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Production and Optimization of Laccase from *Streptomyces lavendulae*

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A B S T R A C T

The process parameters influencing the production of extracellular laccases by *Streptomyces lavendulae* were optimized in submerged fermentation. It was made to screen, enhance and production of laccase enzyme produced by the consortium of laccase producing *Streptomyces lavendulae*. To date, laccases connect mostly been independently and characterized from flora and fauna of fungi and unaided fungal laccases are used currently in biotechnological applications. In contrast, minute is known just approximately bacterial laccases, although recent immediate assume ahead in the combined genome analysis suggests that the enzymes are widespread in bacteria. Since bacterial genetic tools and biotechnological processes are skillfully conventional, therefore developing bacterial laccases would be significantly important. Laccase activity was determined by measuring the oxidation of guaiacol at 530 nm. Laccase activity was maximum when manage at the following conditions, 60 hrs. incubation, 30°C temperature, and pH-5, 2% nitrogen sources, 3 % peptone and beef extract and 2 % carbon sources, glucose and sucrose in the production medium. This research summarizes the distribution of laccases among bacteria, and able to producing maximum laccases at the most favorable conditions.

Introduction

Laccase has wide substrate specificity towards aromatic compounds containing hydroxyl and aminegroups. These enzymes were well-known to catalyze the oxidation of a large range of phenolic compounds and aromaticamines. In fungi, they can be found in ascomycetes, deuteromycetes and most white-rot basidiomycetes (Baldrian, 2006). One of the advantages of laccases is that they reach not require hydrogen peroxide for substrate oxidation and otherwise, they use oxygen as a non-limiting electron acceptor (Michizoe *et al.,* 2005). Laccases are ubiquitous enzymes present in higher plants, bacteria, fungi, insects and lichens (Riva 2006; Lisov *et al.,* 2007). Due to their sophisticated redox potential as compared to the natural world or bacterial laccases, fungal ones are implicated in several biotechnological applications (Brijwani *et al.,* 2010).

Laccases are produced by bacteria, fungi and plant. From the point of view of their structure and function, bacterial and fungal laccases have a similar structure; their amino acid sequences are quite dissimilar. Bacterial laccases frequently occur as monomers, whereas certain fungal laccases take place as isoenzymes that in general oligomerize to

form multimeric complexes (Claus, 2004; Sakurai, 2007). In recent years, bacterial laccases have gained higher concentration for their potential in biodegrading environmentally significant phenolic pollutants due to their relatively elevated production rate, high thermostability, and broad pH range, among others (Held, 2005; Hilden, 2009).

Recently some bacterial laccases have also been characterized from *Azospirillum lipoforum, Bacillus subtilis, S. cyaneus and Marinomonas mediterranea*. A lot of roles for laccases in bacterial systems have been recommended and contain roles in melanin production, spore coat resistance against hydrogen peroxide and UV (Jia *et al.,* 2014).

The application and potential of bacterial laccases for bioremediation applications of bacterial laccases very little are recognized. In generally bacteria tolerate a broader range of habitats and grow faster than fungi (Harms *et al.,* 2011). Moreover, in contrast to fungal laccases, some bacterial laccases can be highly active and much more stable at high temperatures, at high pH as well as at high chloride concentrations (Sharma *et al.,* 2007; Bugg *et al.,* 2011; Dwivedi *et al.,* 2011).

The strains *Bacillus atrophaeus* and *Bacillus pumilu* produced laccase enzymes can degrade and or modify lignin and contribute to the release of fermentable sugars from lignocellulose (Huang *et al.,* 2013).

Laccase activity was highest when operated at the following conditions, 72 h incubation, 40°C temperature, and pH-7, 2% glucose as carbon source and 2% peptone as the nitrogen source in the manufacturing medium from *Pseudomonas aeruginosa* (Peter and Vandana, 2014).

Materials and Methods

Bacterial strain

Bacterial strain *Streptomyces lavendulae* MTCC6821was procured from Microbial Type Culture Collection (MTCC) center, Chandigarh, India. The strain was tested for the purity, morphology, and biochemical characteristics. The strains have been tested for laccase producing ability through plate test method. The ability of the bacterial and fungal strains to produce laccase was visualized according to the method of (Kiiskinen *et al.,* 2004).

