4. DISCUSSIONS

4.1. Fermentation of Ampel Bamboo Shoots

Fermentation was one way to preserve vegetables and fruits due to its perishable properties. In this study, fermentation of Ampel bamboo shoot was carried out in salting condition incubated in different temperature. Salting was one basic type of vegetable fermentation (Montet *et al.,* 2014). The addition of salt in the fermentation could provides suitable environment for LAB to grow. Concentrations of salt used in this study were 2.5% and 5%. By using 2-5% of salt concentration, the fermentation of Ampel bamboo shoot was expected to be carried out by series of lactic acid bacteria (www.fao.org).

Besides salt concentration, temperature also contributes as natural parameter that affect the microbial population of vegetable fermentation. The temperature used in Ampel bamboo shoot fermentation was 15^{o}C and 30^{o}C. According to FAO, different bacteria could tolerate different temperatures. Optimum temperature for most bacteria was between 20 to 30^{o}C, while some prefer with higher or colder temperature (15 to 20^{o}C). Most LAB work best at 18 to 22^{o}C, while *Lactobacillus* species favors temperature above 22^{o}C. To reach optimum fermentation, the concentration of salt, pH of the mixture and temperature of fermentation must all be controlled to ensure a good fermentation and to prevent the growth of undesirable bacteria.

The fermentation brought in this study categorized as spontaneous fermentation, because no starter culture was added in the beginning of fermentation. Thus, the presence of LAB in this study was came out spontaneously. *Lactobacillus* sp. *Leuconostoc* sp. and *Pediococcus* sp. were the most LAB which typically involved in the process of spontaneous fermentation (Tudor and Zamfir, 2011). The turbid water in the end of fermentation (Figure 2a and 2b) may caused by the growth of microorganism and the product of metabolism released by microorganism during the process. To identify the LAB strain in Ampel bamboo shoot fermentation, isolation and identification test should be done.

4.2. Isolation of bacteria from Ampel bamboo shoot

Bacterial isolation, purification and identification were the first step for bacteriological studies. Isolation aimed to obtain pure bacterial cultures for the next analysis (Ruangpan and Tendencia, 2004). MRS-agar plates were used for the isolation of bacteria from Ampel bamboo shoot fermentation water. To distinguish acid-producing bacteria (LAB isolates), 1% $CaCO_3$ was added to the MRS-agar plates. Colonies of LAB were identified by the clear zone around each colony as seen in Figure 4. Djie & Wahyudin (2008) in Sharah *et al.* (2015) reported that the clear zones formed as a sign that the isolates had the capability to use glucose to produce organic acid to degrade $CaCO_3$. Single colony then randomly selected from MRS-agar plates and purified by re-plating on MRS-agar plates (Chen *et al.*, 2010). The isolation yielded 35 random isolates which selected for further LAB identification.

4.3. Screening of Lactic Acid Bacteria (LAB) from Ampel bamboo shoot

4.3.1. Cell Morphology and Motility Test

Cell morphology (gram staining and endospore staining) and motility test were done to observe the LAB isolates among the 35 isolates from Ampel bamboo shoot pickle (Salminen *et al.*, 2004). Gram staining was a method to divide cell into two groups (Gram-positive and Gram-negative) based on its cell wall structure. Gram-positive bacteria possess thick cell wall; consist of several layers of mucopeptide (responsible for thick rigid structure) and two types of teichoic acids, while Gram-negative bacteria possess a complex cell wall containing an outer membrane (OM) composed of lipopolysaccharides (LPS), lipoprotein (LP), thin peptidoglycan layer and phospholipids and a middle membrane (MM). The Gram-positive bacteria will appear purple in color (Figure 5a) caused by crystal violet and iodine dye, which entrapped within the thick and highly cross-linked matrix of Gram-positive. On the contrary, Gram-negative did not trapped as much crystal violet in the very first place, since it had very thin peptidoglycan layer, so decolorizer will enhancing loss of the dye complex that were present. This properties made Gram-negative appeared pinkish-red in color (Figure 5b)

(Moyes *et al.*, 2009). Based on Gram staining result, 34 isolates were found as Gram-positive bacteria, while one isolate identified as Gram-negative bacteria.

