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LACCASE PRODUCTION AND SIMULTANEOUS DECOLORIZATION OF SYNTHETIC DYES BY CYANOBACTERIA

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Abstract: Laccase is copper containing polyphenol oxidase that acts on wide range of substrates. It is also known as "Green Catalysts" or "Ecofriendly" enzymes. Laccase has many potential applications including textile dye decolourization, delignification of pulp and effluent detoxification. In the present study, the cyanobacterial strains were screened for laccase production and dye decolorization potential of crude laccase were investigated. Among all selected strains *Lyngbya* NCCU-102 showed activity (34 mU/ml), *Synechocystis* NCCU-370 stood at second position (31 mU/ml). Maximum laccase production was found on 7th day. Laccase activity of studied strains ranged from was 24.9 to 34 mU/ml. In all strains under normal condition cyanobacteria showed low laccase activity but in addition of inducer (guaiacol) it was amplified multiple time (60%-80%). Though, under normal condition highest laccase activity was exhibited by *Synechocystis* NCCU-370 but highest laccase activity induction was shown by *Lygbya* NCCU-102. The crude laccase of *Lyngbya* and *Synechocystis* revealed a promising result on the decolorization of synthetic dyes. About 74.1% of reactive blue 4 were effectively decolorized by *Synechocystis* and 59.45% by *Lyngbya* within 144 hr of incubation. This is first report of freshwater cyanobacteria for the laccase production and its decolorization ability and this will provide a possible way to solve environmental problems.

Keywords: Laccase, guaiacol, Lyngbya NCCU-102, *Synechocystis* NCCU-370, Reactive blue 4, Decolorization.

I. INTRODUCTION

Synthetic dye released in the environment by textile & leather-dyeing, paper printing that cause major threat to the environment. Synthetic dyes have complex aromatic structure that makes them more stable and more difficult to biodegrade. The released of coloured compound from synthetic dyes into the environment may not only affect the photosynthesis in aquatic plants, but also seem to be and produce products which are toxic and mutagenic to living organisms. Decolorization of these dyes by physical or chemical methods including coagulation, ion exchange oxidation and electrochemical method has economic and methodological disadvantages. Biological processes for dye decolorization have received increasing interest owing to their lower cost, higher efficiency and environment friendliness. In nature biological degradation of synthetic dye occur due to release of laccase, lignin peroxidase, magnese peroxidase [1, 2].

Laccases (EC 1.10.2.3); benzenediol: oxygen oxidoreductase) are multicopper-containing enzymes, often extracellular in nature. They use molecular oxygen to oxidise a wide range of aromatic and non aromatic compounds by a radical catalysed mechanism [3, 4]. Due to its low specificity for the reducing substrate its commercial and biotechnological significance is greater [5].

Laccase are typically found in plants and fungi. It is involved in the pigmentation process of spores, virulence factors in fungi, the regeneration of tobacco protoplasts and delignification of plants [6]. In addition laccase activity

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is also reported from bacteria (*Bacillus* sp. [7] which involved in metabolic activity like sporulation [8], resistance to copper [9]. Many studies have proved the efficacy of fungi in degrading and decolorizing synthetic dyes, and this ability was found out to be due to the production of enzymes, especially laccases [10].

Cyanobacteria, the wide-adapting photo-oxygenic prokaryotes, which are self dependent for carbon and nitrogen (certain species), have rarely been investigated for their ligninolytic activity and bioremediation ability.

The mass cultivation of cyanobacteria are simple and less expensive as compared to fungi and bacteria. Cyanobacteria have added advantage of oxygenation of the environment. There are only few report of laccase activity in cyanobacteria in few marine forms like *Phormidium valderianum* and *Oscillatoria boryana*, [11]. Present study was undertaken to explore fresh water cyanobacteria for the laccase production and studied its potential in the decolorization of synthetic dyes.

II. MATERIALS AND METHODS

A. Chemicals and reagents:

All the chemical used were analytical grade and were obtained from Merck, India. Synthetic Dyes (Reactive Blue 4 & RBBR) were purchased from Sigma-Aldrich Chemical Co. (St. Louis,MO, USA).

