Biocatalysis and Agricultural Biotechnology 18 (2019) 100995

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Identification and characterization of two peroxidases from *Lichtheimia corymbifera*

Wafaa M. Abd El-Rahim[a,*], Ahmed Z. Abdel Azeiz[c], Hassan Moawad[a], Michael J. Sadowsky[b,**]

[a] Agriculture Microbiology Department, National Research Centre, Cairo, Egypt
[b] Biotechnology Institute, University of Minnesota, St. Paul, MN, USA
[c] College of Biotechnology, MisrUniversity for Science and Technology (MUST), Egypt

ARTICLE INFO

Keywords:
L. corymbifera
Peroxidase
Azo dye
Direct green
Methyl red

ABSTRACT

The fungus *Lichtheimia corymbifera* was isolated on mineral salt medium (MSM) supplemented with direct green azo dye as a sole carbon source and identified by sequencing the ITS1-ITS4 gene region. This fungus showed lignin peroxidase activity when grown in liquid MSM supplemented with methyl red azo dye as a sole carbon source. The lignin peroxidase was isolated by ammonium sulfate precipitation, and purified by DEAE-SephadexA50 column chromatography and gradient SDS-PAGE separation. The enzyme bands on gels were identified by reaction with pyrogallol and hydrogen peroxide, picked, and hydrolyzed by tryps in followed by amino acids sequencing by LC/MS/MS. Two different peroxidases were identified by homology to entries in the PEAKS database and Uni Prot repository. The first (accession number A0A068RV79) was comprised of 360 AA residues with MW 40.16KD. The second (accession number A0A068S1N8) was comprised of 271 AA with a MW 30.91KD. A kinetic study of the most abundant peroxidase (MW 30.91 KD) showed a maximum enzyme activity (V_{max}) (10.23 mg/ml/20 s) and the lowest K_m value (0.33 μM hydrogen peroxide) at 0.8 μM of hydrogen peroxide and 32 μM of pyrogallol. The kinetics of this enzyme was reported for the first time in this work; furthermore, no previous articles reported the ability of *L. corymbifera* to degrade the azo dyes.

1. Introduction

The peroxidase enzymes are reported as active enzyme involved in the biodegradation of several organic pollutants (Chandrakant and Shwetha, 2011; Osama et al., 2014, 2015). Textile industrial wastewater is a major source of environmental pollution due to presence of textile dye residues (Resmi et al., 2004 and Adedayo et al., 2004, Moawad et al., 2003). Biological treatment of this wastewater represents a challenge to achieve pollution removal in an ecofriendly approach since most azo dyes are of synthetic origin that resists traditional biodegradation reactions (Wafaa et al., 2003a,b, Wafaa et al., 2016).

The textile industry uses three classes of fiber types: 1) cellulosic (cotton, rayon, linen, ramie, and lyocell), 2) protein based (wool, angora, mohair, cashmere and silk) and 3) synthetic materials (polyester, nylon, spandex, acetate, acrylic, in geo and polypropylene) (Ghaly et al., 2014). Several classes of textile dyes are used for dyeing fibers, including: acid dyes, basic dyes, direct dyes, fluorescent dyes, reactive dyes, sulphurous dyes, vat dyes (Kirk-Othmer, 1979). Biodegradation of this diversity of textile dyes often requires use of microbial consortia containing mixtures of microorganisms capable of degrading various dyes. In addition, the use of microbial enzymes for industrial wastewater treatment is an alternative approach due to lower sensitivity to the toxic components of industrial waste, fast reaction rates, safer application, and lower costs. Several studies have been conducted to isolate and identify azo dyes degrading microbes and their enzymes, such as lignin peroxidase (Shaffiqu et al., 2002; Nazaret et al., 2011; Osama et al., 2015), laccases (Volker and Lele, 2009; Yu Huan et al., 2011), dioxygenase (Prateeka et al., 2015) and azoreductases (Huizhong et al., 2005 and Ajay and Vinay, 2012; Osama et al., 2014).

