

## CHAPTER 1

### INTRODUCTION

*Bacillus* spp. Gram-positive bacteria, anaerobes or facultative (capable of growth with or without oxygen). Spoilage food by *Bacillus* spp. is the most common spoilage due to ability of this organism to produce spores can survive high processing temperatures (Ellin, 2007), and This spores are resistant to heat, cold, radiation, desiccation, and disinfectants (Turnbull, 1996). *B. cereus* is the most common *Bacillus* cause food poisoning due this type has ability to produce enterotoxins and emetic toxin, The diarrheal type of food-borne illness related to this type of toxin. *Bacillus subtilis*, *B. pumilus*, *B. licheniformis* and *B. anthracis* other type of *Bacillus* spp. that have been linked to incidents of food-borne illness also can produce enterotoxins (From et al., 2005).

There are different methods to reduce and kill vegetative cell and spore germination of *Bacillus* spp. such as high temperatures, low temperature, irradiation, high hydrostatic pressures, pulsed electric fields, chemical preservative are not generally found naturally in foods or produced by microorganisms (Propionic, sorbic, benzoic acids ). This method sometimes effect of sensory properties of the food (Gupta, 2007). Lactic acid bacteria (LAB) are microorganisms prefer environment rich in carbohydrate such as fermented foods, plants. LAB considered as part of normal microflora in the human and animal bodies that live in gastrointestinal and genitourinary tracts (Florou-Paneri et al., 2013), due to this reason they have been classified as 'Generally Recognized As Safe' (GRAS) (Adimpong et al., 2012).

Lactic acid bacteria (LAB) are Gram-positive, non-spore forming, catalase-negative bacteria, facultative but aero-tolerant, fastidious, acid tolerant and strictly fermentative (Šušková et al., 2010). The genera of LAB include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* (Arimah & Ogunlowo, 2014). LAB divided into two groups according on the end-products formed during the fermentation of glucose, Homofermentative lactic acid bacteria such as *Pediococcus*, *Streptococcus*, *Lactococcus* and some *lactobacilli* produce lactic acid as end-product of glucose fermentation. Heterofermentative group ferment

glucose with lactic acid, ethanol/acetic acid and carbon dioxide (CO<sub>2</sub>) as by-products this group of LAB including *Weissella* and *Leuconostoc* (Rattanachaikunsopon & Phumkhachorn, 2010).

Lactic acid bacteria used in fermentation industries (Bayane et al., 2010) as preservation agents due to ability of these bacteria to exert antimicrobial activity against pathogens (Fraga Coteló et al., 2013), by a decrease in pH as a result of lactic acid and other organic acids (Djadouni & Kihal, 2012). LAB can produce antimicrobial compounds other organic acid such as diacetyl, hydrogen peroxide, and bacteriocin (Savadog et al., 2004).

Bacteriocins are antimicrobial proteinaceous compounds produced by both Gram-positive and Gram negative bacteria (Line et al., 2008). Bacteriocin producing bacteria (BPB) has provided many benefits in improving feed animal (Yusuf & Hamid, 2013). Several studies indicated that LAB starter cultures in fermented vegetables, dairy products, Cheddar cheese, fermented sausage and sourdough are able to produce bacteriocins that function as biopreservatives against food spoilage and pathogenic bacteria (De Vuyst & Leroy, 2007). Examples of bacteriocins are plantaricin 35d produced by *Lactobacillus plantarum* and active against *Aeromonas hydrophila*; bacteriocin ST151BR produced by *Lb. pentosus* ST151BR, and bacteriocin produced by *Lb. paracasei* subsp. *paracasei* was active against *Escherichia coli*; thermophylin produced by *Streptococcus thermophilus* was active against *E. coli*, *Yersinia pseudotuberculosis* and *Y. enterocolitica*. Bacteriocin also inhibited Gram-positive species including several *Bacillus* sp., *Listeria monocytogenes* (Parada et al., 2007). Bacteriocin produced by *Lb. casei* showed inhibitory activity against pathogens including *E. coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Citrobacter freundii* (Rashti & Koohsari, 2015).

Most studies on antimicrobial activity of LAB and their supernatants have concentrated on bacterial vegetative cells and antifungal activity (Muhialdin & Hassan, 2011; Muhialdin et al., 2012). Limited study on *Bacillus* spp. spore germination showed that strains of *Lb. acidophilus* inhibited the germination of bacterial spores of *Bacillus* spp. (Klewicka & Libudzisz, 2004), Spores of *B. cereus* were more resistant to the organic acids compared to vegetative cells (Wong & Chen, 1988). Study on antimicrobial activity of LAB against *Bacillus* spore germination isolated from Malaysia food sources effective against growth of vegetative and spore germination of *Bacillus* spp. is limited. Therefore, the purpose of this study was to isolate and determine the ability of isolated LAB from fermented foods available in Malaysia that can

prevent the growth and spore germination of *Bacillus* spp. for possible application to extend the shelf life of food and make food safe from *Bacillus* spp. poisoning.

The objectives of this study were:

- i. To isolate LAB from three types of-fermented foods available in Malaysia.
- ii. To determine the antimicrobial activity of isolated LAB against *Bacillus* spp.
- iii. To evaluate the sporocidal activity of LAB cells and supernatants against *Bacillus* spp.
- iv. To determine the effect of proteolytic enzymes on the antimicrobial and sporocidal activity of the LAB supernatants against *Bacillus* spp.