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A thermostable humic acid peroxidase from *Streptomyces* sp. strain AH4: Purification and biochemical characterization

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ABSTRACT

An extracellular thermostable humic acid peroxidase (HaP3) was isolated from a *Streptomyces* sp. strain AH4. MALDI-TOF MS analysis showed that the purified enzyme was a monomer with a molecular mass of 60,215.18 Da. The 26 N-terminal residues of HaP3 displayed high homology with *Streptomyces* peroxidases. Optimal peroxidase activity was obtained at pH 5 and 80 °C. HaP3 was stable at pH and temperature ranges of 4–8 and 60–90 °C for 72 and 4 h, respectively. HaP3 catalyzed the oxidation of 2,4-dichlorophenol, commercial humic acid, guiacol, and 2,6-dichlorophenol (50 mM); L-3,4-dihydroxyphenylalanine (40 mM); 4-chlorophenol, 2,4,5-trichlorophenol, and 2,4,6-trichlorophenol (30 mM) in the presence of hydrogen peroxide. Sodium azide and potassium cyanide inhibited HaP3, which indicated the presence of heme components. These properties make HaP3 a potential strong candidate for future application in the elimination of natural humic acids in drinking water.

1. Introduction

Peroxidases [E.C. 1.11.1.x; donor: hydrogen peroxide (H_2O_2) oxidoreductase] are ubiquitous enzymes that require H_2O_2 or other peroxides to oxidize a wide range of reducing substrates. Heme-containing peroxidases are involved in a variety of defense mechanisms against pathogens based on the so-called oxidative burst, a rapid increase in the levels of H_2O_2 and other reactive oxygen species. These enzymes are commonly grouped into plant and animal peroxidase superfamilies (Koua et al., 2009).

The superfamily of plant peroxidases includes evolutionarily related, heme-containing peroxidases of fungal and bacterial origins and has often been further subdivided into three classes based on cellular localization and function. Class I encompasses intracellular enzymes, including yeast cytochrome *c* peroxidase, ascorbate peroxidase (APX) from plants, and bacterial gene-duplicated catalase-peroxidases (Passardi et al., 2007). Class II consists of secretory fungal peroxidases, such as lignin (LiP) and manganese (MnP) peroxidases from *Phanerochaete chrysosporium* (Yang et al., 2011) and *Arthromyces ramosus* peroxidase (ARP). Class III contains secretory plant peroxidases, such as those from horseradish (HRP), barley (BP), and soybean (SBP) (Qiu et al., 2010).

The superfamily of animal peroxidases differs markedly from plant peroxidases and has over 360 entries. A novel superfamily of heme-containing peroxidases, the so-called dye-decolorizing peroxidase (DyP-type) superfamily, has recently been identified (Sugano, 2009). The DyP-type superfamily of peroxidases consists of peroxidases that are unrelated to the superfamilies of known peroxidases and of which only few members have been well characterized, such as YcdB from *Escherichia coli* (Cartron et al., 2007; Sturm et al., 2006), BtDyP from *Bacteroides thetaiotaomicron*, and TfuDyP from *Thermobifida fusca* (van Bloois et al., 2010).

Despite this large amount of data on peroxidases, only few studies have so far reported on the isolation of peroxidases from actinomycetes (Fodil et al., 2011; Gottschalk et al., 2008; van Bloois et al., 2010). In fact, actinomycetes peroxidases have been detected both intra- and extracellularly (Ortiz de Orue Lucana et al., 2004) and are either heme (Ramachandra et al., 1988) or non-heme (Rob et al., 1997) proteins.

An extracellular peroxidase has, for instance, been isolated, purified, and characterized from *Streptomyces viridosporus*, a lignocellulose-degrading actinomycete (Gottschalk et al., 2003; Ramachandra et al., 1988). Nevertheless, little data is currently available on the purification and characterization of peroxidases from *Streptomyces* sp.

Although microbial degradation of humic substances, particularly high-molecular-mass aromatic moieties in humic acid and humin, is known to play an important role in the turnover of humus and the retention of the global carbon cycle (Aulenta

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