According to Ray (2004) bacteria have three morphological forms *i.e.* spherical (cocci), rod shape (bacilli), and comma. The shape of bacteria could be revealed after staining. Salminen *et al.* (2004) wrote that LAB could be divided into rods (*Lactobacillus* and *Carnobacterium*) and cocci (all other genera). Based on the observation result, all isolates were noticed to have rods shape under microscope.

Endospore staining test was done to differentiate between spore forming and non-spore forming bacteria. According to Rahman *et al.* (2015), bacterial endospores could differentiate cells formed within the vegetative cells. Endospore production was important for some bacteria to survive in adverse enviromental conditions. Spore-forming bacteria will have green color from *malachite green* dye and non-spore-forming will have brownish red to pink color. Pundir *et al.* (2013) stated that lactic acid bacteria was non-spore forming bacteria. Table 1 showed that all 35 isolates were categorized as non-spore forming bacteria. Hence, the isolates will appear red under microscope observation (Figure 6).

Motility test were done to determine motile and non-motile bacteria between the isolates. Motility is the ability of living systems to exhibit motion and to perform mechanical work at the expense of metabolic energy (Allen, 1981). Motile bacteria possess flagella to move by itself (UK Standards for Microbiology Investigation, 2016). It will migrate through the medium which becomes turbid, while non-motile bacteria will be confined to the stab inoculums. Table 1 showed 33 isolates were categorized as non-motile bacteria, indicated by growth along the inoculation line, but not further (Figure 7), while two isolates (B7 and B18) could not be identified because it had no visible growth on the medium (Figure 8). Salminen *et al.* (2004) stated LAB is non-motile bacteria. From cell morphology and motility test, 32 isolates were selected for further identification, based on their similarity properties with LAB, which are Gram-positive, rods shape, non-spore forming and non-motile (Pundir *et al.*, 2013)..

4.3.2. Physiological Test

Khalid (2011) wrote that LAB had core group consisting of four genera; *Lactobacillus, Leuconostoc, Pediococcus and Streptococcus*. The recent taxonomic revisions proposed several new genera including *Alloiococcus, Carnobacterium, Enterococcus, Lactococcus,* and *Weissella.* The classification of lactic acid bacteria into different genera was largely based on morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance (Khalid, 2011). In this study, genera of LAB from Ampel bamboo shoot pickle was determined by testing their ability to grow at different level of temperature, pH and NaCl concentration, referring to differential characteristics of LAB by Salminen *et al.* (2004).

The phisiological test result (Table 2) showed that from 32 isolates, 3 of the isolates were identified as *Lactobacillus* and 23 isolates were assumed as *Lactobacillus.* The rest 6 isolates were not determined because they did not show any similar characteristics with LAB genera (Salminen *et al.,* 2004). *Lactobacillus* species had varies response on the temperature parameter, pH 4.4 and 6.5% of NaCl, which mean some species could grow and some could not grow in that environment. The typical characteristic of *Lactobacillus* were they could not grow in pH 9.6 and 18% of NaCl. This study found three isolates which fit with these characteristics. On the other hand, isolates which shown most of the characteristics were assumed as *Lactobacillus* genera. However, some of these isolates also showed characteristic which did not match to *Lactobacillus,* such as they able to grow in pH 9.6, while this characteristic was belong to cocci shape bacteria. This founding were inline with Tanasupawat *et al.* (1992) wrote that some species from *Lactobacillus sp.* has the ability to grow in alkaline condition (pH 9-9.6). This characteristic also refer to aminopeptidase enzyme possessed by LAB, which affect their ability to adapt in their growth environment (De Angelis *et al.*, 2001 in Dewi & Anggraini, 2012). In addition, Pundir *et al.* (2013) described that the characteristics of LAB belonging to genus *Lactobacillus* should be gram positive, rod shaped, non endospore forming, catalase negative, must be acid producing and gas formation may or

may not be there from sugars and moreover LAB should also be pH, temperature, NaCl, bile salt tolerant and lactic acid producers from sugar.

