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Aureobasidium pullulans: The Versatile Microcolonial Fungus for Industrial Production of Natural Products

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Abstract

Aureobasidium pullulans, a yeast-like fungus, exhibits a remarkable capacity for synthesizing an extensive array of bioproducts with diverse industrial applications. This fungus is known to survive in several environmental conditions, making it a robust organism for biotechnological processes. The organism is characterized by its adaptability to various environmental conditions and habitats. It produces an array of extracellular enzymes such as amylases, lipases, proteases, and pectinases, contributing to its role as a versatile biocatalyst. Additionally, A. pullulans synthesize bioactive compounds including pullulan, polyols, melanin, and extracellular polysaccharides, which exhibit pharmaceutical, food, and industrial applications. Pullulan, in particular, stands out as a valuable product with unique properties such as water solubility, film-forming ability, and biodegradability. Recent advancements in genetic engineering and fermentation technology have enhanced the production efficiency of important products in A. pullulans and expanded applications in various domains. A. pullulans has environmental applications, particularly in bioremediation processes. It can degrade a variety of organic pollutants, including hydrocarbons, pesticides, and dyes, through its enzymatic activities. Additionally, A. pullulans has been studied for its role in the bioremediation of heavy metal-contaminated environments by immobilizing metals and facilitating their removal from contaminated sites. Overall, A. pullulans and its wide range of bioproducts are promising resources for sustainable bioprocessing and the development of eco-friendly solutions across diverse industries. This review provides an overview of A. pullulans and its wide range of bioproducts, emphasizing its significance in biotechnology, food, pharmaceuticals, agriculture, and environmental remediation, with recent updates on technologies speeding up the production processes at the industrial scale.

Keywords: Yeast-like fungus, production scale-up, agriculture, pharmaceutical, extracellular polysaccharides

INTRODUCTION

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Members of the genus *Aureobasidium*, a yeast-like fungus, are widely distributed and able to live in a variety of environments and a varied range of habitats, including man-made surfaces, and have a global distribution, across various geographies and climates. *Aureobasidium pullulans* is present in most phyllospheric environments, including in grapevines. It has also been isolated from hypersaline water in salters and rocks [1]. According to available records, this fungus is most common in temperate zones. The Mediterranean regions of France, Egypt, and Italy, and the subtropical tropical regions of Brazil, China, India, Jamaica, Thailand, and Malaysia [2]. *A. pullulans* belongs to the Dothideaceae family and is classified

as an ascomycetous yeast [3, 4]. *A. pullulans var. pullulans, A. pullulans var. subglaciale, A. pullulans var. melanogenum, and A. pullulans var. namibiae* are a variety of *A. pullulans,* that have been found in Arctic environments. Each variety may have specific characteristics or adaptations that allow it to thrive under harsh conditions in the Arctic region [5].

Owing to its wide range of habitats, environmental conditions, and biochemical traits, *A. pullulans* is a prime source for biotechnological applications. *A. pullulans* isolates and strains produce many products of industrial importance and are considered safe for environmental and biotechnological applications. Many compounds, such as volatile organic compounds, hydrolytic enzymes, and antimicrobial compounds, are among the major uses of this fungus in agriculture [6, 7]. There have been reports of *A. pullulans* strains producing highly beneficial industrial rarer enzymes, such as amylases, glucosidases, and xylanases. The product, pullulan (poly-1,6-maltotriose biopolymer), has immense applications in the food, pharmaceutical, and cosmetic industries. It is also known to produce new metabolites, such as siderophores, and biocontrol against post-harvest diseases and single-cell proteins (SCP) [2]. This review discusses the significance of *Aureobasidium* and its various species to produce various products.

MORPHOLOGICAL CHARACTERISTICS

On potato dextrose agar at 37°C, colonies quickly grew to a minimum diameter of 20 mm after five days and appeared smooth. Depending on the (sub) species, their appearance can be shiny, slimy, or matt. The border texture ranges from smooth to raveling [8, 9]. On solid media, isolates are normally black, but some tropical isolates show color variations with pigments in shades of purple, brown, and pink. The type of solid medium used can affect the color of a single isolate. Colonies can become completely or partially dark in the center or certain regions [6]. Cultures become darker because of the development of chlamydospores, which contain melanin pigments. Depending on the habitat and (sub) species, *Aureobasidium* can develop as budding yeast or mycelia with a black or hyaline appearance (Table 1). The recognized species on potato dextrose agar media exhibit the following microscopic traits: smooth, thin-walled hyaline hyphae. Hyaline hyphae can turn dark brown at any moment, ranging from locally in older cultures to quite quickly in all cultures, depending on the (sub) species. These thick-walled hyphae can separate into individual dark cells known as chlamydospores or chlamydoconidia, or function as chlamydospore chains. Undifferentiated, intercalary, or terminal conidiogenous cells were observed on the hyaline hyphae. *A. pullulans* was polymorphic. Depending on the environment, it can develop into budding yeast or mycelia.

Table 1. *Aureobasidium sp.* and their relative habitats.

Aureobasidium sp.	**Habitats**	**Reference**
A. proteae	Protea spp. plants	[10, 11]
A. melanogenum	Hypersaline and marine environments	[12]
A. pullulans	Fruits carposphere	[13, 14]
A. acericola	*Acer pseudosieboldianum* leaves	[15, 16]
A. subglaciale	Glacial habitats	[17]
A. iranianum	Plant endophyte	[18, 19]
A. uvarum	Grape juice	[20]
A. khasianum	Leaves of *Wightia speciosissima*	[21]
A. leucospermi	*Leucospermum spp*. plants	[10]
A. castaneae	Leaves of *Castanea henryi*	[22]
A. mustum	Grape juice	[23]
A. pini	Needles of *Pinus tabuliformis*	[24]
A. aerium	Air	[25]
A. namibiae	Extreme environments	[26]

GENOMICS TRAITS OF *AUREOBASIDIUM SPP.*

Aureobasidium species have intercalary conidiogenous cells for the simultaneous production of single-celled conidia of various sizes and shapes. This type of conidiation has also been found in sporodochial *Kabatiella* species, complicating identification and requiring molecular studies [27]. These fungi demonstrate remarkable phenotypic flexibility, changing between yeast and hyphal forms with different degrees of melanization, even under consistent growth conditions [28]. Traditional morphology-based taxonomy, on the other hand, faces difficulties due to phenotypic heterogeneity, not only between species but also within species. Despite the use of multi-locus DNA sequence analysis, the phylogenetic relationship of *Aureobasidium*-like isolates remains a mystery. *A. pullulans*, once thought to be a species complex with four varieties, have been re-evaluated, and a new unidentified species has been discovered, which is expected to include a number of former *A. subglaciale* isolates. Taxonomical identification of *Aureobasidium spp*. is important for exploiting their biotechnological and biocontrol potential, particularly considering the pathogenic characteristics reported in *A. melanogenum* [29].

