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REVIEW ARTICLE

STREPTOMYCES TYROSINASE: PRODUCTION AND PRACTICAL APPLICATIONS

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Abstract

Tyrosinases (monophenol, *o*-diphenol:oxygen oxidoreductase, EC (1.14.18.1) are copper-containg enzymes which catalyze 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 studied in a lesser extent.

Keywords: *Streptomyces* spp., phenol oxidases, tyrosinases

Introduction

The *Actinomycetes* are Gram positive bacteria having high G+C (>55%) content in their DNA. *Actinomycetes* were originally considered to be an intermediate group between bacteria and fungi but now are recognized as prokaryotic organisms.

The majority of actinomycetes are free living, saprophytic bacteria found widely distributed in soil, water and colonizing plants. Several species of *Streptomyces* genus produces bioactive molecules like antibiotics, pigments and many extracellular enzymes as glucose isomerase, amylase, cellulases and proteases. Their capacity to produce tyrosinase was studied in a lesser extent.

In addition, this group of actinomycetes is also able, when are cultivated on organic media, to synthesize and excrete dark pigments, melanin or melanoid, which are considered as an useful criteria in taxnomic studies (Zonova, 1965; Arai and Mikami, 1972).

Phenol oxidases are of great interest for many applications in biotechnology, food processing, medicine, and the textile and pulp and paper industry. The ability to oxidize various small molecular weight phenolic compounds in biopolymers, and the high reactivity of the primary oxidation products, also provide a basis for the wide application potential of tyrosinases and laccases.

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Tyrosinase and laccase catalyze oxidation of substrate using molecular oxygen as a terminal electron acceptor with concomitant reduction of oxygen to water. Tyrosinases are found in prokaryotic and eukaryotic microbes, in mammals, invertebrates and plants. The most extensively investigated tyrosinases are, however, from mammals (Kwon et al., 1987, 1988; Spritz et al., 1997; Kong et al., 2000b).

Streptomyces tyrosinases are the most thoroughly characterized enzymes of bacterial origin (Della-Cioppa *et al*., 1998a and 1998b; Matoba *et al.*, 2006). The first bacterial tyrosinases have been purified from cell extracts of *Streptomyces nigrifaciens* (Nambudiri et Bhat, 1972) and *Streptomyces glaucescens* (Lerch et Ettlinger, 1972).

Biotechnological conditions for tyrosinase production at streptomycetes

Numerous investigations have revealed that the production of tyrosinase by a microorganism in a growth medium is regulated by such factors as the genetics of the microorganism, the composition of the medium, the growth duration and temperature, pH, the presence of biosynthetic inhibitors, the density of tyrosinase-producing cells and the presence of enzyme inducers (Katz and Betancourt, 1988). Some microorganisms are capable of producing extracellular tyrosinases which are synthesized intracellularly prior to their transport and secretion into the growth medium (Bauman *et al.*, 1976). During bacterial growth in a complex medium, the enzymatic activities are induced in the stationary phase.

Screening tests applied to 13 strains of *Streptomyces sp.*, isolated from different samples of soils withdrawn from East Antarctica showed that 73 % of the strains have a good potential for producing tyrosinase. Analysis based on quantitative criteria that estimated the exogenous tyrosinase activity, released in the cultural medium after 72 h of submerged cultivation on liquid Gauze medium containing 1g/L tyrosine, put into evidence two strains with tyrosinase activity 2.0 and 1.19 times higher than the strain *Streptomyces* MIUG 4.88, which was used as control (Bahrim and Negoita, 2007; Bahrim *et al*., 2004).

In streptomycetes, tyrosinase biosynthesis is conditioned by the presence into the fermentative medium of tyrosine, the enzyme substrate, and Cu^{2+} ions as the enzyme major constituents. The biosynthesis process is regulated through induction in the presence of L-metionine and L- isoleucine, while NH_4^+ ions, as nitrogen source in the mineral media, act as repressors (Held T., Kutzner H., 1990; Ikeda K. *et.al*, 1996).

For most bacterial tyrosinases it is not known whether they are produced constitutively or inducibly. Tyrosinase synthesis by *Streptomyces glaucescens* is surprisingly not induced by tyrosine, but by other amino acids like phenylalanine, methionine and leucine (Baumann *et al.*, 1976).