Peroxidase mode of action: Peroxidases are enzymes that catalyze reactions of hydrogen peroxide. The peroxidases mode of action was discussed in many research articles (Jose et al., 2001; Nigel, 2004 and Dunford and Jones, 2010). The Enzyme catalyzes the reduction of hydrogen peroxide combined with oxidation of organic substrate. The ground state of peroxidase contains ferric atom (Fe + 3) that reacts with hydrogen peroxide and gain one oxygen atom to be converted into Fe +4 intermediate (compound I), which in-turn gains one proton and

---

[*] Corresponding author. Agricultural Microbiology Department, National Research Centre (NRC), 33 El Bohouth St., Dokki, P.O. 12622, Cairo, Egypt.
[**] Corresponding author. BioTechnology Institute, University of Minnesota, 1479 Gortnet Ave, 140 Gortner Labs, St. Paul, MN, 55108, USA.
E-mail addresses: wafaa10m@hotmail.com (W.M. Abd El-Rahim), sadowsky@umn.edu (M.J. Sadowsky).

https://doi.org/10.1016/j.bcab.2019.01.033
Received 26 August 2018; Received in revised form 10 December 2018; Accepted 18 January 2019

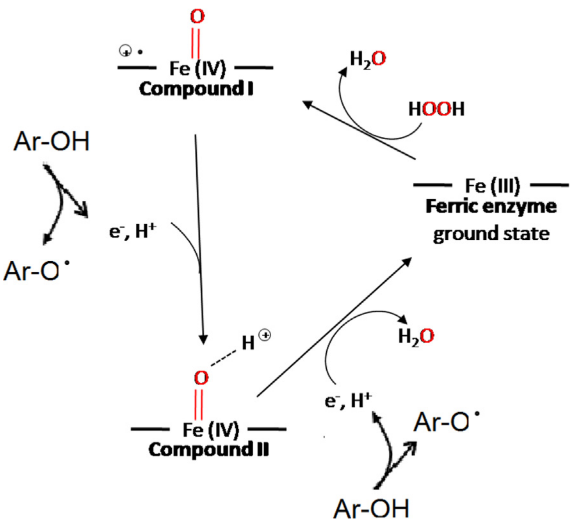

Fig. 1. The peroxidase mode of action for oxidation of phenolic compounds.

electron from the organic substrate (usually phenols or amines) and converted to intermediate compound (II). This compound is converted into the ground state again by gaining one proton and electron from another substrate molecule (phenolic compound) to form one water molecule and converted into the ground state again (Fig. 1). On the other hand, the phenolic compound is converted into radical that can be polymerized to form oligomer of the substrate molecule. For that reason, peroxidases are used for polymers synthesis (Frank and Isabel, 2012).Where AH is the substrate molecule.

In this study we attempted to isolate efficient azo dye degrading fungi from textile dye users in Egypt. The identified fungi produced variety of azo dye-degrading enzymes which were subsequently isolated, identified, and characterized.

2. Materials and methods

2.1. Soil and water samples

Three soil samples were collected from different locations in Egypt (Kafer El-Shekh, Suez Canal and Cairo, Egypt), two water samples from Suez Canal and Kafer EL-Shekh beach, and one wastewater sample from Mardiny textile staining factory, 10thof Ramadan City, Egypt and one soil sample from San Joauin valley, USA.

2.2. Microbial media

The Mineral Salt Medium (MSM) was prepared as described by (Chao et al., 2006) with modification. The medium was supplemented with 0.5 g/l of yeast extract as a source of growth factors and100 ppm of the azo dyes methyl red or direct green. This medium was used in liquid or solid form. Potato dextrose (PDS) agar (difco) was also used.

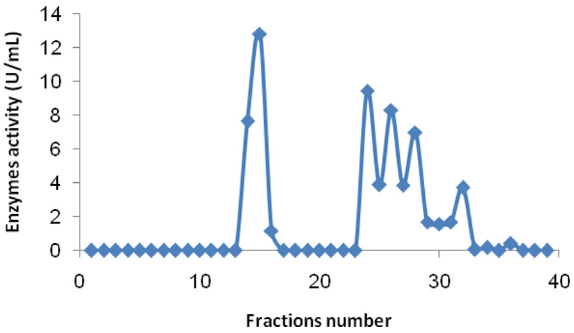

Fig. 2. Lignin peroxidase activities of the fractions collected from the DEAE-Sephadex column fractionation of *L. corymbifera* crude enzyme precipitate.

2.3. Fungi isolation

Ten g of the soil sample was suspended in 100 ml of sterilized saline solution (0.85% NaCl) and shacken for 1 h. Molten and cooled MSM medium, containing either commercial direct green azo-dye (100 ppm) or the industrial wastewater as a sole source of carbon, was poured into petri dishes. One ml from each soil suspension was separately spread onto PDA. Cultures were incubated at 28 °C for 5 d. Colonies that appeared on the medium were picked, and purified by streaking on the same medium.