4.4. Screening Probiotic Potentials of LAB

De Vrese (2001) in Savadogo *et al.* (2006) described probiotics as viable microbial food supplements, which beneficially influence the health of the host by balancing the microbial bacteria in gastrointestinal tract of human or animal. According to Salminen *et al.* (2004), resistance to acid and bile salt were some criteria for probiotic LAB selection. In present study, the resistance to acid and bile salt were tested to 32 isolates from Ampel bamboo shoot pickle, which already confirmed as lactic acid bacteria based on previous morphology, motility and physiological test.

Acid tolerance test performed in pH 3 and pH 7 as seen in Figure 9. The result showed that 26 isolates were able to grow at both pH condition. According to Setyawardani *et al.* (2011), LAB tolerances against acids were due to their abilities to keep the constant, higher alkalinity of cytoplasm relative to that of extracellular condition. The acid tolerance assay was conducted by adjusting pH to human gastrointestinal pH value. If LAB enter into human body, they will exposed to gastric acid with very low pH level, around 2 for the empty stomach and 3 for the full one. The LAB tolerance against acids was very important to withstand initial stress in the stomach, on the other side; acid condition in the long run was required by the LAB as a carrier of food such as in yoghurt (Minellia *et al.*, 2004 in Setyawardani *et al.*, 2011).

From bile salt tolerance test (Table 3), it could be known that 10 isolates (A7, A8, A11, A12, A15, A16, B1, B6, B12 and B13) were able to grow at 0.3% and 0.5% of bile salts (Figure 10). These isolates were classified as high bile salt tolerance. The rest of isolates were not able to grow at both or one of the conditions. The survival in bile salt condition explained by Setyawardani *et al.* (2011), found that several strains of *Lactobacillus* were able to hydrolyze bile salt by using specific enzymes (bile salt hydrolysis) that had the capability to decrease the solubility of bile salt, which in turn, decreased or eliminated the toxic effect of the bile salt to the LAB. This case was one of the factors,

why some LAB isolates were capable of surviving at bile salt condition. The inability in survival of LAB at bile salt condition was due to the ability of bile salt to destroy the main components of cell membrane, fat and fatty acids, of the LAB and in turn, it affects the permeability of cell membrane and viability of LAB (Succi *et al.*, 2005 in Setyawardani *et al.,* 2011). According to Havenaar *et al.* (1992), tolerance of bile salts was an essential requirement for colonization and metabolic activity of LAB in the small intestine of human or animal. These properties will help LAB to reach the gastro intestinal tract and contribute in balancing its microflora (Tambekar and Bhutada, 2010).

The probiotic potential assay resulting 8 isolates (A7, A8, A11, A12, A15, A16, B1 and B13) were adapted to grow at all acid and bile salts conditions, means that they had high potential as probiotics. Most of the isolates were recognized as *Lactobacillus* strain (Table 2). Salminen *et al.* (1998) in Savadogo *et al,* (2006) stated that the predominant population of lactic acid bacteria in the upper gastrointestinal tract is the *Lactobacillus* species, which may colonize the mucosal surface of the duodenum as well as the stomach. Pundir *et al.* (2013) also reported that within the group of LAB, *Lactobacillus* species were most commonly utilized group of microorganisms for their potential beneficiary properties as probiotics. These two findings support that LAB isolates from Ampel bamboo shoot were potential to act as probiotic.

