



American Journal of
Food Technology

ISSN 1557-4571



Academic
Journals Inc.

www.academicjournals.com



Research Article

Plantaricin IIA-1A5 from *Lactobacillus plantarum* IIA-1A5 Retards Pathogenic Bacteria in Beef Meatball Stored at Room Temperature

¹K.W. Kia, ¹I.I. Arief, ¹C. Sumantri and ^{1,2}C. Budiman

¹Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University (IPB), Jl. Kampus Dalam IPB Darmaga, Bogor, 16680, Indonesia

²Biotechnology Research Institute, Universiti Malaysia Sabah, Jl. UMS 88400, Kota Kinabalu, Sabah, Malaysia

Abstract

This study aims to demonstrate the efficiency of antibacterial activity of plantaricin IIA-1A5 in meatball when stored at room temperature. The results revealed addition of plantaricin IIA-1A5 to meatball reduced population of contaminant pathogenic bacteria of *Staphylococcus aureus* and *Escherichia coli*. Furthermore, the application of 0.3% plantaricin IIA-1A5 extends the shelf life of the meatball to up to 20 h at room temperature based on its microbiology quality, as stated by the Indonesian National Standard for meatball products. The ability of plantaricin IIA-1A5 in retarding the growth of these pathogenic bacteria is comparable to that of 0.3% nitrite, which has been widely used. In addition, A_w and pH values of meatball in the absence (or in the presence) of nitrite or plantaricin did not significantly vary. This implies that reducing the population of pathogenic bacteria was indeed affected by nitrite or plantaricin IIA-1A5. Overall, this result demonstrated that the use of plantaricin IIA-1A5 as a nitrite replacer is promising.

Key words: Plantaricin, meatball, antibacterial activity, *Lactobacillus plantarum* IIA-1A5, food safety

Received: November 28, 2015

Accepted: January 02, 2016

Published: February 15, 2016

Citation: K.W. Kia, I.I. Arief, C. Sumantri and C. Budiman, 2016. Plantaricin IIA-1A5 from *Lactobacillus plantarum* IIA-1A5 retards pathogenic bacteria in beef meatball stored at room temperature. Am. J. Food Technol., 11: 37-43.

Corresponding Author: C. Budiman, Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University (IPB), Jl. Kampus Dalam IPB Darmaga, Bogor, 16680, Indonesia

Copyright: © 2016 K.W. Kia *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Foodborne diseases badly impact public health in both developed and developing countries. Each year, these types of diseases affect about 6-8 million people in the United States alone, with more than 9,000 death cases reported. In Indonesia, foodborne diseases are also considered a major public health problem and there are cases reported every year in almost all districts throughout the country. Supraptini (2002) reported that, on average, 2,800 people suffered from foodborne diseases, 24 of which died every year. To the best of our knowledge, there is no report that exists on economy loss due to these cases in Indonesia. However, this is thought to have a serious impact economically.

Foodborne diseases are considered side effects of the attempts to fulfill human nutrition requirements from foodstuffs. Meat and its processed products are undoubtedly considered nutritious foodstuffs that are good sources of protein (Malav *et al.*, 2015). However, pathogenic microorganism contaminations might occur during the production, processing, distribution or preparation of these foodstuffs. Some well-known pathogenic bacteria meat and its products included Salmonella, *Escherichia coli*, Campylobacter and *Staphylococcus aureus*. Supraptini (2002) clearly mentioned that these bacteria are considered common sources for foodborne illnesses in Indonesia.

Meatball, known as bakso in Indonesia, is considerably the most popular meat product in the country. It is mainly served by street vendors and is stored and prepared at room temperature, hence contamination risk is very likely. Indonesian National Standards (SNI) for meatball product defines meatball as ball-shaped food products made by at least 50% meat, with or without allowable additional ingredients (Badan Standarisasi Nasional, 1995). In Indonesia, the main materials used for meatball production include meat, tapioca flour and garlic. In addition to these main materials, some additional ingredients are added for flavoring and preservation purposes.

