

Antibacterial Activity of Lactic Acid Bacteria Isolated from Gastrointestinal Tract of ayam Kampung

by Tyas Rini Saraswati

Submission date: 21-Jan-2020 03:26PM (UTC+0700)

Submission ID: 1244364972

File name: acteria_Isolated_from_Gastrointestinal_Tract_of_ayam_Kampung.pdf (789.64K)

Word count: 3435

Character count: 17406

PAPER • OPEN ACCESS

8

Antibacterial Activity of Lactic Acid Bacteria Isolated from Gastrointestinal Tract of “Ayam Kampung” Chicken Against Food Pathogens

To cite this article: Siti Nur Jannah *et al* 2018 *J. Phys.: Conf. Ser.* **1025** 012082

View the [article online](#) for updates and enhancements.

Related content

- [Antimicrobial activity of lactic acid bacteria isolated from bekasam against staphylococcus aureus ATCC 25923, escherichia coli ATCC 25922, and salmonella sp](#)
Melia Sari, Dwi Suryanto and Yurnaliza
- [Antibacterial activity study of Attacus atlas cocoon against Staphylococcus aureus and Escherichia coli with diffusion and dilution method](#)
Aminah, E R Nugraheni and A Yugatama
- [Modeling the effect of Rose Bengal on growth and decay patterns of Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus](#)
M-Ali H. Al-Akhras, Mohammad Al Shorman, Majed M. Masadeh et al.



IOP | ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

Antibacterial Activity of Lactic Acid Bacteria Isolated from Gastrointestinal Tract of “Ayam Kampung” Chicken Against Food Pathogens

Siti Nur Jannah, Tyas Rini Saraswati, Dwi Handayani, Sri Pujiyanto

Department of Biology, Faculty of Science and Mathematics, Diponegoro University
Jl. Prof. Soedharto, SH, Tembalang, Semarang 50275, Indonesia
E-mail: nurjannah.suroso@gmail.com

Abstract. Food borne disease results from ingestion of water and wide variety of food contaminated with pathogenic organisms. The main causes of food borne diseases are bacteria, such as *Escherichia coli* and *Staphylococcus aureus*. The objective of this study was to determine antimicrobial activity of lactic acid bacteria (LAB) isolated from local chicken gastrointestinal tract with an emphasis on their probiotic properties. The colonies of bacteria that producing clear zone on MRSA plus 0.5% CaCO₃, Gram-positive and catalase-negative were isolated as lactic acid bacteria. Some of the strains (10 isolates) were tested for their ability to inhibit growth of *Escherichia coli* and *Staphylococcus aureus*, and for acid pH and bile salt tolerance. The results showed that the all selected isolates producing antimicrobial compounds inhibits the growth of *Escherichia coli* and *Staphylococcus aureus*, both in the supernatant and supernatant plus 2M NaOH, and still growing in medium condition with pH 2.0 and 0.1% bile salt. It revealing the potential use of the lactic acid bacteria from chicken gastrointestinal tract for probiotics in food.

Keywords: Probiotics, Lactic acid bacteria, antimicrobial activity, chicken gastrointestinal tract

1. Introduction

The growing number of food-borne illness outbreaks caused by several pathogens and their enterotoxins are very concern in the field of food safety and regulatory. Several reports indicate that foodborne pathogens are one of the main causes of death in the world. Control of these foodborne enteric pathogens is a challenge for food industry and public health agency.

Livestock harbor a diverse collection of bacteria, especially in the gastrointestinal tract. The chicken gastrointestinal tract can harbor in excess of 500 bacterial species at concentrations of 10¹⁰ cells/g of digesta. The majority of bacterial are native microflora of the gut that beneficial to the host (Bjerrum *et al*, 2006). Nevertheless, there are several species that exhibit pathogenic or toxigenic effects in both humans and livestock. These pathogens can colonize in the environment of the gastrointestinal tract. The use of antibiotics in poultry as Antibiotics growth promoters (AGPs) in poultry feed can improve performance and efficiency and protect poultry from various pathogenic gut microbes. But this has led to the potential development of antibiotic resistance in humans after long-term consumption. The prohibition of antibiotic (AGPs) in animal feed is an important factor that causes ecological changes microbes in the gastrointestinal tract of broiler chickens, resulting in many outbreaks of necrotic enteritis. To increase chicken productions and still provide a safe livestock



product for consumption, alternative antibiotics or medicines in the livestock industry should be employed, for example by using probiotics.

