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Agar-Plate Screening for Acid Dyes Decolourization by Three Strains of *Pleurotus eryngii* Isolated from Tunceli, Elazig and Hakkari Province of Turkey

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Many synthetic dyes present in industrial wastewaters are resistant to degradation by conventional treatments. In this study, the ability to decolourize two textile dyes by *Pleurotus eryngii* strains collected from geographically different habitats of Turkey was evaluated on agar plates. For all strains, growth and decolourization halos were determined on malt extract agar plates containing 0.1 and 0.2 g/L of acid red 183 or acid green 25. Each *P. eryngii* strain showed certain decolourization capacities and was able to decolourize both acid red 183 and acid green 25 on the malt extract agar plates, but not to the same extent. Both dyes at all concentrations were found to be toxic for *P. eryngii* growth. The presence of the dyes in plates reduced the mycelial growth of all strains in comparison with the control culture growing in the medium without dyes. A positive correlation was found between the mycelial growth rate and the decolourization ability.

Key Words: *Pleurotus eryngii*, Decolourization, Acid red 183, Acid green 25.

INTRODUCTION

Out of many contaminants present in wastewater, such as acids, bases, toxic organic and inorganic dissolved solids and colours, colours are considered the most undesirable and are mainly caused by dyes[1]. Dyes are synthetic aromatic water-soluble dispersible organic colourants, having potential application in various industries. The dyestuff usage has been increased day by day because of tremendous increase of industrialization and man's urge for colour[2]. The textile industry utilizes about 10000 different dyes and pigments. The worldwide annual production of dyes is over 7×10^5 tons[3-5].

Commonly applied treatment methods for colour removal from dye-contaminated effluents consist of integrated processes involving various combinations of biological, physical and chemical decolourization methods[6,7]. These integrated treatment methods

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are efficient but not cost-effective. Conventional wastewater treatment plants (WWTP) relying on activated sludge systems are not adequate for the treatment of textile mill effluents, neither on site nor after dilution with domestic wastewater at the sewage works. Activated sludge and other types of bioreactors fail to remove sufficiently colour, COD, N, surfactants and other micro-pollutants present in the textile effluents[8,9].

White-rot basidiomyctes include a group of fungi able to degrade lignin from woody and non-woody plant tissues, often with limited degradation of cellulose[10]. Anextracellular enzymatic system, which includes different oxidoreductases, is involved in this biodegradation process together with low-molecular mass metabolites and activated oxygen species[11]. These enzymes are also able to degrade aromatic pollutants causing environmental problems[12].

Recently, extensive research on basidiomycetous fungi has been conducted with aim to isolate new organisms with tremendous secretion of ligninolytic enzymes as well as enzymes with properties important for their industrial and environmental application[13,14]. The fungi selected for the present study were *P. eryngii* and *P. eryngii* var. ferulae which are edible white rot fungi, frequently consumed and distributed in the Mediterranean, Central Europe, Central Asia, North Africa[15] and Turkey.

In this study, the strains was screened for textile dye decolourization in agar plates. The purpose of this research is to evaluate the ability of *Pleurotus eryngii* strains from the ecophysiological point of view. Therefore, *P. eryngii* strains originally isolated from three different environments.

EXPERIMENTAL

The *Pleurotus eryngii* strains used for this work were isolated from different habitats from Turkey (Table-1). They belong to the culture collection of Microbiology Research Laboratuary, Dicle University, Diyarbakir, Turkey. The stock cultures were maintained on 2 % (w/v) malt extract agar (MEA) at 4 ºC. The mycelium from stock culture was transferred to MEA plates and incubated at 25 ºC for 7 days. Mycelial plugs (3 mm diameter) from peripheral region of actively growing culture. Two textile dyes used in this work acid red 183 (CI = 18,800; m.w. = 587.87 g/mol; maximum wavelength = 493 nm molar absorption coefficient = 9013 L/mol cm) and acid gren 25 (CI = 61,570 MW = 622.59 g/mol; maximum wavelength = 641 nm; molar absorption coefficient = 9687 L/mol cm) were obtained from Aldrich and used without any further purification. Chemical structures of the dyes are illustrated in Fig. 1.