4.5. Determination of Antimicrobial Activity of LAB

The ability of LAB to produce antimicrobial substances has long been used to preserve foods (Salminen *et al.,* 2004). The antimicrobial activity of LAB against pathogens was also contributed to the probiotic potential to combat with gastrointestinal microbial pathogens (De Vuyst & Leroy, 2007). In this study, LAB isolates from Ampel bamboo shoot were evaluated for its antimicrobial activity against pathogenic Gram-negative bacteria (*E. coli* FNCC 0091) and Gram-positive bacteria (*L. monocytogenes* FNCC 0156 and *S. aureus* FNCC 0047) (Kabadjova *et al.,* 2000 in Todorov and Dicks, 2005). Agar-well diffusion method was used to determine antimicrobial activity of the isolates.

Table 4 showed that all 32 LAB isolates had inhibitory activity against all pathogen indicator strain. The inhibitory effects were attributed to the production of antimicrobial compounds such as lactic, acetic acid and hydrogen peroxide (Soomro *et al.*, 2002). The production of organic acids (lactic, acetic and propionic acids) as end products of LAB was very important to provides an acidic environment which unfavorable for the growth of many pathogenic and spoilage microorganisms (De Vuyst *et al.*, 2004 in De Vuyst & Leroy, 2007). Largest inhibitory activity was performed by isolate B6 against *L. monocytogenes FNCC 0156* (Table 4) which categorized as Gram-positive bacteria. Doores (1993) in Ross *et al.* (2002) explained that the mechanism of antimicrobial effect of acid was by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic functions. The cell wall structure of Gram-negative bacteria had outer membrane consist of lipopolysaccharides (LPS), phospolipids, and lipoproteins. These components provides barrier to antimicobial substances which makes LAB tends to had less inhibitory activity against the Gram-negative bacteria (Ray, 2004). However, acids still have a very broad mode of action and inhibit both Gram-positive and Gram-negative bacteria as well as yeast and moulds. Ramachandran *et al*, (2012) stated that LAB from different sources possess antimicrobial properties at different extend.

Certain strains of LAB were further known to produce bioactive molecules such as ethanol, formic acid, fatty acids, hydrogen peroxide, diacetyl, reuterin, and reutericyclin. Many strains also produced bacteriocins and bacteriocin-like molecules that displayed antibacterial activity, as well as antimicrobial peptides that may also contribute to food preservation and safety (De Vuyst & Leroy, 2007). Apparently, each antimicrobial compound produced during fermentation provides an additional hurdle for pathogens and spoilage bacteria to overcome before they can survive and/or proliferate in a food or beverage (Ross *et al.*, 2002)

4.6. Bacteriocin Inhibitory Activity and Identification of LAB species

Bacteriocins of LAB constitute a heterogeneous group of ribosomally synthesized and extracellularly released proteins or peptides that display antibacterial activity against not

only closely related LAB species but also other Gram-positive bacteria, including food spoilage and pathogenic bacteria (O'Sullivan *et al.*, 2002 in Alemu *et al.*, 2006). Crude bacteriocin from LAB isolates were obtained by adjusting pH of cell-free supernatant of the isolates. Adjustment of the cell-free supernatant to pH 6.0 aimed to prevent the inhibitory effect of lactic acid (Todorov and Dicks, 2006). According to Abubakar and Arpah (2015), generally bacteriocin production was occurring in liquid media, for example MRS-B. Wang *et al.* (2010) stated that changing components of the medium could alter the production of bacteriocin. Therefore, the optimum media compositions for bacteriocin production were evaluated in this study.

In this study, bacteriocin inhibitory activity of 32 LAB isolates from Ampel bamboo shoot pickle were observed. Before analysis, all isolates were cultured in MRS-B media supplemented with carbon and nitrogen source, incubated for 24 hours. Supplementation to culture media with addition of carbon source (sucrose) and nitrogen source (peptone and yeast extract) in MRS-B media were done to induce the bacteriocin production of the isolates. Using the same media composition, Desmukh and Thorat (2013) found that high bacteriocin activity of *Lactobacillus* was recorded in pH 6.0. The bacteriocin antimicrobial inhibitory analysis were observed using agar-well diffusion method against indicator pathogen bacteria *E. coli* FNCC 0091, *L. monocytogenes* FNCC 0156 and *S. aureus* FNCC 0047.