The *Aureobasidium* genus is thought to contain roughly 50 taxa, including species and variations, based on information from the literature and the Index Fungorum database, with new species being described regularly [Cabi Database Index Fungorum 2022]. Other species are receiving increasing attention as curiosity develops because of their adaptable and valuable qualities. *A. iranianum,* an endophytic black yeast, has been reported to be present in healthy bamboo stems, with larger conidial dimensions than *A. melanogenum*. Compared to *A. pullulans, A. thailandense sp.* nov. exhibits a distinct type I intron in 18S rRNA. *A. acericola sp*. nov. was obtained from deciduous Korean Acer *Pseudosieboldianum* wood in 2021, and a new species, *A. aerium*, was detected in Beijing's air microbiome survey [25, 30]. Only two *Aureobasidium spp*., A. pullulans and A. subglaciale, have been reported as biological control agents, *A. pullulans* and *A. subglaciale* [31].

In terms of genomic attributes, research has concentrated on genome analysis not only for phylogenomics but also for investigating their biotechnological potential, stress-tolerance, and virulence aspects. De novo genome sequencing and analysis of the four *A. pullulans* variants resulted in their reclassification as separate species. Their genome ranges in size from 25.4 Mbp to 29.6 Mbp, with 10,000 to almost 12,000 predicted proteins encoded. Using genomic data, the predicted secretome revealed a wide range of extracellular enzymatic activities shared by common protein families with biotechnological potential [32, 33].

Potential virulence factors, such as lipases and proteases, have been identified, necessitating caution in plant applications [34, 35]. Sugar transporters, siderophore synthases, stress-tolerance genes, and pullulan synthesis capacities are known to contribute to adaptation and stress resistance.

Population genetic investigations have revealed that *A. pullulans* is a generalist in a variety of settings, but *A. subglaciale* has a more habitat-specific population structure, implying clonality and possible biocontrol advantages [32, 31].

BIOPRODUCTS FROM *AUREOBASIDIUM SPP.*

Pullulans

Pullulan, a linear extracellular polysaccharide (EPS), is formed by connecting repeating maltotriose units via α-(1,6)glycosidic bonds. The primary producer of pullulan is *Aureobasidium pullulans (A. pullulans),* a fungus that resembles yeast. [36, 12]. It is renewable and non-carcinogenic and has diverse applications in food engineering, environmental engineering, and biomedicine [37, 38, 3]. The distinct α-D-glucan linkage pattern provides pullulan with exceptional attributes such as solubility, oxygen barrier efficiency, film-forming ability, and structural elasticity, making it suitable for wound healing, drug delivery, and gene targeting [39, 37, 40, 41, 27]. The excellent properties of the material were attributed to intramolecular and intermolecular polyhydroxyl interactions. High-molecular-weight

pullulan, characterized by its elongated polysaccharide chain and augmented hydroxyl content, shows potential for biomedical applications and the development of shape memory polymers (SMPs). (SMPs) are a class of materials that can return to their original shape after deformation [22, 42].

However, recent manufacturing of pullulan-based hard capsules has utilized commercial pullulan, sourced from Hayashibara Co., Ltd., Japan, characterized by mean molecular weights of 1.0 × 105 or 2.0 × 105 Da [43]. The restricted application of pullulan in various sectors stems from its low production yield and low-molecular-weight [44]. To mitigate these limitations, extensive research has focused on bioprocessing and strain modification techniques aimed at augmenting both the molecular weight and yield. Furthermore, the advent of efficient genome editing methodologies established in 2019 has facilitated the targeted regulation of pertinent enzymes and genes in the relevant biosynthesis pathways in many *Aureobasidium spp*. strains [45]. For instance, a mutant strain named AMY-PKS-11 was developed by removing duplicated AMY1 and PKS1 genes. This resulted in the production of pigmented free pullulan heavy Mw 3.2×10^5 Da [9, 25]. Another strain, triple mutant DT15, yielded 46.2 g/L pullulan with a molecular weight of 3.02×10^5 Da in flasks and 58.14 g/L pullulan in a 10-L fermenter, while the wild-type strain P16, produced 65.5 ± 3.5 g/L pullulan with laser molecular weight [44, 46, 47]. Consequently, further research is warranted to augment pullulan production and refine its chemical properties through synthetic biology-driven molecular modifications of the producer strains.

Method of Extraction of Pullulan

This study presents a systematic and comprehensive approach to optimizing pullulan production via fermentation. Methodical strategy: initial optimization of key fermentation parameters individually before integrating them sequentially in their experiments. The composition of the initial fermentation medium, with precise concentrations of the ingredients at pH 5.0, was designed to support pullulan production. Inoculation with *A. pullulans* at a specific cell density followed by incubation at various time intervals and temperatures facilitated a thorough examination of their influence on pullulan yield. Notably, the investigation of the effect of pH on pullulan production involved systematic pH adjustments within a defined range using precise control mechanisms, yielding valuable insights into optimal pH conditions. Furthermore, supplementation of the growth medium with additional carbon and nitrogen sources at controlled concentrations enhanced pullulan production, demonstrating the positive impact of nutrient supplementation. The study employed a non-stirred fed-batch fermentation process in a well-defined fermenter setup with meticulous attention paid to operational parameters, sterilization procedures, and incubation conditions. An innovative aspect was the dynamic management of fermenter volume through incremental replenishment, which ensured consistent conditions throughout fermentation. Additionally, the controlled supply of sterile air during specific incubation periods underscores the precision of the experimental design [4, 36, 48, 49].

