycle glutathione and scavenge more free radicals. Nicotinamide adenine dinucleotide phosphate, a coenzyme involved in anabolic processes, is renewed by glucose-6-phosphate (NADP), resulting in a reducing environment [124]. These two enzymes do not directly eliminate free radicals but rather provide support to the other endogenous antioxidants.

Third-line antioxidants recognize, degrade, and remove oxidized or damaged proteins, DNA, and lipids to prevent their accumulation, which can be toxic to cells. These include DNA repair enzyme systems (polymerases, glycosylases, and nucleases) and proteolytic enzymes (proteinases, proteases, and peptidases) found in both the cytosol and mitochondria of cells. The third lineage includes direct and indirect repair systems [125,126].

The fourth line of defense is the adaptive response to oxidative stress. This line uses the signals the cell receives about the formation or action of ROS and transports the appropriate antioxidants to the right place. The adaptive response depends on changes in gene expression or increased translation of a pre-existing mRNA to induce the synthesis of antioxidant enzymes. This leads to increased resistance to the stressors [126].

5.2.1. Enzymatic Antioxidants

The antioxidant enzymes SOD, CAT, and GPx play a crucial role in the ability of biological systems to fend off attacks from the free radicals. SOD (**EC** 1.15.1.1.) eliminates

$^{\bullet}O^{-}{}_{2}$ radicals in a "ping-pong" mechanism. The enzyme catalyzes the dismutation of two molecules of $^{\bullet}O^{-}{}_{2}$ to hydrogen H_2O_2 and O_2, which limits $^{\bullet}O_2{}^{-}$ availability [Reaction 3]. This situation led to the reduction of iron and a decrease in the amount of H_2O_2 formed by the Haber–Weiss/Fenton reaction [127,128].

$$^{\bullet}O_2^- + {}^{\bullet}O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \text{ [Reaction 3]}$$

The enzyme was discovered in 1969 by Joe McCord and Irwin Fridovich and has since been the subject of much research. Initially, three types of SOD were identified, and now there are a known four. The first and most obvious similarity between these enzymes is that they all contain redox-active metal ions in the active center: $Fe^{2+/3+}$ in Fe-SOD, $Mn^{2+/3+}$ in Mn-SOD, $Cu^{1+/2+}$ in Cu/Zn-SOD, and $Ni^{2+/3+}$ in Ni-SOD [128–133]. Manganese- and Fe-SOD are evolutionarily older than Cu/Zn-SOD and are derived from the same precursor enzyme [134]. In later stages of phylogenetic development, they diverged, and their amino acid sequences formed two distinct groups. The evolution of the Cu/Zn SOD family proceeded independently, resulting in a significant departure from the characteristics of the initial two enzymes.

The distribution of SOD isoenzymes across various organisms is indicative of both the developmental stage of the organism and the specific cellular organelles with which these enzymes are associated [135,136]. Cu/Zn-SOD is predominantly present in eukaryotic organisms, while Fe-SOD is typically found in prokaryotic organisms. Mn-SOD is observed in both prokaryotic and eukaryotic organisms, and Ni-SOD is specifically located in cyanobacteria and aquatic eukaryotes. Additionally, extracellular SOD (EC-SOD) is produced in the tissues of higher eukaryotes, playing a crucial role in the detoxification of superoxide radicals generated from external sources [137]. These isoenzymes exhibit distinct localization within cellular components [138]. Cu/Zn-SOD is found in the cell wall, cytosol, chloroplasts, and peroxisomes; Fe-SOD is present in the cytosol of prokaryotic organisms and within the chloroplasts of plants; Mn-SOD is located in mitochondria and peroxisomes.

Fe-SOD is the most ancient SOD metalloenzyme with a high degree of homology with Mn-SOD. There are two recognized categories of Fe-SOD: homodimeric protein structures with an approximate molecular weight of 20 kDa, which incorporate 1–2 g-atoms of iron in their active site, and tetrameric forms with a molecular weight ranging from 80 to 90 kDa, containing 2–4 g-atoms of iron in their active site.

In bacterial cells, manganese superoxide dismutase (MnSOD) is localized in the cytoplasm, whereas in eukaryotic cells, it is found within the mitochondria. MnSOD can exist as either a homotetramer or a homodimer, with molecular weights of approximately 96 kDa and 48 kDa, respectively. Each form comprises four or two identical subunits, with each subunit containing a single manganese atom.

Cu/Zn-SOD is predominantly found in the cytosolic compartment of eukaryotic cells [139]. However, exceptions exist, as Cu/Zn-SOD has been identified in certain prokaryotic organisms and within the intermembrane space of mitochondria, albeit in considerably lower concentrations than its iron- and manganese-containing counterparts. In prokaryotes, it is frequently observed as periplasmic SOD, indicating its potential function as a defensive mechanism for pathogenic bacteria against oxidative stress generated by mammalian macrophages. The molecular weight of Cu/Zn-SOD is approximately 32 kDa, consisting of two identical subunits (homodimer), with each subunit incorporating one atom of copper and one atom of zinc [137]. The coordination of these metal ions at the active site is facilitated by the histidine residue at position 61. The inclusion of zinc ions is likely crucial for maintaining the structural integrity of the enzyme. In periplasmic

bacterial SOD, both monomeric and dimeric forms are present, with the dimeric forms exhibiting distinct characteristics compared to those found in eukaryotic systems.

The presence of Ni-SOD can be attributed to evolutionary adaptations that enable the utilization of nickel as an essential redox metal.

This adaptation arose in response to the diminished availability of iron in oceanic environments, a consequence of photosynthetic processes. Within the SOD family, this particular enzyme is distinctive, characterized by its unique amino acid sequence and specific spectroscopic properties. The enzyme is structured as a homohexamer, consisting of six identical subunits, each formed from monomers comprising 120 amino acids, resulting in a molecular mass of approximately 13 kDa, with each subunit containing one atom of nickel.

EC-SOD shares homology with intracellular Cu/Zn-SOD and is characterized as a tetrameric glycoprotein that incorporates a Cu/Zn prosthetic group [137]. This enzyme is released into the interstitial spaces of tissues as well as extracellular fluids, including plasma, lymph, and synovial fluid, in both humans and animals. It exhibits a binding affinity for the plasma membrane and heparin-associated components of the extracellular matrix. Notably, EC-SOD has been identified in certain instances within plants and prokaryotic organisms. Its primary role is to catalyze the dismutation of superoxide radicals that are generated extracellularly.

Catalase (EC 1.11.1.6) is an enzyme present in nearly all aerobic organisms and a variety of microaerophilic microorganisms, such as lactic acid bacteria, which thrive in low-oxygen environments [140,141]. This enzyme serves a crucial protective function for aerobic organisms by decomposing H_2O_2 generated within cells before it can escape into the surrounding environment. Although CAT may not be critical for every cell type, it significantly enhances the organism's resilience against various oxidants. Historically, it was believed that CAT was primarily located in peroxisomes; however, recent findings indicate its presence in mitochondria, cytosol, and chloroplasts as well. CAT found in peroxisomes originates from the nucleus and requires transport to reach these organelles. Within the peroxisomes, the individual monomers assemble into tetramers, and the heme group is incorporated during this process.

CAT is an enzyme characterized by the presence of porphyrin and is composed of four identical subunits, forming a homotetrameric structure. Each subunit has a molecular weight of 60 kDa, resulting in a total molecular mass of 240 kDa for the complete enzyme, and comprises approximately 500 amino acids. This enzyme is recognized for its high catalytic efficiency. It facilitates the breakdown of H_2O_2 into water and oxygen, while also interacting with hydrogen donors such as methanol, ethanol, and phenol, utilizing one mole of peroxide in the process. The active site of the enzyme contains either manganese or iron ions.

