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Bioactive enzymes from Marine and Mangrove fungi - Overview.

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Abstract

Microbial cells produce a variety of enzymes and help in microbial growth and respiration including other cellular activities. At times, these enzymes may themselves become fermentation products, so that one of them is specifically interested in obtaining high level of the enzymes. The use of enzymes in food preservation and processing predates modern civilization. Fermentation of common substrates such as fruits, vegetables, meat and milk provide a diverse array of food in the human diet. Beer, wine, pickles, sausage, salami, yogurts, cheese and buttermilk are all fermented products. Fungi are good candidate for employing them in degrading refractory substrates, cellulose, lignin, chitin, keratin and other substrates. Fungi like *Aspergillus nige*r and *A. oryzae* are regarded as safe by the food and drug administration.

Fungi play an important role in decomposition of natural substrates in marine and mangrove ecosystem. The fungi from mangroves are mainly used in enzyme technology, biochemical, agricultural, pharmaceutical, molecular biology and other applied research fields.

Keywords: Enzymes, Fungi, Marine and Mangrove ecosystem.

Introduction

Fungal biotechnology has become an integral part of human welfare. Nature represents a formidable pool of bioactive compounds and is more than ever a strategic source for new and successful commercial product. Among the microorganisms, fungi are well recognized to produce a wide variety of most valuable pharmaceutical chemicals, agrochemicals and industrial products. Recent advances made in genomics, proteomics and combinatorial chemistry show that nature maintains compounds that are the essence of bioactivity, within the host and environment. So the major challenging task is to explore the unexplored fungal wealth in our country and reveal their potential applications.

The screening of marine fungi for novel bioactive compounds has yielded several novel metabolites, some of which are being commercially developed for medicinal or agricultural use. Sadly the data generated by pharmaceutical companies in screening for bioactive compounds if often 'lost' to science due to the need for industrial secrecy. Fungal enzymes are widely used in industry and, many vitamins and food supplements rely on fermentation processes using terrestrial fungi. Due to their slow growth rates it is unlikely that marine fungi will replace their faster – growing terrestrial counterparts in this respect.

Many important industrial products are now produced from fungi using fermentation technology. A wide range of enzymes are excreted by fungi and play an important role in the breakdown of organic materials and many of these enzymes are now produced commercially. Most of these enzymes are used in food processing. Fungi are good candidate for employing them in degrading refractory substrates, cellulose, lignin, chitin, keratin and other substrates. Fungi like *Aspergillus nige*r and *A. oryzae* are regarded as safe by the food and drug administration.

Microbial cells produce a variety of enzymes and help in microbial growth and respiration including other cellular activities. At times, these enzymes may themselves become fermentation products, so that one of them is specifically interested in obtaining high level of the enzymes (Bell et al., 1972). Qualitative screening of degrading enzymes in marine fungi was reported by Rohrmann and Molitoris (1992).

The use of enzymes in food preservation and processing predates modern civilization. Fermentation of common substrates such as fruits, vegetables, meat and milk provide a diverse array of food in the human diet. Beer, wine, pickles, sausage, salami, yogurts, cheese and buttermilk are all fermented products. Irrespective of their origin, these fermented food products are, in fact, result of the enzymatic modification of constituents in the substrate. The use of enzymes in food industry also involves a range of effects including the production of food quality attributes such as flavors and fragrances and control of colour, texture, and appearance besides affecting their nutritive value.

Fungal biotechnology

Amylase from fungi

The decomposition of starch by marine fungi was demonstrated by Barghoorn (1944) for the representative of the genera Ceriosporopsis, Corollospora, Lulworhtia, Phialophorophoma and Zalerion and by Nilsson (1974) for Humicola alopallonella. Denaturation of bacterial and fungal - amylase by heat acid and urea was also investigated in the presence of added calcium ions (Hagihara et al., 1956).

Iqbal and Zafar (1994) reported a new matrix petiolat felt- sheath of palm (Livistona chinesis) immobilize the cells of Aspergillus niger for the production of aplha amylase. Growth of immobilized culture was 19% greater than free cells. Different types of immobilized cell systems have also been used for Glucoamylae (GA) production (Li et al., 1984; Fiedurek and Szczdrak, 1995; Shimada et al., 1998; Ariff and Webb, 1996). Mycelia of A. niger were immobilized on various seeds such as wheat rye, barley, mustrad etc., for GA production. Enzyme productivity was 1.6 times higher in immobilized system than by free cells (Fiedurek and Szczdrak, 1995). Shimada et al. (1998) described a system using immobilized cells of Saccharomyces cerevisiae for the production of GA. The system was capable of accumulating high quantity of GA. Ariff and Webb (1996) compared the influence of different fermenter configuration and modes of operation on GA production by *Aspergillus awamori.*

Goto et al. (1998) studied the amylase from fungi easy to manufacture then the amylases from the bacteria and Streptomyces. Among the fungi *Aspergillus oryzae* and *Aspergillus niger* have been well studied. Arora et al. (2000) studied that the potato waste was fermented by *Rhizopus oryzae* under solid substrate fermentation and yielded a dry biomass of 25 g containing 17- 18% protein and 70% dry matter digestibility (in - vivo) and 3.2 g crude alpha amylase enzyme. Mangrove derived fungi (35 sp.) was screened for amylase activity using starch agar and all the fungi showed zone of clearance on starch agar plates (Sivakumar and Ravikumar, 2006).