The post-fermentation process involved a methodical procedure aimed at isolating and quantifying pullulan, the target EPS. Initially, upon completion of fermentation, the culture medium was subjected to heat treatment at 100°C for 15 min in a water bath to deactivate the residual microbial activity and ensure processing safety. After cooling to room temperature, centrifugation of the medium at 12,000 rpm and 4°C for 10 min effectively separated the pullulan-containing supernatant from the cells and solid particles. To precipitate pullulan, a specific volume (3 ml) of the supernatant was transferred to a test tube, and cold ethanol (absolute or 95%) was added (6 ml), inducing polysaccharide precipitation upon thorough mixing. The mixture was stored at 4°C for 12 h to facilitate precipitation. Subsequently, the residual ethanol was removed, and the precipitated pullulan was dissolved in deionized water (3 ml) at 80°C. Following dissolution, the solution was dialyzed for 48 hours to effectively remove small molecules and impurities. Purified exopolysaccharide was precipitated again with cold ethanol. The resulting precipitate was weighed [1, 3]. This comprehensive isolation and quantification methodology demonstrates a well-executed approach for obtaining purified pullulan from fermentation cultures, ensuring the accuracy and purity of pullulan measurements, thereby enhancing the reliability of research involving this EPS.

A. pullulans produces numerous products that have undergone isolation, characterization, and testing for diverse biological and non-biological applications. The most common are antimicrobials, enzymes, polyesters, polysaccharides, heavy oils, and siderophores [6].

The versatile and beneficial properties of pullulan, an edible polymer, have received certification for safe use in food products in various countries. This underscores the unique ability of pullulan to form clear films or coatings that possess several advantageous characteristics such as being impermeable to oxygen, resistant to oil, and endowed with strong mechanical properties [4, 50–52]. These attributes make pullulan films a promising option as novel and versatile packaging materials. Furthermore, moderate molecular weight pullulan produced by *A. pullulans* BL06ΔPMAs was used to preserve celery, cabbages, and mangos. After being coated with pullulan and left at room temperature for 15 days, the coated vegetables and fruits retained their freshness and maintained an appealing appearance, in stark contrast to the control group which exhibited shrinkage and wrinkling. The data on weight loss further emphasized the effectiveness of the pullulan coating, reducing it by 12.5% for celery cabbages and 22.0% for mangoes compared to the control group. Overall, the valuable potential of pullulan as a food coating material presents practical evidence of its ability to enhance the shelf-life of perishable items such as vegetables and fruits. This application not only safeguards against dehydration and spoilage but also demonstrates a sustainable approach to reducing food waste and improving food quality [25, 53].

β-mannanases

Bacteria, fungi, yeast, and plants have all been used to isolate β-mannanases [54]. Fungi primarily produces extracellular enzymes that catalyze a variety of lignocellulosic biomass degradation reactions. Two forms of β-mannanase were obtained from *Aureobasidium spp.* [55].

The xylanolytic yeast strain, *A. pullulans*, has a diverse enzyme repertoire capable of complete galactomannan and galacto-glucomannan breakdown. The enzymes had different roles and cellular localizations; endo-1,4-mananase was extruded into the fluid culture, whereas -mannosidase remained completely intracellular, -galactosidase and -glucosidase, on the other hand, were identified in both extracellular and intracellular compartments [56, 57]. Only extracellular-mannanase and intracellular-mannosidase were inducible. When grown in galactomannan-rich media, as opposed to other carbon sources, the synthesis of these enzymes increased by a factor of 10 to 100. Surprisingly, galactomannan, galacto-glucomannan, and -1,4-manno-oligosaccharides co-induced both extracellular -mannanase and intracellular -mannosidase, with -1,4-mannobiose as the likely natural inducer. Glucose, mannose, and galactose effectively inhibit the synthesis of these enzymes. In addition, the synthetic glycoside methyl-D-mannopyranoside was discovered to be a non-metabolizable inducer of both -mannosidase and -mannanase [17, 58, 59].

This study found that certain enzymes might be induced under certain conditions. When yeast was grown on galactomannan-rich medium, compared to other carbon sources, the synthesis of these enzymes increased significantly.

Laccase

Laccases play a variety of biological functions, including the morphogenesis of fungi, regulation of fungal pathogenicity [5], and participation in the carbon cycle by effective breakdown of lignin. Laccases with biotechnological significance are mostly produced by Ascomycetes, Basidiomycetes, and Deuteromycetes [60]. Physiological fungal functions are also mediated by laccases.

Laccases, notably those found in *A. pullulans*, play a variety of roles. Laccases, classified as polyphenol oxidases [EC 1.10.3.2], are widely used in bioremediation and industrial applications. Laccases are known for their role in lignin breakdown; however, they also serve other purposes. The

study examined 41 strains of *A. pullulans* and discovered unique laccase production patterns when compared to lignin-degrading fungi. Laccases from phylogenetic clade 5 strains produce vinaceous pigments, demonstrate optimal activity at 50–60°C, and remain stable at that temperature for an hour, separating them from enzymes found in lignin-degrading fungi. Details of laccase purification, glycosylation, and molecular weights for individual strains are included in the investigation, demonstrating variances in coloration and enzymatic characteristics [61, 62]. 41 strains of *A. pullulans* showed distinct laccase production patterns compared to those of lignin-degrading fungi. Laccases from phylogenetic group 5, which are distinguished by the synthesis of dark vinaceous pigments, perform best at temperatures ranging from 50°C to 60°C. Furthermore, these laccases are stable at this temperature for an extended period of time, distinguishing them from enzymes found in lignin-degrading fungi. This study examined several characteristics of laccases, such as purification, glycosylation, and molecular weight, in different strains of *A. pullulans.* In summary, this study sheds light on the diverse roles of laccases, with emphasis on those found in *A. pullulans.* This emphasizes their vast functioning, use, and distinctive characteristics that set them apart from other lignin-degrading fungal laccases.

Xylanase

Xylan, the principal polysaccharide component of hemicelluloses, is composed of D-xylose units linked via β-1,4-glycosidic bonds in the main chain with various carbohydrate side chains [63]. Xylanases are the primary component of hemicellulose in the cell walls of both hardwoods. Rice bran was used as the xylan source for xylanase production from *A. pullulan* CCT 1261. Using statistical design, the ideal medium composition and culture conditions were determined. After successive cultivation to increase the xylanase output, the final model was validated. The conditions that yielded the highest xylanase production (82.2 U/mL) were found to be pH 7.0, 28°C, 3.6 g/L $(NH_4)_2SO_4$, 1.5 g/L yeast extract, and 61.9 g/L rice bran. Compared to cultivation conditions, xylanase activity increased 7-fold using these approaches. Finally, this study indicated the possibility of long-term use of rice bran byproducts, which could result in cost savings. The importance of xylanases as a necessary carbohydrate in the modern period emphasizes their structural components and role in hemicellulose within the cell walls. Here, we describe the synthesis of xylanase from the *A. pullulans* strain CCT 1261, utilizing rice bran as a xylan source. These findings suggest that rice bran byproducts can be used to produce xylanase in the long term. This method can result in cost savings, while effectively utilizing readily available resources.

