Isolation and Identification of Fungi for Decolorization of Synthetic Dyes

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ABSTRACT

For decolorization of synthetic dyes, Isolate fungi were investigated for the decolorization of 8 industrial dyes. One fungus isolated from textile wastewater collected from Banweol industrial complex, Korea showed excellent ability for removing synthetic dyes. Internal Transcribed Spacers (ITS) sequencing result was confirmed as the new *Basidomycetes species. HUE05-1* The optimal decolorizaton conditions were pH5, 30℃ and aerobic condition. *HUE05-1* was completely decolorized all dyes in both solid and liquid condition. The result is decolorization effect at Reactive Orange 16; 97.12%, Reactive Blue 19; 92.09%, Reactive Blue 49; 97.04%, Reactive Yellow 145; 95.53%, Acid Orange 10; 99.18%, Acid Violet 43; 98.73%, Acid Blue 350; 94.71%, Disperse Blue 106; 90.07%.

Key Words: White rot fungi, textile waste water, dye, decolorization, Identification

I. Introduction

Synthetic dyes are extensively used in textile dyeing, paper printing, color photography, pharmaceutical, food, cosmetic, and other industries. Approximately 10,000 different dyes and pigments are used industrially, and over 0.7million ton of synthetic dyes are produced annually worldwide. It is estimated that 10~15% of the dyes are lost in the effluent during such dyeing processes[1]. Major classes of synthetic dyes and many of them are toxic or even carcinogenic compounds with long turnover times[2]. Therefore, the discharge of highly colored synthetic dye effluents from those industries can result in serious environmental pollution problems.

Many physical and chemical methods have been used for the treatment of dye-containing effluents. Adsorption is the most widely used method at present due to its convenience and efficiency [3]. The most commonly used adsorbent for color removal is activated carbon, but it is relatively expensive and the synthetic dyes are not degraded[4]. Biological methods being simple to use and low in cost have been the main focus in recent studies on dye biodegradation[5, 6].

The use of White-rot fungi, *Phanerochaete chrysosporium* and *Tictoporia sp.*, to decolorize the lignin containing pulp and paper wastewater was reported as early as in 1980. Since then, *P. chrysosporium* has been examined for decolorization of pulp mill wastewaters and various dyes by many researchers[7]. Other white- rot fungi including *Trametes versicolor*, *Coriolus versicolor* and *Funalia trogii* were also capable of decolorizing dyes[8].

II. Materials and method

1. Used dyes

Eight different synthetic dyes (Reactive Orange 16, Reactive Blue 19, Reactive Blue 49, Reactive Yellow 145, Acid Orange 10, Acid Violet 43, Acid Blue 350, and Disperse Blue 106) were used.

2. Isolation of fungi

Fungal strains were isolated from textile wastewater collected from Banweol industrial complex, Korea. The 10 fold diluted solution was cultured on MGYM (Yeast extract 1.0g, Glucose 2.0g, $MgSO_4 \cdot 7H_2O$ 0.14g, KH_2PO_4 0.5g, K_2HPO_4 1.0g, NaCl 0.4g, D.W 1ℓ, pH5.0±0.2, Dye 0.1g) agar plate containing dyes at 30±1℃ for 10 days. Taxonomic identification including morphological and cultural characterization was examined.

3. Strain identification by ITS sequencing

Universal primer ITS5 (5'-GGAAGTAAAAGTCGT AACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used for ITS sequencing. PCR condition was denaturation, 94℃, 40sec; annealing, 55℃ 40sec; extension 72℃, 1min 30cycle, final extension 72℃ 15min. ABI Prism 310 Genetic analyzer (PE Applied Biosystem) conducted analysis of base sequencing. And then it was compared National Center for Biotechnology Information (NCBI)'s Basic Local Alignment Search Tool (BLAST)

4. Decolorization test with synthetic dyes

1) Solid test

The isolate strain inoculated on Potato dextrose agar and incubated until extensive mycelium growth occurred. They were divided into $1cm^2$ pieces, and were placed on the center of agar plate containing MGYM with 100mg/l dyes. Then, clear zone size was measured.

2) Liquid test

Isolated strain was pre-grown in Potato dextrose broth for seeding.
Then, MGYM broth containing 100mg/l dye was incubated at 30℃, pH5 and 150rpm after 4%(v/v) seeding. The culture solutions were centrifuged at 4,000rpm for 10 min. The supernatant of the centrifuged

samples was read at absorbance maximum (λmax) of dyes used 436nm for RY145, 592nm for RB19, 580nm for AV43, 628nm for RB49, 502nm for RO16, 580nm for DB106, 622nm for AB350, 478nm for AO10 using shimadzu UV-mini1240 model spectrometer. The dye free medium was used as blank. The decoloriztion efficiency of different isolates was expressed as per the following Eq.

$$Decolorization(\%) = \frac{(I-F)}{I} \times 100$$

I = initial absorbance.