2.4. Identification of fungi

The fungal isolates were identified by morphological characteristics and by using a combination of PCR amplification of rDNA genes corresponding to unique sequences within the Internally Transcribed Spacer (ITS) regions ITS1-4. Fungal DNA was extracted by using an UltraClean Microbial DNA isolation kit (MOBiolaboratory, USA) according to the manufacturer's instruction. The primers used were: ITS1: TCCGTAGGTGAACCTGCGG andITS4: TCCTCCGCTTATTGATATGC. The PCR conditions were: 95 °C, 10 min; 30 × (95 °C, 30 s; 57 °C, 45 s; 72 °C, 1 min 30 s); 72 °C, 10 min; 4 °C forever. PCR products were separated by agarose gel electrophoresis and purified using ExoSAP-IT® PCR Product Cleanup kits (Affymetrix-USB, city state) were used as per instructions of manufacturer. The samples were sequenced at the University of Minnesota Genomic Center (UMGC), St. Paul, MN. The obtained sequences were trimmed followed by BLAST analysis using the NCBI-BLAST web-tool: http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome.

2.5. Inoculum preparation

A 10 ml aliquot of sterilized saline solution (0.85% NaCl) was added to a 5-day-old agar slant and the fungal mycelia were suspended by use of a needle.

2.6. Enzyme production

Liquid MSM containing 20 ppm of methyl red was inoculated with

Table 1
Source of isolated fungal strains.

Sample	Number of fungal isolates	Isolate identification
MSM containing industrial wastewater	6	*Aspergillus oryzae AoKSA13-04*and ORF355,*Aspergillusniger A40, Aspergillus terreus ATCC 1012 and CSV2, and Aspergillus fumigatus FJAT-31052*
Soil sample from San Joauin valley, USA	4	*Aspergillus fumigatus* S4518S and MSEF106, *Aspergillus nigers* 211, *Aspergillus flavus* AfKSA13-06
Soil and water samples from Kafer EL-Shekh and the Suez canal (Egypt)	9	*Aspergillus terreus* FJAT-31011, FJAT-31038, E18S, FJAT-31011, and CV2, *Aspergillus flavus* FJAT-31042, *Aspergillus fumigatus* FJAT-31052, *Aspergillus alabamensis* CBS and *Lichtheimia corymbifera* 1-5-f-9

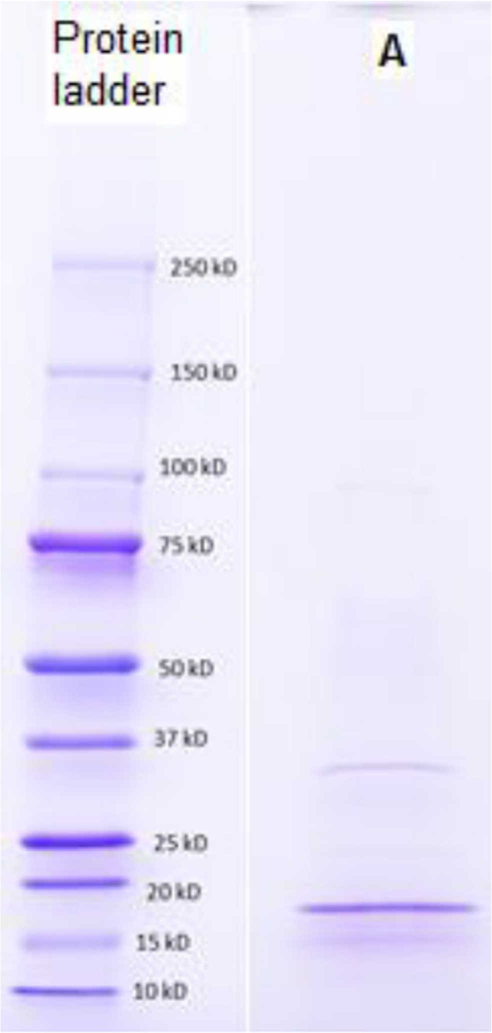

Fig. 3. Gradient SDS-PAGE analysis of the active fractions of the *L. corymbifera* lignin peroxidase showed three bands at ~ 36, 18 and 15KD.

