NURSYAFIQAH BINTI AHMAD GHAZALI AS201 FSG UiTM 2022

IDENTIFICATION OF *Bacillus licheniformis* FROM SOIL USING CULTURE-BASED METHOD AND BIOINFORMATIVE TOOLS FOR *ccpA* GENE

NURSYAFIQAH BINTI AHMAD GHAZALI

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

AUGUST 2022

This Final Year Project entitled "Identification of *Bacillus licheniformis* from soil using culture-based method and bioinformative tools for *ccpA* gene" was submitted by Nursyafiqah Binti Ahmad Ghazali 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

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

En. Syukri Bin Noor Azman
Project Coordinator
B. Sc. (Hons.) Biology
Faculty of Applied Sciences
University Teknologi MARA
02600 Arau
Perlis

Pn. Zalina Binti Zainal Abidin
Programme Coordinator
AS201
Faculty of Applied Sciences
Universiti Teknologi MARA
02600 Arau
Perlis

Date: 16th AUGUST 2022

TABLE OF CONTENTS

ABSTRACT

IDENTIFICATION OF *Bacillus licheniformis* FROM SOIL USING CULTURE-BASED METHOD AND BIOINFORMATIVE TOOLS FOR *ccpA* GENE

Bacillus species is a Gram-positive bacterium which usually found in soil. *Bacillus* species can also be found in mangrove soil. Mangrove soil able to support diverse group of microbial communities due to its characteristics that contain high rate of organic matter. This study aims to isolate *Bacillus licheniformis,* that have potential in various bioactivity such as plastic degradation and bioremediation. The identification of bacteria was usually done using culture-based methods such as culture media and biochemical tests. However, this method was laborious and time-consuming. Thus, the bioinformative tools were used in this study to specifically identify *Bacillus licheniformis*. Various genes can be found in *Bacillus licheniformis,* and one of the genes is *ccpA* gene. *ccpA* gene is the specific gene of *Bacillus licheniformis* that exhibit many functions such as biofilm formation and spore production. Bioinformative tools help analyse specific genes by designing specific primers to identify the *ccpA* gene of *Bacillus licheniformis*. Designing a specific primer pair is a critical step in amplifying PCR products. These bioinformative tools used in this study are BLAST, ClustalW, Primer3, and *In-silico* PCR amplification. Firstly, the nucleotide sequence of the *ccpA* gene was aligned in BLAST to compare a query DNA sequence with the database of other strains sequences. Next, the unique region within *ccpA* gene was found based on the alignment sequence using ClustalW. Based on the unique region within the *ccpA* gene, a pair of primer, (forward and reverse) was designed. The concentration of bacteria was higher in mangrove soil compared to commercialised soil. Morphology and microscopy analysis showed the characteristics of *Bacillus* species which are Gram positive, rod-shaped and appeared purple when viewed under microscope (1000x total magnification). Further identification using Microgen *Bacillus*-ID kit showed highest percent probability of *Bacillus licheniformis* with 99.41% for mangrove soil and 89.97% for commercialised soil. The primer of *ccpA* gene consists of 60 % G/C content, length of sequence was 16 bp, and the melting temperature was 60 ºC. After that, the primers were tested for its functionality to amplify and also to determine the specificity using *in-silico* PCR amplification. The outcome showed that the primers could detect *Bacillus licheniformis* with the expected amplicon size of 207 bp. In conclusion, the identification method of *Bacillus licheniformis* using bioinformative tools was a practical method for designing specific primers and PCR amplified *ccpA* gene reactions.

Keywords: *Bacillus licheniformis*, mangrove soil, Polymerase chain reaction (PCR), Bioinformative tools, *ccpA* gene