Based on Table 5, it has been observed that 23 of 32 isolates showed bacteriocin inhibitory activity against pathogenic bacteria. The inhibitory activity obtained in this research was ranged between 13.10 - 3274.99 mm^2 mL^{-1}. Each isolate gave different response to each media treatment and indicator pathogens. Isolate A18 and B2 were found able to produce bacteriocin after cultured in MRS-B without supplementation. This occurrence showed that those isolate gained enough nutrient by inoculated in MRS-B media. DeMan Rogosa Sharpe Broth (MRS-B) was known as common and effective for growth of *Lactobacillus* species. Iyapparaj *et al.* (2013) reported that MRS medium were favoured maximum bacteriocin production from *Lactobacillus* sp. MUS3IR than other culture media (Nutrient broth, Luria broth, *Lactobacillus* selection broth, Tryptic Soy broth, Brain-Heart infusion broth). Previously reports also evidenced

that MRS medium is a better medium for cell growth and bacteriocin production (Ten *et al.*, 1994 in Iyapparaj *et al.*, 2013)

Some LAB isolates which had no bacteriocin activity when cultured in MRS-B, showed better result when induced by carbon and nitrogen supplementation. Isolate A14, A17, A22, A24 and B16 were produced bacteriocin when grown in MRS-B supplemented with carbon source only (Table 5). Sucrose was used in this study as carbon source for LAB growth. Sucrose was type of sugar, which have been the traditional and most commonly used sweeteners in the dairy industry. It also known as nutrient and energy source for living things. Commonly, glucose was considered as carbon source by all microorganisms due to its size, rapid uptake, utilization and cellular energy conversion. However, some bacteria have a complete enzymatic machine that allows them to use complex carbohydrates; for example, *E. faecium* that shows a variable sucrose fermentation pattern (Audisio *et al.*, 2001 in Wang *et al.*, 2010). Hayek & Ibrahim (2013) also observed that LAB species had different preferences to sugar, which could influence growth and functional properties of LAB.

In this study, highest bacteriocin inhibitory activity (3274.99 ± 2932.35 mm^2 mL^{-1}) were performed by B16 with supplementation of 2% sucrose. The similar result were found by Khalil *et al.* (2009) that bacteriocin from *Bacillus megaterium* 22 had higher values of bacteriocin activity obtained in the presence of sucrose in MRS-B medium. Bacteriocin from *L. lactis* observed by Indriati *et al.* (2014) cultured in CM medium supplemented with sucrose yielded higher activity then supplemented with other sugar. The properties of sucrose as carbohydrate source was able to result in high biomassa and bacteriocin production. This may happen probably corresponded with sugar consumption, because sucrose was consumed faster than lactose or glucose when present as sole carbohydrate source (Neysen *et al.*, 2003 in Indriati *et al.*, 2014). These foundings allegedly revealed that sucrose could be the best alternative sugar to glucose to yield higher bacteriocin activity.

Thirteen isolates (A7, A8, A12, B4, B5, B6, B8, B9, B10, B11, B14, B15 dan B19) showed bacteriocin production after inoculated in MRS-B with combination of carbon

