IDENTIFICATION OF POLYSTYRENE DEGRADING BACTERIA AND POTENTIAL ENZYME ACTION FROM *ZOPHOBAS MORIO* FOR IMPROVEMENT AND SUSTAINABLE BIODEGRADATION OF WASTE

TAN KIAN MENG

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DEDICATION

Special dedication to my loving parents,
and brother,
all my teachers and friends,
my fellow lab-mates and faculty members

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Praise

Him

Daily!

(P.H.D.)

ABSTRACT

Polystyrene (PS) is a widely used petroleum-based plastic polymer. However, it is recalcitrant towards degradation and expected to remain longer in the environment which leads to a major waste problem. Moreover, conventional PS disposal and waste management consume massive amounts of chemicals and energy which have deleterious effects on the environment. Therefore, the aim of this research is to explore an alternative approach for PS biodegradation by identifying PS-degrading bacteria and the potential enzyme action isolated from the gut of PS feeding insect, *Zophobas morio*. The bacteria that capable of degrading PS was isolated and presumed identified and characterised as *Bacillus megaterium*. The viability and metabolic activity of *Bacillus megaterium* to utilise PS as sole carbon source were validated through indicator redox probe 2,3,5-triphenyltetrazolium chloride. Biodegradability of *Bacillus megaterium* corroborated by biofilm quantification, FESEM and FTIR analysis. Biofilm formed and morphological changes induced by degradation were clearly viewed via FESEM and the chemical bonds alteration was detected by FTIR analysis. The expressed PDE were profiled with SDS-PAGE and the three most visible bands were obtained at 20, 45 and 60 kDa. The protein sequencing results revealed the 20 kDa protein (Isochorismatase), 45 and 60 kDa (insignificant matches). The *de novo* sequencing of 60 kDa protein showed similarity to trypsin (*Bos Taurus*), whilst BLAST results revealed that it is a serine hydrolase enzyme. In summary, the 60 kDa protein is a multi-domain protein, with one domain functioning identified as a hydrolase. The whole protein is a hypothetical protein, with no annotated function. Thus, the results revealed unidentified, perhaps novel protein. Furthermore, PS enzymatic degraded products were identified through GC/MS analysis as part of the complementary study. The conversion pathways of PS to various aromatic and non-aromatic compounds were also proposed. The findings of this research provide insight into PS biodegradation and its potential application in waste management and polymer industries, thus encouraging the use of green and sustainable technology.

ABSTRAK

Polistirena (PS) adalah polimer plastik berasaskan petroleum yang digunakan secara meluas. PS mempunyai ketahanan yang tinggi terhadap degradasi dan berkekalan lama dalam persekitaran telah menimbulkan masalah persekitaran utama. Selain itu, pengurusan konvensional pelupusan PS memerlukan banyak bahan kimia dan tenaga, memberi kesan buruk kepada alam sekitar. Tujuan penyelidikan ini adalah untuk meneroka pendekatan alternatif untuk biodegradasi PS dengan mengenal pasti bakteria yang mendegradasikan PS dan potensi tindakan enzim terpencil dalam usus serangga *Zophobas morio.* Spesies bakteria yang mampu mengurai PS telah dikenalpasti dan dicirikan sebagai *Bacillus megaterium*. Aktiviti metabolik *Bacillus megaterium* menggunakan PS sebagai sumber karbon telah disahkan melalui 2,3,5-triphenyltetrazolium klorida. Keupayaan biodegradasi *Bacillus megaterium* disahkan melalui kuantifikasi biofilm, analisasi FESEM dan FTIR. Biofilm yang terbentuk dan perubahan morfologi yang disebabkan degradasi jelas dilihat melalui FESEM dan perubahan ikatan kimia dikesani dengan FTIR. Enzim degradasi PS terhasil telah diprofil dengan SDS-PAGE dan tiga jalur yang ternyata diperolehi pada 20, 45 dan 60 kDa. Keputusan penjujukan protein menunjukkan 20 kDa protein (Isochorismatase), 45 dan 60 kDa (pemandanan tidak nyata). Penjujukan *de novo* bagi protein 60 kDa menunjukkan persamaan dengan trypsin (*Bos Taurus*), keputusan BLAST mendedahkan bahawa ia adalah enzim hidrolase serine. Secara keseluruhannya, protein 60 kDa adalah protein pelbagai domain, dengan satu domain dikenalpasti berfungsi sebagai hidrolase. Keseluruhan protein adalah protein hipotesis, tanpa fungsi anotasi buat sementara ini. Keputusan penjujukan *de novo* mendedahkan informasi baru, kemungkinan satu protein baru yang masih belum dikenalpasti. Tambahan pula, produk enzim PS telah dikenalpasti melalui analisasi GC/MS sebagai sebahagian daripada kajian pelengkap. Laluan penukaran PS kepada pelbagai sebatian aromatik dan bukan aromatik turut dicadangkan. Hasil kajian ini menggalakkan potensi penggunaan teknologi hijau dan lestari dalam pengurusan sisa dan industri polimer.

