

1. INTRODUCTION

1.1. Background of Research

Indonesia which belongs to tropical country has many kinds of bamboo species. Some bamboo species have high economic value and could be processed as edible product *e.g.* *Bambusa vulgaris* (Bambu Ampel) (Chongtham *et al.*, 2011). Bamboo shoots are often consumed as pickle due to their perishability. Pickle is a traditional fermented food made of vegetables. Pickling can be done using lactic acid bacteria (LAB) which are natural microbia associated with bamboo shoots, controlled temperature, and salt concentrations to regulate the fermentation process (Atlas, 2006). During fermentation process of vegetables, LAB are influenced by several factors including initial sugar concentration, pH, salt concentration, and temperature (Fleming *et al.*, 1985). Salt concentration and temperature are the most important factors affecting vegetables fermentation, since vegetables fermentation occurs mainly by the microorganisms naturally present in raw vegetables (Hutkins, 2006). Fermentation of Ampel Bamboo Shoots Pickle under different salt concentrations and temperatures were evaluated by Armando (2016) and Mariana (2016). It has been reported that LAB isolated from Ampel Bamboo Shoot Pickles which were fermented in 2.5% of salt concentration at 15°C for 5 days and in 5.0% of salt concentration at 30°C for 4 days had shown antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

Different synthetic chemicals are continuously used during the processing of food in order to avoid contamination of foodborne pathogens and increase the shelf-life. Nowadays, consumers concerns regarding the safety of synthetic chemicals used to preserve foods and their side effects are increasing. Therefore, there has been growing interest in identifying natural and safe antibacterial compounds from various natural sources *e.g.* LAB. LAB have been widely used for preservation of fermented products due to their ability to produce antimicrobial compounds including bacteriocins (Soomro *et al.*, 2002). Bacteriocins of LAB are considered as safe additives (food-grade) and useful to inhibit spoilage as well as pathogenic microorganisms either in foods or feed.

Therefore, bacteriocins could be natural replacement for synthetic food preservatives (Ennahar *et al.*, 1999).

Bacteriocin production is usually performed in complex growth medium. Although this medium promote relatively high bacteriocin concentration levels, its high cost limits its suitability for a large-scale production. Therefore, by-product from the food industry such as whey was used as culture medium for bacteriocin production in order to reduce cost (Mirhosseini & Emtiazi, 2011). Jozala *et al.*, (2011) investigated the effect of different milk whey concentrations as culture medium to produce bacteriocin. It has been found that the optimal bacteriocin production was achieved in milk whey medium with concentration of 100 gL⁻¹. However, Guerra *et al.*, (2001) in Khay *et al.*, (2012) showed that bacteriocin production on whey medium was lower than that obtained on deMan Rogosa Sharpe (MRS) medium due to its lack of some essential nutrients for growth and bacteriocin production compared to those of MRS medium.

Further studies showed that the supplementation of carbon and nitrogen sources are needed to optimize the bacteriocin production on whey medium. Guerra *et al.*, (2007) in Jozala *et al.*, (2011) showed that supplementation of glucose into milk whey could be an appropriate alternative for increasing bacteriocin production. Based on Ananou *et al.*, (2008) it has been found that the key factor for optimal bacteriocin production in whey-based substrate was addition of 10 gL⁻¹ glucose. In addition, Todorov & Dicks (2005^b) showed that optimal bacteriocin production by *Lactobacillus plantarum* ST194BZ isolated from Boza (traditional fermented beverage from Belogratchik) was recorded in the presence of tryptone (20 gL⁻¹), combination of tryptone and yeast extract (1:0.6). Furthermore, Daba *et al.*, (1993) and Amiali *et al.*, (1998) in Goulhen *et al.*, (1999) has showed that optimal bacteriocin production was achieved in whey permeate medium supplemented with 20 gL⁻¹ of yeast extract. Mirhosseini & Emtiazi (2011) investigated that supplementation of yeast extract on cheese whey medium was useful for bacteriocin production.

The research about the effect of initial fermentation conditions (salt concentration and temperature) on characteristic of LAB isolated from Ampel Bamboo Shoots Pickle

particularly in their ability to produce bacteriocin is still limited. Furthermore, the research about effect of supplementation of carbon and nitrogen sources on whey medium towards bacteriocin inhibitory activity of LAB isolated from bamboo shoot pickle is also still limited. Therefore, this study is aimed to investigate the effect of initial fermentation conditions (salt concentration and temperature), carbon and nitrogen supplementation on whey medium towards bacteriocin production of LAB isolated from Ampel Bamboo Shoots Pickle, and also to find out the isolate with optimal bacteriocin inhibitory activity to inhibit pathogenic bacteria.

