



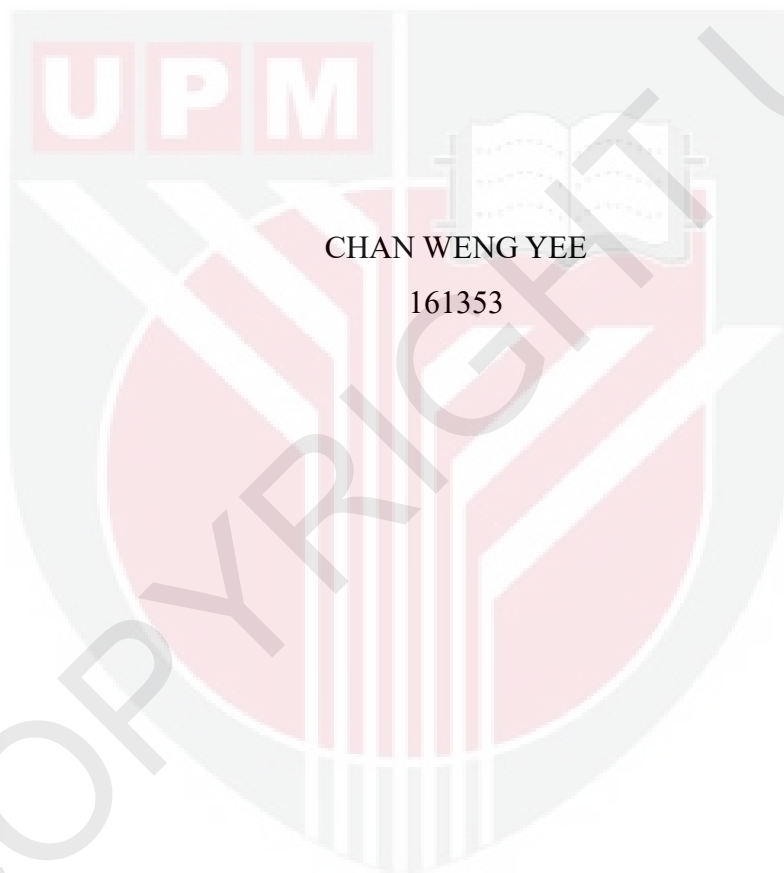
UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND CHARACTERIZATION OF *STREPTOCOCCUS* FROM
DENTAL PLAQUE SAMPLES**

CHAN WENG YEE

FBSB 2015 97

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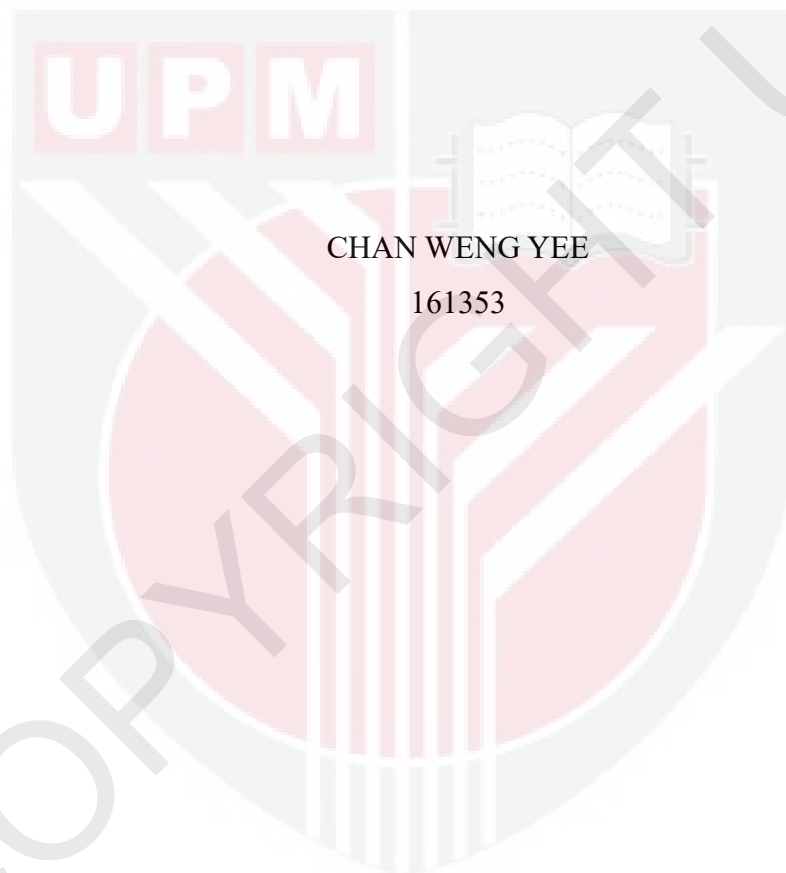


