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Preliminary Studies on Laccase Production by *Chaetomium globosum* an Endophytic Fungus in *Glinus lotoides*

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Abstract: The effects of the carbon and nitrogen sources, initial pH and incubation temperatures on laccase production by the endophytic fungus *Chaetomium globosum* were evaluated. The optimal temperature and initial pH for laccase production by *Chaetomium globosum* in submerged culture were found to be 30°C and 6.5, respectively. Glucose or maltose and peptone were the most suitable carbon and nitrogen source for laccase production. Under optimal culture medium, the maximum laccase activity was determine to be 10, 21 μ ml, which was approximately three times higher than that in basal medium.

Key words: Chaetomium globosum · Endophytic fungus · Laccase production · Submerged cultures

INTRODUCTION

Laccase, a kind of polyphenol oxidase containing copper atoms, can oxidize an array of organic and inorganic substrates, including mono., di., and polyphenols, aminophenols, methoxyphenols as well as metal complexes. Laccase can be found in plants, insects and bacteria, but its major sources is fungi. In fungi it is associated with many biological functions such as lignin degradation, removal of potentially toxic phenols in addition to paper industry [1], pigment synthesis and phytopathogensis and fungal virulence [2. Laccase is considered to be a potentially important enzymes for industries.

As a less investigated microorganisms "hidden" within host plants, endophytes are obviously a rich and reliable source of bioactive metabolites with huge medical, agriculture and industrial potentials [3]. Although the enzymes vary from isolate to another, all the endophytic fungi tested all synthesize *in vitro*, the enzyme necessary for penetrating and colonizing their plant hosts [4]. Such enzymes including pectinases, xylanase, cellulases, lipases, protinases and phenol oxidase have been documented with some endophytes [3]. However, little information has been reported about laccase production for degrading lignin by endophytic fungi up to date. The objective of the present work is to describe the conditions for laccase production by *Chaetomium globosum*, an endophytic fungus in *Glinus lotoides*, various physicochemical parameters were examined to optimize laccase production.

MATERIALS AND METHODS

A-microorganism: *Chaetomium globosum,* is an endophytic fungus isolated from fresh leaves of an apparently healthy *G. lotoides* collected in March 2006 in Wadi Allaqi, Aswan, Egypt. Endophytic strains were isolated following the procedure described [5,6]. The stock culture was maintained on a potato – dextrose agar (PDA) slant. Slants were incubated at 25°C for 5 days and stored at 4°C.

B-growth and laccase production media: Basal medium (MSB) was used for fungal growth. The seed culture grown in 250 ml flask containing 100 ml of mineral salts broth (MSB) containing: sucrose, 5g; yeast extract, 2.5g; acetic acid, 0.6ml; KH_2PO_4, 2g; $MgSO_4$ $7H_2O$, 1g; $CaCl_2$ $2H_2O$, 0.1g; Thiamine hydrochloride, 0.01g; polysorbate 80, 1g; trace elements solution, 10ml in 1 liter distilled water (initial pH 4.8), and incubated at 25°C on a rotary shaker incubator at 150 rpm for 4 days.

C-laccase production: To 100ml production medium per 250 ml conical flask capacity, only 1 disc (5 mm diam.) of the agar growth loaded with fungal spores of 5-7 days old

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was added and then the flask incubated at 25°C on a rotary shaker incubator at 150 rpm for 6 days. In order to optimize the culture condition, the initial pH in the MSB medium was adjusted to desired value by addition of either 0.1 M HCl or 2.5 M NaOH to reach the proposed pH values as (1, 2, 3, 4, 5, 6, 7, 8). The flasks were incubated at various temperatures (20, 25, 30, 35,40 45°C). Glycerol, glucose, cellobiose, lactose, maltose, beef extract, carboxymethyl cellulose were studied as carbone source in relation to expression of laccase activity. Yeast extract, ammonium nitrate, sodium nitrate, Alanin, Peptone, L-asparagine and glycine were used in this study to select the most suitable for maximum nitrogen source. For a time course study, the flasks were incubated at 30°C on a rotary shaker at 150 rpm for 16 days. At one day interval, the contents of each flask were filtered through Whatman filter paper No. 1 and the filtrate was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant thus obtained was treated as the enzyme extract. All experiments were performed at least in triplicate to ensure reproductive similarity.

D-Laccase enzyme assay: The fungal biomass was harvested by filtration through filter paper and biomass yield was determined in the terms of oven-dried (80°C for 24 h) mycelium weight. The protein in the filtrate was estimated by method of Amr and Sheded [7]. Laccase activity was determined with 2,2`-azino-bis (3-ethylbenzthiazoline-6-sulfonate) ABTS as the substrate [8]. One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 μ mol of ABTS per min at 25°C [9].