TABLE-1
FUNGAL STRAINS USED IN THIS STUDY

Fungal species	Substrate/habitat and collection region
Pleurotus eryngii (H)	Hakkari Province, Turkey
Pleurotus eryngii (T)	*Eryngium* spp., *Ferula communis* Tunceli-Mazgirt Province, Turkey
Pleurotus eryngii (E)	*Eryngium* spp., *Ferula communis* Elazig Province, Turkey

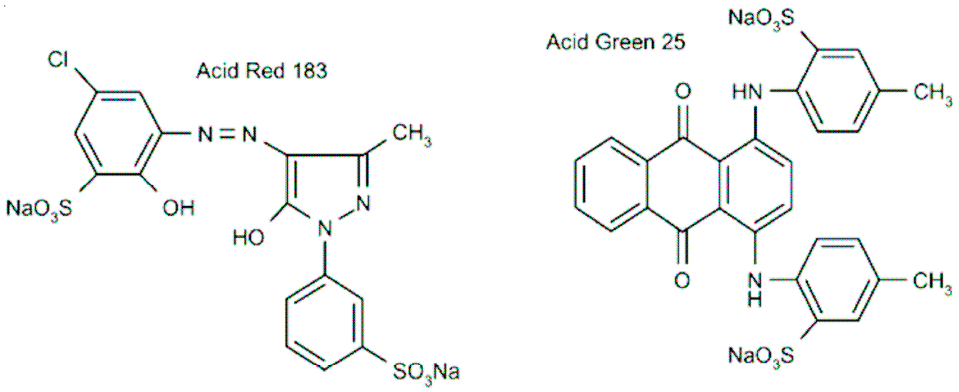

Fig. 1. Chemical structures of the acid dyes

Decolourization activity tested on solid media. For this aim, mycelial plugs (5 mm diameter) were inoculated into the center of Petri dishes (90 mm diameter) containing 0.1 and 0.2 g/L of acid red 183 or acid green 25, in triplicate. The plates were incubated at 25 ºC in the dark until they were completely colonized with the fungus or for a maximum period of 20 days. The diameters (cm) of the decolourization and growth halos were determined in two perpendicular directions of the plate[16]. Plates containing the dye but not inoculated served as control. All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA). The data presented are the averages of the results of three replicates with a standard deviation. To compare the decolourization ability of fungi, the data were analyzed by analysis of variance (ANOVA). Mean separations were performed using Duncan's multiple range test.

RESULTS AND DISCUSSION

Three different *P. eryngii* strains were tested for decolourization (Figs. 2-5) and radial growth rate (Table-1) on agar plates containing 0.1 and 0.2 g/L of acid red 183 or acid green 25 (Table-2). All strains were able to grow on solid media in the presence of the dyes. Out of the tested strains, it is found that the highest decolourization zone for acid red 183 in *P. eryngii* (E) and for acid green 24 in *P. eryngii* (T) (Figs. 2-5). For acid red 183 at 0.1 g/L concentration the maximum decolourization zone by *P. eryngii* (E) and *P. eryngii* (T) were 5.63 and 4.60 cm, respectively within 20 d (Fig. 2). For acid green 25 at 0.1 g/L concentration the maximum decolourization zone by *P. eryngii* (T) and *P. eryngii* (E) were 4.86 and 3.60 cm, respectively within 20 d (Fig. 4). *P. eryngii* (H) produced slower decolourization and only 4.30 cm decolourization halo was observed in agar plates with 0.1 g/L acid red 183 after 20 d. However, 3.56 and 3.20 cm were decolourized in agar plates with 0.1 g/L acid green 25 and 0.2 g/L acid green 25 by *P. eryngii* (H), respectively, after 20 d (Figs. 4 and 5). It was also observed that different *P. eryngii* strains revealed statistically ($p < 0.05$) different results in decolourization ability.