Probiotics are living microorganisms administered in sufficient quantities that have beneficial effects on host health (FAO / WHO 2002). Microbes that are often used as probiotics are the group of lactic acid bacteria (LAB). They are non-pathogenic and classified as generally recognized as safe (GRAS) microorganisms, have been widely used in fermented foods, acid and bile salt tolerance, and produce antimicrobial substances (Jannah, *et al.* 2014, Patterson and Burkholder 2003, Mojgani *et al.* 2007; Torshizi *et al.* 2008; Vargas-Rodriguez *et al.* 2013). These bacteria produced antimicrobial agents such as acids, hydrogen peroxide and bacteriocins and have great potential as food bio-preservatives

Commercial probiotics have been widely available in the market, but its potential effectiveness in tropical country like Indonesia has not been widely developed. In the present study was conducted to isolate of lactic acid bacteria (LAB) from gastrointestinal tract from "Ayam Kampung" chickens and to assess their probiotic activity against food pathogenic microorganisms.

2. Material and methods

The materials used in the study were liquid and solid digesta samples taken from digestive tract 2 groups of chickens by age (2 adults, age 7-8 months and 2 young chickens age of 1 week). *Escherichia coli* and *Staphylococcus aureus* that obtained from DUCC, biology department of Diponegoro University.

2.1 Isolation and enumeration of Lactic acid bacteria from 'Ayam Kampung' Chicken gastrointestinal tracts

Samples were collected from 4 chickens that taken from Mranggen district, center of Java, Indonesia. The Lactic acid bacteria were isolated by dilution and plating on the selective media for Lactobacilli, De Man, Rogosa and Sharpe (MRS) agar and 0.5% Calcium carbonate (CaCO_3) added and then serial dilution and plating of bacterial cultures was conducted until 10^8 dilution. The plates were incubated anaerobically by inserting an anaerobic indicator strip and Anaerocult A (Merck, Germany) in anaerobic jar at 37°C for 48 h. The Lactic acid bacteria was presumptively identified by their ability to grow well on MRS medium and showing halo zone around the colonies, Gram-positive, and catalase-negative. Representative colonies of all morphologies were taken randomly and purified on the same media and then stored in 20% glycerol at -20°C .

2.2 Antimicrobial activity assay

To detect the presence of antimicrobial activity produced by LAB was determined by agar diffusion method (Taheri *et al.* 2009, Heravi *et al.*, 2011). LAB culture was grown in liquid MRS at 37°C for 24 hours to achieve cell concentration of 10^8 CFU / ml. The cell free supernatant of bacterial cultures obtained by centrifugation. Bacterial cell cultures and cell-free supernatant of LAB isolates were determined its antimicrobial activity against indicator bacteria (*Escherichia coli* and *Staphylococcus aureus*). Supernatants from overnight cultures of the LAB strains were neutralized to pH 6.5 to 7.0 with 2M NaOH. A total of 0.1% bacterial culture indicator with a cell density of 10^6 CFU / ml was suspended in 100 ml of solid Nutrient Agar medium, then poured into a petri dish, and after solid was made well using a sterile pasteur pipette. A total of 40 μl of bacterial culture or supernatants were included in the well. After incubation at 37°C for 24 h, a clear zone around the well was measured.

2.3 Acid tolerance.

Isolates of LAB were subcultured in MRS broth for 24 h at 37°C before experimental use. The cells from 1 ml MRS culture were harvested by centrifugation (7500g, 5 min), and washed two times in sterile phosphate-buffered saline, pH 7.0. Washed cell pellets were then suspended in the same buffer with cell density (at OD 600 nm) of 0.5-0.7. The cells were resuspended in PBS by lowering pH to 6.5, 4 and 2 followed by incubation at 37°C in each set of pH for 90 min (Taheri *et al.* 2009). Total

viable count was determined before and after incubation period by total plate count method on MRS agar under aerobic conditions for 48 h at 37° C.

2.4 Bile tolerance

Isolates of LAB were subcultured in MRS broth for 24 h at 37 °C before experimental use. The cells of LAB (1 ml) from were collected by centrifugation (7500g, 5 min), washed twice in PBS solution containing 0.3%, 0,1, 0.05, 0.08, 0% Ox-bile (SIGMA) and incubated at 37 °C for 5 h. The optical density (OD) at 600 nm was measured and compared to a control culture (Taheri et al. 2009). Experiments of acid and bile tolerance were repeated two times each with duplicate analysis.

