UNIVERSITI PUTRA MALAYSIA

BIODEGRADATION OF WASTE AND PURE CANOLA OIL BY COLD-ADAPTED *Rhodococcus* sp. AQ5-07 FROM ANTARCTICA

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FBSB 2020 15

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By

SALIHU IBRAHIM

Thesis Submitted to the school of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the degree of Doctor of Philosophy

June 2020

DEDICATION

I hereby dedicate this thesis to the Almighty Allah who has been my help, sustainer, provider, guide, encouragement, keeper and my all in all throughout the course of my studies and also to my parents (Alhaji Ibrahim Umar and Hajiya Ramatu Ibrahim) whose prayers and support has kept me going. Finally to my brothers and sisters who are always by my side and ready to render any kind of support and assistance.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairperson: Siti Aqlima Ahmad, PhD
Faculty: Biotechnology and Biomolecular Sciences

With the increase in human activities in Antarctic region, the possibility of vegetable oil spillage becomes unavoidably high. The removal of oils including canola oil from the environment and wastewater using biological approaches is needed since the thermal process of oil degradation is ineffective and requires the use of high temperatures. More so, thermal degradation leads to the generation free radicals. Thus, in this present study, the potential canola oil-degrading ability of cold-adapted *Rhodococcus* sp. AQ5-07 from Antarctica was evaluated using one-factor-at-a-time (OFAT), and response surface method (RSM). The bacterial growth kinetics was examined and screened for biosurfactant production. The ability of *Rhodococcus* sp. AQ5-07 to degrade canola oil in the presence of different heavy metals and xenobiotics was also tested. Furthermore, canola oil degradation pathway was investigated via whole genome analysis. *Rhodococcus* sp. strain AQ5-07 was found to have a high canola oil-degrading ability over other strains tested. Considerable degradation (78.60% and 70.14%) of 3% waste and pure canola oil was achieved by this bacterium when incubated with 1.0 g/L ammonium sulphate, 0.3 g/L yeast extract, pH 7.5 and 10% inoculum at 10°C over 72 h incubation period. Optimisation of the medium conditions using response surface methodology (RSM) resulted in an increase in both oil degradations (87.61% and 86.34%). Three out of the 10 heavy metals tested namely mercury (Hg), cadmium (Cd) and silver (Ag) had a significant effect on canola oil degradation at 1 ppm. The IC_{50} values of the waste canola oil (WCO) for Hg, Cd and Ag were found to be 0.3866, 0.4539 and 0.3217 ppm, respectively. Meanwhile, for the pure canola oil (PCO), the IC_{50} was found to be Hg (0.2495), Cd (0.5452) and Ag (0.3088), respectively. This shows that the strain can also withstand 10 mg/L acrylamide, 50 mg/L phenol and 0.5% (v/v) diesel, respectively, for both oils. The bacterium was also found to produce high biosurfactant with beta haemolysis, high cellular hydrophobicity of 89.5% and 83.5% in hexadecane and tetradecane, respectively. The strain displayed 55 mm drop collapse with an oil displacement of 48 mm. Emulsification test (E_{24}) revealed the highest emulsification index of 92% in hexadecane. Haldane and Yano model best describes the kinetics of the strain growth, the calculated constants for Haldane model such as maximum growth rate (μ_{max}), half saturation constant for maximal growth (K_s), and growth inhibition constant (K_i) tolerated were 0.142 h^{-1}, 7.743% (v/v) and 0.399% (v/v), respectively, whereas those for Yano model were 0.096 h^{-1}, 3.0% (v/v) and 4.527% (v/v), respectively. Lastly, the bacterium genome was sequenced using whole genome sequencing (WGS). The WGS was assembled into a draft genome of 6,753,902 bp with G+C content of 62.4% and 828.79x sequencing depth of coverage. It comprises 4 contigs with sizes of 81,655, 134,292, 214,483 and 6,323,472 bp. The smaller contigs could represent plasmids. The coding sequences (CDSs) contained a total of 6,350 annotated genes, 53 tRNA and 15 rRNA. The WGS showed that *Rhodococcus* sp. AQ5-07 was closely related to *Rhodococcus erythropolis* NBRC 15547 and *Rhodococcus qingshengii* JCM 15477, respectively. Analyses revealed the presence of lipase genes responsible for lipid degradation. This study therefore confirmed the possible use of cold-adapted *Rhodococcus* sp. AQ5-07 in biodegradation of canola oil-polluted Antarctic soils at low temperature, as it is disease free, eco-friendly, easier and cheaper.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIODEGRADASI SISA DAN MINYAK CANOLA TULEN DENGAN DISESUAIKAN SEJUK *Rhodococcus* sp. AQ5-07 DARI ANTARCTICA

