International Biodeterioration & Biodegradation 65 (2011) 470–478

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Purification and characterization of two extracellular peroxidases from *Streptomyces* sp. strain AM2, a decolorizing actinomycetes responsible for the biodegradation of natural humic acids

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ARTICLE INFO

Article history:
Received 5 December 2010
Received in revised form
15 January 2011
Accepted 25 January 2011
Available online 18 February 2011

Keywords:
HaP
Peroxidase
Humic acids
Lignin
Streptomyces
Biodegradation

ABSTRACT

Two extracellular humic acids peroxidases called HaP1 and HaP2 were isolated from the *Streptomyces* sp. strain AM2 and, based on MALDI-TOF MS analysis. The purified enzymes were determined as monomers with molecular masses of 40,351.11 and 25,175.19 Da, respectively. The N-terminal amino acid sequences of HaP1 and HaP2 were identified, and their optimum pH values were determined as 6 and 7.5, respectively. Standard 2,4-dichlorophenol (2,4-DCP) assays showed that both enzymes had maximal activity at 55 °C. HaP2 was stable at 55 °C for more than 24 h and had a half-life of 90 min at 65 °C. Although the catalytic properties of HaP1 and HaP2 were nearly identical, their stabilities and Reinheitzahl (RZ) values were substantially different. Both peroxidases were found to be heme proteins that catalyzed the oxidation of a wide range of substrates in the presence of hydrogen peroxide (H_2O_2), with HaP2 exhibiting a broader range of substrate specificity. The characterization of peroxidase activity revealed activity against humic acids, guiacol, 2,4-DCP, L-3,4-dihydroxyphenylalanine, and 2,4,5-trichlorophenol as well as other chlorophenols in the presence of H_2O_2. However, the inhibition of peroxidase activity by the addition of potassium cyanide and sodium azide also indicated the presence of heme components in the tertiary structure of these enzymes.

1. Introduction

Humic substances (HS) are the most widespread and ubiquitous natural nonliving organic materials in terrestrial and aquatic environments and represent the major fraction of soil organic matter (Basfar et al. 2009). Moreover, HS comprise a physically and chemically heterogeneous mixture of biogenic, relatively high-molecular-mass compounds with mixed aliphatic and aromatic natures (Soleimani et al. 2010). Although the microbial degradation of HS – particularly of high-molecular-mass, aromatic moieties in humic acid and humin – plays an important part of humus turnover and is essential for maintaining the global carbon cycle (Albers et al. 2009), little work has been conducted on the microorganisms that decompose and recycle humic matter.

During the last few decades, various peroxidases have been frequently used as alternative options to harsh chemicals in a wide array of industrial and developmental processes. Of particular interest to the aims of the present study, peroxidases offer a promising and resourceful class of enzymes for industrial applications. Peroxidases [E.C. 1.11.1.x; donor: H_2O_2 oxidoreductase] are ubiquitous enzymes utilizing H_2O_2 or other peroxides to oxidize a second reducing substrate. Heme-containing peroxidases are involved in a variety of defense mechanisms toward pathogens based on the so-called oxidative burst, in which the levels of H_2O_2 and other reactive oxygen species (mainly superoxide) rapidly increase (Davies et al. 2008). These enzymes are commonly grouped in two major superfamilies: "plant" and "animal" peroxidases (Koua et al. 2009). The "plant peroxidases" superfamily also includes evolutionarily related, heme-containing peroxidases from fungi and bacteria and has been further subdivided into three classes based on cellular localization and function (Welinder et al. 1992). Class I represent intracellular enzymes, including yeast cytochrome *c* peroxidase, ascorbate peroxidase (APX) from plants, and bacterial gene-duplicated catalase-peroxidases (Passardi et al.

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doi:10.1016/j.ibiod.2011.01.009