CHARACTERISTICS, STABILITY AND ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA (*Leuconostoc sp*) ISOLATED FROM BROILER'S CAECUM DURING STORAGE

S. Sumarsih, B. Sulistiyanto and C. I. Sutrisno
Faculty of Animal Agriculture, Diponegoro University
Tembalang Campus, Semarang 50275, Central Java-Indonesia
Corresponding E-mail: ssumarsih71@gmail.com

Received November 15, 2011; Accepted February 10, 2012

ABSTRAK

Bakteri asam laktat (BAL) merupakan mikrobia alami yang biasa digunakan sebagai agensia fermentasi. Tujuan penelitian adalah untuk mengkaji karakteristik, stabilitas dan sifat antimikrobia isolat bakteri asam laktat *(Leuconostoc sp)* yang diisolasi dari sekum ayam broiler selama penyimpanan. Penelitian menggunakan rancangan acak lengkap dengan 4 perlakuan (penyimpanan 0, 2, 4 dan 6 minggu) dengan 12 ulangan. Parameter yang diamati adalah total , kestabilan sifat bakteri asam laktat (*Leuconostoc sp*). Hasil penelitian menunjukkan bahwa total *Leuconostoc sp* nyata (P<0,05) menurun dari 8 x 10^7 menjadi 1 x 10^3 Colony Forming Unit (CFU)/mL selama penyimpanan. Isolat *Leuconostoc sp* tetap stabil sifatnya selama penyimpanan. Aktivitas anti mikrobia *Leuconostoc sp* nyata (P<0,05) menurun dari 9,94 menjadi 8,68; 7,23 dan 6,14 mm selama 2, 4 dan 6 minggu penyimpanan.

Kata Kunci : bakteri asam laktat, Leuconostoc sp, penyimpanan, sekum ayam broiler

ABSTRACT

Lactic acid bacteria (LAB) is one of the natural microbe which widely used as fermentation agents. The purpose of this study was to examine the characteristics, stability and antimicrobial properties of lactic acid bacteria *(Leuconostoc sp)* isolated from broiler's caecum during storage. This research was conducted by the Complete Randomized Design with 4 treatments (time of storage: 0, 2, 4 dan 6 weeks) with 12 replications. Parameters observed were total number and stability properties of *Leuconostoc sp* isolate which known as lactic acid bacteria. The results obtained total number of *Leuconostoc sp* isolate were significantly (P<0.05) decreased from 8 x 10^7 to 1 x 10^3 Colony Forming Unit (CFU)/mL during storage. Stability properties of *Leuconostoc sp* isolate were not affected by the time of storage. Antimicrobial activity of *Leuconostoc sp* were significantly (P<0.05) decreased from 9.94 to 8.68, 7.23 and 6.14 mm during storage at 2, 4 and 6 weeks.

Key Words: lactic acid bacteria, Leuconostoc sp, storage, broiler's caecum

INTRODUCTION

Lactic acid bacteria (LAB) is one of the natural microbe that is widely used as fermentation agent. Their capabilities in lowering pH due to the lactic acid produced could give a beneficial effect to the fermented product. The process of fermentation by LAB are depend on activities and proliferation of bacteria, lactic acid-producing bacteria (Rahayu, 2003).

Based on their characteristics, LAB are characterized as Gram positive, usually non-motile, non-sporulating bacteria that produce lactic acid as a major or sole product of fermentative metabolism (Malaka and Laga, 2005). Classified LAB can be isolated from temperate regions according to their morphology, physiology and molecular characters (Holt *et al.*, 1999).

Lactic acid-forming organisms are divided into two species, namely: 1) homofermentatif species capable of changing form the 95% lactic acid, 2) heterofermentatif species, a group that produces lactic acid in small amounts and the resulting product is ethyl alcohol, acetic acid, formic acid and carbon dioxide (Fuller, 2001). Lactic acid bacteria on fermentation of carbohydrates produce lactic acid which can

lower the pH. The decrease of pH can inhibit the growth of other microorganisms, particularly bacterial pathogens (Huang *et al.,* 2004). The potency of LAB to inhibit the growth of pathogenic bacteria is caused by the availability of antimicrobial compound, especially lactic acid.