TABLE-2
EFFECT OF AR 183 AND AG 25 DYES ON MYCELIAL GROWTH IN PETRI DISH ON MEDIUM CONTAINING 0.1 g/L OR 0.2 g/L DYE

Fungi	Growth 10 day (cm)*		Growth 20 day (cm)*	
	Control**	With 0.1 g/L AR 183	Control**	With 0.1 g/L AR 183
P. eryngii (H)	4.63 ± 0.52	3.46 ± 0.51	7.32 ± 0.63	5.03 ± 0.68
P. eryngii (E)	4.86 ± 0.58	3.93 ± 0.66	8.56 ± 0.60	6.33 ± 0.56
P. eryngii (T)	3.80 ± 0.72	2.93 ± 0.55	7.40 ± 0.55	5.10 ± 0.60
	Control	With 0.2 g/L AR 183	Control	With 0.2 g/L AR 183
P. eryngii (H)	3.12 ± 0.60	2.80 ± 0.60	5.20 ± 0.49	4.60 ± 0.70
P. eryngii (E)	4.22 ± 0.63	3.46 ± 0.63	6.12 ± 0.58	5.86 ± 0.58
P. eryngii (T)	2.96 ± 0.72	2.46 ± 0.60	5.26 ± 0.56	4.70 ± 0.52
	Control	With 0.1 g/L AG 25	Control	With 0.1 g/L AG 25
P. eryngii (H)	3.20 ± 0.60	2.90 ± 0.26	5.20 ± 0.51	4.33 ± 0.57
P. eryngii (E)	3.10 ± 0.84	2.46 ± 0.89	5.58 ± 0.23	4.55 ± 0.49
P. eryngii (T)	4.23 ± 0.89	3.46 ± 0.55	5.99 ± 0.55	5.60 ± 0.51
	Control	With 0.2 g/L AG 25	Control	With 0.2 g/L AG 25
P. eryngii (H)	3.21 ± 0.25	2.46 ± 0.25	4.20 ± 0.55	3.60 ± 0.36
P. eryngii (E)	2.15 ± 0.32	1.86 ± 1.02	5.10 ± 0.62	4.23 ± 0.60
P. eryngii (T)	3.24 ± 0.60	2.83 ± 0.32	5.89 ± 0.45	5.03 ± 0.85

*Radial growth measured as a diameter of mycelial colony on MEA medium. **Control culture growing in the medium without dyes. AR-183 = Acid red-183, AG-25 = Acid green-25

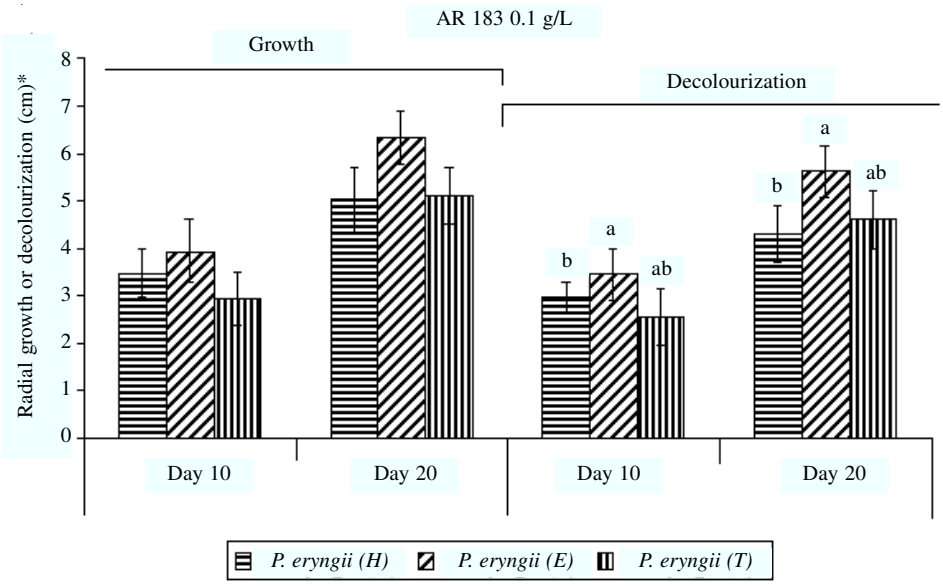

Fig. 2. Mycelial growth of fungi and decolourization of acid red in petri dish on medium containing 0.1 g/L acid red 183 dye. Different letters above the bar on the same day are statistically different by Duncan's multiple range test ($p < 0.05$). *Radial growth measured as a diameter of mycelial colony on MEA medium containing 0.1 g/L acid red 183. Decolourization measured as a diameter of decolourized zone on a Petri dish on MEA medium containing 0.1 g/L acid red 183

