



**UNIVERSITI PUTRA MALAYSIA**

***EFFECT OF pH, TEMPERATURES AND CARBON SOURCES ON  
FORMATION OF BIOFILM IN *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 “Effect Of pH, Temperatures And Carbon Sources on Formation of Biofilm in *Xanthomonas oryzae* Pv. *oryzae* ” is prepared by Nurul Amalina binti Zulkiflee 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 Horticultural Science.

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## TABLE OF CONTENTS

CONTENTS	PAGE
ENDORSEMENT	i
ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	vii
ABSTRACT	viii
ABSTRAK	ix
CHAPTER 1 : INTRODUCTION	1
CHAPTER 2 : LITERATURE REVIEW	
2.1: Biofilm	3
2.1.1 : Advantages of Biofilm	4
2.2: Biofilm Formation, Growth and Development	5
2.2.1 : Biofilm Formation	5
2.2.2 : Quorum Sensing Molecules in Biofilm	7
2.2.3: Biofilm Behaviour	8
2.3 : Extracellular Polymeric Substances (EPS) in Biofilms	8
2.4: Environmental factors influencing biofilm development	10
2.4.1 : Effect of pH on Biofilm Formation	10
2.4.2 :Rheological and adhesive properties of	11
	11

biofilms

2.4.3 : Effect of Temperature on Biofilm Formation 12

2.4.4 : Effect of carbon sources on Biofilm formation 12

2.5 : Microtiter Dish Biofilm Formation Assay

CHAPTER 3 : MATERIALS AND METHODS 15

3.1: Growth of Bacteria Cultures 15

3.2 : Treatment 15

3.4 : Growing of Biofilm 17

3.5 : Staining the Biofilm 18

3.6 : Quantifying the Biofilm 19

3.7 : Statistical Analysis 20

CHAPTER 4 :RESULTS AND DISCUSSION 24

4.1 : Effect of pH on Biofilm Formation 24

4.2 : Effect of Temperatures on Biofilm Formation 26

4.3 : Effect of Carbon Sources on Biofilm Formation 28

CHAPTER 5 : CONCLUSION 30

REFERENCES 31

APPENDICES 35

## LIST OF TABLES

TABLE	TITLE	PAGE
3.1	The pH level of the media	16
3.2	The conditions of each batch experiments	17





## LIST OF FIGURES

<b>FIGURE</b>	<b>TITLE</b>	<b>PAGE</b>
2.1	The pattern of biofilm formation	6
3.1	Each well are stained by using 0.1% Crystal Violet solution	19
3.2	Multiskan GO machine (Thermo-Scientific)	19
3.3	Experimental layout for pH treatment.	21
3.4	Experimental layout for temperature treatment.	22
3.5	Experimental layout for carbon sources treatment.	23
4.1	Optical Density of Biofilm Production at different pH of the media.	24
4.2	Optical Density of Biofilm Production at different temperature of the media.	26
4.3	Optical Density of Biofilm Production at different carbon sources of the media	28

## LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AHL	Acyl homoserine lactone
°C	Celcius
CRD	Completely Randomized Design
CV	Crystal Violet
et al	et alia 'and others'
EPS	Extracellular polymeric substance matrix / Exopolysaccharides
g	Gram
Hr	Hours
LSD	Least Significant Differences
M	Molar
μl	Microlitre
μg	Miligram
ml	Mililitre
ppm	Parts per million
PSA	Peptone Sucrose Agar
Xoo	<i>Xanthomonas oryzae pv. oryzae</i>

## ABSTRACT

A biofilm is any group of microorganisms in which cells stick to each other and often these cells adhere to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Hence, the objective of this study was to determine the effect of pH, temperatures and carbon sources on biofilm formation in *Xanthomonas oryzae* pv. *oryzae* (Xoo), the causative agent of bacterial leaf blight disease of rice. To achieve the research objective, peptone sucrose agar (PSA) and microplate titer method are used to assess biofilm formation. For the pH treatment, it was indicated that the optimum pH media for biofilm production was at pH 7. For temperature treatment, it was indicated that the optimum temperature for biofilm production was at 30°C. Biofilm formation also increased when high glucose content in the medium. So, it can be conclude that, pH, temperatures and type of carbon sources will be significantly influence of biofilm formation in *Xanthomonas oryzae* pv. *oryzae* (Xoo).

