1. INTRODUCTION

1.1. Background of research

Bamboo shoots is the one of food items which are abundant in Indonesia. In the world there are about 1.300 bamboo species, and 145 of them are indigenous species from Indonesia (Kencana *et al.*, 2012 in Putra *et al.*, 2013). Some bamboo species have high economic value and could be processed as edible product that preferred among people around the world. One of them is "Ampel" bamboo (*Bambusa vulgaris*) shoot. Fresh bamboo shoots are seasonal, perishable in nature and short-lived. Fermentation is one of the ways to preserve bamboo shoots, while the process also brings out typical taste as vegetable pickles. Bamboo shoots pickles is part of Indonesian diet, but aside from see it as only a nourishment, the fermentation process opens more potential benefit in microbial technology. Fermentation of bamboo shoot can occur spontaneously by natural lactic bacterial surface microflora, since such vegetable could provide a natural medium for lactic acid fermentation (Swain *et al.*, 2014). Food fermentations by lactic acid bacteria (LAB) have a great economic value and contribute in improving human health, due to their potential uses as bio-preservatives and probiotics because of their abilities to produce several functional metabolites, like bacteriocins (Hutkins, 2006).

According to Rahayu (2003) young bamboo shoots (*Bambusa sp*.) can be processed into salted fermented bamboo shoots by trimming, slicing, and mixing bamboo shoots with (2-3%) brine, placed into sealed container and incubated for several weeks. Salt concentration, pH, nutrient and temperature are the important factors of the succession of LAB fermentation and metabolism. In previous study, Armando (2016) and Mariana (2016) evaluated fermentation of Ampel bamboo shoots pickle. The studies reported that LAB isolated from Ampel Bamboo Shoot Pickles, which fermented in 2.5% of salt concentration at 15ºC for 5 days and in 5.0% of salt concentration at 30ºC for 4 days had shown antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. The antimicrobial activity of LAB took role on its preservatives effect in the fermentation process, which was attributed to the production of bacteriocins and other

antimicrobial compounds such as lactic, acetic acid and hydrogen peroxide (Soomro *et al.*, 2002). The study by Armando (2016) and Mariana (2016) already revealed the succession of LAB species in fermented Ampel bamboo shoot but did not investigate the presence of bacteriocin-producing LAB in the process.

Bacteriocins produced by LAB have attracted special interest as potential alternative safe commercial food preservatives. LAB have been used as food and feed preservatives for centuries, and bacteriocin-producing LAB could replace chemical preservatives for the prevention of bacterial spoilage and the outgrowth of pathogenic bacteria in food products (Chen *et al.*, 2010). In order to reach optimum bacteriocin production, LAB need appropriate biochemical and biophysical environment to grow and express normal metabolic activities. The biochemical environment conditions were made by nutrients in the culture media. For LAB cultivation, supplementary nutrients like carbon source and the widely used complex nitrogen sources such as yeast extract, beef extract and peptone are important necessities for growth (Tantjana & John, 2002 in Kuntiya *et al.*, 2010). According to Todorov (2008), the presence of sucrose as carbon source could induce bacteriocin production from LAB strain isolated from fermented milk, In addition, Desmukh and Thorat (2013) found that supplementation with 1% of peptone and 0.8% of yeast extract yielded maximum bacteriocin production of *Lactobacillus rennanquilfy WHL 3*. Based on the potential of these nutrients, this study will evaluate the effect of sucrose and nitrogen (peptone and yeast extract) supplementation in culture medium, also different initial fermentation conditions (salt concentration and temperature) towards bacteriocin production of LAB isolated from Ampel bamboo shoot pickle.