Nitrites, formaldehyde and sodium borate (borax) are additional ingredients used in meatball production with the aim of increasing the shelf life of meatball. While, Indonesian authorities strictly prohibit formaldehyde and borax, the allowable concentration of nitrites in food is up to 200 ppm in the country. In other countries, the allowable concentration is between 20-200 ppm. Nitrites have been introduced as preservative agents for food centuries ago (Cassens, 1995). This substance has been initially used as a curing ingredient for fixing the characteristic of color and for creating a unique flavor profile that is distinguishable from products not containing nitrite. However, the European Food Safety

Authority (EFSA Panel on Food Additives and Nutrient Sources added to Food, 2010) stated that nitrites as curing agents are also classified as preservative agents, i.e., compounds inhibiting microorganisms. This is based on the ability of nitrites to inhibit *Clostridium botulinum* in model systems and challenge studies. Furthermore, the application of nitrite in meat and its products has been shown to inhibit pathogenic bacteria, including *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Bacillus cereus* and Salmonella (Milkowski *et al.*, 2010; Tompkin, 2005). This ability promotes nitrite to be widely used as a preservative agent to prolong the shelf-life of meat and its products (Sindelar and Milkowski, 2011).

Despite its advantages as an antimicrobial agent, a high frequency of nitrite consumption might badly impact human health. The lethal dose of nitrite for mice and dogs was 250 and 330 mg kg⁻¹ b.wt.⁻¹, respectively. For humans, for safety and health reasons, the amount of daily nitrite consumption is limited to 0.4 mg kg⁻¹ b.wt. Arief *et al.* (2012) reported that nitrite consumption of 150 ppm for a longer period yielded a carcinogenic effect. Therefore, attempts to develop better Generally Recognized as Safe (GRAS) preservative agents for meat and its products are unavoidable.

Bacteriocin produced by lactic acid bacteria (LAB) has been known to display antimicrobial properties against some Gram-positive and-negative bacteria (Sapatnekar *et al.*, 2010). It is essentially a ribosomal peptide which is regulated directly by some genes in genomes of LAB (Galvez *et al.*, 2007). The use of bacteriocin in food preservation has been shown to reduce the contamination of pathogenic bacteria and prolong shelf-life of products (Hata *et al.*, 2010; Savadogo *et al.*, 2006). In terms of safety, bacteriocin is considerably safe, since it is degradable by proteolytic enzymes in the digestive tract (Cleveland *et al.*, 2001; Arief *et al.*, 2015).

Plantaricin is a bacteriocin member specifically produced by *Lactobacillus plantarum* and mainly inhibited Gram negative pathogenic bacteria (Gong *et al.*, 2010; Abo-Amer, 2007). Plantaricin are generally small in size, thermostable and chemically cationic or hydrophobic (Holo *et al.*, 2001). *Lactobacillus plantarum* also included as probiotic that has functional properties such as probiotic for culture starter of fermented sausage (Afiyah *et al.*, 2015).

Plantaricin IIA-1A5 has been previously isolated from Indonesian BAL of *L. plantarum* IIA-1A5 (Arief, 2011, 2015). This plantaricin has been shown to inhibit the growth of *E. coli*, Salmonella and *S. aureus*, making plantaricin IIA-1A5 a promising preservative agent to replace the use of nitrite. The application of plantaricin IIA-1A5 in food system, however, remains limited. The study on meatball stored at low temperatures clearly demonstrated the ability of this

plantaricin to retard the growth of pathogenic bacteria (Arief *et al.*, 2012). However, broadening the application of plantaricin IIA-1A5 is crucial.

Despite the effect of plantaricin IIA-1A5 on meatball having been investigated (Arief *et al.*, 2012) its effect on meatball stored at room temperature remains unknown. Indeed, Indonesian meatball are mainly served at room temperature, which is more risky against pathogenic bacteria contamination.

In this study, highlighted the effect of the introduction of plantaricin IIA-1A5 on meatball upon storage for up to 20 h at room temperature. It is found that plantaricin IIA-1A5 displays comparable ability to reduce the contamination of *Salmonella*, *E. coli* and *S. aureus* in meatball during storage periods to that of 0.3% nitrite. Overall, plantaricin IIA-1A5 is a promising preservative agent for meat products replacing the use of nitrite.

MATERIALS AND METHODS

Production and purification of plantaricin IIA-1A5:

According to Arief *et al.* (2015), the production of plantaricin IIA-1A5 from *L. plantarum* IIA-2C12 culture was performed. Secreted plantaricin IIA-1A5 was then purified from the medium by ammonium sulfate precipitation and cation exchange chromatography (Arief *et al.*, 2013).

