CHARACTERIZATION OF A NOVEL BACTERIAL DEHALOGENASE FROM COW DUNG

SITI NURUL FASEHAH BINTI ISMAIL

UNIVERSITI TEKNOLOGI MALAYSIA

CHARACTERIZATION OF A NOVEL BACTERIAL DEHALOGENASE FROM COW DUNG

SITI NURUL FASEHAH BINTI ISMAIL

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Bioscience)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

.

MARCH 2018

Specially dedicated

To my lovely mother and my late father,

Siti Khadijah Draman & Ismail Jusoh

who gave me endless love, trust, constant encouragement and great source of inspiration.

To my dear husband,

Dr. Arman Shah Abdullah

for being very understanding and supportive in keeping me going, enduring the ups and downs during the completion of this thesis. I am truly thankful for having you in my life.

To my adorable babies,

Ayra Shazia Putri Binti Arman Shah & Ayrin Shazwina Binti Arman Shah

ACKNOWLEDGEMENT

First and foremost, I would like to thank Allah the Almighty for His mercies and kindness in granting the means for achieving the goals of this work. My sincere and profound gratitude goes to my supervisor, Professor Dr. Fahrul Zaman Huyop for his excellent supervision, valuable advice and continuous encouragement at all stages of the work.

I would also like to thank to students and laboratory staff in Malaysia Genome Institute (MGI) Bangi, Faculty of Biosciences and Medical Engineering, UTM and Universiti Pendidikan Sultan Idris for their help in my research work. Special thanks to my beloved parents and brothers for their endless contributions and encouragements. I am also indebted to my husband, Dr. Arman Shah for his continuous supports and sacrifices. Finally, I would also like to express my sincere appreciation to all who had helped me.

ABSTRACT

The large quantities of the halogenated compound for example 2,2-dichloropropionic acid (2,2DCP) in the environment may lead to health problems in humans and pollution due to their toxicity and recalcitrance, respectively. Interestingly, previous studies have indicated that cow dung was proven to degrade pollutants. Hence, such animals feeding on a daily diet of halogen contaminated forage may influence the microflora in their digestive tract. Bacterial species from cow dung able to utilize 2,2DCP is yet to be reported. Therefore, the purpose of this study was to isolate, identify and characterize dehalogenase bacteria from cow dung. Four bacteria were isolated which are SN1, SN2, SN3, and SN4. Strain SN1 was observed with rapid growth in 20 mM 2,2DCP liquid minimal media, and was used for further experiments such as growth in different concentration of 2,2DCP, High-performance liquid chromatography (HPLC), Biolog GENIII, 16S rRNA analysis, characterization of purified enzyme, kinetic analysis, Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and amplification of dehalogenase gene. The growth of strain SN1 in various concentrations (10 mM, 20 mM, 30 mM and 40 mM) of the substance was evaluated. The study found the bacteria grew particularly well in 20 mM 2,2DCP with the highest chloride ion released (39.5 µmolCl^-/mL) while exhibiting a remarkably short doubling time of 3.85 h. The utilization of 2,2DCP was also confirmed by detection of 20 mM 2,2DCP depletion in the growth medium containing strain SN1 measured using HPLC. The result showed 98.6 % utilization of 2,2DCP in the growth medium. Species identification via Biolog GENIII system and 16S rRNA analysis was performed and identified strain SN1 as *Bacillus cereus.* Further investigations on dehalogenase enzyme were done by using purified enzyme of *Bacillus cereus* SN1. The molecular weight of the purified enzyme was 25 kDa by SDS-PAGE. The enzyme characteristics revealed it was optimum at pH 6 and 30 °C. It also has low K_m value of 0.2 mM. The dehalogenase peptide was identified by LC-MS/MS with 18% sequence coverage to haloacid dehalogenase, *Bacillus cereus* (strain 03BB102). Moreover, Group I and Group II dehalogenase primers were used to amplify dehalogenase gene and the band only appeared for Group I. The dehalogenase gene fragment amplified was designated "DehSN1" and belongs to Group I dehalogenase since it has 75 % similarity with Group I dehalogenase (DehE). Five conserved residues were identified as Asn33, Tyr117, Cys42, Ala120 and Asp136. As a conclusion**,** this is the first reported case of a *Bacillus* sp. isolated from cow dung capable of utilizing 2,2DCP. Therefore, further assessment of its ability to degrade other types of haloalkanoic acids merits special consideration.

ABSTRAK

Kuantiti sebatian halogen yang besar seperti asid 2,2-dikloropropionik (2,2DCP) dalam persekitaran menyebabkan masalah kesihatan di kalangan manusia dan pencemaran disebabkan ianya toksik dan tegar. Menariknya, kajian lepas menyatakan bahawa najis lembu boleh menguraikan pencemaran. Oleh itu, haiwan yang memakan makanan hariannya yang tercemar dengan halogen, akan mempengaruhi mikroflora di dalam saluran penghadamannya. Spesis bakteria daripada najis lembu yang berupaya menggunakan 2,2DCP belum pernah di laporkan. Oleh itu, tujuan kajian ini adalah untuk mengasingkan, mengenalpasti dan mencirikan dehalogenase dari bakteria najis lembu. Empat jenis bakteria telah diasingkan seperti SN1, SN2, SN3 dan SN4. Strain SN1 mempamerkan pertumbuhan pantas dalam media minima cecair 20 mM 2,2DCP, dan ia digunakan untuk eksperimen seterusnya seperti pertumbuhan dalam kepekatan 2,2DCP yang berbeza, kromatografi cecair berprestasi tinggi (HPLC), Biolog GENIII, analisis 16S rRNA, pencirian enzim tulen, analisis kinetik, kromatografi cecair-spektrometri jisim (LC-MS/MS) dan amplifikasi gen dehalogenase. Pertumbuhan strain SN1 dalam variasi kepekatan bahan (10 mM, 20 mM, 30 mM and 40 mM) telah dikaji. Kajian ini mendapati bakteria tumbuh dengan baik dalam 20 mM 2,2DCP dengan perlepasan ion klorida yang tertinggi (39.5 μmolCl^-/mL) disamping menunjukkan masa berganda yang singkat iaitu 3.85 jam. Pengunaan 2,2DCP juga dibuktikan dengan pengesanan pengurangan 20 mM 2,2DCP di dalam media pertumbuhan yang mengandungi strain SN1 yang diukur dengan HPLC. Hasil menunjukkan penggunaan 2,2DCP adalah sebanyak 98.6 % di dalam media pertumbuhan. Pengenalpastian spesis melalui sistem Biolog GENIII dan analisis 16S rRNA telah dijalankan dan strain SN1 dikenalpasti sebagai *Bacillus cereus*. Kajian selanjutnya terhadap enzim dehalogenase adalah dengan menggunakan enzim tulen dari *Bacillus cereus* SN1. Berat molekul enzim yang telah ditulenkan adalah 25 kDa melalui SDS-PAGE. Ciri-ciri enzim menunjukkan ia optima pada pH 6 dan 30 °C. Ia juga mempunyai nilai K_m yang rendah iaitu 0.2 mM. Peptida dehalogenase dikenalpasti melalui LC-MS/MS dengan liputan jujukan sebanyak 18 % dengan *Bacillus cereus* (strain 03BB102). Selain itu, primer dehalogenase Kumpulan I dan Kumpulan II digunakan untuk amplifikasi dehalogenase dan jalur hanya muncul pada Kumpulan I. Gen dehalogenase yang telah diamplifikasi dinamakan "DehSN1" dan ia tergolong dalam dehalogenase Kumpulan I memandangkan ia mempunyai persamaan 75 % dengan dehalogenase Kumpulan I (DehE).5 residu telah dikenalpasti sebagai Asn33, Tyr117, Cys42, Ala120 dan Asp136. Sebagai kesimpulannya, ini adalah laporan yang pertama mengenai *Bacillus* sp. yang diasingkan daripada najis lembu yang mampu menggunakan 2,2DCP. Oleh itu, kajian selanjutnya perlu dipertimbangkan secara khusus tentang kebolehannya untuk menguraikan asid haloalkanoik berlainan jenis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2,2DCP	-	2,2-dichloropropionic acid
BLAST	-	Basic Local Alignment Search Tool
et al.	-	and friends
PCR	-	Polymerase Chain Reaction
sp.	-	Species
bp	-	Base pairs
rpm	-	Renovation per minute
nm	-	nanometre

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The ever growing human population has resulted in the mass production of a myriad of halogenated compounds pertinently used in pharmaceutical and agricultural management (herbicides, fungicides, insecticides) as well as intermediates in organic solvents etc. However, the large presence of such substances in the environment may lead to undesirable consequences pertaining to health problems in humans and pollution due to their toxicity and recalcitrance, respectively (Pee and Unversucht, 2003). This problem has been further exacerbated by the fact that such halogenated chemicals i.e. 2,2-dichloropropionic acid (2,2DCP) has been extensively use as the prevailing active components in the production of herbicides (Dalapon) for weed management in agricultural practices. Pertinently, it has been indicated in literature that the heavy reliance on the chemical means to control weed in agricultural sectors may cause long term adverse effects to our existing fragile ecosystem (Reyes-Franco *et al.*, 2006; Srivinasan *et al.*, 2009). Therefore, alternative methods that would overcome such disadvantages need to be suggested. In this context, the enzymatic degradation of 2,2-dichloropropionic acid using dehalogenases may prove to be a promising alternative means to the prevailing chemical route to control weed in agriculture. The continuing search for novel biocatalysts is a matter of great interest as such only a fraction of the microbial species present in nature has been reported, encompassing only 0.2 to 0.6 % bacteria, 5 % fungi and a maximum of 24 % of known

algae (Wubbolts *et al*., 2000). Therefore, such available wealth of undiscovered microorganisms out there has led in concerted efforts in search of novel microorganism with "unnatural" abilities to synthesize compounds that are not normally produced by "normal metabolic" routes (Adamczak and Krishna, 2004; Gomes and Steiner, 2004).