The expression of the *Streptomyces castaneoglobisporus* tyrosinase is favoured by methionine and copper (Ikeda *et al.*, 1996), and the transcription of the *Streptomyces michiganensis* tyrosinase is induced by copper and repressed by ammonium (Held and Kutzner, 1990). Methionine is also the inducer of the tyrosinase from *Streptomyces antibioticus* (Betancourt *et al.*, 1992; Katz and Betancourt, 1988).

Enzyme structure

Tyrosinases (monophenol, o-diphenol:oxygen oxidoreductase, EC 1.14.18.1), often also called polyphenol oxidases, are copper containing metalloproteins. Copper proteins are typically classified to different classes, based on optical and electron paramagnetic resonance (EPR) spectroscopic features. Binding of dioxygen in the copper proteins includes mononuclear (type 1), dinuclear (type 3) and trinuclear (combination of type 2 and type 3) copper centres.

Type 1 and 3 coppers show absorption maxima at about 600 and 330nm, 345 nm, respectively, whereas type 2 copper has undetectable absorption (Gerdemann *et al*., 2002; Solomon et al., 1996). Type 1 and 2 coppers show an EPR spectrum, whereas type 3 copper gives no EPR signal due to a pair of copper ions which are antiferromagnetically coupled (Makino *et al.*, 1974; Bento *et al.*, 2006).

These enzymes are known as type 3 copper proteins having a diamagnetic spin-coupled copper pair in the active centre (Lerch *et al.*, 1986). Both copper atoms (CuA and CuB) are coordinated by three conserved histidine residues (fig.2) (Klabunde *et al.*, 1998), which are located in a "four α-helix bundle". During the catalytic cycle the 'type 3 copper centre' can adopt different functional forms: the *oxy*-state [Cu(II)-O_2^{2-}-Cu(II)], *deoxy*-state [Cu(I) Cu(I)], *half-met* state [Cu(I) Cu(II)] and the *met* state [Cu(II)-OH^--Cu(II)].

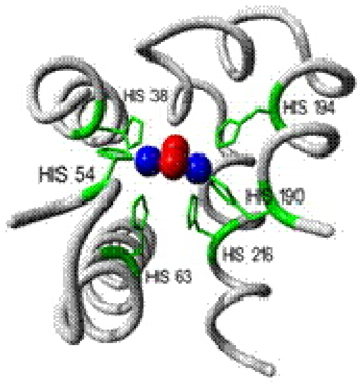

Figure 1. *Dioxygen binding and orientation of tyrosine at the active site of oxy form of Streptomyces tyrosinase. Coppers: blue, histidines: green, dioxygen molecule: red*

Some species of *Streptomyces* produce a melanin-like pigment. A gene that is responsible for the synthesis of melanin-like pigment has been cloned from a few *Streptomyces* species, such as *Streptomyces antibioticus* (Katz *et al.*, 1983; Bernan *et al.*, 1985), *Streptomyces glaucescens* (Huber *et al.*, 1985; Hintermann *et al.*, 1985), *Streptomyces lavendulae* (Kawamoto *et al.*, 1993), and *Streptomyces castaneoglobisporus* (Ikeda *et al.*, 1996). In these species the melanin operon consists of two parts: *mel*C1 which codes for a small helper protein and the tyrosinase structure gene *mel*C2.

Genetic and biochemical studies predominantly whith *Streptomyces antibioticus*, have shown that the *mel*C1 product MelC1 is responsabile for incorporation of Cu(II) into apotyrosinase, MelC2 (Lee *et al.*, 1988). In this case, the incorporation of copper has been suggested to be mediated through a complex formed between MelC1 and apotyrosinase (Chen *et al.*, 1992).

The crystal structure of *S. castaneoglobisporus* tyrosinase was established as a complex with the caddie protein ORF378, which consists of a six-stranded β-sheet and a single α-helix (Matoba *et al.*, 2006). ORF378 is suggested to act as C-terminal domain, as in catechol oxidase, shielding the active site. After dissociation of the caddie protein, the active site becomes accessible to substrates (Matoba *et al.*, 2006; Decker *et al.*, 2006).

