ISOLATION AND CHARACTERIZATION OF MESOPHILE PLA-DEGRADING BACTERIA FROM LANDFILL SOIL

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MASTER OF SCIENCE

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SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis, and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Asid polilaktik (PLA) ialah biopolimer yang diperkenalkan sebagai pilihan alternatif untuk plastik berasaskan petroleum. Walau bagaimanapun, apabila PLA dibuang di tapak pelupusan sampah, ia tidak akan terurai sepenuhnya kerana kadar penguraian optimum berlaku dalam keadaan termofilik. Objektif kajian ini adalah untuk mengisolasikan dan mencirikan bakteria yang diambil dari tapak tanah pelupusan sampah yang mampu menguraikan PLA pada suhu mesofilik. Sampel tanah diambil dari tapak pelupusan sampah di Pekan, Pahang, dan proses pengasingan dilakukan melalui pencairan bersiri sehingga faktor pencairan (DF) 10^{-10}. Keputusan menunjukkan bahawa dua isolat, yang ditetapkan sebagai B8A dan A10B, telah dipilih dan dikenal pasti sebagai *Brevibacillus parabrevis* dan *Renibaterium salmoninarum*, masing-masing. Daripada keputusan, kedua-dua isolat dicirikan lagi dalam kajian ini. Pencirian isolat bakteria telah dijalankan melalui kajian morfologi, pewarnaan Gram, ujian katalase, kehadiran enzim protease, dan aktiviti enzim relatif. Isolat B8A mempunyai ciri morfologi punctiform, termasuk ketinggian rata dan jidar melengkung. Manakala isolat A10B dicirikan secara morfologi sebagai mempunyai bentuk bulat, ketinggian rata dan keseluruhan jidar. Selain itu, isolat-isolat B8A dan A10B juga adalah gram-positif dan katalase-positif. Apabila dicoret pada agar susu skim, kedua-dua isolat memaparkan pembentukan zon halo yang mengesahkan kehadiran enzim protease yang dirembeskan oleh bakteria. A10B menunjukkan aktiviti enzim relatif tinggi iaitu 3.86, manakala isolate B8A lebih rendah sedikit pada 1.33. Pengasingan B8A dan A10B kemudiannya dicirikan dengan memerhatikan lengkung pertumbuhan dan aktiviti enzim bagi enzim protease, lipase, dan amilase. Bagi ujian enzim, enzim protease menunjukkan aktiviti tertinggi masing-masing pada 120.45 ± 1.04 U/mL dan 114.49 ± 0.37 U/mL untuk isolat A10B dan B8A. Manakala, aktiviti lipase untuk A10B ialah 9.23 ± 0.02 U/mL dan 15.36 ± 0.01 U/mL untuk B8A. Aktiviti enzim yang paling rendah ialah amilase, dengan 6.56 ± 0.003 U/mL dan 3.17 ± 0.001 U/mL, masing-masing, untuk A10B dan B8A. Di bawah keadaan yang dioptimumkan (kandungan lembapan 55 ± 5 %, pH 7.5 ± 0.5), pencilan telah diuji untuk kebolehbiodegradasian PLA dalam tanah untuk tempoh 8 minggu. Ujian biodegradasi PLA yang ditanam di dalam tanah yang disuntik dengan bakteria A10B mencatatkan penurunan berat badan sebanyak 32.6%, manakala bakteria B8A mencatatkan penurunan berat badan sebanyak 37.8% daripada berat awal PLA. Filem PLA ini dihantar untuk analisis *Scanning Electron Microscopy* (SEM) dan dibandingkan dengan filem awal, yang menunjukkan tanda-tanda penguraian pada permukaan PLA, oleh itu membuktikan keupayaan kedua-dua bakteria untuk menguraikan PLA dalam tanah tertimbus. Kesimpulannya, penyelidikan ini menunjukkan bahawa kedua-dua bakteria *Brevibacillus parabrevis* dan *Renibaterium salmoninarum* mempunyai potensi untuk merendahkan PLA pada suhu mesofilik, yang menunjukkan bahawa penyelidikan lanjut dalam bidang ini boleh membantu membangunkan teknik bioremediasi baru, meningkatkan kaedah semasa dan membangunkan laluan baharu untuk memulihkan PLA polimer menggunakan bakteria.

ABSTRACT

Polylactic acid (PLA) is a biopolymer introduced as an alternative to petroleum-based plastics. However, when PLA is discarded in landfills, it will not degrade entirely because it is optimally degraded in thermophilic conditions. Therefore, the isolation and characterization of PLA-degrading bacteria from landfill soil were conducted. The objectives of this study were to isolate and characterize PLA-degrading bacteria from local landfill soil at mesophilic temperature. The soil sample was collected from a landfill site in Pekan, Pahang, and the isolation process was done via serial dilution up to a dilution factor (DF) of 10^{-10}. The results revealed that two isolates, designated as B8A and A10B, were selected and identified as *Brevibacillus parabrevis* and *Renibaterium salmoninarum,* respectively. From the results, both isolates were further characterized in this study. The characterization of bacterial isolates was conducted through morphological studies, Gram staining, the catalase test, the presence of the protease enzyme, and relative enzyme activity. Isolate B8A has punctiform morphological characteristics, including a flat elevation and a curled margin. In comparison, isolate A10B was morphologically characterized as having a circular form, flat elevation, and entire margin. Isolates B8A and A10B were also gram-positive and catalase-positive. When streaked on skim milk agar, both isolates displayed halo zone formation, confirming the presence of the protease enzyme secreted by the bacteria. A10B showed a high relative enzyme activity of 3.86, while isolate B8A is slightly lower at 1.33. Isolates B8A and A10B were further characterized by observing the growth curves and enzyme activities for protease, lipase, and amylase enzymes. For the enzyme assays, the protease enzyme shows the highest activity at 120.45 ± 1.04 U/mL and 114.49 ± 0.37 U/mL for isolates A10B and B8A, respectively. Meanwhile, lipase activity for A10B was 9.23 ± 0.02 U/mL and 15.36 ± 0.01 U/mL for B8A. The lowest enzyme activity was amylase, with 6.56 ± 0.003 U/mL and 3.17 ± 0.001 U/mL, respectively, for A10B and B8A. Under optimised conditions (55 ± 5 % moisture content, pH 7.5 ± 0.5), the isolates were tested for their PLA biodegradability in soil for a duration of 8 weeks. The biodegradation test of PLA buried in soil inoculated with isolate A10B recorded a weight loss of 32.6%, while isolate B8A recorded a weight loss of 37.8% from the initial PLA weight. These PLA samples were sent for SEM analysis and compared to the initial film, which showed decomposition signs on the PLA surface, therefore proving the ability of both isolates to degrade PLA in buried soil. In conclusion, this research demonstrates that both bacteria *Brevibacillus parabrevis* and *Renibaterium salmoninarum* have the potential to degrade PLA at mesophilic temperatures, which suggests that further research in this field may help develop novel bioremediation techniques, enhance current methods, and develop new pathways for recovering PLA polymers using bacteria.

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