F = absorbance of decolorized medium.

Ⅲ. Results

1. Isolation of fungi

Taxonomic identification including morphological and cultural characterization was examined. Isolate fungi has a many pore hole and a cell wall. It was a diaphragm (septum) and it was become accomplished with the thin thread shape. To the diaphragm it will be able to confirm the small projection shape in the diaphragm region 1 thing or 2 nuclear to be included.

2. Strain identification

The ITS sequence of isolated strain is shown in Fig. 1. It was compared with National Center for Biotechnology Information (NCBI)'s Basic Local Alignment Search Tool (BLAST). The isolated strain was identified *Bacidomysetes spp. HUE05-1.*

3. Decolorization test with synthetic dyes

1) Solid test

Decolorization began with formation of clear zone around the colonies. Complete decolorization was then achieved (Fig. 2.). *Bacidomysetes spp. HUE05-1* efficiently decolorized different activities, 8 dyes after 4days (Table. 1.).

2. Liquid test

The Decolorization results on liquid are shown in Fig. 3. RB19 and DB106 were the fast decolorization. They complete decolorization was achieved within 2days after inoculation. All dyes showed decolerization over the 90% after 4days.

> *HUE05-1*

GTTCAGCGGGTAGTCCTACCTGATTTGAGCTCAGAGTTCAGATGTATTGT
CCCGTGAAGGCGGTTAGAAGCGCGTACTTCACATACCANTCGANGGCAGN
GCAGATAATTATCACGCTGAAGCGACCGGTAACGTCCGCACTAATGCATT
TCAGAGGAGTCGACTCGCGAGAGCCGACACGACCTCCAAGTCCAAGCCTT
GCAGTAACAAAACTGTAAGCTTGAGAATTCCATGAGACTCAAACAGGCAT
GCTCCTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGATT
CACTGAATTCTGCAATTCACATTACTTATCGCATTTCGATGCGTTCTTCA
TCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAGTTGCGT
TATCCGCAAATAAAGACATTCTATAACTGAAGCGTTTGTAGTAAGATAAC
ACCTACAAGTGCACGCGATATCAGACGTACGTACTAGCCGCGAAGCCAGC
CTCCGTCACAAAGCCANCCTTACATGCGATATGTGCACAGAAGTTGAGAG
TGGATGAGANTAGGTGTGCATCATGCCTTGCGGCCAGCAACAACCTNACC
AAAANCTCGATAATGTCCTCCGCGTATTATCAATAAGCGGAGG