B. Culture collection and maintenance:

All Seven fast growing cyanobacterial cultures of *Lyngbya* NCCU-102, *Phormidium* NCCU-104, *Oscillatoria* NCCU 369, *Gleocapsa gelatinosa* NCCU 430, *Chrococcus* NCCU-207, *Plectonema sp.* NCCU-104, *Synechocystis* NCCU-370 were procured from the National Centre for Culture Collection and Utilization of Blue Green Algae, Indian Agriculture Research Institute (IARI), New Delhi and were grown in 500 ml Erlenmeyer flask containing 200 ml BG-11 medium. Experimental cultures were incubated in identical condition at light intensity 2000 ± 200 lux; photoperiod 12:12 h light: dark; temperature 30 ± 1°C and pH 7.4. The cultures were swirled twice a day for aeration and mixing of nutrients. The supernatants were collected by centrifugation at 6000g for 8-10 minutes and used for the enzyme assay.

C. Laccase Activity:

The Laccase activity was assayed at room temperature by using 10mM Guaiacol in 100 mM sodium acetate buffer (pH 5.0).The reaction mixture contained 3ml acetate buffer, 1ml Guaiacol and 1ml enzyme source. The change in the absorbance of the reaction mixture containing guaiacol was monitored at 470 nm for 10 mins of incubation using UV Spectrophotometer. Enzyme activity is measured in U/ml which is defined as the amount of enzyme catalyzing the production of one micromole of colored product per min per ml [12].

D. Induction of Laccase in culture:

Laccases activity in actively growing cultures was induced by 100 µM of Guaiacol added on zero day and cyanobacterial cells allowed to grow for 10 days. Here the culture without guaiacol was considered as control

E. Protein estimation: The whole protein content of the cell extracts of each strain was estimated by the method described by Lowry et al., (1951) using bovine serum albumin as standard.

F. Dye Decolorization:

The cyanobacteria culture was supplemented with Reactive Blue 4 (50 mg/l) and RBBR (200 mg/l) on third day of inoculation. Samples were taken at 24 hr interval and centrrfuge at 5000rpm for 20 mins. The decrease of color intensity in cell free supernatant was analyzed spectrophotometrically at $\lambda = 495$ nm for Reactive Blue 4 and at $\lambda = 595$ for RBBR. Percent dye decolourization will calculated according to formula:

$$D=100(A_{ini} - A_{obs}) / A_{ini}$$

where D is decolorisation (in %), A_{ini} initial absorbance and A_{obs} Observed absorbance [13].

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G. Statistical analysis:

The results were expressed as mean ± standard deviation. Dunnett's multiple range test was applied and differences among the means were analyzed by ANOVA using Graph Pad Prism version-6.1 (Graph Pad Software, San Diago, CA, USA.

III. RESULTS AND DISCUSSIONS

Laccase production was observed in all the cyanobacterial strains that peaked in 7 day old culture day ranged from 9.2 mU/ml to 25.37 mU/ml. Highest laccase activity exhibited by *Synechocystis* NCCU-370 (Fig.1). So far laccase activity have been reported in cyanobacterium *Phormidium tenue* [14].

