UNIVERSITI TEKNOLOGI MARA

ANTIBACTERIAL ACTIVITY OF *Lactobacillus* spp. ISOLATED FROM FERMENTED FOOD PRODUCTS AGAINST *Porphyromonas gingivalis*

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ABSTRACT

Fermented foods and drinks such as cheese, cultured milk, yogurt, pickles and tempeh contain lactic acid bacteria such as *Lactobacillus* that promote a lot of benefits to the human. The emergence of antibiotic resistance of oral pathogens brings a concern of the effect of *Lactobacillus* spp. on oral disease especially in the case of periodontitis. *Porphyromonas gingivalis* is the causative agents for periodontitis. Five samples of fermented foods and drinks which are cheese, cultured milk, yogurt, pickles and tempeh were collected from the local supermarket. de Man Rogosa and Sharpe (MRS) medium was used to isolate the *Lactobacillus* spp. The pure colonies were further identified through the morphological, biochemical tests and molecular methods. The sequences obtained from 16S rDNA gene sequencing were analyzed in BLAST and compared with the biochemical test identification. All isolates were investigated for the antagonistic activities on *P. gingivalis* ATCC 33277 through agar well diffusion method. One way ANOVA was employed to analyze the diameter of inhibition zones between the isolates. The presence of inhibitory substances was also determined through agar well diffusion method. Isolation of *Lactobacillus* from five samples found a total of 41 isolates that showed the characteristics of *Lactobacillus*. Biochemical tests led to the identification of the five probable species comprising *Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus reuteri* and *Lactobacillus suebicus*. Comparison of sequencing analysis and biochemical test identification exhibited that all isolates from cheese, cultured milk, yogurt and pickle samples showed 98-100% similarity. Meanwhile all isolates from tempeh sample were identified as *L. fermentum* with 91-98% similarity Isolate *L. plantarum* displayed the highest mean diameter of inhibition zone which was 14.00$\pm$0.58 mm and followed by *L. rhamnosus* and *L. paracasei* where the diameters of the inhibition zones were 11.67$\pm$0.33 mm and 11.00$\pm$0.58 mm, respectively. Isolates of *L. fermentum* were the least effective in suppressing the growth of *P. gingivalis* where the diameters of both species were 9.83$\pm$0.17 mm. There was significance difference of inhibition zones ($p<0.05$) between the species. All isolates also produced organic acids to inhibit *P. gingivalis*. As a conclusion, *Lactobacilli* spp. were successfully obtained from all samples. From identification of the biochemical tests and 16S rDNA, the isolates can be identified as *L. plantarum, L. rhamnosus, L. paracasei* and *L. fermentum*. All isolates of *Lactobacillus* were able to suppress the growth of *P. gingivalis* and produced organic acids. For recommendations, it is suggested to carry out phylogenetic study of isolated *Lactobacillus* in the future study to examine the relatedness among species. Isolation of *Lactobacillus* from more samples of fermented products should be carried out. Also, in the future, a further analysis such as the structure of the cell wall, the ability to aggregate and adhere on the oral mucosa should be explored on the potential *L. plantarum*.

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CHAPTER ONE
INTRODUCTION

1.1 Background of Study

Periodontal diseases are inflammation that occurs at the supporting tissues of teeth caused by *Porphyromonas gingivalis* where it is one of the prominent periodontal pathogens (Tokutomi *et al.*, 2015). The anaerobiosis life of *P. gingivalis* makes it one of the major periodontal pathogen in an adult's inflammatory periodontal disease (Nichols *et al.*, 2004). Previous study found that *Aggregatibacter actinomycetemcomitans* and *P. gingivalis* are able to adhere and invade oral epithelial cells and can even grow intracellularly (Kulik *et al.*, 2008). The current treatments for periodontal disease are antibiotic and surgical procedures such as bone surgery, splints and soft tissue graft (JADA-Journal of American Dental Association, 2005). The use of antibiotic such as tetracycline and chemical plaque control such as chlorehexidine are potentially giving a harmful side effect like allergic symptoms (Meurman, 2009). These dental caries and periodontal disease treatment need the patients to invest significant costs. To reduce the oral health care amount, the use of viable *Lactobacilli* is encouraging (Meurman, 2009).

Lactic acid bacteria such as *Lactobacilli* are Generally Recognized as Safe (GRAS) bacteria that have been used extensively as a starter culture in the fermented foods production (Gharaei-Fathabad and Eslamifar, 2011). A growing interest involving the live *Lactobacilli* among the foods and health markets sector is due to the emergence of antibiotic resistant pathogens (Forouhandeh *et al.*, 2010). This has force the market to develop more therapeutic and bio-preservatives products as alternative to antibiotic (Osuntoki *et al.*, 2008). *Lactobacilli* have successfully capture worldwide attention due to its ability in the prevention, control and treatment of diseases and in health maintenance (Osuntoki *et al.*, 2008). Viable *Lactobacilli* are reported to produce antimicrobial metabolites that are capable to suppress oral pathogens from causing periodontal diseases (Jain and Sharma, 2012). The ability of the viable *Lactobacilli* in adhering to the oral mucosa and suppressing the growth of periodontal pathogens (Haukioja *et al.*, 2006) helps the maintenance of the microecological balance in the