Protease from fungi

Pisano et al. (1964) screened 14 marine fungi for their geletinase activity and found such activity in the culture filtrate of 13 isolates, *Halosphaeria mediosetigera* produced the highest level of gelatinase. Sguros et al. (1973) concluded that the Culcitalana achtaspora, *Halosphaeria mediosetigera* and *Humicola alopallonella* were probably insignificantly proteolytic, lipolytic, nucleolytic or ligninolytic.

The extracellular protease production was studied in many Aspergillus species. Klapper et al. (1973a,b) reported about this enzyme production and the factors affecting their synthesis and release from *A. oryzae* NRRL 2160. Fukushima et al. (1989) studied continuous protease production in a carbon limited chemostat salt tolerant *A. oryzae*. In the same way Battaglino et al. (1991) also reported the culture requirements for the production of protease by *Aspergillus oryzae* in solid-state fermentation.

Alkaline protease production from *Aspergillus niger* was also reported by Singh et al. (1973); Bathomeuf et al. (1992). Monod et al. (1991) also studied the enzyme production in *Aspergillus fumigatus*, *A. sojae* by Nausho and Ohara (1971), *A. nidulans* by Stevens (1985), Cohen (1973) and *A. melleus* (Luisetti et al., 1991). Malathi and Chakraborty (1991) reported about the production of alkaline protease from new *Aspegillus flavus* strain by solid substrate fermentation.

Dahot (1993) reported that *Penicillium expansum* was grown on 1% rice husk fine powder medium and along with 1% glucose, raffinose, maltose and molasses and corn steep liquor for the production of protease. The other Penicillium species like *P. lilacinces* and *P. griseofulvin* was also known to produce alkaline protease reported by Kitano et al. (1992). Dozie et al. (1994) studied the production of alkaline protease by *Chrysosporium keratinophilum.*

Aspergillus ustus (NIOCC 20) producing the highest amounts of the enzyme was selected for further studies. The growth yield was substantial at 30 C and 50 C at 1 bar and elevated hydrostatic pressures. The fungus produced alkaline, cold-tolerant protease when grown at 30 C and 1 bar pressure. The enzyme was active at combinations of 30 C and 50 C and 300 bar pressure. The enzyme was totally inhibited in the presence of 2 mM PMSF suggesting it to be a serine protease (Damare, et al., 2006).

Cellulase from fungi

Barghoorn (1944) was first to use marine Ascomycetes and Deuteromycetes to demonstrate their ability to grow on wood flour and regenerated cotton cellulose by measuring the rate of radial growth on agar medium. The clearing of cellulose – contianing agar by 14 marine fungi was also used by Henningsson (1976) as a measure of cellulase and xylanase production. Nilsson (1974) employed several methods to assay the enzymatic activities 0f 36 wood inhabitating fungi, among them one marine species, namely, *Humicola alopallonella*. 12 of these fungi unable to degrade pure cellulose substrates in culture but produced characteristic soft-rot patterns, namely cavities in the secondary cell walls of wood.

A number of fungal species are known to produce cellulase enzymes. Among these are the Ascomycetes such as Neurospora and Trichoderma. Shoemaker et al. (1983) reported a variety of cellulase produced from Trichoderma. These enzymes are also produced by Sporotrichum, Humicola, Thermoascus, *Trichoderma ressei* and *T. koningii. Penicillium funiculosum* is a potent cellulase producer, has been studied earlier for various applications including cellulolysis of various cellulose substrates (Dighe et al., 1987; Betrabet and Paralikar, 1977).

Cellulolytic enzyme system can be produced by a number of different fungi, such as white rot fungi (Uzcategui et al., 1991; Thompson et al., 1998) soft rot - fungi (Kubicek et al., 1990) and anaerobic fungi (Barichievic and Calza, 1990).

Pectinase from fungi

Raghukumar et al. (1994) have investigated degradative enzyme pectin lyase production by fungi isolated from detritus of the leaves of the mangrove *Rhizophora apiculata.* Twenty-one higher filamentous fungi isolated from *Spartina alterniflora* and other salt march substrata were shown to capable of degrading cellulose, lipids, starch including pectin compounds (Gessner, 1980).