Meatball preparation:

Meatball were prepared based on Arief *et al.* (2012) using beef, tapioca flour, ice blocks, salt, mixed spices and preservatives (bacteriocin and nitrite). Briefly, 400 g of beef was ground in the presence of 5% (w/w) salt and 15% (w/w) ice (the total percentage was based on the weight of beef). Twenty percent (w/w) of tapioca flour was then added into the mixture, along with spices (2% (w/w) garlic and 2% (w/w) pepper). Bacteriocin was introduced by the addition of 0.3% (v/v) plantaricin IIA-1A5 in the first grinding, together with salt mixture. As positive control, 0.3% (w/w) nitrite was added in the second grinding. They were then formed into meatball in 60°C boiled water. The meatball samples were packed aerobically in a polyethylene bag, stored at room temperature and taken for physical and microbiological analysis every 5 h (0, 5, 10, 15 and 20 h).

Microbial analysis of meatball (Total of microbe, *Staphylococcus aureus*, *Salmonella* and *Escherichia coli*):

The experiment was performed based on the study by Arief *et al.* (2012) and AOAC (2005). Briefly, 5 g of meatball were suspended in 45 mL of 0.85% (w/v) autoclaved NaCl. The medium used for microbial analysis was prepared by pouring plate count agar (PCA, Merck), bird parker afar (BPA, Merck),

Xylose Lyxin Dexoycolate Agar (XLDA) and eosin methylene blue agar (EMBA, Merck) for the Total Plate Count (TPC), *S. aureus*, *Salmonella* and *E. coli* respectively. Regarding TPC, the sample was diluted up to 10⁻⁵ dilution, in which 10⁻³ to 10⁻⁵ dilutions were plated in PCA. As for *S. aureus*, *Salmonella* and *E. coli* test, 10⁻¹ to 10⁻³ dilutions were plated in plates with a specific medium for each bacterium. All plates were then incubated at 37°C for 24 h.

Antibacterial activity of plantaricin IIA-1A5: The experiment was performed based on the research by Hata *et al.* (2010) and Arief *et al.* (2012) by using the agar well diffusion method. Briefly, 10⁶ CFU mL⁻¹ stock of *E. coli* (as a model pathogenic bacteria) was spread on to Muller Hinton Agar (MHA/Difco) media. Fifty microliters of plantaricin IIA-1A5 was poured into 6 mm-diameters well on agar. The plate was then incubated at 37°C for 24 h. The area of inhibition was calculated from the diameters of the inhibition zones. These experiments were carried out in triplicate.

Physical properties analysis

pH and water activity: The experiment was conducted based on the research of AOAC (2005) and Arief *et al.* (2012) with slight modifications. Ten percent (w/v) of meatball suspension was formulated in distilled water. The pH meter (Hanna Instrument) was used to the measure pH value of meatball suspension. As for water activity (A_w), LabSwift A_w meter (Novasina, UK) was used for measurements.

Experimental design and statistical analysis: All data were statistically analyzed by analysis of variance (Anova) with Duncan as a *post hoc* test (Steel *et al.*, 1996). For this purpose, a completely randomized design was used in the experiment.

RESULTS AND DISCUSSION

Antibacterial activity of plantaricin IIA-1A5: Table 1 demonstrates remarkable antibacterial activity of plantaricin IIA-1A5 towards *E. coli* and *Staphylococcus aureus*. The diameter of inhibition zone for *E. coli* and *S. aureus* in the presence of plantaricin IIA-1A5 was about 10 cm. Pan *et al.* (2009) reported that antibacterial activity is considered when the diameter is greater than 6 mm. Therefore, despite the

Table 1: Antibacterial activity of plantaricin IIA-1A5

Tested bacteria	Treatments	Inhibition zone diameter
<i>Staphylococcus aureus</i>	Control	0.00±0.00 ^b
	Plantaricin IIA-1A5	10.39±1.80 ^a
<i>Escherichia coli</i>	Control	0.00±0.00 ^b
	Plantaricin IIA-1A5	10.25±1.88 ^a

^{a,b}Different letters following values indicate statistical significance

antibacterial activity towards both pathogenic bacteria showing no difference ($p > 0.05$), the data might imply plantaricin IIA-1A5 most likely exhibited antibacterial activity with a wide spectrum for both Gram negative and positive bacteria. Previously was also reported that plantaricin IIA-1A5 displayed inhibition effect towards *Salmonella thypimurium* ATCC 14028 (Arief *et al.*, 2012). A wide spectrum of plantaricin has also been shown by plantaricin MG from *L. plantarum* KLDS1.0391 (Gong *et al.*, 2010). Therefore, it is probably a common feature for plantaricin to exhibit wide spectrum, cross-Gram, antibacterial activity.