Measurement of growth

The growths of bacterial strain was inoculated in nutrient broth and grown at 37 °C and 180 rpm in an orbital shaker. The strain was sub-cultured @ 1:100 in 50 ml fresh nutrient broth media in 250 ml Erlenmeyer flasks and grown for 12 hrs. Aliquots were withdrawn at hourly intervals and the optical densities were measured using spectrophotometer at 600 nm. The un-inoculated media was used as a blank.

Laccase activity

The activity of laccase *in vivo* determined by spectrophotometric tests using phenolic substrates and by monitoring the colored oxidation products. Laccase activity was determined by measuring the oxidation of guaiacol at 530 nm. The reaction mixture was containing 10 mM guaiacol and 100mM citrate-phosphate buffer (pH5.6).

Absorbance for blank was measured at 470 nm while that of test samples was measured at 530 nm. Protein concentration was determined by the method of (Lowery *et al.,* 1951) with bovine serum albumin. The following formula was used for determination of enzyme activity.

Optimization of culture conditions for enzyme production

A range of process parameters that move the enzyme production were optimized greater than a broad range. The entire adopted for standardization of process parameters was to examine the effect of an individual parameter and to incorporate it at the standardized level previously standardizing the neighboring-door parameter. The effects of organic and inorganic nitrogen sources, carbon sources, regulate in the period, temperature, pH, was studied.

Effect of incubation period on enzyme production

To find out the effect of incubation period on enzyme production, fifty ml of nutrient broth (NB) culture media was taken in 250 ml Erlenmeyer flasks. The flasks were sterilized, cooled to room temperature, and inoculated with fresh bacterial culture of *Streptomyces lavendulae* culture was incubated at 120 rpm at different time intervals, namely 24, 48, 60, 72, 96, 120 and 144 hrs. respectively at 30 °C. This culture was used as inoculums for laccase production studies. The contents of the flasks were centrifuged at 10000 rpm for 10 min at 4 °C and the supernatant was used to assay the enzyme activity at 450 nm. Laccase activity was assayed using the procedure described previously. The sample which is showing high activity considered as 100 % activity.

Effect of temperature on enzyme activity

Environmental temperature is a factor to which the biomass is an inescapable subject matter since cell temperature should become equal to the temperature of culture medium. The media inoculated with fresh bacterial culture of *Streptomyces lavendulae* culture was incubated at 120 rpm at different temperature 25, 30, 35, 40, 45, and 50 °C respectively for 60 hrs. This culture was used as inoculums for laccase production studies. The contents of the flasks were centrifuged at 10000 rpm for 10 min at 4 °C and the supernatant was used to assay the enzyme activity at 450 nm. The sample which is showing high activity considered as 100 % activity.

Effect of pH on enzyme activity

The power of hydrogen ions on biological actions is linked to their hydrogen ion concentration on enzyme activity. The fresh media subculture using the bacterial culture of *Streptomyces lavendulae* culture and pH were adjusted in each of the flasks from 4, 4.5, 5, 6, 7, and 8 (using HCl or NaOH) was incubated at 120 rpm at 30 °C respectively for 60 hrs. The contents of the flasks were centrifuged at 10000 rpm for 10 min at 4 °C and the supernatant was used to assay the enzyme activity at 450 nm.

Effect of carbon and nitrogen sources on enzyme activity

The nature and sum of carbon and nitrogen sources in the culture medium are significant for the growth and construction of laccase by bacterial. The production medium enriched with varying of carbon sources, specifically, glucose, maltose, sucrose, and starch with the final concentrations (2 %) and varying of inorganic and organic nitrogen sources, specifically, ammonium sulphate, sodium nitrate, peptone and beef extract with the final concentrations (2 %)pH was adjusted 5 incubated at 120 rpm at 30 °C respectively for 60 hrs. The contents of the flasks were centrifuged at 10000 rpm for 10 min at 4 °C and the supernatant was used to assay the enzyme activity at 450 nm. The sample which is showing high activity considered as 100 % activity.

Results and Discussion

Primary screening of the strains became finished through plate assay method. At the strong agar media for isolation, the different isolates might be outstanding through their color and morphology (Fig. 1). The bacterial tradition became investigated for the lignolytic enzyme, laccase pastime with the aid of using guaiacol technique. A easy screening approach was accompanied in organize to hit upon laccase generating bacteria on strong media containing 0.02% guaiacol as an indicator turned into placed into effect for screening of laccase generating with the aid of bacteria, expand an extreme reddish brown colour in the medium across the bacterial colony vicinity as laccase signs (Ang *et al.,* 2010). The appearance of the reddish brown area inside the medium resulted from the oxidative polymerization of guaiacol (Mabrouk *et al.,* 2010).

