ISOLATION AND BIODEGRADATION OF 3-CHLOROPROPOINIC ACID BY *Aspergillus Aculeatinus* M1

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ABSTRACT

Halogenated organic compounds are found from industrial and agricultural products. They are dangerous environmental pollutants due to their toxicity and persistency in nature. The main objectives of this study are to isolate and identify fungi from dead wood which able to utilize 3-chloropropionic acid as a sole source of carbon and energy. 3-chloropopionic acid (3CP) is used as a herbicide. Fungi *Aspergillus* was isolated from a dead wood sample taken from UTM plantation. The molecular analysis, of the isolated fungus had 100% identity to the *Aspergillus aculeatinus*. Therefore, it was designated as *Aspergillus aculeatinus* M1. The fungi were able to grow in minimal medium contain 20mM 3-chloropropoinic acid as sole carbon source and energy. The growth was measured based on growth in 3-chloropopionic acid minimal. *Aspergillus aculeatinus M1* showed the best growth on 20mM 3-chloropropionic acid but concentration of above 20 mM3-chloropropionic acid was toxic. The current study demonstrates the ability of fungal to growth on 3-chloropropionic acid as their sole source of carbon and energy.

ABSTRAK

Sebatian organik halogen boleh didapati daripada produk-produk perindustrian dan pertanian. Sebatian ini amat merbahaya terhadap alam sekitar kerana sebatian ini sangat toksik dan sukar untuk terurai oleh alam semula jadi. Objektif utama kajian ini adalah untuk mengasingkan dan mengenalpasti kulat daripada kayu mati yang mampu menggunakan asid 3-kloropropionik sebagai sumber karbon dan tenaga. Asid 3-kloropropionik (3CP) kebiasaannya digunakan sebagai herbisid. Kulat *Aspergillus* telah diasingkan daripada sampel kayu mati yang diambil dari ladang UTM. Selepas melalui analisis molekul, kulat tersebut telah dikenalpasti sebagai sepsis Aculeatinus Aspergillus. Oleh itu, ia telah dilantik sebagai *Aspergillus aculeatinus M1*. Kulat boleh membiak dalam keadaan asid sederhana yang mengandungi kepekatan asid 3-kloropropionik pada 20mM sebagai sumber karbon dan tenaga. Pertumbuhan kulat diukur berdasarkan pertumbuhan dalam asid 3-Chloropopionic minimum. *Aspergillus aculeatinus M1* menunjukkan pertumbuhan terbaik adalah pada kepekatan 20mM asid 3-kloropropionik tetapi akan menjadi toksik jika kepekatan adalah melebihi 20mM. Kajian menunjukkan asid 3-kloropropionik di dalam 3CP boleh digunakan sebagai sumber tunggal karbon dan tenaga untuk pertumbuhan kulat.

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CHAPTER I

INTRODUCTION

1.1 Overview

Due to industrial, agricultural and domestic activities, pollutants are exposed to the environment. These industrial pollutants such as pesticides, herbicides, pharmaceutical products and cosmetic products are having negative effects on human and ecology, which their existence in drinking water or food can cause cancer, mutagen and acute effect on human (Drivon and Ruppin, 1998). Furthermore, it can persist for a long time considering the physical and chemical properties of these compounds.

There are many methods that have been used to treat water and soil contaminated with pollutants, including the physical, chemical and biological treatment. However, the biological treatment is considered a favorable method relating to the economy, environmental friendly and ecological background. Biological treatment involves using microorganisms, including fungi, bacteria and algae to biodegrade Lin *et al*. (2011). Fungi and bacteria are commonly used as biological agents in bioremediation of contaminated air, water and soil. Many researchers have been isolated bacteria in laboratory that capable of utilize pollutants as their sole sources of carbon and energy (Janssen et al., 2005, Olaniran et al., 2001,

Schwarzeet al.,1997). Recently, researchers discovered certain species of fungi that have the ability to biodegrade pollutants such as insecticide, herbicide, fungicide, solvents,creosote, coal tar, and hydrocarbon fuels (Pervizpour and Huyop, 2013). Halogenated aliphatic compounds are one of the most important groups of xenobiotics which introduced into the environment through pesticide, herbicide and insecticide. 3-chloropropionic acid (3CP) is a halogenated compound, which often included in pesticide or synthetic of pharmaceuticals (Jing and Huyop, 2007). (3CP) belongs to the class of chlorinated mono carboxylic acid.

