UNIVERSITI PUTRA MALAYSIA

ASSESSMENT ON DETECTION OF BIOFILM IN ESCHERICHIA COLI, ERWINIA CAROTOVORA, BACILLUS SUBTILIS AND XANTHOMONAS ORYZAE PV. ORYZAE

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BY

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A project report submitted to Faculty of Agriculture, University Putra Malaysia,

in fulfilment of the requirements of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Sciences.

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ENDORSEMENT

This project entitled ‘‘Assessment on detection method of biofilm in *E.coli, Bacillus subtilis, Erwinia carotovora and Xanthomonas oryzae pv oryzae* ’’ is prepared by Muhammad Aisamuddin Bin Mohd Rifaie and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT 4999 (Final year project) for the award of the degree of Bachelor of Horticultural Science.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLE

LIST OF ABBREVIATIONS

BHIA	Brain Heart Infusion Broth
ºC	Celcius
CRA	Congo Red Agar
CV	Crystal Violet
et al	et alia 'and others'
EPS	Extracellular polymeric substance matrix / Exopolysaccharides
g	Gram
Hr	Hours
µl	Microliter
µg	Microgram
ml	Mililiter
MC	Microtiter Plate
NA	Nutrient Agar
NB	Nutrient Broth
nm	Nanometer
PBS	Phosphate Buffer Saline
ppm	Parts per million
PSA	Peptone Sucrose Agar
PSB	Peptone Sucrose Broth
TM	Tube Method
TSB	Trypticose Soy Broth
Xoo	*Xanthomonasoryzaepv. oryzae*

ABSTRACT

Biofilm is a colony of microorganism that attached to a particular surface and covered by the extracellular polymeric substance (EPS). Biofilm can be produced by microorganism such as bacteria, algae and fungi. Most bacteria reproduced in the free state but generally the bacteria attached to a surface and form colonies started from a single cell and developed into multilayers and live in colonies that more complex. The objectives of this study were to identify type of bacteria which can produce biofilm and evaluating the best method for detection biofilm formation. The methods used in this presentstudy are microtiter plate (MC), tube method (TM) and congo red agar method (CRA). In total, four type of isolates bacteria such as *Escherichia coli, Bacillus subtilis, Erwinia carotovora* and *Xanthomonas oryzae* pv.*oryzae* were subjected to biofilm detection. The microtiter plate method was considered to be the best method compared to TM and CRA. The microtiter plate method was found more consistent in producing results in this experiment, more quantitative and reliable method in detection of biofilm forming microorganisms compared to TM and CRA. In contrast, TM and CRA methods were less consistent and sensitive in detecting biofilm forming microorganisms.

ABSTRAK

Biofilm sebuah koloni mikroorganisma yang melekat pada permukaan tertentu dan dilindungi oleh bahan polimer extracellular (EPS). Biofilm boleh dihasilkan oleh mikroorganisma seperti bakteria, alga dan kulat.Kebanyakan bacteria dihasilkan dalam keadaan bebas tetapi secara umumnya bacteria melekat pada permukaan dan membentuk koloni bermula daripada satu sel tunggal dan berkembang menjadi berlapis dan hidup dalam koloni yang lebih kompleks.Objektif kajian ini adalah untuk mengenal pasti jenis bakteria yang boleh menghasilkan biofilm dan mengenal pasti kaedah yang terbaik untuk pengesanan pembentukan biofilm. Kaedah yang digunakan dalam kajian ini ialah 'microtiter plate' (MC), kaedah tiub (TM) dan congo agar merah (CRA). Sebanyak empat jenis bacteria telah digunakan untuk pengesanan biofilm. Bakteria yang digunakan ialah *Escherichia coli, Bacillus subtilis, Erwinia carotovora* dan *Xanthomonas oryzae pv.oryzae*. Kaedah' microtiter plate' telah dianggap sebagai kaedah terbaik berbanding dengan kaedah tiub (TM) dan Congo agar merah (CRA).kaedah 'microtiterplate' adalah kaedah yang lebih kuantitatif dan boleh dipercayai untuk mengesan biofilm yang membentuk mikroorganisma berbanding dengan TM dan CRA kerana keputusan konsisten diperolehi bagi setiap percubaan yang dilakukan. Sementara itu, kaedah TM dan CRA telah didapati kurang konsisten dan sensitiviti.

CHAPTER 1

INTRODUCTION

Bacterial biofilms are cellular consortia in which cells are embedded in an extracellular matrix at close proximity to one another. Such consortia are generally studied to assess particular properties of biofilms attached to solid surfaces, but they occur also as multicellular aggregates, flocks and granules suspended in the aqueous phase in many habitats.Bacterial biofilm can be commended by single species, however, usually they are mixed species consortia in natural and artificial system, They are ranging from highly diverse communities phylogenetic to one numerically and functionally dominant restricted number of species (Tujula et al., 2006)

Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. Initially, the first colony is weak and reversible adhesionvia van der Waals forces. If the colony are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili. Some species are not able to attach to a surface on their own but are able to anchor themselves to the matrix or directly to earlier colonists. Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. The final stage of biofilm formation is known as development; this is the stage the biofilm has established and changes only in shape and size. In addition, bacterial biofilm can be resistant to antibiotic as they developed.

In this study, *E.coli*, *Erwinia carotovora* and *Xanthomonas oryzae pv.oryzae* are being tested. *E. coli* is a pathogenic bacteria that that can cause wide range of illness symptoms to human, animal and plant. Beside that, *Erwinia carotovora and Xanthomonas oryzae* also a plant pathogenic that harm worldwide crops over the year. The fourth bacteria that has been tested was *Bacillus subtilis. B.subtilis* was a plant antagonistic bacteria and used as a soil inoculant in agricultural practices

There are various methods to detect biofilm production. These include the microtiter plate (TCP), Tube method (TM), Congo Red Agar method (CRA), bioluminescent assay, piezoelectric sensors ,and fluorescent microscopic examination. In regards, three detection methods for bacterial biofilm were used in the present study such as microtiter plate method, tube method and congo red agar method. The microtiter plate method is considered as primary and important method in detecting biofilm formation at the early stage. Besides that, the tube method and congo red agar method are considered in this study due to their simplicity easiest analysis to detect biofilm formation.(Freeman et al., 1989).

Therefore, the objectives of this present study were to evaluate and comparing between microtiter plate method, tube method and congo red agar method for detection of biofilm. and to determine the biofilm producer among tested bacteria.

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