There are four types of CATs, categorized based on the characteristics of their active sites:

- The first type encompasses the typical or monofunctional catalases, which consist of four identical subunits. Each subunit contains either heme b or heme d, both of which are prosthetic groups that incorporate iron within their active sites. The molecular weight of each subunit ranges from 55 to 84 kDa. This category of CATs is the most prevalent in the natural world and demonstrates catalytic activity across a broad spectrum of pH levels. The heme group situated in the active site is positioned deep within the tetrameric structure. Monofunctional catalase enzymes operate through a two-step mechanism to facilitate the breakdown of H_2O_2. Initially, a single molecule of H_2O_2 is oxidized by the enzyme, resulting in the formation of ferryl porphyrin, referred to as compound I [Reaction 4]. Subsequently, in the second phase, compound

I further oxidizes another molecule of H_2O_2, yielding molecular oxygen and water as products [Reaction 5].

$$\text{Enzyme } (Fe^{3+}\text{Por}) + H_2O_2 \longrightarrow \underset{\text{(ferryl porphyrin)}}{\text{Compound I } (Fe^{4+} - \text{Por}^{+\bullet} = O)} + H_2O \quad \text{[Reaction 4]}$$

$$\text{Compound I } (Fe^{4+} - \text{Por}^{+\bullet} = O) + H_2O_2 \longrightarrow \text{Enzyme } (Fe^{3+}\text{Por}) + H_2O + O_2 \quad \text{[Reaction 5]}$$

- The second category of enzymes, known as catalase-peroxidases, demonstrates a bifunctional nature, possessing both catalase and peroxidase activities. However, their activity levels are significantly lower, by two to three orders of magnitude, compared to conventional catalases. These enzymes typically exist as dimers or tetramers, with each subunit having an approximate molecular mass of 80 kDa, and they contain heme, which serves as a prosthetic group with iron. Catalase-peroxidases have been identified in a variety of organisms, including bacteria, archaea, fungi, and even more complex eukaryotes. The catalytic mechanism they employ mirrors that of conventional CATs, comprising the same two distinct stages.
- The third category encompasses manganese-containing CATs. In contrast to the previous two categories, these catalases lack heme as a prosthetic group and instead utilize manganese ions at their active sites. Mn-CATs are structured as hexamers, with each subunit exhibiting a molecular mass ranging from 28 to 35 kDa, and they contain two manganese ions within their active center. They operate through a two-step mechanism that is similar to that of heme-containing enzymes [Reactions 6 and 7]. During the first phase, H_2O_2 acts as the oxidizing agent, while in the subsequent phase, it serves as the reducing agent.

$$H_2O_2 + Mn^{2+} - Mn^{2+}(2H^+) \longrightarrow Mn^{3+} - Mn^{2+}I + 2H_2O \quad \text{[Reaction 6]}$$

$$H_2O_2 + Mn^{3+} - Mn^{3+} \longrightarrow Mn^{2+} - Mn^{2+}(2H^+) + O_2 \quad \text{[Reaction 7]}$$

Glutathione peroxidase (GPx) is an enzyme that contains selenium and is classified under the peroxidase category (EC 1.11.1.19). This enzyme utilizes glutathione (GSH) to facilitate the conversion of hydrogen peroxide (H_2O_2) into water and to reduce organic peroxides to their corresponding alcohols in mammalian systems. The GPX family comprises eight distinct isoforms (GPX1 to GPX8), among which GPX1, GPX2, GPX3, GPX4, and GPX6 are recognized as selenoproteins with significant antioxidant properties. Notably, GPX6 is uniquely expressed in humans. Despite the importance of these enzymes, comprehensive investigations into the functions of the entire GPX family in the context of oxidative stress responses remain insufficient. To date, only a limited number of studies have systematically explored the involvement of GPX in oxidative stress [142].

Reductase enzymes, including glutathione reductase (GTR) and thioredoxin reductase (TRR), are integral components of the secondary antioxidant defense system. These enzymes facilitate the continuous production of NADPH, which is essential for counteracting reactive oxygen species (ROS) [143]. The presence of NADPH is crucial for mitigating the harmful effects of toxic substances that may enter aerobic cells. Additionally, NADPH can also be generated through the pentose phosphate pathway, aided by the enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase [144,145].

5.2.2. Non-Enzymatic Antioxidants

Natural compounds without enzymatic characteristic include vitamins, flavonoids, carotenoids, glutathione, plant polyphenols, melatonin, bilirubin, curcumin, and polyamines. Some of them are water-soluble, and in most cases, localized in the cytosol or cytoplasmic matrix, while others are fat-soluble and are present in cell membranes. Vitamins

are complex organic compounds essential for the body in minimal quantities. Among these, antioxidant vitamins such as A, E, and C play a crucial role in neutralizing reactive oxygen species and exhibit a diverse array of biological functions. They contribute to cellular protection against genomic alterations by mitigating DNA damage caused by oxidative stress.

Vitamin A, also known as retinol, is a fat-soluble vitamin classified within the retinoid family. This family comprises 20 distinct compounds characterized by a methyl-substituted cyclohexenyl and a tetraene side chain, which contains four double bonds. Beta-carotene serves as the precursor to vitamin A, undergoing hydrolysis to form retinol in the gastrointestinal system. The mechanism through which vitamin A exerts its antioxidant effects involves the neutralization of peroxyl radicals (LOO•). This is achieved by the addition of these radicals to the β-ionone ring of retinol, resulting in the formation of a highly conjugated retinol-carbon radical. This intermediate compound is relatively stable and, under typical physiological conditions, does not possess sufficient reactivity to promote further lipid peroxidation [146].

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin that was first isolated in 1928. It serves as an essential trace element necessary for the proper metabolism of aerobic cells [146]. Within the cytosol, vitamin C engages directly with reactive species such as $^{\bullet}O_2^-$ and HO•, thereby protecting cellular membranes against oxidative damage [125]. The antioxidant mechanism of vitamin C operates through its role as an electron donor, functioning as a reducing agent that can neutralize oxidized compounds. Upon interaction with a free radical, vitamin C donates an electron, resulting in the formation of the ascorbyl radical (AscR), which exhibits greater stability compared to other free radicals. The AscR can subsequently neutralize more reactive radicals, including OH•. In a further reaction, AscR can donate an additional electron to another reactive radical, leading to the production of dihydroascorbic acid. Notably, a single molecule of ascorbic acid has the capacity to neutralize two free radicals.

Vitamin E is a group of compounds that includes four tocopherols and four tocotrienols, with alpha- and gamma-tocopherols being the most abundant in human tissues. The antioxidant mechanism of vitamin E operates through its interaction with free radicals, resulting in the formation of a tocopheroxyl radical along with a non-radical by-product [146]. In the cellular context, vitamin E serves several critical functions: it quenches peroxyl radicals produced during lipid peroxidation, protects polyunsaturated fatty acids in cell membranes, assists in the neutralization of singlet oxygen, and participates in reactions with peroxynitrites. It is recognized as the most potent antioxidant in the lipid environment, and its efficacy is greatly enhanced when combined with other antioxidants such as vitamin C, selenium, and beta-carotene [147].

Carotenoids represent a diverse class of red-orange pigments found in plants. These pigments are characterized as fat-soluble chromophores that utilize a system of conjugated double bonds to absorb light within the wavelength range of 400 to 500 nm [148]. More than 600 different carotenoids have been identified, with six being particularly prominent: alpha-carotene, beta-carotene, lycopene, lutein, beta-cryptoxanthin, and zeaxanthin. The antioxidant properties of carotenoids are attributed to their polyene structure, which enables them to interact effectively with reactive oxygen species, including singlet oxygen [149]. Alterations to this polyene structure, such as modifications in the number of conjugated double bonds or the introduction of oxygen-containing functional groups, can significantly influence the reactivity of carotenoids.

Polyphenols represent a significant category of secondary metabolites derived from plants, encompassing approximately 10,000 distinct structures. The increasing interest in polyphenols is attributed to their potential in mitigating oxidative stress and asso-

ciated diseases. This group is classified into two primary categories: flavonoids and non-flavonoids [150]. Non-flavonoid polyphenols consist of phenolic acids, such as hydroxybenzoic and hydroxycinnamic acids, as well as stilbenes and lignans.

Flavonoid polyphenols function as exogenous antioxidants and are characterized by a C6-C3-C6 structural framework, which consists of two aromatic rings linked by a three-carbon bridge. These metabolites play a crucial role in various cellular functions, influencing pigmentation, aroma, flavor, growth, and reproductive processes. The principal dietary flavonoids are categorized into six groups based on the configuration of the C ring: anthocyanidins, flavan-3-ols, flavanols, flavones, isoflavones, and flavanones [143]. The process by which these antioxidants exert their effects involves the transfer of a hydrogen atom from a flavonoid to a free radical, resulting in the formation of a phenoxy radical. This phenoxy radical is characterized by its stability, which effectively halts the progression of the chain reaction. Subsequently, the phenoxy radical may engage with another free radical, leading to the production of a quinone. Alternatively, rather than donating a hydrogen atom, the flavonoid can also provide an electron to the free radical, resulting in the generation of a stable cation radical.

