Review Article

A Noval Approach for Treating Cancer by Using Laccases from Marine Fungi

R. Jaya lakshmi*, D. Siva Mallika, S. Jeevan Amos, K. Kasturi
Department of Biotechnology, Acharya Nagarjuna University, Guntur, India.
***Corresponding author's E-mail:** jaya.rudramalla@gmail.com

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ABSTRACT

Marine sources play a vital role in the form of pharmaceutical care and for the discovery of new molecular structures. Recently marine organisms attain importance of new therapeutics, or beneficial dugs especially for cancer. Marine derived fungi provide plenty of biologically active structurally unique, secondary metabolites. In recent years the use of enzymes in the diverse fields has been of greater importance. In nature many of such potential enzymes are widely distributed. laccases are one among them, which are most studied and oldest enzymatic systems. Among these, fungi accounted as the most important group of laccase producers. At present fungal laccases are used in biotechnological applications. Laccase from marine fungi is novel and an advanced approach for treating cancer because laccases have the capability to destroy the cancer cells so, it can be treated for the development of new preparations for the therapy of tumour. This review mainly focus on the various sources, properties, production and potential applications of laccases.

Keywords: marine fungi, laccase, cancer, potential applications.

INTRODUCTION

Fungal biotechnology or 'mycotechnology' has advanced considerably in the last five decades. During the decade from 1977 to 1987 about 2500 new metabolites were reported from a variety of marine organisms. This information has clearly demonstrated that the marine environment is an excellent source of novel chemicals. Marine products have been used for medicinal purposes in China, India, the Near East and Europe since ancient times.[1] Pharmaceutical market sectors is growing continuously and rapidly. But, still the new drug discovery is push and demand. The reason behind this more and more upcoming disorders and growing number of drug–resistant infectious disease. The marine resources are nowadays widely studied, because marine sources decrease the side effects. Ocean covers more than 70% of the world surface. The attempt and efforts to extract drugs from the sea started in the late 1960s. The ocean is considered to be a great reservoir of biodiversity. The adaptability of marine-derived fungi to oceanic conditions can be considered an attractive point in the field of fungal marine biotechnology.

Cancer continues to be one of the major causes of death worldwide and only modest progress has been made in reducing the morbidity and mortality of this disease.[2] Cancers may be caused by three ways, namely, incorrect diet, genetic predisposition, and via the environment. Cancer may also be initiated by carcinogens, radiation, tobacco smoke, chemicals or infectious agents, particularly some viruses. As long as 20–30 years may take to develop cancer. As many as 95% of all cancers are caused by life style, and Current estimates from the American Cancer Society and from the International Union against Cancer indicate that 27 million cases with 17 million deaths 2030.[3] The search for new biomedical from marine organisms resulted in the isolation of approximately 10000 metabolites, many of which showed anti cancerous and pharmacological properties. A broad spectrum of biological activities has been detected, such as, antifungal, toxic, cytotoxic, antimitotic, neurotoxic, antibiotic, antiviral and antineoplastic. In recent years, new targets have been added to the familiar and general screening, e.g. AIDS, anti-inflammation, Alzheimer disease, immunosuppression, ageing processes and some tropical diseases.[4]

Unique properties of marine environment and their relevance to marine fungi

A consideration of the unique features of the marine environment is important for marine biotechnology for several reasons: 1) The physical factors that influence the marine fungi most are a) salinity and pH, b) low water potential, c) high concentrations of sodium ions, d) low temperature, e) oligotrophic nutrient conditions and f) high hydrostatic pressure.2) A good understanding of the ecosystem will help prospect for novel genes and 3) biotechnological production processes are influenced by the special adaptations of organisms to their environment. In the oceans, fungi live as saprophytes, parasites and symbionts on various matrices such as sea, sand, logs, water, as well as algae and other microorganism.[5]

Marine derived fungi have been rich sources of structurally novel and biologically active secondary metabolites, and become an important resource for new chemicals in drug discovery.[6,7] Marine fungi compare with terrestrial fungi that have become an important target group for pharmaceuticals and enzymes. They have been isolated from deep-sea sediments.[8,9] Because marine fungi form an ecological, and not a taxonomic group.

