# ประสิทธิภาพของฟังไจกลุ่ม White-rot ในการกำจัดสีย้อมกลุ่มรีแอคทีฟ

## Efficiency of White-rot Fungi on the Decolorization of Reactive Dyes

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## บทคัดย่อ

สีย้อมกลุ่มรีแอคทีฟนิยมใช้ในโรงงานอุตสาหกรรมฟอกย้อม แต่สีย้อมเหล่านี้ทนต่อการย่อยสลายทางชีวภาพ จึงตกค้างในสิ่งแวดล้อมและก่อให้เกิดผลกระทบต่อระบบนิเวศ ซึ่ง white-rot fungi เป็นจุลินทรีย์ที่มีรายงานว่าสามารถย่อยสลายสารประกอบเชิงซ้อนต่างๆ รวมทั้งสีย้อมได้ ดังนั้น การศึกษาครั้งนี้ จึงทำการศึกษาเกี่ยวกับประสิทธิภาพในการกำจัดสีย้อม Reactive blue 19 และ Reactive blue 171 ของ white-rot fungi จำนวน 11 ชนิด ได้แก่ *Pleurotus sajor-caju* (Fr.) Sing., *Lentinula edodes* (Berk.) Pegler., *Flammulina velutipes* (Curt.ex Fr.) Sing., *Ganoderma lucidum* (Leys. Ex Fr.) Karst., *Ayricularia polytricha* (Mont.) Sacc., *Lentinus strigosus* (Schwein.) Fr. Pegler., *Lentinus squarrosulus* Mont., *Agrocybe cylindracea* (DC. Ex Fr.) Maire., *Volvariella volvacea* (Bull. ex Fr.) Sing., *Pleurotus eryngii* (Cand.ex.Fr.) quel.และ *Coprinus fimetarus* (L) Fr. และศึกษาชนิดและปริมาณของเอนไซม์ที่เกี่ยวข้องกับการย่อยสลายสีย้อม ผลการศึกษา พบว่า *P. sajor-caju* (Fr.) Sing. ที่เพาะเลี้ยงในอาหารเลี้ยงเชื้อเหลวที่เติมสีย้อม Reactive blue 19 และ Reactive blue 171 ที่ความเข้มข้น 50 ppm สามารถย่อยสลายสีย้อม Reactive blue 19 และ Reactive blue 171 ได้สูงสุดถึง 89.29% และ 70.67% ตามลำดับในระยะเวลา 9 วัน และเมื่อนำ *P. sajor-caju* (Fr.) Sing. มาเพาะเลี้ยงในอาหารเลี้ยงเชื้อที่เติมสีย้อมสีย้อมผสม (Reactive blue 19 และ Reactive blue 171) ในอัตราส่วน 1:1 พบว่า *P. sajor-caju* (Fr.) Sing. สามารถย่อยสลายสีย้อมได้ 45% ในระยะเวลา 19 วัน สำหรับการศึกษาปริมาณของเอนไซม์กลุ่ม Ligninolytic พบว่า *Pleurotus sajor-caju* (Fr.) Sing. สร้างเอนไซม์ Lignin peroxidase (LiP) มากที่สุดถึง 222.79 มิลลิยูนิตต่อมิลลิกรัมโปรตีน และสร้างเอนไซม์ Manganese peroxidase (MnP) ได้ 62.54 มิลลิยูนิตต่อมิลลิกรัมโปรตีน ส่วนเอนไซม์ Laccase (Lac) สร้างได้น้อยที่สุด (38.55 มิลลิยูนิตต่อมิลลิกรัมโปรตีน) จากผลการศึกษาสรุปได้ว่า *P. sajor-caju* (Fr.) Sing. มีประสิทธิภาพมากที่สุดในการกำจัดสีย้อมกลุ่มรีแอกทีฟ ซึ่งสามารถนำ *P. sajor-caju* (Fr.) Sing. ไปสู่การประยุกต์ใช้ในการบำบัดสีย้อมกลุ่มรีแอกทีฟในน้ำเสียจากโรงงานอุตสาหกรรมฟอกย้อมได้

