# MOLECULAR DETECTION OF *rpoB* GENE IN *Bacillus licheniformis* VIA POLYMERASE CHAIN REACTION ASSAY

**AHMAD NOR AIMAN BIN MOHAMAD MURAD**

**BACHELOR OF SCIENCE (Hons.) BIOLOGY**
**FACULTY OF APPLIED SCIENCES**
**UNIVERSITI TEKNOLOGI MARA**

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**AHMAD NOR AIMAN BIN MOHAMAD MURAD**

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This Final Year Project Report entitled **"Molecular Detection of *rpoB* gene in *Bacillus licheniformis* via Polymerase Chain Reaction (PCR) assay"** was submitted by Ahmad Nor Aiman Bin Mohamad Murad in partial fulfilment of the requirements for the Degree of Bachelor of Science (Hons.) Biology, in the Faculty of Applied Sciences, and was approved by

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Dr. Roziana Mohamed Hanaphi
Supervisor
B.Sc. (Hons.) Biology
Faculty of Applied Sciences
Universiti Teknologi MARA
02600 Arau
Perlis

_____

Muhammad Syukri Noor Azman
Project Coordinator
B.Sc. (Hons.) Biology
Faculty of Applied Sciences
Universiti Teknologi MARA
03600 Arau
Perlis

_____

Dr. Rosyaini Afindi Zaman
Coordinator of Programme
B.Sc. (Hons.) Biology
Faculty of Applied Science
Universiti Teknologi MARA
03600 Arau
Perlis

Date: _____

## ABSTRACT

### MOLECULAR DETECTION OF *rpoB* GENE IN *Bacillus licheniformis* VIA POLYMERASE CHAIN REACTION ASSAY

*Bacillus* species is a rod shaped, Gram-positive bacteria which are abundantly found in various soil environments such as landfill, mangrove and agricultural soils. The bacteria are able to thrive in landfill site due to the high availability of organic and inorganic waste compared to other soils. The aims of this study were to isolate and detect *Bacillus licheniformis* which possess high potential in bioactivities such as plastic degradation and bioremediation of waste. The common method such as culture media and biochemical tests were used for identification of bacteria isolated from the soils. The landfill soil sample have the highest concentration of bacteria ($42.046 \times 10^{10}$ CFU/ml) compared to mangrove and agricultural soils. The *Bacillus* detected from these soil samples were *Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Paenibacillus macerans* and unidentified *Bacillus*. Among all the *Bacillus* spp. obtained, *B. licheniformis* known to have potential in environmental related bioactivities. The molecular approach was used to specifically identify *Bacillus licheniformis* with the use of bioinformatics tools and PCR. The target organism of *Bacillus licheniformis* was detected using *rpoB* gene. *rpoB* gene is a specific gene of *Bacillus licheniformis* which responsible for spore production mechanism against heat and encoding beta (β) subunit for most RNA polymerase. The bioinformatics tools used in this study was BLAST, ClustalW, OligoNucleotide and *In-silico* PCR amplification. The primers obtained was forward primer (5'-GCGTCGGTGATGAGGTTG-3') and reverse primer (5'-CGTCTTTTACAAGGCGTTCG-3'). The PCR had carried out optimization of parameters for annealing temperature ($T_{a)}$ and concentration of $MgCl_2$. The results obtained the amplicons at 162 bp with optimal annealing temperature of 55.6 ºC and $MgCl_2$ concentration of 4 mM. In conclusion, the identification of *Bacillus licheniformis* using PCR assay method via *rpoB* gene was a rapid, specific and practical method to be used for the species detection.



**Keywords:** *Bacillus licheniformis*, Polymerase chain reaction (PCR), PCR optimization, *rpoB* gene, Bioinformatics tools, landfill soil, mangrove soil

## TABLE OF CONTENTS