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ORIGINAL ARTICLES

UV-Mutagenesis in Some White Rot Fungi for Increasing Decolorization of Textile Dyes

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ABSTRACT

Three different strains of white rot fungi ,i.e, *Pleurotus ostreatus, Phanerochaete chrysosporium* and *Trametes versicolor* were evaluated for decolorization of six textile dyes. *P. ostreatus* showed more effective for dyes decolorization followed by *P. chrysosporium.* So, they were selected and mutagenized using UV as a physical mutagen in order to induce genetic variations. The majority of *Pleurotus ostreatus* mutants can decolorize dyes with efficiency higher than the wild type strain especially Acid fast red and Disperse violet 31 dyes, while most of *P. chrysosporium* mutants exhibited negative decolorization effects especially Reactive black 5 and Methylene blue dyes. Frequency analysis of UV mutants according to dye decolorization showed higher decolorization values and the development of positive mutants of *P. ostreatus* compared to *P. chrysosporium*. *P. ostreatus* mutants 7/30 and 14/30, since mutant 7/30 has ability to decolorize efficiently Methyl orange and Congo red while (14/30) are the most effective methods, decolorize Acid fast red, Reactive black 5, Methylene blue and Disperse violet 31.

Kew words: White rot fungi, UV-Mutagenesis, decolorization, textile dyes.

Introduction

Synthetic dyes are extensively used in textile dyeing, paper printing, color photography, pharmaceutical, food, cosmetic, and leather industries (Kuhad *et al*., 2004). The traditional textile finishing industry consumes about 100L of water to process about 1kg of textile materials (Couto, 2009). Large scale production and extensive application, synthetic dyes can cause considerable environmental pollution and are serious health-risk factors (Forgacs *et al*., 2004).

There is a great need to develop an economic and effective way of dealing with the textile dyeing waste in the face of the ever-increasing production activities (Park *et al*., 2007). Government legislation is becoming more and more stringent, especially in more developed countries, regarding the removal of dyes from industrial effluents (Kuhad *et al*., 2004). Over the last decade, azo dyes that could breakdown to carcinogenic aromatic amines have been largely phased out in Europe (Pinheiro *et al*., 2004).

Fungi, especially white rot fungi have nonspecific ligninolytic enzyme system that includes manganese peroxidase, lignin peroxidase and laccase can degrade a wide range of dyes. Many white-rot fungi have been studied for their decolorization ability (Sathiya moorthi *et al*., 2007; Vasdev, 2011 and Hadibarata, 2013). *Pleurotus* spp. and *Phlebia* spp. are being evaluated for dye decolorization to look for friendly solutions for decolorization of industrial wastewaters (Casieri *et al*., 2008). The realization of these potentials would be considerably enhanced, however, if genetic methods for selecting strains with superior capacities were available (Homolka *et al*., 1995).

Random mutation was introduced for white rot fungi using X rays (Gold *et al*., 1982), UV-irradiation (Cacchio *et al*., 2003 and Yashvant *et al*., 2013) and Gamma irradiation (Chang *et al*., 2003). In some non-sporulating filamentous fungi, protoplasts are a suitable starting material for mutagenesis (Homolka, 1988). In less sensitive mycelial fungi, mutagenesis of hyphal fragments (Vijaya *et al*., 2009) is a method of choice. Toyomasu *et al*., (1986) induced mutation using protoplasts from *Pleurotus ostreatus* and *Pleurotus salmoneo-stramineus* by UV. Vijaya *et al*., (2009) obtained mutants strains of *Pleurotus ostreatus* with the objective of enhancing their ligninolytic activity after exposure of mycelium growing in agar plates to UV light. UV irradiation for mutant production from some white rot fungi has been reported. Strains have been produced which were altered in cellulase, xylanase, mannanase, amylase, phenoloxidase and lignin peroxidase (LiP) activity, LiP regulation and nutrient requirements. In some cases, such as cellulase-deficient mutants of *Phanerochaete chrysosporium*, an increase in ligninolytic activity was reported (Addleman *et al*., 1995). Protoplast preparation and UV-irradiation of mycelial fragments were used to study the variability of production of laccase, peroxidase and manganese-dependent peroxidase (MnP) involved in lignin degradation in *Pleurotus*

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ostreatus and *Lentinus tigrinus*. After protoplasting, the variability of production of all enzymes increased substantially (Homolka *et al*., 1995).