1.2. Literature review

1.2.1. Lactic Acid Bacteria (LAB)

Lactic acid bacteria constitute a group of bacteria that have morphological, metabolic and physiological similarities, and also relatively closely related phylogenetically. The common characteristics of LAB were Gram-positive, fermentative, catalase negative,

facultative anaerobes, non-sporeforming, non-respiring cocci or rods, low mol% G _ C, non-motile and acid-tolerant (Hutkins, 2006; Salminen *et al.*, 2004). At present, bacterial species from 12 genera were included in a group designated as lactic acid bacteria because of their ability to metabolize relatively large amounts of lactic acids from carbohydrates. The genera include *Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Lactobacillus, Enterococcus, Aerococcus, Vagococcus, Tetragenococcus, Carnobacterium, Weissella,* and *Oenococcus* (Ray, 2004).

Based on their carbohydrate metabolism, LAB could be divided into two groups which are homofermentative and heterofermentative bacteria. In the homofermentative pathway, more than 90% of the sugar substrate was converted exclusively to lactic acid. In contrast, the heterofermentative pathway results in about 50% lactic acid, with the balance as acetic acid, ethanol, and carbon dioxide. Lactic acid bacteria possessed one or the other of these two pathways (*i.e.*, they are obligate homofermentative or obligate heterofermentative), although there were some species that have the metabolic wherewithal to perform both (facultative homofermentative) (Hutkins, 2006). The homofermentative group composing *Lactocococcus, Pediococcus, Enterococcus, Streptococcus* and some lactobacilli, while the members of heterofermentative group include *Leuconostoc, Weissella* and some lactobacilli (Franz *et al.*, 1999 in Ross *et al.*, 2002)

Lactic acid bacteria were often described as being fastidious with complex nutritional requirements, and indeed, there were species that will grow only in nutrient-rich, well-fortified media under optimized conditions (Hutkins, 2006). For LAB cultivation, supplementary nutrients like carbon source and the widely used complex nitrogen sources such as yeast extract, beef extract and peptone are important necessities for growth (Tantjana & John, 2002 in Kuntiya *et al.*, 2010). According to De Vuyst (1994) in Todorov (2008), LAB were also known for their production of antimicrobial compounds, including bacteriocins or bacteriocinlike peptides

1.2.2. Bamboo Shoots

Bamboo shoots are the young bamboo plants that have just emerge from the ground, generally 20-30 cm long (Choudhury *et al.*, 2012). The bamboo shoots in fermented form were consumed as one of favourite traditional foods by different ethnic communities. Bamboo shoots had low fat and rich in protein, amino acids, minerals, fibre and carbohydrates. Phytosterols which presence in young shoots provides youthful feeling, athletic energy, and longevity to regular consumers (Bao, 2006 in Nongdam & Tikendra, 2014).

According to Choudhury *et al.* (2012), bamboo shoots were consumed as food items that best consumed in middle of growing season as shoots harvested during the beginning or end of growing season tend to be either weaker or overdeveloped. The bamboo shoots could be further processed for consumption in fresh or fermented forms after harvest. They were popular food items and used to prepare different traditional dishes in many Asian countries like China, Korea, Japan, Thailand and Taiwan (Nongdam & Tikendra, 2014)

1.2.3. Important Role of Lactic Acid Bacteria in Bamboo Shoots Fermentation

Fermentation was one of the oldest method preservation method which depends on the biological activity of microorganisms. It was associated to the production of a range of metabolites to suppress the growth and survival of undesirable microflora in foodstuffs (Ross *et al.*, 2002). According to Savadogo *et al.* (2006), LAB took important role in food fermentation to obtain products which characterized by hygienic safety, storage stability and attractive sensory properties. LAB were used as natural or selected starters in fermentations to produce lactic and acetic acids (acidification). In fermented vegetables, LAB purposed to enhance digestibility, increases the vitamin levels and vitamin bioavailability. These microorganism also contributed to organoleptic properties and the preservation of fermented products by in situ production of antimicrobial substances such as lactic and acetic acid, hydrogen peroxide, bacteriocins etc. (De Vuyst & Vandamme, 1944 in Tudor *et al.*, 2011).

