UNIVERSITI PUTRA MALAYSIA

ISOLATION OF BACTERIOPHAGE FROM SOIL SAMPLE

HAFIZAH ALIAS

FP 2016 66

ISOLATION OF BACTERIOPHAGE FROM SOIL SAMPLE

HAFIZAH BINTI ALIAS

DEPARTMENT OF AGRICULTURE TECHNOLOGY

FACULTY OF AGRICULTURE

UNIVERSITY PUTRA MALAYSIA

2015/2016

ISOLATION OF BACTERIOPHAGE FROM SOIL SAMPLE

BY

HAFIZAH BINTI ALIAS

A project report submitted to Faculty of Agriculture, University Putra Malaysia, in fulfillment of requirement of PRT 4999 (Final Year Project) for the award of degree of Bachelor of Horticultural Science

FACULTY OF AGRICULTURE

UNIVERSITY PUTRA MALAYSIA

2015/2016

DECLARATION

This project report entitled Isolation of Bacteriophage from Soil Sample is prepared by Hafizah Binti Alias and submitted to the Faculty of Agriculture in fulfillment of requirement of PRT4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science

Student's name: Student's signature:

Hafizah Binti Alias

Certified by:

Dr. Tan Geok Hun

Supervisor

Department of Agriculture Technology

Faculty of Agriculture

University Putra Malaysia.

Date :

ACKNOWLEDGEMENT

I would like to thank Allah SWT because of His blessing and mercy I can finish my final year project on time. To finish this project I need to learn many new things because I need to do my project in the laboratory. After finish this project I already improve my skill to work in laboratory.

First of all, of course I would like to thank my supervisor for final year project Dr. Tan Geok Hun who always patient by help me during my study and give me advice to make sure I understand what I need to do in this project. I am really thankful because she had sacrificed her energy and time by sharing her knowledge and helps me to solve my problems when I faced difficulties to finish my project.

Next my appreciation goes to my parents Hj. Alias B. Ismail and Hjh. Zainah Bt. Abdullah who always listen to my problem and always give me moral support to finish this thesis. They also gave me the financial support to finish this project. I am really grateful to have a parents and sibling who always give me support on what I have done.

Not to be forgotten, I also want to thanks to science officer Mrs Musliyana, assistant of science officer Mrs Nor Umaira and Miss Qamariah for allowing me to do my experiment in the Microbial Culture Collection Unit (UNiCC), Institute of Bioscience. They are always patient and help me to finish my experiment without their help and advice I cannot finish my project on time. Last but not least, I would to thank to all my friends who always want to help me a lot during my study. Finally, I want to say thanks for those who help me directly or indirectly during my study. May Allah bless all of you.

TABLE OF CONTENTS PAGE

LIST OF TABLE

LIST OF FIGURES

ABSTRACT

Bacteriophage also known as phage, is a virus that attack to a specific bacteria and replicates inside bacteria cell to produce more phages. Bacteriophage can be found in sewage, soil, water and food. This virus kills bacteria by lysis the host cell. There are two cycles of phage which are Lytic Cycle and Lysogenic Cycle. The benefits of phage are they can be used to kill bacteria, as replacement of antibiotic, biological control agent, and phage application in food industry. Phage can also survive in any condition because they have ability to reproduce quickly, high level of specificity and long term survivability. Phages were isolated and partially purified using PEG/ NaCl. The titers of phages were determined by plaque assay methods. The soil sample has different population of bacteriophage. Phage is isolate from soil sample to determine what type of phage in soil. From this study, the characteristic of phage showed that the phage is a type of lytic phage because it has similarities with T-type phage.