The mechanism of antibacterial activity of plantaricin has been reported to possess a bacteriostatic (Lewus *et al.*, 1992; Atrih *et al.*, 2001) or bactericidal agent (Yeates and Padilla, 2002; Gonzalez *et al.*, 1994; Zhou *et al.*, 2007). It is likely that plantaricin IIA-1A5 employs bactericidal activity, since it disrupts the cell membrane and promotes the release of ions, proteinaceous and genetic materials (Arief *et al.*, 2015). The bacterial activities towards Gram negative and positive are considerably different. The cell structure of both bacteria might account for this discrepancy. The cell wall of Gram positive has a thicker peptidoglycan layer which is dominantly composed of lipoteichoic acid (LTA). The LTA is the target recognition of bacteriocin, facilitating the absorption of bacteriocin in the cell wall of Gram positive bacteria (Kjus *et al.*, 2002).

Microbial quality of meatball during preservation: Table 1 presents the population of total microbe Total Plate Count (TPC), *S. aureus*, *E. coli* and Salmonella during storage of up to 20 h. Fresh meatball (0 h) are considerably free from contamination, as indicated by the absence colonies that grew in all plates for total microbe (TPC), *S. aureus*, Salmonella and *E. coli*. In terms of microbial quality as regulated by Indonesian National Standard for Meatball Products (SNI 01-3818-1995) (Badan Standarisasi Nasional, 1995), the meatball in this state is considerably safe to be consumed.

Upon storage, the total microbes in meatball without the addition of 0.3% plantaricin or 0.3% nitrite (control meatball) are significantly increased up to 4.4 log CFU⁻¹ at 20 h of storage ($p < 0.05$). Bacterial colonies can be observed since 5 h storage at room temperature, which gradually increase with time. However, this population remains considerably safe to be consumed based on SNI 01-3818-1995. This standard states that the maximum population of total microbes is 5.0 log CFU⁻¹. In the presence of 0.3% nitrite, a common antibacterial substance is used in commercial meatball, population of total microbes in the meatball is significantly

lower than that in control meatball. Up to 20 h storage at room temperature, the population of total microbes in the presence of 0.3% nitrite was 1.18 CFU g⁻¹, which is definitely safe to be consumed. Interestingly, when plantaricin IIA-1A5 is introduced in meatball, the population of total microbes (2.78 CFU g⁻¹) was also significantly lower than that in meatball control during storage of 20 h at room temperature. Despite the population of total microbe with 0.3% plantaricin IIA-1A5 being considerably higher than that of meatball with 0.3% nitrite, the population is also considerably safe to be consumed. In terms of safety, this result indicates that both nitrite and plantaricin IIA-1A5 remarkably retarded the population of total microbes in meatball, making the final populations safe to consume.

Despite TPC resulting in a safe microbial population of all meatball, TPC is a non-selective medium which may include some pathogenic bacteria that is greatly harmful to humans, even with a low population. A selective medium was then used in our experiment to investigate the presence of pathogenic bacteria in meatball, including *Escherichia coli*, Salmonella and *S. aureus*.

Table 2 shows that *E. coli* starts to be observed in meatball control with a population of 2.82 CFU g⁻¹ after 10 h of storage at room temperature. Based on SNI, this population is considerably safe to be consumed, since it is lower the maximum *E. coli* population allowed in meatball products (3.0 CFU g⁻¹). The population increased up to 4.19 CFU g⁻¹ after 20 h of storage. In the presence of 0.3% plantaricin IIA-1A5, interestingly, there is no colony of *E. coli* that grew at the plate until 20 h of storage at room temperature. Meanwhile, at 20 h of storage in the meatball with 0.3% nitrite, *E. coli* was observed to have a population of 0.33 CFU g⁻¹, which remains lower than that of the maximum population allowed for consumption. Despite both nitrite and plantaricin IIA yielding a lower *E. coli* population than the maximum population allowed by the standard, this result indicated that plantaricin IIA-1A5 inhibits *E. coli* that is much stronger than nitrite at the given concentration. The ability of plantaricin IIA-1A5 to retard the growth of *E. coli* is in agreement with Abdelbasset and Djamilia (2008) and Arief *et al.* (2012, 2013). *Escherichia coli* has been known to be a spoilage-causing bacteria in foodstuffs, including meatball (Ali *et al.*, 2010). The consumption of this bacterium in certain limits may promote diarrhea and other kinds of foodborne diseases.