**คำสำคัญ** : การกำจัดสี สีย้อมกลุ่มรีแอคทีฟ Lignin peroxidase, Manganese peroxidase, Laccase

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## ABSTRACT

Reactive dyes are widely use in textile dyeing industry but they resist to biodegrade and persist in the environment. The contamination of reactive dyes in the environment cause adverse effects on the ecosystem. White-rot fungi are one of the microbial groups that have been reported to degrade the various complex compounds including synthetic reactive dyes. This, study focused on the screening of a potent white-rot fungus that is able to degrade reactive blue 19 and reactive blue 171. White-rot fungi used to assess their ability to degrade reactive dyes namely, *Pleurotus sajor-caju* (Fr.) Sing., *Lentinula edodes* (Berk.) Pegler., *Flammulina velutipes* (Curt.ex Fr.) Sing., *Ganoderma lucidum* (Leys. Ex Fr.) Karst., *Ayricularia polytricha* (Mont.) Sacc., *Lentinus strigosus* (Schwein.) Fr. Pegler., *Lentinus squarrosulus* Mont., *Agrocybe cylindracea* (DC. Ex Fr.) Maire., *Volvariella volvacea* (Bull. ex Fr.) Sing., *Pleurotus eryngii* (Cand.ex.Fr.) quel. and *Coprinus fimetarus* (L) Fr. In addition, quantitative analysis of ligninolytic enzymes produced by a potent white-rot fungus was determined. The results showed *P. sajor-caju* (Fr.) Sing. cultivated in minimal liquid medium supplemented with either 50 ppm of of reactive blue 19 or 50 ppm of reactive blue 171 exhibited strongly potential to decolorize both reactive blue dyes. The percentages of reactive blue 19 and of reactive blue 171 decolorization by *P. sajor-caju* (Fr.) Sing. were 89.29% and 70.67%, respectively at 9 days of incubation period. In mixture of both reactive blue dyes at concentration 50 ppm (1:1 ratio), *P. sajor-caju* (Fr.) Sing. was moderately able to degrade mixed reactive blue dye by 45% of degradation at 19 days of incubation period. Moreover, the levels of lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) produced by *P. sajor-caju* (Fr.) Sing. were 222.79, 62.54 and 38.55 mU/mg protein, respectively. These findings suggested that the efficiency of *P. sajor-caju* (Fr.) Sing. on the decolorization of reactive dyes could be applied for treatment of wastewater from textile dyeing industry.


**Keywords** : Decolorization, Reactive dyes, Lignin peroxidase, Manganese peroxidase, Laccase

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## INTRODUCTION

Textile mills discharge large volumes of effluents that are colored due the presence of synthesis dyes. Among the various classes of dyes, reactive dyes are the only colorants designed to bond covalently with the fabrics. Reactive dyes contain chromophoric groups and reactive groups that from covalent bonds with the fibers. In particular, the release of azo dyes into the environment in effluent form dye-utilizing industries has become a major concern in wastewater treatment, since some azo dyes or their metabolites may be mutagens or carcinogens (1). There are many different methods for removal of color. (2). Current non-biological methods may successfully accomplish dye removal but could be very expensive because of high chemical usage, costly infrastructure and high operating expenses (3). Biological treatment technologies in most cases give the highest degree of

confidence from an engineering and economic practicability point of view (4) and being a low cost, environmentally friendly and publicly acceptable treatment technology (5). This research focused on the screening of white-rot fungi for biodegradation of reactive dyes. Activity of ligninolytic enzymes including, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) produced by a potent white-rot fungus was determined.

## METHODS

### 1. Microorganism and cultivation condition

Eleven whit-rot fungal strains were purchased from Thailand Mushroom Culture Collection (TMCC) Department of Agriculture, Thailand. For experiments, fungal strains were grown on PDA plates at 28 °C 7 for days. Fungi were maintained on PDA slants and stored at 4 °C and subcultured every month.

