



In broth: 250 ml Erlenmeyer flask containing 50 ml artificial media [4] composed of Glucose 10 g, asparagine 1.0 g, yeast extract, 0.5 g  $MgSO_4 \cdot 7H_2O$  and  $FeSO_4 \cdot 7H_2O$  and double distilled water 1 litre and amended with natural lignin substrates such as wheat bran (1 gm/100 ml) was sterilized by autoclaving at 121 psi for 15 minutes and then after autoclaving media was inoculated with culture treated EtBr with different concentration were incubated at 30°C for 7 days. After 7 days, for laccase production, protein estimation and fungal biomass in artificial liquid culture medium.

### Enzyme assay method

Filtered extract was analyzed for extracellular laccase activity using DMP as a substrate. The 1.0 ml assay solution containing, 1.0 mM DMP in 50 mM sodium malonate buffer (pH-4.5) at 37°C,  $\epsilon = 468$  nm and molar extinction coefficient of 49.6 mM/cm [12]. The UV/Vis spectrophotometer ofermo Scientific Evolution 201 model was used for absorbance measurement. One enzyme unit produced 1µmol of the product per minute under the specified assay conditions.

### Protein estimation

The extracellular Protein estimation was done by following the methodology of Lowry et al. [13].

### Biomass

The fungal biomass was determined by oven drying the mycelium at 80°C for 24 h. All assays were performed in triplicates and results are reported as average of three replicates.

### Statistical analysis

All the experiments were carried out in triplicate and data were expressed in mean, standard deviation (SD) and SEM.

## Results and Discussion

Figure 1 shows the extracellular laccase production from *Pleurotus citrinopileatus* when initially grown with different concentration of EtBr. Laccase activity (19.67 U/ml) was higher in 1.5 µg/ml as compared to control (8.85 U/ml). But according to Perlman and Mahler [14], they reported that ethidium bromide which acts as an effective mutagen they bring about quantitative conversion of facultative anaerobic yeast cells cytoplasmic petites mutant that could be resulted into the characteristic respiratory deficiency. They also reported that in case of aerobic yeast

**Figure 1:** Extracellular laccase production from *P. citrinopileatus* culture pre-treated with EtBr at different concentrations (0, 0.5, 1.5, 3, 6, 12, 24 µg/ml) on MEA media. The graph shows that laccase activity increases significantly with increasing EtBr concentration, peaking at 1.5 µg/ml.

**Figure 2:** Extracellular laccase activity: Growth of EtBr pre-treated *P. citrinopileatus* on MEA devoid of EtBr as well as repeated treatment with EtBr on MEA media supplemented with different concentration of EtBr.

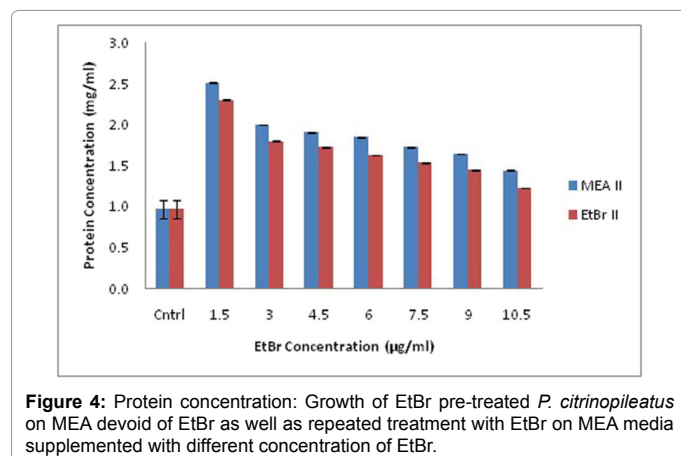
**Figure 3:** Biomass production: Growth of EtBr pre-treated *P. citrinopileatus* on MEA devoid of EtBr as well as repeated treatment with EtBr on MEA media supplemented with different concentration of EtBr.

cells when treated with EtBr, it blocks the formation of cytochromes  $a$ ,  $a_3$  and did not show the induced petite mutation.

In case of animal cell lines, Gomez-Diaz et al. [15] reported that when treated with EtBr animal cell line HL-60 leads to the degradation of mitochondrial DNA and as well as mitochondrial respiratory chain that in turn resulted into the increased glycolysis as well as various other enzymes that would provide activation energy to overcome the oxidative stress threshold.

Effect of EtBr on the extracellular production of laccase, which is an oxidoreductase enzyme they enhanced the production of laccase to a specific concentration i.e., at 1.5 µg/ml (Figure 1) due to the oxidative/respiratory stress in the EtBr treated culture. As the concentration increases the extracellular laccase production decreases in agreement with Dhawan et al. [16] and as shown in Figure 1 that could be the detrimental effect of EtBr on the cell growth. Similar results were found by Pinto et al. [17]. They reported the EtBr that induces mutation from wild type to the cytoplasmic respiratory deficient petite in yeast cells. Interestingly, higher concentration of EtBr causes toxic effect on the yeast cells.

In second attempt, pre-treated EtBr culture of *P. citrinopileatus* were again regrown on MEA plates devoid of EtBr, laccase activity (35.05 U/ml), biomass (210 mg) and protein concentration (2.5 mg/ml) were found to be highest in 1.5 µg/ml as compared to control (Figures 2-4). In the above attempt, drastic fourfold increase in extracellular laccase production in artificial media.



In the third attempt, pre-treated EtBr culture of *P. citrinopileatus* were again regrown on MEA plates supplemented with different concentration of EtBr, laccase activity (16.29 U/ml), biomass (88.1 mg) and protein concentration (2.3 mg/ml) were found to be highest in 1.5 µg/ml as compared to control (Figures 2-4). Due to the detrimental effect of repeated treatment with EtBr, according to Desjardins et al. [18].

The induction or increase in laccase activity by EtBr confirms a regulatory role of this aromatic compound in laccase production. Previously, various research groups have reported that elevated production of laccase by EtBr could possibly be due to respiratory stress induced in the cell. However, more work is needed to elucidate the molecular mechanism underlying regulation of laccase gene transcription by EtBr.

## Conclusion

EtBr treated culture of *P. citrinopileatus* stimulates the extracellular laccase production which in turn can bioremediate the harmful existing pollutants such as xenobiotic compounds, dyes, and heavy metals. The extracellular laccase enzymes are more efficient and effective in bioremediation than the intracellular enzymatic system. As it the green solution, it is economically very important. Besides laccase have many applications such as in cosmetics, bleaching of pulp in paper industry, food, beverages, and various other biotechnological applications.

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