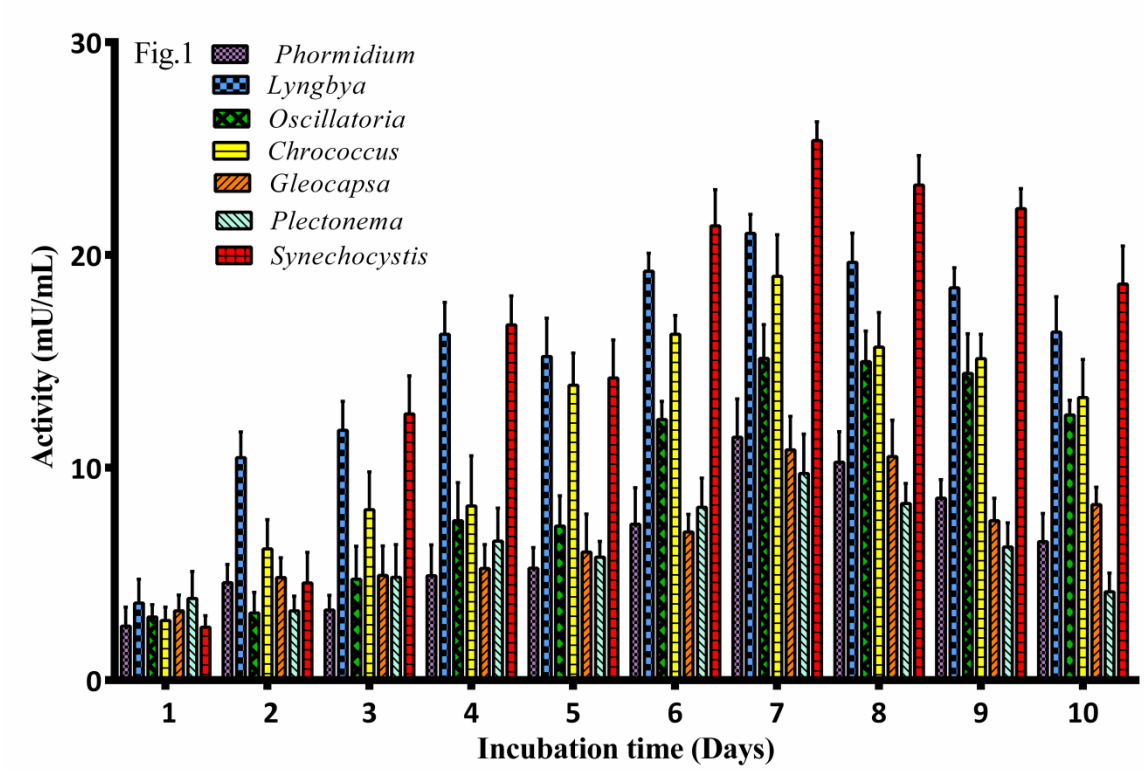

Fig. 1. Laccase activity in cyanobacteial culture filtrate in control condition

Addition of inducer (guaiacol) in culture medium on zeroth day of inoculation resulted in induction of laccase activity (Fig. 2). The percentage enhanced ranged from 24.92 to 34. 34.12 mU/ml. Though, under normal condition highest laccase production was exhibited by *Synechocystis* NCU-370 but highest laccase activity induction in presence of guaicol was shown by *Lygbya* NCCU-102. The differences in laccase activity among different strain are likely because of their genotyic variations. Previous studies have been carried out on the effect of laccase production induced by elicitors. Guaiacol supplementation (1 mM) enhances laccase production from 2- to 232-fold in different fungi. They have found maximum stimulatory effect in *Phlebia spp.* followed by *P. ostreatus* [15]. It has reported

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that marine cyanobacteria *Phormidium tenue* induced laccase production to manifold after addition of 100 µM of Guaiacol, tannic acid, caffeic acid, copper [14].

