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Identification of the potential of microbial combinations obtained from spent mushroom cultivation substrates for use in textile effluent decolorization

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HIGHLIGHTS

- Spent substrate from three mushrooms varied in population and diversity of microbes.
- Two fungi and three bacteria exhibited appreciable activities of laccase, MnP and LiP.
- Presence of glucose and sucrose and 30 °C temperature supported higher decolorization.
- Wheat straw and paddy straw worked as best immobilizing agents for two fungi.
- Consortium of two fungi and three bacteria led complete effluent decolorization.

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ABSTRACT

The study presents variation in microbial population of *Agaricus bisporus*, *Pleurotus sajor-caju* and *Volvariella volvacea* spent substrates (SMS) along with ligninolytic enzymes activity and textile effluent decolorization potential of microorganisms isolated from these. The effect of temperature, pH, carbon sources and immobilizing agents on effluent decolorization using different combinations of these microorganisms has also been studied. SMS of *P. sajor-caju* harbored highest population and diversity of bacteria and fungi compared to other SMSs. *Schizophyllum commune* and *Pezizomycotina* sp. from *P. sajor-caju* SMS, exhibited highest activities of laccase (11.8 and 8.32 U mL^{-1}) and lignin peroxidase (339 and 318 U L^{-1}), while *Pseudomonas fluorescens* of Manganese peroxidase. Highest decolorization was in presence of glucose and sucrose at 30 °C, and microbial consortium comprised of the immobilized forms of *S. commune* and *Pezizomycotina* sp. on wheat straw and broth cultures of *P. fluorescens*, *Bacillus licheniformis* and *Bacillus pumilus*.

1. Introduction

Synthetic dyes are used in a wide range of industries like textile, pharmaceutical, cosmetic, paper printing and food (Ali et al., 2008). Out of these, textile industry is one of the important and leading sectors, which produces excessive wastewaters. Composition of textile wastewaters (effluents) depends upon textile processing stage, thus these vary in wastewater amount, pollutant types and their concentrations. The pollutants found in textile effluent are contributed mainly by the natural impurity in fibers and the chemical materials used in different processes. Different dyes are in vogue in the textile industries – acid and 1:2 metal complex dyes are used for polyamide; alkaline for acrylic; direct for viscose; dispers high temperature for polyester and reagent non-continuous; reagent continuous and vat for cotton fibers (Correira et al., 1994). Sansapol EM and Sandogen EH are used for stabilization of the color. The dyes are added either in ppm or g L^{-1} and the color is measured by spectrophotometery. The common characteristics of textile wastewaters are high chemical oxygen demand (500–1500 mg L^{-1}), high biological oxygen demand (100–500 mg L^{-1}), high temperature (28–31 °C), high pH (9–10), high total suspended solids (>100 mg L^{-1}), high total dissolved solids (>2100 mg L^{-1}), high electric conductivity (>400 μmhos cm^{-1}), sulfate (300–400 mg L^{-1}), high total alkalinity (800–1000 mg L^{-1}), phenol and the colors contributed by different dyes (Demir et al., 2000). However, the values of these constituents decrease in a treated effluent (BOD > 100 mg L^{-1}, COD > 500 mg L^{-1}, electrical conductivity > 400 μmhos cm^{-1}, sulfate > 300 mg L^{-1}, total alkalinity > 800 mg L^{-1} and pH near neutral) (Garg and Kaushik, 2008). Important pollutants in textile wastewater are especially the organics, followed by color, toxic materials, inhibitor compounds, active substances, chlorine compounds, salt and dying substances (Sandhya and Swaminathan, 2006).

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During processing, up to 40% of the total dyestuffs used found their way in wastewater (Vaidya and Datye, 1982), producing a highly colored wastewater that affects , transparency and gas solubility in water bodies (Faraco et al., 2009). Other attributes like mutagenic, carcinogenic and toxic effects, high volume and effluent composition (Faraco et al., 2009), and their resistance towards the conventional methods of wastewater treatment (Banat et al., 1996) further make these problematic industrial wastes. Currently several physico-chemical methods are in vogue for decolorization of these wastewaters, but these have the limitations of high cost, high salt content in the effluent and difficulty in treating concentrated waste. Therefore, cost and efficiency of materials to be used in dye decolorization is a matter of concern for environmental scientists. Being eco-friendly and economically viable (Joe et al., 2008), white rot fungi have been recognized as an efficient tool, degrading dyes with the involvement of their extracellular lignin-modifying enzymes (Pointing, 2001).

Spent Mushroom Substrate (SMS) released after mushroom cultivation, contains residual mushroom biomass along with a rich population of heterotrophic fungi and bacteria. It can act as an inexpensive source of phenol oxidases (Fermor et al., 2000). SMS also has the ability to chemically adsorb organic and inorganic pollutants, while diverse category of microbes it harbors, have the capability of biologically breaking down the organic xenobiotic compounds present in soil and water (Ahlawat et al., 2010). The extracellular ligninolytic enzymes and microbes from SMS of different mushrooms (Ahlawat and Singh, 2011), and mushroom mycelia, especially of *Pleurotus florida*, *Pleurotus ostreatus*, *Pleurotus flabellatus* and *P. sajor-caju* have been reported to have role in dye decolorization activities (Faraco et al., 2009; Ahlawat and Singh, 2009, 2011). Being an inexpensive source of phenol oxidases and different types of microorganisms, the SMS itself has the ability to adsorb and catabolize the pollutants present in textile effluent. The physical structure of SMS, and the enzymes and microorganisms present in it work coherently for decolorization of coloring dyes. However, the decolorization potential of SMS depends upon activities of its enzymes and microorganisms, which require optimum cultural conditions for exerting their best potential against the textile effluent. With this background the study presents variations in microbial population of spent substrate of three different mushrooms and textile effluent decolorization potential of microorganisms isolated from these SMSs when applied singly or as consortia as affected by temperature, carbon sources and immobilization media.