ABSTRAK

Bakteriofaj juga dikenali sebagai faj, ia merupakan sejenis virus yang akan menyerang bakteria tertentu dan replikasi dalam sel bakteria untuk menghasilkan faj yang banyak. Bakteriofaj boleh ditemui di dalam kumbahan, tanah, air dan makanan. Virus ini akan membunuh bakteria melalui proses lysis pada sel perumah. Terdapat dua kitaran faj iaitu Kitaran Lytik dan Kitaran Lysogenik. Antara kebaikan faj ialah ia boleh digunakan untuk membunuh bakteria, sebagai pengganti antibiotik, sebagai ejen kawalan biologi, dan penggunaan faj di dalam industri makanan. Faj boleh hidup dalam pelbagai keadaan kerana ia mempunyai kemampuan untuk membiak dengan cepat, tahap pengkhususan yang tinggi dan boleh hidup dalam jangka masa yang lama. Faj akan diasingkan dan dibersihkan dengan menggunakan PEG/NaCl. Pengiraan phage akan ditentukan melalui kaedah plak assay. Sampel tanah ini mempunyai populasi faj yang berbeza. Faj diasingkan dari sample tanah untuk mengetahui jenis fai apa di dalam tanah. Dari kajian ini, ciri-ciri yang ditunjukkan oleh fai menunjukkan bahawa faj tersebut dari jenis lytik faj kerana ia mempunyai persamaan denagn faj jenis T.

CHAPTER 1

1.0 INTRODUCTION

Bacteriophage also known as phages, are simply viruses that only infect bacteria cell (Jeremy and Simon, 2004). They have genes that can be transferred from one bacterium to another and at the same time it will directly produce more phages. They have either RNA or DNA enclosed in a protein coat. The infection of phage will occur by attaching itself to a specific receptor on bacteria's surface and release nucleic acid into the bacteria cell. There are only specific groups of bacteria that can be target hosts for phage. This bacterium is often subset of one species but several related species can be infected by the same phage.

Phages have two life cycle which are lytic (virulent) or lysogenic (temperate). Phages have some specialties that help them survive. But there are some physical and chemical factors that can affect the growth and death of bacteriophages by studying the plaque assay technique. When phages infect a liquid culture of bacteria the plaques will appear. The plaque is a clear zone in the lawn own of bacteria on the plate and it can be seen by naked eyes. Plaque assay technique is used to estimate the number of phage particles by counting the plaques that arise from a known volume of phage suspension.

Bacteriophage can be used in many sector because it have many benefits such as it can be used to kill bacteria, can be used as alternative antibiotics and phage therapy in medical field, and biological control in agriculture field. Thus, this study will be conducted to isolate of bacteriophage from soil sample.

1.1 Problem Statement

Soil is an important factor influencing the productivity in agriculture. Soil is a medium for growth and supply nutrient requirement for plant development. Most soil contains four basic components which are 45% of mineral particles, 25% of air, 25% of water and 5% of organic matter. For 5% of organic matter there are some sub-divisions which are 80% of humus, 10% of roots and 10% of organisms (Tom *et al*, 2015). Bacteriophage is one of the organisms in soil. It can give benefits to the soil, plant, human and animal.

Phages are virus that will help to kill bacteria by penetrating the cell wall. The population of the phages can increase through two types of cycle which are virulent and temperate. Virulent phage is a strictly lytic. It will kill bacteria cells and release their progenies. For temperate phage, it will enter bacterial cell that have been infected. It will alter the genotype of host cell, and start to replicate itself before completely destroying the host.

From discovery of Frederick Twort and Felix d'Herelle in 1915 and 1917, bacteriophages have been studied in many laboratories worldwide and have been used in most of practical application sector. From the study many advantages of phage were found, one of the advantages of phage in agriculture sector is it can be used as a biological control agent because it is an environmental friendly. For example, phage can be used to control some disease such as leaf blight at paddy, tomato and pepper that was caused by bacteria *Xanthomonas sp*. (Jones *et al*., 2012)

However, the used of phage is not commercialized yet in Malaysia. With all the characteristics phages have, it has the potential to be applied in agriculture as biocontrol agent because it have benefits for environment and at the same time it can solve the disease caused by bacteria. Thus, this study will be conducted to study the isolation of bacteriophage from soil sample. The objective of this study is to isolate bacteriophage from soil sample.