A Gram positive pathogenic bacterium, *S. aureus* has been observed in meatball control after 5 h of storage at room temperature (Table 2). The population started from 0.87-3.35 log CFU g⁻¹ from 5-20 h of storage time at room temperature. The SNI 01-3818-1995 suggests that the

Table 2: Microbial quality of meatball stored at room temperature with addition of plantaricin IIA-1A5 and nitrite

	Treatments	Storage time (h)				
		0	5	10	15	20
TPC (log CFU g ⁻¹)	Control	0.00 ^a	2.60 ^{cd}	3.14 ^b	3.39 ^b	4.44 ^{1a}
	Ni	0.00 ^a	0.00 ^a	0.00 ^a	0.33 ^f	1.18 ^e
	Pln	0.00 ^a	0.00 ^a	2.46 ^d	2.46 ^d	2.78 ^c
<i>Staphylococcus aureus</i> (log CFU g ⁻¹)	Control	0.00 ^d	0.87 ^c	1.98 ^b	2.43 ^b	3.35 ^a
	Ni	0.00 ^d	0.00 ^d	0.00 ^d	0.43 ^c	0.33 ^c
	Pln	0.00 ^d	0.00 ^d	0.00 ^d	0.67 ^c	1.30 ^c
<i>Escherichia coli</i> (log CFU g ⁻¹)	Control	0.00 ^f	2.41 ^d	2.82 ^c	3.49 ^b	4.19 ^a
	Ni	0.00 ^f	0.00 ^f	0.00 ^f	0.00 ^f	0.33 ^e
	Pln	0.00 ^f	0.00 ^f	0.00 ^f	0.00 ^f	0.00 ^f
Salmonella (log CFU g ⁻¹)	Control	Negative	Negative	Negative	Negative	Negative
	Ni	Negative	Negative	Negative	Negative	Negative
	Pln	Negative	Negative	Negative	Negative	Negative

Meatball without addition of nitrite or plantaricin IIA-1A5 is designated as a control, Ni and Pln correspond to meatball with addition of 0.3% nitrite or 0.3% plantaricin IIA-1A5, respectively. The values are means of at least three independent replications with standard error less than 10%, ^{a-g}Different letters following values indicate statistical differences

maximum population of *S. aureus* in meatball is 2 log CFU g⁻¹, which indicates that meatball control is considerably safe in terms of *S. aureus* risk to be consumed after being stored up to 10 h at room temperature. When meatball control is stored longer than 10 h at room temperature, the population of *S. aureus* exceeds the allowed population for consumption (more than 2 log CFU g⁻¹).

The presence of 0.3% nitrite significantly retarded the growth of *S. aureus* compared to meatball control. The colonies were observed after 15 h of storage at room temperature with a population of 0.43 log CFU g⁻¹ and kept constant statistically ($p > 0.05$) up to 20 h of storage time. Interestingly, 0.3% plantaricin IIA-1A5 displayed the ability to inhibit a population of *S. aureus* as strong as 0.3% nitrite. The colonies were first observed after 15 h of storage at room temperature and the population was statistically kept stable until 20 h of storage ($p > 0.05$). The population of *S. aureus* in meatball with the addition of 0.3% plantaricin or 0.3% nitrite is lower than the maximum level allowed by the standard (2 log CFU g⁻¹). It strongly indicated that until 20 h storage at room temperature, meatball with nitrite or plantaricin IIA-1A5 are considerably safe to be consumed. It is worthy to mention that the ability of plantaricin IIA-1A5 to inhibit *S. aureus* is comparable to that of nitrite (at given concentration), demonstrating promising potential use of plantaricin as a nitrite replacer.

