

UNIVERSITI TEKNOLOGI MARA

**INHIBITION OF *Salmonella*
typhimurium BIOFILM BY
COMMERCIAL DISINFECTANTS**

NUR ANISAH BINTI JOHARI

MSc

February 2022

ABSTRACT

Salmonellosis is a foodborne disease caused by *Salmonella typhimurium*; a Gram-negative bacterium that can form biofilm in infected hosts. The effectiveness of existing commercial disinfectants against *Salmonella* biofilm especially on biomass, viability and biochemical composition of *S. typhimurium* biofilm needs to be further investigated for biofilm control. The objective of this study was to evaluate the effects of commercial disinfectants against biomass, viability and biochemical composition of *S. typhimurium* biofilm. *S. typhimurium* ATCC14028 biofilm was developed in the absence and presence of commercial disinfectants. Sodium hypochlorite, sodium dodecyl-benzene sulfonate, benzalkonium chloride and chloroxylenol-based disinfectants were evaluated in the range between 0.78% and 25%. Biofilm biomass, biofilm viability and biofilm biochemical composition were determined using crystal violet assay, resazurin assay and FTIR-ATR spectroscopy respectively. Results demonstrated that all commercial disinfectants effectively inhibited *S. typhimurium* biofilm. Treatment with chloroxylenol-based disinfectant significantly ($p < 0.05$) reduced biofilm biomass and biofilm viability at all tested concentrations. Correlation between biofilm biomass and biofilm viability in the presence of chloroxylenol-based disinfectant was also found to be significant with $r = 0.857$ and its half-maximal biofilm inhibitory concentration (BIC_{50}) showed the lowest values of 5.06%. Biochemical composition of *S. typhimurium* biofilm treated with chloroxylenol-based disinfectant showed changes in FTIR spectral peaks in lipid (1460 cm^{-1} , 1400 cm^{-1}) and protein (1550 cm^{-1} , 1460 cm^{-1} , 1400 cm^{-1}). The findings of the present study suggest that the most efficient agent against *S. typhimurium* biofilm is a chloroxylenol-based disinfectant.

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful

Alhamdulillah, all gratitude to Allah for the strengths and His blessing in helping me finish my thesis. I want to convey my thank you to my supervisor, Dr. Mohd Fakharul Zaman Raja Yahya for his guidance and constant support during this project. His recommendation and remarks throughout the experimental and thesis works have contributed to the success of this research.

I also would like to extend my appreciation to the research coordinator, Dr. Azani Salleh for her support and assistance with my master's thesis. My appreciation goes to my co-supervisor Dr. Umi Marshida Abd Hamid for her assistance in supervising me for this master's thesis. My acknowledgment also extends to all the lab assistants and staff of the School of Biology for their cooperation.

Sincere thanks to all my friends for their generosity and moral support throughout the research project. Thank you for the friendship and memories.

Finally, my thesis is dedicated to the loving memories of my very dear late father and mother, who had the foresight and determination to educate me. This piece is dedicated to them both. Finally, my deepest appreciation towards my siblings for their prayers and encouragement. To those who indirectly helped me finish this research project, your goodwill means a lot to me. Thank you very much.

Nur Anisah Johari

TABLE OF CONTENTS

	Page
CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF SYMBOLS	xiii
LIST OF ABBREVIATIONS	xiv
LIST OF NOMENCLATURE	xv
CHAPTER ONE INTRODUCTION	1
1.1 Research Background	1
1.2 Problem Statement	3
1.3 Objectives of The Study	4
1.4 Significance of The Study	5
1.5 Scope and Limitation of The Study	5
CHAPTER TWO LITERATURE REVIEW	6
2.1 <i>Salmonella</i>	6
2.1.1 <i>Salmonella</i> Serovars	6
2.1.2 <i>S. typhimurium</i>	8
2.1.3 Pathogenicity	8
2.1.4 Resistance Mechanisms of <i>S. typhimurium</i> Against Disinfectants	9
2.2 Disinfectants	10
2.2.1 Sodium Hypochlorite	13
2.2.2 Sodium Dodecyl-benzene Sulfonate (SDBS)	14
2.2.3 Benzalkonium Chloride (BAC)	15
2.2.4 Chloroxylonol	16

2.3	<i>S. typhimurium</i> Biofilm	17
2.3.1	Developmental Stages of Biofilm	18
	2.3.1.1 <i>Attachment of Cell</i>	19
	2.3.1.2 <i>Formation of Microcolony</i>	19
	2.3.1.3 <i>Biofilm Maturation and Formation</i>	20
	2.3.1.4 <i>Detachment and Dispersion of Biofilm</i>	21
2.3.2	Components of Biofilm	21
2.3.3	Biochemical Composition of <i>S. typhimurium</i> Biofilm	22
2.3.4	<i>S. typhimurium</i> Attachment on Abiotic Surfaces	24
2.4	Assessments Method for Biofilm	24
2.4.1	Microbiological Method	25
2.4.2	Chemical Method	25
2.4.3	Physical Method	27
2.4.4	Fourier Transform Infrared (FTIR) Spectroscopy	27
	2.4.4.1 <i>Principle of FTIR Spectroscopy</i>	29
	2.4.4.2 <i>FTIR Difference Spectroscopy</i>	32
	2.4.4.3 <i>Application of FTIR in Microbiology</i>	37
2.5	Control and Removal Strategies of Biofilm	39
2.6	Changes of Biochemical Composition of Biofilm Following Treatment of Disinfectants or Chemicals	40
CHAPTER THREE RESEARCH METHODOLOGY		41
3.1	Materials	41
	3.1.1 Raw Materials	41
	3.1.2 Chemicals	41
	3.1.3 Media	43
	3.1.4 Apparatus	43
3.2	Methods	43
	3.2.1 Preparation of Microorganisms	43
	3.2.2 Preparation of Disinfectants	44
	3.2.3 Pellicle Assay	44
	3.2.4 Biofilm Formation in 96 Well Microplate	44
	3.2.4.1 <i>Cystal Violet Assay</i>	44
	3.2.4.2 <i>Resazurin Assay</i>	44