In conclusion, the significance of xylanases, their structural composition, their involvement in hemicellulose, and the optimization of xylanase synthesis using *A. pullulans* CCT 1261 and rice bran. The use of statistical designs for optimization and the resulting increase in xylanase production are the major points raised in the discussion.

Protease

Proteases play a crucial role in the breakdown of diverse protein sources, thereby facilitating the creation of valuable applications and products. However, the potential of proteases derived from ocean-originating yeasts remains relatively unexplored [64, 65]. *A. pullulans* strain proteases can hydrolyze proteins from bioactive peptides with ACE-inhibitory and antioxidant properties. The purified protease from strain HN2-3 exhibited exceptional ACE-inhibitory activity (88.3%) and antioxidant activity (82.8%) when applied to crude bioactive peptides sourced from shrimp muscle (*Trachypenaeus curvirostris*) and Spirulina powder (*Arthrospira platensis*), respectively. *Yarrowia lipolytica* cells expressing the cloned *cDNAALP2* gene display protease activity and produce bioactive peptides from many types of proteins. This investigation underscores the importance of genetic amplification and expression of protease genes in marine yeast. The utilization of yeast cells for bioactive peptide production represents a promising avenue for potential biotechnological applications.

SOME OTHER BIOPRODUCTS FROM *AUREOBASIDIUM SPP.*

Antimicrobial Compounds

Many *Aureobasidium* isolates produce compounds that inhibit the growth of microorganisms. *Botrytis cinerea* on the surface of table grapes, was reportedly reduced by *A. pullulans*. The exclusion of fungal adhesion sites by bacteria and yeasts, competition for resources, and the generation of lytic enzymes and antagonistic metabolites have been suggested as the proposed mechanisms for this antifungal activity. It is likely that *A. pullulans* secrete glucanase and chitinase, which hydrolyze molds. Furthermore, a strain of *A. pullulans* was shown to contain antibacterial compounds that inhibit the growth of Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas fluorescens* [6]. Cyclic deosipeptides contain a product called *aureobasidin* (molar mass ranging from 1071 to 1148 Da). Research on factors influencing their activity and production has indicated that glucose boosts the amino acid composition, and antifungal activity of the culture medium can have a variable function in some situations; aureobasidin has been reported in the majority of cases [66].

Several studies have focused on the properties and activities of various isolates of the fungus *A. pullulans*, especially those from the phyllosphere. These isolates have been discovered to produce a wide range of antimicrobial compounds. Furthermore, it is suspected that *A. pullulans* produces antagonistic chemicals or enzymes that directly hinder the growth of other fungi.

Siderophores

Many microorganisms produce siderophores, which are low-molecular-weight iron-chelating ligands, to scavenge iron from resource-scarce environments, particularly marine habitats. It has been established that whereas bacteria produce hydroxamate and catecholate siderophores, yeasts solely produce hydroxamate-type compounds [67, 1]. Iron (Fe_3^+ and Fe_2^+ ions) was solubilized and secluded by the molecules. Siderophores are highly intriguing compounds with potential for use in biotechnology as well as in the fields of medicine, agriculture, and the environment. Studies have revealed the role of siderophores in the biocontrol of plant and human infections. *A. pullulans* (HN6.2) produces siderophores [68].

Poly(β-L-malic acid) (PMA)

The biopolyester poly(-L-malic acid) (PMA) holds promise in the pharmaceutical sector, particularly for drug delivery. Their water solubility, biodegradability, and biocompatibility make them particularly intriguing. Moreover, certain fungi such as *Aureobasidium spp.*, have been found to produce PMA. Initial screening revealed the synthesis of PMA by *Aureobasidium*, prompting further investigation of *A. pullulans* strains [37]. The most productive strain was *A. pullulans* CBS 591.75, producing 9.8 g/L PMA (0.11 g/g glucose) in the stirred tank bioreactor. The structure of PMA was elucidated by 1H- and 13C-nuclear magnetic resonance spectroscopy [44]. In a 5-L fermenter, *A. pullulans* CCTCCM 2012223 achieved a maximum PMA yield of 44 g/L under low nitrogen concentrations (2 g/L NH_4NO_3). Nitrogen limitation was shown to enhance PMA synthesis by regulating the target of the rapamycin (TOR) signaling pathway in *A. pullulans.* [47].

Liamocins

Several strains of *Aureobasidium sp.* have developed heavy oils in their growth medium with an unknown structure. It was discovered that the heavy oil was active and capable of preventing the development of mammalian cancer cell lines. They were then given the name ‘liamocins’. Their structure consists of three or four 3,5-dihydroxy-decanoic ester groups attached to a single, partly o-acetylated mannitol headgroup [44]. *A. pullulans* RSU 9 and RSU 21 from Thailand have anticancer activity against breast and cervical cancer cell lines, but not against a normal human cell line. Liamocins, a complex group of chemicals generated by some strains of *Aureobasidium sp.,* have been shown to inhibit the proliferation of malignant mammalian cell types.

Single-cell Protein

Protein-rich foods may be made using biomass and proteins harvested from cultures of fungi, bacteria, and algae. Edible unicellular or multicellular bacteria are referred to as SCPs. These products are of high quality and safe for consumption by both humans and animals, and efforts to grow SCP using both autotrophic growth and agricultural and food waste have been quite successful.

Fungal proteins offer greater benefits than those derived from bacteria or algae. Essential amino acids and nucleic acid components were abundant in *A. pullulans* isolates. *A. pullulans* with a high protein content may be particularly significant in the creation of single cells of proteins by converting waste items, including starch, xylan, protein, and cellulose, into cell proteins [64, 65].

SCPs have been successfully cultivated using methods such as autotrophic development and the exploitation of agricultural and culinary waste. Fungal proteins have specific benefits over bacterial and algal proteins as protein sources. *A. pullulans* isolates a form of fungus, are particularly rich in vital amino acids and nucleic acids [69].

