UNIVERSITI TEKNOLOGI MARA

CHARACTERIZATION OF *Corynebacterium pseudotuberculosis* BIOFILM

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MSc

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Caseous lymphadenitis (CLA) is a ruminant disease caused by *Corynebacterium pseudotuberculosis*, a Gram-positive facultative intracellular pathogen. To date, the biofilm formation by *C. pseudotuberculosis* is not well understood. The present work was performed to characterize *C. pseudotuberculosis* biofilm. Determination of biochemical composition, morphology, antimicrobial susceptibility pattern and whole-cell protein expression of *C. pseudotuberculosis* biofilm was carried out using Raman spectroscopy, field emission scanning electron microscopy (FESEM), microplate biofilm assay and sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) respectively. Results showed that the 24-h-old biofilm was characterized by Raman spectral peaks at 615 cm^{-1} (CCC symmetric bend phenyl ring), 668 cm^{-1} (Valine) and 825 cm^{-1} (Ring breath Tyr.) whilst the 48-h-old and 72-h-old biofilms were characterized by Raman spectral peaks at 1400 cm^{-1} (COO- sym.), 1450 cm^{-1} (COO- sym.), 1581 cm^{-1} (Ring breath Trp.), 1650 cm^{-1} (COO- asym.) and 1725 cm^{-1} (C-O str.). Raman spectra also revealed the biochemical heterogeneity in *C. pseudotuberculosis* biofilm. FESEM images clearly showed the biofilm cells which were surrounded by the extracellular matrix. Treatment with nalidixic acid, streptomycin, tetracyclin, ethylenediaminetetraacetic acid (EDTA) and dimethyl sulfoxide (DMSO) significantly ($p < 0.05$) inhibited the viability of *C. pseudotuberculosis* biofilm. The major protein bands of *C. pseudotuberculosis* biofilm were found to be in the range between 33.7 kDa and 150 kDa. Differential protein expression in *C. pseudotuberculosis* biofilm was observed following the treatment with antimicrobial agents. The present study suggests that the biochemical composition of *C. pseudotuberculosis* biofilm may vary across different developmental stages. Meanwhile, nalidixic acid, streptomycin, tetracyclin, EDTA and DMSO may be useful in the treatment of CLA.

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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	**x**
LIST OF FIGURES	**xi**
LIST OF ABBREVIATIONS	**xiv**
CHAPTER ONE: INTRODUCTION	**1**
1.1 Background of Study	1
1.2 Problem Statement	4
1.3 Significance of the Study	5
1.4 Objectives of the Study	6
1.5 Scope and Limitation of the Study	6
CHAPTER TWO: LITERATURE REVIEW	**7**
2.1 Caseous Lymphadenitis (CLA)	7
2.1.1 Epidemiology of Caseous Lymphadenitis (CLA)	9