3. Results and discussion

3.1 Population of culturable Lactic Acid Bacteria

Base on the conventional method by culturing, the population of lactic acid bacteria from the “Ayam Kampung” chicken gastrointestinal tract is found between 6.3 to 8.9 log CFU g⁻¹ and the highest amount is found in the Crop (Table 1), followed by respectively by cecum, ileum and colon. The pH scale ranges from 4.5 to 7. From the data showed no correlation between the amount of LAB with substrate acidity. This is caused by the amount of LAB is not only influenced by pH values, but there are many other factors, such as diet, age, the presence of obstructions in the intestine and the different physiological functions in organ systems (Dumonceaux *et al.*, 2006). According to Barrow (1992), factors affecting the growth of microbes in the chicken intestines are age, immune response, feed and antibiotic administration.

Table 1. The number and pH scale of culturable lactic acid bacteria isolated from ‘Ayam Kampung’ Chickens gastrointestinal tract

Sample	Chicken A		Chicken B		Chicken C		Chicken D	
	Log CFU g ⁻¹	pH	Log CFU g ⁻¹	pH	Log CFU g ⁻¹	pH	Log CFU g ⁻¹	pH
Crop	8.04	6.1	8.85	5.5	7.23	5.1	8.45	4.5
Ileum	7.08	6.4	7.49	6.7	7.60	6.3	7.18	6.1
Cecum	8.66	6.1	8.15	7.0	7.89	6.4	7.83	6.0
Colon	7.57	7.0	6.40	6.5	7.64	6.0	7.38	6.1

Thirty-seven LAB colonies were randomly selected based colony morphological differences and the presence of clear zones around colonies grown on MRS solid medium with 0.5% CaCO₃ added (data not shown). The morphology of the colony is almost identical and uniform with round shape, white, creamy, brownish, pink, 0.5 - 4 mm in diameter, convex surface, flat edge or fiber, shiny surface, and soft texture. All isolates exhibited special characteristics of lactic acid bacteria, such as Gram positive, negative catalase reactions and non endospore forming bacteria. We found that most of the rod-shaped bacterial cells (30 isolates) and 7 isolates are round-shaped. Salminen and Wright (1993) states that *Lactobacillus* has rod-shaped cells, some of which are usually very long and some are round bars. The morphology appearance are a single cell or on short to long chains, facultative anaerobes, Gram positive, most immobile species and mesophilic.

3.2 Antimicrobial activity

Isolates of LAB as a candidate probiotic expected to work in the digestive tract of the host, so it should be selected based on several criteria. The bacteria can live in conditions of limited oxygen, can compete with the microbes resident and pathogens for getting a place and nutrients in the gut, can resist to barriers in the gastrointestinal tract such as acidic pH and the presence of bile salts whose activity can damage bacterial cell walls, and produce defense enzymes and metabolites. *Escherichia coli* and *Staphylococcus aureus* bacteria were used as indicator bacteria because these bacteria are pathogenic bacteria in the food. Antimicrobial activity showed by lactic acid bacteria from the challenge test is characterized by a clear zone found around the disc paper.

The antimicrobial activity of 10 isolates of LAB (in the form of LAB culture, cell-free supernatant and cell-free neutralized supernatant with 2N NaOH) against *E. coli* and *S. aureus* are shown in Table 2. The result showed that all bacterial cultures and supernatants have antimicrobial activity against *E. coli* and *S. aureus*. Most of isolates showed stronger antagonisms against *E. coli* than to *S. aureus*. Neutralized supernatant still showed antibacterial activity against *E. coli* and for *S. aureus*. This indicated that the inhibition of LAB is not only caused by acids produced but also the other metabolites like bacteriocins.

Table 2. Antimicrobial activity data of 10 selected isolates LAB

Strain	Source	Inhibitory zone (mm)					
		against <i>E. coli</i>			against <i>S. aureus</i>		
		A	B	C	A	B	C
BUB 3	Colon	17.6 ± 1.1	10.5 ± 0.7	9.5 ± 0.5	8.7 ± 0.5	8.0 ± 1.2	8.5 ± 1.3
CUB1	Colon	15.3 ± 0.3	8.5 ± 1.0	9.1 ± 1.2	8.3 ± 0.7	7.6 ± 0.9	6.6 ± 0.5
BUB 4	Colon	15.0 ± 1.3	8.5 ± 0.5	9.5 ± 0.9	8.0 ± 0.6	8.0 ± 0.6	8.5 ± 0.5
DC1	Crop	18.7 ± 0.5	13.5 ± 0.7	9.0 ± 0.6	9.3 ± 0.6	8.0 ± 0.5	8.7 ± 1.0
DS1	Cecum	11.2 ± 0.5	7.5 ± 0.6	10.4 ± 0.5	9.2 ± 0.5	7.7 ± 0.5	9.0 ± 0.5
CC1	Crop	10.4 ± 1.0	8.3 ± 0.6	9.5 ± 0.5	8.0 ± 0.6	7.0 ± 0.6	8.4 ± 0.6
CI4	Ileum	12.5 ± 0.5	9.5 ± 0.5	8.4 ± 0.5	7.5 ± 1.1	6.9 ± 0.5	6.6 ± 0.6
CC2	Crop	14.6 ± 0.6	7.3 ± 0.6	14.3 ± 0.6	13.7 ± 1.5	7.3 ± 1.2	6.3 ± 0.6
AS2	Cecum	13.6 ± 0.6	9.3 ± 0.6	11.3 ± 1.2	9.3 ± 0.6	8.3 ± 0.6	10.0 ± 2.0
AC1	Crop	15.3 ± 0.6	8.3 ± 0.6	9.3 ± 1.2	7.7 ± 1.2	18.7 ± 0.6	8.3 ± 0.6