### 2. Screening of a potent white-rot fungus for biodegradation of reactive dyes

Primary screening on minimal medium agar plate, 11 strains of white-rot fungi were assessed their biodegradable ability of reactive blue19 (RB19) and reactive blue171 (RB171) as previously mentioned by Nyanhongo et al. (2002) (6). Secondary screening in liquid minimal medium, four mycelial plugs (8 mm diameter) of the potent fungal strains from primary screening were inoculated into 50 ml of liquid minimal medium amended with either RB 19 or RB 171 by specific concentration at 50 ppm. All flasks were incubated at 28 °C with continuous shaking at 150 rpm. The dyes were measured by UV-VIS spectrophotometer at wavelength 592 of RB19 and at wavelength 604 of RB171 for 9 days and were collected at 1, 3, 5, 7 and 9 days. The percentage of dye removal was calculated as following equation.

$$\% \text{ Decolorization} = \frac{C_0 - C_t}{C_0} \times 100$$

Where; $C_0$ = initial concentration of dyes

$C_t$ = observed concentration of dyes

### 3. Effect of mixed reactive dye on the dye removal by a white-rot fungus

The four mycelial plugs (8 mm diameter) of fungal strain was inoculated in 50 ml liquid minimal medium added with mixed reactive dye solution (RB19 and RB171 at ratio 1:1) to give a final concentration at 50 ppm. All flasks were incubated at 28 °C with continuous shaking at 150 rpm for 19 days and were collected at 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19 days. No dye amended mixed reactive blue solution was used as control experiment. Decolorization in liquid minimal medium of fungal cells was determined by measuring the absorbance at wavelength 602 nm. Dye concentration was calculated from the calibration curve. Dye removal was calculated similar to equation in method no. 2.

**4. Quantitative determination of ligninolytic enzymes produced by white-rot fungus**

Laccase (Lac) activity was determined by measuring the oxidation of 2,6- dimethoxyphenol (DMP) according to the method of Paszczynski et al. (1991)(7). Manganese peroxidase (MnP) activity was assayed for the oxidation of 2,6- dimethoxyphenol (DMP) by using the modified method of Eibes et al. (2005) (8). Lignin peroxidase (LiP) activity was analysed by the oxidation of veratryl alcohol according to the method of Magalhães et al., (1996) (9). The total protein in culture filtrate was determined by coomassie blue protein assay according to the method of Bradford (10). Total protein in sample could be calculated by using the following equation. Specific activity of all ligninolytic enzymes was expressed as per mg of total protein.

$$\text{Total protein (mg/ml)} = \frac{A_{595} \text{ of sample x Standard concentration (mg/ml)}}{A_{595} \text{ of standard}}$$

## RESULTS AND DISCUSSION

**1. Primary screening of a potent white-rot fungal on agar plate**

The highly potential white-rot fungi that able to degraded both RB19 and RB171 on agar plates were *P. sajor-caju* (Fr.) Sing., *L. squarrosulus* Mont. and *C. fimetarus* (L) Fr. (Table 1). Chagas et al. (2001) (11) found that *P. sajor-caju* was able to decolorize some azo dye including, amaranth, new coccine and orange G. However, the decolorization of tartrazine on agar plate could not be observed.

**Table 1** Dye decolorization by tested white-rot fungi on agar plate after 14 days of incubation

| White-rot fungi | Synthesis reactive dyes | |
|---|---|---|
| | Reactive blue 19 | Reactive blue 171 |
| *Pleurotus sajor-caju* (Fr.) Sing. | + | + |
| *Lentinula edodes* (Berk.) Pegler. | + | + |
| *Flammulina velutipes* (Curt.ex Fr.) Sing. | – | + |
| *Ganoderma lucidum* (Leys. Ex Fr.) Karst. | + | + |
| *Ayricularia polytricha* (Mont.) Sacc. | – | – |
| *Lentinus strigosus* (Schwein.) Fr. Pegler. | + | + |
| *Lentinus squarrosulus* Mont. | + | + |
| *Agrocybe cylindracea* (DC. Ex Fr.) Maire. | + | + |
| *Volvariella volvacea* (Bull. ex Fr.) Sing. | – | + |
| *Pleurotus eryngii* (Cand.ex.Fr.) quel. | + | + |
| *Coprinus fimetarus* (L) Fr. | + | + |