1 ml of the fungus suspension (O.D. = 1). The flasks were incubated at 28°C for 2 d on rotary shaker at 150 rpm.

2.7. Enzymes assay

Manganese peroxidase was determined from the formation rate of purpurgallin at 520 nm from the reaction between pyrogallol and hydrogen peroxide. A molar extinction coefficient of ε 520 = 12 mg/ml was used (Bourbonnais and Paice, 1988). One unit of lignin peroxidase was defined as the amount of enzyme which catalyzes the production of one milligram of purpurogallin in 20 s at 28 °C at a pH of 6.0.

2.8. Protein determination

Protein concentration was determined by using the Folin reagent (Lowry et al., 1951) and the specific activity was calculated as enzyme units/mg protein.

2.9. Enzyme purification

The fungal biomass was separated by centrifugation from 100 ml culture at 5000 × *g* for 10 min at 15 °C. The enzyme was precipitated from the supernatant by 100% saturated ammonium sulfate (Fischer scientific, enzyme grade) by stepwise addition of 1 g portions of ammonium sulfate with continues stirring in an ice bath. The solution was left over night at 5 °C. The crude enzyme precipitate was separated by centrifugation at 5000 × *g* for 15 min under cooling at 15 °C. The enzyme precipitate was dialyzed against 50 mM phosphate buffer, pH 6. The product was then fractionated by DEAE-sephadex (Biorad) column chromatography step-wisely elution with 30 ml portions of different mixture of phosphate buffet, pH 6 (A) and 1 M NaCl solution (B) (from 100% A, to 0% A, with 10% incremental decrease in the % of solution A). Forty fractions (10 ml each) were collected. Both manganese peroxidase and soluble proteins were determined in the collected fractions. The subsequent fractions that showed enzyme activity were combined followed by enzyme precipitation by ammonium sulfate and dialysis.

2.10. Electrophoresis analysis

The enzyme precipitate was separated by SDS-PAGE gradient gel electrophoresis cell under cooling at 15 °C. A protein ladder (250KD-10KD) was used. Two gels were prepared, the first was stained by

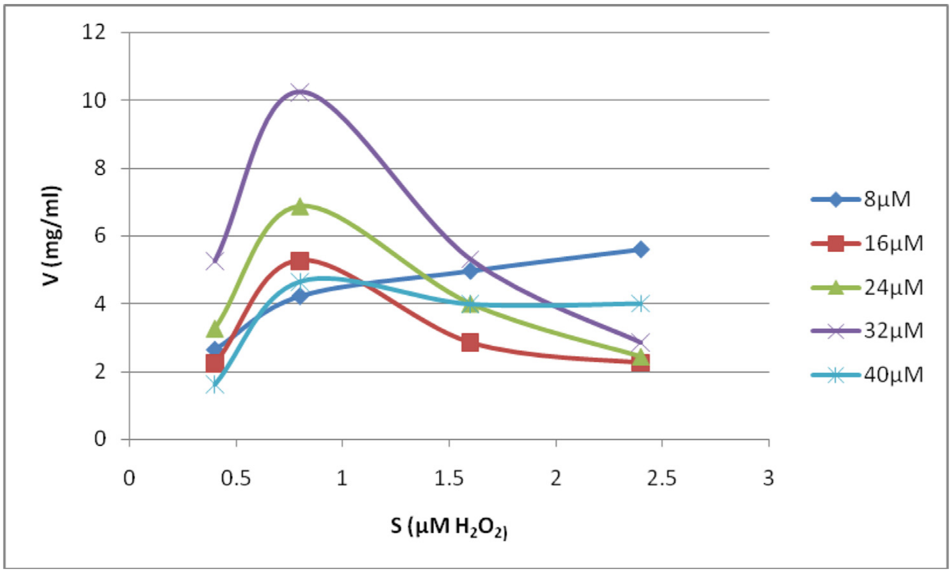

Fig. 4. The Michaelis Menten plot of hydrogen peroxide concentrations as a substrate (S) versus the enzyme velocity expressed as mg of purpurgallin produced per one ml of the reaction mixture/20 s. (V) at different pyrogallol concentrations.

coomassie and the second was stained by soaking the gel in a 100 ml mixture of 0.8% pyrogallol (70 ml), 29.5 ml of 50 mM phosphate buffer, pH 6 and 500 µl of 1% (V/V) hydrogen peroxide solution. The gel was incubated for 2 h at 28 °C. The enzyme band appearing was brown on a transparent background.

