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Screening of Fungi for Decolorization of Wastewater from Pulp and Paper Industry

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ABSTRACT

Sixty-four fungal strains collected from decomposed woods in Khao-Yai National Park were evaluated for their ability to decolorize the wastewater from pulp and paper industry. Among the strains collected, KAPI03, KAPI04, KAPI13, KAPI25, KAPI30, KAPI39 and KAPI50 were able to grow well on agar containing synthetic lignin indicating that they could utilize synthetic lignin as nutrient for their growth. However, when they were tested for their effectiveness on decolorization of the wastewater from pulp and paper mill, it was indicated that strains KAPI25, KAPI39 and KAPI50 were the most efficient strains able to decolorize the pulping wastewater by 54.4, 54.9 and 53.7%, respectively. These strains were identified by basic morphology as the Genus *Trichaptum* (KAPI25), *Datronia* (KAPI39) and *Trametes* (KAPI50).

Key words: decolorization, white-rot fungi, pulp and paper, wastewater

INTRODUCTION

The pulp and paper mill is a major industrial sector utilizing a huge amount of lignocellulosic materials and water during the manufacturing process. Recently, the impact of this industry on the environment has been closely examined. Even though the color of effluent from pulp and paper industry does not directly affect the environment, it extremely causes psychological trouble for people living in those areas. A number of researches demonstrated that the effluent from pulping process contains lignin compounds as major degraded compounds. These compounds are largely removed from the woody raw material in an alkali pulping stage. Some of these degradation products have exhibited toxicity and mutagenicity and may be accumulated in the tissues of animals, fish and human beings (Bajpai *et al.*, 2000).

Typically, wastewater treatment is able to improve the quality of wastewater discharged from any of the production processes, however, the color of the effluent is usually still intense according to lignin compounds. This is obvious when the effluent released to environments such as river or stream. Removal of lignin compounds from the effluent is therefore needed (Kirk and Cullen, 1998). This could be achieved by using microorganisms that are able to completely degrade polymeric lignins. A number of studies have provided information on white-rot fungi that can secrete enzymes for degrading natural lignin as summarized by Kondo (1998). For example, Fukuzumi and his coworkers (1980) was the first

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group who studied on utilization of white-rot fungi for wastewater treatment. They fed varieties of the fungi with wastewater from pulping process containing essential nutrients and found that from total of 35 fungal strains, *Tinctoporia* sp. was the best for wastewater decolorization. Furthermore, Eaton *et al.* (1980) carried out similar experiments using *Phlebia brevispora*, *P.subserialis*, *Poria cinerascens* and *Trametes versicolor*. Results demonstrated that the tested strains efficiently decolorized the wastewater. Another experiment was done by Livernoche and his partners in 1983. They screened and isolated the white-rot fungi that were able to decolorize wastewater from pulp bleaching process. From 15 strains, only six strains: *T. versicolor*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Polyporus versicolor* and unidentified strain, showed ability of wastewater decolorization. In this experiment, microorganisms from natural sources were screened for their capable to produce ligninolytic enzyme in order to decolorize the effluent from pulp and paper production process.

MATERIALS AND METHODS

1. Analysis of wastewater quality

Wastewater of pulping process from the Pulp and Paper Company in Khanchanaburi province was used in this study. Chemical Oxygen Demand (COD) was analyzed by closed reflux method. Color and turbidity of the samples were determined by using HUCH DR/2010 spectrophotometer. pH value and temperature were determined by using model 220 pH-conductivity meter. Dissolved Oxygen (DO) was analyzed by using DO HANNA HI 9143 Microprocessor Auto Cal DO meter. Total solid and suspended solid were analyzed according to the standard method for the Examination of Water and Wastewater, APHA. AWWA. Edition 19th, 1992.

2. Isolation of fungal strains (Modified from Watling)

From Figure 1, the mushroom samples were collected from Khao-Yai National Park, Nakornnayok province. The standard method, called drop spore or shoot spore technique, was used for fungal spore isolation. The technique used was aimed to obtain single spore directly shot from fresh mushroom samples onto Potato Dextrose agar (PDA; pH 5-6) containing antibiotics (0.5 g/L chloramphenical, 0.3 g/L penicillin and 1.3 g/L streptomycin).

The agar plates containing the mushroom samples were then incubated at 30°C for 24 hours. The resulting spores were observed as groups which would be afterwards isolated using needle under microscope and placed onto PDA agar containing antibiotics. The growing fungal mycelium was subcultured until the purified fungal strains were obtained. The selected strains were identified according to their basic morphology.