1.2. Literature Review

1.2.1. Lactic Acid Bacteria (LAB)

LAB are group of Gram-positive, cocci or rod, non-spore forming, non-motile, catalase negative, anaerobic but aerotolerant, which produce lactic acid as the main end product of carbohydrate fermentation. The classification of LAB into different genera is based on morphology, mode of glucose fermentation, configuration of the lactic acid produced, growth ability at different temperature, high salt concentrations, and acid or alkaline tolerance (Salminen *et al.*, 2004; Hutkins, 2006). According to Hutkins (2006), LAB can be divided into two types based on the results of fermentation, namely:

- **Homofermentative**
Homofermentative LAB convert glucose to lactic acid through glycolytic Embden-Meyerhoff pathway. This type of LAB includes *Lactococcus lactis*, *Pediococcus sp.*, *Streptococcus thermophilus*, *Lactobacillus belveticus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*.
- **Heterofermentative**
Heterofermentative LAB convert glucose to lactic acid, ethanol, acetic acid, and CO₂ through phosphoketolase pathway. This type of LAB has been widely used in food fermentations. Included are *Lactobacillus mesentroides* subsp. *cremoris*, *Lactobacillus mesentroides* subsp. *mesentroides*, *Leuconostoc lactis*, and *Leuconostoc kimchii*.

LAB have been widely used for preservation of fermented products due to the production of antibacterial substances such as organic acids, hydrogen peroxide, diacetyl, and bacteriocins (Albano *et al.*, 2007 in Chanprasert & Gasaluck, 2011). The acid produced by LAB during the fermentation process will lead to a decrease in pH of fermented foods. Low pH value can inhibit the growth of some pathogens and spoilage microorganisms (Karovicova *et al.*, 1999 in Sayin & Alkan, 2015). LAB are found in milk, meat, and fermented products (vegetables and beverages) (Parada *et al.*, 2007).

The genus *Lactobacillus* is the largest of genus included in LAB and widespread in nature. Many species of *Lactobacillus* have been found in food industry. Generally, *Lactobacillus* are the most acid tolerant among LAB. Therefore, a lot of *Lactobacillus* species have been found in many spontaneous lactic fermentations such as vegetable fermentations (Salminen *et al.*, 2004). Armando (2016) and Mariana (2016) examined Ampel Bamboo Shoots Pickle and showed that *Lactobacillus* was the major genus. It has been reported that 79 of 90 total isolates from Ampel Bamboo Shoots Pickle were identified as *Lactobacillus* according to their morphology characteristics.

1.2.2. Bamboo Shoots Pickle

Bamboo belongs to *Gramineae* family and spreads over the tropical, subtropical, and temperate climate area (Darmayanti *et al.*, 2014). Some bamboo species have high economic value and could be processed as edible product *e.g.* *Dendrocalamus asper* (Bambu Betung), *Gigantochloa atter* (Bambu Legi), and *Bambusa vulgaris* (Bambu Ampel) (Chongtham *et al.*, 2011). Bamboo shoots are known for their nutritional value and delicious taste. It has been reported that bamboo shoots are rich in nutrients, mainly protein, carbohydrate, and minerals, but have low fat content. Bamboo shoots also contain phytosterols and a high amount of fiber that have cholesterol-lowering and anticarcinogenic activity. Therefore, bamboo shoots could be called as nutraceuticals or natural medicines (Chongtham *et al.*, 2011).

Bamboo shoots are characterized by perishability in natural condition. Fermentation is one of the most common methods of preserving bamboo shoots. Fermented bamboo

shoots could have longer shelf-life for 6 months than unfermented bamboo shoots (Pandey *et al.*, 2012). A variety of fermented bamboo shoots had been prepared in the Himalayan region for many centuries. Tamang *et al.*, (2008) mentioned that *mesu*, *soidon*, *soibum*, and *soijim* were some kind of fermented bamboo shoots which produced by LAB fermentation. In addition, Choudhury *et al.*, (2012) also mentioned *khorisa-tenga*, *amil hendua* (fermented bamboo shoots from India), *alu tama* (fermented bamboo shoots from Bhutan), and *naw mai dong* (fermented bamboo shoots from Thailand) were produced by LAB fermentation.