RESULTS AND DISCISSION

The shake flask cultures using the basal medium (MSB) showed the ability of endophyte *Chaetomium globosum* in *G. lotoides* to produce appreciable amounts of laccase extracellularly. Maximum enzyme production reached on 5 days in *Chaetomium globosum,* while the biomass maximum was achieved during 4 – 6 days (Figures 1 and 2). The production maximum was also attained earlier as compared to some white rot fungi as *phlebia floridensis* 20th day [10] and *trametes pubescens* 13th day [9], while the maximum production of laccase from *monotospora sp* an endophytic fungus was on the 8th day and the mycelial dry weight was between (4 – 7 days) [11]. In order to improve laccase production by this organism a range of pH, temperature, the carbon and nitrogen were chosen for optimization as these have been reported to influence laccase production by other microorganisms [11,12]. The effect of pH and temperature on laccase activity of *Chaetomium* are shown in (Figures 3 and 4).

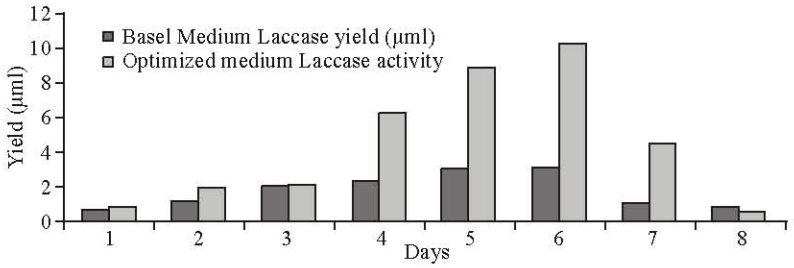

Fig. 1: The Lassaca yield on Basal and optimized media

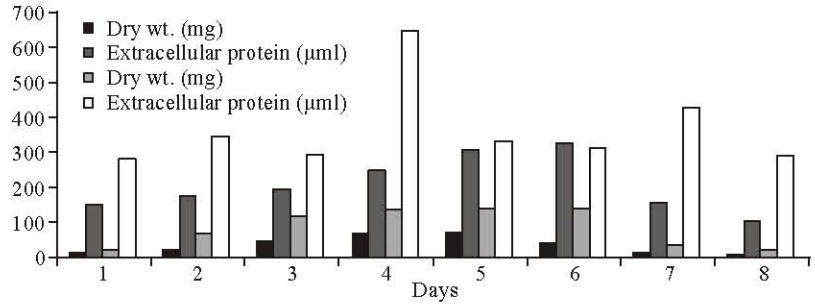

Fig. 2: Dry weight (mg) and Extracellular protein in (μml) on basal and optimized media

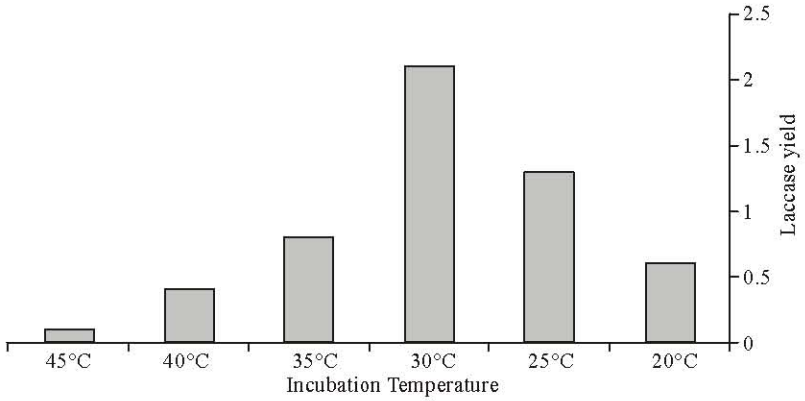

Fig. 3: Diffrent incubation temperature in relation to laccasa production by Chaetomium globosum