Current research on fermentation process showed that LAB is known to play a role in the process of fermentation and preservation of food (Rahayu, 2003). *Pediococcus acidilactici F-11* is known to produce bacteriocin as biocontrol agent *E. Coli* and *S. aureus* (Rahayu *et al.,* 2004). *Lactobacillus* sp and *Leuconostoc sp* were LAB with potential as probiotics (Purwandhani and Rahayu, 2003) and its stability can be maintained during storage by dry cell preparation as a probiotic powder (Hartati and Harmayani, 2006).

Lactic acid bacteria are also known as the hypercholesterolemia prevention agent reflected in increased HDL cholesterol and LDL cholesterol reduction in Winstar rat (Harmayani, 2002) and broiler (Sumarsih *et al*., 2010). Acid-producing microbe in chicken manure is known comes from the caecum (Harimurti *et al.*, 2005). Lactic acid bacteria (*Lactobacillus sp* and *Leuconostoc sp*) can be used as a probiotic isolated from broiler intestinal tract. The *Leuconostoc* spesies has been considered as a potential pathogen especially initial immunocompromized host (Ling, 1992).

There are many technological challenges in the development of LAB as fermentation agent. These challenges include selection of LAB, levels of addition, safety consideration, processing effect on viability, enumeration, storage stability and sensory impact. LAB isolate can be stored before used as a starter and probiotic. Storage isolates simplest way for investment in the medium or liquid medium and stored at low temperature. There are many extrinsic and intrinsic factors that affect stability of probiotics during storage, including acidity (pH), temperature, oxygen, water activity, lack of nutrients, microbial antagonism, presence of inhibitors (Vasiljevic and Shah, 2008).

Numerous studies have been conducted on the stability of LAB and probiotics. Research in protection of LAB to maintain the viability and stability have been carried out, particularly using micro-encapsulation using several materials, such as Ca alginate and maltodextrin, as well as the methods of micro-encapsulation (Ngatirah *et al.,* 2009; Banyuaji *et al.,* 2009). Characteristic, stability and antimicrobial activity of LAB can change during storage (Champagne *et al.,* 2008). There is a need to understand and improve survival of these cultures during storage at refrigerated temperatures. Therefore, the objective of this study was to examine the characteristics, stability properties and antimicrobial activity of lactic acid bacteria isolated from broiler's caecum during storage.

MATERIALS AND METHODS

Materials

This investigation was conducted at Feed Technology Laboratory of Animal Agriculture Faculty, Diponegoro University. Materials were broiler's caecum and MRS medium (de-Mann Rogossa and Sharp). MRS agar consisted of 15 g of agar; 10 g of ocsoid pepton; 5 g of yeast extract; 2 g of K_2HPO_4; 2 g of diamonium sitrat; 20 g of glucosa; 1 g of tween 80; 5 g of natrium asetat; 0.58 g of $MgSO_4.7\ H_2O$, 0.28 g of $MnSO_4.H_2O$, 10 g of meat extract and 1000 mL of aquadest.

Isolation Methods

Leuconostoc sp was isolated from broiler's caecum with MRS medium-$CaCO_3$ 1% of added 10 ppm Syclo-hexamide to suppress the growth of yeast and 10 ppm of Na Azida to suppress microaerob (Rahayu, 2003). Broiler's caecum 10 g were taken aseptically and homogenized in 90 ml of NaCl solution. Serial dilution up to 10^{-7} were prepared and appropriate dilutions were plated onto MRS medium-$CaCO_3$ 1% of added 10 ppm syclo-hexamide. All plates were incubated at 37°C for 48 hours. LAB can be observed from clear zones around the colonies which indicated the dissolving of $CaCO_3$ by an acid.