The objectives of this study can be summarized as follows: Screening of different species of white rot fungi to evaluate their ability to decolorize different textile dyes; genetic improvement of the efficient strain(s) using UV mutagenesis protocol; evaluation of the ability of genetically modified strains for dye decolorization using their high ligninolytic enzymes production.

Materials and Methods

1. Materials:

1.1. White-rot fungi strains:

Table 1: Code number, name and source of white rot fungi strains used in this study.

Code No.	Strain	Source
1	*Phanerochaete chrysosporium*	National Regulatory Research Institute (NRRI) 6361
2	*Pleurotus ostreatus*	Faculty of science, Ain shams university
3	*Trametes versicolor*	Faculty of science, Ain shams university

1.2. Textile dyes:

Six textile dyes were selected with consideration of prevalence in the textile industry. The chemical structures and peak absorbance wavelength of these dyes are listed below. All these dyes were obtained from s. d. fine-chemical ITD, and their molecular formula, molecular weight, concentration are listed in table (2).

Table 2: Molecular formula, molecular weight and concentration of dyes.

Dye	Type	Molecular formula	Molecular weight	Absorbance (nm)	Concentration (mg/l)
Methyl orange	monoazo	$C_{14}H_{14}N_3NaO_3S$	327.33	470	6.8
Acid fast red	diazo	$C_{18}H_{13}N_3Na_2O_8S_2$	509.430	531	67
Reactive black 5	diazo	$C_{26}H_{21}N_5Na_4O_{19}S_6$	991.82	595	100
Congo red	diazo	$C_{32}H_{22}N_6Na_2O_6S_2$	696.7	492	20
Methylene blue	heterocyclic	$C_{16}H_{18}N_3ClS$	319.82	667	3.3
Disperse Violet 31	heterocyclic	$C_{26}H_{18}N_2O_4$	422.43	545	100

1.3. Media:

1.3.1. Malt extract agar medium (MEA):

Malt extract, 30 g/l; Mycological peptone , 5 g/l ; Agar, 20 g/l.

1.3.2. Liquid culture medium:

Sucrose , 5 g/l; Ammonium sulphate , 0.5 g/l; Yeast nitrogen base without amino acid and ammonium sulphate, 1.7 g/l ; L-asparagine, 1 g/l; pH 4.5.

1.3.3. Nutrient salt medium:

Glucose	10 g/l
Ammonium phosphate	0.2 g/l
Potassium phosphate	1 g/l
Magnesium sulphate	0.5 g/l
Potassium chloride	0.5 g/l
Ferrous sulphate	0.005 g/l
Thiamine hydrochloride	0.001 g/l
pH	6

1.3.4. MMP medium:

Malt extract	10 g/l
Mycological peptone	5 g/l
3'-(*N*-morpholino)-propanesulfonic acid (MOPS)	10mM
pH	7

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2. Methods:

2.1. Screening for dye decolorization in liquid medium:

Fungal strains grown on malt extract agar plates were used to inoculate 50 ml of the above liquid culture medium in 250 ml flasks, then incubated at 30°C in a rotary shaking adjusted at 150 rpm. After six days, stock solution aliquots of dyes were added to medium. The initial concentrations of dyes in medium were mentioned in table 2, (Fu and Viraraghavany, 2001). Periodically during the fungal dye decolorization process, percent color removal in liquid was calculated.

2.2. Percent color removal in liquid medium:

The color measurement method developed by Strickland and Perkins (1995) was used in this study. This method is used to measure the decolorization effect in a dye solution containing one dye or several dyes. For the decolorization effect of a dye solution containing a single dye, decolorization was measured spectrophotometerically at the wavelength of peak absorbance of each dye (table 2) using a Shimadzu UV-VIS recording spectrophotometer model UV-240. The decolorization percentage calculated by the following formula: Decolorization (%) = (ABT-AAT /ABT) x 100 ; AAT = absorbance after treatment; ABT = absorbance before treatment.

2.3. Induction of mutation:

2.3.1. Pleurotus ostreatus:

Protoplast suspension from *Pleurotus ostreatus* were suspended in 0.7 M mannitol. For UV irradiation, Petri dishes containing protoplast suspension was exposed to UV light (30W Philips germicidal lamp) at a distance of 15 cm in dark for 30, 45 and 60 sec. To avoid photoreactivation, treated protoplasts were kept in dark for about 1 hour. Protoplasts were then diluted with 0.7M mannitol and plated on a malt extract medium containing osmotic stabilizer. The irradiated protoplasts and untreated control, in parallel, were suitably diluted with osmotic stabilizer solution and plated for regeneration on solid agar medium (Toyomasu *et al*., 1986).

