CHAPTER 1

INTRODUCTION

1.1Research Background

Periodontal disease, a type of chronic dental problem is a serious disease that affects around 50% of adults and adolescents globally (Nazir, 2017). It is caused by the pathogenic invasion that leads to the deterioration of the tissues surrounding the teeth and subsequently causes tooth detachment (Michaud et al., 2017). Several prominent periodontal pathogens are identified as *Porphyrmonas gingivalis, Aggregatibacter actinomycetemcomitans,* and *Tannerella forsythia*. Amongst the stated pathogens, *P. gingivalis*is considered to be the keystone pathogen that shifts the balance of oral microbiota and eventually disrupts the regulation of the immune responses (Curtis et al., 2020).

Personal oral hygiene such as tooth brushing and interdental cleaning is important to prevent the growth of pathogenic bacteria and the formation of plaque on the supragingival line. In addition to that, professional mechanical cleaning such as scaling and root planing can remove the formed calculus on supragingival and subgingival lines but is unable to prevent the recolonization of these pathogens (Geisinger et al., 2019). Thus, the administration of antibiotics is important to prevent the recolonization of pathogens. The common drugs administered are metronidazole, amoxicillin-metronidazole combination, and azithromycin. Other than antibiotic drugs, antibacterial mouthwash chlorhexidine is commonly prescribed to periodontal patients as the other choice of treatment (Pretzl et al., 2019).

Current antimicrobial treatments have a few drawbacks such as staining on the teeth surfaces and the reduction of palatal sensitivity by antibiotics metronidazole and amoxicillin (Borges et al., 2017). Moreover, the formation of biofilm by *P. gingivalis*with other periodontal pathogens reduces the efficiency of antibiotic treatment and

imposed the risk of the pathogens developing resistance towards antimicrobial drugs (Gerits et al., 2017). Due to this concern, there is an urgent need for research and development of supportive antimicrobial treatments in preventing the colonization, biofilm formation, and recolonization of periodontal pathogens. A few approaches have been suggested to be developed as supportive treatment for periodontal disease including the application of probiotics.

Probiotic is a beneficial group of microorganisms that when consumed in an adequate amount, will confer health benefits (Martín & Langella, 2019). Probiotics from various sources such as fermented food, agricultural and dairy products are widely investigated for their beneficial effects. A lot of prominent studies have shown the benefits of probiotics for gut health and the balance of microbiota in the stomach, the ability to modulate the immune system, alleviate topical problems and studies for the prevention of urogenital infections (Abatenh, 2018; Pujia et al., 2017). There are also studies of various probiotics strains for health benefits such as neurological benefits, endocrine disease management and the production of specific bioactive for medicinal purposes (Sanders et al., 2018).

Probiotics potentials in the dental field are based on a few benefits that can be developed for dental health improvements. This includes the ability to inhibit the attachment of related pathogens to the host tissue, modulation of immune responses to dental infections, and production of substances that can be antagonistic towards pathogenic bacteria (Allaker & Stephen, 2017). Probiotic has also been explored for the potential to combat biofilm formation by dental pathogens and disrupt the bacteria-to-bacteria complex communication (Pujia et al., 2017). The interest to study the benefits of probiotics had extended to their activity against periodontal-related pathogens and their mechanism of action. Currently, various clinical trials and fundamental research investigated the efficacy of probiotics as a potential approach in assisting the management of the periodontal disease(Pujia et al., 2017). Nonetheless, the underlying mechanism of action is largely unclear due to the complicated condition of oral microbiota and the lack of specificity in probiotic action on the pathogens (Mahasneh & Mahasneh, 2017).

1.2 Problem Statement

The discovery and development of antimicrobials are needed as a supportive treatment to control the advancement of periodontal pathogens in periodontal disease. Despite the availability of clinical treatments for periodontal disease, recolonization of periodontal pathogens is still a major issue. To counter the situation, antibiotics are administered to control the colonization and recolonization of periodontal pathogens. However, the administration of antibiotics drugs or antibacterial mouthwash should be done with careful considerations due to the risk of antibiotic-resistant pathogens and oral discomforts such as teeth staining, reduction of taste, dry mouth, and oral ulcers in some cases. Additionally, the administration of antimicrobial treatments raised the concern of multi-drug resistant bacteria. Other than that, periodontal pathogens formed biofilm upon colonization which limits the efficacy of antimicrobial treatments. The presence of biofilm acted as protective layers that prevent the pathogens from being exposed to the antimicrobial treatment. Due to these problems, it is important to seek antimicrobial sources that can assist the inhibitions of periodontal pathogen, namely *P. gingivalis.*

In this study, probiotic is chosen as the supportive antimicrobial treatment to inhibit the keystone pathogen and combat biofilm formation due to its vast potential for oral health. It is timely to investigate the efficacy of probiotic actions on periodontal disease keystone pathogen, *P. gingivalis* and to assess the gene expression upon probiotics treatment to develop methods that is effective to combat *P. gingivalis* colonization and biofilm formation.

1.3 Research Question

This study aims to understand the following research questions:

1. Will *L. rhamnosus* ATCC 7469 cell-free supernatant (CFS) exhibit antimicrobial activity against *P. gingivalis*?
2. Will *L. rhamnosus* ATCC 7469 CFS inhibit the biofilm formation of *P. gingivalis*?
3. What molecular pathway is disrupted in the gene expression of *P. gingivalis*upon the treatment with *L. rhamnosus* ATCC 7469 CFS?

1.4 Objectives

The main objective of this study is to investigate the molecular mechanism of antimicrobial activity of *L. rhamnosus* ATCC 7469 CFSagainst periodontal pathogen *P. gingivalis.*

1. To determine the antimicrobial activity of *L. rhamnosus* ATCC 7469 CFS by antimicrobial assays.
2. To assess the anti-biofilm activity of *L. rhamnosus* ATCC 7469 CFS against *P. gingivalis.*
3. To investigatethe effect of *L. rhamnosus* ATCC 7469 CFS on *P. gingivalis* gene expression.

1.5 Significant of the Study

The outcomes of this study are to provide new knowledge and understanding of probiotics as a supportive antimicrobial treatment against *P. gingivalis*. The development of probiotics as one method of supportive treatment is crucial to combat *P. gingivalis* biofilm formation andrecolonization besides countering the drawbacks of current antimicrobial treatments such as the emergence of antibiotics resistant strain and various oral discomforts observed in some patients. Other than that, the application of potential antimicrobials to suppress the identified genes might be a subject of interest in future studies.