3. Preliminary screening of fungal strains capable of wastewater decolorization

The pure fungal cultures were cultivated on PDA agar for 5-7 days at 30°C. After that, five pieces of 1-cm agar containing fungal mycelium were inoculated into 100 ml of Glucose Yeast Extract (GYE) broth with shaking speed of 135 rpm for 7 days. The growth pattern of fungus and color of media were determined. The strains that grew well and did not cause darken the color of medium broth were selected and tested for their effectiveness for decolorization of wastewater.

Figure 2 showed the selected fungi cultured on PDA agar for 5-7 days at 30°C. After that, five pieces of 1-cm agar containing fungal mycelium were subjected into 100 ml of the effluent from pulp and paper process supplemented with essential nutrients (glucose 10 g, K_2HPO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4.7\ H_2O$ 0.01 g and NH_4NO_3 1.75 g per effluent 1 L). The fungal strains were grown at 30°C with shaking

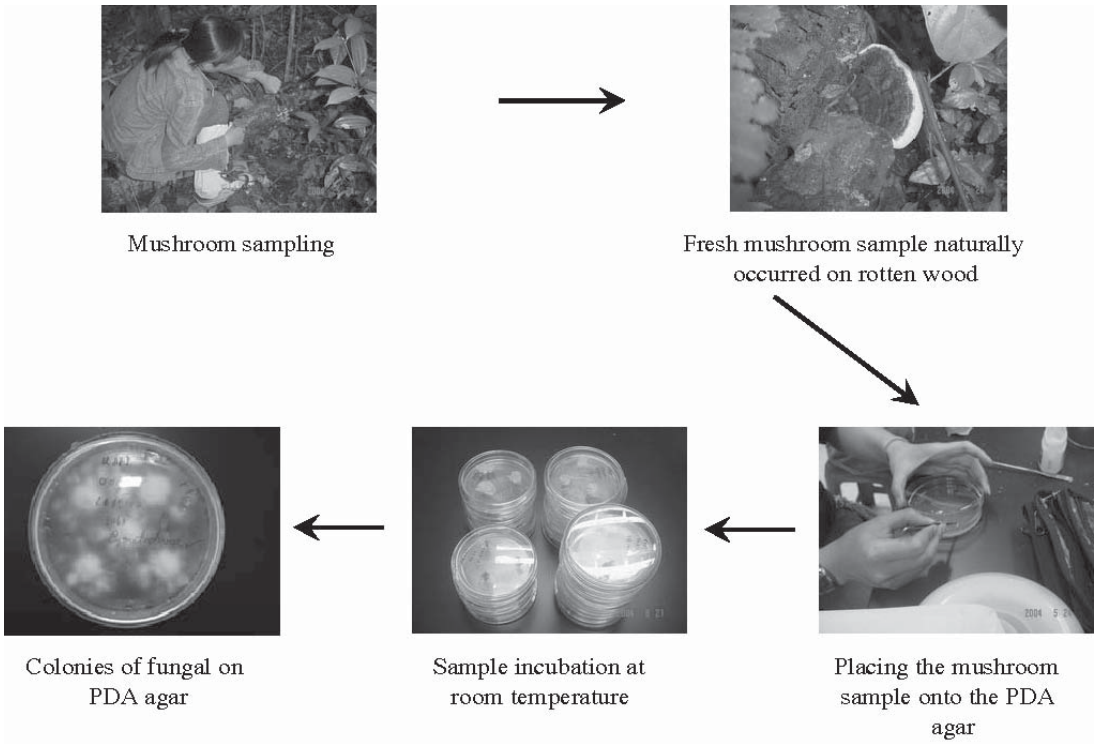

Figure 1 Isolation of fungal strains.