2.3.2. *Phanerochaete chrysosporium*:

For UV irradiation, spores suspended in sterile distilled water were irradiated in Petri dish at room temperature (30°C) in the dark under mild agitation. The source of the UV radiation was a (30-W) lamp. After irradiation for 30, 60 and 90 sec. at a distance of 15 cm, plates were kept for 60 min in the dark to allow full expression of the change induced by UV light. The irradiated spores and untreated control, in parallel, were suitably diluted with sterile distilled water and plated on a malt extract medium. Growing colonies were counted against the control plates of the same dilution and transplanted onto a slant for genetic analysis (Gold *et al*., 1982).

Results and Discussion

The textile industries use large amounts of toxic dyes that pose a threat when discharged to the environment. The treatment of dye containing wastewater by white rot fungi has shown promising results, but needs to be enhanced to find higher decolorization efficiency for all or most textile dyes used in this study.

1. Evaluation of different fungal strains for dye decolorization:

The efficiency of three white rot fungi for dye decolorization in liquid medium containing a single dye was investigated The initial dye concentrations were approximately 6.8, 67, 100 , 20, 3.3 and 100 mg/L for Methyl orange, Acid fast red, Reactive black5, Congo red, Methylene blue and Disperse violet 31, respectively (Fu and Viraraghavany, 2001). Table (3) illustrate the differences ability between three white rot fungal strains for dye reduction in liquid medium during three incubation periods of fungal treatment. Results showed that *Pleurotus ostreatus*, is the most effective strain for dye decolorization followed by *Phanerochaete chrysosporium*. After 72 hours of incubation, *Pleurotus ostreatus*, have the ability to decolorize Reactive black 5, Congo red and Disperse violet 31 with decolorization percentages of 89.56, 91.19 and 91.52, respectively. In fact, *Phanerochaete chrysosporium* exhibited nearly the same decolorization trend for Congo red and Disperse violet 31 dyes since more than 90% and 85% color removal were obtained, respectively. However, among six textile dyes used in this study (Table 3), Methyl orange, Acid fast red and Methylene blue were more resistant to

decolorization. Color removal percentages were 33.16, 21.94 and 44.12, respectively for *Phanerochaete chrysosporium*, while it were 44.29, 50.33 and 42.49 respectively for *Pleurotus ostreatus* after the same incubation period of 72 hours.

The differences in the dye decolorization capacity have been related to fungal variations, the molecular complexity of dyes as well as culture conditions (Levin *et al.*, 2004 and Machado *et al*., 2006). The differences in the decolorization characteristics for the individual dyes are attributed to the dissimilarity in specificities, structures and complexity, particularly on the nature and position of subsistent in the aromatic rings and the resulting interactions with the azo bond of different dyes as reported by many authors (Eichlerova, 2006; Vijaykumar *et al*., 2007).

However, *Pleurotus ostreatus, and Phanerochaete chrysosporium* were selected for treatment by UV light to improve their ability to decolorize some textile dyes compared to its original strain.

Table 3: Screening for dye decolorization by three white rot fungi with different dyes.

Dyes	Methyl orange			Acid fast red			Reactive black 5			Congo red			Methylene blue			Disperse violet 31		
Incubation periods / Fungal strains	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Pleurotus ostreatus	30.86	35.17	44.29	24.86	30.89	50.33	78.27	84.37	89.56	88.27	91.19	91.19	42.49	42.49	42.49	88.16	89.40	91.52
Phanerochaete chrysosporium	20.42	26.39	33.16	12.67	13.61	21.94	23.25	39.77	52.72	85.46	85.46	90.42	14.83	18.52	44.12	69.44	79.66	85.20
Trametes versicolor	14.55	17.95	43.42	2.91	2.91	2.91	21.87	36.99	36.99	22.37	26.48	30.95	8.47	22.26	22.26	4.29	9.01	11.70

2. Pleurotus ostreatus mutagenesis:

Table (4) showed effect of UV light on the survival frequency of *P. ostreatus* protoplasts. Results showed that when protoplast suspension of *Pleurotus ostreatus* was exposed to UV-light for 30, 45 and 60 seconds, survival percentages rapidly decreased by increasing exposure time and were 28.8 and 7.6 after 30 and 45 seconds, respectively, as well as it dropped down to zero percent after 60 seconds. This may be due to sensitivity of the protoplasts to UV. The prolonged incubation causes uncontrolled DNA damage which results in cell death. A suitable incubation limit for good mutagenesis to occur can be determined by selection process (Gopinath *et al*., 2009).