and nitrogen source (Table 5). Some of them yielded better result by the presence of peptone or yeast extract, and combination of both. Different nitrogen source yielded different result for each isolate as well. Peptones are water-soluble protein hydrolysates, containing peptides, amino acids, and inorganic salts as well as other compounds, such as lipids, vitamins, and sugars, which was a good source of nitrogen, carbon and other nutrition for microorganism culture (Davami *et al.,* 2015). While yeast extract contains peptides, amino acids, nucleotides and other soluble components of yeast cells (Chae *et al*., 2001). Based on Table 5, supplementation with yeast extract induced more inhibitory activity against pathogens by the isolates. However, there were no significant differences between peptone and yeast extract on their supplementation to support bacteriocin production. Ramachandran *et al.* (2012) in their study found that compared to other nitrogen source (urea, yeast extract, potassium nitrate, ammonium chloride and sodium nitrate), peptone was observed to have significant effect on bacteriocin production of *L. lactis*. Similar results have been reported for the production of plantaricin 423, in which case optimal production was obtained in MRS broth supplemented with bacteriological peptone, followed by casamino acids, tryptone and meat extract (Verellen *et al.,* 1998 in Todorov & Dicks, 2006). However, in some earlier reports, it was found that different nitrogen sources such as yeast extract were effective to enhance bacteriocin production (Bing Han *et al.*, 2011; Meera & Devi, 2012 in Ramachandran *et al.,* 2012). The variation of result may happened because another factors had influenced the bacteriocin production of the isolates. According to Todorov & Dicks (2004) in Ramachandran *et al.* (2012), besides nutrient source, pH, temperature inoculums size, and other environment factors were strongly affect LAB bacteriocin production. Thus, the enviromental factor, such as temperature and pH should be optimized for maximum bacteriocin production in future study.

From Table 5, it also could be seen that the number of isolates code B (13 isolates) has more inhibitory activity rather than code A (10 isolates), which were differentiate by its initial fermentation conditions. This result apparently showed that initial fermentation condition (salt concentration and temperature) affect the ability to produce bacteriocin on LAB isolates from Ampel bamboo shoot fermentation. Fermentation condition B, which performed in higher temperature (30^{o}C) and higher salt concentration (5.0%)

more facilitated the growth of bacteriocin-producing LAB during the process. Similar result was obtained by Flemming (1991) in Salminen *et al.* (2004), who found that *Ln. mesentroides* isolated from fermentation at higher temperature had better bacteriocin inhibitory activity than those isolated from colder temperature.

Based on Table 6, it could be known that various species were exist in both fermentation condition. This condition may caused by the natural fermentation method, which enable both desirable and associated microorganisms to grow during the process. Besides, the natural microbial flora in the raw materials may not always be the same that lead to difficulty to produce a product with consistent characteristics over a long period of time (Ray, 2004). Surprisingly, same LAB species from different fermentation had different bacteriocin inhibitory activity after cultivated in supplemented media. *Lb. fermentum I* (A14, A17 and A18) generally could be induced by most of the carbon and nitrogen supplementation, but *Lb. fermentum I* (B3) could be induced only when supplemented with combination of carbon and all nitrogen source. From this result, it also known that *Lb. fermentum I* from fermentation A had better bacteriocin activity than from fermentation B. According to Harris (1998) in Salminen *et al.* (2004), higher temperature could alter fermentation process to homofermentative. Since *Lb. fermentum* categorized as hetero-fermentative LAB (Adebayo *et al.*2014), lower temperature (fermentation A) could be a better condition for growth of *Lb. fermentum 1*, which may affected its potential to produce bacteriocin. Ray (2004) explained that the species and the strains of a species growing under similar conditions differ greatly in the amount of bacteriocin production. A nutritionally rich medium is always better, and for many bacteriocins, growing the strains in a fermentor under a controlled terminal pH can produces more bacteriocin.

Vogel *et al.* (1993) in De Vuyst & Leroy (2007) stated that the antibacterial spectrum of bacteriocin frequently includes spoilage organisms and food-borne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus*. From all 32 isolates, only 4 isolates: A14 (*Lactobacillus fermentum 1*), A17 (*Lactobacillus fermentum 1*), A18 (*Lactobacillus fermentum 1*) and B9 showed bacteriocin inhibitory activity against all indicator pathogens *E. coli* FNCC 0091, *L. monocytogenes* FNCC 0156 and *S. aureus*

FNCC 0047. Besides, other isolates showed inhibitory activity against one or two pathogens only. Optimum bacteriocin inhibitory activity was found in bacteriocin B9 which was able to inhibit all pathogens when cultured in MRS-B supplemented with 2% sucrose and 0.8% yeast extract, while largest inhibitory activity were performed by B16 against *S. aureus* FNCC 0047 (Gram-positive pathogen) but it had no bacteriocin activity against *E. coli* FNCC 0091 and *L. monocytogenes* FNCC 0156. Most isolates also showed inhibitory activity against *S. aureus* FNCC 0047 more than against *E. coli* FNCC 0091 and *L. monocytogenes* FNCC 0156.