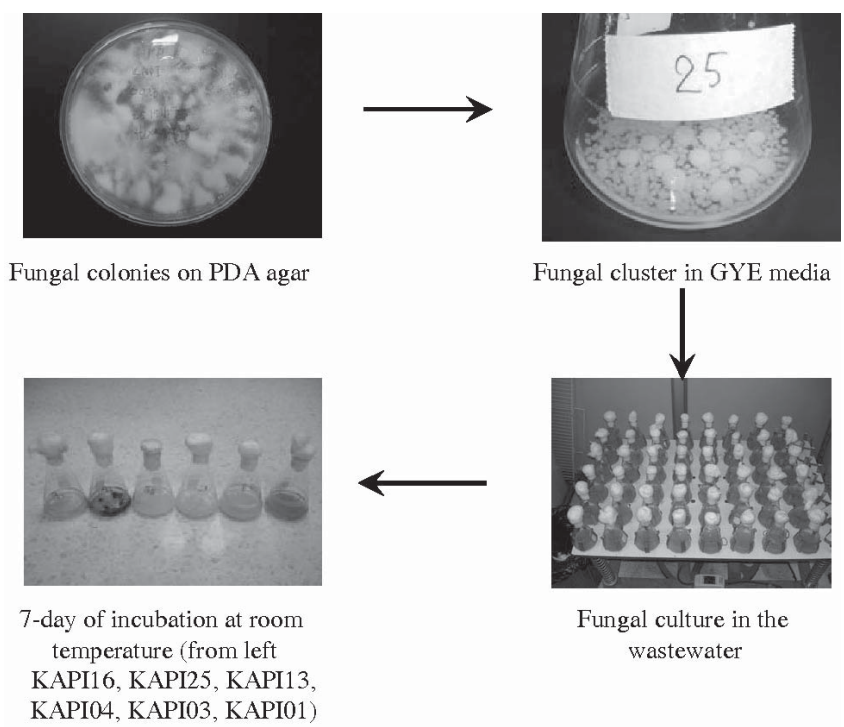

Figure 2 Preliminary screening of fungal strains capable of wastewater decolorization.

speed of 135 rpm for 7 days. Samples were collected daily for 4 days. Before analysis, samples were centrifuged to obtain supernatant and the analysis were carried out as described in section 1.

Alternatively, preliminary screening of the fungi could be done by growing the fungal strains on PDA agar containing 1% synthetic lignin at 30°C for a period of time. The strains that were capable of decolorizing the wastewater from pulp and paper industries would be theoretically able to survive and grow well on this agar.

RESULTS AND DISCUSSION

1. Analysis of wastewater quality

Table 1 shows the quality of wastewater from pulping process which was used for further experiments.

2. Isolation of white-rot fungal strains

Sixty-four fungal samples from the National Park, including white-rot fungi and brown-rot fungi designated as KAPI01 to KAPI64. These strains were kept on PDA agar in screw-capped test tubes at 20°C asp culture stocks.

3. Preliminary screening of fungal strains capable of wastewater decolorization

After cultivation with shaking for 7 days, seven fungal strains grew very well and did not darken the color of medium broth i.e. KAPI03, KAPI04, KAPI13, KAPI25, KAPI30, KAPI39 and KAPI50 whereas others could not grow in the same medium and did not change the color of the medium. This led to the conclusion that when these seven strains were used for wastewater treatment, they themselves will not produce any coloring agent which could affect the color of wastewater.

Table 1 Quality of wastewater from pulping process.

Parameter	Quality of wastewater
pH	5.2
Temperature (°C)	24.1
DO (mg/L)	4.75
Suspended solid (mg/L)	75
Total dissolved solid (mg/L)	4,065
COD (mg/L)	1,154
Color (Pt Co)	1,317

* According to the Department of Industrial Works, Ministry of Industry

The selected strains were tested for their effectiveness on decolorization of wastewater. It was shown that three out of seven tested strains (KAPI25, KAPI39 and KAPI50) were the most efficient strains capable of lightening the wastewater color (Figure 3). The strains KAPI25, KAPI 39 and KAPI50 decreased the wastewater color by 54.4, 54.9 and 53.7% from 1,317 Pt Co to 601, 594 and 610 Pt Co, respectively. This was consistent with the experiment of Nagarathnamma *et al*. (1999) that *Ceriporiopsis subvermispora* CZ-3, a wood degrading white-rot fungus, showed ability for decolorization of the wastewater from kraft pulp bleaching. However, the mechanism is unknown and needs more research works. In contrast, the COD value of treated effluent increased from 1,080 mg/L to 1,500-2,000 mg/L. This might be due to a number of nutrients which were added for accommodating the fungal growth as shown in Figure 4.

The three fungal strains from previous step were cultivated on PDA agar containing 0.1% synthetic lignin at room temperature for 5 days in order to confirm their capability of growth in lignin. Figure 5 demonstrated that these three strains, KAPI25, KAPI39 and KAPI50, were able to grow well in lignin as observed by their growing mycelium whereas other strains could not grow on the same medium.