Identification Methods

Identification of LAB based on morphological characters, biochemical and physiological. Morphological parameters included cell shape and coloring grams. Biochemical parameters was the catalase test. Physiological parameters included clear zone at colony of LAB. Cells form colonies of bacteria were observed in LAB isolates grown on MRS at room temperature for 2 - 3 days (Harimurti *et al.*, 2005).

Gram staining method was conducted by coloring method (Seely *et al.,* 2001). Catalase test was performed by dripping a solution of hydrogen peroxide on mikrobia culture, in which a positive

reaction if the CO_2 bubbles appear. Clear zone was observed in colonies of lactic acid bacteria isolates grown on MRS-1% $CaCO_3$.

Storage and Starter Preparation

Storage of LAB isolates is done by entering in 0.1 ml of isolates in MRS Broth medium in a test tube and stored on refrigerator at 10 ºC temperature with storage time according to treatment. Culture *Leuconostoc sp* was entirely transferred to the erlenmeyer containing 50 mL of MRS Broth medium and then incubated at 37°C for 48 hours. Two drops of the incubated MRS Broth medium were inoculated into 10 mL of solution with 10% (w/v) skim milk and has sterilized at a temperature of 12°C for 15 minutes. After incubation 37°C for 48 hours, the culture was used as parent culture.

The parent culture was inoculated into the same medium with the addition of 3% glucose. The culture was incubated 37°C for 24 hours in order to obtain working culture which was ready to be used.

The stability of LAB was measured by pour plate method using MRS agar. MRS agar was used for enumeration total *Leuconostoc sp*. One mL of appropriate serial dilutions of each sample was pour plate onto the sterile media. After 24 h incubation at 37°C, the colonies that appeared on the plates were counted and the cfu/mL was calculated.

The antimicrobial activity of LAB against *Escherichia coli* was performed by the wall diffusion assay (Lade *et al.*, 2006). *Leuconostoc sp* culture were grown in MRS broth at 37°C for 24 hours. *Escherichia coli* were grown in nutrient broth at 37°C for 24 hours. Ten mL of nutrient soft agar inoculated by 50 µL broth culture of *Escherichia coli.* MRS agar poured on petri dish and allow to solidity. Then overlaid with nutrient broth and in placed at a temperature of 4°C for 1 hour. Fifty µL of *Leuconostoc sp* culture was filled and incubated at 37°C for 24 hours. *Leuconostoc sp* gave clear zones when have antimicrobial activity against *Escherichia coli.* The diameter of the inhibition zone was measured.

Parameters Measured and Statistical Analysis

Experiment was conducted according to completely Randomized Design (CRD) consisting 4 treatments and 12 replications. The treatments applied were , T0 = 0-week storage as control, T1, T2 and T3 was time storage for 2, 4 and 6 weeks.

The observed parameters included the parameters of phenotypic characters of isolates of LAB (Seely *et al.*, 2005), total LAB in accordance with the method Fardiaz (1993) and Characteristics of LAB's isolates during storage. The data were analyzed by variance (ANOVA) to determine the influence of treatment and further tests performed Duncan's Multiple Areas to know the difference between treatments (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

Phenotypic characters of isolates could be observed from morphological, biochemical and physiological appearance of LABs colony (Holt *et al.,* 1999). The LAB isolates were classified into the genera *Streptococcus, Leuconostoc, Pediococcus* and *Lactobacillus* based on their morphology and biochemical characters (Nair and Surendran, 2005). Table 1 shows phenotypic characters of *Leuconostoc sp* isolate from broiler's caecum.

Twelve isolates (L1 to L12) appeared the coccus cells, positive to gram staining reaction, negative to catalase-test and there are Clearly Zone around the colony. It is strongly suspected for a genus of *Leuconostoc* (Ling, 1992; Harimurti *et al.*, 2005).