According to Pandey *et al.*, (2012) bamboo shoots can be processed into pickle by cutting into small pieces (2 cm), boiling, drying in the air for 1 hour, and adding salt. The bamboo shoots are transferred into a sterile glass container. The mustard oil is heated until smoke comes, cooled for 10 minutes and poured into the container. The pickle is stirred once a day and kept unopened for a week. Panjaitan (2012) reported that salting technique in fermented pickles are dry salting and brine salting. Dry salting is process of making pickle by adding a 50 g amount of crystal salt per kg raw material, while brine salting is process of making pickle by soaking the raw material in 5.3-10.5% of saline solution. In addition, Tamang & Sarkar (1996) reported the processing of *mesu* using traditional method. The shoots are cut into pieces (1-2 cm) and transferred into a glass bottle, tightly packed to maintain an airtight environment during fermentation. The material is incubated at ambient temperature (20-25°C) for 7 to 8 days.

1.2.3. Important Role of Lactic Acid Bacteria in Pickle Fermentation

Pickle is a traditional fermented food made of vegetables. Pickling is known as a conversion of sugar to acid by lactic acid bacteria which are natural bacteria. As a result from pickling, vegetable will have longer shelf-life, translucent appearance, firm texture, and pickle flavor. Fermentation of vegetable can occur spontaneously by natural lactic acid bacteria, such as *Lactobacillus sp.*, *Leuconostoc sp.*, and *Pediococcus sp.* (Karovicova *et al.*, 1999 in Sayin & Alkan, 2015). Mheen & Kwon (1984) in Zhou *et al.*, (2014) also showed that diverse groups of LAB which have been isolated from fermented vegetables are *Leuconostoc*, *Lactobacillus*, *Lactococcus*, *Pediococcus*, etc.

According to Holzapfel (1997) and Leroy & De Vuyst (2004) in Con & Karasu (2009), LAB have an important role in the fermentation of pickle because they provide a rapid acid accumulation in the raw material by producing lactic and several organic acids. Furthermore, LAB can also produce various aroma component, bacteriocins, and exopolysaccharides which contribute to the development of some characteristic properties such as taste, visual appearance, texture, shelf-life, and safety. Following to Albano *et al.*, (2007) in Chanprasert & Gasaluck (2011), it has been found that during fermentation process LAB also produce antibacterial substances against food spoilage and food-borne pathogens such as organic acids, hydrogen peroxide, diacetyl, and bacteriocins.

It has been reported by Tamang & Sarkar (1996) that LAB which play important role in fermentation of *mesu* (fermented bamboo shoots from North East Himalayan) were *Pediococcus pentosaceus*, *Lactobacillus brevis*, and *Lactobacillus plantarum*. Furthermore, Chen *et al.*, (2010) reported that LAB which were isolated, characterized, and identified from *jiang-sun* (fermented bamboo shoots from Taiwan) were *Lactobacillus plantarum*, *Enterococcus faecium*, and *Lactococcus lactis* subsp. *lactis*. Choudhury *et al.*, (2012) also showed that *naw mai dong* (fermented bamboo shoots from Thailand) contained *Lactobacilli*, *Leuconostoc* and *Pediococci*. In addition, Dhavises (1972) in Alemu *et al.*, (2006) examined fermented bamboo shoots and reported that the major species were *Lactobacillus brevis*, *Lactobacillus plantarum*, and *Pediococcus pentosaceus*. These LAB species were similar to the predominant species in fermented bamboo shoots from India (Tamang & Sarkar, 1996).

1.2.4. Bacteriocins

Bacteriocins are peptide compounds which are synthesized by ribosomes of bacteria to inhibit other bacteria growth. Many bacteriocins have narrow killing spectrum which inhibit only closely related strains (Cleveland *et al.*, 2001). It has been reported that Gram-negative bacteria were mostly resistant to bacteriocins of LAB (Zhou *et al.*, 2014). Bacteriocins are considered as safe additives (food-grade), useful to inhibit spoilage and pathogenic microorganisms either in foods or feed. Therefore, bacteriocins

could be natural replacement for synthetic food preservatives (Ennahar *et al.*, 1999). According to their biochemical and genetic characteristics, bacteriocins produced by LAB can be classified into three major classes *i.e.* class I (lantibiotics), class II (non-lantibiotics), and class III (large heat-labile proteins) (Parada *et al.*, 2007).