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced intracellularly by the pineal gland in mammals. Its antioxidant properties are realized through both direct and indirect mechanisms [151]. In the direct detoxification of reactive oxygen species, melatonin donates one or more electrons to free radicals (such as OH•), facilitating their transformation into stable, non-radical forms. This action is particularly effective in curtailing lipid peroxidation and neutralizing singlet oxygen. The indirect antioxidant effects involve the activation of key antioxidant enzymes (GPx, glutathione S-transferase, and CAT, as well as the stimulation of glutathione production.

Ubiquinones possess a 1,4-benzoquinone framework, with coenzyme Q10 being the most biologically active variant. This compound, resembling a vitamin, is fat-soluble and exhibits antioxidant properties while playing a crucial role in the electron transport chain involved in ATP synthesis. Its antioxidant mechanism is linked to the safeguarding of unsaturated fatty acids within cellular membranes from oxidative damage caused by peroxides, the neutralization of reactive carbonyl species, and its synergistic interactions with other antioxidants, such as vitamin E [152].

Glutathione is a tripeptide composed of three amino acids: cysteine, glutamine, and glycine. It functions primarily as an intracellular antioxidant, with approximately 90% of its presence in the reduced form and only 10% in the disulfide form. The antioxidant mechanism of glutathione involves its role as a substrate for various enzymes that facilitate the detoxification of reactive metabolites produced by the interaction of ROS with DNA, proteins, and other biomolecules. Additionally, certain enzymes utilize glutathione to neutralize potentially harmful substances, including estrogens and xenobiotics. Furthermore, glutathione serves as an endogenous antioxidant, effectively neutralizing free radicals. It also plays a crucial role in regenerating the oxidized forms of ascorbic acid, alpha-tocopherol, and ubiquinones.

6. Low Temperature-Induced Oxidative Stress in Antarctic Fungi

Among the mentioned harsh parameters of growth in Antarctica, the temperature plays an important role in the realization of oxidative stress in fungi [1,123]. The cold temperatures result in a decline in the metabolic activity of microorganisms, which leads to a reduction in the utilization of ATP and the accumulation of electrons at specific points within the respiratory chain (see Figure 1) [153]. The situation gives rise to a rapid increase in the production of ROS [Figure 8].

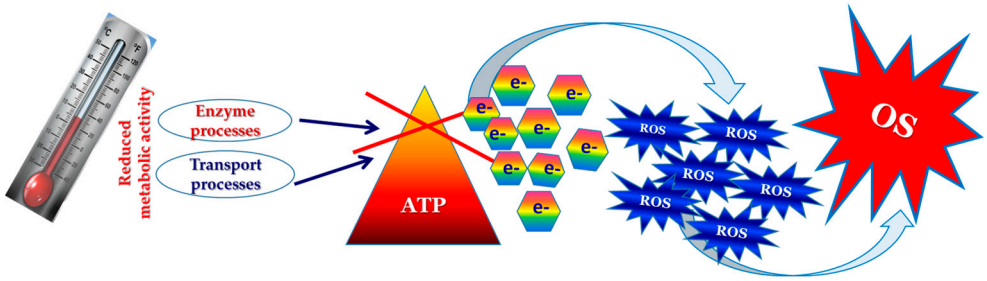


Figure 8. Molecular mechanism of cold stress realization.

There are data that support the modification of metabolic pathways to ensure some species survive under cold stress conditions. The findings suggest the presence of a multifaceted interaction between physiological and biochemical mechanisms that contribute to the adaptation of these species to low-temperature environments [154]. Reduced metabolic activity in response to cold stress has been observed in the fungal strains *P. chrysogenum* [155], *Mrakia psychrophila* [156], and *Mortierella isabellina* [157]. Tsuji [50] demonstrated significant metabolite changes in the carbon-metabolite pathway (glycolysis, pentose-phosphate pathway, and TCA cycle) in the Antarctic basidiomycetous yeast *Mrakia blollopis* grown at 10 and 3 °C. Proteomic analysis of fungal strains belonging to the genus *Pseudogymnoascus* revealed a positive correlation between protein levels and transcription levels in certain pathways when subjected to low-temperature stress, indicating an up-regulation of chaperones and pathways associated with energy metabolism [11,154]. Moreover, the expression of cold-responsive genes, such as the FRO1 gene, as well as the accumulation of ROS, can be influenced by the disruption of mitochondrial function in plants [158]. Suzuki and Mittler [159] propose a model that elucidates the relationship between temperature stress and ROS production. This model is primarily based on the disruption of cellular homeostasis and the uncoupling of metabolic processes.

The scientific literature contains only a very small number of studies that make a connection between low temperatures and oxidative stress in fungi (Table 2). As shown by Zhang et al. [160], a rapid reduction in growth temperature from 30 to 10 °C increased H_2O_2 levels in *S. cerevisiae*, coinciding with increased transcripts of genes responsible for antioxidant defense. Similar results have been reported for the *S. cerevisiae* BY4743 strain growth at low temperatures (10–15 °C) [161]. The authors suggested that the suboptimal temperature caused an enhanced level of ROS and induction of the antioxidant response. Temperature changes induced ROS generation in plant pathogen *A. flavus* [162]. A transient temperature downshift from 30 °C to 10 °C or 4 °C resulted in a significant increase in $^{\bullet}O_2^-$ and H_2O_2 levels within both the intact cells and mitochondria of the Antarctic strains *Penicillium* sp. 161 and *A. glaucus* 363 [163]. The reduction in temperature to 4 °C resulted in a more pronounced elevation of ROS levels compared to the conditions at 10 °C. Furthermore, the mesophilic strain *A. glaucus* 363 exhibited a more vigorous ROS production process in contrast to the psychrotolerant *Penicillium* sp. 161. Similar results have been reported for the Antarctic strains *P. griseofulvum* P29 and *P. chrysogenum* P27 [164], as well as for *P. waksmanii* m12 and *P. olsonii* p14 [165]. An association between cold stress and ROS generation has been reported on two strains of the cold-adapted oleaginous yeast *Rhodosporidium kratochvilovae* [166]. In the psychrophilic fungus *Pseudogymnoascus pannorum*, increased levels of unsaturated fatty acids allow membrane function to be maintained under high ROS levels at low temperatures [76].

Table 2. Biomarkers of Cold-Induced Oxidative Stress in Antarctic Fungi.

Strain	Sources	Response	References
P. griseofulvum P29	Terra Nova Bay	ROS generation	[164]
P. chrysogenum P27	Terra Nova Bay	ROS generation	[164]
A. glaucus	*Livingston island*	ROS generation	[163]
Penicillium sp. 161	Livingston island	ROS generation	[163]
Rhodosporidium kratochvilovae	Cold-adapted yeast	ROS generation	[166]
P. waksmanii m12 *P. olsonii* p14	Terra Nova Bay Casey Station	ROS generation	[165]
A. fumigatus I-9	Livingston island	Accumulation in trehalose and glycogen	[167]
Geomyces pannorum	Signy Island	Changes in lipid composition	[168]
Metschnikowia bicuspidata	King George Island	Changes in lipid profile	[169]
Mrakia blollopis	King George Island	Changes in lipid composition	[169]
P. olsonii p14	Casey Station	Increase in carbonylated proteins	[52,87,170]
P. waksmanii m12	Terra Nova Bay	Increase in carbonylated proteins	[52,87,170]
A. glaucus	Livingston island	Increase in carbonylated proteins	[163]
Penicillium sp. 161	Livingston island	Increase in carbonylated proteins	[163]
A. fumigatus I-9	Livingston island	Increase in carbonylated proteins, lipid peroxidation	[167]
P. griseofulvum	Terra Nova Bay	Increase in carbonylated proteins, lipid peroxidation	[40,171]
Cryomyces antarcticus	Not specified Antarctic site	Changes in DNA	[172]
Wickerhamomyces anomalus	King George Island	Changes in lipid profile	[169]
Goffeauzyma gastrica	King George Island	Changes in lipid profile	[169]