Total numbers of LAB's during storage are presented in Table 2. Table 2 shows that the total numbers of LAB's decreased during storage. At 0 weeks of storage, the the total numbers of LAB's were highest. Isolates of *Leuconostoc sp* stored at 0 week has a higher total number, that was 8×10^7 CFU/mL compared to isolates of L*euconostoc sp* stored at 2, 4 and 6 weeks that only 2×10^6; 2×10^4 and 1×10^3 CFU/mL. At 0 week of storage, the total *leuconostoc sp* were highest. At 0 weeks storage conditions of lactic acid bacteria is still in the logarithmic phase of growth and cell division occurs very quickly with nutritional support growth media. Hartati and Harmayani (2006) stated that the logarithmic phase bacterial cells will grow and divide exponentially until the maximum number supported by environmental factors such as environmental conditions, nutrients and temperature.

Storage at week 2, 4 and 6 significantly ($P<0.05$) lower total number of *Leuconostoc sp*. Decreasing in the total number of *Leuconostoc sp* due to the storage temperature of 10ºC in

Table 1. Phenotypic Characters of *Leuconostoc sp* Isolated from Broiler's Caecum

Characteristics	Code of Isolate											
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12
Coccus shape	+	+	+	+	+	+	+	+	+	+	+	+
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test	-	-	-	-	-	-	-	-	-	-	-	-
Clearly Zone	+	+	+	+	+	+	+	+	+	+	+	+

Table 2. Total Numbers of LAB's During Storage

Time of Storage	Numbers of LAB's (cfu/g)
0 weeks	$8 \times 10^{7\ a}$
2 weeks	$2 \times 10^{6\ b}$
4 weeks	$2 \times 10^{4\ c}$
6 weeks	$1 \times 10^{3\ d}$

Different of superscripts in the same column indicates significantly difference ($p<0.05$)

refrigerator was not fully inhibit the activity of the metabolism so that the availability of nutrients in the medium diminished and unable to support further growth of bacteria. Lidya and Djenar (2000) added that the death caused bacteria deteriorating environment mainly by the growing accumulation of toxic metabolic results of bacteria cells.

Stability of LAB is influenced by such of things as the number of early starters who were inoculated, the end result of fermentation metabolites harvesting, drying, osmotic pressure, oxygen availability, freezing and stress during the manufacturing processe of product (Siuta and Goulet, 2001). During storage, lactic acid which can lower the pH so it can affect cell viability. There was a linear relationship decrease in the number of cells microbe with decrease pH of growth media (Wang, 2005)

Temperature is a key factor that affects the stability of microorganism during storage (Lourens-Hattingh and Viljoen, 2001). Higher temperatures accelerate microbial metabolism which in impedes microbial survival (Liu and Tsao, 2010). LAB are exposed to a number of stress conditions, such as low and high temperature, low pH and low water activity, which cause membrane and cell wall damage, inhibition of active transport, retention of nutrients, morphological changes and loss of stability. Bacteria have developed adaptive strategies to face the challenges of changing environments, and to survive under conditions of stress (Abee and Wouters,1999). Stress adaptation of microbial cells enables the cells to survive better when they are subsequently exposed to the same stress or other types of stresses.

Lactic acid bacteria have been shown to induce adaptive response after exposing to some stresses.. *Lactobacillus lactis* ssp. *lactis* showed increased resistance to freezing stress at 10°C (Panoff *et al*., 1995). Bâati *et al.* (2000) reported that preincubation of *Lactobacillus acidophilus* at low temperature (22°C) for 6 h led to development of cryotolerance during freezing treatment at -80°C for 24 h.

Total LAB can be maintained with respect to environmental conditions during storage. Short-term storage of isolates stored in refrigerator temperature 4ºC while in the long-term culture bacteria should get special treatment, for example by encapsulated (Hoog, 2005) or freeze dried (Kurtman *et al*., 2009).