The prevalence of Salmonella in ground beef is considerably high (Erol, 1999; Heredia *et al.*, 2001; Zhao *et al.*, 2001; Siriken, 2004). However, in this study, Salmonella was not detected (negative) on meatball stored up to 20 h at room temperature, either in the absences or in the presence of preservative agents (nitrite or plantaricin). Salmonella in beef

products usually originates from cross contamination of external sources (workers, environment, tools, etc., Siriken *et al.*, 2009). The negative result of Salmonella observation in this experiment might be caused by the sanitary property of meatball preparation, which is free from Salmonella. In addition, boiling treatment during meatball preparation might also be responsible for the elimination of Salmonella from the final product.

Altogether, microbial analysis of meatball in the absence or presence of nitrite or plantaricin IIA-1A5 demonstrated the ability of plantaricin IIA-1A5 to retard pathogenic bacteria (*E. coli*, *S. aureus* and Salmonella) in a manner that is as effective as nitrite. Plantaricin IIA-1A5 was able to maintain microbial quality of meatball until 20 h of storage at room temperature, in which the population of the pathogenic bacteria was considerably low, hence, the final product was safe to be consumed. This result was similar with Sihombing *et al.* (2015) that proved that plantaricin IIA-1A5 could be used as biopreservative on fresh beef.

Physical properties of meatball: Water content in the food system is a critical factor for the survival of microorganisms, especially the amount of free water that can be utilized by them to ensure growth. This free water is defined as water activity (A_w). Table 3 shows the A_w of meatball in the absence or presence of preservative agents (nitrite or plantaricin IIA-1A5).

Table 3 demonstrates that the addition on 0.3% nitrate or 0.3% plantaricin IIA-1A5 did not seriously affect A_w ($p > 0.05$). In addition, during storage of up to 20 h at room temperature, there was no serious effect on A_w as indicated by A_w values upon the storage ($p > 0.05$). The same evidence was observed

Table 3: Physical quality of meatball stored at room temperature with addition of plantaricin IIA-1A5 and nitrite

Treatments	Storage time (h)				
	0	5	10	15	20
Water activity					
Control	0.90	0.90	0.90	0.90	0.91
Ni	0.90	0.90	0.90	0.90	0.89
Pln	0.90	0.90	0.90	0.89	0.89
pH					
Control	6.29	6.29	6.29	6.29	6.29
Ni	6.21	6.21	6.21	6.21	6.20
Pln	6.17	6.17	6.17	6.17	6.17

Meatball without addition of nitrite or plantaricin IIA-1A5 is designated as a control, Ni and Pln correspond to meatball with addition of 0.3% nitrite or 0.3% plantaricin IIA-1A5, respectively. The values are means of at least three independent replications with standard error less than 10%

on pH values, in which pH values of meatball were similar for control with the addition of 0.3% nitrite or 0.3% plantaricin IIA-1A5. When the meatball were kept at room temperature for 20 h, their pH values did not significantly change ($p > 0.05$).

These results are interesting in terms of the ability of plantaricin IIA-1A5 to retard the aforementioned pathogenic bacteria. Water activity and pH values have been reported to play important roles in microorganism survival. In general, low A_w values would retard the growth of microorganism, since the amount of water to be used in their metabolism is limited. Meanwhile, certain pH values might seriously damage the cells of microorganisms due to the change in membrane permeability, ion transport and so on. Since A_w and pH values of the meatball are similar, these two factors might not be responsible for the inhibition of *S. aureus* and *E. coli* in the meatball in this study. The differences in the population of *S. aureus* and *E. coli* of meatball in 0.3% nitrite meatball and 0.3% plantaricin IIA-1A5 meatball are likely due to the presence of nitrite or plantaricin in the meatball. Meanwhile, these preservative agents did not play an important role in the inhibition of Salmonella, since this bacterium was not observed in all meats.

CONCLUSION

This study demonstrated the ability of plantaricin IIA-1A5 from *Lactobacillus plantarum* IIA-1A5 to reduce population of total bacteria, *S. aureus* and *E. coli* in the meatball. Up to 20 h, the population of these bacteria are lower than maximum population allowed by Indonesian National Standard for Meatball Products (SNI 01-3818-1995) for safety consumption. This ability comparable to of nitrite, an antibacterial agents widely used by meatballs vendor in Indonesia. This suggests promising application of plantaricin IIA-1A5 as a nitrite replacer for meatballs.