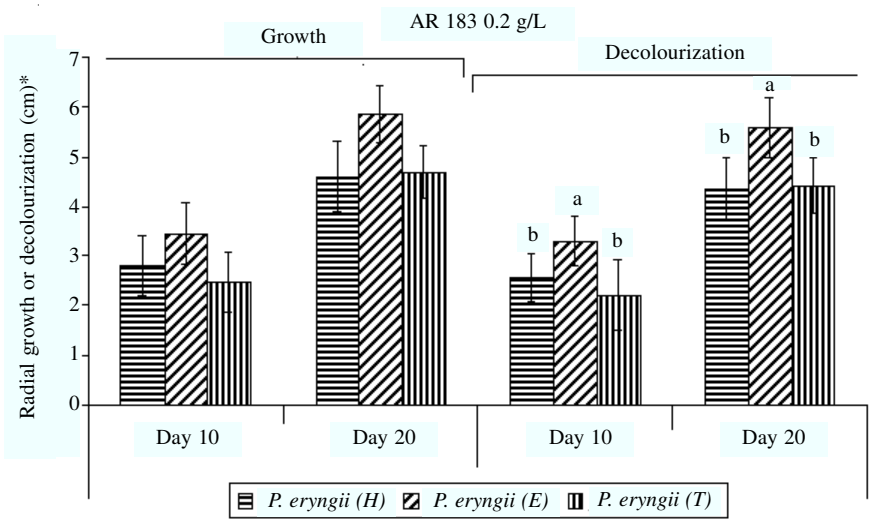

Fig. 3. Mycelial growth of fungi and decolourization of acid red in petri dish on medium containing 0.2 g/L acid red 183 dye. Different letters above the bar on the same day are statistically different by Duncan's multiple range test ($p < 0.05$). * Radial growth measured as a diameter of mycelial colony on MEA medium containing 0.2 g/L acid red 183. Decolourization measured as a diameter of decolourized zone on a Petri dish on MEA medium containing 0.2 g/L acid red 183

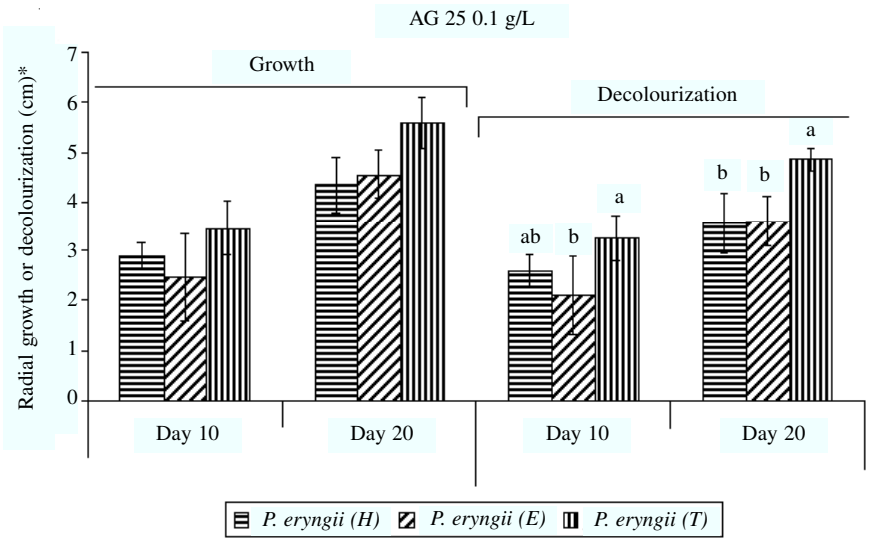

Fig. 4. Mycelial growth of fungi and decolourization of acid green-25 in petri dish on medium containing 0.1 g/L acid green 25 dye. Different letters above the bar on the same day are statistically different by Duncan's multiple range test ($p < 0.05$). *Radial growth measured as a diameter of mycelial colony on MEA medium containing 0.1 g/L acid green 25. Decolourization measured as a diameter of decolourized zone on a Petri dish on MEA medium containing 0.1 g/L acid green 25