Hence, the hunt for such biocatalysts specific for a particular application remains a challenge. To date, two common approaches are used to select microorganisms suited for a particular task. The first approach involves screening for novel microorganisms by taking advantage of the largely unexplored biodiversity; and the second option is screening for new activities amongst the existing microorganisms. These approaches may also include works in the improvement of catalytic properties of known enzymes through protein engineering, either through molecular biology or direct protein modification (Hamid *et al.*, 2011). Moreover, chances of finding enzymes with extraordinary properties is fairly reasonable considering numerous reports pertaining to microbial isolation studies (Jing *et al.*, 2008a; Roslan *et al.*, 2011; Abel *et al.*, 2012b; Bagherbaigi *et al.*, 2013; Niknam *et al.*, 2014). Such findings have since open doors on explorations of different properties of dehalogenases from countless microorganisms capable of hydrolyzing 2,2-dichloropropionic acid (Jing *et al.*, 2008a; Roslan *et al.*, 2011; Abel *et al.*, 2012b; Bagherbaigi *et al.*, 2013; Niknam *et al.*, 2014).

Over the years, microorganisms displaying interesting properties have been isolated from extreme environments *viz.* high salt concentration or temperature as well as in polluted water bodies (Edbieb *et al*., 2016; Hamid *et al*., 2010a). Interestingly, several studies have indicated that the dung of a cow may prove to be a rich source of pollutant degrading microflora bacteria (Joshi and Pandey, 2011; Geetha and Fulekar, 2008; Randhawa and Kullar, 2011). In effect, previous studies have shown that the major portion in the daily diet of ruminant animals mostly consisted of forage (Hendriks, 2012; Camboim *et al.*, 2012a, 2012b) which may contain naturally occurring halogenated compounds (Siuda and De Bernardis, 1973; Fuge, 1988). Hence, it could not be ruled out that such animals feeding on a daily diet of halogen contaminated forage may inadvertently influence the microflora in their digestive tract.

Pertinently, recent studies have supported the hypothesis that the microflora in the digestive tract of cattle may develop special adaptations to counteract the effects of halogenated chemicals in their diet (Singh and Fulekar, 2007; Hendriks, 2012; Camboim *et al.*, 2012a, 2012b; Arunkumar and Chandrasekaran, 2013).

Reports by Hendriks (2012) and Camboim *et al*. (2012a) indicated that bacteria found in the digestive tract of herbivores which fed on naturally occurring halogenated compound monofluoroacete in gifblaar (*Dichapetalum cymosum*) were effective in treating cows affected by the toxicity of monofluoroactetae (Hendriks, 2012; Camboim *et al.*, 2012a, 2012b). A previous work by Singh and Fulekar (2007) described several microorganisms namely *Pseudomonas* sp., *Streptococcus* sp., *Sarcina* sp., *Escherichia coli*, *Penicillium* sp., *Rhizopus* sp., *Mucor* sp. and *Nocardia* sp. isolated from the dung of cow were effective in degrading phenol. Correspondingly, endosulfan (Arunkumar and Chandrasekaran, 2013) and palm oil degrading microorganisms (Ojonoma and Udeme, 2014) isolated from similar sources have been reported. Therefore, this study will be focussing in the ability of microorganisms that will be isolated from cow dung to utilize 2,2-dichloropropionic acid as a carbon source and to characterise further the isolate.

1.2 Problem Statement

Halogenated compounds are commonly used as herbicides, pesticides, insecticides and antibiotics. The extensive use of halogenated compounds led to harmful effects on human and natural environment because of their toxicity and difficult to be degraded. Microorganisms cause natural degradation of the halogenated compounds to become less toxic compounds. Nevertheless, natural bioremediation is a slow process and needs to be enhanced by the action of the potential microorganisms. Example of naturally occurring halogenated compound, monofluoroacete found in the plant, gifblaar (*Dichapetalum cymosum*) in the South African which resulted in acute death of ruminants, mostly cattle (Minnaar *et al*., 2000). The young leaves of this plant

are most toxic during spring and autumn. The lethal oral dose for cattle is 0.15 mg (kg bodyweight)$^{-1}$. Therefore, the bacteria were used to reduce toxicity of monofluoroactetae (Hendriks, 2012). In addition, cow dung has potential bacteria that can degrade pollutants especially pesticides (Randhawa and Kullar, 2011). Current research reported that cow dung have the great influences to degrade crude oil and pesticides (Ikuesan *et al*., 2015; Khan and Manchur, 2015).

Considering the abundance of forage fed cattle in the rural areas of Peninsular Malaysia, specific studies on isolating dehalogenase producing microbes from cow dung merits certain consideration. This present investigation attempted to isolate, characterize and identify such microorganism from the dung of free range cow capable of utilizing 2,2-dichloropropionic acid as sole source of carbon, consequently analyse and identify its dehalogenase. It is pertinent to highlight here that isolation studies on bacterial species from cow dung able to utilize 2,2-dichloropropionic acid is yet to be reported in Malaysia.

1.3 Objectives

i. To isolate bacteria that capable to degrade 2,2-dichloropropionic acid from cow dung.

ii. To identify and characterize the isolated bacteria based on growth experiment on different concentration of 2,2-dichloropropionic acid (2,2DCP), Biolog GEN III MicroPlate and molecular analysis.

iii. To purify and characterize the dehalogenase by enzyme assay analysis, possibly to identify new kind dehalogenase.

1.4 Scope of Study

The work involves isolation of the bacteria from cow dung followed by bacterial identification and growth establishment on 2,2-dichloropropionic acid as a carbon source. The best colony grow on 2,2-dichloropropionic acid will be further analysed by the preparation of the crude extract from the cells grow on 2,2-dichloropropionic acid and assay the dehalogenase. It is hope that new kind of dehalogenases will be isolated from this study.

1.5 Significances and Original Contributions of This Study

A few studies of using cow dung to degrade some of pollutants such as benzene, phenol and pesticide were reported by Singh and Fulekar (2010), Singh and Fulekar (2009) and Geetha and Fulekar (2008) respectively. Yet, no research of using cow dung to degrade halogenated compound or 2,2-dichloropropionic acid was reported. Therefore, this research was focused on identifying the bacteria from cow dung that can degrade 2,2-dichloropropionic acid. This study also provides new knowledge on the degradation of α-haloalkanoic acids through the use of an exceptional dehalogenase producing bacteria. The properties of the α-haloalkanoic acid dehalogenase isolated in this study and 2,2-dichloropropionic acid product were evaluated which were important to recognize their catalytic properties. Amplification and analysis of α-haloalkanoic acid dehalogenase gene sequence was carried out from the isolated bacteria using Group I and Group II primers proposed by Hill *et al*. (1999). Thus, the group of dehalogenases was identified and the conserved gene sequence were known that was important for identifying key residues that responsible for dehalogenating capability. The information here, may be valuable in the future for precise prediction of the interaction between the active sites residues of the α-dehalogenase isolated from this novel bacteria with 2,2-dichloropropionic acid.

This research will contribute to safe the cost of current bioremediation method by using the cow dung which is readily available. This research will reveal ability of halogenated compound to be degraded enzymatically, and to reduce its toxicity. The cow dung is highly potential to bio-remediate many pollutants because it contains various beneficial microorganisms to degrade pollutants. Therefore, the adverse effect of pollutants on environment can be reduced and safe the future.

REFERENCES

Aanniz, T., Ouadghiri, M., Melloul, M., Swings, J., Elfahime, E., Ibijbijen, J., Ismaili, M. and Amar, M. (2015). Thermophilic Bacteria in Moroccan Hot Springs, Salt Marshes and Desert Soils. *Brazilian Journal of Microbiology*. 46(2), 443-453.