Table 3 shows the stability of character of isolates that has been identified as the *Leuconostoc sp*. The characteristics LAB remained stable during storage until 6 weeks. Table 3 also shows that the isolates have coccus cells shape, a positive gram reaction, catalase test is negative and there was allegedly a clear zone. It was stated by Ling (1992) that *Leuconostoc sp* has the characteristics of coccus cells shape, gram-positive reactions, negative catalase test and produce lactic acid. Storaging at 6 weeks at 10ºC temperature properties of *Leuconostoc sp* remained stable due to the mutation did not occur

Table 3. Characteristics of *Leuconostoc sp* during Storage

Characteristics	Code of Isolate											
	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12
0 weeks												
Coccus shape	+	+	+	+	+	+	+	+	+	+	+	+
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+
Catalase-test	-	-	-	-	-	-	-	-	-	-	-	-
Clearly Zone	+	+	+	+	+	+	+	+	+	+	+	+
2 weeks												
Coccus shape	+	+	+	+	+	+	+	+	+	+	+	+
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+
Catalase-test	-	-	-	-	-	-	-	-	-	-	-	-
Clearly Zone	+	+	+	+	+	+	+	+	+	+	+	+
4 weeks												
Coccus shape	+	+	+	+	+	+	+	+	+	+	+	+
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test	-	-	-	-	-	-	-	-	-	-	-	-
Clearly Zone	+	+	+	+	+	+	+	+	+	+	+	+
6 weeks												
Coccus shape	+	+	+	+	+	+	+	+	+	+	+	+
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+
Catalase-test	-	-	-	-	-	-	-	-	-	-	-	-
Clearly Zone	+	+	+	+	+	+	+	+	+	+	+	+

Table 4. The Antimicrobial Activity of LAB's During Storage

Time of Storage	Average Diameter of Inhibitin Zone of LAB's (mm)
0 weeks	9.94 [a]
2 weeks	8.68 [b]
4 weeks	7.23 [c]
6 weeks	6.14 [d]

Different of alphabetic superscripts in the same column means significantly difference ($p<0.05$)

during storage bacteria cells. This result was similar to Kurtmann *et al.* (2009). According to Kurtmann *et al.* (2009), stability character of LAB can be maintained during storage. Test of inhibition of *Leuconostoc sp* against the bacteria *Escherichia coli* showed that all isolates tested could inhibit the growth of *Escherichia coli* (Table 4). Table 4 shows that the antimicrobial activity of LAB's decreased during storage. Storage at week 2, 4 and 6 significantly ($P<0.05$) lower antimicrobial activity of *Leuconostoc sp.* At 0 weeks of storage, the antimicrobial activity of LAB's were highest. Isolates of *Leuconostoc sp* storage at 0 weeks has a wider zone of inhibition that is equal to 9.94 mm compared with isolates L*euconostoc sp* storage at 2, 4 and 6 weeks that only 8.68; 7.23 and 6.14 mm. These suggests that isolates *Leuconostoc sp* storage at 0 weeks better able to inhibit the growth of *Escherichia coli* compare to other time of storage.

Lade *et al.* (2006) classifies bacterial isolates inhibitory zone on the growth of LAB in 3 criteria, namely moderate inhibition (covering in

area 6 - 9 mm), strong inhibition (10-14 mm width) and very strong inhibition (15-18 mm of width). Inhibition test of *Leuconostoc sp* isolated included in the criteria moderate inhibition.

The ability of *Leuconostoc mesenteroides* SM-22 and *Pediococcus acidilactici* F-11 could inhibit psikrofil and phatogen bacteria isolated from fresh meat and fresh milk (Rahayu *et al*., 2004). Antimicrobial substances produced by Lactic acid bacteria (LAB) are used in association with selective insensitive starter to inhibit competitive microflora (Scannell *et al*., 2000).