The oxidative burst of ROS formed under cold exposure causes significant injury in aerobic fungal cells. However, only a few studies have reported on the damage caused by cold stress to the main biological molecules of fungi, especially those found in Antarctica. It is well-known that cold stress leads to the process of lipid peroxidation in fungi and thus alters the fluidity of lipid-containing membranes and disrupts membrane-bound processes [3]. Schade et al. [173] reported the accumulation of the reserve carbohydrates, i.e., glycogen and trehalose. The experimental findings indicate that low temperatures result in an increase in unsaturated lipid content [168]. Specifically, a psychrotrophic isolate of *Geomyces pannorum* cultivated at 5 °C exhibited a modified lipid profile in comparison to the same isolate grown at 15 °C. Unsaturated fatty acids are very sensitive to oxidative degradation [174]. For example, the grey mold fungus *Botrytis cinerea* demonstrated oxidative lipid damage under low-temperature growth [175]. A similar response to suboptimal growth temperatures has been documented for the strains *Humicola marvinii*, *Geomyces pannorum*, and *Mortierella elongata*, isolated from Signy Island, Antarctica [168]. Short-term temperature downshift from 25 to 10 °C caused a significant increase in the malondialdehyde (MDA) level (a marker for lipid peroxidation degree) in Antarctic strain *P. griseofulvum* P29 [171,176].

Modification in lipid composition has been reported as a component of the survival strategy employed by cold-adapted fungi. In psychrophilic yeasts, such as species from the

genera *Candida*, *Leucosporidium*, and *Torulopsis*, the fatty acids present exhibit a lower degree of saturation compared to those found in mesophilic yeasts. Furthermore, a reduction in incubation temperature serves to further enhance this unsaturation [177,178]. At lower temperatures, there is an increased production of long-chain polyunsaturated fatty acids (LCPUFAs), including eicosapentaenoic, arachidonic, and docosahexaenoic acids [179]. These compounds form hydrophobic boundaries between lipid bilayers, thereby preventing ROS damage and the intake of H_2O_2 into cells. Consequently, LCPUFAs have been identified as playing a crucial role in the protection of cells from oxidative stress under these lower temperature conditions. Moreover, the shortening of fatty acid chain lengths has been detected as a response to cold stress [180].

Changes in lipids are significantly associated with the stability of membrane structures in fungi and their integrity under low-temperature conditions [154]. The authors found a decreased phospholipid metabolism in the Antarctic isolate *Pseudogymnoascus* spp. (sp3) in response to cold stress. The Antarctic strain of *Geomyces pannorum* exhibited enhanced membrane fluidity due to alterations in the phospholipid composition of its membrane lipids when exposed to low temperatures [181]. Sterols serve an essential structural and regulatory function within eukaryotic cell membranes, affecting their fluidity. They intercalate within the fatty acid chains, generally contributing to the stabilization and fortification of the membrane lipid bilayer [180]. Using transcriptomic analysis, Su et al. [182] demonstrated an up-regulation of genes associated with the biosynthesis of unsaturated fatty acids in *Mrakia psychrophile* grown at 4 °C. This suggests that the cold adaptation mechanism of the obligatory psychrophilic fungus involves the synthesis of unsaturated fatty acids to preserve membrane fluidity. Data on the fungus *Thermomyces dupontii* revealed a lipid-mediated fungal response to cold stress [183].

In the context of low-temperature stress, cold-adapted fungi demonstrated significant damage in the tertiary structures of molecules such as enzymes and other functional proteins, which compromise their functionality and may lead to degradation. Similar data on the cold stress response of fungi isolated from Antarctica are described in previous publications of our group. Research demonstrated that protein carbonyls serve as reliable biomarkers for evaluating protein oxidation, which is a consequence of temperature stress. The long-term growth of Antarctic strains *P. olsonii* p14 and *P. waksmanii* m12, along with the temperate strain *Penicillium* sp. t35, under suboptimal temperature conditions, resulted in a significant increase in protein carbonyl concentrations [87]. A six-hour temperature downshift applied to exponentially growing cultures of these strains led to an observable increase in the number of carbonyl groups in cell proteins throughout the entire period. The mesophilic strains (*P. waksmanii* m12 and *Penicillium* sp. t35) exhibited a more substantial increase in the content of damaged proteins compared to the psychrotolerant strain (*P. olsonii* p14) [170]. Thus, the cold-adapted Antarctic psychrotolerant strain showed enhanced tolerance to cold stress in contrast to the mesophilic strains. The stationary-phase cultures also demonstrated a remarkable increase in carbonylated protein content, but to a greater extent [52]. It could be assumed that the exponential-phase cells exhibit higher resistance to cold stress.

A remarkable increase in carbonylated protein content has also been evaluated in two Antarctic strains, particularly the psychrotolerant *Penicillium* sp. 161 and mesophilic *A. glaucus* 363, when subjected to temperatures of 4 °C or 10 °C [163]. The same typical response to oxidative stress regarding protein damage by low-temperature exposure has been reported for *P. griseofulvum* [171,176].

The modification of DNA is also significant in understanding the interplay between low temperatures and oxidative stress. Cells may either accumulate DNA molecules that have been damaged by reactive oxygen species (ROS) or successfully repair them to their

original state [184]. The psychrophilic strain *Cryomyces antarcticus* exhibited an increase in the GC content of its genes, as well as in the bases located at the third positions of codons, when subjected to extremely low temperatures during growth [172].

7. Cold Stress Response of Antarctic Fungi and Adaptation

We have summarized a compendium of research reports on the antioxidant cell response data for fungi with a geographical origin of Antarctica (Table 3).

Table 3. Antioxidant cell response of Antarctic fungi.

Strain	Sources	Response	References
Morphological adaptation			
Cryomyces antarcticus	McMurdo Dry Valleys, Southern Victoria Land,	Meristematic growth	[75]
Cryomyces minteri	Alatna Valley, Victoria Land	Meristematic growth	[75]
Friedmanniomyces endolithicus	Victoria Land	Meristematic growth, Melanization	[185]
Friedmanniomyces endolithicus	Northern Victoria Land	Meristematic growth	[75]
Penicillium sp. 1-6-4	Livingston island	Intercalary growth chlamydospores	[47]
Friedmanniomyces endolithicus	Northern Victoria Land	Morphological changes	[155]
Mrakia blollopis *Mrakiella niccombsii*	Vestfold Hills area of Davis Base, Antarctica	Morphological changes of cells, hyphae, colonies	[186]
Penicillium commune 161	Livingston Island	Ultrastructural changes	[187]
Penicillium griseofulvum	Terra Nova Bay	Ultrastructural changes	[171]
Cryomyces antarcticus	McMurdo Dry Valleys	Ultrastructural changes	[188]
Physiological Changes			
Penicilliumgriseofulvum	Terra Nova Bay	Decrease in biomass content	[171]
Penicillium olsonii p14 *Penicillium waksmanii* m12	Casey Station Terra Nova Bay	Decrease in biomass content	[38,52,87,170]
Pichia pastoris	Cold-adapted	Decrease in biomass content	[189]
Pseudogymnoascus roseus	Mars Oasis, Alexander Island	Decrease in biomass content	[190]
Volvariella volvacea	Cold-adapted	Decrease in biomass content	[191]
Mortierella elongata	Signy Island	Trehalose accumulation	[168]
Volvariella volvacea	Soil samples	Trehalose accumulation	[192]
Penicillium olsonii p14	Casey Station	Trehalose and glycogen accumulation	[52,87,170]
Aspergillus fumigatus I-9	Livingston Island	Trehalose and glycogen accumulation	[167]
Aspergillus glaucus	Livingston Island	Trehalose and glycogen accumulation	[163]