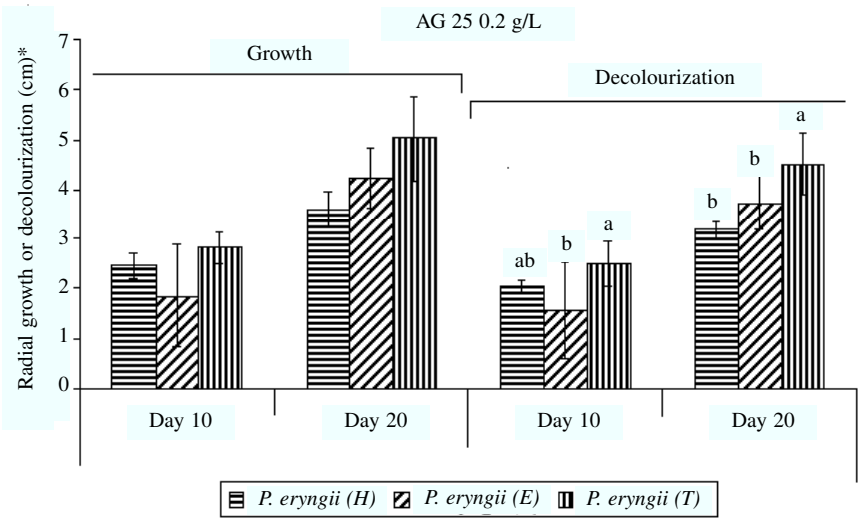

Fig. 5. Mycelial growth of fungi and decolorization of acid green-25 in petri dish on medium containing 0.2 g/L acid green 25 dye. Different letters above the bar on the same day are statistically different by Duncan's multiple range test ($p < 0.05$). *Radial growth measured as a diameter of mycelial colony on MEA medium containing 0.2 g/L acid green 25. Decolorization measured as a diameter of decolorized zone on a Petri dish on MEA medium containing 0.2 g/L acid green 25

For screening of basidiomycete strains for decolourization. The majority of authors used a dye concentration of 0.1-0.3 g/L or lower in their studies[17-19]. Present results showed a positive correlation between the mycelial growth rate and the decolourization ability measured as the diameter of the decolourized zone. The radial growth diameter mostly exceeded the diameter of the decolourized zone. It seems likely that the strong growth reduction in the presence of both dyes caused their poor decolourization.

It is also observed differences between decolourization of structurally different dyes (acid red 183 and acid green 25) for each strain. A fungus capable of decolourizing one dye has different capacities for other dyes. Similarly Paszcynski *et al.*[20] and Spadaro *et al.*[21] reported that for some dyes the slow decolourization rate could be attributed to the complexity of their chromophores, but the overall complexity alone is not an indicator of the difficulty of decolourization of a particular dye.

Dye toxicity for fungal growth was determined by comparing mycelial growth in dye containing (0.1-0.2 g/L) media with control groups containing only MEA but no dye. Maximum radial growth value (7.40 cm) was detected for *P. eryngii* (T) in control groups in such a way that increased dye concentrations have caused a decrease in radial growth value (Table-1). This radial growth values indicate the toxic effects of both dyes on *P. eryngii* growth. Apohan and Yesilada[22] reported that astrazon blue and red dyes inhibit growth of *F. trogii* and *S. aureus* on solid medium

in a concentration dependent manner. Ramsay and Nguyen[23] reported that reactive blue 15, Congo red and reactive black 5 were partially toxic for *C. versicolor* growth but Amaranth and Tropoeolin has no toxic effect for fungal growth.

Conclusion

The ability of white-rot fungi to degrade a wide variety of environmentally persistent pollutants indicates their potential use in antipollution treatments. None of present three *P. eryngii* strains have been used previously for aromatic recalcitrant compounds was not reported before. There is a need to develop fungal strains which are capable of decolourizing dye wastewater. Present results indicate that these two white-rot fungi could be used in bioprocesses to remove colour from industrial effluents or to treat coloured solid residues. It was found that *P. eryngii* was able to decolourize acid red 183 and acid green 25 dyes. Present findings can serve as an important base for the development of an economical as well as simplified biological treatment for treating toxic dyes by using white rot fungi. The results indicate that *P. eryngii* can easily decolourize some synthetic dyes, which could be promising for biotechnological purposes.

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