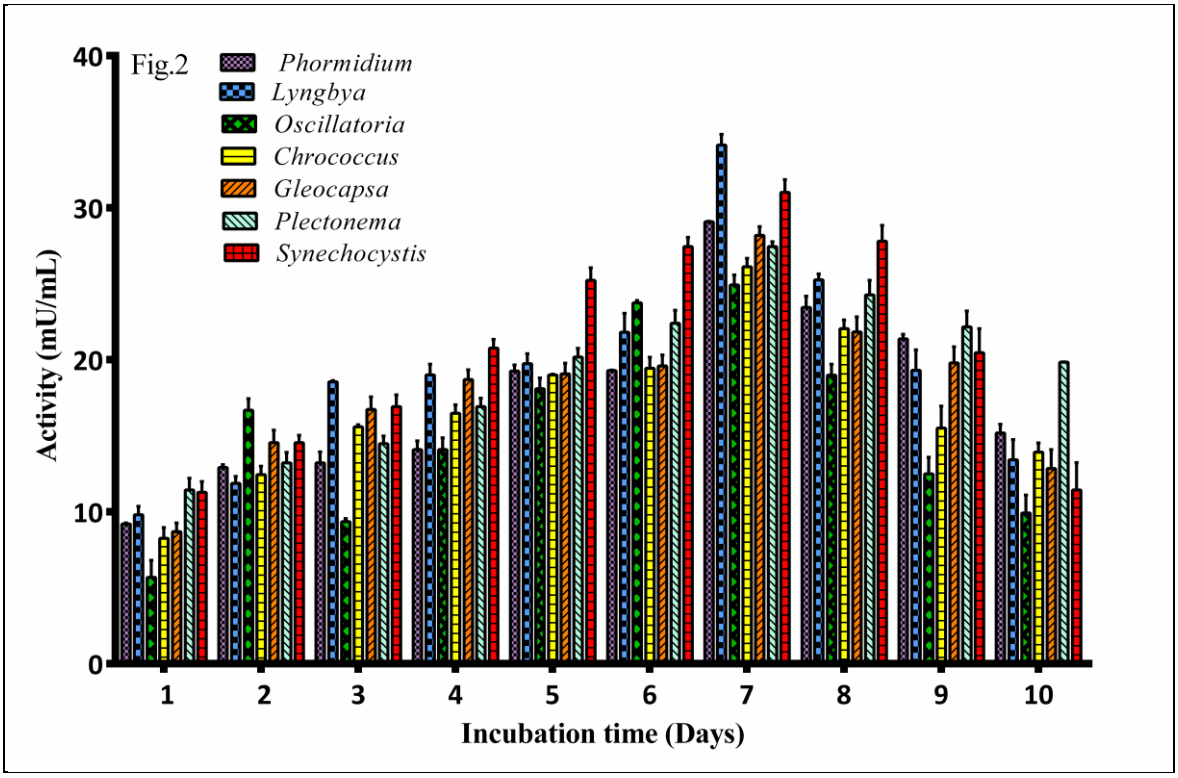

Fig. 2 Laccase activity in cyanobacterial culture filtrate with guaiacol induction

Fig. 3(a) shows the results on the effect of guaiacol on the production of laccase enzyme in cynobacteria. It was found that there was a significant increase (ANOVA $p < 0.0001$) in all strains except *Synechocystis NCCU-370* ($p < 0.001$) and about 60-80% increased in the laccase activity found in actively growing cultures induced by guaiacol as compared to control. Guaiacol act as good inducer in some strains of bacteria even at a low concentration (0.1%) induced laccase production from 33 to 56 mU/L after 48 hr of incubation [16].

In order to investigate whether the addition of guaiacol in culture media have any effect on the total protein content of cell , the whole protein content of cell has been estimated on the 7^{th} day. We also observed that there was an significant increase in the protein content ($p < 0.001$) in all the tested strains as compared to control (Fig. 3b) and according to [17], this increase in protein may be because of the de novo synthesis of phenol-degrading enzymes and stress-related proteins in response to aromatic compounds Much study has been carried out on the efficacy of fungi to produce laccase and its various application like in degradation and decolorization of synthetic dye. Till now there is no report on the cyanobacterial laccase except in *Phormidium teneu* which showed the presence of laccase [14].

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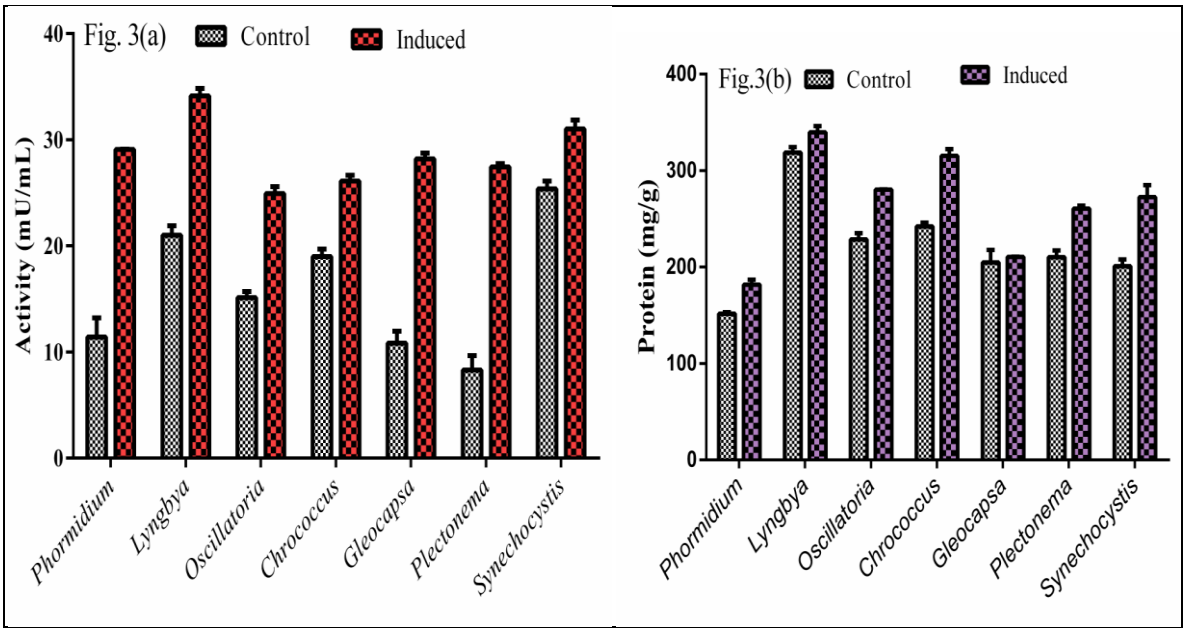