## ABSTRAK

Biofilm adalah kumpulan mikroorganisma di mana sel melekat antara satu sama lain dan sering sel-sel ini melekat di permukaan. Sel-sel yang melekat ini kerap tertanam di dalam matriks sendiri yang dihasilkan daripada bahan polimer extracellular (EPS). Oleh itu, objektif kajian ini adalah untuk menentukan kesan pH, suhu dan sumber karbon pada pembentukan biofilm di *Xanthomonas oryzae pv. oryzae* (Xoo), ejen penyebab bakteria penyakit Hawar daun padi. Bagi mencapai objektif kajian, pepton agar sukrosa (PSA) dan kaedah 'microtiter plate' digunakan untuk menilai pembentukan biofilm. Bagi rawatan pH, ia telah menunjukkan bahawa pH media optimum untuk pengeluaran biofilm adalah pada pH 7. Bagi rawatan suhu, ia telah menunjukkan bahawa suhu optimum untuk pengeluaran biofilm adalah pada 30 °C. Pembentukan biofilm juga meningkat apabila kandungan glukosa yang tinggi di dalam media. Jadi, ia boleh membuat kesimpulan bahawa, pH, suhu dan jenis sumber karbon akan mempengaruhi pembentukan biofilm di *Xanthomonas oryzae pv. oryzae* (Xoo).

## CHAPTER 1

### INTRODUCTION

Biofilms are the aggregations of bacteria that adhere to a surface and to each other, excreting an extracellular matrix, forming nutrient channels, and regulating gene expression to maintain the community of bacteria. A biofilm consists of cells immobilised at a substratum and frequently embedded in an organic polymer matrix of microbial origin. Biofilms are a biologically active matrix of cells and extracellular substances in association with a solid surface (Robinson *et al.*, 1990). Biofilms are sessile microbial communities growing on surfaces, frequently embedded in a matrix of extracellular polymeric substances. A biofilm may be described as a microbially derived sessile community characterised by cells that attach to an interface, embedded in a matrix of exo-polysaccharide which demonstrates an altered phenotype. Microcolonies are discrete matrix enclosed communities of bacterial cells that may include cells of one or many species.

Depending on the species involved, the micro-colony may be composed of 10–25% cells and 75–90% extracellular polymeric substances (EPS) matrix.

Extracellular polymeric substances are generally responsible for the structural and functional of biofilm and are considered as the key components that determine the physicochemical and biological properties of biofilms. The EPS by microbial cells depends on the pH, carbon sources in the culture medium and the cultivation temperature. In this study, *Xanthomonas oryzae* pv. *oryzae* was tested *in vitro*. *Xanthomonas oryzae* pv. *oryzae* (xoo) is a gram negative bacterium that causes a

serious blight disease in rice, turf and sedges. In recent time, bacterial blight of rice is highly destructive to high yielding cultivars in tropical regions especially in Asia. Among the bacterial diseases, bacterial leaf blight or bacterial blight is one of the most dangerous diseases of rice in Asia. There was very few research was carried out to investigate the biofilm formation on bacteria that infect plants, especially *Xanthomonas oryzae* pv. *oryzae*. This is due to its self-protective structure to defend against the invasion of pathogens, any biological or chemical treatments hardly overcome this infectious biofilm structure. Biofilms display unique characteristics that increase resistance to host immune mechanisms (Saini *et al.*, 2011). Thus, the objective of this study were to determine the effect of pH media, temperatures and of availability of carbon sources in the media on biofilm formation in *Xanthomonas oryzae* pv. *oryzae*.

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