Table 3. *Cont.*

Strain	Sources	Response	References
Physiological Changes			
Humicola marvinii	Signy Island	Trehalose and glycogen accumulation	[168]
Penicillium griseofulvum	Terra Nova Bay	Trehalose and glycogen accumulation	[40,171]
Penicillium sp. 161	Livingston Island	Trehalose and glycogen accumulation	[163]
Penicillium waksmanii m12	Terra Nova Bay	Trehalose and glycogen accumulation	[52,87,170]
Metabolic Adaptation			
Aspergillus fumigatus I-9	Livingston Island	Modification in enzyme profiles	[167]
Friedmanniomyces endolithicus	Northern Victoria Land	Modification in protein profiles	[155]
Pseudogymnoascus sp. (sp. 3)	Not specified Antarctic site	Modification in enzyme profiles	[154]
Penicillium waksmanii m12	Terra Nova Bay Casey Station	Re-routing of glycolysis into the PPP	[165]
Penicillium olsonii p14	Terra Nova Bay Casey Station	Re-routing of glycolysis into the PPP	[165]
Penicillium waksmanii m12	Terra Nova Bay Casey Station	Metabolite changes	[193]
Penicillium olsonii p14	Terra Nova Bay Casey Station	Metabolite changes	[193]
Aspergillus glaucus	Livingston Island	Metabolite changes	[163]
Penicillium sp. 161	Livingston Island	Metabolite changes	[163]
Pseudogymnoascus pannorum	Not specified Antarctic site	Metabolite changes	[76]
Pseudogymnoascus sp. (strain SF-7351)	King George Island	Metabolite changes	[194]
Pseudogymnoascus spp. strain	South Shetland Islands	Metabolite changes	[55]
Mrakia psychrophila	Syowa station	Reduced metabolic activity	[50]
Mrakia blollopis	Syowa station	Changes in carbon metabolism	[50]
Penicillium olsonii p14	Casey Station Terra Nova Bay	Changes in carbon metabolism	[165]
Penicillium waksmanii m12	Casey Station Terra Nova Bay	Changes in carbon metabolism	[165]
Pseudogymnoascus sp. (sp.3)	Not specified Antarctic site	Decrease in phospholipid metabolism	[154]
Activation of antioxidant enzyme defence			
Aspergillus glaucus	Livingston Island	SOD, CAT	[43,88,163]
Penicilliumcommune	Livingston Island	SOD, CAT	[43,88,187]
Cladosporium cladosporioides	Livingston Island	SOD, CAT	[43]
Cladosporium herbarum	Livingston Island	SOD, CAT	[43]
Cladosporium oxysporum	Livingston Island	SOD, CAT	[43]

Table 3. *Cont.*

Strain	Sources	Response	References
Activation of antioxidant enzyme defence			
Epicoccum nigrum	Livingston Island	SOD, CAT	[43]
Monodictys austrina	Livingston Island	SOD, CAT	[43]
Penicillium aurantiogriseum	Livingston Island	SOD, CAT	[43]
Penicilliumdierckxii	Livingston Island	SOD, CAT	[43]
Penicillium italicum	Livingston Island	SOD, CAT	[43]
Penicillium olsonii	Livingston Island	SOD, CAT	[43]
Penicillium waksmanii	Livingston Island	SOD, CAT	[43]
Pseudogymnoascus pannorum	Livingston Island	SOD, CAT	[43]
Rhizopus sp.	Livingston Island	SOD, CAT	[43]
Penicillium rubens III11-2	Livingstone Island	CAT	[53]
Penicillium olsonii p14	Terra Nova Bay	SOD, CAT	[52]
Penicillium waksmanii m12,	Terra Nova Bay	SOD, CAT	[52]
Aspergillus fumigatus I-9		CAT	[167]
Penicillium cyclopium	Spitsbergen	CAT	[195]
Rhodotorula sp. USM-PSY62	Casey Station	CAT	[196]
Volvariella volvacea	Cold-adapted	GPx	[197]
Penicillium sp.	Not specified Antarctic site	Mn-Peroxidase	[198]
Molecules with antioxidant and scavenging properties			
Aspergillus versicolor	Deep-sea sediments	Phenolic compounds	[199]
Penicillium citreonigrum SP-6	Antarctic Great Wall Station	Phenolic compounds	[200]
Cryomyces antarcticus	Victoria Land	Melanin	[201]
Knufia petricola A95	Cold adapted	Carotenoids	[202]
Rhodosporidium kratochvilovae	Cold adapted	Carotenoids	[166]
Penicillium citreonigrum SP-6	Antarctic Great Wall Station	Diketopiperazine	[200]
Geomyces pannorum	Terra Nova Bay	Phenolic compounds	[181]
Goffeauzyma gastrica	King George Island	Antifreeze proteins	[169]
Goffeauzyma gastrica	King George Island	Ergosterol	[169]
Leucosporidium creatinivorum	King George Island	Ergosterol	[169]
Vishniacozyma victoriae	King George Island	Ergosterol	[169]
Exophiala xenobiotica	Galindez Island	Pigments	[203]
Rhodotorula mucilaginosa	King George Island	Pigments	[203]

7.1. Morphological Modification Caused by Cold Stress

The morphological adaptations of cold-adapted fungi are well-suited for surviving in challenging environmental conditions. The phenomenon of meristematic growth, which

involves a shift toward isodiametric expansion, serves as a specific adaptation to stress, establishing a stable characteristic among fungi that thrive in extreme environments. These fungi demonstrate a high degree of adaptability to significant environmental stressors, particularly low temperatures, through mechanisms such as melanization and the promotion of meristematic development [185]. Such growth helps maintain an optimal volume-to-surface ratio, effectively limiting their interaction with external stressors [76,204]. Meristematic development of hyphae has been reported for black yeast-like fungus *Exophiala dermatitidis* [204,205], *Friedmanniomyces endolithicus* [185,206], rock-inhibiting Antarctic fungi [207], etc. Fourteen fungal species isolated from Livingston Island, Antarctica, demonstrated morphological peculiarities growing at low temperatures [47]. The prevalence of melanized species indicates the advantageous function of melanin in their survival in extreme conditions. It has been observed that certain colonies exhibit multicellular meristematic clusters, in addition to formations characterized by concentric rings that display variations in density and thickness. Furthermore, some colonies present rings composed of sterile mycelium. In certain instances, the colony surface is marked by irregularities, featuring convoluted wrinkles and pronounced radial furrows. The mycelium of *Penicillium* sp. 1-6-4 isolated at a temperature of 4 °C exhibited a significant presence of intercalary, swollen, and thick-walled cells that develop into chlamydospores, known as long-term survival structures. The significance of chlamydospores for survival at low temperatures has been evidenced in the case of the plant pathogen *Zymoseptoria tritici* [208]. In the microbial communities of soils found in the Antarctic, Arctic, and Alpine regions, fungi exhibiting dark septate hyphae are the most prevalent [6].

Considerable ultrastructural changes have been observed in the cells of *P. griseofulvum* following a short-term temperature downshift to 6 °C [188]. Analysis via transmission electron microscopy (TEM) indicated an increase in vacuolar volume, focal damage to the cell wall, and structural changes in the mitochondria. Moreover, ultrastructural findings revealed evidence of plasma membrane turnover, characterized by frequent deep infoldings of the plasmalemma that penetrate the cytoplasm, extending toward the center of the hyphae. The study of two Antarctic fungal strains under extreme cold conditions revealed that the mesophilic *A. glaucus* 363 exhibited more substantial ultrastructural changes in both its cells and conidia compared to the psychrotolerant *P. commune* 161 [187]. Scanning electron microscopy (SEM) results suggest that the psychrotolerant strain has a significantly enhanced ability to adapt and thrive in such harsh environments. This assertion is further substantiated by the findings of Sato et al. [209], who observed ultrastructural changes in the mitochondrial matrix and rupture of nuclear membranes in a temperature-sensitive mutant of *Schizosaccharomyces pombe* following a temperature decrease from 20 °C to 4 °C. A related trend has been identified in various studies. Psychrophilic yeasts of the genera *Mrakia* and *Mrakiella*, sourced from glacial regions in Antarctica and the Alps, reveal marked differences in their ultrastructure compared to mesophilic yeasts [186]. Onofri et al. [35] also observed changes in the morphological characteristics of Antarctic fungi subsequent to several freeze–thaw cycles.

7.2. Physiological Modification Caused by Cold Stress

It is evident that to overcome any type of stress, including cold stress, changes in cell physiology are required, which subsequently result in positive selection [7]. Adaptation at the physiological level may involve individual strategies or a combination of them. One of the initial responses to a reduction in the growth temperature of microorganisms is a cessation of growth. This is followed by an acclimation phase, which is a period of latency preceding the restoration of cell growth [210]. During this phase, there is a transient

inhibition of the synthesis of the majority of cellular proteins, most likely at the level of translation.

