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FUNGI AS POTENTIAL SOURCE OF ENZYMES AND BIOACTIVE METABOLITES FOR ORGANIC SYNTHESIS AND MEDICINE

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Abstract. Fungi produce a large number of enzymes, including haloperoxidases. These enzymes are ubiquitous metalloenzymes that catalyse a variety of enantioselective oxygen-transfer reactions with hydrogen peroxide or alkyl peroxides. Advances have recently been made in using these enzymes to prepare, under controlled conditions, chiral organic molecules that are valuable for the synthesis of a wide range of useful com-pounds. The application of biocatalytic methods in asymmetric organic synthesis is of great interest as an alternative to chemical procedures employing chiral auxiliaries. Among the secondary metabolites which are found in fungi, many of these compounds show anti-cancer and other properties.

Key words: Fungi, enzymes, synthesis, bioactive, metabolites, medicine.

Introduction. Terrestrial and marine fungi and/or fungal endophytes are known to produce a diverse array of novel secondary metabolites, some of them featuring hitherto unprecedented new carbon frameworks, and many of them exhibiting interesting biological and pharmacological properties [1-3]. Higher fungi, in particular Basidiomycetes with their impressive metabolic diversity, open new avenues for the biotechnological production of many flavour and volatile molecules formerly thought to be plant-typical [4], including lipids and fatty acids [5, 6], cellulases [7], α- and β-glucosidases [8], laccase [9], lipase [10],

phospholipase [11, 12], lignin peroxidase, тyrosinase, peroxidase [13, 14], haloperoxidases [15], and other enzymes [16] and unusual natural compounds [17, 18].

Fungal enzymes. Fungal cellulases are hydrolytic enzymes that catalyze total hydrolysis of cellulose into sugars. These enzymes are produced by various groups of microorganisms, plant and animals. The cellulases from fungal species also find application in environmental bioremediation, food industry and molecular biology. Research work on cellulase has been done over the last ten decades, but there is no exclusive review available on the cellulases from fungal species [8, 19, 20]. The cellulolytic activity of fungi with special emphasis on the large extracellular enzyme complex called the cellulosome has been described [21]. The cellulosome is composed of a scaffolding protein, which is attached to various cellulolytic and hemicellulolytic enzymes, and this complex allows the organisms to degrade plant cell walls very efficiently. The enzymes include a variety of cellulases, hemicellulases, and pectinases that work synergistically to degrade complex cell-wall molecules [21].

Fungal α- and β-glucosidases catalyze the selective cleavage of glucosidic linkages and are an important class of enzymes having significant prospects in industrial biotechnology. These are classified in family 1 and family 3 of glycosyl hydrolase family. Therefore, α- and β-glucosidases are prospective toolbox in bioethanol production, and in the near future, it might be successful in meeting the requirements of alternative renewable sources of energy. β-glucosidases have been isolated from the fungus *Trichoderma* and other species [8, 22].

Laccases are blue multi-copper oxidases, which catalyze the monoelectronic oxidation of a broad spectrum of substrates, for example, *ortho*- and *para*-diphenols, polyphenols, aminophenols, and aromatic or aliphatic amines, coupled with a full, four-electron reduction of O_2 to H_2O. Hence, they are capable of degrading lignin and are present abundantly in many white-rot fungi. Interest in laccases has increased recently because of their potential use in the detoxification of pollutants and in bioremediation of phenolic compounds. These fungal enzymes can convert wood, plastic, paint, and jet fuel among other materials into nutrients. Some of these enzymes have already been harnessed in pulp and paper processing and in the synthesis of fine chemicals [9, 23].

Fungal lipases are class of enzymes which catalyze the hydrolysis of long-chain triglycerides. Some important lipase-producing fungal genera include *Aspergillus*, *Penicillium*, *Rhizopus*, *Candida*, etc. Current fermentation process techniques such as batch, fed-batch, and continuous mode of lipase production in submerged and solid-state fermentations were described recently [10, 24].

Phospholipases are enzymes that degrade phospholipids through hydrolytic cleavage of carboxy- and phospho-diester bonds. The enzymes are classified as phospholipases A_1, A_2, C or D depending on the site of hydrolysis at the sn-1 or sn-2 acyl ester bond, at the glycerol phosphate bond or at the glycerol phosphate-base phosphodiester bond, respectively. These enzymes have evolved to hydrolyse phospholipids at an organized lipid–aqueous interface. The use of phospholipases in industrial processes has grown hand-in-hand with our ability to clone and

express the genes in microbial hosts with commercially attractive amounts. Further, the use in industrial processes is increasing by optimizing the enzymes by protein engineering [11, 12, 25].

