

**BIOSYNTHESIS OF
POLYHYDROXYALKANOATE BY *Massilia
haematophila* UMTKB-2 USING OPTIMIZED
CULTURE PARAMETERS AND ITS POLYMER
CHARACTERIZATION**

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**MASTER OF SCIENCE
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JONG TSE KIUN

**Thesis Submitted in Fulfillment of the Requirement for the
Degree of Master of Science in the School of Marine and Environmental
Sciences
Universiti Malaysia Terengganu**

2018

DEDICATION

I dedicated this thesis

*To my big family members;
(For your endless support)*

*To my supervisor;
Associate Professor Dr. Kesaven A/L Bhubalan
(For your guidance, caring and encouragement)*

*To my Co-supervisor;
Professor Dr. Amirul Al-Ashraf Abdullah
(For your coaching and support)*

*Last but no least
To laboratorial colleagues of the Marine Biotechnology Laboratory (PPSMS, UMT)
To laboratorial colleagues of the Bioprocess Department (Ipharm, NIBM, Penang)
To laboratorial colleagues of the Laboratory 318 (SBS, USM)*

And

To my beloved friends

(Every moment of this thesis holds every memorial of all of you)

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu
In fulfillment of the requirement for the degree of Master of Science

BIOSYNTHESIS OF POLYHYDROXYALKANOATE BY *Massilia haematophila* UMTKB-2 USING OPTIMIZED CULTURE PARAMETERS AND ITS POLYMER CHARACTERIZATION

JONG TSE KIUN

2018

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Co- Supervisor : Professor Amirul Al-Ashraf Abdullah, PhD
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Polyhydroxyalkanoate (PHA) is a type of biodegradable polymer that is synthesized by some bacteria under limited nutrient and excess carbon source. The production of PHA by *Massilia* sp. remains mostly unexplored even though some previous studies had highlighted its ability to accumulate P(3HB). In this study, chemical and physical culture parameters were screened for P(3HB) and P(3HB-*co*-3HV) copolymer production by a local aquatic bacteria, *Massilia haematophila* UMTKB-2. This strain had shown proficiency in producing homopolymer, P(3HB), using glucose as a carbon source and urea as a nitrogen source. The potential of this bacteria to accumulate copolymer has never been reported. It was found that *M. haematophila* UMTKB-2 could produce 4.40 g/L of P(3HB-*co*-3HV) with 4 mol% 3-hydroxyvalerate (3HV) monomer using glucose and 1-pentanol as carbon source and precursor respectively. The P(3HB-*co*-3HV) production was enhanced through the optimization of fermentation parameters using the response surface methodology (RSM) in shake flasks. The production of P(3HB-*co*-3HV) by *M. haematophila* UMTKB-2 using optimal conditions (0.07 wt% of 1-pentanol concentration, 176 rpm of agitation rate, and 122h of incubation time), had yielded 5.0 g/L of P(3HB-*co*-3HV) with 7 mol% of 3HV monomer, were higher than the value obtained from un-optimized conditions (0.06 wt% of 1-pentanol concentration, 200 rpm of agitation rate, and 108 h of incubation time). In comparison with non-optimized conditions, a 14 % increment of copolymer concentration and two-fold increment of 3HV composition were observed.

The copolymer produced in this study was better than P(3HB) in terms of physical and thermal properties. The P(3HB-*co*-3HV) has lower crystallinity, lower melting temperature and higher elongation of break than P(3HB). The P(3HB-*co*-3HV) produced was determined as a block copolymer using nuclear magnetic resonance (NMR). In conclusion, this study is a platform for further development of large-scale P(3HB-*co*-3HV) production using *M. haematophila* UMTKB-2.

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

BIOSINTESIS POLYHYDROXYALKANOATE OLEH *Massilia haematophila* UMTKB-2 MENGGUNAKAN PARAMETER KULTUR YANG OPTIMA DAN PENCIRIAN POLIMERNYA.

JONG TSE KIUN

2018

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Pusat : Marin dan Sains Persekitaran