A drastic effect of low temperatures on the growth of Antarctic fungal cultures was found in *Pseudogymnoascus roseus* [190], *Mrakia blollopis* SK-4 and TKG1-2 [50], and *P. griseofulvum* [171]. In addition, a decrease in biomass content has been documented in fungal strains isolated from soil samples in three areas of Antarctica: Casey Station, Terra Nova Bay, and South Georgia, due to both long-term and short-term exposure to low temperatures [38,87,170]. A comparative analysis of the cell response of Antarctic fungi to a reduction in temperature, focusing on two thermal classifications, psychrotolerant and mesophilic, indicated a complete cessation of growth or a marked reduction in biomass [163]. This effect was notably more significant in the mesophilic fungus than in the psychrophilic ones. A similar trend has been observed in the Antarctic strains *P. olsonii* p14 and *P. waksmanii* m12, as well as in the temperate strain *P. rugulosum* t35, when exposed to the temperature downshift during the stationary growth phase [52]. The reduction in biomass, known as cold-shock-induced autolysis, is linked to the activation of genes that encode peptidoglycan hydrolases, which facilitate the process of autolysis [210]. Similar findings have been reported for various microorganisms exposed to cold stress, such as *Pichia pastoris* [189], *Volvariella volvacea* [191], etc. The phenomenon of low-temperature autolysis coincided with reduced glucose consumption [37,52,163,171].

The findings presented by Chang et al. [211] indicated that the low-temperature autolysis in *V. volvacea* resulted from both intracellular and extracellular interactions. The authors proposed that the variations in metabolites and genes associated with malate metabolism played a role in supplying electrons to the electron transport chain. However, prolonged exposure to low temperatures led to an inadequate intracellular energy supply, ultimately resulting in cold-induced damage.

The accumulation of trehalose and glycogen in microbial cells under adverse growth conditions can serve as indicators of stress [11,212–214]. Trehalose is a vital storage compound found in both the vegetative cells and spores of fungi, and it is recognized as the most commonly occurring disaccharide among fungal species [6]. Its role as a universal stress protectant in the cytosol is significant, particularly in its ability to stabilize membranes during episodes of dehydration [214]. The stabilizing properties of trehalose can be attributed to the formation of a strong hydrogen bonding network that links biomolecules to the sugar–water matrix [215]. This connection inhibits the unfolding of biomolecules, as it involves a reconfiguration of the surrounding medium.

Glycogen serves as the common carbohydrate storage and energy reservoir in fungi, especially during periods of stress. This polysaccharide consists of glucose units connected by α, 1–4 glycosidic bonds, with additional α, 1–6 branching points. The metabolism of glycogen is governed by a complex network of interactions involving numerous genes and biochemical pathways. Key to this metabolic process are five critical enzymes: ADP-glucose pyrophosphorylase (GlgC), glycogen synthase (GlgA), glycogen branching enzyme (GlgB), glycogen phosphorylase (GlgP), and glycogen debranching enzyme (GlgX) [216,217]. Studies have shown that the activation of genes responsible for glycogen synthesis and degradation can occur simultaneously, which may lead to no net increase in glycogen within cells exposed to diverse forms of stress, such as heat shock [213,216]. Although this transcription pattern is also observed when subjected to low temperatures, the results demonstrate a substantial accumulation of glycogen in yeast and fungal cells.

Cold stress led to the accumulation of trehalose and glycogen and triggered the pathways involved in their biosynthesis [11]. The evidence for the increased content of these carbohydrates in response to low-temperature treatment of many fungal species was observed [218]. As an illustration, the Antarctic fungi *P. commune* 161 and *A. glaucus*

363 [88,163], as well as *P. griseofulvum* [171], demonstrated enhanced glycogen and trehalose content when exposed to both short-term and long-term cold stress. In addition, the Antarctic fungal strains *P. olsonii* p14 and *P. waksmanii* m12 demonstrated a significant increase in intracellular trehalose and glycogen levels after being subjected to low temperatures. It is noteworthy that exponentially growing cells experienced a more pronounced enhancement in reserve carbohydrate levels than their counterparts in the stationary growth phase [52]. In contrast, the late cold response in *S. cerevisiae* triggered the accumulation of the carbohydrate reserves trehalose and glycogen [173].

Furthermore, the intracellular content of trehalose was significantly greater in the temperature-tolerant strain *V. volvacea* (VH3) relative to the temperature-sensitive strain *V. volvacea* (V23) when both were grown at optimal temperatures [192]. During exposure to low-temperature stress, the expression levels of the trehalose-6-phosphate phosphatase (*TPP*) gene and the trehalose phosphorylase (*TP*) gene in VH3 were found to be significantly higher than those in V23. The authors indicated that the *TP* gene might be a pivotal component of trehalose metabolism, potentially promoting trehalose synthesis in response to low-temperature stress.

7.3. Metabolic Adaptation Response

In recent years, there has been a significant rise in the volume of research focused on the effect of low temperature on protein modifications through the application of proteomic methodologies [11]. Research conducted by Tesei et al. [155] on the proteomic profiles of fungi that inhabit black rocks under conditions of low-temperature stress demonstrated an increase in the total number of protein spots. The findings revealed significant changes in high molecular mass protein spots, particularly those ranging from 70 to 170 kDa, in the species *Friedmanniomyces endolithicus* and *Coniosporium perforans*. Conversely, the analysis of *Exophiala jeanselmei* exhibited alterations in protein patterns at a lower molecular mass range of 25 to 100 kDa. Exposure of fungus *E. dermatitidis* to low-temperature stress resulted in a decline in proteins associated with metabolic activity, particularly those involved in general carbon metabolism. A comprehensive array of proteins engaged in energy metabolism pathways underwent down-regulation, encompassing malate synthase, malate dehydrogenase, acetyl-coenzyme A synthetase, and glyceraldehyde-3-phosphate dehydrogenase [219]. The proteomic study on the low-temperature stress response indicated a positive correlation between protein levels and transcription levels in certain pathways due to an increase in chaperones and energy metabolism pathways. As an illustration, a comparable modification in protein profiles was observed in response to both short-term and long-term low-temperature exposure to *Flammulina velutipes* [156]. Furthermore, Abu Bakar et al. [154] demonstrated a substantial modification in the levels of various metabolic enzymes and ribosomal proteins in Antarctic isolates of *Pseudogymnoascus* spp. Through pathway enrichment analysis, the authors further demonstrate diversity in the pathways associated with cold stress responses, particularly those related to metabolism and translation processes. Of interest are the studies conducted by Chen et al. [183], which reported for the first time the involvement of tryptophan-based metabolic reprogramming in the response of *T. dupontii* to cold stress. The response mechanisms of the psychrophilic strain *Mrakia psychrophile* [182], *Flammulina velutipes* [156], and *Mortierella isabellina* [157] to low-temperature stress encompass several physiological changes. These include a desaturation of fatty acids, an accumulation of glycerol, an increase in the expression of glutamine synthetase and MFS transport proteins, and a decrease in the expression levels of citrate synthase, succinyl Co-A ligase, pyruvate decarboxylase, and ribosomal proteins.

The impact of a significant reduction in growth temperature on carbon metabolism has been investigated in two Antarctic strains, one psychrotolerant and one mesophilic, along-

side a temperate mesophilic strain [165]. Findings indicated a pronounced elevation in the activity of hexokinase (HK), the enzyme that initiates glycolysis. A similar increase was noted for glucose-6-phosphate dehydrogenase (GPDH), which catalyzes the initial reaction in the pentose phosphate pathway (PPP), as well as glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which plays a crucial role in energy production. A key conclusion drawn from this study is that low-temperature stress prompts a shift in carbohydrate metabolism from glycolysis toward the PPP, contributing to the survival mechanisms at suboptimal temperatures. Additionally, it is noteworthy that the enhancement in the activity of these three enzymes has been markedly more pronounced in cells during the exponential growth phase compared to those in the stationary phase.