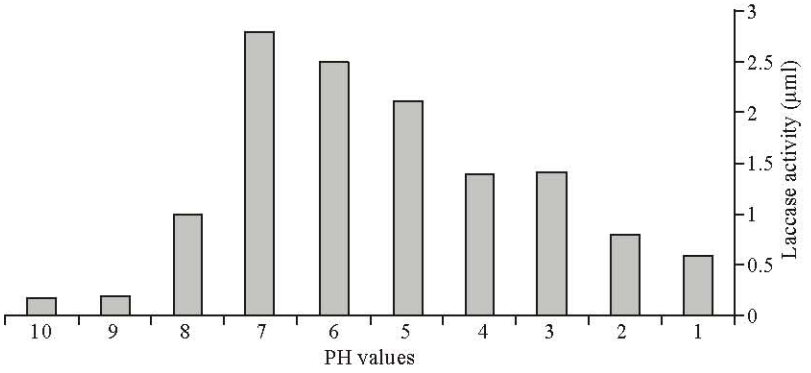

Fig. 4: Various pH values in relation to laccasa production

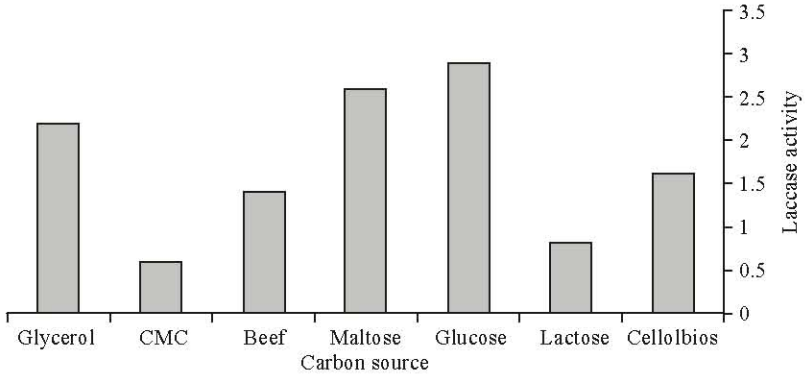

Fig. 5: Effect of various carbin sources on laccasa production by Chaetomium globosum at 30°C

The optimum initial pH and incubation temperature were determined to 7.0 and 30 °C respectively. The fungus produced the enzyme on all the carbon sources tested (Fig. 5), however the substrates were rapidly utilized by the organism resulted in high levels of laccase activity as is evident for glucose or maltose, both yielding comparatively high level of laccaes (10.21 µ ml) when using glycerol, similar activity were obtained but was slower consumed than glucose. Both lactose and carboxymethyl cellulose are only poorly utilized for growth, resulted in low laccase levels (Fig. 5).

Another factor essential for efficient laccaes production by fungi can be the nitrogen source used for cultivation. Optimum enzyme production was achieved when using peptone (Fig. 6). Interestingly, ammonium nitrate and sodium nitrate reduced growth as well as laccase production. When using asparagines as the

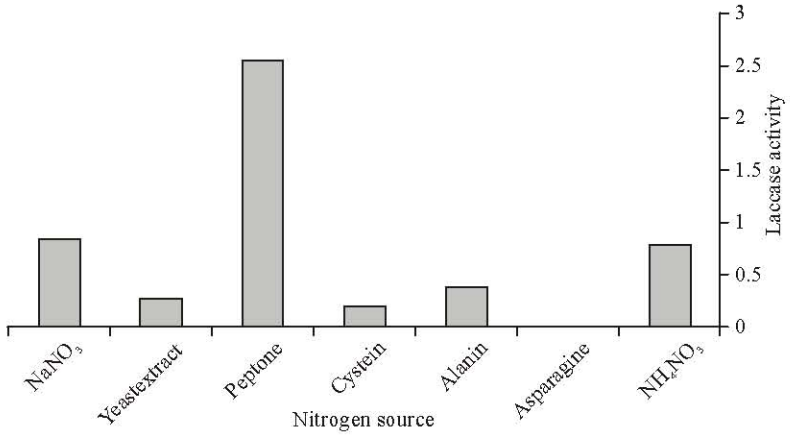

Fig. 6: Effect of various nitrogen sources on laccasa production by Chaetomium globosum at 30°C

sole nitrogen source both growth and laccase secretion were negligible. In comparison to fungal plant pathogens and fungal soil isolates, endophytic fungi grow in different habitats and require distinct levels of nutrition [11,4].

Optimum condition for laccase production appear to be quite different to those typically reported for other fungi. Clealy, the most obvious difference is that the addition of (g/l): 3 glucose and 10 pepton were more favorable than the addition of 5 sucrose and 2.5 yeast extract, production of laccase in optimized medium could be increased more than 3-fold compared to basal medium (MSB). This confirms the importance of carbon and nitrogen sources (that is C / N ratio was 0.3 / 1). However, this is a controversial subject and some authors have found that laccase activity increased under N- limitation and higher C/N in cultures [13,14]. As compared to laccase production determined by oxidation of ABTS in cultures of the much-studied white rot fungi such as *Trames* spp. [15,16], *Cheatomium globosum* proved to be a high laccase producer. In conclusion, the results presented here indicate that, higher laccase activity by the endophytic *Cheatomium globosum*, the potential for screening and optimizing more endophytic fungi for laccase production may be exploited in lignin degradation and other biotechnological applications under suitable conidions.

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