Table 4: Number of the isolated colonies and survival percentages of *Pleurotus ostreatus* and *Phanerochaete chrysosporium* exposed to UV- light.

Strain	Exposure time (sec)	Dilution factor	Average of colonies No.	% survival
Pleurotus ostreatus	0 (control)	10^{-1}	66	100
	30		19	28.8
	45		5	7.6
	60		0	0
Phanerochaete chrysosporium	0 (control)	10^{-3}	272	100
	30		148	54.4
	60		36	13.2
	90		13	4.8
	120		0	0

However, in some non-sporulating filamentous fungi, protoplasts are a suitable starting material for mutagenesis (Joh *et al*., 2004). Vijaya *et al*., (2009) obtained mutant strains of *Pleurotus ostreatus* with the objective of enhancing their ligninolytic activity after exposure of mycelium growing in agar plates to UV light for 10, 20, 30 40, 50 and 60 minutes.

3. Phanerochaete chrysosporium mutagenesis:

The dose-response analysis for the UV mutagenesis of *Phanerochaete chrysosporium* was done and the percent of survival values are shown in Table 4. The dosage was expressed in terms of time of exposure to the UV light and varied in the range 30-120 sec. to analyze the survival capacity and mutation effect.

Data presented in Table (4) showed that 148, 36 and 13 colonies were recovered after UV treatment for 30, 60 and 90 second, respectively. No colony development was observed after 120 sec. Cell death may be due to the penetration of UV light through the cell membrane and destruction of intracellular organelles, which result in protein structure damage. The occurrence of damage to the DNA depends on the probability of the exposure of UV light and sometimes this may not happen due to high cellular density (Gopinath *et al.*, 2009).

However, many investigators (Homolka *et al*., 1995; Cacchio *et al*., 2003) used UV mutagenesis to improve enzymatic activities in white rot fungi. Other research groups have been successful in obtaining *Trichoderma* mutants by means of UV-mutagenesis (Szekeres *et al*., 2004; Hatvani *et al*., 2006; Besoain *et al*., 2007).

4. Decolorization efficiency of UV mutants:

Mutants obtained through random mutagenesis approach using UV light were employed for dye decolorization. Potential strains were selected based on their decolorization ability compared to parental wild type strain.

5. Analysis of UV mutants according to dye decolorization:

The frequency analysis of 30 mutants obtained from UV mutagenesis are presented in Table (5). The mutants showed decolorization percentages more than the value obtained for wild type strain, were considered as positive mutants.

Data presented in Table (5) and Fig. (1) showed the positive and negative *Pleurotus ostreatus* mutants. The largest numbers of positive mutants were recorded after Acid fast red and Disperse violet 31 decolorization along with all incubation periods (Fig. 1). Moreover, incubation period of 48 hours is more suitable for Methyl orange (18 mutants), Acid fast red (24) and Disperse violet 31(28) while 72 hours of incubation enhanced Reactive black 5 (16), Congo red (16) and Methylene blue (17). It is surprising to notice from Table (5) that the same numbers of positive mutants, *i.e.* 16 mutants were obtained after incubation with Congo red, Reactive Black 5, Acid fast red and Methyl orange for 72 hours.

Decolorization of Disperse violet 31 and Reactive black 5 dyes by *Pleurotus ostreatus* mutants in comparison with the original strain was presented in Fig.(2). These dyes are used widely in the textile industries.

Table 5: Frequency analysis of *Pleurotus ostreatus* and *Phanerochaete chrysosporium* UV mutants according to dye decolorization.