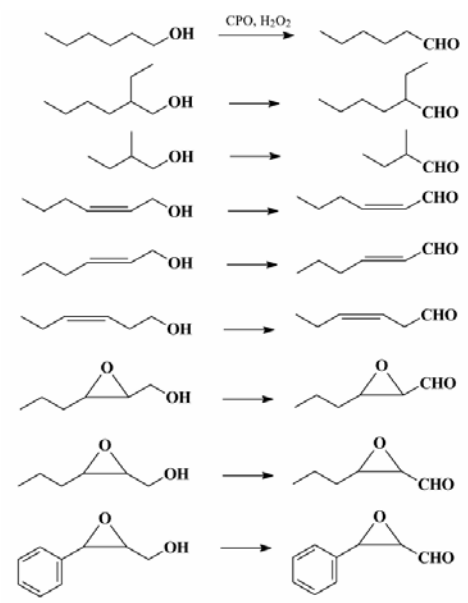
Lignin is the most abundant renewable source of aromatic polymer in nature, and its decomposition is indispensable for carbon recycling. It is chemically recalcitrant to breakdown by most organisms because of the complex, heterogeneous structure. The white-rot fungi produce an array of extracellular oxidative enzymes that synergistically and efficiently degrade lignin. The major groups of ligninolytic enzymes include lignin peroxidases, manganese peroxidases, versatile peroxidases, and laccases. Lignin peroxidases have the unique ability to catalyze oxidative cleavage of C-C bonds and ether (C-O-C) bonds in non-phenolic aromatic substrates of high redox potential. Manganese peroxidases oxidize Mn^{+2} to Mn^{+3}, which facilitates the degradation of phenolic compounds or, in turn, oxidizes a second mediator for the breakdown of non-phenolic compounds. Versatile peroxidases are hybrids of lignin peroxidase and manganese peroxidase with a bifunctional characteristic [13, 14, 26].

Tyrosinases are copper-containg enzymes which catalyse the o-hydroxylation of monophenols and subsequent oxidation of o-diphenols to quinones. The enzymes are involved in the pigmentation and are important factors in wound healing and primary immune response. Tyrosinases are found in prokaryotic and eukaryotic microorganisms, in mammals, invertebrates and plants. *Streptomyces* is included in the Streptomycetaceae family and represents one of the most important genus of the Actinomycetales order due to its impressive number of species and their practical role. Members of this genus were deeply studied because of their capacity to produce antibiotics and enzymes of industrial importance as glucose isomerase, protease, amylase, xylanase, while their capacity to produce tyrosinase was reported [27].

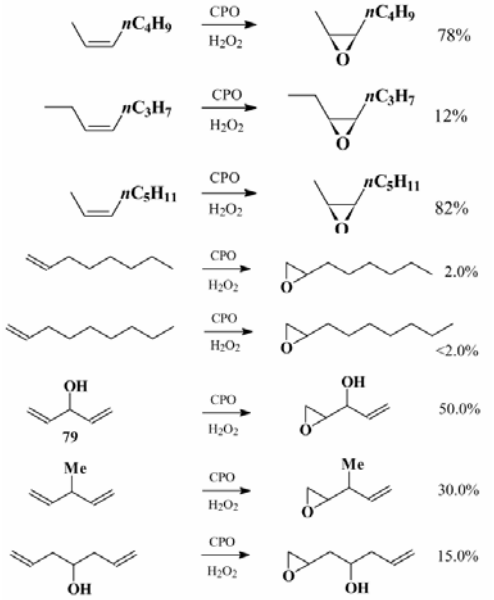
Biological systems have evolved haloperoxidase enzymes to catalyze the oxidation of chloride, bromide and iodide by hydrogen peroxide. Three classes of haloperoxidases have been identified. The first is a class of enzymes found in bacteria without a prosthetic group. The second is heme containing peroxidases such as chloroperoxidase (CPO) first discovered in the marine fungus *Caldariomyces fumago* in 1966. The third class of haloperoxidases is vanadium-containing peroxidases that require a vanadate ion (VO_4^{-3}). Vanadium peroxidase was first discovered in the brown alga Ascophyllum nodosum in 1984 but since then it has also been found in lichen *Xanthoria parietina* and in fungi [15].

CPO from the fungus *C. fumago* also catalysed the oxidation of primary alcohols selectively to the corresponding aldehydes (Scheme 1) in a biphasic system of hexane or ethyl acetate and a buffer (pH = 5.0) [28].

The CPO-catalysed epoxidation recently discovered by Colonna et al. [29] and Allain et al. [30] proceeds in high chemical and optical yields. Highly enantioselective epoxidation of the disubstituted alkenes with hydrogen peroxide catalysed by CPO provided the R epoxides preferentially. All the data support the view of oxygen delivery from the ferryl oxygen directly to the substrates (Scheme 2).



Scheme 1



Scheme 2

Fungal bioactive metabolites. Fungi-derived natural products have been an excellent source of pharmaceuticals as well. Fungi produce metabolites belonging to highly diverse structural classes, including aromatic compounds, amino acids, alkaloids, anthracenones, butenolides, cytochalasans, macrolides, naphthalenones, pyrones, terpenes, peptides, etc.

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Резюме

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ГРИБЫ КАК ПОТЕНЦИАЛЬНЫЕ ИСТОЧНИКИ ФЕРМЕНТОВ
И БИОЛОГИЧЕСКИ АКТИВНЫХ МЕТАБОЛИТОВ,
ИСПОЛЬЗУЕМЫХ В ОРГАНИЧЕСКОМ СИНТЕЗЕ И МЕДИЦИНЕ

Грибы продуцируют большое количество биологически активных соединений, антибиотиков, а также ферментов: целлюлазы, a- и b-глюкозидазы, лакказы, липазы, фосфолипазы,

лигнин пероксидаза, пероксидазы, в том числе и галопероксидазы. Многие из этих ферментов используются в органическом синтезе для селективного окисления или восстановления органических соединений. Среди вторичных метаболитов, которые находятся в грибах, многие из этих соединений показывают анти-раковые, анти-бактериальные и многие другие активности. Многие грибы обладают не только ценными пищевыми, но и лечебными свойствами. В последние десятилетия грибами стали интересоваться как источником антибиотических, лекарственных средств, специфических ферментов. Все эти вопросы будут рассмотрены в лекции.

Ключевые слова: грибы, ферменты, синтез, биологически активные метаболиты.