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LIST OF SYMBOLS AND ABBREVIATIONS

APS	-	Ammonium persulfate
ASTM	-	American Society for Testing and Materials
BLAST	-	Basic Local Alignment Search Tool
BSA	-	Bovine serum albumin
C=C	-	Carbon-carbon double bonds
-C=O	-	Carbonyl group
C-H	-	Carbon hydrogen bonds
C-OH	-	Alcohol group
C–OR	-	Ester bond
CYP450s	-	Cytochrome P450 enzymes
dH_2O	-	Distilled water
EPS	-	Expanded polystyrene
ETS	-	Electron transport system
FESEM	-	Field Emission Scanning Electron Microscopy
$FeSO_4 \cdot 7H_2O$	-	Iron sulphate
FTIR	-	Fourier Transform Infrared Spectroscopy
GC/MS	-	Gas Chromatography Mass Spectrometry
H_2O_2	-	Hydrogen peroxide
HIPS	-	High impact polystyrene
HPLC	-	High pressure liquid chromatography
K_2HPO_4	-	Dipotassium phosphate
kDa	-	Kilo Dalton
KH_2PO_4	-	Potassium phosphate

LB	-	Luria broth
LCFBM	-	Liquid carbon free basal medium
LC-MS	-	Liquid chromatography mass spectrometry
LDPE	-	Low density polyethylene
LLE	-	Liquid-liquid extraction
$MgSO_4.7H_2O$	-	Magnesium sulphate
$MnSO_4 \cdot H_2O$	-	Manganate sulphate
MS	-	Mass spectrometry
NaCl	-	Sodium chloride
NATA	-	National Association of Testing Authorities
NCBI	-	National Centre for Biotechnology Information
NH_4NO_3	-	Ammonium nitrate
NIST	-	National Institute of Standards and Technology
PAH	-	Polyaromatic hydrocarbons
PCR	-	Polymerase chain reaction
PDB	-	Polystyrene degrading bacteria
PDE	-	Polystyrene degrading enzyme
PE	-	Polyethylene
PET	-	Polyethylene terephthalate
PS	-	Polystyrene
RSM	-	Response surface methodology
SDS	-	Sodium dodecyl sulphate
SDS-PAGE	-	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SIM	-	Selected ion monitoring
TEMED	-	Tetramethylethylenediamine
TPF	-	Triphenyl formazan
TTC	-	Triphenyltetrazolium chloride
XPS	-	Extruded polystyrene
$ZnSO_4 \cdot H_2O$	-	Zinc sulphate

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CHAPTER 1

INTRODUCTION

1.1 Overview of research

Since the 1950s, the production of petroleum-based synthetic plastic has outpaced that of almost every other material. This is due to its versatility and functionality eventually the global usage of petroleum-based synthetic plastics has increased yearly. Expanded polystyrene (EPS) foam, commonly known as Styrofoam, is one of the main products of polystyrene (PS) has contributed to 'white pollution'- one of the major marine pollutions. Increasing landfill costs and decreasing landfill space are forcing consideration of alternative options for the disposal of PS materials. Apparently, PS alone has silted up one-third of the world's landfills (Savoldelli *et al*., 2017), it is rather urgent to look for a green and cost-effective alternative for PS degradation.

This thesis investigates the ability of the polystyrene feeding insect, *Zophobas morio* (Superworm) to utilise and assimilate polystyrene as a carbon source. It is believed the insect larva's gut microbiome plays a vital role in assimilating the synthetic plastic material. It has drawn the research's attention to identify the polystyrene degrading bacteria (PDB) and its potential enzyme action for the improvement and sustainable biodegradation of polystyrene waste.