Abel, E., Ibrahim, N. and Huyop, F. (2012a). Identification of *Serratia marcescens* SE1 and Determination of Its Herbicide 2,2-Dichloropropionate (2,2-DCP) Degradation Potential. *Malaysian Journal of Microbiology*. 8(4), 259-265.

Abel, E., Pakingking, R. V., Pagador, G., Wint, M. T. and Huyop, F. (2012b). Characteristics of Dehalogenase from Bacteria Isolated from the Gut of Pond-Reared Rohu (*Labeo rohita*) Juveniles in Myanmar. *Advances in Bioscience and Biotechnology* . 39(2012), 353-361.

Adamczak, M. and Krishna, S. H. (2004). Strategies for Improving Enzymes for Efficient Biocatalysis. *Food Technology and Biotechnology*. 42 (4), 251-264.

Adams, G. O., Tawari-Fufeyin, P. and Ehinomen, I. (2014). Laboratory Scale Bioremediation of Soils From Automobile Mechanic Workshops Using Cow Dung. *Journal of Applied and Environmental Microbiology* . 2, 128-134.

Agrawal, N. and Shahi, S. K. (2015). An Environmental Cleanup Strategy-Microbial Transformation of Xenobiotic Compounds. *International Journal of Current Microbiology and Applied Sciences* .4(4), 429-461.

Allison, N. (1981). *Bacterial Degradation of Halogenated Aliphatic Acids*. PhD. Thesis, Trent Polytechnic, Nottingham, United Kingdom.

Allison, N., Skinner, A.J. and Cooper, R.A. (1983) The Dehalogenases of a 2,2-Dichloropropionate Degrading Bacterium. *Journal of General Microbiology*. 129, 1283-1293.

Almaki, J. H., Nasiri, R., Soon, W. T. and Huyop, F. Z. (2016). Identification of Novel Bacterial Species Capable of Degrading Dalapon Using 16s rRNA Sequencing. *Jurnal Teknologi*. 78(6), 77-82.

Amini, S., Zulkifly, A. H., Wen-Yong, W. and Huyop, F. (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.

Alomar , D., Hamid, A. A. A. , Khosrowabadi , E., Gicana, R. G., Lamis, R. J., Huyop, F. and Hamid, T. H. T. A. (2014). Molecular Characterization of Monochloroacetate-Degrading *Arthrobacter* sp. Strain D2 Isolated from Universiti Teknologi Malaysia Agricultural Area. *Bioremediation Journal* .18 (1), 12-19.

Arunkumar, S. and Chandrasekaran, R. (2013). Bioremediation of Endosulfan in Surface Soil Treatment Using Microbial Consortia. *Global Research Analysis*. 2(8),151-153.

Bachas-Daunert, P.G., Sellers, Z.P. and Wei, Y. (2009). Detection of Halogenated Organic Compounds Using Immobilized Thermophilic Dehalogenase. Analytical and Bioanalytical Chemistry. 395(4), 1173-1178.

Bagherbaigi, S., Gicana, R. G., Lamis, R. J., Nemati, M. and Huyop, F. (2013). Characterisation of *Arthrobacter* sp. S1 That Can Degrade α and β-Haloalkanoic Acids Isolated from Contaminated Soil. *Annals of Microbiology* 63, 1363–1369.

Bahadure, S., Kalia, R. and Chavan, R. (2013). Comparative Study of Bioremediation of Hydrocarbon Fuels. *International Journal of Biotechnology and Bioengineering Research* . 4 (7) ,677-686.

Batumalaie, K., Khalili, E., Mahat, N. A., Huyop, F. and Wahab R. A. (2017). A Statistical Approach for Optimizing the Protocol for Overexpressing Lipase KV1 in *Escherichia coli*: Purification and Characterization. *Biotechnology and Biotechnological Equipment*. 32(1), 69-87.

Bakermans, C., Tsapin, A. I., Souza-Egipsy, V., Gilichinsky, D. A. and Nealson, K. H. (2003). Reproduction and Metabolism at −10°C of Bacteria Isolated from Siberian Permafrost. *Environmental Microbiology*. 5, 321–326.

Bakermans, C., and Nealson, K.H. (2004). Relationship of Critical Temperature to Macromolecular Synthesis and Growth Yield in *Psychrobacter cryopegella*. *Journal of Bacteriology*. 186, 2340–2345.

Barth, P. T., Bolton, L. and Thomson, J. C. (1992). Cloning and Partial Sequencing of an Operon Encoding Two *Pseudomonas putida* Haloalkanoate Dehalogenases of Opposite Stereospecificity. *Journal of Bacteriology* . 174, 2612-2619.

Berger, F., Morellet, N., Menu, F. and Potier, P. (1996), Cold Shock and Cold Acclimation Proteins in the Psychrotrophic Bacterium *Arthrobacter globiformis* SI55. *Journal of Bacteriology* .178, 2999–3007.

Bergman, J. G. and Sanik, J. Jr. (1957). Determination of Trace Amounts of Chlorine in Naphtha. *Analytical Chemistry*. 29, 241-243.

Berry, E.K.M., Allison,N., Skinner,A.J. and Cooper,R.A. (1979). Degradation of the Selective Herbicide 2,2-Dichloropropionate (Dalapon) by a Soil Bacterium. *Journal of General Microbiology*. 110, 39-45.

Boricha, H. and Fulekar, M. (2009). *Pseudomonas plecoglossicida* As a Novel Organism for the Bioremediation of Cypermethrin. *Biology and Medicine*. 1(4),1-10.

Bradford, M. (1976). A Rapid and Sensitive Method for the Quantification of Micrograms Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*. 72, 248-254.

Brokamp A., Happe, B. and Schmidt, F.R.J. (1997). Cloning and Nucleotide Sequence of a D,L-Haloalkanoic Acid Dehalogenase Encoding Gene from *Alcaligenes xylooxidans* sp. *denitrificans* ABIV. *Biodegradation*. 7, 383-396.

Cairns, S. S., Cornish, A. and Cooper, R. A. (1996). Cloning, Sequencing and Expression in *Escherichia coli* of Two *Rhizobium* sp. Genes Encoding Haloalkanoate Dehalogenases of Opposite Stereospecificity. *European Journal of Biochemistry*. 253,744-749.

Camboim, E. K. A., Tadra-Sfeir, M. Z., Souza, E. M., Pedrosa, F. O., Andrade, P. P., McSweeney, C. S., Riet-Correa, F. and Melo, M. A. (2012a). Defluorination of Sodium Fluoroacetate by Bacteria from Soil and Plants in Brazil. *The Scientific World Journal*. 2012,1-5.

Camboim, E. K. A., Almeida, A. P., Tadra-Sfeir, M. Z., Junior, F. G., Andrade, P. P., McSweeney, C. S., Melo, M. A. and Riet-Correa, F. (2012b). Isolation and Identification of Sodium Fluoroacetate Degrading Bacteria from Caprine Rumen in Brazil. *The Scientific World Journal*. 2012, 1-6.

Chintalapati, S., Kiran, M.D. and Shivaji, S. (2004). Role of Membrane Lipid Fatty Acids in Cold Adaptation. *Cellular and Molecular Biology* . 50, 631–642.

Chojniak, J., Jałowiecki, Ł., Dorgeloh, E., Hegedusova, B., Ejhed, H., Magnér, J., and Płaza, G. (2015). Application of the BIOLOG System for Characterization of *Serratia marcescens ss marcescens* Isolated from Onsite Wastewater Technology (OSWT). *Acta Biochimica Polonica*. 62(4), 709-805.

Corpet, F. (1988). Multiple Sequence Alignment with Hierarchical Clustering. *Nucleic Acids Research*. 16(22), 10881-10890.

Dalmaso, G.Z.L., Ferreira, D. and Vermelho, A.B. (2015). Marine Extremophiles: A Source of Hydrolases for Biotechnological Applications. *Marine drugs* . 2015(13), 1925-1965.

Diez, A., Prieto, M.I., Alvarez, M.J., Bautista, J.M., Puyet, A. and Pertierra, G. (1996). Purification and Properties of a High-Affinity L-2-Haloacid Dehalogenase from *Azotabacter* sp. Strain RC26. *Letters in Applied Microbiology*. 23, 279–282.

Dobranic, J.K. and Zak, J.C. (1999). A Microtiter Plate Procedure for Evaluating Fungal Functional Diversity. *Mycologia* .91, 756–765.

Dominguez, K. and Ward, W.S. (2009). A Novel Nuclease Activity that is Activated by Ca^{2+} Chelated to EGTA. *Systems Biology in Reproductive Medicine*. 55 (5–6), 193–99.