Biocontrol Agent

In horticulture and agriculture, *A. pullulans* has shown promising potential as a biocontrol agent. Some strains of *A. pullulans* can efficiently inhibit plant infections through a variety of processes, such as resource competition, the synthesis of antifungal metabolites, and the stimulation of plant defense responses. Furthermore, A. pullulans has been shown that *A. pullulans* colonize plant surfaces, creating a barrier that prevents pathogen invasion [60]. *A. pullulans* is capable of controlling a broad variety of plant diseases caused by bacterial pathogens and fungi such as *Botrytis cinerea, Alternaria spp., and Fusarium spp*. It is a desirable alternative for sustainable crop protection because of its low environmental impact and compatibility with integrated pest management techniques [70, 71, 4]. *A. pullulans* has been emphasized as a key factor in both the carposphere and phyllosphere, displaying adaptation and robustness [41, 1, 72].

AUREOBASIDIUM SPP. USE IN FOOD INDUSTRIES

A. pullulans holds substantial promise for biotechnological applications in the food industry. Strains of *Aureobasidium spp.* are recognized for their production of vital enzymes, such as lipase, amylase, mannose, protease, cellulose, transferases, and xylanase, which are important organisms in food technology. Many enzymes such as polygalacturonase, endo-1,4-β-xylanase, β-xylosidase, and pectin lyase are valuable for clarifying fruit pulp and juices, particularly for enhancing processes within the vitivinicultural sector. In vitiviniculture, *A. pullulans* is a prevalent microbe commonly found in grapevine environments. Its impact on wine composition and fermentation has been extensively studied, with pectinolytic activity identified as a notable characteristic that positively influences the color and aroma of the resulting wines. Additionally, *A. pullulans* can influence the concentration of volatile compounds such as terpenes in wines. Its biotechnological potential extends to starch saccharification, bread, and baking processing, wherein *Aureobasidium spp.*-derived glucoamylase and α-amylase enzymes have been applied [23].

CONCLUSION AND FUTURE PROSPECTS

Unlike bacteria, yeast and fungi have not been used in biotechnology to the same extent. The strains of *A. pullulans* that have the potential to be used as yeast-like fungi in various industries are listed along with those that have the potential to be used in biotechnology. *A. pullulans* can be found everywhere in the world and in a variety of environments; thus, they probably offer a wide range of activities. Isolates from harsh environments may contain polyextremophilic metabolites and enzymes that can be exploited under harsh conditions present in industrial operations.

A. pullulans and its metabolites are used directly in the food and medical industries. Through a variety of mechanisms, the fungus also demonstrates a beneficial effect in mycotoxin biocontrol, the most

notable of which is binding and/or absorption. Certain strains of *A. pullulans* produce valuable commercial enzymes but are not limited to mannanases, proteases, β-glucosidase, cellulases, lipases, amylases, and xylanases. Other important molecules that may be used in biotechnology include liamocins, siderophores, and poly(β-L-malic acid). Additionally, the fungus exhibits a high concentration of critical amino acid and nucleic acid components, suggesting a possible single-cell protein source that needs to be investigated further. This fungus has demonstrated excellent biocontrol against diseases that affect plants.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

1. Singh R, Gaur R, Bansal S, Biswas P, Pandey PK, Jamal F, et al. Aureobasidium pullulans-an industrially important pullulan producing black yeast. Int J Curr Microbiol Appl Sci. 2015;4(10):605-622.
2. Xiao Q, Lim LT. Pullulan-alginate fibers produced using free surface electrospinning. Int J Biol Macromol. 2018;112:809-817. DOI: 10.1016/j.ijbiomac.2018.02.005. PubMed: 29410269.
3. Singh RS, Kaur N. Understanding response surface optimization of medium composition for pullulan production from de-oiled rice bran by Aureobasidium pullulans. Food Sci Biotechnol. 2019;28:1507-1520. DOI: 10.1007/s10068-019-00585-w. PubMed: 31695950.
4. Singh RS, Saini GK. Biosynthesis of Pullulan and Its Applications in Food and Pharmaceutical Industry. In: Satyanarayana T, Johri B, editors. Microorganisms in Sustainable Agriculture and Biotechnology. Springer, Dordrecht; 2012. https://doi.org/10.1007/978-94-007-2214-9_24.
5. Zhu M, Zhang L, Yang F, Cha Y, Li S, Zhuo M, et al. A recombinant β-mannanase from Thermoanaerobacterium aotearoense SCUT27: Biochemical characterization and its thermostability improvement. J Agric Food Chem. 2020;68:818-825. DOI: 10.1021/acs.jafc.9b06246. PubMed: 31845578.
6. Bozoudi D, Tsaltas D. The multiple and versatile roles of Aureobasidium pullulans in the vitivinicultural sector. Fermentation. 2018;4(4):85.
7. Cao W, Chen X, Luo J, Yin J, Qiao C, Wan Y. High molecular weight β-poly(L-malic acid) produced by A. pullulans with Ca^{2+} added repeated batch culture. Int J Biol Macromol. 2016;85:192–9. DOI: 10.1016/j.ijbiomac.2015.12.056. PubMed: 26721383.
8. Chaturvedi S, Sarethy IP. Virtual screening of compounds from microcolonial Fungal Strain TD-062 obtained from the Thar Desert of India. Curr Trends Biotechnol Pharm. 2021;15(1):62–6.
9. Chen G, Zhu Y, Zhang G, Liu H, Wei Y, Wang P, et al. Optimization and characterization of pullulan production by a newly isolated high-yielding strain Aureobasidium melanogenum. Prep Biochem Biotechnol. 2019;49(6):557–66. DOI: 10.1080/10826068.2019.1591988. PubMed: 30957655.
10. Crous PW, Summerell BA, Swart L, Denman S, Taylor JE, Bezuidenhout CM, et al. Fungal pathogens of Proteaceae. Persoonia. 2011;27:20–45. DOI: 10.3767/003158511X606239. PubMed: 22403475.
11. Shrief R, El-Kholy RM, Rizk MA, Zaki MES. Prevalence of tetracycline resistant genes in Staphylococcus aureus isolates from surgical site infections Egypt. Biosci Biotechnol Res Asia. 2019;16(1):229–50. DOI: 10.13005/bbra/2740.