The strain *Streptomyces lavendulae* MTCC6821 that was capable of producing laccase enzymes was selected as the best strain for future works. The growth pattern of *Streptomyces lavendulae* in nutrient broth is shown in figure 2. The strain *Streptomyces lavendulae* was growth pattern showed that this strain is not growth defective.

The incubation duration of laccase production indicated that the maximum enzyme yield became performed at 60 hr. of incubation. Some of the time, a gradual boom in the enzyme activity was referred to on the starting time of incubation length and the maximum enzyme interest was attained at 60 hr in figure 3.

The most laccase pastime turned into located at 30 °C at 60 h of incubation in figure 4. a few of the temperature, a slow increase in the enzyme activity become referred to on the starting time of incubation period and the maximum enzyme pastime was attained at 30 °C for 60 hrs of incubation in figure 4. However the manufacturing enzyme hobby was declined on the better incubation temperature of 60 °C figure 4.

Fig.1 Bacterial growth on Nutrient agar (NA) and screening of laccase production; **A:**bacterial culture is stricken on Nutrient agar (NA) incubated at 37 °C for overnight. **B:**Using solid media containing 0.02% guaiacol as indicator compound after 3 days of incubation at 25 °C. The oxidative polymerization of guaiacol to reddish brown zones in the medium by positive strain

A. *Streptomyces lavendulae* B. *Streptomyces lavendulae*

Fig.2 Bacteria strain does not exhibit defective growth in *in-vitro* culture media. *Streptomyces lavendulae* strains were grown in broth media. Aliquots were taken out at one-hour intervals and optical density was measured at 600 nm. Data is presented as mean ± S.D. (n = 3)

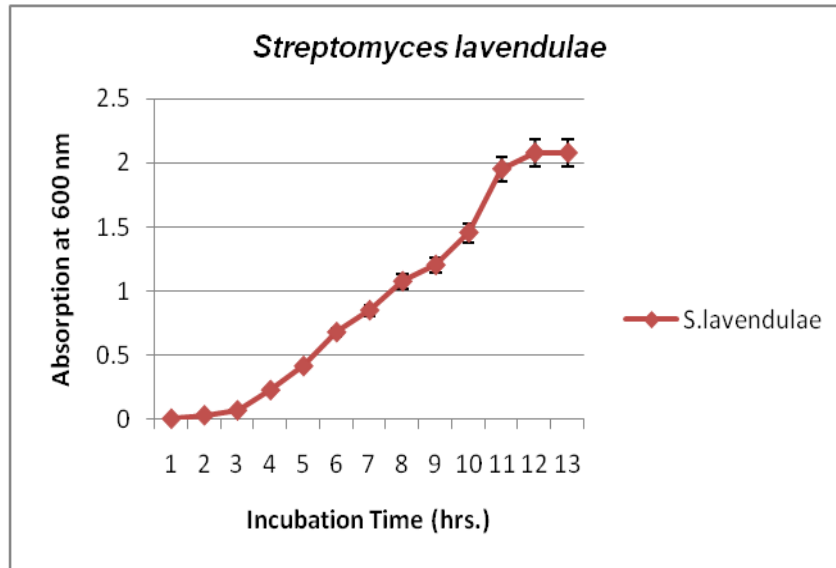


Fig.3 Effect of the incubation period on laccase production. The crude laccase activity from *Streptomyces lavendulae* using guaiacol oxidation method. Laccase activity was measured using phosphate buffer (50mM, pH 5.0). The error bars in the figure indicate the relative standard deviation

