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INDUCTION OF EXTRACELLULAR ENZYME PRODUCTION

Tadjieva Khosiyat Sultanovna,
Kosimova Hilola Tokhtapulatovna
Tashkent State Medical University
hosiattadzieva@gmail.com
kasimovakhilola@gmail.com

Abstract
Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds consisting of two or more fused benzene rings. Interest in the mechanisms of biodegradation and the fate of PAHs in the environment is due to their ubiquity, resistance to degradation, accumulation in soil and sediment, and toxic, mutagenic, and carcinogenic properties. Some PAHs are classified as priority pollutants by the U.S. and several other national environmental agencies. PAHs are hydrophobic compounds; their stability within ecosystems is a consequence of their low solubility in water. The discharge and accidental release of PAHs into the environment poses a serious problem, especially when the biodegradative activity of natural microflora is insufficient to remove or neutralize pollutants. Currently, active research is underway to improve technologies for the bioremediation of soils and water contaminated with PAHs. The use of microorganisms represents a highly effective and relatively inexpensive technology for the detoxification of these hazardous pollutants, which can replace traditional methods. The development and effective use of bioremediation requires a comprehensive study of degrader organisms, metabolic pathways for PAH degradation, the enzyme systems that catalyze them, and the environmental conditions necessary to optimize the destruction of these compounds. Currently, the ability to metabolize PAHs has been identified in a number of bacteria, algae, cyanobacteria, and fungi. PAH-degrading fungi can be divided into two groups: non-ligninolytic and ligninolytic. The most active are fungi that naturally destroy the lignin component of wood (ligninolytic). These include mainly wood-dwelling (white rot fungi) and soil-dwelling litter-decomposing saprotrophs basidiomycetes and some species of ascomycetes. The enzymatic system of these fungi, which catalyzes lignin degradation, is extracellular, nonspecific, and oxidative, which allows them, in addition to the natural substrate, to metabolize a wide range of pollutants and their mixtures, giving them a significant advantage over bacteria and non-ligninolytic fungi. The main ligninolytic enzymes are lignin peroxidase, Mn-peroxidase, hybrid peroxidase (in the English version, versatile peroxidase) and laccase. Repression of enzyme synthesis does not occur when concentrations of substances are reduced to a level that is ineffective for their induction, and therefore they can degrade even low concentrations of pollutants.

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Introduction

The aim of this study is to identify the main patterns and mechanisms of regulation of PAH metabolism by ligninolytic fungi, and to characterize and function the enzymes that catalyze different stages of degradation.

To achieve this goal, the following tasks were formulated**:** and study of the influence of cultivation conditions on the degradation of three (phenanthrene, anthracene, fluorene) and four-ring (pyrene, chrysene, fluoranthene) PAHs and the production of ligninolytic enzymes by Pleurotus fungi ostreatus DI and Agaricus sp. F-8. Identification of major metabolites. Detection of emulsifying agent production during PAH degradation by P. ostreatus DI. Purification and determination of molecular (molecular weight, subunit composition, absorption spectra) and catalytic (pH optima, Km, Vmax, kcat for monoaromatic substrates, activity towards PAHs) properties of extracellular laccases from submerged and solid-phase cultures of P. ostreatus DI and Agaricus fungi sp . F-8.

Results of the Study

The combined role of laccase and peroxidase of the fungus in the degradation of these xenobiotics was established. Both enzymes can catalyze the oxidation of PAHs, which suggests their interchangeability in the initial attack of the molecules of these substances. It was shown that peroxidase is involved in the utilization of the formed metabolites. For the first time, the formation of an emulsifying substance was detected during the degradation of PAHs by P. ostreatus DI, correlating with the production of peroxidase and ensuring the availability of a hydrophobic substrate for the fungus. For the first time, a peroxidase with unusual catalytic properties that distinguish it from the typical hybrid peroxidase of B. fumosa and other known ligninolytic fungi was isolated from the white rot fungus P. ostreatus DI. peroxidases. The ability of the studied peroxidases to oxidize a wide range of synthetic dyes of the anthraquinone series was discovered, which significantly expands the catalytic capabilities of these enzymes. Surface and intracellular forms of laccases and peroxidase from P. ostreatus DI have been isolated and partially characterized for the first time. The potential for the surface forms of these enzymes to participate in the initial attack of a PAH molecule has been demonstrated. It has been discovered that the catalytically active intracellular forms of laccases and peroxidase not only serve as precursors to the extracellular and surface forms but also participate in the utilization of PAH metabolites entering the cell. The ability of ligninolytic fungi to metabolize a wide range of petroleum hydrocarbons has been established, both under experimental conditions and under conditions of real pollution.

Based on an analysis of literature data and a comparison with the results of our own research, conclusions were drawn for the first time, using P. ostreatus DI as an example, about the main physiological and enzymological features of PAH degradation by ligninolytic fungi. Degradation of three- and four-ring PAHs by the white rot fungus P. ostreatus DI and the soil saprotroph Agaricus sp. F-8. occurs according to the same scheme with the formation of quinones in the first stage. The formation and subsequent utilization of phthalic acid in the final stages of PAH metabolism by the fungus P. ostreatus DI suggests its inclusion in the main

metabolism. Lactase is produced in the early stages of PAH degradation by P. ostreatus DI, leading to the formation of quinones, while peroxidase is produced in later stages, leading to the utilization of the resulting metabolites. There is a dependence of the "depth" of PAH degradation on the set of ligninolytic enzymes produced by the fungus: the accumulation of quinones under conditions of laccase production and the formation and subsequent utilization of quinones under conditions of laccase and peroxidase production. The production of the emulsifying substance by P. ostreatus DI depends on the solubility of the studied PAHs and correlates with the production of peroxidase by this fungus. Extracellular forms of laccases and peroxidases isolated from submerged cultures of P. ostreatus DI can oxidize PAHs, suggesting their interchangeability in the initial attack on the molecule. Peroxidase oxidizes the metabolites of PAH degradation, whereas these compounds are unavailable to laccase. "Yellow" forms of extracellular laccases isolated from solid-phase cultures of P. ostreatus DI and Agaricus sp. F-8, are capable of oxidizing PAHs without a mediator in the reaction mixture. Peroxidase P. ostreatus DI has unique catalytic properties that distinguish it from the hybrid peroxidase B. fumosa 137 Karst and other known fungal ligninolytic enzymes. The molecular and catalytic properties of the extracellular and "surface" forms of laccases coincide and differ from those of the intracellular forms. Presumably, the intracellular forms are catalytically active precursors of the "surface" and extracellular forms.

Conclusions

The obtained data on the metabolic pathways, the enzymes that catalyze them, and the degradation products of PAHs are of significant interest to specialists in microbiology, biochemistry, and biotechnology. Information regarding the isolation, purification, and catalytic properties of the main ligninolytic enzymes is important for elucidating the mechanisms of their function and for creating biocatalysts based on them. The experimental data and methodological approaches presented in this study can be used by biological and biotechnology organizations engaged in xenobiotic degradation research and technology development, as well as in teaching biochemistry and microbiology courses at universities.

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