12. Wei X, Liu GL, Jia SL, Chi Z, Hu Z, Chi ZM. Pullulan biosynthesis and its regulation in Aureobasidium spp. Carbohydr Polym. 2021;251:117076. DOI: 10.1016/j.carbpol.2020.117076. PubMed: 33142619.
13. Di Francesco A, Ugolini L, Lazzeri L, Mari M. Production of volatile organic compounds by Aureobasidium pullulans as a potential mechanism of action against postharvest fruit pathogens. Biol Control. 2015;81:8–14. DOI: 10.1016/j.biocontrol.2014.10.004.
14. Di Francesco A, Ugolini L, D'Aquino S, Pagnotta E, Mari M. Biocontrol of Monilinia laxa by Aureobasidium pullulans strains: Insights on competition for nutrients and space. Int J Food Microbiol. 2017;248:32–8. DOI: 10.1016/j.ijfoodmicro.2017.02.007. PubMed: 28242420.
15. Leonowicz A, Cho NS, Luterek J, Wilkolazka A, Wojtas-Wasilewska M, Matuszewska A, et al. Fungal laccase: Properties and activity on lignin. J Basic Microbiol. 2001;41:185–227. DOI: 10.1002/1521-4028(200107)41:3/4<185::aid-jobm185>3.0.co;2-t. PubMed: 11512451.
16. Li X, Qian P, Wu SG, Yu HY. Characterization of an organic solvent-tolerant lipase from Idiomarina sp. W33 and its application for biodiesel production using Jatropha oil. Extremophiles. 2014;18:171–8. DOI: 10.1007/s00792-013-0610-0. PubMed: 24276735.
17. Zhang L, Liu J, Zheng X, Zhang A, Zhang X, Tang K. Pullulan dialdehyde crosslinked gelatin hydrogels with high strength for biomedical applications. Carbohydr Polym. 2019;216:45-53. DOI: 10.1016/j.carbpol.2019.04.004. PubMed: 31047081.
18. Arzanlou M, Khodaei S. Aureobasidium iranianum, a new species on bamboo from Iran. Mycosphere. 2012;3(4):404–8. DOI: 10.5943/mycosphere/3/4/2.
19. An JM, Shahriar SMS, Hasan MN, Cho S, Lee YK. Carboxymethyl cellulose, pluronic, and pullulan-based compositions efficiently enhance antiadhesion and tissue regeneration properties without using any drug molecules. ACS Appl Mater Interfaces. 2021;13(14):15992–6006. DOI: 10.1021/acsami.0c21938. PubMed: 33797224.
20. Onetto CA, Schmidt SA, Roach MJ, Borneman AR. Comparative genome analysis proposes three new Aureobasidium species isolated from grape and other fruits and vegetables. Fungal Genet Biol. 2021;151:103573. DOI: 10.1016/j.fgb.2021.103573. PubMed: 34091066.
21. Prabhugaonkar A, Pratibha J. Aureobasidium khasianum (Aureobasidiaceae) a novel species with distinct morphology. Phytotaxa. 2018;374(3):257–62. DOI: 10.11646/phytotaxa.374.3.7.
22. Jiang N, Fan X, Tian C. Identification and characterization of leaf-inhabiting fungi from Castanea plantations in China. J Fungi. 2021;7:64. DOI: 10.3390/jof7010064. PubMed: 33477575.
23. Onetto CA, Borneman AR, Schmidt SA. Investigating the effects of Aureobasidium pullulans on grape juice composition and fermentation. Food Microbiol. 2020;90:103451. DOI: 10.1016/j.fm.2020.103451. PubMed: 32336373.
24. Jiang H, Chen TJ, Chi Z, Hu Z, Liu GL, Sun Y, et al. Macromolecular pullulan produced by Aureobasidium melanogenum 13–2 isolated from the Taklimakan desert and its crucial roles in resistance to the stress treatments. Int J Biol Macromol. 2019;135:429–36. DOI: 10.1016/j.ijbiomac.2019.05.190. PubMed: 31145957.
25. Chen S, Zheng H, Gao J, Song H, Bai W. High-level production of pullulan and its biosynthesis regulation in Aureobasidium pullulans BL06. Front Bioeng Biotechnol. 2023;11:1131875. DOI: 10.3389/fbioe.2023.1131875. PubMed: 36777253.
26. Zajc J, Černoša A, Sun X, Fang C, Gunde-Cimerman N, Song Z, et al. From glaciers to refrigerators: The population genomics and biocontrol potential of the black yeast Aureobasidium subglaciale. Microbiol Spectr. 2022;10. DOI: 10.1128/spectrum.01455-22. PubMed: 35880866.
27. Nishimura T, Shishi S, Sasaki Y, Akiyoshi K. Thermoresponsive polysaccharide graft polymer vesicles with tunable size and structural memory. J Am Chem Soc. 2020;142:11784–90. DOI: 10.1021/jacs.0c02290. PubMed: 32506909.
28. Di Francesco A, Zajc J, Stenberg JA. Aureobasidium spp.: Diversity, versatility, and agricultural utility. Horticulturae. 2023;9(1):59. DOI: 10.3390/horticulturae9010059.
29. Černoša A, Sun X, Gostinčar C, Fang C, Gunde-Cimerman N, Song Z. Virulence traits and population genomics of the black yeast Aureobasidium melanogenum. J Fungi (Basel). 2021;7(8):665. DOI: 10.3390/jof7080665. PubMed: 34436204.

