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Research paper

Optimization of conditions for decolorization of azo-based textile dyes by multiple fungal species

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ABSTRACT

Wastewater from textile industries contains azo dye residues that negatively affect most environmental systems. The biological treatment of these wastes is the best option due to safety and cost concerns. Here we isolated and identified 19 azo dye-degrading fungi and optimized conditions resulting in enhanced degradation. The fungi belonged to five species of *Aspergillus* and a single *Lichtheimia* sp. All fungi were evaluated for their ability to decolorize 20 azo dyes. While the most easily transformable azo dye was direct violet (decolorization ranged from 71.1 to 93.3%), the most resistant to decolorization was fast green azo dye. The greatest degradation potential of azo dyes (direct violet and methyl red) was optimized using the most promising four fungal strains and changing media glucose concentration, nitrogen source, and micronutrients. Biomass, lignin peroxidase, and laccases production were also determined in the optimization studies. The decolorization of both azo dyes by the four fungal strains was greatly enhanced by glucose supplementation. The fungal strains were not able to produce lignin peroxidases in the absence of organic nitrogen source. Both yeast extract and casamino acid supplementation enhanced decolorization of direct violet and methyl red dyes and production of lignin peroxidase by the fungal strains. In contrast, the laccases were absent in the similar medium enriched with the same organic nitrogen sources.

1. Introduction

Industrial wastewater released from textile plants often contains residual azo-dyes, which are considered as environmental hazardous materials. These dyes are difficult to treat biologically, mainly due to their synthetic origin and complex aromatic structures. Chemical methods remain the major wastewater treatment procedures used in these factories, and often result in the production of other forms of chemical pollutants. Consequently, a lack of remediation technologies in industrial dye plants results in the discharge of wastewater into the environment, which affects plant, animal, and human health. One major problem in the treatment of textile industrial wastewater is due to the variation in the types and chemistry of textile dyes. This is in part due to the staining requirements of different fiber types:1) cellulose (cotton, rayon, linen, ramie, and lyocell), 2) protein (wool, angora, mohair, cashmere and silk) and 3) synthetic fibers polyester, nylon, spandex, acetate, acrylic, ingeo and polypropylene (Ghaly et al., 2014). The textile dyes used to treat these fibers are generally classified as acid, basic, direct, fluorescent, reactive, sulphurous, or vat dyes (Kirk-Othmer, 1979).

Several investigations have been done to examine the biodegradation of azo-dyes by either bacteria or fungi isolated from textile dye effluent. Manikandan et al. (2012) isolated *Achromobacter xylosoxidans* GRIRKNM11 from a textile dye effluent site able to decolorize turquoise blue dye (100 mg/L) within 48 h. Similarly, Hassan et al. (2013) isolated and identified indigenous bacteria from textile dye effluent and tested them for decolorization of the azo dyes novacron, viz orange W3R, red FNR, yellow FN2R, blue FNR and navy Joshi et al. (2015) studied degradation of methyl red (MR) and carbolfuchsin (CF) by *Proteus* spp., *Pseudomonas* spp. and *Acinetobacter* spp. bacteria and its consortium isolated from textile effluents. The consortium showed the highest potential in decolorizing MR and CF, up to 100% and 96%, respectively, within 24 h. Karthikeyan et al. (2015) tested the efficacy of *Pleurotus platypus* in decolorization of two textile dye effluents.

Variation in textile dye types and chemistries prevent the use of a single microorganism to treat all wastewaters containing industrial dyes. Moreover, since there are many types of azo dyes, only relatively few isolated microorganism have been tested for decolorization of a

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Table 1
Absorbance maxima (λ-max in nm) for the 20 tested azo dyes.

Dye No.	Azo-dye	λ-max (nm)	Dye No.	Azo-dye	λ-max (nm)
1	Reactive red	520	11	Tartrazin	460
2	Direct blue	584	12	Naphthol blue black	626
3	Direct red	546	13	Trypan blue	626
4	Direct violet	626	14	Janus green	626
5	Reactive blue	626	15	Alirazin yellow	410
6	Reactive orange	512	16	Evans blue	660
7	Fast green	418	17	Brilliant green	627
8	Methyl red	480	18	Safranin	500
9	Crystal violet	600	19	Pararosaniline	550
10	Alura red	491	20	Poneau S	567

limited number of azo dyes or textile dye effluents. Therefore, there is a need for obtaining multiple azo dye-degrading microorganisms to be used for biological treatment of wastewater produced from different textile plants. In the present study, we aimed to isolate and identify multiple azo dye- degrading microorganisms and optimize the degradation and decolorization process by altering growth conditions that favored enzyme production.

2. Materials and methods

2.1. Azo dyes

Twenty azo dyes (Table 1) were used in this study. These included direct blue 71, reactive blue 4, reactive orange, fast green, methyl red, crystal violet, alura red AC, tartrazine, naphthol blue black, trypan blue, janus green B, alizarin yellow R, evans blue, brilliant green, safranin, pararosaniline, and ponceau S. The dyes were obtained from Sigma-Aldrich (St. Louis, MO, USA). Direct red 80, cibacron brilliant Red 3B-A, and direct violet 51 were obtained from Santa Cruz Biotechnology (Dallas, TX, USA).