4. Identification of selected white-rot fungal strains

The results showed that the selected fungal strains KAPI25, KAPI39 and KAPI50 possessedbasic morphology to the Genus

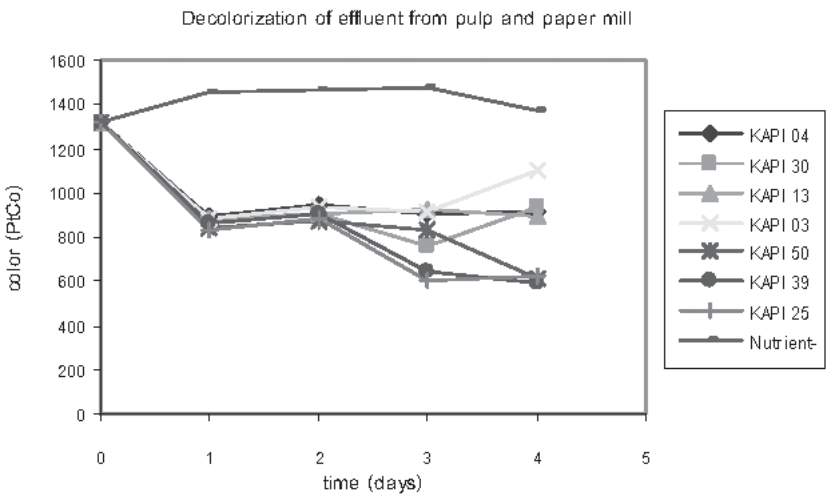

Figure 3 Effectiveness of fungal strains on decolorization of treated effluent.

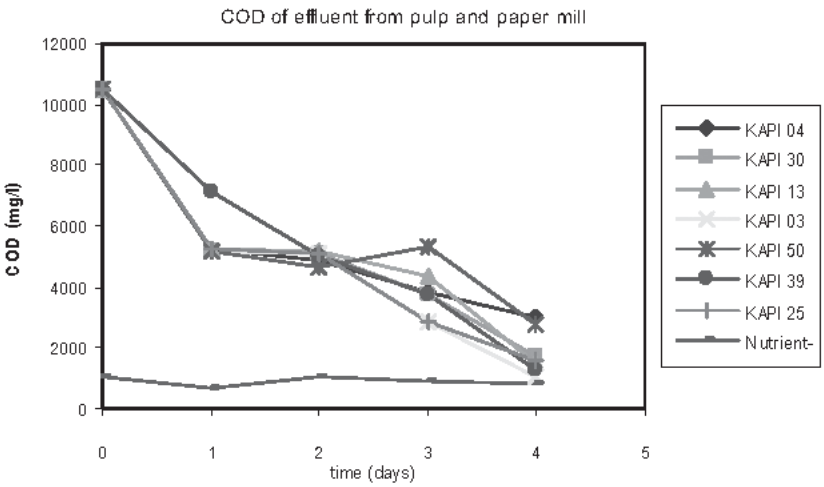

Figure 4 Effectiveness of fungal strains on reduction of COD of the treated effluent.

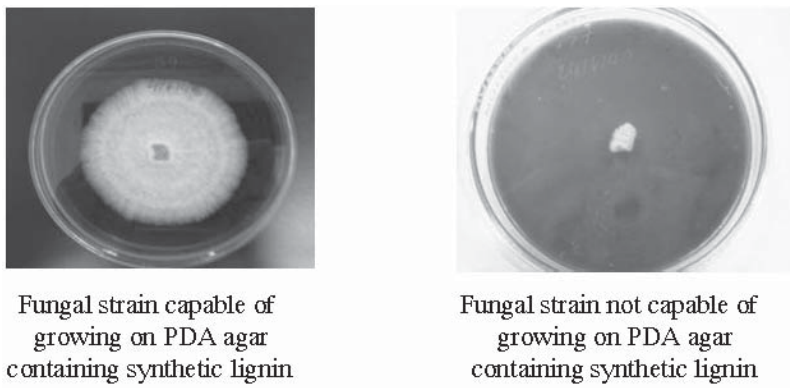

Figure 5 Capability of fungal strains on growing on PDA agar containing synthetic lignin.

Trichaptum sp., *Datronia* sp. and *Trametes* sp., respectively. Their characteristics were as follows:

- *Trichaptum* sp. (Ryvarden, 1636)

Polyporaceae, Polyporales, Agaricomycetidae, Basidiomycetes, Basidiomycota, Fungi

Fruitbodies, annual, resupinate and effused to pileate with imbricate narrow pilei or with an elongated narrow pileus over an effused and decurrent porelayer, pilus hispid to adpressed tomentose, blackish, grey or dirty, white, hymenophore irpicoid, lamellate to poroid, mostly pale brownish to light violet when actively growing, tubes brownish, context distinctly duplex, lower part dense and dark, upper part white and loose. White spores cylindrical, often slightly bent, smooth, hyaline thin-walled and non-amyloid.