2.11. LC/MS/MS amino acids sequencing

The enzyme band from the gel was digested by using trypsin according to the method described by Shevchenko et al. (1996). The solution after proteolytic digestion was analyzed by LC-MS/MS on Orbitrap Velos mass spectrometer. The data were interpreted by using the software package PEAKS. This analysis was performed at the Center for Mass Proteomics (CMSP), University of Minnesota, USA.

2.12. Kinetics study

The Michaelis Menten graph Enzyme velocity (V) versus substrate concentration (S) was plotted at several concentrations of hydrogen peroxide and pyrogallol. The reaction mixture solutions were prepared in 1 ml containing: H_2O_2 (0.4, 0.8, 1.6, 2.4, 3.2 µM) and pyrogallol (8, 16, 24, 32, 40 µM) in phosphate buffer pH of 6.0. The reaction was initiated by addition of hydrogen peroxide and the OD was measured at 520 nm after 20 s intervals for 1 min.

3. Results and discussion

3.1. Isolation and identification of azo dye-degrading fungi

In A previous study conducted in our lab, 19 fungal isolates were obtained from MSM containing industrial wastewater, soil sample from San Joauin valley, USA, Soil and water samples from Kafer EL-Shekh and the Suez canal (Egypt). The identification of these fungal isolates using the ITS1 and ITS4 genes showed that the isolated fungi belonged to five *Aspergillus* species (*A. niger*, *A. fumagatus*, *A. flavus*, *A. oryzae* and *A. terrus*). In addition one strain was identified as *Lichtheimia corymbifera* as illustrated in Table 1.

Since no information is available in the "Protein Data Bank" and BRENDA databases for peroxidases enzymes from *L. corymbifera*, this fungus was selected for enzyme identification and characterization. The fungus L. *corymbifera* belongs to the Mucorales order of the Zygomycetes class. It is a saphrophytic fungus causing human mucormycosis infections Dea et al. (2009), Woo et al. (2012) and Evangelia et al. (2013).

3.2. Enzyme production and activity determination

The fungus grown in MSM supplemented with methyl red azo dye showed production of lignin peroxidase with a specific activity of 0.027U/mg protein, which increased after precipitation by ammonium sulfate to 0.031 U/1 mg protein.

3.3. Enzyme purification

The DEAE-Sephadex column fractionation showed the presence of lignin peroxidase activity in fractions number 14–16, 24–28, and fraction number 32 (Fig. 2). The gradient SDS-PAGE followed by coomassie staining showed three bands with MW of 36, 18 and 15 KD (Fig. 3). These bands were picked up for amino acids sequencing analysis by LC/MS/MS.

3.4. Amino acids sequencing

The amino acids sequences of the three protein bands were analyzed and compared with the reference proteins in the PEAKS database done with a search of *Lichtheimia corymbifera,* this was downloaded from UniProt repository on Aug 16, 2016. The search showed similarity with two peroxidases. The first, accession number A0A068RV79, was comprised of 360 AA residues with a MW of 40.17KD. The second, accession number A0A068S1N8, was comprised of 271 AA residues with a MW 30.915KD.

3.5. Determination of enzyme kinetics

Since no information was available for the kinetics of *L. corymbifera* peroxidase enzymes, the most abundant peroxidase (MW 30.915KD) was selected for kinetics studies. Effect of hydrogen peroxide and pyrogallol concentrations on the enzyme activity showed that the highest enzyme activity was obtained at 0.8 µM of hydrogen peroxide at all of the tested pyrogallol concentrations. However, a pyrogallol concentration of 8 µM showed highest enzyme activity (5.59 mg/ml) at the highest hydrogen peroxide concentration (2.4 µM). The maximum enzyme activity ($V_{max.}$) (10.23 mg/ml/20 s) and the lowest K_m value (0.332 µM hydrogen peroxide) were obtained at 0.8 µM of hydrogen peroxide and 32 µM of pyrogallol (Fig. 4). This result reflects the inhibition effect of hydrogen peroxide for *L. corymbifera* peroxidase at concentrations greater than 0.8 µM.Also, the result reflects that the highest enzyme activity and lowest Km value were obtained when the ratio of both enzyme substrates (pyrogallol/hydrogen peroxide) is 40.

