UNIVERSITI TEKNOLOGI MARA

SCREENING AND ISOLATION OF POLYHYDROXYALKANOATES (PHA)-PRODUCING BACTERIA FROM LANDFILL BY USING COCOA POD HUSKS AS CARBON SOURCE

NURFARAHANA JASMIN BINTI MOHAMAD YUSUF

Dissertation submitted in partial fulfilment
of the requirements for the degree of
Master of Science
(Applied Biology)

Faculty of Applied Sciences

April 2018

ABSTRACT

Polyhydroxyalkanoates (PHA) are bioplastics, produced by various bacteria as food and energy reservoir. PHA is an alternative for synthetic plastic because they are environmentally friendly and can be degraded naturally by microorganisms. One of the important factors for the growth of PHA producing bacteria is an excess of carbon supply. In order to reduce the overall cost of PHA production, a low cost pure substrate, which is cocoa pod husks (CPH) was used as a carbon source. The objectives of this study were to isolate and screen PHA producing bacteria from landfill samples which are leachate and soil, to identify the PHA producing bacteria by using morphological characterization and *16s rDNA* gene sequencing and to determine the best percentage of CPH that can be used as a carbon source for PHA producing bacteria. PHA producing bacteria from leachate and soil from landfill in Jeram, Selangor were screened by using Nile Blue A staining method. Two potential PHA producers with the brightest fluorescence under UV light from each samples were isolated and characterized by using morphological and molecular identification. Results of morphological identification shows all bacterial isolates have a rod shape and have a capsule, three bacterial isolates have an endospore while the remaining does not have endospore. Three out of four were Gram positive bacteria and the remaining was Gram negative bacteria. These isolates were confirmed of their identity as *K. pneumoniae, B. cereus* and *B. toyonenis* using *16s rDNA* gene sequencing. Different concentration of CPH, which are 2% (w/v), 5% (w/v), 7% (w/v) and 10% (w/v) were used to study the best percentage of CPH that can be used as carbon source. PHA accumulation was the highest at 7% (w/v) for all bacterial species tested and lowest at 10% (w/v) CPH except for *B. toyonensis.*

ACKNOWLEDGEMENT

Alhamdulillah, first of all, I would like to thank Almighty Allah for everything in my life, who bless to all for his divide throughout my life. I am really grateful because I managed to complete this research within the time given with the help of many sources.

Foremost, I would like to use this opportunity to express the greatest gratitude to my supervisor, Dr Wan Razarinah Binti Wan Abdul Razak and my co-supervisor, Dr Aziyah Binti Abd. Aziz for their guidance, advices and encouragement. Thank you for the patience and ideas in assisting me with this project.

My sincere thanks also goes to my parent, Mohamad Yusuf Bin Aman, Sabariah Binti Ibrahim and all my friends especially Nasrul Nafis bin Ibrahim for their support and advices. Without them, it would be very difficult to overcome the challenges. This project would not have been possible without the support, critique, guidance and direction from those who associated with my academic and personal life.
Last but not least, I would like to thanks Fundamental Research Grant Scheme (FRGS) for the financial support for this research. The completion of this research could not have been possible without the assistance of many people who names may not all be numerated. Thank you to all people who directly or indirectly involved in order to complete this research.

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 Background of Study

Tons of plastic that have been extensively used in our daily lives caused environmental problems. These plastics petrochemical-based plastics, which cannot be decomposed by natural processes (Verlinden *et al.*, 2007; Dietrich, 2016). It takes up to 400 years for the plastics to decompose (Parker, 2017). Parker (2017) reported that over 8.3 billion metric tons of plastics have been produced from six decades ago and from the amount, about 6.3 billion metric tons have become environmental waste. From that, less than ten percent of plastics has been successfully recycled and the remaining is loaded up in landfills. Thus, overconsumption and overproduction of non-degradable polymer has raised concerns and increased the desire for production of biodegradable plastics especially from renewable sources (Ntaikou, 2014).

Nowadays, bio-polyester or bio-based plastics have captured attention because they has high potential in the future towards sustainable environment and can be naturally decomposed by microorganisms in natural environments (Lee *et al.*, 2016). There are many types of biodegradable plastics and one of them is Polyhydroxyalkanoates (PHA), one of the well known polymers for production of biodegradable plastics, which can be acquired from microorganisms or plants (Dietrich, 2016). For the production of biodegradable polymer, there are two components needed which are carbon sources for the growth of bacteria and PHA-producing bacteria (Santhanam, 2010). PHA is favoured because they have similar physiochemical properties as synthetic plastics and can be degraded in environment (Motamedi, 2015).

PHA are environmentally friendly, biodegradable and also biocompatible bioplastics, that can be used as an alternative for synthetic plastics (Reddy *et al.*, 2003). PHA serves as energy and carbon storage material in the bacterial cells that can be used during limited carbon supply (Koller *et al.* 2013). Chen (2009) reported that PHA can be used in a variety of disposable packaging goods and may also have high-value applications in medicine and pharmaceutical industry. One of the advantages of PHAs compared to other biodegradable plastic is that PHA can