Donnelly, C. and Murphy, C.D. (2009) Purification and Properties of Fluoroacetate Dehalogenase from *Pseudomonas fluorescens* DSM 8341. *Biotechnology Letters*. 31, 245-250.

Drancourt, M. Berger, P. and Raoult, D. (2004) Systematic 16S rRNA Gene Sequencing of a Typical Clinical Isolates Identified 27 New Bacterial Species Associated with Humans. *Journal Clinical Microbiology*. 42, 2197–2202.

Drienovska, I., Chovancova, E., Koudelakova, T., Damborsky, J. and Chaloupkova, R. (2012). Biochemical Characterization of a Novel Haloalkane Dehalogenase from a Cold-Adapted Bacterium. *Applied and Environmental Microbiology*. 78,4995-4998.

Eichler, J. (2003). Facing Extremes: Archaeal Surface-Layer (Glyco-) Proteins. *Microbiology*. 149, 3347–3351

Edbeib, M. F. A., Wahab, R. A. and Huyop, F. (2016). Characterization of an α-Haloalkanoic Acid–Degrading *Pseudomonas aeruginosa* MX1 Isolated from Contaminated Seawater. *Bioremediation Journal*. 20(2), 89-97.

Edbeib, M. F., Wahab, R. A., and Huyop, F. (2017). Isolation and Characterization of a Novel 2,2-DCP-Degrading *Achromobacter* sp. MX2 Isolated from Contaminated Seawater of Desaru Beach, Southern Malaysia. *Journal of Engineering and Applied Sciences*. *12*(17), 4306-4309.

Fanali, S., Haddad, P.R., Poole, C. and Lloyd, D.K. (2013). *Liquid Chromatograph Applications.* United Kingdom: Elsevier.

Fetzner, S.R. and Lingens, F. (1994). Bacterial Dehalogenases. *Microbology Reviews*. 58, 641-685.

Foy, C. L. (1975). *The Chlorinated Aliphatic Acids.*In P.C. Kearney and D.D. Kaufman (Ed.) *Herbicides- Chemistry, Degradation and Mode of Action.* (pp. 399-452). New York: Dekker.

Friedman, T. E. and Haugen, G. H. (1943). Pyruvic Acid II. Determination of Keto-Acids in Blood and Urine. *Journal of Biological Chemistry* .147, 415-422.

Fuge, R. (1988). Sources of Halogens in the Environment, Influences on Human and Animal Health. *Environmental Geochemistry and Health*. 10(2), 51-61.

GE Healthcare Bio-Sciences AB (2016). *Ion Exchange Chromatography Principles and Methods.* [Brochure]. Sweden: General Electric Company

Geetha, M. and Fulekar, M. H. (2008). Bioremediation of Pesticides in Surface Soil Treatment Unit Using Microbial Consortia. *African Journal of Environmental Science and Technology*. 2(2), 036-045.

Gilbert, J.A., Hill, P.J., Dodd, C.E. and Laybourn-Parry, J. (2004) . Demonstration of Antifreeze Protein Activity in Antarctic Lake Bacteria. *Microbiology*. 150, 171–180.

Gilbert, J.A., Davies, P.L. and Laybourn-Parry, J. (2005). A Hyperactive, Ca^{2+}-Dependent Antifreeze Protein in an Antarctic Bacterium. *FEMS Microbiology Letter*. 245: 67–72.

Godinho, L. M. and de Sá-Nogueira, I. (2011). Characterization and Regulation of a Bacterial Sugar Phosphatase of the Haloalkanoate Dehalogenase Superfamily, AraL, from *Bacillus subtilis*. *The Febs Journal* . 278(14), 2511–2524.

Goldman, P. (1972). Enzymology of Carbon-Halogen Bonds. *Degradation of Synthetic Organic Molecules in the Biosphere*. 147-165.

Gomes, J. and Steiner, W. (2004). Extremophiles and Extremozymes. *Food Technology and Biotechnology*. 42 (4), 223-235.

Gyorkos, J., Denomme, M.A., Leece, B., Homonko, K., Valli, V. and Safe, S.F. (1985). Reconstituted Halogenated Hydrocarbon Pesticide and Pollutant Mixtures Found in Human Tissues: Effects on the Immature Male Wistar Rat After Short-Term Exposure. *Canadian Journal of Physiology and Pharmacology*. 63(1), 36–43.

Hadeed, H. M., Shweash, M. and Huyop, F. (2014). Isolation and Characterization of Putative *Burkholderia* sp. HY1 from Mud That Able to Utilize 2,2-Dichloropropionate. *European International Journal of Science and Technology*. 3(9), 222-236.

Haggblom, M.M., Fennell, D.E., Ahn, Y.B., Ravit, B. and Kerkhof, L.J. (2006). *Anaerobic Dehalogenation of Halogenated Organic Compounds: Novel Strategies for Bioremediation of Contaminated Sediments*. *Soil and Water Pollution Monitoring, Protection and Remediation*. Netherlands: Springer Netherlands

Hamid, A. A. A., Rolando, S. H., Pakingking Jr., V. and Huyop, F. (2010a). Identification of *Pseudomonas* sp. Strain S3 Based on Small Subunit Ribosomal RNA Gene Sequences. *Biotechnology*. 9 (1), 33-40.

Hamid, A. A. A., Hamdan, S., Ariffin, S. H. Z. and Huyop, F. (2010b). 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.

Hamid, T. H. T. A., Hamid, A. A. A., Zulkifly, A. H., Hamdan, S., Ariffin, S. H. Z. and Huyop, F. (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.

Hamid, A. A. A., Wong, E. L., Joyce-Tan, K. H., Shamsir, M. S., Hamid, T. H. T. A., and Huyop, F. (2013). Molecular Modelling and Functional Studies of the Non-Stereospecific α-Haloalkanoic Acid Dehalogenase (DehE) from *Rhizobium* sp. RC1 and Its Association with 3-Chloropropionic Acid (β-Chlorinated Aliphatic Acid). *Biotechnology and Biotechnological Equipment*. 27(2), 3725-3736.

Hamid, A. A. A., Hamid, T. A. T. H., Wahab, R. A., Omar, M. S. S. and Huyop, F.(2015). An S188V Mutation Alters Substrate Specificity of Non-Stereospecific α-Haloalkanoic Acid Dehalogenase E (DehE). *PLoS ONE*. 10(3),1-21.

Hardman, D.J. (1991). Biotransformation of Halogenated Compounds. *Critical Reviews in Biotechnology*. 11: 1-40.

Hareland, W. A., Crawford, R. L., Chapman, P. J. and Dagley, S. (1975). Metabolic Function and Properties of a 4-Hydroxyphenylacetic Acid 1-Hydroxylase from *Pseudomonas acidovorans*. *Journal of Bacteriology*. 121, 272-285.

Hasan, A. K. M. Q.,Takada, H., Koshikawa, H., Liu, J-Q, Kurihara, T., Esaki, N. and Soda, K. (1994). Two Kinds of 2-Halo Acid Dehalogenases from P*seudomonas* sp. YL Induced by 2-Chloroacrylate and 2-Chloropropionate. *Bioscience, Biotechnology, and Biochemistry*. 5(9), 1599-1602.

Hasan, K., Fortova, A., Koudelakova,T., Chaloupkova, R., Ishitsuka, M., Nagata,Y., Damborsky, J. and Prokop, Z. (2011). Biochemical Characteristics of the Novel Haloalkane Dehalogenase DatA, Isolated from the Plant Pathogen *Agrobacterium tumefaciens* C58. *Applied and Environmental Microbiology*. 77(5), 1881–1884.

Helgason, E., Økstad, O.A., Caugant, D.A., Johansen, H.A., Fouet, A., Mock, M., Hegna, I. and Kolstø, A.B. (2000) *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis* - One Species on the Basis of Genetic Evidence. *Applied and Environmental Microbiology*. 66, 2627–2630.

Hendriks, C. B. S. (2012). *The Role of Endophytes in the Metabolism of Fluorinated Compounds in the South African Dichapetalaceae*. M.Sc. Thesis. University of Pretoria, Pretoria.

Hill, K. E., Marchesi, J. R. and Weightman, A. J. (1999). Investigation of Two Evolutionarily Unrelated Halocarboxylic Acid Dehalogenase Gene Families. *Journal of Bacteriology*. 181(8), 2535–2547.

Horikoshi, K. (1999). Alkaliphiles: Some Applications of Their Products for Biotechnology. *Microbiology and Molecular Biology Reviews* . 63,735–750.

Huang, J.Y., Xin, Y.J. and Zhang, W. (2010) Isolation and Characterization of Haloacid Degrading Bacteria from Marine Sponge *Hymeniacidon perleve. Fisheries Science.* 29, 166–171.