REFERENCES

- AOAC., 2005. Official Methods of Analysis of AOAC International. 18th Edn., AOAC International, Gaithersburg, MD., USA., ISBN-13: 978-0935584752.
- Abdelbasset, M. and K. Djamil, 2008. Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk Raib. Afr. J. Biotechnol., 7: 2908-2914.
- Abo-Amer, A.E., 2007. Characterization of a bacteriocin-like inhibitory substance produced by *Lactobacillus plantarum* isolated from Egyptian home-made yogurt. ScienceAsia, 33: 313-319.
- Afiyah, D.N., I.I. Arief and C. Budiman, 2015. Proteolytic characterization of trimmed beef fermented sausages inoculated by Indonesian probiotics: *Lactobacillus plantarum* IIA-2C12 and *Lactobacillus acidophilus* IIA-2B4. Adv. J. Food Sci. Technol., 8: 27-35.
- Ali, N.H., A. Farooqui, A. Khan, A.Y. Khan and S.U. Kazmi, 2010. Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. J. Infect. Dev. Countries, 4: 382-388.
- Arief, I.I., 2011. Characterization of indigenous lactic acid bacteria from beef as probiotic and identification by 16S rRNA gene sequencing. Ph.D. Thesis, Bogor Agricultural University, Indonesia.
- Arief, I.I., B.S.L. Jenie, T. Suryati, G. Ayuningtyas and A. Fuziawan, 2012. Antimicrobial activity of bacteriocin from indigenous *Lactobacillus plantarum* 2c12 and its application on beef meatball as biopreservative. J. Indonesian Trop. Anim. Agric., 37: 90-96.
- Arief, I.I., J.T. Suryati, Z. Wulandari and E. Andreas, 2013. Isolation and characterization of plantaricin produced by *Lactobacillus plantarum* strains (IIA-1A5, IIA-1B1, IIA-2B2). Media Peternakan: J. Anim. Sci. Technol., 36: 91-100.
- Arief, I.I., C. Budiman, B.S.L. Jenie, E. Andreas and A. Yuneni, 2015. Plantaricin IIA-1A5 from *Lactobacillus plantarum* IIA-1A5 displays bactericidal activity against *Staphylococcus aureus*. Beneficial Microbes, 6: 603-613.
- Atrih, A., N. Rekhif, A.J.G. Moir, A. Lebrihi and G. Lefebvre, 2001. Mode of action, purification and amino acid sequence of plantaricin C19, an anti-Listeria bacteriocin produced by *Lactobacillus plantarum* C19. Int. J. Food Microbiol., 68: 93-104.
- Badan Standarisasi Nasional, 1995. Bakso daging. Standar Nasional Indonesia 01-3818-1995, Jakarta, Indonesia.
- Cassens, R.G., 1995. Use of sodium nitrite in cured meats today. Food Technol., 49: 72-80.
- Cleveland, J., T.J. Montville, I.F. Nes and M.L. Chikindas, 2001. Bacteriocins: Safe, natural antimicrobials for food preservation. Int. J. Food Microbiol., 71: 1-20.

