UNIVERSITI PUTRA MALAYSIA

ISOLATION AND ENZYMATIC STUDIES OF DYE-DEGRADING WHITE-ROT FUNGUS

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FBSB 2013 34

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

December 2013

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Assoc. Prof. Mohd Yunus Abd Shukor, PhD

Faculty : Biotechnology and Biomolecular Sciences

Dye is present continuously in the environment. They are designed to be permanent and resistant to degradation by their physical and chemical properties. Therefore, increasing discharge and improper management of solid and liquid industrial dye wastes have generated a great concern among industrialists and the scientific community due to their negative effects. To date, effective method to treat recalcitrant dye is still lacking, to ensure safe disposal of dye effluents. In this regards, this study had been designated to isolate and screen dye degrading white rot fungi from local environments in Peninsular Malaysia; to identify the selected white rot fungus that showed the best degrading ability; to evaluate the degradation of azo dye by the selected white-rot fungus and determine its optimum conditions; and to partially purify and partially characterize the ligninolytic enzyme. Thirty nine white rot fungi (WRF) from soil and wood samples were isolated in Selangor, Kelantan, Pahang and Terengganu and tested for their capability to degrade textile azo dyes (Orange G (C.I. 16230), Ponceau 2R (C.I. 16450), Amaranth (C.I. 16185), Trypan Blue (C.I. 23850) dan Direct Blue 71 (C.I. 34140). Thirty-three isolates showed positive results with varying degrees of dye degradation. Two isolates (Isolate 4-UPM and Isolate 17-UPM) from Universiti Putra Malaysia (UPM) campus in Selangor were selected for further studies owing to their ability to completely decolourize all the azo dyes within the shortest time. Qualitative study on defined solid media showed Isolate 17-UPM and Isolate 4-UPM were capable of degrading all five dyes under nitrogen-limiting conditions, with glucose as the source of energy. When cultured in two-stage liquid medium for quantitative screening, Isolate 17-UPM degradation rate was in the range of 96 to 99% of 0.2 g/L while Isolate 4-UPM showed a range of 38 to 96 % of all the tested azo dyes. Both isolates degraded the dyes within one to ten days at different rates. Isolate 17-UPM and Ponceau 2R were used for further studies. Overall, the degradation rates of Isolate 17-UPM in agitated

cultures were higher by nearly ten times compared to static cultures. Ponceau 2R was degraded optimally when incubated between 35 to 40°C in agitated cultures at the initial pH of 6.

The assays for lignin modifying enzymes involved in the azo dye degradation showed the presence of laccase only, while lignin peroxidase and manganese peroxidases were absent. There was a significant correlation between the laccase activity profile in agitated liquid cultures and the azo dye degradation profile where both optimum temperature and initial pH were 40°C and pH 6, respectively. The laccase produced by Isolate 17-UPM during azo dye degradation was partially purified using DEAE Cellulose anion exchanger and ZorbaxR GF-250 gel filtration column. The partial purified enzyme showed a $K_{m\ (app)}$ value of 0.28 mM, $V_{max\ (app)}$ value of 100 µmol/min.ml, optimum temperature activity at 40 to 50°C and pH 3.0 to 5.0 when 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) was used as the substrate. It was also shown to be most stable at room temperature and pH 6.0 to 7.0.The Isolate 17-UPM was further characterized at molecular level through ITS region gene sequencing. The internal transcribe spacer region of the isolated DNA was amplified by PCR using the primers recognized as, primer ITS 1F and ITS 4. Isolate 17-UPM was identified as *Coriolopsis* sp. strain aff17. In this study, a white-rot fungus capable of degrading azo dyes was isolated, identified and optimized for dye degradation, and the enzyme involved was partially purified and characterized.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

PEMENCILAN DAN KAJIAN ENZIM KULAT REPUT-PUTIH YANG MENGURAI PEWARNA

Oleh

AHMAD AFIF ABDUL AZIZ

Disember 2013

Pengerusi: Prof. Madya. Mohd Yunus Abd Shukor, PhD

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Pewarna wujud secara berterusan dalam alam sekitar. Ia direka untuk menjadi kekal dan tahan penguraian kepada sifat-sifat fizikal dan kimia pewarna tersebut. Oleh itu, peningkatan pelepasan dan salah pengurusan sisa pewarna industri pepejal dan cecair telah menjadi satu kebimbangan besar di kalangan usahawan-usahawan dan komuniti saintifik disebabkan oleh kesan negatif mereka. Setakat ini, kaedah yang berkesan untuk merawat sisa pewarna masih kurang, untuk memastikan pelupusan selamat efluen pewarna. Oleh itu, kajian ini telah direka untuk memencil dan menyaring kulat reput putih dari persekitaran tempatan di Semenanjung Malaysia yang boleh mengurai pewarna; untuk mengenal pasti kulat reput putih yang dipilih; untuk menilai keupayaan kulat reput putih penguraian pewarna azo yang dipilih dan menentukan syarat-syarat alam sekitar yang optimum; dan untuk penulenan separa dan pencirian separa enzim pengubah lignin. Tiga puluh sembilan kulat reput putih dari sampel tanah dan kayu di Selangor, Kelantan , Pahang dan Terengganu telah dipencilkan dan diuji keupayaan untuk menguraikan pewarna tekstil azo Orange G (CI 16230), Ponceau 2R (CI 16450), Amaranth (CI 16185), Trypan Blue (CI 23850) dan Direct Blue 71 (CI 34140). Tiga puluh tiga pencilan menunjukkan hasil yang positif dengan pelbagai peringkat penguraian pewarna. Dua pencilan (Isolat 4-UPM dan Isolat 17-UPM) dari Universiti Putra Malaysia (UPM) kampus di Selangor telah dipilih untuk kajian lanjut berdasarkan keupayaan untuk menguraikan keseluruhan warna pewarna azo dalam masa paling singkat. Kajian kualitatif di media pepejal menunjukkan Isolat 17-UPM dan Isolat 4-UPM mampu menguraikan kesemua lima pewarna dalam keadaan nitrogen yang terhad, dengan glukosa sebagai sumber tenaga. Apabila dikultur dalam medium cecair dua peringkat bagi penyaringan kuantitatif, kadar penguraian Isolat 17-UPM adalah dalam lingkungan 96 hingga 99

