RpoN-DEPENDENT ADAPTATION OF *Burkholderia xenovorans* LB400 FOR BIODEGRADATION AND BIOREMEDIATION OF DIBENZOFURAN

NOOR FAIZUL HADRY BIN NORDIN

THESIS SUBMITTED IN FULFILMENT OF THE

REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF BIOLOGICAL SCIENCES

FACULTY OF SCIENCE

UNIVERSITY OF MALAYA

2012

ABSTRACT

Alternative sigma subunit-54 (RpoN) forms holoenzyme complex when associated with core RNA Polymerase (RNAP) to specifically recognise and initiate transcription of specific sets of genes in response to environmental stimuli. RpoN has important role in many major adaptive responses in bacteria and is involved in various physiological responses such as pathogenesis, quorum sensing and bioremediation. The main focus of this study is to gain insight into the role of alternative sigma factor-54 (RpoN) of *Burkholderia xenovorans* LB400 in degradation of dibenzofuran via biphenyl degradation pathway. Additionally, this study also investigated the ability of *Burkholderia cenocepacia* J2315 in utilisation of dibenzofuran. The single knockout mutants of *rpoN* genes were established using pKNOCK suicide vector series resulting two *rpoN* mutants of *Burkholderia xenovorans* LB400; NRPLB [(*rpoN1* mutant) and NRP2LB (*rpoN2* mutant)] and one *rpoN* mutant of *Burkholderia cenocepacia* J2315 [NRPJ (*rpoN* mutant)]. The physiological and metabolic responses analyses were conducted to differentiate the single-gene knockout mutants from their wildtype strains; *Burkholderia xenovorans* LB400 and *Burkholderia cenocepacia* J2315. The physiological response analysis demonstrated that the ability of the mutants NRPLB and NRP2LB to form biofilm were not affected with inactivation of *rpoN* genes. However, the biofilm formation in NRPJ was reduced indicating the involvement of *rpoN* gene in formation of biofilm in *Burkholderia cenocepacia* J2315. Inactivation of *rpoN2* gene does not affect motility of NRP2LB (*rpoN2* mutant). However, inactivation of *rpoN1* gene significantly reduced motility of NRPLB (*rpoN1* mutant). Metabolic response analysis shows that *rpoN* genes play an important role in utilisation of nitrogenous compound even though the effects are depending on the species of the nitrogen. The altered nitrogen utilisation profile when using ammonium, histidine, asparagines, nitrate, glutamine and alanine as sole nitrogen source in

single-gene knockout mutants indicate that *rpoN* genes of *Burkholderia xenovorans* LB400 and *Burkholderia cenocepacia* J2315 are active and functional for nitrogen utilisation. The ability of *Burkholderia xenovorans* LB400 and *Burkholderia cenocepacia* J2315 in degrading ortho-substituted PCBs such as dibenzofuran was also determined. Degradation studies of dibenzofuran showed significant differences between wildtype *Burkholderia xenovorans* LB400, *Burkholderia cenocepacia* J2315 and their single-gene knockout mutants. Degradation rate was found higher in NRP2LB (*rpoN2* mutant) compared to wildtype *Burkholderia xenovorans* LB400 but reduced significantly in NRPLB (*rpoN1* mutant). This result was supported by gene expression analysis where RpoN-dependent *bphA* gene that encodes for biphenyl dioxygenase was highly expressed in NRP2LB (*rpoN2* mutant) thus enhanced the degradation of dibenzofuran via biphenyl degradation pathway. This result indicates the important role of *rpoN1* gene in *Burkholderia xenovorans* LB400 in degradation of dibenzofuran. Simple phytotoxicity assay showed that byproducts from degradation of dibenzofuran by wildtype *Burkholderia xenovorans*, NRPLB (*rpoN1* mutant) and NRP2LB (*rpoN2* mutant) is less toxic towards the test species compared to dibenzofuran. Furthermore, the degradation byproducts from NRP2LB (*rpoN2* mutant) was able to enhanced the growth of *Sorghum saccharatum* compared to control (water).

ABSTRAK

Sigma subunit alternatif-54 (RpoN) membentuk kompleks holoenzim apabila bergabung dengan Polymerase RNA teras (RNAP), untuk mengenali dan memulakan transkripsi set gen yang khusus sebagai tindak balas terhadap rangsangan persekitaran. RpoN memainkan peranan penting dalam banyak tindak balas penyesuaian utama dalam bakteria dan terlibat dalam pelbagai tindak balas fisiologi seperti patogenesis, penderiaan kuorum dan bioremediasi. Fokus utama kajian ini adalah untuk memahami peranan sigma faktor alternatif-54 (RpoN) *Burkholderia xenovorans* LB400 dalam degradasi dibenzofuran yang menggunakan laluan degradasi bifenil. Sebahagai tambahan, kajian ini juga menyiasat keupayaan *Burkholderia cenocepacia* J2315 dalam menggunakan dibenzofuran. Mutan-mutan 'knockout' gen-tunggal *rpoN* telah dibina menggunakan siri pKNOCK vektor menghasilkan dua mutan *rpoN Burkholderia xenovorans* LB400 [NRPLB (mutan *rpoN1*) dan NRP2LB (mutan *rpoN2*)] dan satu mutan *rpoN Burkholderia cenocepacia* J2315 [NRPJ (mutan *rpoN*)]. Analisis tindak balas fisiologi dan metabolik telah dijalankan untuk membezakan mutan-mutan 'knockout' gen-tunggal dari strain jenis liar; *Burkholderia xenovorans* LB400 dan *Burkholderia cenocepacia* J2315. Analisis tindak balas fisiologi menunjukkan bahawa keupayaan mutan NRPLB dan NRP2LB untuk membentuk biofilm adalah tidak terjejas dengan penyahaktifan gen *rpoN*. Walau bagaimanapun, pembentukan biofilm oleh NRPJ (mutan *rpoN*) adalah berkurangan, menunjukkan gen *rpoN* adalah terlibat dalam pembentukan biofilm oleh *Burkholderia cenocepacia* J2315. Penyahaktifan gen *rpoN2* tidak menjejaskan motiliti NRP2LB (mutan *rpoN2*). Walaubagaimanapun, penyahaktifan gen *rpoN1* mengurangkan motiliti NRPLB (mutan *rpoN1*) dengan ketara. Analisis tindak balas metabolik menunjukkan bahawa gen *rpoN*