In the previous research related to 3-CP done by Mesri et al. (2009), Pseudomonas sp. B6p were isolated from paddy soil agricultural to biodegrade 3CP. Recently, researches that using fungi as an alternative method in treating of waste water contaminated with organic matter have received a great concern related to many factors including the widespread and availability in aquatic sediments, acidic oil seeps and water surfaces.

This study focussed on the isolation and identification of microorganisms than can utilize 3-chloropropionica acid. In detail, the goal of the study is the identification of the fungi isolate from dead wood that can degrade 3-chloropropionica acid.

1.2 Problem Statement

The widespread usage of chloroaliphatic hydrocarbon compounds such as chlorinated compound in industries have resulted in extensive pollution of the environment and the ground water. Chlorinated aliphatic compounds represent one of the most vital groups of industrially produced chemicals. Numerous of these

compounds hardly degrade in the environment. Aerobic biodegradation by using microorganisms such as bacteria and fungi to degrade the chloroaliphatic hydrocarbon compounds to carbon dioxide, water, and hydrogen chloride more efficient than anaerobic or chemical processes. Therefore, the aerobic biodegradation by using microorganism such as fungi is preferable as it less cost and biological effectiveness.

1.3 Objective and Research Goal

The main goal of the current research was to isolate and identify 3-chloropropionic acid degrading fungi. Therefore, in achieving this goal, the following objectives were considered:

i. To isolate fungi from the nature which has the ability to degrade 3CP.

ii. To identify and characterize the isolated fungus by their morphological appearance and genetic sequence analysis.

iii. To observe the degradation capability of the isolated fungus.

1.4 Scope of the Study

The scope of this research included the molecular identification of fungi isolated from UTM plantation an agricultural area. The 3-chloropropoinic acid was used as the sole source of carbon. In order to identify fungi, ITS region and

Then phylogeny tree will be constructed to observe the evolutionary relationship distance of the isolated fungus to the nearest known fungi by BLAST search analysis.

1.5 Research Significance

The significance of this research is to isolate, characterize and identify the fungus that can biodegrade the toxic chlorinated compound. This fungus has the ability to secrete dehalogenase enzyme which can reduce the toxicity and carcinogenicity of these compounds in the ecosystem.

1.6 Summary

In chapter one, the detailed introduction of this study was explained. In the second chapter will explain the background of this research. Then, the problem statement of the study is formulated and mentioned in the sequel. Also, the research goal and objectives are presented in the next section. In the sequel, the significance and scope of the research are described. Finally, the outline of the thesis is explained. In the next chapter, the literature review of the research is described.

REFERENCES

Alexander, M. (1981). *Biodegradation Of Chemicals Of Environmental Conern,*Science,211,132-138.

Alexopoulos, C.J., Mims, C.W. and Blackwell,M. (1996). *Introductory Mycology, John Wiley And Sons Press*.

Abel S.E.R., F.M.S. Hidayathul, F. Huyop and D.D. Roslan (2011). *Identification of 2,2 dichloropropionic acid degrading bacteria in soil using 16S rRNA method.* Universiti Malaysia Terengganu 10th International Annual Symposoum – UMTAS 2011. 11-13 July 2011, Permai Hotel, Kuala Terengganu, Malaysia. (ORAL).

Barth, P. T ., Bolton, L. and Thomson, . C. (1992). *Cloning And Partial Sequencing Of An Operon Encoding Two pseudomonas putida Haloalkanoate Dehalogenases Of Opposite Stereospecifity*. Jounal Of Bacteriology, 174,2612-2619.

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., Rapp, B. A. and Wheeler, D. L. (2002). *GenBank. Nucleic Acids Res.* (30): 17—20.

Bollag, J. M. and Alexander, M. (1971). *Bacterial Dehalogenation Of Chlorinated Aliphatic Acid. Soil. Biol.* And Biochem. , 3,91-96.

Bergman, J.G. and J. Sanik, (1957). *Determination of trace amounts of chloride in naphtha. Anal. Chem. (*29): 241-243.

Bhatt P, Kumar MS, Mudliar S, and Chakrabarti T (2007). *Biodegradation of chlorinated compounds-A review.* Crit. Rev. Environ. Sci. Technol. 37: 165-198.