Dyes		Methyl orange			Acid fast red			Reactive black 5			Congo red			Methylene blue			Disperse violet 31		
Incubation periods / Fungal strains		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
pleurotus ostreatus	**Positive mutants	8	18	16	23	24	16	6	12	16	14	9	16	13	16	17	27	28	27
	*Negative mutants	22	12	14	7	6	14	23	18	14	16	20	12	17	14	12	3	2	3
Phanerochaete chrysosporium	**Positive mutants	13	10	6	4	4	1	0	0	0	14	16	13	2	4	0	1	0	0
	*Negative mutants	17	20	14	25	26	29	30	30	30	15	13	17	28	26	30	29	30	30

More than wild type**; Less than wild type*

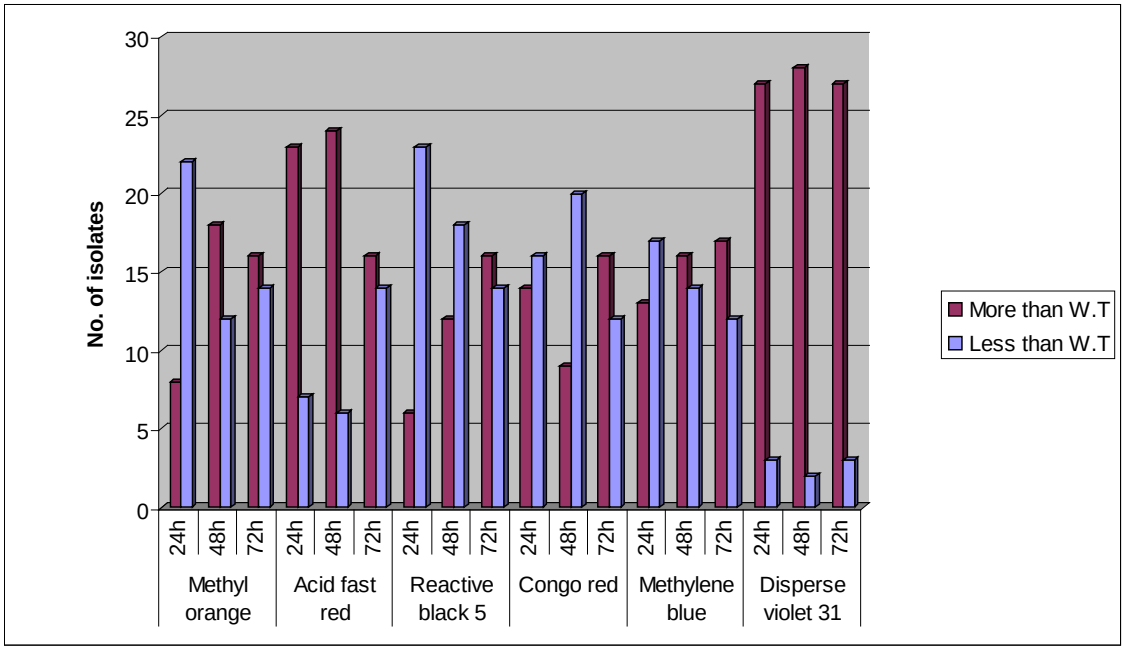

Fig. 1: Frequency distribution of *Pleurotus ostreatus* UV mutants according to dye decolorization.