Fig. 3(a) Laccase production in culture filtrate on 7th day Fig. 3(b) Effect of Guaiacol on Protein of on the 7th day

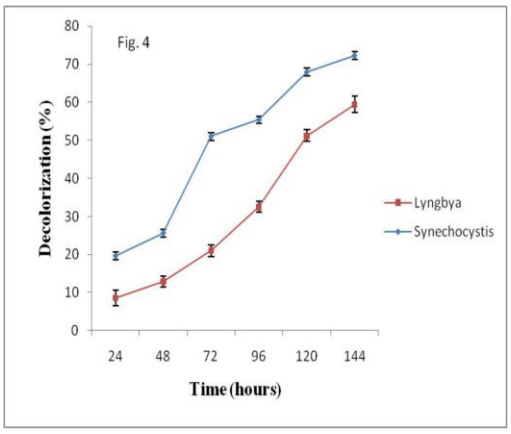

Fig.4. Decolorization of Reactive Blue 4 by the crude laccase of *Lyngbya* NCCU-102 and *Synechocystis* NCCU-370

Enzyme based decolorization is an efficient method and of current interent in industrial effluent treatments [18]. Laccase mediated dye decolorization has been described with crude and purified form from many fungi, however no study has been reported in cyanobacteria. In the present study, the dye decolorization ability of crude laccase from *Lyngbya* NCCU 102 & *Synechocystis* NCCU-370 was assayed using synthetic dye RBBR and Reactive Blue 4 (RB4). From the results we observed a detectable level of decolorization of RB4 within 24 hrs. The rate of decolorization increased with incubation time (Fig.4). About 74.1 of reactive blue 4 were effectively decolorized by *Synechocystis* and 59.45% by Lyngbya at 144 hr. Previous studies in fungus *Trametes versicolor* showed that at same conc. of 50 mg/l of Reactive Blue 4, the decolorization rate was found to be 73% at 144 hr of incubation [19]. It was also observed that *Synechocystis* NCCU-370 has good decolorization potential as compared to *Lyngbya* NCCU-107 under normal condition which suggested rate of decolorization is directly propotional to laccase production. However, RBBR was not decolorized by either of crude laccase

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of cyanobacterial strain. It might be possible because RBBR is strong anthracene derivative which may required a mediator for its decolorization .Most previous studies focused on the use of crude laccase for treatment of synthetic dyes [20].

IV. CONCLUSION

The present study reveals that *Lynbya* NCCU is potent strain for the laccase production which showed the maximum activity under however other strain were also showed it presence. There is constitutive production of laccase in cyanobacteria but the value for laccase activity were significantly low which was increased many folds (60%- 80 %) in presence of inducer guaiacol. To the best of our knowledge our study, marked the first report on the presence of laccase production in freshwater cyanobacteria and dye decolorization potential of crude laccase. Their biodegradative potentials can be exploited to deal with the problem of synthetic dyes's pollution and explore new horizons for further research.

V. ACKNOWLEDGEMENT

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