Huang, J., Xin, Y., Cao, X. and Zhang, W. (2011). Phylogenetic Diversity and Characterization of 2-Haloacid Degrading Bacteria from the Marine Sponge *Hymeniacidon perlevis*. *World Journal of Microbiology and Biotechnology*. 27, 1787–1794.

Hurst, L.D. and Merchant, A.R. (2001). High Guanine-Cytosine Content is not an Adaptation to High Temperature: A Comparative Analysis Amongst Prokaryotes. *Proceedings of the Royal Society B: Biological Sciences*. 268, 493–497.

Huyop, F., Yusn, .Y., Ismail, M., Wahab, R. A. and Cooper, R. A .(2004). Overexpression and Characterisation of Non-Stereospecific Haloacid Dehalogenase E (DehE) of *Rhizobium* sp. *Asia-Pacific Journal of Molecular Biology and Biotechnology*. 12(1&2), 15-20.

Huyop, F. and Cooper, R. (2012). Degradation of Millimolar Concentration of the Herbicide Dalapon (2,2-Dichloropropionic Acid) by *Rhizobium* sp. Isolated from Soil. *Biotechnology and Biotechnological Equipment*. 26(4), 3106-3112.

Ibiene, A. A., Orji, F. A., Ezidi, C. O. and Ngwobia, C. L. (2011). Bioremediation of Hydrocarbon Contaminated Soil in the Niger Delta Using Spent Mushroom Compost and Other Organic Wastes. *Nigerian Journal of Agriculture, Food and Environment*. 7(3), 1-7.

Ikuesan, F. A., Boboye, B. E. and Adetuyi, F. C. (2015). Cow Dung as Inoculum Carrier for the Degradation of Crude Oil. *Sky Journal of Microbiology Research*. 3(4), 47-54.

Illanes, A. (Ed.). (2008). *Enzyme Biocatalysis: Principles and Applications*. Springer Science and Business Media.

Isah, A. A., Mahat, N. A., Jamalis, J., Attan, N., Zakaria, I. I., Huyop, F. and Wahab, R. A. (2017). Synthesis of Geranyl Propionate in a Solvent-Free Medium Using *Rhizomucor miehei* Lipase Covalently Immobilized on Chitosan-Graphene Oxide Beads. *Preparative Biochemistry and Biotechnology*. 47, 199-210.

Ishikawa, Y. and Mihara, M. (2013). Changes in Surviving Microorganism in Cow Manure with Adding Lime Nitrogen. *International Journal of Environmental and Rural Development*. 2013 (4-2), 33-37.

Ismail, S. N., Taha, A. M., Jing, N. H., Wahab, R. A., Hamid, A. A., Pakingking Jr., R. V. and Huyop, F. (2008). Biodegradation of Monochloroacetic Acid (MCA) by a Presumptive *Pseudomonas* sp. Strain R1 Bacterium Isolated from Malaysian Paddy (Rice) Field. *Biotechnology* . 7(3), 481-486.

Jesenská, A., Pavlová, M., Strouhal, M., Chaloupková, R., Těšínská, I., Monincová, M., Prokop, Z., Bartoš, M., Pavlík, I., Rychlík, I., Möbius, P., Nagata,Y. and Damborský, J. (2005). Cloning, Biochemical Properties, and Distribution of Mycobacterial Haloalkane Dehalogenases. *Applied and Environmental Microbiology*. *71*(11), 6736–6745.

Jia, Z. and Davies, P.L. (2002). Antifreeze Proteins: An Unusual Receptor–Ligand Interaction. *Trends in Biochemical Sciences* .27, 101–106

Jing, N.H. (2007). *Isolation of Local Bacterial Capable of Degrading Halogenated Compounds and Analysis of Putative Haloacid Permease Gene*. Degree of Master, Universiti Teknologi Malaysia, Skudai.

Jing, N. H. and Huyop, F. (2007a). Dehalogenation of Chlorinated Aliphatic Acid by *Rhodococcus* sp. *Asia Pacific Journal of Molecular Biology and Biotechnology*. 15, 147-151.

Jing, N. H. and Huyop, F. (2007b). Identification of a *Methylobacterium* sp. Strain HN2006B by 16S rRNA Gene Analysis with the Ability to Degrade the Herbicide Dalapon. *Borneo Science* (*The Journal of Science and Technology)*. 20, 1-7.

Jing, N. H. and Huyop, F. (2008). Degradation of 3-Chloropropionate (3CP) by *Rhodococcus* sp. Strain HJ1. *Borneo Science* (*The Journal of Science and Technology)*. 22,30-38.

Jing, N. H., Wahab, R. A., Taha, A. M., Rashid, N. A. A. and Huyop, F. (2008a). A further Characterization of 3-Chloropropionic Acid Dehalogenase from *Rhodococcus* sp. HJ1. *Research Journal of Microbiology*. 3(6),482-488.

Jing, N. H., Taha, A. M., Pakingking Jr., R. V., Wahab, R. A. and Huyop, F. (2008b). Dehalogenase from *Methylobacterium* sp. HJ1 Induced by the Herbicide 2,2-Dichloropropionate (Dalapon). *African Journal of Microbiology Research*. 2, 32-36.

Jing, N. H., Sulaiman, F. H., Wahab, R. A., Pakingking Jr., R. V., Rashid, N. A. A. and Huyop, F. (2008c). Purification and Properties of a Non-Stereospecific Dehalogenase Enzyme E (DehE) from *Methylobacterium* sp. HJ1. *African Journal of Microbiology Research* . 2,187-191.

Jing, N. H., Wahab, R. A., Hamdan, S. and Huyop, F. (2010). Cloning and DNA Sequence Analysis of the Haloalkanoic Permease Uptake Gene from *Rhizobium* sp. RC1. *Biotechnology* . 9(3), 319-325.

Johns, G.C. and Somero, G.N. (2004). Evolutionary Convergence in Adaptation of Proteins to Temperature: A4-Lactate Dehydrogenases of Pacific Damselfishes (*Chromis* spp.). *Molecular Biology and Evolution*. 21,314–320.

Jones, D. H. A., Barth, P. T., Byrom, D. and Thomas, C. M .(1992). Nucleotide Sequence of the Structural Gene Encoding a 2-Haloalkanoic Acid Dehalogenase of *Pseudomonas putida* Strain AJ1 and Purification of the Encoded Protein. *Journal of general microbiology*. 138, 675-683.

Joshi, G. K., Kumar, S. and Sharma, V. (2007). Production of Moderately Halotolerant, SDS Stable Alkaline Protease from *Bacillus cereus* MTCC 6840 Isolated from Lake Nainital, Uttaranchal State, India. *Brazilian Journal of Microbiology*. 38(4).

Joshi, P. A. and Pandey, G. B. (2011). Screening of Petroleum Degrading Bacteria from Cow Dung. *Research Journal of Agricultural Sciences*. 2(1), 69-71.

Kamboh, A.A., Rajput, N., Rajput, I.R., Khaskheli, M. and Khaskheli, G.B.. (2009). Biochemical Properties of Bacterial Contaminants Isolated from Livestock Vaccines. *Pakistan Journal of Nutrition*. 8, 578-581.

Kawasaki, H., Tsuda, K., Matsushita, I. and Tonomura, K. (1992). Lack of Homology between Two Haloacetate Dehalogenase Genes Encoded on a Plasmid from *Moraxella species* Strain B. *Journal of General Microbiology*. 138,1317-1323.

Kawasaki, H., Toyama, T., Maeda, T., Nishino, H. and Tonomura, K. (1994). Cloning and Sequence Analysis of a Plasmid Encoded 2-Haloacid Dehalogenase Gene from *Pseudomonas putida* No. 109. *Bioscience, Biotechnology, and Biochemistry* . 58, 160-163.

Kearney, P. C., Kaufman, D. D. and Beall, M. L. (1964). Enzymatic Dehalogenation of 2,2- Dichloropropionate. *Biochemical and Biophysical Research Communications* . 14, 29-33.

Khan, S. and Manchur, A. (2015). Activated Cow-Dung Slurry as a Tool of Pesticides Bioremediation. *Journal of Microbiology and Biotechnology Research*. 5(2), 12-17.

Khosrowabadi, E. and Huyop, F. (2014). Screening and Characterization of Several 2,2-Dicholoropropionic Acid–Degrading Bacteria Isolated from Marine Sediment of Danga Bay and East Coast of Singapore Island. *Bioremediation Journal* .18(1), 20-27.

Kornberg, H. L. and Morris, J. G. (1965). The Utilization of Glycollate by *Micrococcus denitrifcans*: The Beta-Hydroxyaspartate Pathway. *Biochemical Journal*. 95,577-586.

Kranz, R., Hafer, K. W., and Richards, E. (2006). *Identifying Unknown Bacteria Using Biochemical and Molecular Methods*. Washington: Washington University in Saint Louis.

