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Decolourising activity of laccase enzyme from *Trametes* sps against textile dyes

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Abstract

The various treatment methods currently available for dye removal primarily depend on physical and /or chemical principles. Most of these technologies however suffer several short comings, costly infrastructure requirements and high operating expenses. White rot fungi produce non – specific extracellular oxidative enzymes to initiate degradation of lignin. Moreover white rot fungi are known to be able to tolerate a high concentration of toxic substances than bacteria. *Trametes* sps has a widespread distribution. Most of the white rot fungal strains produce laccase as the main enzyme during dye decolourisation process. At present laccase mediated effluent treatment has received greater attention and searching for the potential laccases in order to cope with this demand is an important task in the area of dye degradation.

Key words: Textile dyes, *Trametes* sps, Laccase enzyme, dye decolourising activity.

Introduction:

Wide range of dye and dye stuffs used in textile manufacturing are xenobiotic compounds. In recent years the colour of the dye effluents discharged into receiving waters has become a serious environmental problem. Unless properly treated these dyes may significantly affect photosynthetic activity in aquatic light owing to reduced light penetration and may also be toxic to some forms of aquatic life. Diverse group of synthetic dyes are mostly toxic, mutagenic and carcinogenic. Moreover they are very stable to light, temperature and microbial attack, making them recalcitrant compounds

Conventional waste water treatment systems are often inefficient and existing physical and chemical technologies are expensive, time consuming and methodologically demanding. Currently one of the possible alternatives for treatment of textile effluents is the use of lignolytic fungi, which can oxidize a wide spectrum of organic pollutants including synthetic dyes (Heinfling et al., 1997). Synthetic dyes are extensively used in several industries including textile, paper, printing, cosmetics and pharmaceuticals. There are many structural varieties such as acidic, basic, azo, diazo, anthraquinone based and metal complex dyes.

On the basis of dyeing process textile dyes are classified as reactive dyes, direct dyes, disperse dyes, acid dyes and basic dyes. It is estimated that 10 -15 % of the dyes are lost in the effluent during dyeing process (Zollinger et al., 1987). Many dyes are difficult to decolourise due to their complex structure and synthetic origin. Decolourisation of textile dye effluent does not occur when treated aerobically by municipal sewage systems. Brightly coloured, water soluble, reactive and acid dyes are the most problematic as they tend to pass through conventional treatment systems unaffected (Willmott et al., 1998).

Dye decolourisation using microbial enzymes has received attention in recent years due to its efficient application (Abadulla et al., 2000). White rot fungi have been demonstrated for decolourisation of synthetic dyes mediated by their lignolytic enzymes such as lignin peroxidise, manganese peroxidise and laccase. Generally the white rote fungi contain either of the above or all the three types. Most white rot fungal strains produce laccase as the main enzyme during dye decolourisation process. One of the advantages associated with laccases is that they do not require H2O2 for substrate oxidation unlike peroxidases and moreover they have broad substrate specificity (Saito et al., 2003). Laccase

is a multi- copper bearing lignolytic enzyme. It is widely distributed in the higher plants, some insects,a few bacteria and fungi. Most of the known laccases are of fungal origin, in particular from the white rot fungi. These fungi secrete lignolytic enzymes, which generate radical species that cause complete biodegradation of lignin polymers. Because of the complex structure of lignin, the biodegradation system is highly non – specific. Therefore lignolytic enzymes can be used in the degradation of environmental pollutants that differ structurally. This has attracted interests in its possible use in waste water treatment and bioremediation. In this study laccase enzyme was produced from Tremetes species using wheat bran as substrate. The extracellular enzyme was evaluated for its decolourisation capacity against recalcitrant dyes.

Materials and Methods

Culture

Strains of *Trametes* sps was obtained from University of Madras and it was cultivated in potato dextrose agar medium. It was maintained at 4^0C in slants.

Dyes used

The azo dyes used in this study drimaren blue, foron blue, foron yellow red, optilan red and lanasyn yellow were obtained from Clarient Ltd, Thiruppur, Tamilnadu.