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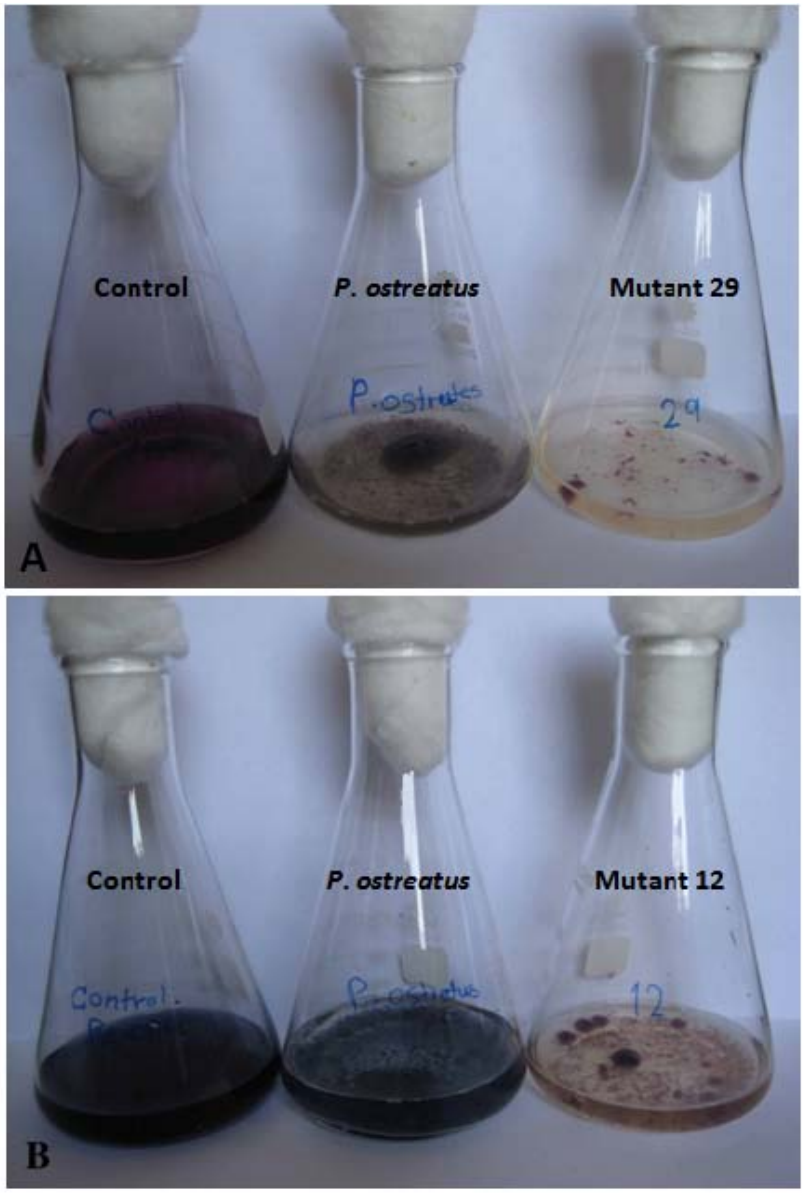

Fig. 2: Decolorization of two various textile dyes by *Pleurotus ostreatus* mutants in comparison with the original strain and control. The tested dyes were as follows: (A) Disperse violet 31 (B) Reactive black 5.

On the other hand, Data presented in Table (5) and Fig. (3) showed the positive and negative *Phanerochaete chrysosporium* mutants on dye removal. The largest number of negative mutants (30 mutants) was obtained after incubation with Reactive Black 5 at all incubation periods followed by Disperse violet 31 since only one positive mutant obtained after 24 hours. Concerning Congo red decolorization, 14, 16 and 13 mutants exhibited positive effect after 24, 48 and 72 hours, respectively, while 13, 10 and 6 positive mutants were obtained after the same incubation periods with Methyl orange dye.

In general, it can be concluded that, *Pleurotus ostreatus* mutants showed higher decolorization values compared to *Phanerochaete chrysosporium.* However, development of positive or negative mutants may be due to secretion of some enzymes responsible for decolorization. Due to the mutagenesis, some enzymes were suppressed, whereas certain enzymes production was stimulated (Gopinath *et al*., 2009).

Anyhow, random mutagenesis is used to induce mutation in organisms for better distinctiveness (Kamath *et al*., 2008). Improvement of the microbial strain by subjecting the genetic material to physical and chemical mutagenic agents offers furthermost opportunity for cost reduction without significant capital outlay (Lotfy *et al*., 2007). However, since various levels of positive mutants were observed, the higher order mutants were selected for further protoplast fusion studies.