memainkan peranan yang penting dalam penggunaan sebatian bernitrogen walaupun kesannya bergantung kepada spesis nitrogen tersebut. Profil penggunaan nitrogen yang berubah dalam mutan-mutan 'knockout' gen-tunggal apabila menggunakan ammonium, histidina, asparagina, nitrat, glutamina dan alanina sebagai sumber nitrogen tunggal menunjukkan bahawa gen *rpoN* *Burkholderia xenovorans* LB400 dan *Burkholderia cenocepacia* J2315 adalah aktif dan berfungsi untuk penggunaan nitrogen. Keupayaan *Burkholderia xenovorans* LB400 dan *Burkholderia cenocepacia* J2315 mendegradasi PCBs orto-tertukarganti seperti dibenzofuran telah juga ditentukan. Kajian degradasi dibenzofuran menunjukkan perbezaan yang signifikan antara *Burkholderia xenovorans* LB400 jenis liar, *Burkholderia cenocepacia* J2315 jenis liar dan mutan-mutan 'knockout gen-tunggal mereka. Kadar degradasi didapati lebih tinggi dalam NRP2LB (mutan *rpoN2*) berbanding jenis liar *Burkholderia xenovorans* LB400 tetapi ianya dikurangkan dengan ketara dalam NRPLB (mutan *rpoN1*). Keputusan ini telah disokong oleh analisis ekspresi gen di mana *bphA*-bersandarkan RpoN, yang mengkodkan biphenyl dioxygenase diekspres berlebihan dalam NRP2LB (*rpoN2* mutan) dan ianya meningkatkan degradasi dibenzofuran melalui laluan degradasi biphenyl. Keputusan ini menunjukkan peranan penting gen *rpoN1* bagi *Burkholderia xenovorans* LB400 dalam pendegradasian dibenzofuran. Ujian kefitotoksikan mudah menunjukkan bahawa produk sampingan daripada degradasi dibenzofuran oleh *Burkholderia xenovorans* jenis liar, NRPLB (mutan *rpoN1*) dan NRP2LB (mutan *rpoN2*) adalah kurang toksik terhadap spesis yang diuji berbanding dengan dibenzofuran. Tambahan pula, produk sampingan degradasi dari NRP2LB (mutan *rpoN2*) dapat meningkatkan pertumbuhan *Sorghum saccharatum* berbanding kawalan (air).

ACKNOWLEDGEMENTS

First and foremost I would like express my sincere appreciation to both of my supervisors, Assoc Prof. Dr. Amir Feisal Merican and Dr. Yusrizam Sharifuddin for their help, guidance and constant support during the course of this PhD program. I would like to thanks Prof. Dr. Martin Buck and Dr. Robert H. A. Coutts of Imperial College London for their support and advice during the early stage of this project.

I am greatly indebted to International Islamic University Malaysia and Ministry of Higher Education for the scholarship and financial assistance for my PhD. Also thanks to IPPP, University of Malaya and RMC, International Islamic University Malaysia for PPP and FRGS research grants for this study, respectively.

I extend my special thanks to Ms Chan Lee Choo that helps me in the lab, Mr Ahmad Muzammil and Mr Mohd Nizam Yusof for their assistance in conducting GCMS analysis. I would also like to thank my friends and colleagues at CRYSTAL, University of Malaya and Department of Biotechnology, IIUM for their helps and assistance.

Many thanks to my families especially my parents; Tuan Haji Nordin Dahlan and Puan Kamariah Musa and my mother in law, Puan Hajjah Atijah Che Awang for their financial and moral support.

The most thanks naturally go to my wife Rasinah Zakaria without whom I would not have been able to complete this work. I have to thank her for being with me in my every ups and downs that sometimes postponing some of her own goals, for supporting me in every aspect of life and for patiently hearing about my problems in research. Finally, I would like to give my special thanks to my beloved daughters; Noor Batrisyia Auni and Noor Wareesha Ayra. Their patience with me alone should earn them credit for much of the success of this work. Trips have been cancelled and vacations postponed all in the name of science. Their endurance has been exemplary and without them, none of this would mean anything.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

PCBs	Polychlorinated biphenyl
BPDO	Biphenyl dioxygenase
µg	Microgram
mL	Milliliter
m/z	Mass-to-charge ratio
cm	Centimeter
EtBr	Ethidium Bromide
O.D.	Optical density