Screening of extracellular laccase activity

Solid state fermentation method for the production of laccase from *Trametes* sps.

Wheat bran was obtained from local mill and used as substrate for solid state fermentation. The average particle size of the rice bran was 300 – 1400 µm. Basal medium was added to 20 grams of wheat bran. After sterilization 0.5ml of spore suspension was aseptically added to the bran and mixed thoroughly. Various process parameters influencing the enzyme production like moisture content, temperature and pH were analysed.

Enzyme extraction

Laccase was extracted by suspending the fermented wheat bran in 50mM sodium acetate buffer, pH 5 and it was mixed at 700 rpm for 30 minutes. The suspended material and

the fungal biomass were separated by centrifugation (10,000 g for 10 minutes). The clarified supernatant was used as the enzyme source.

Purification of laccase

Purification was done by ammonium sulphate saturation, dialysis, column chromatographyand SDS – PAGE as per standard methods.

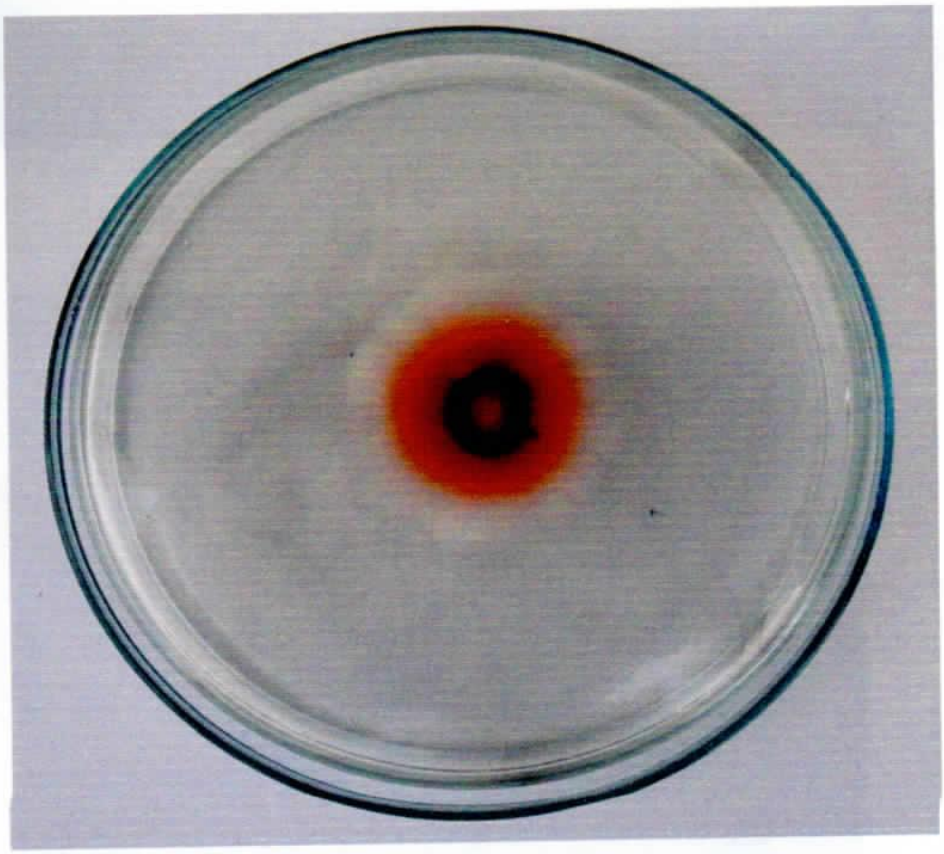
Results

Screening for laccase on ABTS amended agar plate assay

Screening of local fungi for ligninolytic activities was performed using *Trametes* sps . *Trametes* sps exhibited a fast and large oxidation of ABTS on agar plates as demonstrated by the dark reddish brown colour appeared in the plates.(Plate 1)