**Note**: (+) decolorization, (–) no decolorization

**2. Secondary screening of a potent white-rot fungus in liquid minimal medium**

The results of screening the potent white-rot fungus on the decolorization synthesis dyes in liquid minimal medium showed that *P. sajor-caju* (Fr.) Sing. was the most potent white-rot fungus for decolorization of reactive dyes. The percentages of RB19 and of RB171 decolorization by *P. sajor-caju* (Fr.) Sing. were 89.29% and 70.67%, respectively at 9 days of incubation period. *C. fimetarus* (L) Fr. revealed moderately decolorizing activity to RB19 and RB171 at 39.52% and 55.69%, respectively. As 3 days of incubation, *L. squarrosulus* Mont. showed weakly decolorizing activity of RB19 (27.63%) and RB171 (43.63%). The results of decolorization by 3 strains of white-rot fungi showed in Figure 1 and Figure 2.

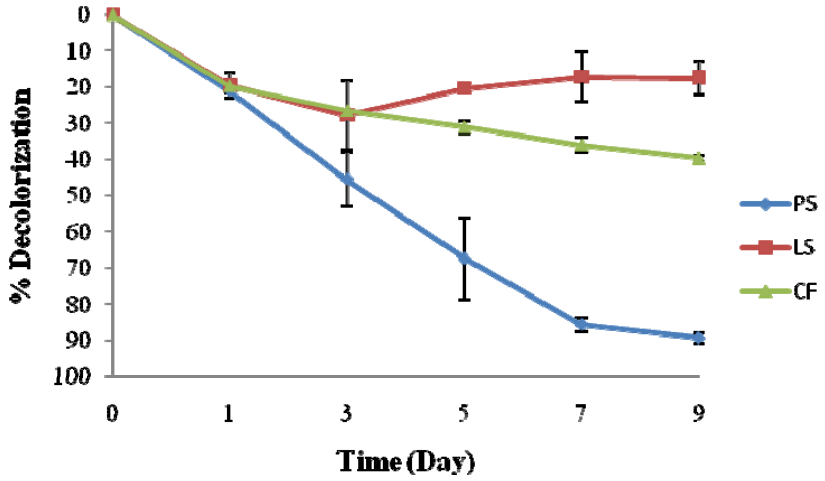


**Figure 1** Decolorization of reactive blue 19 by white-rot fungi cultivated in liquid minimal medium (PS = *P. sajor-caju* (Fr.) Sing.,LS = *L. squarrosulus* Mont., CF = *C. fimetarus* (L) Fr.)