Class I lantibiotics are small (<5 kDa) heat-stable peptides, containing unusual amino acids such as lanthionine, methyl lanthionine, dehydrobutyrine, and dehydroalanine. Class II non-lantibiotics are small (<10 kDa) heat-stable peptides, containing regular amino acids. Class III bacteriocins are large (>30 kDa) heat-labile peptides (Cleveland *et al.*, 2001). Class I and II bacteriocins are the most studied since they are widespread among LAB and most likely to be used in food applications. A fourth class which consists of large complexes with other macromolecules has been proposed (Klaenhammer, 1993 in Cleveland *et al.*, 2001). Each bacteriocin has different inhibition profile against food spoilage or food-borne pathogens. It has been reported that bacteriocin production is affected by several factors including carbon and nitrogen sources, and fermentation conditions, such as pH, temperature, and agitation (Kumar *et al.*, 2015). In general, bacteriocin is closely associated with the growth of bacterial culture since it is released during the growth of bacteriocin-producing cultures and its efficiency decreases slowly at the end of bacterial growth due to protease degradation (Hur *et al.*, 2000 in Thirumurugan *et al.*, 2015).

In the previous study, the presence of bacteriocin-producing LAB in Thai fermented bamboo shoots has been evaluated (Rattanachaikunsopon & Phumkhachom, 2000 in Alemu *et al.*, 2006). However, the LAB isolated from fermented bamboo shoots did not show bacteriocin activity against *Leuconostoc mesentroides* TISTR 473. In contrast, Alemu *et al.*, (2006) showed that LAB strain N1-33 which isolated from the same source had broad spectrum of bacteriocin activity against *Leuconostoc mesentroides* TISTR 473, other Gram-positive food spoilage, and pathogenic bacteria.

1.2.5. Whey as Culture Medium for Bacteriocin Production

Whey is a by-product of the dairy industry and it contains rich nutrients such as lactose, soluble proteins, and mineral salts. Whey has been treated as waste and represents an important disposal and pollution issue because of its high biological and biochemical oxygen demand. However, whey was found to be used as culture medium for bacteriocin production (Mirhosseini & Emtiazi, 2011), although the yield was lower than that obtained on MRS medium due to its lack of some essential nutrients for growth and bacteriocin production (Guerra *et al.*, 2001 in Khay *et al.*, 2012). Guha *et al.*, (2013) also reported that whey contains lesser amount of carbon and nitrogen sources. Therefore, supplementation is needed to enhance the bacteriocin production on whey medium.

Guerra *et al.*, (2007) in Jozala *et al.*, (2011) showed that supplementation of glucose into milk whey could be an appropriate alternative for increasing bacteriocin production. Based on Ananou *et al.*, (2008) it has been found that the key factor for optimal bacteriocin production in whey-based substrate was addition of 10 gL⁻¹ glucose. Todorov & Dicks (2005^a) also reported that higher glucose levels supplemented on medium stimulated the bacteriocin production of *Lactobacillus rhamnosus*. Ogunbanwo *et al.*, (2003) evaluated the effect of supplementation for bacteriocin production by *Lactobacillus brevis* OG1. It was observed that higher bacteriocin production was achieved with addition of 1% of glucose and 2-3% of yeast extract.

According to Todorov & Dicks (2005^a), bacteriocin production of *Lactobacillus rhamnosus* was stimulated by the presence of tryptone as nitrogen source. Todorov & Dicks (2005^b) also showed that optimal bacteriocin production by *Lactobacillus plantarum* ST194BZ isolated from Boza (traditional fermented beverage from Belogratchik) was recorded in the presence of tryptone (20 gL⁻¹), combination of tryptone and yeast extract (1:0.6). Other result was found by Mirhosseini & Emtiazi (2011), supplementation of yeast extract on cheese whey medium was useful for bacteriocin production. In addition, Enan & Al Amri (2006) reported that supplementation of 1.5% of yeast extract on whey permeate yielded more bacteriocin

plantaricin UG1 by *Lactobacillus plantarum* UG1. Yeast extract has also been reported to induce bacteriocin productions, namely mesenterocin, pediocin AcH, and nisin.

1.3. Objectives

The objectives of this study are to examine the effect of initial fermentation conditions (salt concentration and temperature), carbon and nitrogen supplementation on whey medium towards bacteriocin production of LAB isolated from Ampel Bamboo Shoots Pickle, and also to find out the isolate with optimal bacteriocin inhibitory activity to inhibit pathogenic bacteria, *Escherichia coli* (FNCC 0091), *Listeria monocytogenes* (FNCC 0156), and *Staphylococcus aureus* (FNCC 0047).