Oleh

SALIHU IBRAHIM

Jun 2020

Pengerusi: Siti Aqlima Ahmad, PhD
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Dengan peningkatan aktiviti manusia di kawasan Antartika, kemungkinan untuk berlaku pertumpahan minyak masak menjadi tinggi dan tidak dapat dielakkan. Penyingkiran minyak termasuk minyak canola dari persekitaran dan air sisa menggunakan pendekatan biologi diperlukan kerana proses termal degradasi minyak tidak berkesan dan memerlukan penggunaan suhu tinggi. Lebih-lebih lagi, degradasi terma membawa kepada penjanaan radikal bebas. Oleh itu, dalam kajian ini, keupayaan merendahkan minyak kanola *Rhodococcus* sp yang disesuaikan dengan sejuk. AQ5-07 dari Antartika dinilai menggunakan one-factor-at-a-time (OFAT), dan kaedah permukaan tindak balas (RSM). Kinetik pertumbuhan bakteria diperiksa dan disaring untuk pengeluaran biosurfaktan. Keupayaan *Rhodococcus* sp. AQ5-07 untuk menurunkan minyak kanola dengan adanya logam berat dan xenobiotik yang berbeza juga diuji. Selanjutnya, jalan degradasi minyak canola disiasat melalui analisis keseluruhan genom. *Rhodococcus* sp. AQ5-07 didapati mempunyai keupayaan yang tinggi untuk mengurai minyak kanola berbanding dengan isolat yang lain. Penguraian yang tinggi (78.60% dan 70.14%) adalah daripada 3% sisa dan minyak kanola tulen telah dicapai oleh bakteria ini apabila ianya diinkubasikan dengan 1.0 g/L amonium sulfat, 0.3 g/L ekstrak yis, pH 7.5 dan 10% inokulum pada 10°C dalam tempoh inkubasi 72 h. Pengoptimuman keadaan media menggunakan kaedah statistikal gerak balas permukaan (RSM) menghasilkan peningkatan dalam kedua-dua penguraian minyak (87.61% and 86.34%). Tiga daripada 10 logam berat yang diuji iaitu merkuri (Hg), kadmium (Cd) dan perak (Ag) mempunyai kesan yang signifikan terhadap penguraian minyak canola pada 1 ppm. Oleh itu, kepekatan logam berat ini bervariasikan daripada 0 - 1 ppm untuk dilihat di mana kepekatan setiap logam akan memberi kesan. IC_{50} bagi sisa minyak kanola (WCO) Hg, Cd dan Ag didapati masing-masing 0.3866, 0.4539 dan 0.3217 ppm. Sementara itu, bagi minyak kanola tulen (PCO), IC_{50} didapati Hg (0.2495), Cd (0.5452) dan Ag (0.3088). Ini menunjukkan bahawa strain ini juga mampu bertahan pada 10 mg/L akrilamida, 50 mg/L fenol dan 0.5% (v/v) diesel, masing-masing untuk kedua-dua jenis minyak. Bakterium ini juga didapati mampu menghasilkan biosurfaktan yang tinggi dengan hemolisis beta, hidrofobisiti selular yang tinggi 89.5% dan 83.5% dalam heksadekana

