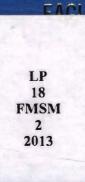
ISOLATION OF POLYHYDROXYALKANOATE PRODUCING BACTERIA FROM SEDIMENT COLLECTED AT SETIU

MUHAMMAD AMIRRUL RASYID BIN AHMAD



EACULTY OF MARITIME STUDIES AND MARINE SCIENCE UNIVERSITI MALAYSIA TERENGGANU

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Final Research Project Report Declaration and Verification Form



DEPARTMENT OF MARINE SCIENCE FACULTY OF MARITIME STUDIES AND MARINE SCIENCE UNIVERSITI MALAYSIA TERENGGANU

DECLARATION AND VERIFICATION REPORT FINAL YEAR RESEARCH PROJECT

It is hereby declared and verified that this research report entitled: Isolation of Polyhydroxyalkanoate Producing Bacteria from Sediment Collected at Setiu by Muhammad Amirrul Rasyid Bin Ahmad Matric no. UK22831 have been examined and all errors identified have been corrected. This report is submitted to the Department of Marine Science as partial fulfillment towards obtaining the Degree Bachelor of Science (Marine Biology), Faculty of Maritime Studies and Marine Science, Universiti Malaysia Terengganu.

Verified by:

Principal Supervisor Dr. Kesaven Bhubalan

Official stamp:

Date: 13/6/2013

DR. KESAVEN BHUBALAN LECTURER DEPARTMENT OF MARINE SCIENCE FACULTY OF MARITIME STUDIES AND MARINE SCIENCE UNIVERSITI MALAYSIA TERENGGANU (UMT) 21030 KUALA TERENGGANU Isolation of Polyhydroxyalkanoate Producing Bacteria from Sediment Collected at

Setiu

By Muhammad Amirrul Rasyid Bin Ahmad

Research Report submitted in partial fulfilment of the requirement for the degree of Bachelor of Science (Marine Biology)

Department of Marine Science

Faculty of Maritime Studies and Marine Science

UNIVERSITI MALAYSIA TERENGGANU

2013

This project should be cited as :

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List of abbreviations and symbol

% mol	Mol percent
μ	Micro
C ₁₀	Carbon-1()
C ₁₂	Carbon-12
C ₁₄	Carbon-14
CaCl ₂	Calcium chloride
CDW	Cell dry weight
СМЕ	Caprylic methyl ester
СоА	Coenzyme-A
CoCl ₂ ·6H ₂ O	Cobalt (II) chloride hexahydrate
CrCl ₃ ·6H ₂ O	Chromium chloride hexahydrate
CuSO ₄ ·5H ₂ O	Copper sulfate pentahydrate
FeCl ₃	Iron (III) chloride
G	Gram
H ₂ O	Water
H_2SO_4	Sulphuric acid
KH ₂ PO ₄	Potassium dihydrogen phosphate
kPa	Kilo pascal
L	Liter
MCL-PHA	Medium chain length PHA
Mg	Milligram