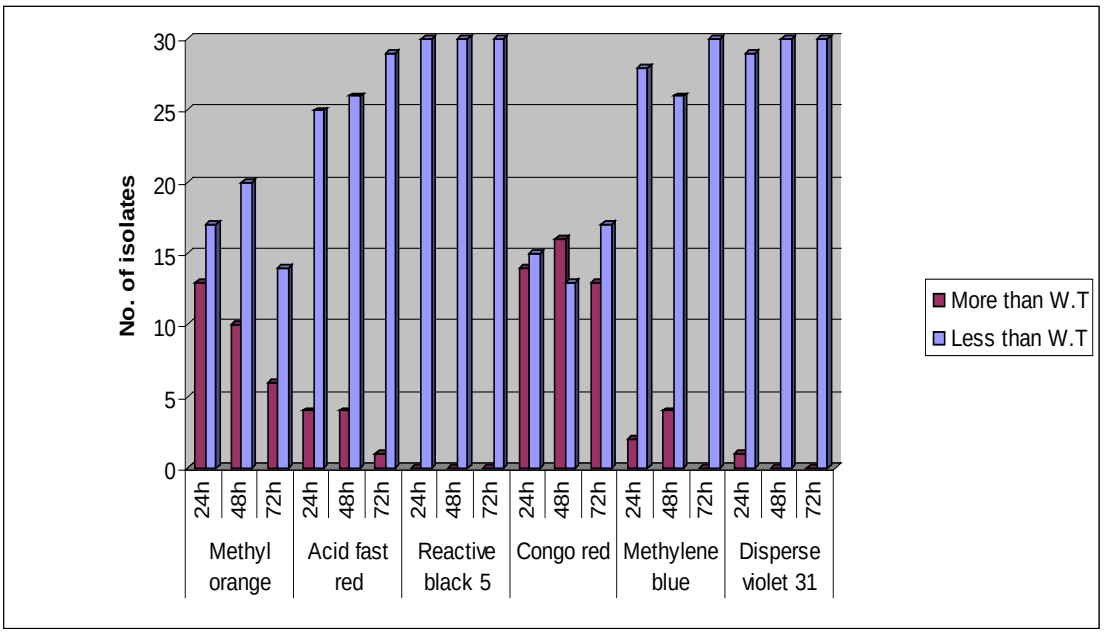

Fig. 3: Frequency distribution of *Phanerochaete chrysosporium* UV mutants according to dye decolorization.

References

Addleman, K., T. Dumonceaux, M.G. Paice, R. Bourbonnais and F.S. Archibald, 1995. Production and Characterization of Trametes versicolor mutants unable to bleach hardwood kraft pulp. Appl. Environ. Microbiol., 61: 3687-3694.

Besoain, X.A., L.M. Pérez, A. Araya, L. Lefever, M. Sanguinetti and J.R. Montealegre, 2007. New strains obtained after UV treatment and protoplast fusion of native *Trichoderma harzianum*: their biocontrol activity on *Pyrenochaeta lycopersici* Electro. J. Biotec., 10: 604-617.

Cacchio, P., E. Fantauzzi and A. Lepid, 2003. Mutagenesis and screening for cellulose (endo-1,4-β-D-glucanase) deficient strains of the white-rot basidiomycete *Trametes trogii* Berk. Ann. Microbiol., 53:105-115.

Casieri, L., G.C. Varese, A. Anastasi, V. Prigione, K. Svobodová, M.V. Filipello and Č. Novotný, 2008. Decolorization and detoxication of reactive industrial dyes by immobilized fungi *Trametes pubescens* and *Pleurotus ostreatus*. Folia Microbiol., 53: 44-52.

Chang, H.H., Y.K. Lee, J.S. Kim, K.S. Lee and K.S. Cho, 2003. Mutation spectrum of manganese (II) peroxidase gene in the *Pleurotus ostreatus* mutants induced by gamma radiation. J. Microbiol., 41: 52-57.

Couto, S.R., 2009. Dye removal by immobilized fungi. Biotechnol. Adv., 27: 227-235.

Eichlerova, I., L. Homolka and F. Nerud, 2006. Ability of industrial dyes decolorization and ligninolytic enzymes production by different *Pleurotus* species with special attention on *Pleurotus calyptratus*, strain CCBAS 461. Process. Biochem., 41: 941-946.

Forgacs, E., T. Cserhati and G. Oros, 2004. Removal of synthetic dyes from wastewaters: a review. Environ. Int., 30: 953-971.

Fu, Y. and T. Viraraghavan, 2001. Fungal decolorization of dye waste waters: a review. Bioresource Technol., 79: 251-262.

Gold, M.H., T.M. Cheng and M.B. Mayfield, 1982. Isolation and complementation studies of auxotrophic mutants of the lignin-degrading basidiomycete *Phanerochaete chrysosporium.*Appl. Environ. Microbiol., 44: 996-1000.

Gopinath, K.P., S. Murugesan, J. Abraham and K. Muthukumar, 2009. *Bacillus sp*. mutant for improved biodegradation of Congo red: Random mutagenesis approach. Bioresour. Technol., 100: 6295-6300.

Hadibarata, T., 2013. Effect of metals on amaranth decolorization by white-rot fungus *Pleurotus eryngi* F019.J. Biol. Sci., 13: 550-554.

Hatvani, L., L. Manczinger, L. Kredics, A. Szekeres, Z. Antal and C. Vagvolgyi, 2006. Production of *Trichoderma* strains with pesticide-polyresistance by mutagenesis and protoplast fusion. Antonie Van Leeuwenhoek, 89: 387-393.