Polyhydroxyalkanoate (PHA) adalah sejenis polimer yang boleh diuraikan, boleh dihasilkan oleh bakteria walaupun dalam keadaan nutrient yang kurang dan dalam keadaan suasana karbon yang berlebihan. Penghasilan PHA oleh genus *Massilia* masih belum diterokai walaupun beberapa kajian menunjukkan keupayaannya untuk mengumpul PHA. Dalam kajian ini, parameter kimia dan fizikal telah dikaji untuk pengeluaran P(3HB) dan kopolimer P(3HB-co-3HV) oleh bakteria akuatik tempatan, *Massilia haematophila* UMTKB-2. Bacteria ini menunjukkan kecekapan untuk menghasilkan homopolimer, P(3HB) dengan menggunakan glukosa sebagai sumber karbon and urea sebagai sumber nitrogen. Potensi bakteria ini untuk penghasilan kopolimer tidak pernah dilaporkan. *M. haematophila* UMTKB-2 telah didapati boleh menghasilkan 4.40 g/L P(3HB-co-3HV) dengan monomer 3HV 4 mol% menggunakan glukosa dan 1-pentanol. Penghasilan P(3HB-co-3HV) dipertingkatkan melalui pengoptimuman parameter penapaian dengan menggunakan statistik, yang bernama metodologi permukaan tindak balas dalam botol yang goncang. Pengeluaran P(3HB-co-3HV) oleh *M. haematophila* UMTKB-2 menggunakan keadaan optimum (kepekatan 1-pentanol 0.07 wt%, kadar agitasi 176 rpm, dan masa pengeraman 122 h) iaitu 5.0 g/L P(3HB-co-3HV) dan 7 mol% daripada 3HV monomer, lebih tinggi berbanding dengan keadaan tidak dioptimumkan (kepekatan 1-pentanol 0.06 wt%, kadar agitasi 200 rpm, dan masa pengeraman 108 h). Sebagai perbandingan dengan keadaan tidak dioptimumkan, peningkatan telah diperhatikan dengan 14% dalam

kepekatan kopolimer dan dua kali ganda dalam komposisi 3HV masing-masing. Kopolimer yang dihasilkan dalam kajian ini adalah lebih baik daripada P(3HB) dari segi sifat fizikal and haba. P(3HB-*co*-3HV) mempunyai penghabluran dan suhu lebur yang lebih rendah dan pemanjangan untuk putus yang lebih tinggi berbanding dengan P(3HB). P(3HB-*co*-3HV) yang dihasilkan telah ditentukan sebagai copolimer blok dengan menggunakan nuklear magnetik resonans (NMR). kesimpulannya, kajian ini merupakan platform selanjutnya bagi penghasilan P(3HB-*co*-3HV) berskala besar menggunakan *M. haematophila* UMTKB-2.

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“The more I learn, the more I realize how much I don't know”

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APPROVAL

I certify that an Examination Committee has met on 27th June 2018 to conduct the final examination of Jong Tse kiun, on his Master of Science thesis entitled “**Biosynthesis of polyhydroxyalkanoate by *Massilia haematophila* UMTKB-2 using optimized culture parameters and its polymer characterization**” in accordance with the regulations approved by the Senate of Universiti Malaysia Terengganu. The Committee recommends that the candidate be awarded the relevant degree. The members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that that the thesis is based on my original work except for quotations and citations, which have been duly, acknowledge. I also declare that is has not been previously or concurrently submitted for any other degree at UMT or other institutions.

JONG TSE KIUN

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LIST OF ABBREVIATIONS

| | |
|---|-------------------------------------|
| $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ | Calcium chloride dihydrate |
| Cm | Centimeter |
| $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ | Cobalt(II) sulfate heptahydrate |
| $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ | Copper(II) chloride dihydrate |
| COA | Coenzyme |
| HCl | Hydrochloric acid |
| $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ | Iron(II) sulfate heptahydrate |
| g | gram |
| g/L | Gram per liter |
| GPa | Gigapascal |
| h | Hour |
| KDa | Kilodalton |
| kPa | Kilopascal |
| KH_2PO_4 | Potassium dihydrogen phosphate |
| mol% | Percentage of monomer |
| mL | Milliliter |
| mm | Millimeter |
| mL/min | Milliliter per minute |
| mg | Milligram |
| mg/mL | Milligram per milliliter |
| min | Minute |
| MPa | Megapascal |
| $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ | Manganese(II) chloride tetrahydrate |

| | |
|--------------------------------------|--|
| MgSO ₄ ·7H ₂ O | Magnesium sulphate heptahydrate |
| N | Normality |
| n.m | Nanometers |
| Na ₂ HPO ₄ | Disodium hydrogen phosphate |
| NH ₄ Cl | Ammonium chloride |
| Na ₂ SO ₄ | Sodium sulfate anhydrous |
| NADPH | Nicotinamide Adenine Dinucleotide Phosphate Hydrogen |
| psi | Pounds per square inch |
| PTFE | Polytetrafluoroethylene |
| rpm | Revolutions per minute |
| US\$ | US dollar |
| wt % | Percentage of weight |
| ZnSO ₄ ·2H ₂ O | Zinc sulfate dihydrate |
| μL | Microliter |
| % (w/v) | Percentage of weight per volume |
| % (v/v) | Percentage of volume per volume |
| °C | Degree celcius |
| °C/min | Degree celcius per min |

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