min	Minute
mL	Millilitre
MS Agar	Mineral salt agar
MSM	Mineral salt medium
Na ₂ HPO ₄	Disodium hydrogen phosphate
Na ₂ SO ₄	Sodium sulphate
NH ₄ Cl	Ammonium chloride
NiCl ₂ ·6H ₂ O	Nickel chloride hexahydrate
NMR	Nuclear magnetic resonance
NR Agar	Nutrient rich agar
NRM	Nutrient rich medium
°C	Degree Celcius
P(3HB)	poly(3-hydroxybutyrate)
P(3HB) P(3HB- <i>co</i> -3HHx)	poly(3-hydroxybutyrate) poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyhexanoate)
P(3HB- <i>co</i> -3HHx)	poly(3-hydroxybutyrate-co-3 hydroxyhexanoate)
P(3HB- <i>co</i> -3HHx) P(3-HB- <i>co</i> -3HHx).	poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3-hydroxyhexanoate)
P(3HB- <i>co</i> -3HHx) P(3-HB- <i>co</i> -3HHx). P(3HB- <i>co</i> -3HV)	poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3-hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyvalerate)
P(3HB- <i>co</i> -3HHx) P(3-HB- <i>co</i> -3HHx). P(3HB- <i>co</i> -3HV) P(3HD)	poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3-hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyvalerate) poly (3-hydroxydecanoate)
P(3HB- <i>co</i> -3HHx) P(3-HB- <i>co</i> -3HHx). P(3HB- <i>co</i> -3HV) P(3HD) P(3HDD)	poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3-hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyvalerate) poly (3-hydroxydecanoate) poly(3-hydroxydodecanoate)
P(3HB- <i>co</i> -3HHx) P(3-HB- <i>co</i> -3HHx). P(3HB- <i>co</i> -3HV) P(3HD) P(3HDD) P(3HHp)	poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3-hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyvalerate) poly (3-hydroxydecanoate) poly(3-hydroxydodecanoate) poly (3-hydroxyheptanoate)
P(3HB- <i>co</i> -3HHx) P(3-HB- <i>co</i> -3HHx). P(3HB- <i>co</i> -3HV) P(3HD) P(3HDD) P(3HHp) P(3HHx)	poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3-hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyvalerate) poly (3-hydroxydecanoate) poly(3-hydroxydodecanoate) poly (3-hydroxyheptanoate) poly (3-hydroxyheptanoate)
P(3HB- <i>co</i> -3HHx) P(3-HB- <i>co</i> -3HHx). P(3HB- <i>co</i> -3HV) P(3HD) P(3HDD) P(3HHp) P(3HHx) P(3HHx- <i>co</i> -3HO)	poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3-hydroxyhexanoate) poly(3-hydroxybutyrate- <i>co</i> -3 hydroxyvalerate) poly (3-hydroxydecanoate) poly(3-hydroxydodecanoate) poly (3-hydroxyheptanoate) poly (3-hydroxyhexanoate) poly (3-hydroxyhexanoate- <i>co</i> -3-hydroxyoctanoate)

PCR		Polymerase Chain Reaction
rpm		Revolution per minute
SCL-PHA		Short chain length PHA
TCA		Tricarboxylic acid
wt %		Percentage per weight
В	Ξ.	Beta

List of appendices

Appendix I	Report of gas chromatography of strain A22 10)-6 1.
Appendix II	Report of gas chromatography of strain A22 10) ⁻⁶ 2.

ABSTRACT

Gram negative strains, A 22 10⁻⁶ 1 and A 22 10⁻⁶ 2 were isolated from sediment collected at Setiu. Both mangrove strains potential are polyhydroxyalkanoate (PHA) producer after shown positive results in screening for PHA production with Nile red stain when emitted pink fluorescence under UV light. Biosynthesis of PHA by both strains were carried out with glucose, fructose and oleic acid as carbon sources. GC analysis on dried sample of both strains from biosynthesis enriched with glucose and fructose detected production of medium chain length PHA. Strain A22 10⁻⁶ 1 detected in production of C₁₀, poly(3-hydroxydecanoate) P(3HD), C_{12} , poly(3-hydroxydodecanoate) P(3HDD) and C_{14} , poly(3-hydroxytetradecanoate) P(3HTD) while A 22 10⁻⁶ 2 detected for production of P(3HD) and P(3HTD).

ABSTRAK

Pengasingan bakteria yang menghasilkan polihidroksialkanoat daripada sedimen yang dikumpulkan di Setiu

Strain Gram negatif, A 22 10⁻⁶ 1 and A 22 10⁻⁶ 2 telah diasingkan dari sedimen di persekitaran bakau yang dikumpulkan dari Setiu. Kedua-dua strain adalah berpotensi menghasilkan polihidroksialkanoat (PHA) selepas menujukkan keputusan positif dalam saringan terdadap penghasilan PHA dengan pewarnaan 'Nile red' memancarkan florescen merah jambu di bawah cahaya ultra ungu. Biosintesis PHA oleh kedua-dua strain dilakukan dengan menggunakan glukosa fruktosa dan asid olik sebagai sumber karbon. Analisis gas kromatografi ke atas sampel kering kedua dua strain daripada biosintesis yang diperkaya dengan glukosa dan fruktosa mengesan penghasilan PHA rantaian sederhana panjang. Strain A 22 10⁻⁶ 1 dikesan dalam penghasilan C_{10} , poly(3-hydroxydecanoate) P(3HD), C₁₂, poly(3hydroxydodecanoate) P(3HDD) and C_{14} , poly(3-hydroxytetradecanoate) P(3HTD) manakala A 22 10^{-6} 2 dikesan dalam penghasilan P(3HD) and P(3HTD).