dan tetradekana masing-masing. Ini menunjukkan kejatuhan penurunan (drop collapse) yang positif dengan anjakan minyak (oil displacement) sebanyak 55 mm. Indeks emulsifikasi (E_{24}) mendedahkan indeks pengemulsian tertinggi pada 92% dalam heksadekana. Haldane dan Yano model terbaik menggambarkan kinetik pertumbuhan terikan. Pemalar yang dikira bagi model Haldane seperti kadar pertumbuhan maksima (μ_{max}), pemalar ketepuan tepu untuk pertumbuhan maksimum (K_s), dan kepekatan yang merencat pertumbuhan (K_i) masing-masing adalah pada 0.142 h^{-1}, 7.743% (v/v) dan 0.399% (v/v), manakala bagi model Yano masing-masing adalah 0.096 h^{-1}, 3.0% (v/v) dan 4.527% (v/v). Akhir sekali, bakteria yang dijujuk menggunakan penjujukan keseluruhan genom. WGS telah disusun menjadi draf genom 6,753,902 bp dengan kandungan G+C 62.4% dan 828.79x penjujukan kedalaman liputan (sequencing depth of coverage). Ia terdiri daripada 4 contigs dengan saiz 81,655, 134,292, 214,483 dan 6,323,472 bp. Contigs yang lebih kecil boleh dikira sebagai plasmid. Jujukan pengekodan (coding sequences) (CDSs) mengandungi sejumlah 6,350 gen penjelasan (annotated genes), 53 tRNA dan 15 rRNA. WGS menunjukkan bahawa *Rhodococcus* sp. AQ5-07 berkait rapat dengan *Rhodococcus erythropolis* NBRC 15547 dan *Rhodococcus qingshengii* JCM 15477, masing-masing. Analisis mendedahkan bahawa kehadiran gen lipase yang bertanggungjawab terhadap penguraian lipid. Oleh itu, kajian ini mengesahkan kemungkinan penggunaan *Rhodococcus* sp. AQ5-07 dalam biodegradasi tanah Antartika tercemar minyak canola pada suhu rendah, kerana bebas penyakit, mesra alam, lebih mudah dan lebih murah.

ACKNOWLEDGEMENTS

In the name of Allah, the most gracious and the most merciful who thought man by pen. All thanks to Allah, who has given me the opportunity to see this day, the day I have been dreaming of, may the peace and blessings of Allah be upon his noble prophet Muhammad (SAW).

First of all, I would like to use this opportunity to express my heartfelt thankfulness to my supervisor Assoc. Prof. Dr. Siti Aqlima Ahmad, for her continue advices, support, guidance and care for the successful conduct of this research work despite multiple challenges. I really appreciate your immense contribution and I pray that may God continue to shower blessing on your path. I would also like to express my gratitude to my co-supervisors Assoc. Prof, Dr. Suriana Sabri, Assoc. Prof. Dr. Khalilah Abdul Khalil, Prof. Dr. Peter Convey and Dr. Claudio Gomez-Fuentes for their great contribution, precious suggestions and critical assessment toward smooth conduct of this research, may God continue to bless them and may He light all their ways of achievements. Equally, I wish to express my appreciation to all my colleagues and lab mates in Eco-Remediation Technology Laboratory especially cooking oil degradation team, Khadijah Zahri, Nadhirah Zakaria, Tengku Athirrah, Syazani Darham, Kavilasni Subramaniam, Catrenar de Silva, Ashwini Naganthran, Aida Noor, Rasidnie Wong, Gayathiri Verasoundarapandian, Syuen Lim, Fareez Roslee, Abdussamad Abubakar and Dr. Mansur Abdulrasheed for their help, contributions, support and understanding they rendered during the conduct of this research. More so, to all lecturers and members of staff in Faculty of Biotechnology and Biomolecular Sciences for their support in making this research a successful one.

Words cannot express my gratitude to my wife Fatima Muhammad and daughter Fatima Salihu Ibrahim for their support and encouragement towards the success of this research. They are always beside me during the happy and hard moments to motivate me. I would also like to extend my sincere gratitude to my father Alhaji Ibrahim Umar and mother Hajiya Ramatu Ibrahim for their prayers, love, care, support and generosity. May Allah grant them all his best in Jannatul Firdaus. My gratitude also goes to the entire members of my family for their encouragement, advices and support as well, may Allah bless them all.

My appreciation also goes to Tertiary Education Trust Fund (Tetfund) via Bayero University, Kano for sponsorship and Yayasan Penyelidikan Antartika Sultan Mizan (YPASM) in collaborations with the National Antarctic Research Centre (NARC) for Antarctic research grant and Universiti Putra Malaysia for providing an enabling environment to conduct the research in order to pursue my goals.

APPROVAL

I certify that a Thesis Examination Committee has met on (Date of viva voce) to conduct the final examination of Salihu Ibrahim on his Doctor of Philosophy thesis entitled "**Biodegradation of waste and pure canola oil by cold-adapted *Rhodococcus* sp. AQ5-07 from Antarctica**" in Accordance with the Universities and University College 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 Doctor of Philosophy Degree.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Declaration by graduate student

I hereby confirm that:

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