Nowroozi1 *et al*. (2004) stated that antibacterial activities were done by an agar spot in which only 14.3% of strains known to produce bacteriocin. The inhibitory effect was pointed to bacteriocin not H_2O_2 since there was no oxidizing effect on bacterial cells which will destroy the basic molecular structure of cell proteins (Zalan *et al.*, 2005) and bacteriocin form the pores in the membrane of sensitive cells and deplete the transmembrane potential and/or the pH gradient, resulting in the leakage of cellular materials (McAuliffe *et al*., 2001).

CONCLUSION

Total number of lactic acid bacteria (*Leuconostoc sp*) isolated from broiler caecum decreased in line with the time of storage. The characteristics of lactic acid bacteria (*Leuconostoc sp*) isolate remain stable during 6th week of storage. Antimicrobial activity of *Leuconostoc sp* were decreased during storage.

REFERENCES

Abee, T. and J. A. Wouters. 1999. Microbial stress response in minimal processing. International Journal of Food Microbiology 50:65-91

Bâati, L., C. Fabre-Gea, D. Auriol, and J. P. Blance, 2000. Study of Cryotolerance of *Lactobacillus acidophilus*: Effect of Culture and Freezing Conditions on the Viability and Cellular Protein. International J. Food Microbiol. 59:241-247.

Banyuaji, A., E.S. Rahayu and T. Utami, 2009. Viabilitas *Lactobacillus acidophilus* SNP 2 dalam kapsul dan aplikasinya dalam es krim. J. Agritech. 29(1):171-178

Champagne, C. P. and N. J. Gardner. 2008. Effect of storage in a fruit drink on subsequent survival of probiotic lactobacilli to gastro-intestinal stresses. J. Food Research International 41:539-543.

Fardiaz, S. 1993. Mikrobiologi Pangan I. Gramedia Utama, Jakarta.

Fuller, R. 2001. The chicken Gut Microflora and Probiotic Supplements. J. Poult. Sci. 38:189 -196

Harmayani, E. 2002. Cholesterol reduction effect of probiotic cultures. Proceeding of The 2 nd Indonesian Biotechnology Conference. P.302-308

Hartati, S and E. Harmayani. 2006. Preparasi sel kering *Lactobacillus sp* DAD 13 dan kestabilannya sebagai bubuk Probiotik. J. Mikrobiologi Indonesia 11(1):1-4

Harimurti, S., E. S. Rahayu, Nasroedin and Kurniasih. 2005. Bakteri asam laktat dari intestin ayam sebagai agensia probiotik. Anim. Prod. 9(2):82-91

Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley and S. T. Willem. 1999. Bergey's Manual of Determinative bacteriology. 9th Edition. Will & Wilk. Baltimore, p 3-6

Hoog, S. 2005. Esencial Microbiology. John Wiley and Sons Ltd, Chichester

Huang, M. K., Y. J. Chory, R. Honde, J. W. Lee, B. Lee and X. Xhao. 2004. Effect of lactobacilli and a acidophilic fungus on the production performance and immune responses in broiler chicken. J Poult. Sci. 83: 788 -795

Kurtmann L., Carlsen, J. Risbo and L. H. Skibsted 2009. Storage stability of freezedried *Lactobacillus acidophilus* (La-5) in relation to water activity and presence of oxygen and ascorbate. Cryobiology 58:175-180.

Lade, H. S., M.P. Chitanand, G. Gyananath and T. A. Kadam. 2006. Studies on some properties of bacteriocins produced by Lactobacillus species isolated from agro-based waste. The Internet J. Microbiol. 2(1):1937-8289

Ling, M. L. 1992. Leuconostoc bacteraemia. J. Singapore Med. 33: 241-243

Liu, S and M. Tsao. 2010. Enhancing stability of lactic acid bacteria and probiotics by Williopsis saturnus var.saturnus in fermented milks J. Nutr. Food Sci. 40(3):314-322

Lourens-Hattingh, A. and B. C. Viljoen. 2001. Yoghurt as probiotic carrier food.