Lipase from fungi

Lawrence (1967) and Barockerhoff and Jensen (1974) have presented its comprehensive reviews. These lipases are being exploited due to their low cost of extractions, thermal and pH stability, substrate specificity, and activity in organic solvents. The chief producers of commercial lipases are *Candida cylindracea, Humicola lanuginose, Rhizopus delemar, R. japonicus, R. niveus* and *R. oryzae* (Godfredson, 1990).

In 1981, one group highlighted the lipolytic activity of thermophilic fungi of paddy straw compost (Satyanarayana and Johri, 1981). Systematic screening strategies were employed by (Bhaduria, 1989). This study reported *Aspergillus niger, Aspergillsus flavus, A. fumigatus* and *Penicillium glaucum* as the potential lipase producers isolated form the kernels of Chironji and Walnut.

Yadav et al. (1997) purified and characterized of a regiospecific lipase from *Aspergillus terreus.* The purified enzyme showed excelled temperature tolerance and was highly thermo stable. The enzyme showed good pH tolerance. Ionic detergents inhibited enzyme activity where as non-ionic detergents stimulated enzyme activity.

Lazer and Schroder (1992) investigated fungal lipases, which degrade lipids from palm oil. Among Mucorales, the lipolytic enzymes of the *moulds Mucor hiemalis, Mucor miehei, Mucor lipolyticus, Mucor pusillus, Rhizopus japonicus , R. arrhizus, R. delear, R. nigricans, R. microsporus* and *R. ch*inesis have been studied.

Kamini et al. (1997) studied the fungal strain isolated from curd. The fungal strain was identified as *Aspergillus niger*. Tributyrin was the substrate for examining lipase production on agar plates. A holozone of 9mm diameter around a colony in the tributyrin agar plate clearly indicated the production of lipase. The initial lipase activity was 8U ml-1 at 72 hrs in the culture supernatant of the basal medium, which indicated the extra cellular nature of the lipase.

Prabhakar et al. (2002) reported the effect of cultural conditions on the production of lipase by Fungi. They found that selected organism *Aspergillus niger, A. flavus, A. japonicus* and a fungi isolated from the contaminated ghee belonging to the genus, Aspergillus spp. were tested for the production of lipase on four different media by submerged fermentation technique.

Xylanase from fungi

Nilsson (1974a) demonstrated a xylanase in *Humicola alopallonella,* whereas mannose was absent. Barghoorn (1944) found that d –xylose, produced by the hydrolysis of xylan, was used by the 8 species of marine fungi tested. Pectin was used as a carbon by the same species Barghoorn (1944). Leightley and Eaton (1977) determined the ability to degrade wood cell wall components of several marine fungi belonging to the genera Cirrenalia, Culcitalana, Halosphaeria, Humicola, Nia and Zalerion. They compared fresh water and terrestrial fungi and found production of cellulase, xylanase and mannanase in all species tested.

Neurospora crassa has also the ability to ferment D – glucose, D- xylose and treated cellulosic substrates directly to ethanol (Deshpande et al., 1984). Most microbial hemicellulolytic system contain beta xylosidase, which has been purified and characterized from many fungi *Aspergillus niger* (Rodonova et al., 1983), *A. fumigatus* (Kitpreechavanich et al., 1986), *Trichoderma viride* (Matsuo and Yasui, 1984b), *Emericella nidulans* (Matsuo and Yasui, 1984a) and Chaetomium trilaterale (Uziie et al., 1985). Screening and production of xylanase enzyme required in the hydrolysis of different xylan was investigated using strains of 35 species of fungi isolated from mangrove samples (Sivakumar and Ravikumar, 2006a).

Phosphatase from fungi

Phosphatases fall into the category of “extracellular enzymes” which are secreted and actively pass through the cytoplasmic membrane, and are associated with the producers. So, their function is involved in chemical communication of microorganisms with the surrounding microenvironment.

Both alkaline and acid phosphatases have been found as external and internal enzymes in microorganisms (Siuda, 1984). There exists a relationship between pH and synthesis and release of phosphatase congregation of organisms producing the enzymes and phosphatase stability and conformation (Herbien and Neal, 1990).

Lignin degrading enzymes from fungi

Lignin is an amorphous high molecular – mass composed of phenylpropane subunits interconnected by variety of non – hydrolysable bonds. The relatively few groups of microorganisms that can degrade the macromolecule. The most efficient degraders are the white rot fungi (Orth and Tien, 1995; Paul and Clark, 1989).

Safari Sinegani et al. (1999) assessed the production of lignin – degrading enzymes by the imperfect fungi *Aspergillus terreus* and *Trichoderma reesei* and yeast in the N-ethyl alanine, benzyl alcohol and benzaldehyde. In contrast to low biomass of the yeast, the Mnp- and LiP activiites of this fungus were much higher of the Deuteromycetes *A. terreus* and *T. ressei.* Among the fungi *A. terreus* reduced pH of its culture media significantly. Laccase activity of *A. terreus* was higher than 2 and 1.35 time of *T. ressei* and the yeast respectively. All the fungi had the highest MnP and LiP activities and fungal biomasses were significantly low in the benzaldehyde treated media.