Plate 1

Plate assay screening for Trametes versicolor
Laccase in ABTS amended plate

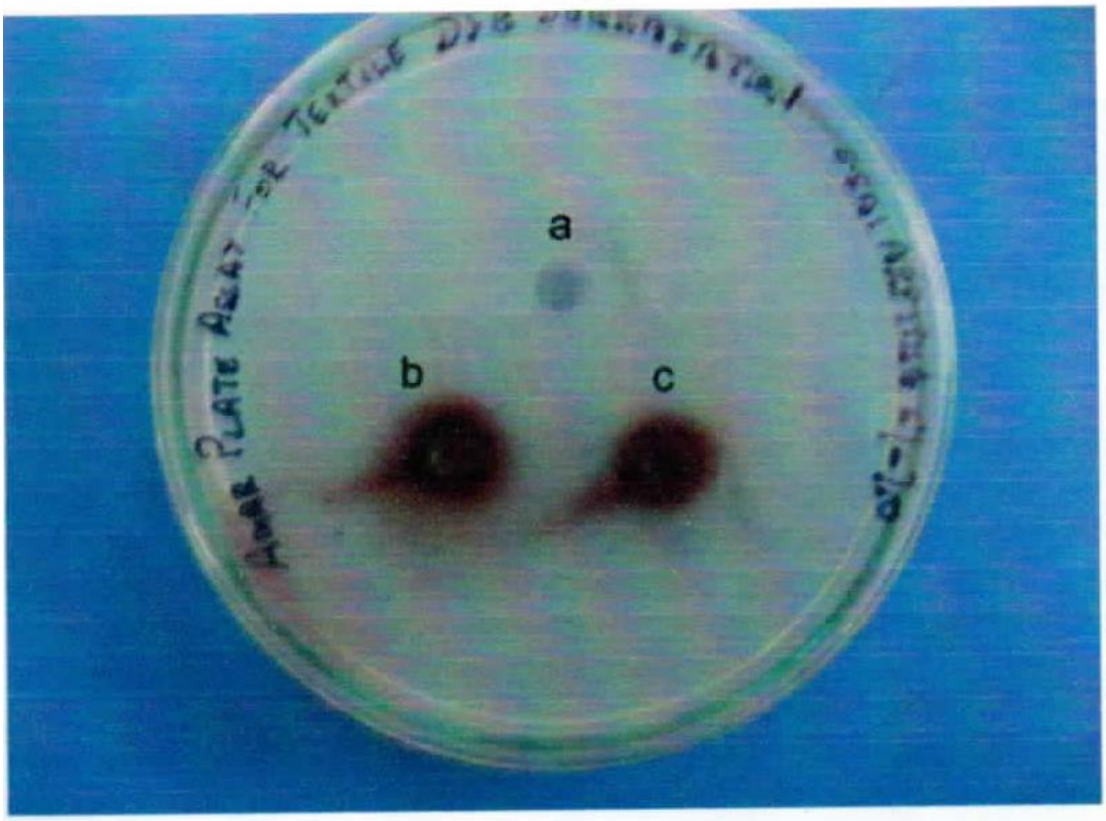


Screening for dye degrading ability on agar plate

Dye decolourisation activity was screened by simple agar plate method amending the reactive dyes drimaren blue, optilan red and lanasyn yellow at a concentration of 50 mg per litre. The mycelial growth covered the agar plate completely on 10^{th} day with 50% decolourisation zone. Further incubation led to complete decolourisation on 14^{th} day with a change from the blue colour to light blue and later colourless. Optilan red dye and lanasyn yellow plate showed no change in colour but good growth of organism on those plates.(Plate 3)

Plate 3

Laccase enzyme assay on agar plate



a - Control

b - Dialysed Laccase 10 µ/ltrs

c - Purified Laccase 10 µ/ltrs

Effect of pH on laccase production

Solid state fermentation was carried out using low nitrogen base, mineral salts with different pH ranges fron 3-7. Maximum enzyme production was observed at pH 7.