2. Methods

2.1. Substrate and textile effluent

Textile effluent was collected from a commercial unit located at industrial area Baddi, Solan (HP), India. It was analyzed for pH and electrical conductivity by using Smart Chem autoanalyzer and HPG System, respectively, both before and after treating with different combinations of bacteria and fungi. SMS used for isolation of effective bacteria and fungi, and agricultural residues used as immobilizing agents were obtained from Directorate of Mushroom Research (DMR), Solan, India. The electrical conductivity of button, oyster and paddy straw mushroom spent substrates varied from lowest of 1.040 $mS\,cm^{-1}$ in paddy straw mushroom (*Volvariella volvacea*) SMS, 2.51 $mS\,cm^{-1}$ in oyster mushroom (*P. sajor-caju*) SMS and highest of 2.90 $mS\,cm^{-1}$ in button mushroom (*Agaricus bisporus*) SMS. The pH of spent substrates varied between 8.2 and 9.0, with lowest of 8.2 in button mushroom SMS and highest of 9.0 in oyster mushroom SMS. The pH and electrical conductivity of textile effluent were 8.9 and 2.845 $mS\,cm^{-1}$, respectively.

2.2. Isolation and identification of SMS microflora

Spent substrate of button, oyster and paddy straw mushrooms were used for the isolation of fungi and bacteria. Fungi and bacteria were isolated using serial dilution and plating method on potato dextrose agar (potato dextrose 24 g, agar agar 20 g, water 1000 ml, pH 7.2) and nutrient agar (beef extract 3 g, peptone 5 g, dextrose 3 g, NaCl 5 g, agar agar 15 g, water 1000 ml, pH 7.2) medium, respectively and incubating at 30 ± 2 °C for 5 days. Well separated colonies were further purified by sub-culturing on respective medium and well grown cultures were stored at 4 °C till further use. Purified fungi and bacteria were identified using 5.8S rRNA and 16S rRNA gene sequencing, respectively, followed by Nucleotide Basic Local Alignment Search Tool (BLASTn) technique (Altschul et al., 1990).

2.2.1. Fungal DNA extraction and 5.8S rRNA gene amplification

All fungal cultures were grown separately on malt extract agar Petri dishes at 30 2 °C for 7 days. The mycelia from individual fungus were scrapped and put in 1.5 ml micro-centrifuge tubes, kept at −85 °C for at least 2 h and freeze dried for 16–18 h. Genomic DNA was extracted from approximately 100 mg of freeze dried fungal mycelia using DNeasy Plant Mini Kit (QIAGEN GmbH, D-40724 Hilden) following manufacturer's protocol.

The polymerase chain reaction (PCR) primer ITS-1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) developed by White et al. (1990) were used to amplify the ITS region along with 5.8S rDNA. PCR amplification was performed in a reaction mixture of 50 µl, containing 0.2 µl *Taq* DNA polymerase (5 $U\,\mu l^{-1}$), 5 µl 10X PCR buffer (100 mM Tris–HCl, pH-8.3, 15 mM $MgCl_2$, 250 mM KCl), 5 µl dNTP mix (2.0 mM each), 1 µl each of ITS-1 and ITS-4 primers (0.01 mM), 1 µl glycerol (5%), 2 µl $MgCl_2$ (25 mM) and 2 µl of genomic DNA (50 ng). PCR reaction was performed in PCR Master Cycler Gradient in 36 cycles each of 95 °C for 1 min, 50 °C for 30 s, 72 °C for 1 min 20 s and final elongation at 72 °C for 10 min with lid heating option at 104 °C. The presence and yield of amplicons was ascertained on 2.0% agarose gel (w/v) prepared in 1% TBE. Gel electrophoresis was carried out at 90 V for 1.30 h in 0.5X TBE buffer. Staining was done with ethidium bromide and the gel was visualized and photographed using Bio Imaging System (Gene Genius, Syngene).

2.2.2. Bacterial DNA extraction, quantification and 16S rRNA gene amplification

Bacterial isolates were grown separately in 10 ml nutrient broth filled in 20 ml screw caped tubes at 30 ± 2 °C for 2 days. For extraction of total genomic DNA, 1.5 ml log phase broth culture was used and DNA was extracted using BACTOZOL™ Bacterial DNA Isolation Kit (Molecular Research Centre, Inc., Cincinnati, OH). DNA concentration in extracted samples was determined by running 5 µl of each sample on 1.2% agarose gel (w/v) at 90 V for 1 h in 0.5% TBE buffer along with quantification marker. Gel was visualized using Bio Imaging System (Gene Genius, Syngene). Alternatively, the DNA concentration and purity were also checked by measuring the extinction at A_{260}/A_{280} on UV visible spectrophotometer. The concentration was calculated assuming that one A_{260} unit is equal to 50 µg of double stranded DNA/ml.

PCR amplification of 16S rRNA gene was performed by employing forward primer (5′-GGA TCC AGA CTT TGA TYM TGG CTC AG-3′) and reverse primer (5′-CCG TCA ATT CMT TTG AGT TT-3′). PCR reaction was performed in a reaction mixture of 50 µl, containing 0.2 µl *Taq* DNA polymerase (5 $U\,\mu l^{-1}$), 5 µl 10X PCR buffer (100 mM Tris–HCl, pH-8.3, 15 mM $MgCl_2$, 250 mM KCl), 5 µl dNTP mix (2 mM each), 1 µl each of forward and reverse primer (0.01 mM), 1 µl glycerol (5%), 2 µl $MgCl_2$ (25 mM) and 2 µl of genomic DNA (50 ng). The reactions were performed in a PCR Master