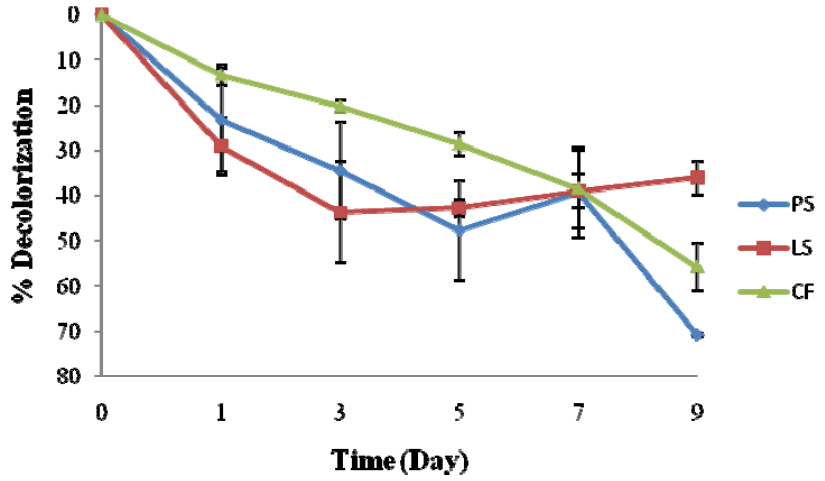


**Figure 2** Decolorization of reactive blue 171 by white-rot fungi cultivated in liquid minimal medium (PS = *P. sajor-caju* (Fr.) Sing.,LS = *L. squarrosulus* Mont., CF = *C. fimetarus* (L) Fr.)

**3. Decolorization of mixed reactive dyes by a potent white-rot fungus in minimal liquid medium**

Among 3 strains of tested white-rot fungi, *P. sajor-caju* (Fr.) Sing. showed the strongly decolorization of mixed reactive blue dyes (45%) as shown in Figure 3. The study of Eichlerová et al. (2006) (12) reported that *Pleurotus calyptratus* was able to decolorize orange G (91%) and remazol brilliant blue R (85%) in liquid culture.

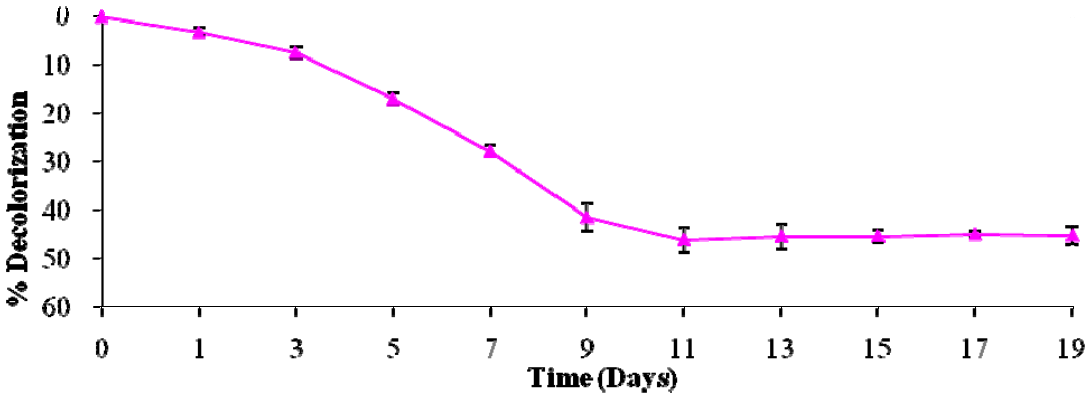


**Figure 3** Efficiency of *P. sajor-caju* (Fr.) Sing. on the decolorization of mixed reactive blue dyes

**4. Quantitative determination of ligninolytic enzymes produced by a potent white-rot fungus**

Fungal discs of *P. sajor-caju* (Fr.) Sing. was inoculated in minimal liquid medium supplemented with mixed reactive blue dyes at final concentration 50 ppm (at ratio 1:1). The results found that *P. sajor-caju* (Fr.) Sing. produced Lignin peroxidase enzyme (LiP) in the range between 24.59 – 222.79 mU/mg protein (Figure 4). The yield of LiP peaked at 13 days of incubation period (222.79 mU/mg protein). MnP were sharply increased at approximately 48.66 mU/mg protein at 9 days and continued maximally at 13 days (62.54 mU/mg protein). In addition, the level of Lac activity produced by *P. sajor-caju* (Fr.) Sing. was 38.55 mU/mg protein at 13 days. According to the study of Eichlerová et al. (2006) (12) reported that the production of Lac and MnP by *P. erygnii* were 108.6 and 1.02 U/L, respectively. *P. erygnii* produced a substantially higher amount of Lac in Kirk medium.