Normally, Gram-positive bacterial strains were more sensitive to bacteriocin, so the spectrum or range of which can vary greatly. Stevens *et al*. (1991) explained that bacteriocins were not frequently active against Gram-negative bacteria. The outer membrane of Gram-negative bacteria acts as a permeability barrier for the cell. It is responsible for preventing molecules such as antibiotics, detergents and dyes from reaching the cytoplasmic membrane. However, some isolates (A11, A12, A18, B8, B9, B10 and B11) showed broader inhibitory activity against *E. coli* FNCC 0091. This may caused by different bacteriocin production characteristics of each genus and species (Salminen *et al*.,2004). This exception also could be occurred by the growth rate of the pathogens and the bacteriocin diffusion on media agar. Some studies have already reported bacteriocin activity against gram-negative bacteria. Examples are plantaricin 35d, produced by *Lactobacillus plantarum* and active against *Aeromonas hydrophila*; bacteriocin ST151BR, produced by *Lactobacillus pentosus* ST151BR, bacteriocin from *Lactobacillus* species (*L. plantarum* and *L. lactis*) from vegetable waste that produced a bacteriocin which inhibitted the growth of *Escherichia coli* (Lade *et al.*, 2006; Torodov and Dicks, 2004; Messi *et al.*, 2001 in Parada *et al.*, 2007). Ogunbanwo *et al.* (2003) also found that *L. brevis* OG1 was able to produce bacteriocin, which had a wide inhibitory spectrum towards both Gram-negative and Gram-positive food spoilage and pathogenic bacteria. Although Gram-negative bacteria cells are normally resistant to bacteriocins of lactic acid bacteria, they become sensitive following impairment of the cell surface lipopolysaccharide structure by physical and chemical stresses (Ray, 2004).

Bacteriocin inhibitory activity generally act through depolarization of the target cell membrane or through inhibition of cell wall synthesis and range in specificity from a narrow spectrum of activity (*lactococcins* which only inhibit *lactococci*) to those which have a broad range of activity such as the lantibiotic nisin (Jack *et al.,* 1995 in Ross *et al.,* 2002). Ray (2004) explained that in general, the bacteriocin molecules initially adsorbed on the membrane surface and form transient pores, leading to loss of protein motive force as well as the pH gradient across the membrane. This alters the permeability of the membrane, causing leakage of small nutrient molecules, as well as affecting the transport of nutrients and synthesis of ATP. These changes finally cause the cell to lose viability. In addition, some bacteriocins can cause lysis of sensitive cells.

Low bacteriocin activity (less than 94 AU/OD) (Todorov and Dicks, 2006) were observed in some LAB isolates (Table 5). This low production of bacteriocin may had the correlation with the environment and the growth of the strain isolates. Lejeune *et al.* (1998) in Savadogo *et al.* (2006) revealed that bacteriocin production was associated with LAB's growth. Bacteriocin production usually occurs throughout the growth phase and ceases at the end of the exponential phase (or sometimes before the end of growth). Generally, the parameters that help generate more cell mass produce more bacteriocin molecules. The parameters include nutritional composition, initial and terminal pH and O–R potential of a broth, and incubation temperature and time (Ray, 2004). However, it was not always had the correlation with cell mass or growth rate of the producer strain. A decrease in bacteriocin concentration could be a result of proteolytic activity, aggregation and/or adsorption to the cells (De Rojas *et al.,* 2004 in Wang *et al.,* 2010). From this study, we can know that production of bacteriocin from the LAB isolates from Ample bamboo shoot pickle indicates its application as probiotic and as bio-preservative (Ramachandran *et al.,* 2012).