Marine fungi have attracted great attention as considerable resources only since the late 1980. In the last decade, there has been dramatic increase in the number of preclinical anticancer lead compounds extracted from metabolites of marine derived fungi.[10]

The obligate marine fungi grow, sporulate exclusively in a marine or estuarine habitat, and their spores are capable of germinating in sea water.

About 800 species of obligate marine fungi belonging to the Fungi have been reported so far.[11]

Historical perspectives of laccases

Laccase was first discovered in the sap of the Japanese lacquer tree *Rhus vernicifera,* and it has a characteristic feature as a metal containing oxidase and it was discovered by Bertrand in 1985.[12]

Laccase from fungi *Monocillium indicum* was the first laccase to be characterized from an ascomycete showing peroxidase activity.[13]

Laccase belongs to the small group of enzymes, are called the blue multi copper oxidases. In the biotechnology area, fungal laccases have more applications. They are ranging, removal of phenolics from wines, organic synthesis, biosensors, effluent decolouration and pulp bleaching detoxification, complex medical compounds synthesis and dye transfer blocking functions in detergents and washing powders most have been patented.[14]

Laccase importance for treating cancer

Studied the cytotoxicity activity of laccase from *Abortiporus biennis* using such as MCF-7 (human breast adenocarcinoma) and Hep G2 (human hepatic carcinoma). This may tumor cell inhibit proliferation *in vitro*. The cell growth inhibition data indicate that laccase significantly inhibits growth of leukemia and solid tumour cells.[15] also studied the anticancer activity of laccase from *Agrocybe cylindracea* against different tumour cell lines.

Laccase has many applications like medical diagnosis, industry of pharmaceutical. It plays a vital role in the preparation of anti cancerous drugs.

The use of laccase in pharmaceutical industry is in progress and is growing very fast. Laccases are used for several novel compounds organic synthesis that shows beneficial antibiotic properties, Increasing anti oxidant, antiproliferative activity.[16,17] In fungi, the molecular techniques have now used the identification of regulators of laccase by methods such as insertional mutagenesis and complementation of mutants.

Laccase Production in different Natural Sources

Laccases are wide spread in fungi, higher plants, bacteria and insects. Around 60 fungal strains, belonging to various classes such as *Ascomycetes, Deuteromycetes, Basidiomycetes* and, have been identified to produce laccase.[18]

Some of the Laccase producing organisms

Laccase: fungial sources

The majority of laccases have been attained and characterized from fungi. Some Important and laccase producers of e.g., *Neurispora crassa, Agaricus bisporus, Coprinus cinereus, Botrytis cinerea, Pycnoporus cinnabarinus, Cerrena unicolor, Pleurotus ostreatus ect*. (Table 1)

Table 1: Laccase from different fungal sources

S. No	Fungi	Reference
1	*Pycnoporus cinnabarinus*	19
2	*Trametes hirsute*	20
3	*Lentinus edodes*	21
4	*Cerrena unicolor*	22
5	*Agaricus bisporus*	23
6	*Botrytis cinerea*	
7	*Coprinus cinereus*	24
8	*Neurispora crassa*	25
9	*Phlebia radiate*	26
10	*Pleurotus ostreatus*	27
11	*Picnoporus cinnabarius*	28
12	*Trametes* (Coriolus, Polyporus) versicolor	29
13	*Trichoderma atroviride*	30
14	*Trichoderma. Harzianum*	

Laccase: bacterial sources

Bacterial laccases would be significantly important they are *Azospirillum lipoferum Streptomyces lavendulae Sterptomyces cyaneus Marinomonasmeditterranea***.** (Table 2)

