

Biochemical characterization and identification of extracellular tyrosinase biosynthesis in *Streptomyces antibioticus*

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Tyrosinases are copper-containing enzymes which are ubiquitously distributed in all domains of life and are found in prokaryotic as well as in eukaryotic micro-organisms, and in mammals, invertebrates and plants. A tyrosinase-expressing *Streptomyces antibioticus* was isolated and it is identified based on biochemical tests like urase, phenol red fermentation, nitrate reduction, starch hydrolysis and gelatine given positive results and indole, methyl red vogues proskauer, citrate utilization, carbohydrate utilization, phenyl alanine deamination, casein hydrolysis, colloidal chitin hydrolysis and catalase test negative results indicated that presence of *Streptomyces antibioticus* and tyrosinase. The results obtained after comparative studies indicated that isolation media and methods used in the present study are not only simple and reliable for large-scale bacterial identification but at the same time are more costeffective compared to commercially available diagnostic kits. This newly isolated and characterized tyrosinase may have potential applications in organic synthesis due to its high activity and stability at typically denaturing conditions.

Keywords: *Streptomyces antibioticus*, biochemical tests, tyrosinase.

INTRODUCTION

Tyrosinase (monophenol, o-diphenol: oxygen oxidoreductase, EC 1.14.18.1) is a copper-containing metalloprotein that is ubiquitously distributed in nature. Tyrosinases are found in prokaryotic as well as in eukaryotic microorganisms, and in mammals, invertebrates and plants. Tyrosinase is a mono-oxygenase and a bifunctional enzyme that catalyzes the o-hydroxylation of monophenols and subsequent oxidation of o-diphenols to quinones (Lerch , 1983;and Robb, 1984). The activities are also referred to as cresolase or monophenolase and catecholase or diphenolase activities, respectively. Tyrosinase thus accepts monophenols and diphenols as substrates, and the monophenolase activity is the initial rate-determining reaction (Robb , 1984; and Rodriguez-Lopez et al, 1992).

Hence, tyrosinases gained industrial application potential at food and non-food processes (Ali et al, 1992) as well as in structure engineering of meat derived food products and tailoring polymers in material

science, for instance, grafting of silk proteins onto chitosan via tyrosinase reactions (Rodriguez-Lopez et al;1992 and Anhgileri et al, 2007). In view of the above, there is an increasing interest development of tyrosinases. Tyrosinases are widely distributed in nature and found in prokaryotic, eukaryotic microbes, mammals and plants, traditionally it was produced from plant-derived food products, such as tea, coffee, raisin and cocoa, where they produce distinct organoleptic properties (Bernan ,1985). Microbial tyrosinases production play a important role because of tyrosinases produced in fruits and vegetables are responsible for undesired browning reactions (Celensa et al, 1986;and Dalfard et al,2006), hence methods for regulating tyrosinase activity are essential for effective use in the food industry or search for alternative tyrosinases. Several microbial strains were isolated and characterized for tyrosinase production and most of the microbial strains are restricted at industrial or food application or even pharma sectors mainly due to multi-catalytic functions such as peroxidase and laccases in addition to tyrosinase activity. Few microbial strains characterized so far revealed tyrosinases with multi-catalytic functionality (Dastager et al,2006) and strains

Table 1. Biochemical identification of *Streptomyces antibioticus*

S.No	Biochemical test	Positive/ Negative
1.	Indole	Negative
2.	Methyl red	Negative
3.	Vogues Proskauer	Negative
4.	Citrate Utilization	Negative
5.	Carbohydrate utilization	Negative
6.	Phenyl Alanine De amination	Negative
7.	Urase	Positive
8.	Phenol Red Fermentation	Positive
9.	Nitrate Reduction	Positive
10.	Starch Hydrolysis	Positive
11.	Casein Hydrolysis	Negative
12.	Colloidal Chitin Hydrolysis	Negative
13.	Catalase Test	Negative
14.	Gelatin	Positive

having potential to produce only tyrosinase enzyme without laccase and peroxidase activities has been isolated from soil samples with better tyrosinase production ((Echigo et al,1997;and Freddi et al,2006)).

MATERIALS AND METHODS

Micro organism and its growth conditions

The isolated tyrosinase producing microbial strain, *Streptomyces antibioticus* RSP – T1 is used in this study. The strain was maintained in precultured medium containing nutrient broth – 8.0 g L⁻¹, extract of meat – 10.0 g L⁻¹, peptone – 10.0 g L⁻¹, tryptone – 10.0 g L⁻¹, agar – 20.0 g L⁻¹ and NaCl - 0.5 g L⁻¹ at 37 °C. The microbes were sub-cultured at regular intervals on slants and stored at 4 °C. The production medium adjusted to pH 6.5 consisted of yeast extract - 1.5 g L⁻¹, tryptone - 1.5 g L⁻¹ and NaCl - 5.0 g L⁻¹ along with 0.01 % (w/v).