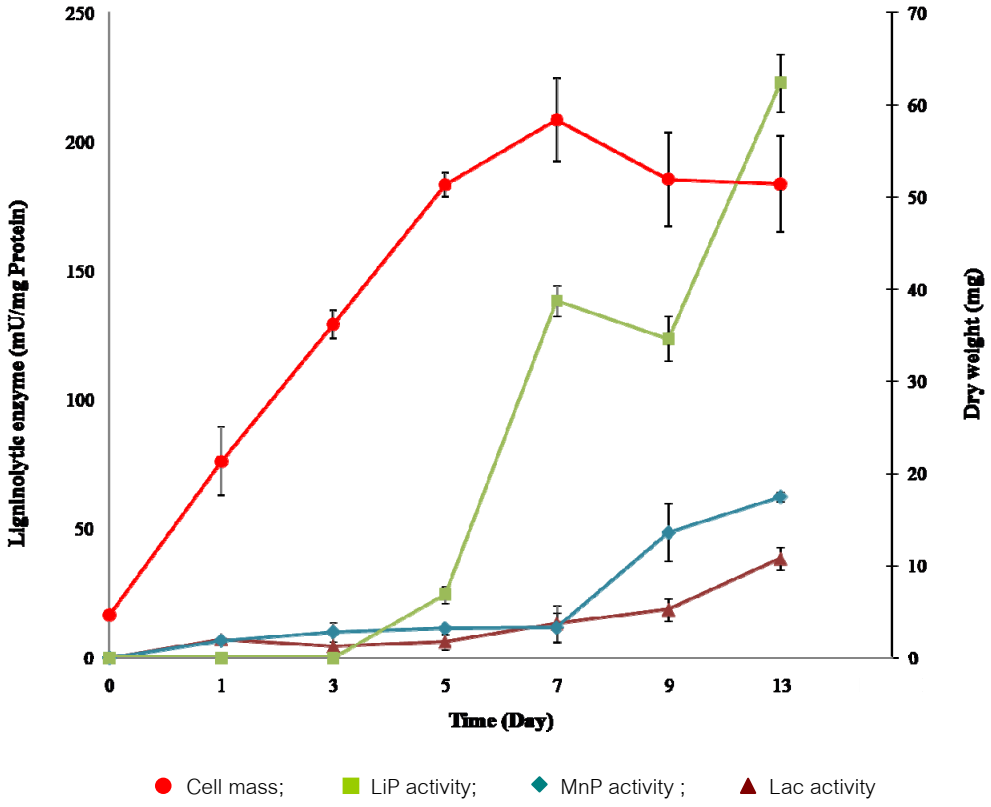


**Figure 4** The levels of ligninolytic enzymes produced by *P. sajor-caju* (Fr.) Sing cultivated in minimal liquid medium amended with reactive blue 19 and reactive blue 171

## CONCLUSION

The efficiency on dye decolorization by 11 strains of white-rot fungi was screened by agar plate assay. Only 8 strains of white-rot fungi showed their ability to decolorize both RB19 and RB171. However, 3 strains of white-rot fungi that exhibited high potential on decolorization in minimal liquid medium were *P. sajor-caju* (Fr.) Sing., *L. squarrosulus Mont.* and *C. fimetarus* (L) Fr. *P. sajor-caju* (Fr.) Sing. was the most potent white-rot fungus for decolorization of RB19 (89.27%) and RB171 (70.67%) at 9 days of incubation period. In addition, the percentage of decolorization of mixed reactive blue dyes by *P. sajor-caju* (Fr.) Sing. was 45%. *P. sajor-caju* (Fr.) Sing. produced the highest amount of LiP activity (222.79 mU/mg protein) than MnP and Lac activities. It could be concluded that the decolorization of synthetic reactive dyes by *P. sajor-caju* (Fr.) Sing. resulted from the presence of higher level of LiP activity. The highest activities of MnP in *P. sajor-caju* (Fr.) Sing. indicate that these enzyme may play an important role in the degradation of dyes.

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