Kumar, L., Awasthi, G. and Singh, B. (2011). Extremophiles: A Novel Source of Industrially Important Enzymes. *Biotechnology*. 10, 121-135.

Lee, S. T., Cook, D. Pister, J. A., Alan J. G., Colegate, S. M., Riet-Correa, F. and Taylor, C. M. (2014). Monofluoroacetate-Containing Plants That are Potentially to Livestock. *Journal of Agricultural and Food Chemistry*. 62, 7345-7354.

Leigh, J. A., Skinner, A. J. and Cooper, R. A. (1986). Isolation and Partial Characterisation of Dehalogenase - Deficient Mutants of a *Rhizobium sp*. *FEMS Microbiology Letters*. 36, 163-166.

Leigh, J. A., Skinner, A. J. and Cooper, R. A. (1988). Partial Purification, Stereospecificity and Stoichiometry of Three Dehalogenases from a *Rhizobium species*. *FEMS Microbiology Letters*. 49, 353-356.

Li, A. and Shao, Z. (2014). Biochemical Characterization of a Haloalkane Dehalogenase DadB from *Alcanivorax dieselolei* B-5. *Plos One*. 9(2), 1-9.

Lim, J., Thomas, T. and Cavicchioli, R, (2000), Low temperature Regulated DEAD-Box RNA Helicase from the Antarctic Archaeon, *Methanococcoides burtonii*. *Journal of Molecular Biology* .297, 553–567.

Lindner, C., Stülke, J. and Hecker, M. (1994). Regulation of Xylanolytic Enzymes in *Bacillus subtilis*. *Microbiology*. 140, 753-757.

Liu, J. Q., T. Kurihara, A. K. Q. Hasan, V. Nardi-dei, H. Kushikawa, N. Esaki, and K. Soda. (1994). Purification and Characterization of Thermostable and Non-Thermostable 2-Haloacid Dehalogenases with Different Stereospecificities from *Pseudomonas* sp. strain YL. *Applied and Environmental Microbiology*. 60, 2389–2393.

López-García, P. (2005). *Extremophiles.Lectures in Astrobiology*.Berlin: Springer-Verlag Berlin Heidelberg.

Manan, F. M. A., Rahman, I. N. A., Marzuki, N. H. C., Mahat, N. A., Huyop, F. and Wahab, R. A. (2016). Statistical Modelling of Eugenol Benzoate Synthesis Using *Rhizomucor miehei* Lipase Reinforced Nanobioconjugates. *Process Biochemistry* .51, 249-262.

Manan, F. M. A., Attan, N., Zakaria, A., Mahat, Keyon A. S. A. and Wahab, R. A. (2017). Enzymatic Esterification of Eugenol and Benzoic Acid by a Novel Chitosan-Chitin Nanowhiskers Supported *Rhizomucor miehei* Lipase: Process Optimization and Kinetic Assessments. *Enzyme and Microbial Technology*. 108, 42-52.

Marais, J. S. C. (1994). Monofluoroacetic Acid, the Toxic Principle of 'Gifblaar' *Dichapetalum cymosum*. *Onderstepoort Journal of Veterinary Research*. 20, 67-73.

Marzuki, N. H. C., Huyop, F., Aboul-Enein, H. Y., Mahat, N. A. and Wahab, R. A. (2015). Modelling and Optimization of *Candida rugosa* Nanobioconjugates Catalysed Synthesis of Methyl Oleate by Response Surface

Methodology. *Biotechnology and Biotechnological Equipment*. 29, 1113-1127.

Mesri, S., Wahab, R. A. and Huyop, F. (2009). Degradation of 3-Chloropropionic Acid (3CP) by *Pseudomonas* sp. B6P Isolated from a Rice Paddy Field. *Annals of Microbiology* . 59(3),447-451.

Miller, J. M., and Rhoden, D. L. (1991). Preliminary Evaluation of Biolog, a Carbon Source Utilization Method for Bacterial Identification. *Journal of Clinical Microbiology*. 29(6), 1143–1147.

Minnaar, P.P., McCrindle, R.I., Naude, T.W. and Botha, C.J. (2000). Investigation of Biological Samples for Monofluoroacetate and *Dichapetalum cymosum* Poisoning in Southern Africa.*Onderstepoort Journal of Veterinary Research*. 67, 27-30.

Morita, R. Y. (1975). Psychrophilic Bacteria. *Bacteriological Review*. 39, 144–167.

Motosugi, K., Esaki, N. and Soda, K. (1982a). Purification and Properties of 2-Haloacid Dehalogenase from *Pseudomonas putida. Agricultural and Biological Chemistry*.46, 837- 838.

Motosugi, K., Esaki, N. and Soda, K. (1982b). Bacterial Assimilation of D- and L-2-Chloropropionates and Occurrence of a New Dehalogenase. *Archives of Microbiology* . 131, 179-183.

Murdiyatmo, U. (1991). *Molecular Genetic Analysis of a 2-Haloacid Halidohydrolase Structural Gene*. PhD. Thesis, University of Kent at Canterbury, Canterbury, United Kingdom.

Murdiyatmo, U., Asmara, W., Tsang, J. S. H., Baines, A. J., Bull, A. T. and Hardman, D. J. (1992). Molecular Biology of the 2-Haloacid Halidohydrolase from *Pseudomonas cepacia* MBA4. *Biochemical Journal*. 284, 87-93.

Muryoi, N., Sato, M., Kaneko, S., Kawahara, H., Obata, H., Yaish, M. W. F., Griffith, M. and Glick, B. R. (2004). Cloning and Expression of afpA, a Gene Encoding an Antifreeze Protein from the Arctic Plant Growth-Promoting Rhizobacterium *Pseudomonas putida* GR12-2 . *Journal of Bacteriology*. 186(17), 5661–5671.

Muslem, W. H., Huyop, F., Zakaria, I. I. and Wahab, R. A. (2015). Isolation and Characterization of a Biodegrading 3-Chloropropionic Acid *Burkholderia cepacia* WH1 Isolated from Abandoned Agricultural Land. *Asia Pacific Journal of Molecular Biology and Biotechnology*. 23(2), 268-279.

Nardi-Dei, V., Kurihara, T., Okamura, O., Liu, J. Q., Koshikawa, H., Ozaki, H., Terashima, Y., Esaki, N. and Soda, K. (1994). Comparative Studies of Genes Encoding Thermostable L-2-Haloacid Dehalogenase from *Pseudomonas* Species Strain YL, Other Dehalogenases and Two Related Hypothetical Proteins from *Escherichia coli. Applied and Environmental Microbiology* .60, 3375-3380.

Nardi-Dei, V., Kurihara, T., Park, C., Miyagi, M., Tsunasawa, S., Soda, K. and Esaki, N. (1999). DL-2- Haloacid Dehalogenase from *Pseudomonas* sp. 113 is a New Class of Dehalogenase Catalyzing Hydrolytic Dehalogenation not Involving Enzyme-Substrate Ester Intermediate. *The Journal of Biological Chemistry* . 274(30), 20977–20981.

Niknam, M. R., Huyop, F. and Wahab, R. A. (2014). Identification and Characterization of *Raoutella ornithilolytica* and Determination of Its Herbicide 2,2-Dichloropropionate (2,2-DCP) Degradation Potential. *Malaysian Journal of Microbiology* . 10(4), 249-254.

Nemati, M., Abdulghader, M. F., Gicana, R. C., Lamis, R. J. S., Ibrahim, M., Hamid, A. A. A. and Huyop, F. (2013). Identification of Putative Cof-Like Hydrolase Associated with Dehalogenase in *Enterobacter cloacae* MN1 Isolated from the Contaminated Sea-Side Area of the Philippines. *Malaysian Journal of Microbiology* . 9(3), 253-259.

Neumann, A. (1998). *Isolation and Cloning of Tetrachloroethene Dehalogenase from Dehalospirillum multivorans*. Germany: Herbert Utz Verlag.

Nichols, C.M., Lardiere, S.G., Bowman, J.P., Nichols, P.D., Gibson, J.A.E. and Guezennec, J. (2005). Chemical Characterization of Exopolysaccharides from Antarctic Marine Bacteria. *Microbial Ecology*. 49, 578–58.

Novak, H.R., Sayer, C., Panning, J. and Littlechild, J.A. (2013a). Characterisation of an L- Haloacid Dehalogenase from the Marine Psychrophile *Psychromonas ingrahamii* with Potential Industrial Application. *Marine Biotechnology*. 15(6), 695-705.

Novak, H.R., Sayer, C., Isupov, M.N., Paszkiewicz, K., DGotz, D., Spragg, A.M. and Littlechild, J.A. (2013b). Marine Rhodobacteraceae L-Haloacid Dehalogenase Contains a Novel His/Glu Dyad That Could Activate the Catalytic Water. *FEBS Journal.* 280, 1664-1680.