Carlile, M. J., Watkinson, S. C. and Gooday, G. W.(2001). *The Fungi, Amsterdam, Elsevier Academic Press.*

Chaudhry, G. R. and Chapalamadugu, S. (1991). *Biodegradation Of Halogenated Organic Compounds.* Microbiology Revolution, 55,59-79.

Commandeur, L. C. M. and Parsons, J. R. (1994). *Biodegradation Halogenated Aromatic Compounds.* Biochemistry Of Microbial Degradation, P423-458.

Drivon, G. and Ruppin, C. (1998). *Process For The Preparation Of 3-Chloropropionic acid.*

Fetzner, S. and Lingens, F. (1994). *Bacterial Dehalogenases: Biochemistry, Genetics And Biotechnological Applications.* Microbial Rev., 58, 641-685.

Fahrul Huyop (2008). *Biodegradation of halogenated alkanoic acid by dehalgenase.* In: Advances in Biosciences and Bioengineering Vol 1. Editors: Zaharah Ibrahim, Haryati Jamaluddin, Shaza Eva Mohamad, Mohd. Shahir Shamsir Omar, Goh Kian Mau, Azman Abdul Samad and Razauden Zulkifli.

Field, J.A., Alvarez, S. R. (2004). *Biodegradability of chlorinated solvents and related chlorinated aliphatic compounds.* Reviews in Environmental Science & Bio/Technology. (3): 185–254

Ghosal, D, You, I, S., Chatterjee, D . K. and Chakrabarty, A. M. (1985). *Microbial Degradation Of Halogenated Compounds.* Science,228,135-142.

Guarro, J., Gene, J. and Stchigel, M. A. (1999). *Development In Fungal Taxonomy.* Clinical Microbiology Reviews, 12, 454-500.

Hoek, C., Mann, D. G. and Jahns H. M. (2005). *Algae AN Introduction To Phycology.* Cambridge, Cambridge University Press.

Huyop, F. Z. and Cooper, R. A. (2003). *A Potential Use Oh Dehalogenase D (Dehd) From Rhizobium Sp. For Industrial Process.* Jurnal Teknology. Jurnal Teknology, 39,1-8.

Hamid, A. A. A and Zulkifly, Nurul Asma Hasliza and Hamdan, Sallehuddin and Ariffin, Shahrul Hisham Zainal and Huyop, Fahrul Zaman (2011). *Purification and properties of a new dehalogenase enzyme from Pseudomonas sp. B6P grow in 3- chloropropionate (3CP).* African Journal of Biotechnology, 10 (4). pp. 610-614.

Hamid ,A.A.A., S. Hamdan, S.H.Z. Ariffin and F. Huyop (2010). *Molecular prediction of dehalogenase producing microorganism using 16S rDNA analysis of 2,2-dichloropropionate (Dalapon) degrading bacterium isolated from volcanic soil.* Journal of Biological Sciences. 10(3):190-199. ISSN 1727-3048. SCOPUS

Janssen, D. B., Dinkla, I. J. T., Poelarends, G. J. and Terpstra, P. (2005). *Bacterial Degradation Of Xenobiotic Cmopounds: Evolution And Distribution Of Novel Enzyme*. A Activities. Environmental Microbiology,7,1868-82.

Janssen,D. B., Pries, F., Ploeg, J. V. D., Kazemier, B., Terpstra, P. and Witholt, B. (1989). *Cloning Of 1,2-Dichloroethane Degradation Genes Of Xanthobacter Autotrophoicus Gjlo And Expression Of The Dhla Gene.* Journal Of Bacteriology,171,6791-6799.

Janssen, D. B., Scheper, A., Dijkhuizen, L. and Witholt,B. (1985). *Degradation Of Halogenated Aliphatic Compounds By Xanthobacter Autotrophicus Gjlo.* Applid And Environmental Microbiology, P673-677.

Janssen, H. L. (1957). *Decomposition Of Chloro-Organic Acids By Fungi.* Nature 1416,1416.

Janseen, H. L. (1963). *Carbon Nutrition Of Some Microorganisms Decomposing Halogen-Substituted Aliphatic Acids.* Acta Agriculture Scandinavica,13,404-412.

Jing, N. H. and Huyop, F. (2007). *Dehalogenation Of chlorinated Aliphatic Acids By Rhodococcus Sp.* Asia Pcific Journal Of Molecular Biology And Biotechnology,15,147-151.