4. Conclusion

The enzymatic treatment of textile industrial wastewater for removal of azo dyes residues is a major goal for several recent researches due to lower sensitivity to the toxic components of industrial wastes, fast reaction and lower cost. Several fungal enzymes have been studied in details; however, the research is conducted to find peroxidases with higher Vmax and lower Km values to achieve higher reaction speed and higher sensitivity to the substrate. Therefore, in the present study *L. corymbifera* was isolated among nineteen fungal isolates, which showed decolorization of direct green azo dye on the solid MSM. The peroxidases of *L. corymbifera* isolate were selected for identification and characterization since no information was available in the "Protein Data Bank" and BRENDA databases for peroxidases enzymes from *L. corymbifera* as well as no previous research articles have been published for the azo dyes degradation by *L. corymbifera.* The LC/MS/MS analysis for amino acids sequencing of the pure enzymes identified two different peroxidases (MW 40.166 KD and 30.915KD). The kinetics study of the most abundant peroxidase (MW 30.915 KD) showed that the maximum enzyme activity (Vmax) (10.23 mg/ml/20 s) and the lowest Km value (0.332 µM hydrogen peroxide) were obtained at 0.8 µM of hydrogen peroxide and 32 µM of pyrogallol.

Acknowledgment

This study was funded, in part, by a grant from the US-Egypt Joint fund, project No 4462, and by a grant from the University of Minnesota Agricultural Experiment Station. The funding sources had no involvement or role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

References

Adedayo, S.J.C., Taylor, W.A., Anderso, M.Y., 2004. Decolorization and detoxification of methyl red by aerobic bacteria from a wastewater treatment plant. World J. Microbiol. Biotechnol. 20, 545–550.

Ajay, Kumar Pandey, Vinay, Dubey, 2012. Biodegradation of azo dye reactive red BL by Alcaligenes Sp. AA09. Int. J. Eng. Sci. 1 (12), 54–60.

Bourbonnais, R., Paice, M.G., 1988. Veratryl alcohol oxidases from the lignin-degrading basidiomycete Pleurotus sajor-caju. Biochem J 255 (2), 445–450 15.

Chandrakant, S. Karigar, Shwetha, S. Rao, 2011. Role of microbial enzymes in the bioremediation of pollutants: a review. Enzym. Res. 11 Article ID 805187.

Chao, Y., Na, L., Xinmin, G., Chuanling, Q., 2006. Cloning of mpd gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil. FEMS Microbiol. Lett. 265, 118–125.

Huizhong, Chen, Sherryll, L. Hopper, Carl, E. Cerniglia, 2005. Biochemical and molecular characterization of an azoreductase from Staphylococcus aureus,a tetrameric NADPH-dependent flavoprotein. Microbiology 151, 1433–1441.

Dea, Garcia-Hermoso, Damien, Hoinard, Gantier, Jean-Charles, Grenouillet, Fre´de´ric, Dromer, Franc¸oise, Dannaoui, Eric, 2009. Molecular and phenotypic evaluation of lichtheimiacorymbifera (Formerly Absidiacorymbifera) complex isolates associated with human mucormycosis: rehabilitation of L. Ramose. J. Clin. Microbiol. 47 (12), 3862–3870.

Dunford, H. Brian, Jones, Peter A., 2010. Peroxidases and Catalases: Biochemistry, Biophysics, Biotechnology and Physiology, second ed. John Wiley and Sons Inc.

Evangelia, Bibashi, Sybren de Hoog, G., Pavlidis, Theodoros E., Symeonidis, Nikolaos, Sakantamis, Athanasios, Walthe, Grit, 2013. Wound infection caused by Lichtheimiaramosa due to a car accident. Medical Mycology Case Reports 2, 7–10.

Frank, Hollmann, Isabel, W. C. E. Arends, 2012. Enzyme initiated radical polymerizations. Polymers 4, 759–793. https://doi.org/10.3390/polym4010759.

Ghaly, A.E., Ananthashankar, R., Alhattab, M., Ramakrishnan, V.V., 2014. Production, characterization and treatment of textile effluents: a critical review. Chemical Engineering & Process Technology 5 (1), 1–18.

Jose, Neptuno Rodríguez-López, Lowe, David J., Hernández-Ruiz, Josefa, Hiner, Alexander N.P., García-Cánovas, Francisco, Thorneley, Roger N.F., 2001. Mechanism of reaction of hydrogen peroxide with horseradish peroxidase: identification of intermediates in the catalytic cycl. J. Am. Chem. Soc. 123 (48), 11838–11847. https://doi.org/10.1021/ja011853.