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2015

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DENTAL PLAQUE SAMPLES



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Dissertation submitted in partial fulfillment of the requirement for the course
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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk '**Isolation and Characterization of *Streptococcus* from Dental Plaque Samples**' telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh **Chan Weng Yee (161353)** sebagai syarat untuk khusus BMY4999 projek.

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Tarikh

ABSTRACT

Dental caries is still a prevalent chronic condition that affecting health in the worldwide population. Other oral diseases, such as infection and tooth loss, are also very common. These diseases normally associated with discomfort and pain, eating and speech difficulties. Periodontal disease is an inflammatory infection on the gums, connective tissues and jaw bone that support the teeth. The root of these complications is formation of dental plaque by specific group of bacteria. Prevention practices, such as brushing teeth twice a day, using dental floss and antibacterial rinse, eating a balanced diet are not practiced. Therefore, the most efficient method to overcome this issue is eliminating the causative agents. This study is to isolate and characterize dental plaque causing bacteria. The bacteria were isolated from dental plaque sample. Preliminary identifications performed were macroscopic, microscopic morphology and biochemical tests. The bacteria isolated were characterized using Restriction Fragment Length Polymorphism (RFLP) and DNA sequencing. Three isolates were selected and identified as *Streptococcus mitis* and *Streptococcus oralis*.

ABSTRAK

Karies gigi masih ialah satu keadaan kronik yang lazim menjejaskan kesihatan manusia di seluruh dunia. Penyakit mulut seperti jangkitan dan kehilangan gigi, juga sangat biasa. Penyakit-penyakit ini biasanya berkaitan dengan ketidakselesaan dan kesakitan, kesukaran makan dan bersuara. Penyakit periodontal ialah jangkitan radang pada gusi, tisu penghubung dan tulang rahang yang menyokong gigi. Akar komplikasi ini adalah pembentukan plak gigi oleh kumpulan bakteria tertentu. Amalan pencegahan seperti memberus gigi dua kali sehari, menggunakan benang gigi dan bilas antibakteria, makan makanan yang seimbang tidak diamalkan. Oleh itu, kaedah yang paling berkesan untuk mengatasi isu ini ialah menghapuskan agen penyebab. Kajian ini adalah untuk memencil dan mencirikan bakteria yang menyebabkan plak gigi. Bacteria telah diasingkan daripada sampel plak gigi. Pengenalan awal dilakukan ialah makroskopik, mikroskopik morfologi dan ujian biokimia. Bacteria terencil telah dicirikan dengan teknik Restriction Fragment Length Polymorphism (RFLP) dan penjujukan DNA. Tiga bakteria telah dipilih dan dikenal pasti sebagai *Streptococcus mitis* dan *Streptococcus oralis*.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celcius
AbpA	Amylase binding protein A
AbpB	Amylase binding protein B
BHI	Brain Heart Infusion
bp	Base pair
Cat.	Catalogue
CBA	Chocolate blood agar
CD83	Cluster of differentiation 83
CD86	Cluster of differentiation 86
dH ₂ O	Distilled water
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxynucleotide
EDTA	Ethylenediaminetetraacetic acid
et al.	And others
GBP	Glucan-binding protein
GTF	Glucosyltransferase
h	Hour
H ₂ O	Water
HA	Hemagglutination
HAEC	Human aortic endothelial cell
HSAmy	Human salivary α -amylase
IgA	Immunoglobulin A
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-12	Interleukin-12
kb	Kilobase
Mb	Megabase
MgCl ₂	Magnesium chloride
MIC	Minimum inhibitory concentration
mM	Millimolar