Jing, Ng Hong and Ab. Wahab, Roswanira and Mohd. Taha, Aishah and Abdul Rashid, Noor Aini and Huyop, Fahrul (2009). *A further characterization of 3-chloropropionic acid dehalogenase from rhodococcus sp.* HJ1. In: International Congress of Malaysian Society for Microbiology (ICMSM 2009), 2009, Park Royal Hotel, Pulau Pinang.

Lin C, Yang L, Xu G. and Wu J. (2011). *Biodegradation and metabolic pathway of β-chlorinated aliphatic acid in Bacillus sp*. CGMCC no. 4196. Appl Microbiol Biotechnol., 90(2): 689-96.

Little, M., and Williams, P.A. (1971). B*acterial Halidohydrolase purification some properties and its modification by specific amino acid reagents. Eur. J. Biochem.* 21(1):99-109.

Mashitah Md. Salim, D.D. Roslan and Fahrul Huyop (2011). *Molecular analysis of dehalogenase gene in genomic DNA of Bacillus megaterium strain GS1 isolated from volcanic area Gunung Sibayak Indonesia.* Journal of Biological Sciences. 11(5):394-398. ISSN 1727-3048. SCOPUS.

Madigan, M. T., Martinko, J. M. and Parker, J. (2000). *Brock Biology Of Microorganism , Prentice Hall.*

Mesri, S., Wahab, R. A. and Huyop, F. Z. (2009). *Degradation of 3-chloropronic acid (3cp) by Pseudomonas sp.* B6P isolated from a rice paddy field. Annals of microbiology, 59, 447-451.

Mcgrath, J. and Harfoot, C. G (1997).*Reductive Dehalogenattion Of Halocarboxylic Acidsy The Phototrophic Genera Rhodospirillum And Rhodopsedudomonas.* Applied And Environmental. Microbiology, 63,3333-3335.

Nathan Szymansk. and Robert A Patterson. (2003). *Effective Microorganisms (EM) and Wastewater Systems.* 0-9579438-1-4.

Ng Hong Jing, Fatin Hanani Sulaiman, Roswanira Ab.Wahab, Rolando V. Pakingking, Noor Aini Abdul Rashid and Fahrul Huyop (2008). *Purification and properties of a non-stereospecific dehalogenase enzyme E (DehE) from Methylobacterium sp. HJ1.* African Journal of Microbiology Research. 2:187-191. ISSN 1996-0808. SCOPUS/WOS IF 0.539.

Ng Hong Jing and Fahrul Huyop (2006). *Dehalogenation of herbicide Dalapon by Methylobacterium sp.* KUSTEM 5th Annual Seminar on Sustainability Science and Management, 2nd-3rd, May 2006. Primula Beach Resort, Kuala Trengganu, Organised by Kolej Universiti Sains & Teknologi Malaysia (KUSTEM). ISBN 983-2888-28-x. pp.262-266.

Ogion, and Ishikawa, H. (1991). *The Longest 18s Ribosomal RNA Ever Known* 202,827-833.

Rieger, P. G., Meier, H. M. Gerle, am., Vagt, U., Groth, T. and Knackmuss, H. J. (2002). *Xenobiotics In The Environmental: Present And Future Strategies To Obviate The Problem Of Biological Persistence.* Journal Of Biotechnology, 94,101-123.

Rittmann, B. and Mccarty, P. (2001). *Environmental Biotechnology: Principles and Application, Mcgraw-Hill.*

Raper, K. B., Fennell, D. (1965). *Compilation Of The Aspergillus Studies in Mycology*,18, p. 1-38.

Roslan ,D. D, Gicana, R.J and Huyop ,F (2011). *Characterisation of Bacillus strains from volcanic area Gunung Sibayak able to degrade 2,2DCP.* African Journal of Microbiology Research. 5(28): 4987-4992. ISSN 1996-0808. SCOPUS/WOS IF 0.539.

Schwarze, R. Brokamp, A. and Schmidt, F. R. J. (1997). *Isolation And Characterization Of Dehalogenase From 2,2-Dichloropropionate-Degrading Soil Bacteria.* Current Microbiology,34,103-109.

Slater , J. H., Bull, A. T. and Hardman, D. J. (1995). *Microbial Dehalogenation Biodegradation.* 6,181-189.