The tyrosinases of bacterial origin are often reported to be extracellular enzymes, involved in melanin production (Claus and Decker, 2006). However, the extracellular bacterial tyrosinases do not have signal sequences, but their secretion is proposed to be assisted by a second protein having a signal sequence (Leu *et al.*, 1992; Tsai and Lee, 1998). Compared to plant and fungal tyrosinases, the bacterial tyrosinases also have a shorter sequence, typically encoding a mature protein of 30 kDa.

Substrates and reaction mechanism of tyrosinase

Tyrosinases are bifunctional enzymes it catalyzes two types of reactions in the presence of molecular oxygen: the *ortho*-hydroxylation of monophenols to its corresponding o-diphenol (monophenolase, cresolase activity) and the oxidation of diphenols to its correspondent *ortho*-quinones (diphenolase, catecholase activity). Quinones are highly susceptible to non-enzymatic reactions, which may lead to formation of mixed melanins and heterogeneous polymers (Lerch, 1983; Robb, 1984), (fig.2).

The catalytic mechanisms behind oxidation of a substrate typically involve formation of a reactive intermediate by the reaction of a reduced Cu^+ centre with molecular oxygen, which may also be incorporated to the substrate (Hatcher and Karlin, 2004).

Monophenols and o-diphenols have been considered as the exclusive tyrosinase substrates for a long time. However, aromatic amines and o-aminophenols have been also recognized as tyrosinase substrates (Claus and Filip, 1990; Gasowska *et al.*, 2004; Lerch, 1995).

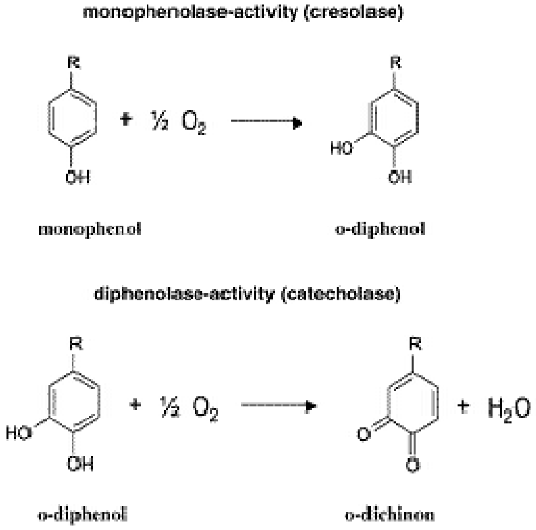

Figure 2. *Enzymatic activities of tyrosinases*

Physiological importance and applications

Tyrosinases are nearly ubiquitously distributed in all domains of life. They are essential for pigmentation and are important factors in wound healing and primary immune response. To date, the information on the physiological role of the tyrosinases in microbes has been limited. However, it has been proposed that melanin has a role in the formation of reproductive organs and spores and in cell wall protection after physical damage (Lerch, 1983).

In soil environments, extracellular tyrosinases are probably involved in the polymerization and detoxification of plant phenolic compounds and the formation of humic matter (Claus and Filip, 1988; Kutzner, 1968; Claus and Filip, 1990).

Melanins bind heavy metals that are otherwise toxic to the cells (Butler and Day, 1998). They also confer protection against oxidants, heat, enzymatic hydrolysis, antimicrobial compounds and phagocytosis and thus can contribute to microbial pathogenesis (Nosanchuk and Casadevall, 2003).

Presently there is an increasing interest in using tyrosinases in industrial applications: in the environmental technology for the detoxification of phenol-containing waste waters (Claus and Filip, 1988) , contaminated soils (Claus and Filip, 1990) and as biosensors for the monitoring of phenols; in cosmetic and food industries, because of either undesirable or beneficial oxidative browning reactions (Mayer and Harel, 1978).

Tyrosinases are also suggested to be potential tools in treating melanoma (Morrison *et al.*, 1985; Jordan *et al.*, 1999, 2001). Furthermore, the role of tyrosinase in neuromelanin production and damage of neurons related to Parkinson's disease has been extensively studied (Greggio *et al.*, 2005). Synthetic melanins have applications as protectives against radiation (UV, X-ray, gamma-ray), cation exchanger, carrier for drugs, antioxidants, antiviral agents and immunogens.

In conclusion, tyrosinases are exceptionally versatile enzymes and more investigations are needed for a better understanding of their physiological importance and to further define their great biotechnological potential.

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