Tyrosinase assay

The quantity of tyrosinase enzyme in the fermentation medium was measured using tyrosine as substrate which is specific for tyrosinase. The produced yellow color intensity was determined at 475 nm wavelength for the amount of tyrosinase present in the sample. One unit is defined as the amount of enzyme that catalyzes the appearance of 1.0 μmol of product per min at 37 °C.

Biochemical Assays

Biochemical tests like urase, phenol red fermentation, nitrate reduction, starch hydrolysis, gelatine, indole,

methyl red vogues proskauer, citrate utilization, carbohydrate utilization, phenyl alanine deamination, casein hydrolysis, colloidal chitin hydrolysis and catalase test were carried out according to the method described by the(Stanier et al, 1989) and were used primarily to distinguish between *Escherichia coli* and *Enterobacter aerogenes strains*.

RESULTS AND DISCUSSION

Though several microbial strains (*Streptomyces nigrificiens* (Gancedo,1998), *Streptomyces glaucescens* (Halaouliet et al,2005), *Streptomyces castaneoglobisporus* (Hong et al,1987), *Streptomyces antibioticus* ((Ikeda et al,1996;and Katz et al, 1983)) and *Streptomyces* sp. (Lantto et al, 2007) were reported in the literature on production tyrosinase, few characterized for laccase and peroxidase free tyrosinase (Echigo et al, 1997). Such tyrosinase without coexistence of laccase and peroxidase activities has significant role in pharma sector, especially in synthesis of L-Dopa as biosynthesis of melanin with tyrosinase is associated with transformation of tyrosine into L-Dopa as an intermediate which is finally converted to indol-5, 6-quinone via dopachrome. Owing to the isolated *S. antibioticus* RSP – T1 strain potential of only tyrosinase synthesis, this strain has been evaluated for improved tyrosinase production by modulating the carbon source requirement during growth of the strain. The results of IMViC reactions and other biochemical tests of *Streptomyces antibioticus* are shown in Table 1. Absence of dark red colour in the amyl alcohol surface layer constituted a negative indole test and confirmed that absence of *E. coli*. In Methyl Red (MR) test, a magenta red color is absence it indicates that absence of *E. coli* (negative test) and yellow color its presence (positive test). The Voges-Proskauer (VP) reaction was

a qualitative test performed to detect the presence of acetyl methyl carbinol: the end products of glucose fermentation. Since the bacterial strains that were tested it produce pink color in the presence of alkali, therefore, these were considered as VP negative. Citrate Utilization, Carbohydrate Utilization, Casein Hydrolysis, Colloidal Chitin Hydrolysis, Catalase Test and Phenyl Alanine Deamination given negative results indicated that absence of *E. coli* and urase, phenol red fermentation, nitrate reduction, starch hydrolysis and gelatin given positive results showed that absence of *E.coli* and presence of *Streptomyces antibioticus*. *Klebsiella* and *Enterobacter* produce more neutral products from glucose (e.g. ethyl alcohol, acetyl methyl carbinol). In this neutral pH the growth of the bacteria is not inhibited. The bacteria thus begin to attack the peptone in the broth, causing the pH to rise above 6.2. At this pH, methyl red indicator is a yellow color (a negative MR test). The reagents used for the VP test are Barritt's A (alpha-naphthol) and Barritt's B (potassium hydroxide). When these reagents are added to a broth in which acetyl methyl carbinol is present, they turn a pink-burgundy color (a positive VP test). This color may take 20 to 30 minutes to develop. *E. coli* does not produce acetyl methyl carbinol, but *Enterobacter* and *Klebsiella* do. The citrate test utilizes Simmon's citrate media to determine if a bacterium can grow utilizing citrate as its sole carbon and energy source. Simmon's media contains bromthymol blue, a pH indicator with a range of 6.0 to 7.6. Bromthymol blue is yellow at acidic pH's (around 6), and gradually changes to blue at more alkaline pH's (around 7.6). Uninoculated Simmon's citrate agar has a pH of 6.9, so it is an intermediate green color. Growth of bacteria in the media leads to development of a Prussian blue color (positive citrate). *Enterobacter* and *Klebsiella* are citrate positive while *E.coli* is negative.

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