International Dairy J. 11:1-17

Malaka, R and A. Laga. 2005. Isolasi dan identifikasi *Lactobacillus Bulgaricus* strain *Ropy* dari yogurt komersial. J. Sains dan Teknologi. 5 (1):50-58

McAuliffe, O., R. P. Ross and C. Hill. 2001. Lantibiotics: Structure, biosynthesis and mode of action. FEMS. Microbiol. Rev. 25: 285–308.

Nair, P. S. and P. K. Surendran. 2005. Biochemical characterization of lactic acid bacteria isolated from fish and prawn. J. Culture Collection. 4:48-52

Ngatirah, M. Ulfah and P.T. Dino. 2009. *Characterization of encapsulated Lactobacillus Dad-13 with several type and concentration of encapsulan materials*. Proceeding Seminar on Lactic Acid Bacteria and Culture Collection, 16-17 January, 2009, Yogyakarta. P 42-54.

Nowroozi1, J., M. Mirzaiil and M. Norouzi. 2004. Study of *Lactobacillus* as probiotic bacteria. Iranian. J. Publ. Health. 33(2):1 -7.

Panoff, J. M., B. Thammavongs, J. M. Laplace, A. Hartke, P. Boutibonnes and Y. Auffry. 1995. Cryotolerance and cold adaptation in *Lactococcus lactis* subsp. *lactis* IL1403. Cryobiology. 32:516-520.

Purwandhani, S. N. and E. S. Rahayu. 2003. Isolasi dan seleksi *Lactobacillus* yang berpotensi sebagai agensia probiotik. Agritech. 23(2): 67-74.

Rahayu, E. S. 2003. Lactic acid bacteria in fermented food of Indonesian origin. Agritech. 23(2):75-84

Rahayu. E. S., E. Harmayani, T. Utami and K. Handini. 2004. *Pediococcus acidilactici F-11* penghasil bakteriosin sebagai agensia biokontrol *E. Coli* dan *S. aureus* pada Sayuran Segar Simpan Dingin. Agritech. 24 (3):113-124

Scannell, A.G.M., R. P. Ross, C. Hill and E. K. Arendt. 2000. An effective lacticin biopreservative in fresh pork sausage. J. Food. Prot. 63:370-375.

Seely, H. W., P. W. Vanden Mark and J. J. Lee. 2001. Microbes in Action. A Laboratory Manual of Microbiology. 4 th Ed. W. H. Freeman and Comp. New York, p. 145 – 146.

Siuta, P. and J. Goulet. 2001. Improving probiotic survival rates (microencapsulation preserves the potency of probiotics microorganism in food system. Food Technol. 55(10):36-42.

Steel, R. G. D. and J. H. Torrie. 1981. Principles and Procedures of Statistics. McGraw-Hill Company Inc., New York

Sumarsih, S., T. Yudiati, C. S. Utama, E. S. Rahayu, E. Harmayani. 2010. The influence of using fish fermented by lactic acid bacteria as feed substitution on serum lipid profile of broilers. J. Indonesian Trop. Anim. Agric. 35(2):125-128

Vasiljevic, T. and N. P. Shah. 2008. Probiotics – from Metchnikoff to bioactives. International Dairy J. 18:714-728

Wang, Y., G. Corrieu and C. Beal. 2005. Fermentation pH and temperature influence the cryotolerance of Lactobacillus acidophilus RD758. J. Dairy Sci. 88:21-29

Zalan, Z., E. Nemeth, A. Barath and A. Halasz. 2005. Influence of Growth Medium on Hydrogen Peroxide and Bacteriocin Production of *Lactobacillus s*trains. Food Technol. Biotechnol. 43(3):219-25