30. Lee DH, Cho SE, Oh JY, Cho EJ, Kwon S. A novel species of Aureobasidium (Dothioraceae) recovered from Acer pseudosieboldianum in Korea. J Asia Pac Biodivers. 2021;14:657–61. DOI: 10.1016/j.japb.2021.08.005.
31. Janisiewicz WJ, Jurick WM, Peter KA, Kurtzman CP, Buyer JS. Yeasts associated with plums and their potential for controlling brown rot after harvest. Yeast. 2014 Jun;31(6):207-18.
32. Gostinčar C, Ohm RA, Kogej T, Sonjak S, Turk M, Zajc J, et al. Genome sequencing of four Aureobasidium pullulans varieties: Biotechnological potential, stress tolerance, and description of new species. BMC Genomics. 2014;15:549. DOI: 10.1186/1471-2164-15-549. PubMed: 24984952.
33. Pinto C, Custódio V, Nunes M, Songy A, Rabenoelina F, Courteaux B, Clément C, Gomes AC, Fontaine F. Understand the potential role of Aureobasidium pullulans, a resident microorganism from grapevine, to prevent the infection caused by Diplodia seriata. Front Microbiol. 2018;9:3047. DOI: 10.3389/fmicb.2018.03047, PubMed: 30619138.
34. Freimoser FM, Rueda-Mejia MP, Tilocca B, Migheli Q. Biocontrol yeasts: Mechanisms and applications. World J Microbiol Biotechnol. 2019;35(9):154. DOI: 10.1007/s11274-019-2728-4. PubMed: 31576429.
35. Gautério GV, da Silva LGG, Hübner T, da Rosa Ribeiro T, Kalil SJ. Maximization of xylanase production by Aureobasidium pullulans using a by-product of rice grain milling as xylan source. Biocatal Agric Biotechnol. 2020;23:101511. DOI: 10.1016/j.bcab.2020.101511.
36. Singh RS, Kaur N, Kennedy JF. Pullulan and pullulan derivatives as promising biomolecules for drug and gene targeting. Carbohydr Polym. 2015;123:190-207. DOI: 10.1016/j.carbpol.2015.01.032. PubMed: 25843851.
37. Feng Z, Chen S, Ahmad A, Chen L, Bai W. Ultra-high molecular weight pullulan-based material with high deformability and shape-memory properties. Carbohydr Polym. 2022;295:119836. DOI: 10.1016/j.carbpol.2022.119836. PubMed: 35988995.
38. Nongkhai SN, Piemthongkham P, Bankeeree W, Punnapayak H, Lotrakul P, Prasongsuk S. Xylooligosaccharides produced from sugarcane leaf arabinoxylan using xylanase from Aureobasidium pullulans NRRL 58523 and its prebiotic activity toward Lactobacillus spp. Heliyon. 2023;9. DOI: 10.1016/j.heliyon.2023.e22107. PubMed: 38034795.
39. Singh RS, Saini GK, Kennedy JF. Pullulan production in stirred tank reactor by a colour-variant strain of Aureobasidium pullulans FB-1. Carbohydr Polym Technol Appl. 2021;2:100086. DOI: 10.1016/j.carpta.2021.100086.
40. Duan Y, Li K, Wang H, Wu T, Zhao Y, Li H, et al. Preparation and evaluation of curcumin grafted hyaluronic acid modified pullulan polymers as a functional wound dressing material. Carbohydr Polym. 2020;238:116195. DOI: 10.1016/j.carbpol.2020.116195. PubMed: 32299553.
41. Ghorbani F, Zamanian A, Behnamghader A, Daliri Joupari MD. Bioactive and biostable hyaluronic acid-pullulan dermal hydrogels incorporated with biomimetic hydroxyapatite spheres. Mater Sci Eng C Mater Biol Appl. 2020;112:110906. DOI: 10.1016/j.msec.2020.110906. PubMed: 32409060.
42. Mounir R, Durieux A, Bodo E, Allard C, Simon JP, Achbani EH, et al. Production, formulation and antagonistic activity of the biocontrol like-yeast Aureobasidium pullulans against Penicillium expansum. Biotechnol Lett. 2007;29:553–9. DOI: 10.1007/s10529-006-9269-2. PubMed: 17216301.
43. Kalantari E, Deopurkar R, Kapadnis B. Antimicrobial activity of indigenous strains of Aureobasidium isolated from Santalum album leaves. Iran J Pharm Res. 2006;5:59–64.
44. Ding Y, Jiang F, Chen L, Lyu W, Chi Z, Liu C, et al. An alternative hard capsule prepared with the high molecular weight pullulan and gellan: Processing, characterization, and in vitro drug release. Carbohydr Polym. 2020;237:116172. DOI: 10.1016/j.carbpol.2020.116172. PubMed: 32241441.
45. Manitchotpisit P, Watanapokasin R, Price NPJ, Bischoff KM, Tayeh M, Teeraworawit S, et al. Aureobasidium pullulans as a source of liamocins (heavy oils) with anticancer activity. World J Microbiol Biotechnol. 2014;30:2199–204. DOI: 10.1007/s11274-014-1639-7. PubMed: 24659335.
46. Zhu X, Gibbons J, Garcia-Rivera J, Casadevall A, Williamson PR. Laccase of Cryptococcus neoformans is a cell wall-associated virulence factor. Infect Immun. 2001;69:5589-5596. DOI: 10.1128/IAI.69.9.5589-5596.2001. PubMed: 11500433.