2.2. Sample collection

Industrial wastewater from a textile plant was collected from the Mardini Fabrics Co., 10th of Ramadan City, Egypt. Three soil and water samples were collected from the Suez Canal and Kafr El Sheikh, in the Nile Delta region of lower Egypt. One soil sample was collected from cultivated soil at San Joaquin valley, near Modesto, California, USA.

2.3. Microbiological media

Mineral salts medium (MSM) was prepared as described by Chao et al. (2006), with slights modifications. The medium contained ($g\ l^{-1}$): NH_4NO_3, 0.5; K_2HPO_4, 1.5; KH_2PO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.2 g; $FeSO_4$, 0.02 g; $CaCl_2$, 0.05; and $CuSO_4$, 0.02 g. The medium was supplemented with 0.5 g/l of yeast extract as a source of growth factors and the azo-dyes, each at a final concentration of 100 μg/ml. The pH of this medium was adjusted to 5.0 for fungi and 7.0 for isolation of bacteria. This medium was solidified by the addition of 1.7% agar, for culture maintenance. Liquid medium was used for testing the decolorization activity of all fungi.

2.4. Isolation of azo dye-degrading microorganisms

A 10 g aliquot of each soil sample was independently suspended in 100 ml sterilized saline solution (0.85% NaCl) and shaken for 1 hat 150 rpm. A 1 ml aliquot of each soil suspension, or water sample, was separately added into a petri dish and 25 ml of molten MSM medium, containing either commercial direct blue or direct green azo-dyes at 100 μg/ml, or the industrial wastewater, was poured into the plate and the contents was mixed well. Plates were incubated at 28 °C for 5 days. Colonies appearing after incubation were picked, purified by streaking multiple times on the same initial isolation medium, and maintained on the same medium. Fungi were isolated in a similar manner except that the medium was adjusted to pH 5.0 prior to use.

2.5. Identification of the fungal isolates

All of the isolated fungi were identified by sequencing the ITS1 and ITS4 gene regions. The primer sequences used were: ITS1: TCCGTAGGTGAAC CTGCGG (Korabecna, 2007) and ITS4: TCCTCCG-CTTATTGATATGC (Korabecna, 2007).The PCR conditions for gene amplification were: 95 °C, 10 min; 29 x (95 °C, 30 s; 52 °C, 2 min; 72 °C, 30 s); 72 °C, 8 min; and 4 °C hold. The PCR products were separated by electrophoresis on 1% agarose gels, and purified using the ExoSAP-It reagent − ExoSAP-IT® PCR Product Cleanup (Affymetrix USA) as instructed by the manufacturer. DNA samples were sequenced at University of Minnesota Genomic Center (UMGC). The sequences obtained were trimmed and analyzed by using BLAST analysis (http://blast.ncbi.nlm.nih.gov).

2.6. Inoculum preparation

Spore suspensions were prepared by washing 3 day-old fungus slants with sterilized saline solution (0.85% NaCl). The OD was measured at 600 nm and adjusted to A 600 nm = 1.0 prior to inoculation.

2.7. Assays for azo dye decolorization activity

A 10 ml aliquot of MSM medium supplemented with 100 μg/ml of the tested azo dye was added to a 50-ml culture tube and separately inoculated with 100 μl ($OD600_{nm}$ = 1.0) of each spore suspension. Tubes were incubated at 150 rpm at 28 °C for 5 days. Three replicates cultures were used for each dye/strain combination. Biomass was separated by centrifugation at 5000 xg for 5 min, and absorbance of the supernatant was measured at the λ-max of each azo dye (Table 1). The λ-max of each azo dye was obtained from scanning a 10 μg/ml solution using an Agilent G1120A spectrophotometer. The initial OD (control) from non-inoculated MSM containing the azo dye was measured and the relative decolorization percentage was calculated as follows:

Percent relative decolorization = 100 − [(OD measured/OD initial)] * 100

The separated fungal biomass was dried at 70 °C until constant weight, usually only a few mg was produced. Since a relatively large amount of dye was used in our assay, and only a small amount of dye can be adsorbed by the limited amount of fungal biomass produced, we did not use dead biomass controls. Moreover, dye decolorization occurred in a fungal-specific manner.

Consequently, we attributed the majority of dye decolorization to biotransformation by living fungal cells.

The biodegradation products of methyl red and direct violet azo dyes by the four selected fungal strains was determined by LC/MS and GC/MS to identify the biodegradation mechanism (unpublished data).

2.8. Enzyme assays

Manganese peroxidases activity was determined by measuring the purpurogallin formation rate at 420 nm, from the reaction between pyrogallol and hydrogen peroxide catalyzed by peroxidase (Bourbannais and Paice, 1988). This was done using the extinction coefficient ε420 nm = 2640 $M^{-1}\ Cm^{-1}$. One unit of peroxidase was defined as the amount of enzyme that catalyzed the production of 1 mg of purpurogallin in 20 s at 25 °C and at pH 6.0. Laccase activity was