% daripada 0.2 g/L manakala Isolat 4- UPM menunjukkan lingkungan 38 hingga 96% bagi semua pewarna azo yang diuji. Kedua-dua pencilan menguraikan pewarna dalam tempoh satu hingga sepuluh hari pada kadar yang berbeza. Isolat 17- UPM dan Ponceau 2R telah digunakan untuk kajian lanjutan. Secara keseluruhan, kadar penguraian Isolat 17-UPM dalam kultur goncang lebih tinggi hampir sepuluh kali berbanding kultur statik. Ponceau 2R telah diuraikan secara optimum apabila dieram di antara 35 hingga 40°C dalam kultur goncang pada pH awal 6.

Pencerakinan untuk enzim-enzim pengubah lignin yang terlibat dengan penguraian pewarna azo hanya menunjukkan kehadiran lakase (E.C. 1.10.3.2) manakala lignin peroksidase (E.C. 1.11.1.14) dan mangan peroksidase (E.C. 1.11.1.13) tidak dapat dikesan. Terdapat hubungan yang signifikan antara profil aktiviti lakase dalam kultur cecair goncang dan profil degradasi pewarna azo di mana suhu dan pH awal optimum bagi kedua-dua adalah masing-masing 40°C dan pH 6. Penulenan separa lakase yang dihasilkan oleh Isolat 17-UPM semasa degradasi pewarna azo melalui penukar anion DEAE Cellulose dan kolum penurasan gel ZorbaxR GF -250. Enzim separa tulen menunjukkan $K_{m\ (app)}$ bernilai 0.28 mM, $V_{max\ (app)}$ bernilai 100 μmol/min.ml, aktiviti suhu optimum pada 40 hingga 50°C dan pH 3.0 hingga 5.0 apabila 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) digunakan sebagai substrat. Ia juga mempamerkan kestabilan tinggi pada suhu bilik dan pH 6.0 hingga 7.0. Isolat 17- UPM terus dicirikan pada peringkat molekul melalui penjujukan rantai gen ITS. Rantai ruangan salinan dalaman DNA yang dipencilkan telah dikuatkan melalui PCR menggunakan primer dikenali sebagai primer ITS-1F dan ITS 4. Isolat 17- UPM telah dikenalpasti sebagai *Coriolopsis* sp. starin aff17. Dalam kajian ini, kulat reput putih mampu menguraikan pewarna azo telah berjaya dipencilkan, dikenalpasti dan dioptimumkan bagi penguraian pewarna, dan penulenan dan pencirian separa enzim yang terlibat.

ACKNOWLEDGEMENTS

Firstly I would like to express my gratitude to Allah for showering me His blessing and consent to make all of this possible. My sincere thanks and wholehearted appreciation to my supervisor Assoc. Prof. Dr. Mohd Yunus Abd Shukor for his invaluable guidance, advice, expertise and motivation throughout my research. My thanks also go to Prof. Dr Mohd Arif Syed for the advice and support on many aspects of this work.

Special thanks to my laboratory mates especially those from lab 204 namely Mohd Haris, Mohd Ezuan, Badrin and Khalizan for being the source of strength, constant sharing of knowledge and advice that will be remembered and more importantly is the friendship we treasure.

Last but not least, I would like to express my warm gratitude to my family especially my mother and father for giving me the strength and unremitting love, not to forget for my lovely wife Dania Aziz for being patient and giving constant encouragement and support throughout the completion of this thesis. I certainly wouldn't be able to achieve what I have today without the unconditional love and support from family and friends. Thank you again.

I certify that a Thesis Examination Committee has met on (16th December 2013) to conduct the final examination of Ahmad Afif Abdul Aziz on his thesis entitled Isolation And Enzymatic Studies Of Dye-Degrading White Rot Fungus in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Masters of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

Declaration by graduate student

I hereby confirm that:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____	Signature:_____
Name of Chairman of Supervisory Committee: **Mohd Yunus Bin Abd Shukor, PhD**	Name of Member of Supervisory Committee: **Mohd Arif Bin Syed, PhD**

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