Slater , J. H., Bull, A. T. and Hardman, D. J. (1997). *Microbial Dehalogenation Of Halogenated Alkanoic Acids.* Advance In Microbial physiology, 38,133-176.

Song, J.S., Lee1, D. H., Lee ,K., and Kim, C.K.(2003). *Characteristics of Several Bacterial Isolates Capable of Degrading Chloroaliphatic Compounds via Hydrolytic Dechlorination.* 41. (4). 277-283.

S. Amini, A.H. Zulkifly, Wong Wen-Yong and F.Huyop (2011). *Molecular identification and characterization of a bacterium that has potential to degrade low concentration of haloalkanoic acid.* Journal of Microbiology Research. 6(6):552-559. ISSN 1816-4935. SCOPUS.

Staub, D.K. and Kohler, H.P.E. (1989). *Microbial Degradation of β-Chlorinated Four-Carbon Aliphatic Acids.* Journal of Bacteriology. 1428-1434.

Stuart Hogg. (2005). *Essential Microbiology.* UK: John Wiley and Sons Ltd.

Somchai Chantsavang (2010). *Application of Effective Microorganisms In A New Hybrid System Of Biogas Production.*

Satou, N., and Ne, M. (1987). *The neighbor-joining method—a new method for reconstructing phylogenetic trees.* Mol. Biol. Evolut. (4): 406–425.

Sepideh Pervizpour and Fahrul Huyop (2013). *Molecular identification and biodegradation of 3-chloropropionic acid (3CP) by filamentous fungi-Mucor and Trichoderma species isolated from UTM agricultural land.* Malaysian Journal of Microbiology. X(X): XX-XX. ISSN (print): 1823-8262, ISSN (online): 2231-7538. SCOPUS. Accepted for publication.

Talaro, K. P. and Talaro, A. (2002). *Foundations In Microbiology,* Mcgraw Hill.

Timmis, K. N. and Pieper,D. H. (1999). *Bacterial Designed For Bioremediation. Trends In Biotechnology*,17,201 -204.

Tan Yea Yusn and Fahrul Huyop (2009). *Degradation of 3-chloropropionic acid by E.coli JM109 expressing dehalogenase (deh) gene used as selection marker*. Biotechnology. 8(3): 385-388. ISSN 1682-296X. SCOPUS.

T.H.T Abdul Hamid, A.A.A. Hamid, A.H. Zulkifly, S. Hamdan, S.H.Z. Ariffin and F. Huyop (2011). *Purification and properties of a new dehalogenase enzyme from Pseudomonas sp.* B6P grow in 3-chloropropionate (3CP). African Journal of Biotechnology 10(4): 610-614. ISSN 1684-5315. SCOPUS.

Tsang, J.S.H. and Pang, B.C.M. (2001). *Mutagenic Analysis of Conserved Residues in Dehalogenese IVa of Burkholderia cepacia MBA4.* FEMS Microbiology Letters. 204.(1): 135-140.

White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). *Amplification and direct sequencing Of Fungal Ribosomal RNA Gene For Phlogenetics.* In M. A. Innis,D.h.Gelfand,J.J Sninsky,and T.J White (eds), PCR Protocols. A Guide to Methods and Application. Academic press, San Diogo, PP.315-324.

Wen-Yong Wong and Fahrul Huyop (2012). *Molecular Identification and Characterization of Dalapon-2,2-dichloropropionate (2,2DCP)-degrading Bacteria from a Rubber Estate Agricultural area.* African Journal of Microbiology Research. 6(7):1520-1526. ISSN 1996-0808. SCOPUS/WOS IF 0.539.

Yokota, T., Fuse, H., Omori, T. and Minoda, Y. (1986). *Microbial Dehalogenation Of Haloalkanes Mediated By Oxygenases Or Halidohydrolase.* Agricul. And Biol. Chem., 50,453-460.

Zhou Sheng, Wei Chaohai, Liao Chaodeng and Wu Haizhen (2008). *Damage to DNA of Effective Microorganisms by Heavy Metals: Impact On Wastewater Treatment*. 1514–1518.

Zulkifly ,A.H, Roslan ,D.D, Hamid, A.A.A, Hamdan, S, Huyop F (2010). *Biodegradation of low concentration of monochloroacetic acid –Degrading Bacillus sp. TW1 isolated from Trengganu water treatment and distribution plant.* Journal of Applied Sciences. 10(22):2940-2944. ISSN 1812-5654. SCOPUS.