REFERENCES

Anonymous 1. Biological world. (2006). Using Phosphate-Buffered Saline (PBS) in Biochemical and Cell Biology Research. Retrieved 24 August 2015 from http://biologicalworld.com/pbs.htm

Anonymous 2. Waksman Foundation for Microbiology .(2003). Bacteriophage Titer Analysis.Retrieved 14 October 2015 from http://www.waksman-foundation.org/labs/rochester/bactertiter.htm

Anonymous 3. World Health Organization .(2015). Antibiotic resistance. Retrieved 14 September 2015 from http://www.who.int/mediacentre/factsheets/antibiotic-resistance/en/

Anonymous 4. Encyclopedia.com .(2003) "Dilution Theory and Techniques." World of Microbiology and Immunology. Retrieved 29 October 2015 from http://www.encyclopedia.com/doc/1G2-3409800168.html

Balogh B .(2006). Characterization and use of bacteriophages associated with citrus bacterial pathogens for disease control. PhD Dissertation, Gainesville, FL: University of Florida.

Barrow, P. A. and Soothill, J. S. (1997). Bacteriophage therapy and prophylaxis: rediscovery and renewed assessment of potential. *Trends Microbiol*. 5, 268-271

Carlton R. (1999). Phage therapy: past history and future prospects. Archivum Immunologiae et Therapiae Experimentalis, 47:5, 267-274, ISSN 0004-069X.

Civerolo E.L. and Keil H.L.(1969). Inhibition of bacterial spot of peach foliage by Xanthomonas pruni bacteriophage. *Phytopathology* 59:1966-7.

D'Herelle F. (1917). Sur un microbe invisible antagoniste des bacilles dysenteriques. *Compt Rend Acad Sci*. 165:373-375.

Douglas, J. (1975). *Bacteriophage*. 1st Published, Chapman & Hall Ltd, London.

Dulbecco R. and Vogt M. (1953). Some problems of animal virology as studied by the plaque technique. Cold Spring Harbor Symp. Quant. Biol., 18, 273-279

Elizabeth K. and Alexander S. (2005). *Bacteriophages in Biology and Application*. CRC Press, USA.

Gill, J. J. and R. F. Young,. (2011). *Therapeutic applications of phage biology*. 3rd Published. Caister Academic Press, Norfolk, UK.

Jeremy W. D. and Simon F. P. (2004). *Molecular Genetics of Bacteria*. 4th Edition. John Wiley & Sons Ltd, England.

Jones J. B., Gary E. Vallad, Fanny B. Iriarte, Aleksa Obradović, Mine H. Wernsing, Lee E. Jackson, Botond Balogh,Jason C. Hong, and M.Timur Momol .(2012). Considerations for using bacteriophages for plant disease control. *Bacteriophage*. 2:4, 208-214.

Kotila J.E. and Coons G.H. (1925). Investigations on the blackleg disease of potato. Michigan Agricultural Experimental Station Technical Bulletin 67:3-29.

Mallmann W.L. and Hemstreet C.J. (1924). Isolation of an inhibitory substance from plants. Agricultural Research. 28:599-02.

Matsuzaki S., Rashel M., Uchiyma J., Ujihara T., Kuroda M., Ikeuchi M., Fujieda M., Wakiguchi J. and Imai S. (2005). Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. Journal of Infection and Chemotherapy, 11:211 – 219, ISSN 1437-7780.

Okabe N. and Goto M. (1963). Bacteriophages of plant pathogens. Annu Rev Phytopathol 1:397-418.

Tom D., Peter K., and Sabrina K. (2015). Cooperative Extension, College of Agriculture, University of Arizona. 2 : 2–4.

Twort F. W. (1915). An investigation on the nature of the ultramicroscopic viruses. Lancet 3:189-241.