Fig. 1. The sequencing of isolated *HUE05-1*

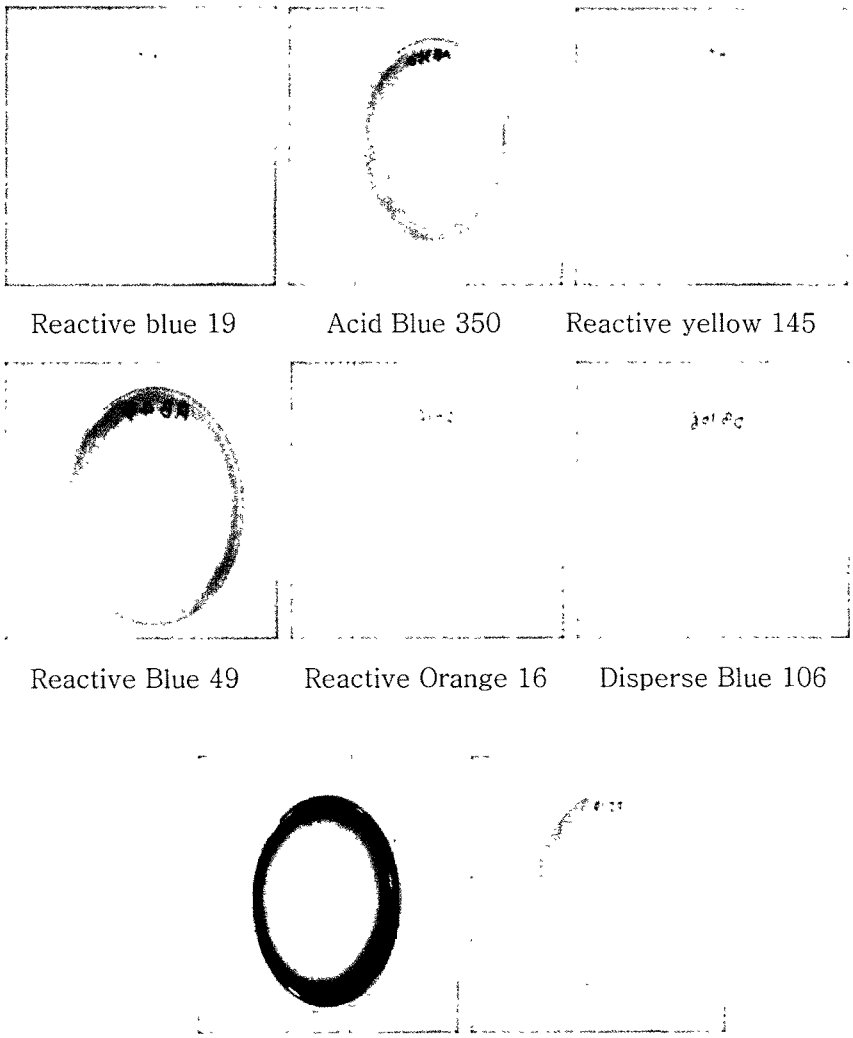

Fig. 2. Decolorization effect of Several Synthetic Dyes by *HUE05-1* (After 3days)

Table 1. Decolorization of various dyes by *HUE05-1* (Solid experiment)

Unit: %

Parameters	1 day			2 day			3 day			4 day		
	25℃	30℃	35℃	25℃	30℃	35℃	25℃	30℃	35℃	25℃	30℃	35℃
AO10	15	33	31	49	63	59	55	86	77	85	100	100
DB106	20	35	31	55	66	59	59	78	68	83	100	92
AV43	18	32	31	41	59	58	62	80	78	83	100	100
AB350	20	25	24	32	45	39	42	51	42	55	63	58
RO16	15	26	31	48	59	65	52	74	74	78	100	100
RB49	22	30	32	46	61	59	62	81	78	85	100	100
RY145	12	18	18	51	61	59	49	65	65	77	91	81
RB19	26	32	36	51	61	59	63	71	79	89	100	100

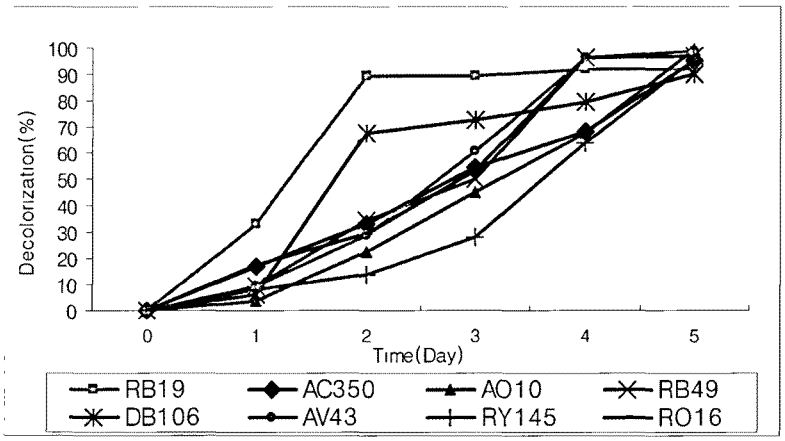

Fig. 3. Decolorization of various dyes by *HUE05-1* at Different day (liquid experiment)

References

1. Zollinger, H., Color Chemistry-Syntheses, Properties, and Applications of Organic Dyes and Pigment. VCH Publishers, New York, 1987

2. Hartman, C. P., Fulk, G. E. and Andrews, A. W., Azo reduction of trypan blue to known carcinogen by a cell-free extract of a human intestinal anaerobe. Mutat. Res. 1978, 58, 125-132.

3. Churchley, J. H., Removal of dye waste color from sewage effluent-the use of a full-scale ozone plant. Wat. Sci. Tech. 1994, 30, 275-284

4. Yeh, R. Y. L., Color different measurement and color removal from dye wastewater using different adsorbents. J. Chem. Tech. Biotechnol. 1995, 63, 55-59

5. Nigam, P., Banat, I. M., Singh, D. and Marchant, R., Microbial process for the decolorization of textile effluent containing azo dye, diazo, and reactive dyes. Proc. Biochem. 1996, 31, 435-442.

6. Seshadri, D., Bishop, P.L. and Agha, A. M., Anaerobic/aerobic treatment of selected azo dyes in wastewater. Waste Manag. 1994, 15, 127-137.

7. Spadora, J. T., Gold, M. H. and Reganathan, V., Degradation of azo dyes by the lignin-degrading fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 1992, 58, 2397- 2401.

8. Yuzhu Fu, T. Viraraghavan, Fungal decolorization of dye wastewaters. Bioresource Technology 2001, 79, 251-262.