In this research, gut bacteria with polystyrene degrading capability were isolated and identified. The isolated PDB was used as inoculum for polystyrene degrading enzyme (PDE) expression in polystyrene induced media. Meanwhile, the functionalities of PDE were characterised by proteomic technologies. The proteomic technologies facilitated the study of the interaction between different proteins and the role they play in the enzymatic degradation of polystyrene**.**

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APPENDIX C

APPENDIX C: LIST OF PUBLICATIONS

1. **Tan, K. M.,** Mohd Fauzi, N. A., Mohd Kassim, A. S., A Razak, A. H., & Kamarudin, K. R. (2020). Isolation and Identification of Polystyrene Degrading Bacteria from *Zophobas morio's* Gut Based on the Nucleotide Sequences of the 16S rRNA Gene: Isolation and Identification of Polystyrene Degrading Strain. *Walailak Journal of Science and Technology (WJST), 18*(8), Article 9118 (11 pages). Retrieved from https://doi.org/10.48048/wjst.2021.9118

2. **Tan, K. M.,** Mohd Fauzi, N. A., Mohd Kassim, A. S., & A Razak, A. H. (2020). *Bacillus megaterium*: A Potential and An Efficient Bio-Degrader of Polystyrene. *Brazilian Archives of Biology and Technology,* 64 **(Accepted Article)**.

3. **Tan, K. M.,** Fauzi, N. A. M. Kassim, A. S. M. & Razak, A. H. A. (2018). Optimization of Polystyrene Biodegradation using Response Surface Methodology (RSM) Measured by Simple Colorimetric Method. *International Journal of Engineering and Technology*, 7, 216–220.

4. **Tan, K.M.,** Fauzi, N.A.M., & Kassim, A.S.M (2017) The Extraction of Protein from Superworm (*Zophobas morio*) Using Saline Treatment (NaCl) Method, *Journal of Engineering and Applied Sciences, Medwell Journals*, 12, 6953, ISSN:1816-949X.

APPENDIX D

APPENDIX D: LIST OF AWARDS

1. Gold Medal Award for Research and Innovation Award, Invention: **"Polystyrene degrading strain and its enzyme production**", Universiti Tun Hussein Onn Malaysia (UTHM), September 2019.

2. Silver Medal Award for International Research and Innovation Award, Invention: **"Polystyrene degrading strain and its enzyme production**", Universiti Tun Hussein Onn Malaysia (UTHM), September 2019.

3. Bronze Medal Award for Research and Innovation Award, Invention: **"Free from Styrofoam**", Universiti Tun Hussein Onn Malaysia (UTHM), November 2017.

4. **Best of the Presenter Award** for a presentation entitled "Optimization of Polystyrene Biodegradation Using Response Surface Methodology (RSM) Measured by Simple Colorimetric Method", 2nd International Conference on Applied Sciences and Industrial Technology (ICASIT, 2018)

VITA

The author was born on February 13, 1992, in Kuala Lumpur, Malaysia. She went to SMK Desa Petaling, Kuala Lumpur for her secondary school and did her high school at SMK Taman Desa, Kuala Lumpur. Upon high school completion, she pursued her degree at Universiti Tun Hussein Onn Malaysia (UTHM) and graduated with Bachelor of Chemical Engineering Technology (Biotechnology) with Honours (First Class Honours) in 2016. Then, she pursues Ph.D study by attending the Postgraduate School of Universiti Tun Hussein Onn Malaysia (UTHM) in 2016. During this period, she was a research assistant under the supervision of Dr. Noor Akhmazillah Mohd Fauzi, co-supervised by Assoc. Prof. Dr. Angzzas Sari Mohd Kassim. She has successfully managed to write and secure a research grant for her Ph.D research; GPPS (U769) under the guidance and the assistance from her supervisors. Throughout Ph.D study, she participated in the in-house 3 Minute Thesis (3MT) presentation. Her research works are recognised by the university when she was awarded with bronze medal I year 2018, silver medal and gold medal in year 2019 in UTHM Research and Innovation Competition. Tan has authored four papers in the field of Microbiology, Engineering and Applied Science. She is currently exploring the world with her expertise in the field of chemical engineering and biotechnology besides being at the forefront to make the world a better place to live in.