Obire, O., Anyanwu, E. C. and Okigbo, R. N. (2008). Saprophytic and Crude Oil Degrading Fungi from Cow Dung and Poultry Droppings as Bioremediating Agents. *Journal of Agricultural Technology* .4(2), 81-89.

Ojonoma, O. L. and Udeme, I. J. J. (2014). Bioremediation of Palm Oil Mill Effluent (POME) Polluted Soil Using Microorganisms Found in Organic Wastes. *The International Journal of Biotechnology* .3(3), 32-46.

Orji, F. A., Ibiene, A. A. and Dike, E. N. (2012). Laboratory Scale Bioremediation of Petroleum Hydrocarbon – Polluted Mangrove Swamps in the Niger Delta Using Cow Dung. *Malaysian Journal of Microbiology* . 8(4), 219-228.

Pandey, K.D., Shukla, S.P., Shukla, P.N., Giri, D.D., Singh, J.S., Singh, P. and Kashyap, A.K. (2004). Cyanobacteria in Antarctica: Ecology, Physiology and Cold Adaptation. *Cellular and Molecular Biology* .50, 575–584.

Parvizpour,S., Hamid, T.H.T.A. and Huyop, F.(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*. 9(1), 120-124.

Phadtare, S. (2004) .Recent Developments in Bacterial Cold-Shock Response. *Current Issues in Molecular Biology* .6,125–136.

Pee, K.H.V. and Unersucht, S. (2003). Biological Dehalogenation and Halogenation Reactions. *Chemosphere*. 52 , 299–312.

Pikuta, E. V., Hoover R. B. and Tang J. (2007). Microbial Extremophiles at the Limits of Life. *Critical Reviews in Microbiology.* 33, 183–209.

Pradhan, P. and Babu, G. K. (2012). Isolation and Identification of Methanogenic Bacteria from Cow Dung. *International Journal of Current Research*. 4, 028-031.

Preston-Mafham, J., Boddy, L. and Randerson, P. F. (2002). Analysis of Microbial Community Functional Diversity Using Sole-Carbon-Source Utilisation Profiles - a Critique. *FEMS Microbiology Ecology.* 42,1-14.

Preiss, L., Hicks, D.B., Suzuki, S., Meier, T. and Krulwich, T.A. (2015). Alkaliphilic Bacteria with Impact on Industrial Applications, Concepts of Early Life Forms, and Bioenergetics of ATP synthesis. *Frontiers in Bioengineering and Biotechnology*. 3, 75.

Randhawa, G. K. and Kullar, J. S. (2011). Bioremediation of Pharmaceuticals, Pesticides, and Petrochemicals with Gomeya/Cow Dung. *International Scholarly Research Network* . 2011,1-7.

Raven, P. H. and Johnson, G. B. (2001). Extremophilic Bacteria and Microbial Diversity. *Biology Sixth Edition* (1-872). United States: The McGraw Hill Companies, United States. pp. 1-872.

Reed, C.J., Lewis, H., Trejo, E., Winston, V. and Evilia, C. (2013). Protein Adaptations in Archaeal Extremophiles. *Archaea*. 2013, 373275.

Reyes-Franco, M. C., Hernandez–Delgado, S., Beas- Fernandez. R., Medina,-Fernandez, M., Simpson, J. and Mayek-Perez, N. (2006). Pathogenic Variability within *Macrophomina Phaseolina* from Mexico and Other Countries. *Journal of Phytopathology*.154, 447- 453.

Riley, M., Staley, J.T., Danchin, A., Wang, T.Z., Brettin, T.S., Hauser, L.J., Land M.L. and Thompson, L.S. (2008). Genomics of an Extreme Psychrophile, *Psychromonas ingrahamii*. *BMC Genomics* . 9, 210.

Roslan, D. D., Gicana, R. G., Lamis, R. J. and Huyop,F. (2011). Characterisation of *Bacillus* Strains from Volcanic Area Gunung Sibayak Able to Degrade 2,2-Dichloropropionic Acid. *African Journal of Microbiology Research* . 5(28), 4987-4992.

Russell, N.J. (1997). Psychrophilic Bacteria—Molecular Adaptations of Membrane lipids. *Comparative Biochemistry and Physiology* .118, 489–493.

Rye, C.A., Isupov, M.N., Lebedev, A.A. and Littlechild, J.A. (2009). Biochemical and Structural Studies of A L-Haloacid Dehalogenase from the Thermophile Archeon *Sulfolobus tokodaii*. *Extremophiles*. 13,179-190.

Sakaff, M.K.L.M., Rahman, A.Y.A., Saito, J.A., Hou, S.B. and Alam, M. (2012). Complete Genome Sequence of the Thermophilic Bacterium *Geobacillus thermoleovorans* CCB_US3_UF5. *Journal of Bacteriology*. 194, 1239–1239.

Saleh, Y. E., El-Fouly, M. Z., Khalil, M. S., and Abo-State, M. A. (1993). Incidence and Characterization of *Bacillus Cereus* Isolated from Egyptian Foods. *Qatar University Science Journal.* 13(1), 75-80.

Salim, M. M., Roslan, D. D. and Huyop, F. (2011). Molecular Analysis of Dehalogenase Gene in Genomic DNA of *Bacillus megaterium* Strain GS1 Isolated from Volcanic Area Gunung Sibayak. *Journal of Biological Sciences*. 11(5), 394-398.

Santillan, Y.M., Moreno, F.P., Garcia, F.P. and Sandoval, O.A.A. (2014). Effect of the Application of Manure of Cattle on the Properties Chemistry of Soil in Tizayuca, Hidalgo, Mexico. *International Journal of Applied Science and Technology.* 4 (3), 67 - 72.

Satyanarayana, T., Raghukumar, C. and Shivaji, S.(2005). Extremophilic microbes: Diversity and perspectives. *Current Science*. 89(1). 78-90.

Schneider, B., Muller, R., Frank, R. and Lingens, F. (1991). Complete Nucleotide Sequences and Comparison of the Structural Genes of Two 2- Haloalkanoic Acid Dehalogenases from *Pseudomonas species* CBS3. *Journal of Bacteriology*. 173, 1530-1535.

Schmidberger, J. W., Wilce, J. A., Weightman, A. J., Whisstock, J. C. and Wilce, M. C. (2008). The Crystal Structure of DehI Reveals a New Alpha-Haloacid Dehalogenase Fold and Active-Site Mechanism. *Journal of Molecular Biology*. 378(1), 284-294.

Siglioccolo, A., Paiardini, A., Piscitelli, M. and Pascarella, S. (2011). Structural Adaptation of Extreme Halophilic Proteins Through Decrease of Conserved Hydrophobic Contact Surface. *BMC Structural Biology*. 11, 50.

Sikkema, S. J., de Bont, J. A. and Poolman, B. (1995). Mechanisms of Membrane Toxicity of Hydrocarbons. *Microbiology Review* . 59(2), 201-222.

Singh, D. and Fulekar, M. H. (2007). Bioremediation of Phenol Using Microbial Consortium in Bioreactor. *Innovative Romanian Food Biotechnology* . 1(30),31-36.

Singh, D. and Fulekar, M. H. (2009). Bioremediation of Benzene, Toluene and O-Xylene by Cow Dung Microbial Consortium. *Journal of Applied Biosciences*. 14, 788 - 795.

Singh, D., and Fulekar, M. H. (2010). Benzene Bioremediation Using Cow Dung Microflora in Two Phase Partitioning Bioreactor. *Journal of Hazardous Materials*. 175, 336-343.

Siuda, J. F. and De Bernadis, J. F. (1973). Naturally Occuring Halogenated Organic Compounds. *Lloydia*. 36(2), 107-143.

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

Smith, J.M., Harrison, K., Colby, J. and Taylor, S.C. (1989). Determination of D-2-Halopropionate Dehalogenase Activity from *Pseudomonas putida* Strain AJ1/23 by Ion Chromatography. *FEMS Microbiology Letters*. 57(1), 71–74.

Smith, J. M., Harrison, K. and Colby, J. (1990). Purification and Characterization of D-2-Haloacid Dehalogenase from *Pseudomonas putida* Strain AJ1/23 *Journal of General Microbiology*. 136, 881-886.

Sufian, N. H. M, Naim, M. A., Hamid, T. H. T. A, Huyop, F. and Hamid, A. A. A. (2015). Isolation and Identification of 3- Chloropropionic Acid Degrading Bacterium from Marine Sponge. *Jurnal Teknologi* . 77(25),71–75.

Srinivasan, A., Wickes, B. L., Romanelli, A. M., Debelenko, L., Rubnitz, J. E., Sutton, D. A., Thompson, E. H., Fothergill, A. W., Rinaldi, M. G., Hayden, R. T. and Shenep, J. L. (2009). Cutaneous Infection Caused by *Macrophomina phaseolina* in a Child with Acute Myeloid Leukemia. *Journal of Clinical Microbiology* . 47, 1969-1972.