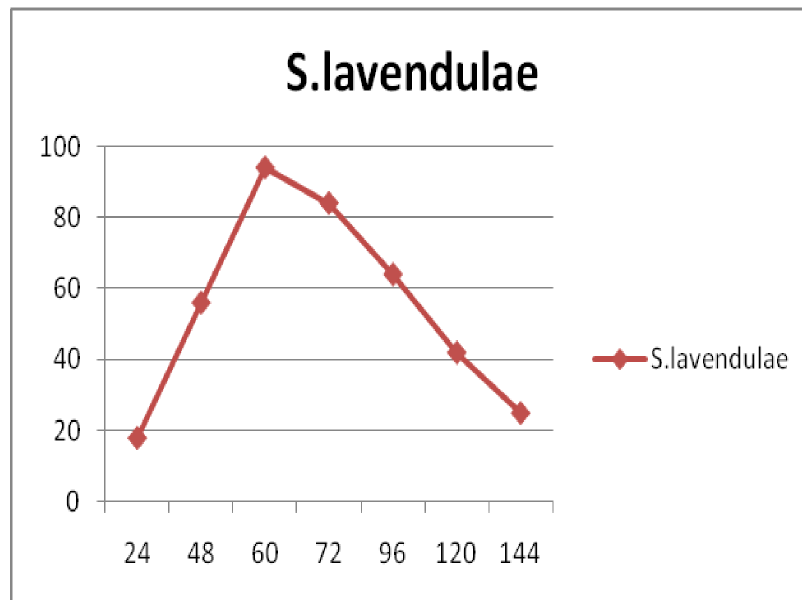


Fig.4 Effect of temperature on laccase production**.** The crude laccase activity from *Streptomyces lavendulae* using guaiacol oxidation method. Laccase activity was measured using phosphate buffer (50mM, pH 5.0). The error bars in the figure indicate the relative standard deviation

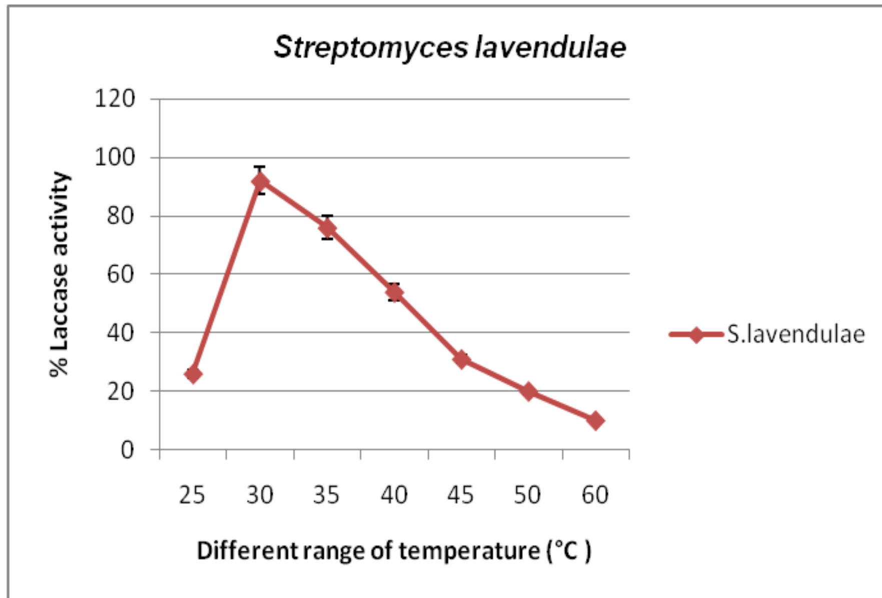


Fig.5 Effect of pH on laccase production. The crude laccase activity from *Streptomyce lavendulae,* using guaiacol oxidation method. Laccase activity was measured using phosphate buffer (50mM, pH 5.0). The error bars in the figure indicate the relative standard deviation