Dhavises (1972) in Alemu *et al.* (2006) examined fermented bamboo shoot and the result showed that the major species, which took role in the fermentation process were *Lactobacillus brevis*, *Lactobacillus plantarum* and *Pediococcus pentosaceus*. These same species also reported to be the predominant species in fermented bamboo shoot of India (Tamang and Sarkar, 1996 in Alemu *et al.*, 2006).

1.2.4. Bacteriocins

The term bacteriocin was used to refer a group of bioactive peptides produced by many bacterial strains from Gram-positive and Gram-negative groups. The bacteriocins produced by many strains of lactic acid bacteria and some propionic acid bacteria are of special interest in food microbiology because of their bactericidal effect normally to different Gram-positive spoilage and pathogenic bacteria and under stressed conditions to different Gram-negative bacteria important in food (Ray, 2004).

Daeschel (1989) in Chen *et al*. (2010) argued that bacteriocins produced by LAB had attracted special interest as potential alternative safe commercial food preservatives. LAB have been used as food and feed preservatives for centuries, and bacteriocin-producing LAB could replace chemical preservatives for the prevention of bacterial spoilage and the outgrowth of pathogenic bacteria in food products. Bacteriocins of food-grade lactic acid bacteria were considered safe food biopreservatives and had the potential to use them to kill sensitive Gram-positive food spoilage and foodborne pathogenic bacteria. Bacteriocins were more effective when used in minimally heat-processed foods in suitable combinations of two or more (*e.g.* nisin and pediocin) (Ray, 2004).

A large number of bacteriocins have been isolated and characterized from lactic acid bacteria and some have acquired a status as potential antimicrobial agents. It was correlated with the antagonistic affect against important food pathogens and considered as potential food preservatives. The important ones were named nisin, diplococcin, acidophilin, bulgarican, helveticins, lactacins and plantaricins. The lantibiotic nisin which is produced by different *Lactococcus lactis* spp. was the most thoroughly studied

bacteriocin to date and the only bacteriocin that is applied as an additive in food worldwide (Delves Broughton *et al.,* 1996; Nettles and Barefoot, 1993 in Savadogo *et al.,* 2006).

1.2.5. Sucrose as Medium for Bacteriocin Production

According to Hayek & Ibrahim (2013), LAB strains had shown preferences among different sugars and thus LAB strains vary in their abilities to ferment different sugars which could affect their growth and functionality. The differences in sugar requirements among LAB strains could be used for enumeration, selection, and identification. Even though glucose is commonly used in LAB media, LAB species and even strains have shown preferences among sugars for optimum growth and metabolic activity. Besides that, the concentration of sugars may also influence the growth and functionality of LAB.

Sucrose, commonly known as table sugar was kind of sugar which present in natural fruits and vegetables. Sucrose could be digested biochemically using invertase and chemically using hydrochloric acid into glucose and fructose monosaccharides(Fieser & Williamson, 1979 in Miloski *et al.,* 2008). Sucrose was one of fermentable sugar. Sucrose may also act as a donor of monosaccharides for exopolysaccharide formation in certain LAB (Salminen *et al.*,2004). It was also known as a source of nutrients and energy. The use of sucrose in microbiology as modification of carbon source in culture media has been studied by researcher in order to find the best medium composition to grow LAB for optimum bacteriocin production. The results were various depends on type LAB strains. Generally, the use of sucrose as carbon source and nitrogen source in media should be maintained properly to reach optimum condition. According to Wang *et al.* (2010), the combination of sucrose, beef extract and soypeptone increase the antimicrobial activity of bacteriocin production by *E. faecium* L17, while combination of sucrose and beef extract only show the decrease in antimicrobial activity.

1.3. Objectives

The objectives of this study are to examine the effect of initial fermentation conditions (salt concentration and temperature), sucrose and nitrogen supplementation on medium culture towards bacteriocin production of lactic acid bacteria isolated from Ampel bamboo shoot pickle, also to find out the isolate with optimum bacteriocin activity to inhibit pathogenic bacteria *Escherichia coli* FNCC 0091, *Listeria monocytogenes* FNCC 0156 and *Staphylococcus aureus* FNCC 0047.