Table 2: Laccase from different bacterial sources

	Bacteria	
1	*Azospirillum lipoferum*	31
2	*Streptomyces lavendulae*	32
3	*Sterptomyces cyaneus*	33
4	*Marinomonasmeditterranea*	34
5	*Aquifex aeolicus*	35
6	*Bacillus subtilis(cotA)*	36
7	*Leptothrixdiscophora* SS1	37
8	*Escherichia coli(yacK)*	38
9	*Pseudomonas maltophila*	39
10	*Pseudomonas putida*	40

Laccase: insect sources

The laccase enzyme has also been characterized in different insects, e.g., *Papilio Drosophila, Diploptera, Bombyx, Calliphora, Musca, Orycetes, Manduca, Lucilia.* (Table 3)

Table 3: Laccase from different insect sources

	Insects	
1	*Bombyx*	41
2	*Calliphora*	
3	*Diploptera*	
4	*Drosophila*	
5	*Lucilia*	
6	*Manduca*	
7	*Musca*	
8	*Orycetes*	
9	*Papilio*	
10	*Phormia*	

Laccase: plant sources

The plants in which the laccase enzyme has been detected include, mango, peach, pine, mung bean, prune lacquer, and sycamore *Acer pseudoptalanu*, *Rhus vernicifera, Aesculus parviflora.* (Table 4)

Table 4: Laccase from different plant source

S. No	Plants	Reference
1	*Acer pseudoptalanu*	42
2	*Rhus vernicifera*	43
3	*Aesculus parviflora*	44
4	*Zea mays*	45

Production of Fungal Laccases

Laccase production is carried out using solid state fermentation (SSF) and submerged fermentation (SmF). Solid state fermentation the organisms grow in the absence and near absence of free flowing water. Whereas in submerged fermentation allows the organisms to grow in the presence of a liquid medium. The two fermentation techniques have their own advantages and disadvantages.

Submerged fermentation is more advantageous because the process can be controlled easily in these systems. The microorganisms are cultured in an oxygen rich liquid medium in this fermentation. When fungal cell grows, formation of mycelium occurred which hinders impeller action, due to this obstruction occurring in oxygen and mass transfer. Broth Viscosity is the major problem associated with the fungal submerged fermentations. For dealing with this problem, different methods have been employed. For obtaining high efficiency, bioreactor functions in continuous manner. The most effective way of producing laccase is Fed-batch operation through which the highest laccase activity can be obtained.

Laccases are generally produced during the secondary metabolism of different fungi growing on natural substrate or in submerged culture.[46] Various cultivation parameters such as carbon, nitrogen source and concentration of microelements effect important for laccase production. Different agricultural wastes such as barley bran, grape seeds,[47] cotton stalk, molasses waste water and wheat bran[48,49] are also used as substrate for laccase production.

Influence of Activators and Inhibitors on Laccase Activity

The activity high in the medium containing maize and rice bran, these are the carbon sources. Nitrogen sources like Yeast extract enhance laccase production, ammonium sulphate in the medium the laccase activity increase many folds.

It has been reported that there are some elements which inhibit the activity of laccase such as azide, thioglycolic acid, and diethyldithiocarbamic acid all inhibited laccase activity. Small anions such as halides, iodide, azide, cyanide, and inhibit laccases activity whereas EDTA affected laccase activity to a lesser extent. Other inhibitors include metal ions, fatty acids, sulfhydryl reagents, hydroxylglycine, kojic acid, and cationic quaternary ammonium detergents.

Purification of Laccase

Various protein purification techniques are frequently employed in purifying laccase. Typical purification protocols involve ultra filtration, ion exchange, gel filtration, electrophoretic, or other hydrophobic interaction and chromatographic techniques.