Effect of moisture level on laccase production

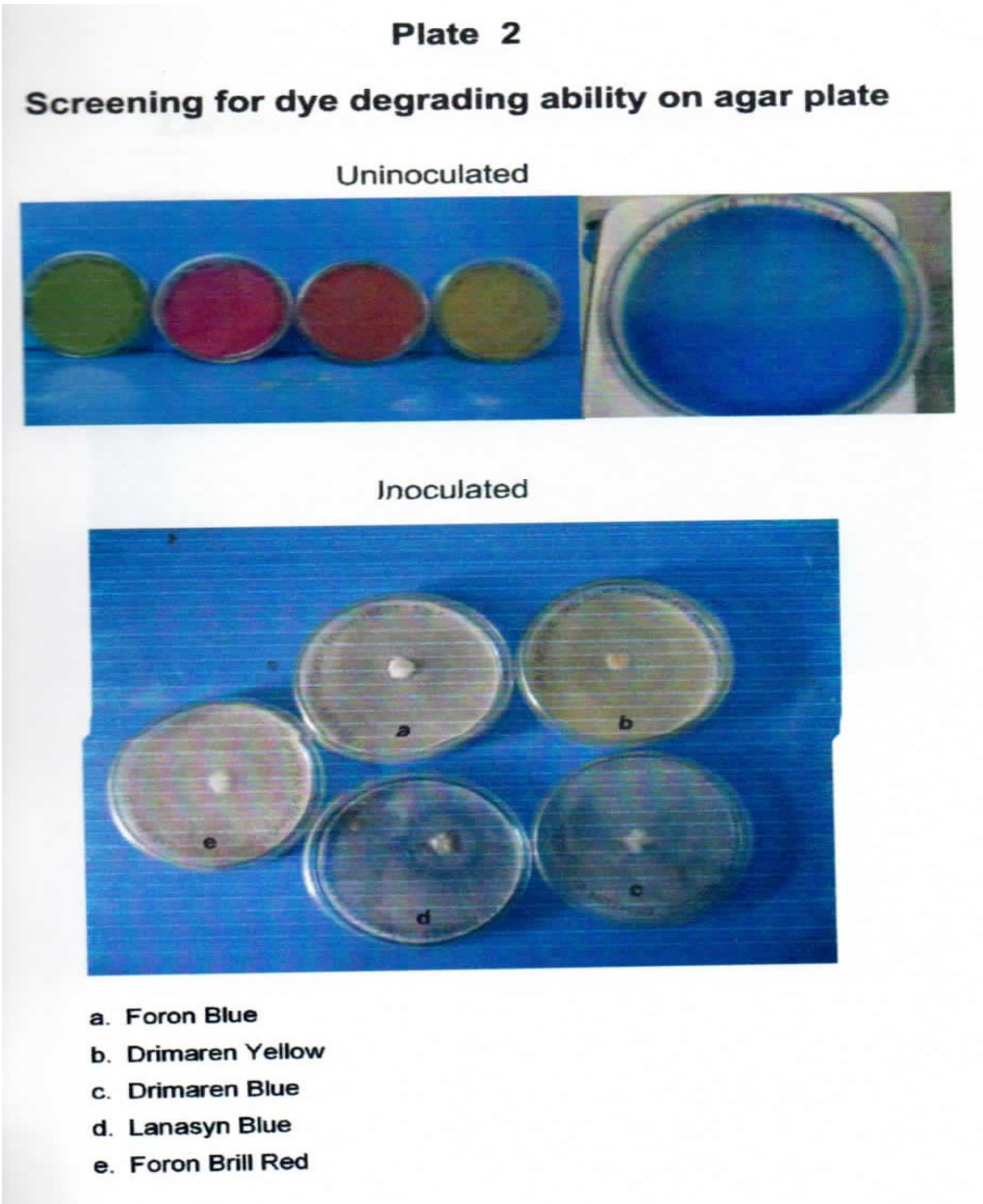
The substrate was moistened using low nitrogen basal mineral salt solution in different ratios (w/v) starting from 1:1, 1:2, 1:3, 1:4 and 1:5. A ratio of 1:4 was found to be best for the production of laccase.