Substrate: On dead unidentified wood

Habitat: Lignicolous, Dry Dipterocarp Forest

- *Datronia* sp. (Ruksawong and Flegel, 2001)

Polyporaceae, Polyporales, Agaricomycetidae, Basidiomycetes, Basidiomycota, Fungi

Fruitbodies, resupinate or reflexed, effused to reflexed margin, imbricate, semicircular, 80-90 mm diam. × 5.0-8.0 mm. thick, villose surface, white to pale yellow or cream color texture, tough but flexible or coriaceous, bicoloured, light brown and dark brown, zonate between two color, dark uneven upper side. White spore, ± cylindrical spores and dendrohyphidia in pore mouths.

Substrate: On dead unidentified wood

Habitat: Lignicolous, Dry Dipterocarp Forest

- *Trametes* sp. (Murrill, 1973)

Polyporaceae, Polyporales, Agaricomycetidae, Basidiomycetes, Basidiomycota, Fungi

Fruitbody; dimidiate, conchate to applanate, 2-3 × 3-5 × 1 cm.; surface smooth, azonate, glabrous, snow-white, pale-isabelline in dried specimens; margin thin, concolorous, entire; context soft, punky-corky, white, 5 mm. thick; tubes 2-4 mm. long, slender, white to pallid within, mouths circular, 5 to a mm., edges thin, firm, entire, glistening, white to slightly discolored. Stipe present, solid, same color surface with fruitbody, white texture, thick and shot.

Substrate: On dead unidentified wood.

Habitat: Lignicolous, Dry Dipterocarp Forest.

CONCLUSION

Sixty-four mushroom samples collected from Khao-Yai National Park, sixty-four of fungal strains including white-rot fungi and brown-rot fungi were obtained. Preliminary screening showed that three strains (KAPI25, KAPI 39 and KAPI50) were able to grow on PDA agar containing synthetic lignin and decolorize the wastewater from pulp and paper process by 54.4, 54.9 and 53.7%, respectively. After being identified by basic morphology, the strains KAPI25, KAPI39 and KAPI50 showed identity to the Genus *Trichaptum* sp., *Datronia* sp. and *Trametes* sp., respectively. However, the treated effluent analysis indicated much higher COD value due to the supplement of nutrients essential for fungal growth. Therefore, further experiments will focus on minimization of nutrients appropriate for fungal growth which will not affect the COD value of the effluent.

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LITERATURE CITED

Bajpai, P., P.K Bajpai and R. Kondo. 2000. **Biotechnology for Environmental Protection in the Pulp and Paper Industry**. Springer-Verlag Berlin Heidelberg, New York. 65-85 p.

Eaton, D., H.-M. Chang and T.K. Kirk 1980. Fungal decolorization of kraft bleach plant effluents. **Tappi** 63: 103-106.

Fukuzumi, T. 1980. Microbial decolorisation and defoaming of pulping waste liquors. pp. 161-171. *In* **Lignin Biodegradation: Microbiology, Chemistry and Potential Applications. Vol. 2**. T.K. Kirk, H.M.Chang and T. Higuchi. Boca Raton, FL, USA: CRC Press.

Kirk, T.K. and D. Cullen. 1998. Enzymology and Molecular Genetics of Wood Degradation by White-Rot Fungi. pp. 273-302. *In* **Environmentally Friendly Technologies for the Pulp and Paper Industry**. A.Y. Raymond and A. Masood. John Wiley & Sons, Inc.

Kondo, R. 1998. Waste Treatment of Draft Effluent by White-Rot Fungi, pp. 515-539. *In* A.Y. Raymond and A. Masood, (eds.). **Environmentally Friendly Technologies for the Pulp and Paper Industry**. John Wiley & Sons, Inc.

Murrill, W. A. 1973. Tropical Polypores. **Bibliotheca Mycologica (BAND 40)**. New York, Verlag Von J. Cramer. 113. p

Nagarathnamme, R., P. Bajpai and P.K. Bajpai. 1999. Studies on decolorization, degradation and detoxification of chlorinated lignin compounds in kraft bleaching effluents by Ceriporiopsis subvermispora. **Process Biochemistry** 34: 939-948.

Ryvarden, L. and I. Johanson 1636. **A Preliminary *Polypore* Flora of East Aflica**. Oslow, Norway, Gronlands Eskefabrikk: 636 p.

Ruksawong, P. and T.W. Flegel. 2001. **Thai Mushrooms and Other Fungi**. National Center for Genetic Engineering and Biotechnology, Bangkok, 268 p.