

UNIVERSITI TEKNOLOGI MARA

**OPTIMIZATION OF
BIFIDOBACTERIUM
PSEUDOCATENULATUM KAKII
CULTIVATION CONDITIONS FOR
EXOPOLYSACCHARIDE
PRODUCTION USING RESPONSE
SURFACE METHODOLOGY**

ALIZA BINTI MAHDI

Disertation submitted in partial fulfillment
of the requirements for the degree of
**Master of Science
(Applied Biology)**

Faculty of Applied Sciences

June 2018

ABSTRACT

Bifidobacteria is gram-positive, non-motile, non-spore-forming and strictly anaerobic bacteria. It belongs to phylum Actinobacteria and able to produce exopolysaccharide (EPS). EPS extracted from the Bifidobacteria are polymers of sugars, long chained polysaccharide that have been shown to give benefit towards human health. This study was conducted with the aim to optimize the EPS production by *Bifidobacterium*, considering the benefits of EPS to the human health. Initially, study was conducted to select *Bifidobacterium* strains with highest EPS production, then optimize the growth conditions, which were pH (range of 5, 6 and 7), incubation temperature (20 °C, 30 °C and 40 °C) and cultivation time (6, 21 and 36 hours), and lastly to characterize the EPS produced by using FT-IR and GC-MS. During the selection of Bifidobacteria strains, results found that *Bifidobacterium pseudocatenulatum* KAKii showed highest EPS production compared to the other two strains (*B. pseudocatenulatum* KAKii and *B. animalis*). While the optimization of the three factors towards *B. pseudocatenulatum* KAKii found that EPS produced crucially depends cultivation time (23.59 hours) rather than pH (5) and temperature (34.75 °C). The validation showed that the predicted (115.89 mg/100mL) and experimental values are not significantly different ($p > 0.05$), indicating that the developed model is fitted well for the optimization. FT-IR spectrum also showed peak that could be attributed to common stretching of hydroxyl group (3435.58 cm^{-1}), symmetric C-H stretching (2088.70 cm^{-1}), stretching of C=O and carboxyl group (1645.74 cm^{-1}) including C-O stretching peak (712.59 cm^{-1}). The FT-IR spectrum supported common pattern of FTIR spectra of EPS extracted from other probiotic indicated that the EPS extraction was successful. GCMS chromatogram on the other hand support previous studies on *B. pseudocatenulatum* to produce acetic acid during EPS production. The result also showed that EPS extracted from the strain was composed of D-glucose, mannose, galactose and maltose thus indicated that EPS was from hetero-exopolysaccharide group.

ACKNOWLEDGEMENT

Firstly, I wish to thank God for giving me the opportunity to embark on my Master of Science and for completing this long and challenging journey successfully. My gratitude and thanks goes to my supervisor, Dr. Khalilah Binti Abdul Khalil, and co-supervisor, Dr. Maslinda Musa for their guidance along the journey. Thank you for the support, patience and ideas in assisting me with this project. I also would like to express my gratitude to all the laboratory assistants who had willingly give me guidance, provided materials and assistance during the experimental process.

My appreciation also goes to all my friends especially Nor Atiqah Binti Jusril who provided me with the knowledge regarding my thesis. Thanks to my friends for helping me with this project. Special appreciation goes to my best friend, Muhammad Aiman bin Mazlan, for his advises and concerns throughout the completion of the thesis.

Finally, this thesis is dedicated to my very dear father and mother for the vision and determination to educate me. This piece of victory is dedicated to both of you. Alhamdulillah.

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 | xiii |
| | |
| CHAPTER ONE: INTRODUCTION | 1 |
| 1.1 Research Background | 1 |
| 1.2 Problem Statement | 3 |
| 1.3 Objectives | 3 |
| 1.4 Significance of Study | 3 |
| | |
| CHAPTER TWO: LITERATURE REVIEW | 5 |
| 2.1 Probiotic | 5 |
| 2.2 Prebiotic | 8 |
| 2.2.1 Galacto-oligosaccharide (GOS) | 8 |
| 2.2.2 Xylo-oligosaccharide (XOS) | 9 |
| 2.2.3 Fructo-oligosaccharides (FOS) | 9 |
| 2.3 Lactic Acid Bacteria | 10 |
| 2.3.1 Adaptation Of Lactic Acid Bacteria In Gastrointestinal Tract | 11 |
| 2.3.2 Acid Tolerance And Cell Membrane Relationship In <i>Bifidobacterium</i> | 12 |
| 2.4 Exopolysaccharide | 13 |
| 2.4.1 Microbial Exopolysaccharide Composition | 16 |
| 2.4.2 Homo-Exopolysaccharide | 18 |
| 2.4.3 Hetero-Exopolysaccharide | 18 |
| 2.5 Biosynthesis of Exopolysaccharide | 20 |

CHAPTER ONE

INTRODUCTION

1.1 Research Background

Bifidobacteria belongs to the phylum of Actinobacteria is a non-motile, strictly anaerobic bacteria, gram-positive and non-spore-forming bacteria (Fabio *et al.*, 2013; Ishibashi *et al.*, 1997). It is one of the bacteria that has been considered as probiotics. This is due to the reason that several strains of *Bifidobacterium* species have been proved to stimulate health promoting activities such as enhancement of resistance against pathogens, reduction of blood cholesterol levels, immunomodulation and others (Jones *et al.*, 2004; Shu and Gill, 2002; Rosenfeldt *et al.*, 2002; Gill and Rutherford, 2001; Seema *et al.*, 2012). Moreover, it has been granted status of Generally Recognised as Safe (GRAS) in food associated with Lactic Acid Bacteria (LAB) and these living microorganisms have also been identified as one of the suitable candidates that are able to produce functional exopolysaccharide (Seema *et al.*, 2012; Laws *et al.*, 2001). There are six genera of LAB species that have been isolated from fermented foods produced from several countries that have also been identified namely *Aerococcus*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Tetragenococcus* and *Weisella*. *Enterococcus* was commonly isolated from crustaceans and fermented fishes. Meanwhile, *Bifidobacteria* can be found in the human gut and also known as commensal bacteria. The population of the bacteria was reported around 3% to 7% of microbiota in adults and up to 91% in newborns according to some reports (Fabio *et al.*, 2013; Miyashita *et al.*, 2012). It is also has been hypothesized as one of the exopolysaccharide-producing bacteria.

Exopolysaccharide (EPS) is a biodegradable polymers, high molecular weight and a long chain polysaccharide synthesized by a wide range of bacteria including LAB such as *Lactobacillus acidophilus*, *Bifidobacterium pseudocatenulatum*, and *Bifidobacterium animalis* (Pinar and Gürcü, 2016). Exopolysaccharide can be divided into two groups based on its chemical composition which are homo-exopolysaccharide and hetero-exopolysaccharide. Homo-exopolysaccharide is composed of repeating units of only single-type monosaccharide while hetero-exopolysaccharide by contrast is a polymer of sugars consisting of repeating units of