Dye decolourisation by purified laccase

The purified enzyme was evaluated for enzymatic decolourisation activity . The enzyme showed 40% decolourisation during 2 hrs incubation in the case of drimeran blue, optilan red and lanasyn yellow (enzme – 25U/ml). The results depicted are the average and standard deviations of three independent experiments.(Plate 2)

Plate 2

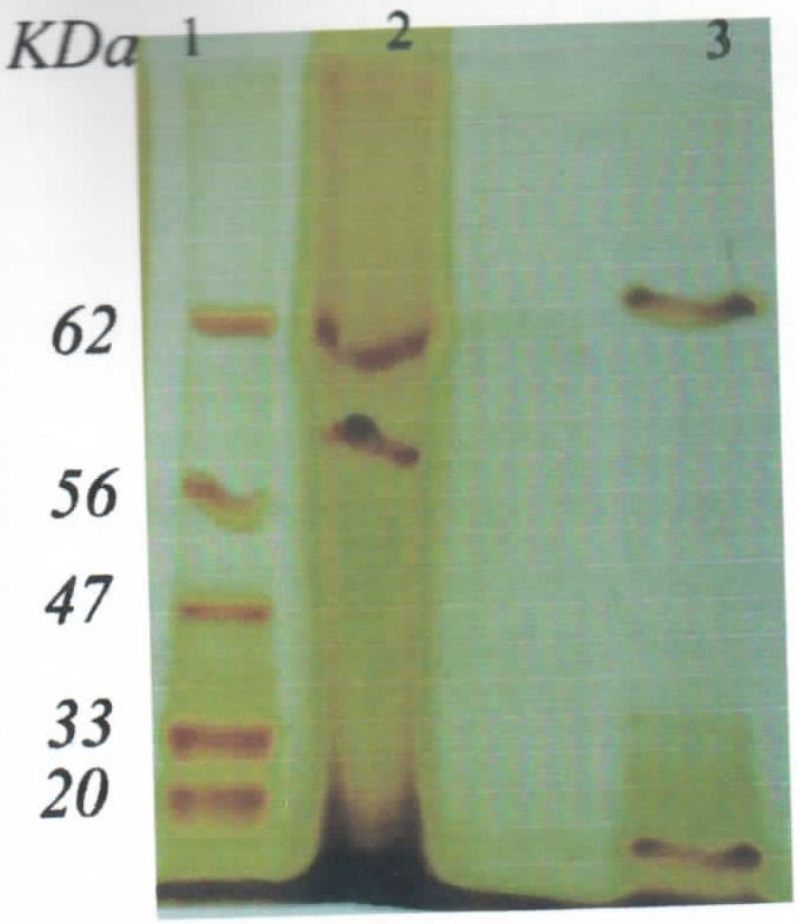
Screening for dye degrading ability on agar plate



a. Foron Blue
b. Drimaren Yellow
c. Drimaren Blue
d. Lanasyn Blue
e. Foron Brill Red

Figure 4

Molecular mass determination of purified laccase by SDS-PAGE



Lane 1- Standard Protein marker
Lane 2 - Dialysis sample
Lane 3 - Purified sample

Discussion

Solid state fermentation has been considered as an efficient method for enzyme production in biotechnological process due to its potential advantages and high yield. In this study solid state fermentation was performed by using wheat bran an agro by product

containing arabino – xylon and phenolic acids as supporting substrate. *Trametes* sps produced high levels of laccase and very low level of manganese peroxidase during the 21 days incubation. The high level of laccase production could be attributed to the presence of ferrulic acid in wheat bran (Hegde et al., 2006). Moreover this is due to the fact that the wheat bran provides the fungus an environment similar to its natural habitat which is conducive for the high secretion of lingo – cellulolytic enzymes. SDS – PAGE indicated the presence of only one acidic isoform of *Trametes* sps laccase (MW 62KDa). Dye decolourisation was possible with the fungal laccase. Similar work using laccase from *Coriolopsis gallica* (Reyes et al., 1999) and *Tramates modesta* (Nyanhongo et al., 2002) have been reported. To conclude, the most economically viable choices available for effluent treatment or decolourisation appear to be the biological system. *Trametes* sps is a promising fungal strain since it produces a high laccase level in the studied condition. Optimisation of laccase production from this fungal strain will have its potential use in decolourisation and detoxification of effluents.

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