It should be noted that the short-term cold stress (4 °C) applied to Antarctic fungi *Penicillium* sp. 161 and *A. glaucus* 363 resulted in the re-routing of carbon metabolism away from glycolysis into the PPP, serving as a stress-resistance mechanism [220]. Additionally, the stress response encompasses changes in the enzymatic activity of the TCA cycle, which acts as a major supplier of precursors, thereby facilitating adaptation to cold stress [165,220].

The ability of fungi to withstand cold temperatures is accelerated by the production of various extracellular hydrolases. Notably, chitinolytic enzymes play a crucial role in the development of the cell wall [48]. Additionally, lipases contribute to the metabolism of fatty acids [221], while proteases facilitate the breakdown of damaged proteins [193]. The response to low-temperature stress in two Antarctic strains is characterized by a multifaceted activation of both biosynthetic and trehalose-degrading pathways, albeit to varying degrees [187]. Notably, the increase in trehalose-6-phosphate synthetase (TPS) during stress is significantly greater than that of neutral trehalase (NT). This disparity results in a substantial accumulation of trehalose during the stress period. Following the alleviation of stress, there is a marked decline in TPS activity, while NT levels remain stable. Consequently, the intracellular concentration of trehalose is determined by a meticulously regulated equilibrium between the expression of synthetic and degradative enzymes [222].

7.4. Cold-Active Enzymes

The survival of fungal strains in conditions of extremely low temperatures is also promoted by so-called cold-active enzymes, which favor their metabolic processes, substrate uptake, development, and reproduction [6]. These enzymes are known to increase the affinity for the substrates by decreasing the activation energy required for the enzyme–substrate complex formation. This is facilitated by a greater flexibility in the enzyme's structure, either in part or as a whole, which enhances the rate of enzymatic reactions at lower temperatures, thereby contributing to reduced energy usage [194].

The International Commission of Enzyme Nomenclature classified the cold-active enzymes that have been discovered thus far into six principal categories: hydrolases, oxidoreductases, transferases, lyases, isomerases, and synthetases. Duarte et al. [9] reviewed the ability of fungi and yeasts from terrestrial and marine Antarctic settings to synthesize such enzymes as part of their survival strategy. The authors presented a wide variety of fungal genera belonging to the phyla *Ascomycota* and *Basidiomycota,* producing enzymes with a low temperature optimum. The analysis encompassed a variety of hydrolases, such as α-amylase, cellulase, chitinase, glucosidase, invertase, lipase, pectinase, phytase, protease, subtilase, tannase, and xylanase, in addition to oxidoreductases, specifically laccase and superoxide dismutase.

Fungi from Antarctic soil and moss samples obtained from various sites in Victoria Land (continental Antarctica) were evaluated for their ability to produce cold-active enzymes, such as lipases, polygalacturonase, pectin lyase, amylase, cellulase, chitinase, phosphatase, glucose oxidase, urease, protease, and RNase [46]. Among these, the psy-

chrotolerant strain of *Lecanicillium muscarium* (CCFEE 5003) emerged as a notable candidate for the production of cold-tolerant extracellular enzymes, particularly those that hydrolyze chitin.

A total of 27 filamentous fungi were isolated from marine sponges obtained from King George Island, Antarctica, and subsequently evaluated as potential sources of cold-active pectinases [223]. Of these, eight demonstrated pectinolytic activity at a temperature of 15 °C. The most effective producer identified was *Geomyces* sp. F09-T3-2, which exhibited optimal enzymatic activity at a temperature that is 10 °C lower than that of commercially available mesophilic enzymes. Singh et al. [224] identified the psychrophilic fungal strain *Truncatella angustata* BPF5 as a promising candidate for the production of pectinase, which holds potential applications in the wine industry. The presence of cold-active enzymes, including SOD [225], catalase [51,53], sialidase [226], lipases [227], invertase [228], chitinolytic enzymes [48], etc., has been detected in fungal strains isolated from Antarctica. The application of metagenomic techniques facilitates the discovery of a greater number of cold-active enzymes [229].

8. Antioxidant Defence in Antarctic Fungi

8.1. Enzymatic Antioxidant Defense

Accelerated ROS production in Antarctic fungi induced by cold stress activates their antioxidant enzyme defense mechanisms. Their ability to survive in extremely cold ecosystems is associated with a cellular response that includes increased levels of SOD, CAT, and GPx. In particular, psychrophilic and psychrotolerant fungal strains isolated from Antarctica show higher enzyme activities compared to mesophilic strains from the same region [52,163]. In contrast, the situation of a drastic decrease in growth temperature led to a more significant increase in enzyme activity in mesophilic Antarctic strains than in their psychrophilic counterparts. Enhanced enzymatic antioxidant defense has also been observed in Antarctic strains *P. olsonii* [170], *P. griseofulvum* [171], and *A. fumigatus* I-9 [167]. Of interest are transcriptomic studies of a psychrophilic strain of *M. psychrophila*, which showed a lack of up-regulation of genes responsible for SOD synthesis at temperatures of 4 °C and 20 °C. Thirty microbial strains isolated from the Bulgarian Antarctic base "St. Kliment Ohridski" on Livingston Island were screened for the synthesis of antioxidant enzymes, specifically SOD and CAT [43]. Species belonging to the genera *Cladosporium*, *Penicillium*, *Monodictys*, *Geomyces*, *Aspergillus*, *Alternaria*, *Lecanicillium*, *Epicoccum*, and *Rhizopus* have been included in the study. The analysis revealed that six of these strains exhibited significantly higher enzyme activity at 15 °C than at 30 °C, with *A. glaucus* strain 363 being recognized as the most proficient producer of cold-active SOD.

An up-regulation of the CAT gene has been observed in the psychrophilic yeast strain *Rhodotorula* sp. (USM-PSY62) when subjected to a temperature downshift to 0 °C. This phenomenon coincides with a rise in ROS concentration and variations in the antioxidant defense mechanisms [196]. Cold-adapted fungi belonging to the species *Chrysosporium pannosum*, *Cylindrocarpon magnusianum*, *Mortierella minutissima*, *P. chrysogenum*, *P. cyclopium*, and *P. islandicum* exhibited increased production of extra- and intracellular CAT at 15 °C compared to 20 °C [195]. Arbuscular mycorrhizal fungi (AMF) have been observed to respond to cold stress by increasing the levels of several antioxidant enzymes, including SOD, CAT, ascorbate peroxidase (APX), and glutathione reductase (GR) [230]. The gpx gene, essential for GPx synthesis in *V. volvacea*, showed expression levels critical for fruiting body formation under low-temperature conditions [197]. Conversely, overexpression of this gene has been shown to enhance fruiting body regeneration in degenerated strains of *V. volvacea* T19. Transcriptomic analysis of *A. alternata* revealed that exposure to cold stress

significantly altered its transcriptional profile, particularly affecting the glutathione and thioredoxin systems [231].

8.2. Non-Enzymatic Antioxidants

In addition to the production of antioxidant enzymes, fungi have been shown to synthesize a wide variety of molecules with antioxidant and scavenging properties. For example, marine fungi produce phenolic compounds, anthraquinones, xanthones, carotenoids, indole derivatives, and alkaloids that participate in their cold stress response [200,232]. Magi et al. [181] reported that the content of phenolic compounds increased under low-temperature conditions, thereby facilitating their ROS scavenging activity. Furthermore, the melanin present in the cells of Antarctic fungi demonstrated remarkable stability as well as significant antioxidant properties, which included a robust ability to scavenge DPPH free radicals [6]. It plays a critical role in protecting injured cells by reducing the presence of ROS. The function of melanin in protecting against cold stress has also been established in *Cryptococcus neoformans* [233] and *Cryomyces antarcticus* [201]. In addition, the elevated carotenoid biosynthesis exhibited a concomitant correlation with the adaptation to low temperatures in the Antarctic yeast *Rhodosporidium kratochvilovae* [166] and rock-inhabiting fungus *Knufia petricola* A95 [202]. The cold adaptation in Antarctic yeasts, isolated from soils in King George Island, correlated with the production of freeze proteins and ergosterols [169].