- EFSA Panel on Food Additives and Nutrient Sources added to Food, 2010. Statement on nitrites in meat products. EFSA J., Vol. 8, No. 5. 10.2903/j.efsa.2010.1538
- Erol, I., 1999. [Incidence and serotype distribution of Salmonella in ground beef in Ankara]. Turk. J. Vet. Anim. Sci., 23: 321-325, (In Turkish).
- Galvez, A., H. Abriouel, R.L. Lopez and N.B. Omar, 2007. Bacteriocin-based strategies for food biopreservation. Int. J. Food Microbiol., 120: 51-70.
- Gong, H.S., X.C. Meng and H. Wang, 2010. Plantaricin MG active against Gram-negative bacteria produced by *Lactobacillus plantarum* KLDS1.0391 isolated from Jiaoke, a traditional fermented cream from China. Food Control, 21: 89-96.
- Gonzalez, B., P. Arca, B. Mayo and J.E. Suarez, 1994. Detection, purification and partial characterization of plantaricin C, a bacteriocin produced by a *Lactobacillus plantarum* strain of dairy origin. Applied Environ. Microbiol., 60: 2158-2163.
- Hata, T., R. Tanaka and S. Ohmomo, 2010. Isolation and characterization of plantaricin ASM1: A new bacteriocin produced by *Lactobacillus plantarum* A-1. Int. J. Food Microbiol., 137: 94-99.
- Heredia, N., S. Garcia, G. Rojas and L. Salazar, 2001. Microbiological condition of ground meat retailed in Monterrey, Mexico. J. Food Protect., 64: 1249-1251.
- Holo, H., Z. Jeknic, M. Daeschel, S. Stevanovic and I.F. Nes, 2001. Plantaricin W from *Lactobacillus plantarum* belongs to a new family of two-peptide lantibiotics. Microbiology, 147: 643-651.
- Kjus, T., C. Lutzow-Holm and O.B. Christensen, 2002. Treatment of panniculitis associated with alpha-1-antitrypsin deficiency with alpha-1-protease inhibitor. Acta Dermato-Venereologica, 83: 462-463.
- Lewus, C.B., S. Sun, T.J. Montville, 1992. Production of an amylase-sensitive bacteriocin by an atypical *Leuconostoc paramesenteroides* strain. Applied Environ. Microbiol., 58: 143-149.
- Malav, O.P., S. Talukder, P. Gokulakrishnan and S. Chand, 2015. Meat analog: A review. Crit. Rev. Food. Sci. Nutr., 55: 1241-1245.
- Milkowski, A., H.K. Garg, J.S. Coughlin and N.S. Bryan, 2010. Nutritional epidemiology in the context of nitric oxide biology: A risk-benefit evaluation for dietary nitrite and nitrate. Nitric Oxide, 22: 110-119.
- Pan, X., F. Chen, T. Wu, H. Tang and Z. Zhao, 2009. The acid, bile tolerance and antimicrobial property of *Lactobacillus acidophilus* NIT. Food Control, 20: 598-602.
- Sapatnekar, N.M., S.N. Patil and B.A. Aglave, 2010. Extraction of bacteriocin and study of its antagonistic assay. Int. J. Biotechnol. Biochem., 6: 865-870.
- Savadogo, A., C.A.T. Ouattara, I.H.N. Bassole and S.A. Traore, 2006. Bacteriocins and lactic acid bacteria-a minireview. Afr. J. Biotechnol., 5: 678-683.
- Sihombing, D.E., I.I. Arief and S. Budiarti, 2015. Application of antimicrobial agents produced by *Lactobacillus plantarum* IIA-1A5 as natural preservative on beef during room temperature storage. Adv. J. Food Sci. Technol., 8: 251-255.
- Sindelar, J.J. and A.L. Milkowski, 2011. Sodium nitrite in processed meat and poultry meats: A review of curing and examining the risk or benefit of its use. AMSA White Paper Series No. 3, November 2011, American Meat Science Association, USA., pp: 1-14.
- Siriken, B., 2004. The presence of *Yersinia enterocolitica* and other *Yersinia* species in ground beef in Aydin, Turkey. Turk. J. Vet. Anim. Sci., 28: 489-495.
- Siriken, B., O. Cadirci, G. Inat and S. Pamuk, 2009. Microbiological examination of meatball, cream cake and Turkish delight (Lokum). J. Anim. Vet. Adv., 8: 2049-2054.
- Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1996. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Edn., McGraw-Hill Book Co. Inc., New York, ISBN-13: 978-0070610286, Pages: 672.
- Supraptini, 2002. Cases of food poisoning and the causes in Indonesia 1995-2000. Health and Development Research Institute, Indonesia.
- Tompkin, R.B., 2005. Nitrite. In: Antimicrobials in Food, Davidson, P.M., J.N. Sofos and A.L. Branen (Eds.). 3rd Edn., CRC Press, Boca Raton, FL., USA., ISBN: 0824740378, pp: 169-236.
- Yeates, T.O. and J.E. Padilla, 2002. Designing supramolecular protein assemblies. Curr. Opin. Struct. Biol., 12: 464-470.
- Zhao, C., B. Ge, J.D. Villena, R. Sudler and E. Yeh *et al.*, 2001. Prevalence of *Campylobacter* spp., *Escherichia coli* and Salmonella serovars in retail chicken, Turkey, pork and beef from the Greater Washington D.C., Area. Applied Environ. Microbiol., 67: 5431-5436.
- Zhou, W., G.R. Liu, P.L. Li, Y.Q. Dai and K. Zhou, 2007. Mode of action of plantaricin L-1, an antilisteria bacteriocin produced by *Lactobacillus plantarum*. Acta Microbiologica Sinica, 47: 260-264, (In Chinese).