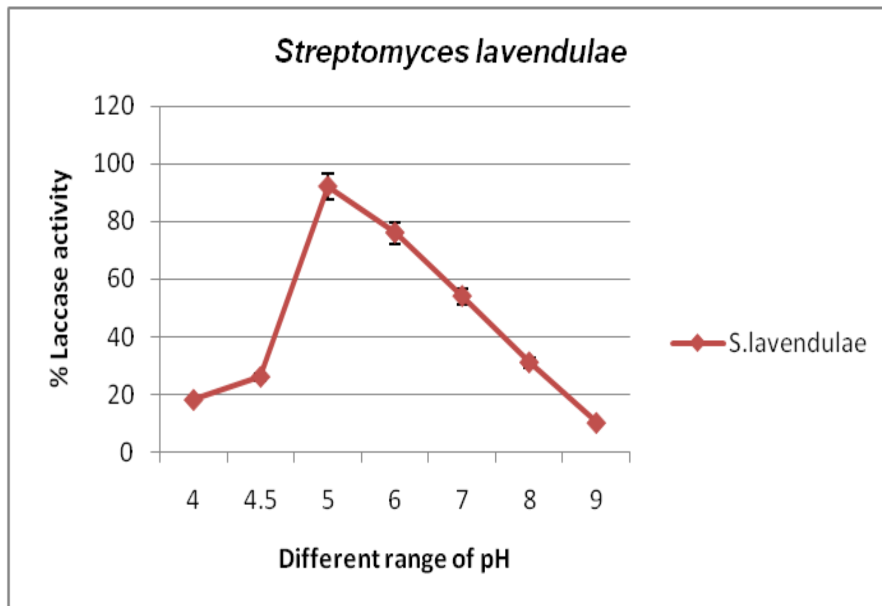
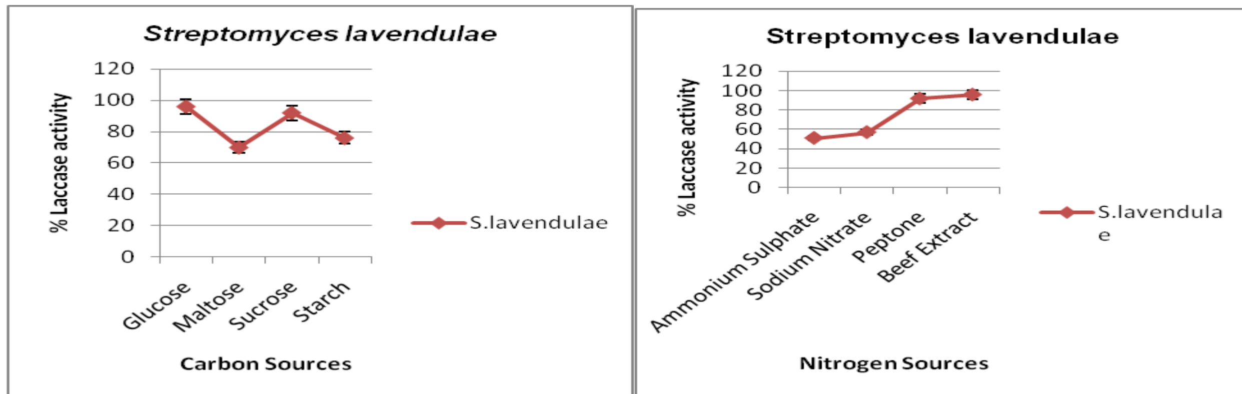


Fig.6 Effect of carbon and nitrogen carbon on laccase production. The crude laccase activity from *Streptomyces lavendulae* using guaiacol oxidation method. Laccase activity was measured using phosphate buffer (50mM, pH 5.0). The error bars in the figure indicate the relative standard deviation



Hydrogen ions concentration (pH) strongly impacts the enzymatic reactions and is receptive to hydrogen ion concentration present in the medium across the cellular membrane (Murugesan *et al.,* 2007). Most laccase activity turned into determined at pH five for *Streptomyces lavendulae*, after a period of 3 hrs (Fig. 5).

Nature and sort of carbon and nitrogen are the most vital elements for any fermentation process (Pandey and Radhakrishnan, 1992). In the present observe, complement of the media with special carbon 2 % resources. a few of the carbon assets examined, 2 % glucose and sucrose have been determined to showcase most enzymatic pastime then starch and maltose in figure 6a. Medium containing peptone confirmed the highest laccase hobby as enzymes are substrate precise. Peptone is the simplified source of protein and may be voluntarily uptake by means of the microorganism. a number of the examined nitrogen assets, 2 % peptone and a couple of % pork extract ended in better laccase manufacturing figure 6b. Even inside the present study, organic nitrogen assets exhibited most activity as compared to inorganic sources (Fig. 6a). *T. villosa* laccase showed stepped forward manufacturing the use of peptone (Morozova *et al.,* 2007). In distinction to that, our findings screen that bacterial stress offers maximum laccase pastime with lactose followed by glucose whereas with maltose it does no longer explicit laccase interest.

In conclusion the optimization of cultural and nutritional parameters for the laccase production by using the *Streptomyces lavendulae* strain in nutrient broth became determined to be a great deal exact than the said values. The boom and high-quality laccase manufacturing of the *Streptomyces lavendulae* was preferred by using acidic pH 5, 2 % carbon and nitrogen resources at 30 °C for 60 hrs incubation of the medium.

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