Properties of Laccases

Urgent need for novel anticancer drugs has paved way for the usage of mushrooms and anti-cancer properties, with their active compounds. Benefits of these medicinal mushrooms have been assessed by various clinical trials.[50] Reported the potential of hispolon, a phenolic compound from *Phellinus linteus* to induce apoptosis of breast cancer cell. Similarly[51] reported the use of polysaccharide extract from *Pleurotus ostreatus* on HT-29 colon cancer cells *Grifola frondosa* or Maitake enhances the efficacy of anti-cancer agent cisplatin, checking the number of decreased immunocompetent cells.[52] *Hypsizygus ulmarius* is rich in antioxidants and proved for its anti-diabetic activity.[53]

For the used medical purposes most compounds of interest are synthesized on an industrial scale and are packaged and administered in ways that maximise the potential benefit.[54] Studied the cytotoxicity activity of laccase from *Abortiporus biennis* using tumour cell lines such as human breast adenocarcinoma and human hepatic carcinoma. The cell growth inhibition data indicate that laccase significantly inhibits growth of leukemia and solid tumour cells.[55] Also studied the anticancer activity of laccase from *Agrocybe cylindracea* against different tumour cell lines.

Schizophyllum commune has yielded Schizophyllan which has been researched clinically for anticancer activity.[56] *Trametes versicolor* have produced protein-bound polysaccharides PSK and PSP (polysaccharopeptide) from different strains of mycelia. PSK is a gastric cancer adjuvant in Japan. Their property to act on a range of substrates and also to detoxify a range of pollutants have made them to be usable for several purposes in many industries including textile, paper, and pulp, petrochemical industries. Recent investigations on marine filamentous fungi looking for biologically active secondary metabolites indicate the tremendous potential of them as a source of new medicines.[57]

Based on the purified proteins, the structure and physico-chemical properties of fungal proteins were studied. More than 100 laccases have been purified from fungi and been more or less characterized yet. An important feature is the high level of glycosylation which may contribute to the high stability of the enzyme.[58] Several laccase isoenzymes have been detected in many fungal species. Until recently, the three-dimensional structure of five fungal laccases has been reported.

Applications of Fungal Laccase

Pharmaceutical sector

Laccase has many applications, like medical diagnosis, and used synthesis of several products in the pharmaceutical industry.[59] The first chemical of the pharmaceutical importance that has been prepared using laccase enzyme is actinocin that has been prepared from 4-methyl-3-hydroxyanthranilic acid. This compound has anticancer capability and works by blocking the transcription of DNA from the tumor cell.[60]

It is also used in the preparation of some important drugs, like anticancer drugs. Laccase can be used in the synthesis of complex medical compounds as anti-inflammatory, anesthetics, sedatives, antibiotics, etc.[61-64] including, vinblastine, mitomycin, penicillin, cephalosporins, and dimerized vindoline. Also, laccase has been reported to possess significant HIV-1 reverse transcriptase inhibitory activity.[65] More recently, cosmetic and dermatological preparations containing proteins for skin lightening have also been developed.[66] Protein engineered laccase may be used to reduce allergenicity.[67]

Commercial sector

Laccases play an important role in different industries like food, cosmetics, synthetic chemistry, soil bioremediation, biodegradation of environmental phenolic pollutants and removal of endocrine disruptors.[68] These enzymes are used for waste detoxification, pesticide degradation, organic synthesis, biosensor and analytical applications.[69]

Laccase is receiving much attention of researchers around the globe. They have been applied to nanobiotechnology, are the developing research field and without additional cofactors, catalyzes electron transfer reactions. Several methods have been improved recently for the immobilization of biomolecule such as self-assembled monolayer, micropatterning, and layer-by layer technique which immobilize laccase and preserve their enzymatic activity. Laccase has also applications in the agriculture area by clearing some explosives in soil. And also added in some cosmetics to reduce their toxicity.[70]

Future Perspectives

This review summarizes that fungal laccases has a great potential application in several areas of Pharmaceutical, and Commercial field. India has one of the highest cancer rates in the world. The frequency of breast cancer is increasing, approximately 80,000 new cases diagnosed annually. This enzyme has become research area for breast cancer. Findings are necessary due to the lack of chemotherapeutic agents of some forms of malignant cancer.

CONCLUSION

In order to control breast cancer one possible alternative is the use of laccase from marine fungi, this review highlights, High concentrations of the laccase enzyme can be produced with proper optimization of various process conditions by using marine fungal sources.

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