DeSouza-Ticlo et al. (2006) studied that the carbon and nitrogen sources in the growth medium play an important role in the production of lignin-degrading enzymes in the white-rot Basidiomyceteous fungi. The role of nutrient nitrogen sources in growth media on production of lignin-degrading enzymes namely laccase, lignin peroxidase and manganese peroxidase as well as on the decolorization of industrial effluents like black liquor, molasses spent wash and textile mill effluents was studied using the Basidiomycetous

fungus NIOCC No.2a isolated from mangrove wood. The amount of extracellular peroxidases increased by several fold in the presence of effluents whereas in their absence they were of negligible quantity. Some of the effluents had an inhibitory effect on laccase production.

Fungal metabolites

Amino acids, Lipids, and Fatty acids from fungi

Schafer and Lane (1957) demonstrated 12 amino acids in Lulworthia sp. and Perters et al. (1975) found the following common to 10 species of marine fungi; alanine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine,serine, threonine, tyrosine and valine.

Lipids are important fungal components both in terms of structure and membrane constitution. Many studies have demonstrated the importance of lipids for development, sporulation and germination and their involvement in various physiological process (Rattray, 1975; van Etten and Gottlies, 1965, Weete, 1980, 1981; Weete et al., 1973).

Most fungi contain 5 to 32% lipids depending on culture conditions, developmental stage and species. The lipid content of spores of many fungi ranges from 5 to 17% dry weight, but spores some species, such as rusts contain up to 35% lipid (Shen, 1966). The major factors influencing the extent of lipid production are the nature and proportion of carbon (C) and nitrogen (N) as nutrients sources in the medium. In fungi in general, lipids have been reported to be important for germination, in addition to having other functions (Cochrane et al., 1963; Owens, 1955; Turain and Bianchi, 1972). Smith and Silverman (1973) reported a 30 to 40% decrease in lipids during the early phase of germination. In a study on *Rhizopus stolonifer* (Weete et al., 1973) was observed that spore having a low concentration of lipid required a new synthesis of lipid during the early stages of spore germination compared to spores with a high concentration of lipid.

Linoleic acid has also been detected in large amounts in *Penicillium atrovenetum,* where it represents 66% of all fatty acids (van Etten and Gottlies, 1965). The study of fatty acid composition has been used for the identification of species of entomopathogenic fungi (Latge and Bievre, 1980; Tyrrell and Weatherston, 1976; Tyrrell, 1967, 1968, 1969). However, studies on lipid and fatty acids at the physiological and genetic level may permit the selection of strains for environmental persistence and expression at the epizootic level and may provide information for their large-scale production and utilization.

A separation of the two conjugated isomers may be obtained using the ability of lipases produced by fungus Geotrichum to selectively hydrolyse the cis – 9, trans – 11 –18; 2 methy ester (Hass, 1999).

Glyceride fatty acids, in particular, oleic, palmitic and linolic acids were isolated from *Corollospora maritima* and *Zalerion maritimum* by Block et al. (1973) and Kirk et al. (1974). A number of these and other fatty acids were also determined in *Buergenerula spatinae* and *Dendtyphiella salina* (Schultz and Quinn, 1973).

Szaniazlo and Mitchell (1971) compared the hyphal wall compositions of marine and terrestrial species of the genus Leptosphaeria and found qualitatively identical compositions in both groups. The walls consisted of glucose, mannose, galactose, glucosamine, amino acids, and traces of galactosamine.

Conclusion

Fungi play an important role in decomposition of natural substrates in marine and mangrove ecosystem. The fungi from mangroves are mainly used in enzyme technology, biochemical, agricultural, pharmaceutical, molecular biology and other applied research fields.

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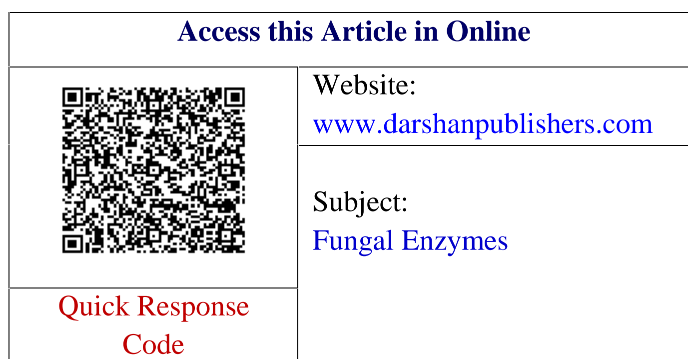

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