Stager, C.E. and Davis, JR.(1992). Automated Systems for Identification of Microorganisms. *Clinical Microbiology Review*. (3), 302-27.

Stringfellow, J. M., S. S. Cairns, A. Cornish, and R. A. Cooper.(1997). Haloalkanoate Dehalogenase II (DehE) of a *Rhizobium* sp.—Molecular Analysis of the Gene and Formation of Carbon Monoxide from Trihaloacetate by the Enzyme. *European Journal of Biochemistry*.250,789–793.

Tadeo, X., López-Méndez, B., Trigueros, T., Laín, A., Castaño, D., Millet, O. (2009). Structural Basis for the Amino Acid Composition of Proteins from Halophilic Archea. *PLoS Biology*. 7,e1000257

Thasif, S., Hamdan, S. and Huyop, F. (2009). Degradation of D,L-2-Chloropropionic Acid by Bacterial Dehalogenases That Shows Stereospecificity and Its Partial Enzymatic Characteristics. *Biotechnology*. 8(2), 264-269.

Topping, A. W. (1992). *An Investigation into the Transposition and Dehalogenase Functions of DEH, a Mobile Genetic Element, from Pseudomonas putida strain PP3*. PhD. Thesis, University of Wales, Cardiff, United Kingdom.

Tsang, J.S.H., Sallis, P.J., Bull, A.T. and Hardman, D.J. (1988). A Monobromoacetate Dehalogenase from *Pseudomonas cepacia* MBA4. *Archives of* Microbiology.150, 441-446.

Tseng, S. H., Lin, Y. J., Chang, P. C., Chou, S. S. and Chang, H. M. (2004). Determination of Residual Dalapon in Sugarcane by Gas Chromatograph Equipped with Electron Capture Detector (GC-E anCD). *Journal of Food and Drug Analysis* .12(3), 238-243.

Umanu, G., Nwachukwu, S. C. U., and Olasode, O. K. (2013). Effects of Cow Dung on Microbial Degradation of Motor Oil in Lagoon Water. *Global Journal of Bio-science and Biotechnology*. 2(4), 542-548.

van den Burg, B. (2003). Extremophiles as a Source for Novel Enzymes. *Current Opinion in Microbiology*. 6(3), 213–218.

van der Meer, J. R. (1997).Evolution of Novel Metabolic Pathways for the Degradation of Chloroaromatic Compounds. *Antonie van Leeuwenhoek*. 71(1-2),159–178.

van Der Ploeg, J., Van Hall, G. and Janssen, D. B .(1991). Characterization of Haloacid Dehalogenase from *Xanthobacter autotrophicus* GJ10 and Sequencing of the *dhlb* Gene. *Journal of Bacteriology* . 173, 7925-7933.

Ventura, M. and Zink, R. (2002). Specific Identification and Molecular Typing Analysis of *Lactobacillus johnsonii* by Using PCR-Based Methods and Pulsed-Field Gel Electrophoresis. *FEMS Microbiol Letter*. 217,141–154.

Vidali M. (2001). Bioremediation. An overview. *Pure and Applied Chemistry*. 73(7), 1163–1172.

Wahab, R. A., Basri, M., Rahman, R. N. Z. R. A., Salleh, A. B., Rahman, M. B. A., Chaibakhsh, N., and Leow, T. C. (2014). Enzymatic Production of a Solvent-Free Menthyl Butyrate via Response Surface Methodology Catalyzed by a Novel Thermostable Lipase from *Geobacillus zalihae*. *Biotechnology and Biotechnological Equipment*. 28(6), 1065-1072.

Walsh, G. (2002). *Proteins: Biochemistry and Biotechnology*. England: John Wiley and Sons Ltd.

Weightman, A. J., Weightman, A. L. and Slater, J. H. (1982). Stereospecificity of 2-Monochloropropionate Dehalogenation by the Two Dehalogenases of *Pseudomonas putida* PP3: Evidence of Two Different Dehalogenation Mechanisms. *Journal of general microbiology*. 131, 1755-1762.

Whalen, J. K., Chang, C., Clayton, G. W. and Carefoot, J. P. (2000). Cattle Manure Amendments Can Increase the pH of Acid Soils . *Soil Science Society of America Journal* . 64, 922-966.

Wong, W. Y. and Huyop, F. (2011). Characterization of a *Labrys* sp. Strain Wy1 Able to Utilize 2,2-Dichloropropionate (2,2-DCP) as Sole Source of Carbon. *African Journal of Microbiology Research*. 5(20), 3282-3288.

Wong, W. Y. and Huyop, F. (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.

Woo, P. C. Y., Ng, K. H. I., Lau, S. K. P., Yip, K.T., Fung, K.T., Leung, K. W., Tam, D. M. W., Que, T.-L. and Yuen, K.-Y. (2003). Usefulness of the MicroSeq 500 16S Ribosomal DNA-Based Identification System for Identification of Clinically Significant Bacterial Isolates with Ambiguous Biochemical Profiles. *Journal Clinical Microbiology*. 41,1996-2001.

Wubbolts, M. G., Bucke, C. and Bielecki, S. (2000). How to Get Biocatalyst. In Straathof, A. J. J. and Adlercreutz, P. (eds). *Applied biocatalyst 2nd edn* (157-297). Amsterdam: Harwood Academic Publishers.

Wysocki, V.H., Resing, K.A., Zhang, Q. and Cheng, G.(2005). Mass Spectrometry of Peptides and Proteins. *Methods*. 35 (3), 211–22.

Xu, Y., Nogi, Y., Kato, C., Liang, Z., Ruger, H.J., De Kegel, D. and Glansdorff, N. (2003). *Moritella profunda* sp. *nov*. and *Moritella abyssi* sp. *nov*., Two Psychropiezophilic Organisms Isolated from Deep Atlantic Sediments.

International Journal of Systematic and Evolutionary Microbiology .53, 533–538.

Yadav, S. J. and Thakare, D. S. B. (2015). Cow Dung for Treatment of Saline Soil and Cow Importance from Vedas. *International Journal of Engineering Research and General Science*. 3(3), 445-452.

Yahaya, N. (2014). *Isolation and Identification of Bacteria Capable to Utilize 2,2-Dichloropropionic Acid from Danga Bay Seawater*. M. Sc. Thesis. Universiti Teknologi Malaysia, Skudai.

Yong, W.,W. (2010). *Isolation, Characterization and Identification of 2,2-DCP Degrading Soil Microorganism from Melaka Rubber Estate*. Bachelor of Degree, Universiti Teknologi Malaysia, Skudai.

Yong, W. W. (2013). *Isolation, Identification and Characterization Of 2,2-Dichloropropionic Acid Utilizing Bacteria*. M.Sc. Thesis. Universiti Teknologi Malaysia, Skudai.

Yu, M., Faan, Y. -W., Chung, W. Y. K., and Tsang, J. S. H. (2007). Isolation and Characterization of a Novel Haloacid Permease from *Burkholderia cepacia* MBA4. *Applied and Environmental Microbiology*. 75 (15), 4874-4880.

Yusn, T. Y. and Huyop, F. (2009). Degradation of 3-Chloropropionic Acid by *E.coli* JM109 Expressing Dehalogenase (deh) Gene Used as Selection Marker. *Biotechnology*. 8(3), 385-388.

Zhang, T., Fan, X., Hanada, S., Kamagata, Y. and Fang, H. H. P. (2006). *Bacillus macauensis* sp. Nov., a Long-Chain Bacterium Isolated from a Drinking Water Supply. *International Journal of Systematic and Evolutionary Microbiology*. 56, 349-353.

Zhang, J., Cao, X., Xin, Y., Xue, S. and Zhang, W. (2013). Purification and Characterization of a Dehalogenase from *Pseudomonas stutzeri* DEH130 Isolated from the Marine Sponge *Hymeniacidon perlevis*. *World Journal of Microbiology and Biotechnology*. 29(10), 1791-1799.

Zhang, J., Xin, Y., Cao, X., Xue, S. and Zhang, W. (2014). Purification and Characterization of 2-Haloacid Dehalogenase from Marine Bacterium *Paracoccus* sp. DEH99, Isolated from Marine Sponge *Hymeniacidon perlevis*. *Journal of Ocean University of China*. 13(1), 91-96.

Zulkifly, A. H., Roslan, D. D., Hamid, A. A. A., Hamdan, S. and Huyop, F. (2010). Biodegradation of Low Concentration of Monochloroacetic Acid-Degrading *Bacillus* sp. TW1 Isolated from Terengganu Water Treatment and Distribution Plant. *Journal of Applied Sciences*. 10(22), 2940-2944.