47. Li Y, Cai H, Zhao M, Zhang H, Feng F. Screening and identification of high-yield thermostable lipase producing microorganisms. Biotechnol Bull. 2015;31:144.
48. Liu NN, Chi Z, Liu GL, Chen TJ, Jiang H, Hu Z, et al. α-amylase, glucoamylase and isopullulanase determine molecular weight of pullulan produced by Aureobasidium melanogenum P16. Int J Biol Macromol. 2018;117:727–34. DOI: 10.1016/j.ijbiomac.2018.05.235. PubMed: 29864535.
49. Wang CB, Jiang N, Tu Y, Zhu YQ, Xue H, Li Y. Aureobasidium aerium (Saccotheciaceae, Dothideales), a new yeast-like fungus from the air in Beijing, China. Phytotaxa. 2022;544:185-192. DOI: 10.11646/phytotaxa.544.2.4.
50. Wang JR, Li YY, Xu SD, Li P, Liu JS, Liu DN. High-level expression of pro-form lipase from Rhizopus oryzae in Pichia pastoris and its purification and characterization. Int J Mol Sci. 2013;15:203-217. DOI: 10.3390/ijms15010203. PubMed: 24368519.
51. Wang M, Danesi P, James TY, Al-Hatmi AMS, Najafzadeh MJ, Dolatabadi S, et al. Comparative pathogenicity of opportunistic black yeasts in Aureobasidium. Mycoses. 2019;62:803-811. DOI: 10.1111/myc.12931. PubMed: 31107996.
52. Wang WL, Chi ZM, Chi Z, Li J, Wang XH. Siderophore production by the marine-derived Aureobasidium pullulans and its antimicrobial activity. Bioresour Technol. 2009;100:2639-2641. DOI: 10.1016/j.biortech.2008.12.010. PubMed: 19162476.
53. Chen TJ, Liu GL, Wei X, Wang K, Hu Z, Chi Z, et al. A multidomain α-glucan synthetase 2 (AmAgs2) is the key enzyme for pullulan biosynthesis in Aureobasidium melanogenum P16. Int J Biol Macromol. 2020;150:1037–45. DOI: 10.1016/j.ijbiomac.2019.10.108. PubMed: 31739016.
54. Mulay YR, Deopurkar RL. Purification, characterization of amylase from indigenously isolated Aureobasidium pullulans Cau 19 and its bioconjugates with gold nanoparticles. Appl Biochem Biotechnol. 2018;184:644–58. DOI: 10.1007/s12010-017-2575-4. PubMed: 28836170.
55. Ibrahim SNMM, Bankeeree W, Prasongsuk S, Punnapayak H, Lotrakul P. Production and characterization of thermostable acidophilic β-mannanase from Aureobasidium pullulans NRRL 58524 and its potential in mannooligosaccharide production from spent coffee ground galactomannan. 3 Biotech. 2022;12:237. DOI: 10.1007/s13205-022-03301-4. PubMed: 36003897.
56. Kremnický L, Biely P. β-Mannanolytic system of Aureobasidium pullulans. Arch Microbiol. 1997;167:350–5. DOI: 10.1007/s002030050454.
57. Leathers TD, Manitchotpisit P. Production of poly(β-L-malic acid) (PMA) from agricultural biomass substrates by Aureobasidium pullulans. Biotechnol Lett. 2013;35:83–9. DOI: 10.1007/s10529-012-1045-x. PubMed: 22955679.
58. Xue SJ, Chen L, Jiang H, Liu GL, Chi ZM, Hu Z, et al. High pullulan biosynthesis from high concentration of glucose by a hyperosmotic resistant, yeast-like fungal strain isolated from a natural comb-honey. Food Chem. 2019;286:123-128. DOI: 10.1016/j.foodchem.2019.01.206. PubMed: 30827584.
59. Zajc J, Cernosa A, Di Francesco A, Casteria R, De Curtis F, Lima G, et al. Characterization of Aureobasidium pullulans isolates selected as biocontrol agents against fruit decay pathogens. Fungal Genomics Biol. 2020;10.
60. Nasr S, Mohammadimehr M, Geranpayeh Vaghei M, Amoozegar MA, Shahzadeh Fazeli SA. Aureobasidium mangrovei sp. nov., an ascomycetous species recovered from Hara protected forests in the Persian Gulf, Iran. Antonie van Leeuwenhoek. 2018;111:1697–705. DOI: 10.1007/s10482-018-1059-z. PubMed: 29511957.
61. Rich JO, Leathers TD, Anderson AM, Bischoff KM, Manitchotpisit P. Laccases from Aureobasidium pullulans. Enzyme Microb Technol. 2013;53(1):33-37. DOI: 10.1016/j.enzmictec.2013.03.015. PubMed: 23683702.
62. Shah SA, Sohail M, Minhas MU, Khan S, Hussain Z, Mahmood A, et al. Curcumin-laden hyaluronic acid-co-pullulan-based biomaterials as a potential platform to synergistically enhance diabetic wound repair. Int J Biol Macromol. 2021;185:350-368. DOI: 10.1016/j.ijbiomac.2021.06.119. PubMed: 34171251.
63. Bhat MK, Hazlewood GP. Enzymology and other characteristics of cellulases and xylanases. 2001.

64. Chi Z, Chi Z, Zhang T, Liu G, Li J, Wang X. Production, characterization and gene cloning of the extracellular enzymes from the marine-derived yeasts and their potential applications. Biotechnol Adv. 2009;27(3):236–55. DOI: 10.1016/j.biotechadv.2009.01.002. PubMed: 19215727.
65. Chi Z, Wang F, Chi Z, Yue L, Liu G, Zhang T. Bioproducts from Aureobasidium pullulans, a biotechnologically important yeast. Appl Microbiol Biotechnol. 2009;82(5):793–804. DOI: 10.1007/s00253-009-1882-2. PubMed: 19198830.
66. Prajapati VD, Chaudhari AM, Gandhi AK, Maheriya P. Pullulan based oral thin film formulation of zolmitriptan: Development and optimization using factorial design. Int J Biol Macromol. 2018;107:2075-2085. DOI: 10.1016/j.ijbiomac.2017.10.082. PubMed: 29074082.
67. Sheng L, Tong Q, Ma M. Why sucrose is the most suitable substrate for pullulan fermentation by Aureobasidium pullulans CGMCC1234? Enzyme Microb Technol. 2016;92:49-55. DOI: 10.1016/j.enzmictec.2016.06.016. PubMed: 27542744.
68. Prasongsuk S, Ployngam S, Wacharasindhu S, Lotrakul P, Punnapayak H. Effects of sugar and amino acid supplementation on Aureobasidium pullulans NRRL 58536 antifungal activity against four Aspergillus species. Appl Microbiol Biotechnol. 2013;97:7821-7830. DOI: 10.1007/s00253-013-5069-5. PubMed: 23828601.
69. Chi Z, Yan K, Gao L, Li J, Wang X, Wang L. Diversity of marine yeasts with high protein content and evaluation of their nutritive compositions. J Mar Biol Assoc U.K. 2008;88(7):1347–52. DOI: 10.1017/S0025315408001938.
70. Merín MG, Morata de Ambrosini VIM. Kinetic and metabolic behaviour of the pectinolytic strain Aureobasidium pullulans GM-R-22 during pre-fermentative cold maceration and its effect on red wine quality. Int J Food Microbiol. 2018;285:18–26. DOI: 10.1016/j.ijfoodmicro.2018.07.003. PubMed: 30015259.
71. Moraes FC, Antunes JC, Forero Ramirez LMF, Aprile P, Franck G, Chauvierre C, et al. Synthesis of cationic quaternized pullulan derivatives for miRNA delivery. Int J Pharm. 2020;577:119041. DOI: 10.1016/j.ijpharm.2020.119041. PubMed: 31978463.
72. Morozova OV, Shumakovich GP, Gorbacheva MA, Shleev SV, Yaropolov AI. “Blue” laccases. Biochemistry. 2007;72:1136–50. DOI: 10.1134/s0006297907100112. PubMed: 18021071.