MMP-1	Matrix MetalloProteinase-1
MR-VP	Methyl Red & Voges-Proskauer
MSA	Mitis-Salivarius agar
MSB	Mitis-Salivarius-Sucrose-Bacitracin
NA	Neuraminidase
ng/μl	nanogram per microlitre
No.	Number
PCR	Polymerase Chain Reaction
pmol/μl	Picomole per microlitre
rDNA	Ribosomal deoxyribonucleic acid
RFLP	Restriction fragment length polymorphism
rpm	Revolutions per minute
RNase	Ribonuclease
RPS	Receptor polysaccharides
spp.	Species (plural)
U/μl	Unit per microliter
w/o	Without
w/v	Weight/Volume percent

CHAPTER 1

INTRODUCTION

Dental plaque is a sticky, colorless or pale yellow film of bacteria that forms on the teeth. It is a type of biofilm that occurs in oral cavity. It is formed by oral bacteria that colonize the enamel of the teeth. It functions as part of the defense system inside the mouth to prevent attachment of other pathogenic microorganisms (Theilade et al., 1982). Dental plaque is formed when food with high carbohydrates, such as sugars and milk are constantly left in the oral cavity. Oral bacteria that live inside the mouth hydrolyze the sugars to produce acids by fermentation processes (Allison, 2003). These oral bacteria are normally attached to the enamel surface, preventing them from flushing away by saliva and food. The layer of biofilm allows entry of sugars into the space between biofilm and enamel, however, it forbid the acids from leaving. These acids are accumulated inside and eventually destroy the enamel, resulting tooth decay. Hence, dental plaque is said to be the beginning of most dental diseases (Taubman & Nash, 2006).

There is a variety of microorganisms present in dental plaque, instead of just one bacterial species. It has been estimated to have 25,000 bacterial species reside in oral cavity, and around 1,000 species exist as part of the dental plaque ecosystem. Among them, the microorganisms that are most studied is mutans streptococci. Most of mutans streptococci are antibiotic resistant (Larsen, 2002; Pratten & Wilson, 1999). They are also capable of surviving in extremely oral environments due to the intake of different types of food that creates a wide range of pH inside the mouth.

Poor oral hygiene can cause various problems. Oral health is a window to our overall health. Poor oral hygiene causes bad breath, dental decay, and tooth loss. If it is left untreated, it can lead to gum disease and mouth cancer. Besides, these microorganisms can migrate to other parts of the body via bloodstream or pharynx. The risk of infective endocarditis-related bacteremia was proven to increase around 4-fold with poor dental health (Lockhart et al., 2009).

Personal oral hygiene is crucial to prevent buildup of dental plaque. It is recommended to brush at least twice a day with a fluoride-containing toothpaste, rinse with antibacterial mouth rinse and floss at least once a day. Having dental checkup every 6 months and eating a balanced diet are also important to keep the teeth healthy. Wearing dental sealants is a commonly applied for children due to high consumption of sugars and poor oral hygiene practice.

Statistics showed that dental caries is still a prevalent problem in the community. Prevention methods such as brush twice daily, floss daily, antibacterial rinse, eat a healthy diet, dental checkup are not practiced. This phenomenon may be caused by lacking of knowledge in personal oral hygiene and dental plaque causing bacteria. To overcome this, the causative agents need to be studied intensively to allow subsequent effective intervention. The hypothesis in this project is dental plaque causing bacteria can be isolated and characterized. The specific objectives of this study are:

1. To isolate dental plaque causing bacteria
2. To characterize dental plaque causing bacteria

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