Kirk-Othmer, 1979. Encyclopedia of Chemical Technology, third ed. Wiley-Inter Science, New York, pp. 1–172.

Lowry, Oh, Rosebrough, N.J., Farr, Al, Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 (1), 265–275.

Moawad, Hassan, Wafaa, M. Abd El-Rahim, Khalafallah, M.A., 2003. Evaluation of biotoxicity of textile dyes using two bioassay tests. J. Basic Microbiol. 43 (3), 218–229.

Nazaret, Hidalgo, Giulia, Mangiameli, Teresa, Manzano, Galina, G. Zhadan, John, F. Kennedy, Valery, L. Shnyrov, Manuel, G., 2011. Roig Oxidation and removal of industrial textile dyes by a novel peroxidase extracted from post-harvest lentil (Lens culinaris L.) stubble. Biotechnol. Bioproc. Eng. 16, 821.

Nigel, C. Veitch, 2004. Horseradish peroxidase: a modern view of a classic enzyme. Phytochemistry 65 (3), 249–259.

Osama, M Darwesh, Moawad, Hassan, Wafaa, M Abd El-Rahim, Olfat, S Barakat, Mohamed, Z Sedik, 2014. Bioremediation of textile reactive blue (RB) azo dye residues in wastewater using experimental prototype bioreactor. Res. J. Pharmaceut. Biol. Chem. Sci. 5 (4), 1203–1219.

Osama, M Darwesh, Moawad, Hassan, Olfat, S Barakat, Wafaa, M Abd El-Rahim, 2015. Bioremediation of textile reactive blue azo dye residues using nanobiotechnology approaches. Res. J. Pharmaceut. Biol. Chem. Sci. 6 (1), 1202–1211.

Prateeka, Borar, Kushan, Chowdhury, Neeraja, Marathe, Parijat, Das, 2015. Isolation and characterization of Aeromonasaquariorum from a dye effluent and its effect in bioaugmentation. IOSR J. Environ. Sci. Toxicol. Food Technol. 9 (2), 10–16.

Resmi, C. Senan, Emilia Abraham, T., 2004. Bioremediation of textile azo dyes by aerobic bacterial consortium. Biodegradation 15, 275–280.

Shaffiqu, T.S., Jegan Roy, J., Aswathi Nair, R., Emilia Abraham, T., 2002. Degradation of textile dyes mediated by plant peroxidases. Appl. Biochem. Biotechnol. 102 (1), 315–326.

Shevchenko, Andrej, Wilm, Matthias, Vorm, Ole, Mann, Matthias, 1996. Mass spectrometric sequencing of proteins from silver-stained polyacrylaminde gels. Anal. Chem. 68, 850–858.

Volker, Madhavi, Lele, S.S., 2009. Laccases: properties and applications. Bioresources 4 (4), 1694–1717.

Wafaa, M. Abd-El Rahim, Moawad, H., Khalafallah, M.A., 2003a. Enhancing the growth of fungal promising strains for rapid dye removal. Fresenius Environ. Bull. 12 (7), 764–770.

Wafaa, M. Abd-El Rahim, Moawad, H., Khalafallah, M.A., 2003b. Microflora involved in textile dye waste removal. J. Basic Microbiol. 43 (3), 167–174.

Wafaa, M. Abd-El Rahim, Enas, M. Mostafa, Moawad, Hassan, 2016. High cell density cultivation of six fungal strains efficient in azo dye bioremediation. Biotechnology Reports 12, 1–5.

Woo, CY Patrick, Leung, Shui-Yee, Antonio, HY Ngan, Susanna, KP Lau, Yuen, Kwok-Yung, 2012. A significant number of reported Absidiacorymbifera (Lichtheimia corymbifera) infections are caused by Lichtheimi aramosa (syn. Lichtheimia hongkongensis): an emerging cause of mucormycosis. Emerg. Microb. Infect. 1, 1–8.

Yu Huan, Liu, Ye, Mao, Lu, Yi, Zhang, Xia, Li, Gang, 2011. Improving the decolorization for textile dyes of a metagenome-derived alkaline laccase by directed evolution. Appl. Microbiol. Biotechnol. 91 (3), 667–675.