Homolka, L., 1988. Preparation, reversion, mutagenesis and intra-species fusion of protoplast of *Oudemansiella mucida*. Folia Microbiol., 33: 298-308.

Homolka, L., I. Voláková and F. Nerud, 1995. Variability of enzymatic activities in ligninolytic fungi *Pleurotus ostreatus* and *Lentinus tigrinus* after protoplasting and UV-mutagenization. Biotechnol. Tech., 9: 157-162.

Joh, J.H., B.G. Kim, W.S. Kong, Y.B. Yoo, K.S. Chu, N.K. Kim, H.R. Park, B.G. Cho and C.S. Lee, 2004. Isolation and characterization of dikaryotic mutants from *Pleurotus ostreatus* by UV irradiation. Microbiol., 32: 88-94.

Kamath, S.B., R. Vidhyavathi, R. Sarada and G.A. Ravishankar, 2008. Enhancement of Carateonids by mutation and stress induced carotenogenic genes in *Haematococcus pluvialis* mutants. Bioresour. Technol., 99: 8667-8673.

Kuhad, R.C., N. Sood, K.K. Tripathi, A. Singh and O.P. Ward, 2004. Developments inmicrobial methods for the treatment of dye effluents. Adv. Appl. Microbiol., 56: 185-213.

Levin, L., L. Papinutti and F. Forchiassin, 2004. Evaluation of Argentinean white rot fungi for their ability to produce lignin-modifying enzymes and decolorize industrial dyes. Bioresour. Technol., 94: 169-176.

Lotfy, W.I., K.M. Ghanem and E.R. El-Helow, 2007. Citric acid production by a novel *Aspergillus niger* isolate: I. Mutagenesis and cost reduction studies. Bioresour. Technol., 98: 3464-3469.

Machado, K.M.G., L.C.A. Compart, R.O. Morais, L.H. Rosa and M.H. Santos, 2006. Biodegradation of reactive textile dyes by basidiomycetous fungi from Brazilian ecosystems. Braz. J. Microbiol., 37: 481-487.

Park, C., M. Lee, B. Lee, S.W. Kim, H.A. Chase, J. Lee and S. Kim, 2007. Biodegradation and biosorption for decolorization of synthetic dyes by *Funalia trogii*. Biochem. Eng. J., 36: 59-65.

Pinheiro, H.M., E. Touraud and O. Thomas, 2004. Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. Dyes and Pigm., 61: 121-139.

Sathiya Moorthi, P., S. Periyar Selvam, A. Sasikalaveni, K. Murugesan and P.T. Kalaichelvan, 2007. Decolorization of textile dyes and their effluents using white rot fungi. Afr. J. Biotechnol., 6: 424-429.

Strickland, A.F. and W.S. Perkins, 1995. Decolorization of continuous dyeing wastewater by ozonation, Text. Chem. Color, 27: 11-15

Szekeres, A., L. Kredics, Z. Antal, F. Kevei and L. Manczinger, 2004. Isolation and characterization of protease overproducing mutants of *Trichoderma harzianum*. FEMS Microbiol. Lett., 233: 215-222.

Toyomatsu, T., T. Matsumoto and K.I. Mori, 1986. Interspecific Protoplast Fusion between *Pleurotus ostreatus* and *Pleurotus salmoneo-stramineus*. Agric. Biol. Chem., 50: 223-225.

Vasdev, K., 2011. Decolorization of triphenylmethane dyes by six white-rot fungi isolated from nature. J. Bioremed. Biodegrad., 2: 128-132.

Vijaya, C., M.A. Singaracharya and L.M. Vijaya, 2009. Use of lignocellulolytic mutants of *Pleurotus ostreatus* in ruminat feed formulations. Bioresources, 4: 142-154.

Vijaykumar, M.H., P.A. Vaishampayan, S.Y. Shouche and T.B. Karegoudar, 2007. Decolourization of naphthalene-containing sulfonated azo dyes by *Kerstersia* sp. strain VKY1. Enzyme Microbial. Technol., 40: 204-211.

Yashvant, P., R. Naraian, K. Sunita, P. Abbasi and V.K. Singh, 2013. A new antibiotic resistant mutant of *Pleurotus sajor-caju* with improved expression of malate dehydrogenase enzyme. IJALS, 6: 36-43.