8.3. The Role of Cold Shock Proteins (CSPs)

Upon exposure to cold temperatures, fungal cells quickly trigger the production of a substantial number of cold shock proteins (CSPs). These proteins play a crucial role in transcription and translation mechanisms [173]. In eukaryotes, CSPs are incorporated as cold shock domains in multidomain proteins, distinguished by their variable sequences at the N- and C-terminal regions. While the function of CSPs in the adaptation of bacteria to low-temperature environments is well understood, the function of cold shock domains in eukaryotes remains to be fully elucidated [234]. Furthermore, there is a paucity of information regarding the contributions of these proteins to fungal growth and survival in low-temperature environments. Louet et al. [234] found that the up-regulation of CSP genes in the rust fungus *Melampsora larici-populina* significantly bolstered its cold stress tolerance. CSPs have been identified in *A. clavatus* and *Metarhizium anisopliae* growing under conditions of extremely low temperatures [235]. The study of Keto-Timonen et al. [236] provides a comprehensive overview of the functions of CSP-family proteins, emphasizing their significance in the stress response mechanisms of enteropathogenic *Yersinia*. In the context of budding yeast cultures, the identification of two distinct groups of genes encoding CSPs has been made. These groups are designated as the "early response to low temperatures" group and the "late response" group. The "early response to low temperatures" group is associated with transcription, transport, and the metabolism of lipids and fatty acids. The second group of genes encodes proteins that support carbohydrate metabolism and the metabolism of trehalose and glycogen. The results of this study demonstrate that yeast cells employ two distinct programs in their response to low temperatures, thereby enhancing their capacity to adapt [173].

9. Genetic Basis of Cell Response to Cold Stress

Research on mycoflora in Antarctica has identified a limited number of endemic psychrophilic species, while the predominant findings indicate the presence of cosmopolitan psychrotolerant taxa [84]. The endemic species have been described as genuine psychrophilic fungi, which can only thrive and reproduce in particular Antarctic conditions [25].

On the other hand, cosmopolitan psychrotolerant fungi are ecotypes that exhibit mesophilic–psychrotolerant behavior, which has developed as an adaptation to the cold Antarctic climate [24]. Various species of the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, and *Rhodotorula*, among others, isolated from Antarctica, are characteristic of temperate environments. Robinson [6] suggested that fungi in Arctic and Antarctic regions might employ a cold avoidance strategy through recolonization in the spring, utilizing spores or hyphal fragments that have been transported from warmer areas. In addition to the morphological, physiological, and metabolic mechanisms of response against oxidative stress noted above, researchers are looking for molecular genetic evidence for the cold tolerance of such species. Zakharova et al. [237] reported that the Antarctic rock-inhabiting fungus *Cryomyces antarcticus*, one of the most stress-resistant eukaryotic microorganisms, transcribes a limited part of its genome as a strategy for cold adaptation. Probably, a significant portion of its genome has been silenced due to an 'energy conservation mode'. The identified proteins are predominantly derived from highly conserved protein families that are indispensable for the maintenance of central metabolism, which is critical for growth and recovery after dormancy [238]. A set of novel proteins, characterized by their remarkable resistance to stress, has been evaluated [239]. Additionally, the first assessment of the draft genome did not uncover any substantial variations in the *C. antarcticus* genome relative to comparative species and mesophilic hyphomycetes [240].

Tesei et al. [156] discovered that a significant decrease in temperature (up to 1 °C) resulted in an increase in the number of proteins in three extremotolerant black fungi: *Coniosporium perforans*, *Exophiala jeanselmei,* and *Friedmanniomyces endolithicus*. This increase was not observed in the mesophilic hyphomycete *P. chrysogenum*. These variations in the cell response to temperature stress could indicate a distinct strategy to survive suboptimal temperatures.

The data on six *Pseudogymnoascus* spp., collected from different biogeographical regions, presented novel findings regarding the reactions to temperature changes in their habitats [241]. The proteomic study revealed no definitive correlation between protein alterations and the geographical backgrounds of the strains. At the same time, gene ontology analysis indicated a significant presence of proteins associated with various protective strategies, such as the maintenance of protein homeostasis, the regulation of energy metabolism, and the initiation of DNA damage response and repair mechanisms.

The research of Gomez-Gutiérrez et al. [173] extended the genetic basis of fungal cell response to oxidative stress. Stajich [242] emphasized the relationship between the ecological niche adaptation and the evolution of the fungal genome. The expansion or contraction of orthologous protein families has been described as one of the most straightforward mechanisms by which organisms adapt to harsh environments. Similar gene modifications contributing to the evolution of fungal genomes have been published for the microcolonial black fungus *Saxispiralis lemnorum* MUM 23.14 [243], *Cryptococcus neoformans* [244], *Mrakia psychrophila* [183], etc. The transcriptomic analysis of *M. psychrophila* grown at 4 °C demonstrated up-regulation of the genes responsible for energy metabolism [183]. At the same time, other genes such as those involved in protein binding, proteasome, spliceosome, and mRNA surveillance were up-regulated at 20 °C. However, further investigation is required to establish the role of fungal gene evolution in the capacity of fungi to thrive under extreme conditions.

10. Concluding Remarks

In recent years, survival in extreme cold temperatures has sparked growing interest in the biodiversity, ecology, and biotechnological potential of Antarctic microorganisms. Furthermore, they are the subject of exobiology as a unique model for evolutionary studies.

Several scientists point out the similarity between the conditions of existence of microorganisms in some regions of Antarctica (e.g., deep ice, rock crevices, frozen soil, etc.) and those on the planet Mars. The Antarctic cold, arid climate, and remoteness from the rest of the Earth's surface are responsible for the preservation of many life forms untouched by human civilization. In addition, low temperatures, short summers, high concentrations of salts and UV radiation, and many other factors have a significant impact on the type of microorganisms that survive in this place.

Despite the extremely negative effect of cold temperatures on biochemical reactions, Antarctic fungi grow at rates similar to those achieved by related species living in temperate climates. This tolerance in conditions of extremely low temperatures is related to the cellular response to oxidative stress induced by the available abiotic factors. Antarctic fungi demonstrate the formation of ROS even at an optimal growth temperature, which is adequate for their survival mechanisms. Lowering the temperature, however, causes accelerated generation of ROS, which leads to the realization of oxidative stress. Data on the induction of oxidative stress in Antarctic fungi after exposure to low temperatures and their response are very scarce. Even less studied are the fungi isolated from the area of the Bulgarian base "St. Kliment Ohridski" on Livingston Island. This review presents data on the biodiversity of filamentous fungi in the Antarctic region and their tolerance to low temperatures.

As is clear from this review, cold stress causes significant oxidative damage to all cellular molecules in Antarctic fungi, such as lipids, proteins, and DNA. This results in substantial changes in the structure and fluidity of cell membranes, modifications to the structures of enzymes and other functional proteins, changes in the profile of bases in the DNA molecule, breakage of DNA chains, and numerous additional effects.

In the course of their evolution, Antarctic fungi have developed efficient survival mechanisms in harsh conditions, specifically at extremely low temperatures. Their survival strategies encompass modifications in cell and colony morphology, such as melanization, and the promotion of meristematic development. The literature documents significant ultrastructural changes, including an increase in vacuolar volume, focal damage to the cell wall, rupture of nuclear membranes, and structural changes in the mitochondria. The physiological adaptation of these organisms may encompass a variety of individual strategies or a synthesis of multiple approaches. Growth retardation, including a decrease in the amount of biomass, has been observed to occur as a result of the cold-shock-induced autolysis process. Furthermore, under cold stress conditions, it has been observed that the metabolic pathways of reserve carbohydrate synthesis and stress protectants trehalose and glycogen are triggered, resulting in their accumulation within the cells. Cold tolerance is facilitated by the presence of cold-active enzymes, which enhance metabolic activities, substrate assimilation, growth, and reproduction of these organisms.

In this review, we have also focused on the antioxidant defense mechanisms employed by Antarctic fungi. They promptly identify and respond to the changes in the level of ROS by inducing protective enzymes that counteract oxidative damage (SOD, CAT, GPx) as well as non-enzyme antioxidants. A significant research direction pertains to the comparison of the cellular response to oxidative stress in strains isolated from Antarctica and those from temperate habitats. The acquisition of such data may contribute to the elucidation of the cold-adapted mechanism.

The knowledge about the cold-stress response of Antarctic fungi is increasing rapidly, and, in the future, studies should be focused on the significance of combined stress exposure.

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