ISOLATION OF LACCASE PRODUCING FUNGI FOR DECOLORIZATION OF AZO DYES

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Laccase enzyme, which has low specificity towards substrates can degrade a wide variety of xenobiotic compounds including synthetic textile dyes which cannot be completely removed by conventional wastewater treatment methods. Therefore, the present study was focused on the isolation of native laccase producing fungi which are more applicable to Sri Lankan textile wastewater treatment processes. Soil samples were collected from four textile wastewater effluent sites in Sri Lanka and enriched with 50 mg L^{-1} of the azo dye mixture contained CI Direct Blue 201 (DB 201) and Moxillan Blue GRL (MB GRL) in 1:1 (v/v) ratio under shaking conditions (100 rpm) at 28 °C for 14 days. Fungi with different morphological features were isolated on potato dextrose agar (PDA) medium and pure cultures were obtained following several inoculations on PDA. The potential laccase secreting fungi were identified by growing the isolated fungi on PDA plates supplemented with 50 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) at pH 4.5. The fungal strains which showed green or purple color halos on agar plates were identified as laccase producing fungi and were then screened for textile dye decolorization potential. In solid medium screening, growing each fungal strain on PDA plates overlaid with 5 mL of PDA with 0.01% (w/v) of the azo dye mixture was used for primary screening of the textile dye decolorizing fungi and then subjected to a liquid medium screening in the mineral salt medium using similar textile dye mixture. All the experiments were carried out in triplicates and controls were maintained under similar conditions without adding fungi. Dye decolorization and the laccase activity were measured by following the standard spectrophotometric method at 605 and 420 nm, respectively. Among the 36 isolated fungal strains, seven strains showed laccase producing activity on agar plates. Among them, three fungal strains identified through 18s rRNA analysis: *Aspergillus niger*, *Curvularia lunata* and *Talaromyces* sp. showed complete decolorization of the azo dye mixture within 12, 12 and 18 h of incubation time respectively, while control showed no decolorization. The activity of the laccase enzyme by *Aspergillus niger*, *Curvularia lunata* and *Talaromyces* sp. increased with incubation time and reached to 254 U ml^{-1}, 171 U ml^{-1} and 136 U ml^{-1} respectively, at the time when complete decolorization was observed suggesting that the laccase enzyme may play a major role in the decolorization process. Therefore, the isolated laccase producing fungal species can be used to treat azo dye contained textile wastewater effluents. Further studies in proteomics are in progress.

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