A : Bacterial culture; B : Supernatant; C = Neutralized supernatant

Five of LAB isolates showing the highest inhibitory zone were selected for further research (Figure 2). The wide spectrum inhibitory activity against challenging food borne pathogens make this isolate desirable for exploring their potential for health benefits in production of functional food.

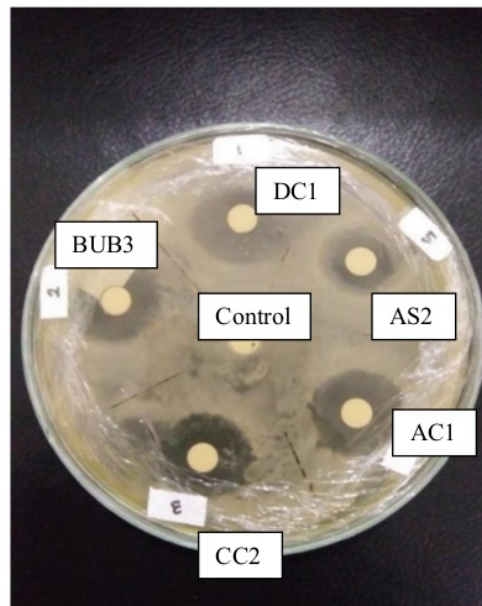


Figure 1. Zone of Inhibition by culture of Lactic acid bacteria isolates against *Escherichia coli*

3.3 LAB Screening for pH and Bile-Salt Tolerance

LAB as candidate probiotics have to survive in the gastrointestinal tract where pH can be as low as 2 and the presence of bile salt. The viability of five LAB isolates showed tolerance to pH 4.0 and 2.0 during 90 minutes of incubation. However, the growth in these conditions was not comparable with that in conventional MRS (pH 6.5), with reductions in counts at least 1-2 logarithmic units compared with controls. Among other LAB isolates, the AC1 isolate had the highest viability at low pH (pH 2.0) with a decrease in the number of isolates by 0.65 log cycles. (Figure 2). The resistance to low pH may be related to the origin of the LAB isolate sample, where AC1 is derived from the crop. Most of bacteria grow slower at low pH, due to the presence of an acid that can damage and decrease its viability. However, LAB has the ability to regulate their cytoplasmic or intracellular pH around neutral pH, even when it is in low extracellular pH during growth or in storage (Konings *et al.*, 1997).

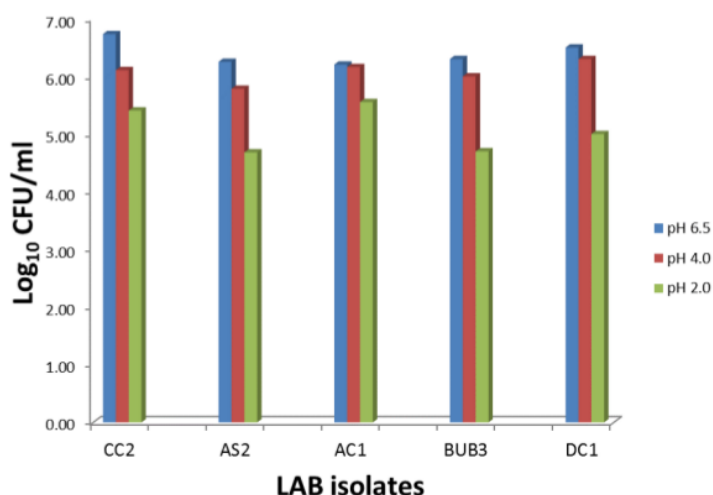


Figure 2. The viability of LAB isolates under acidic conditions.

The survival assay of LAB isolates in the presence of bile salts showed that all isolates did not tolerate in 0.3% bile salt. However, isolates may survive in 0.1% bile salts. The viability of LAB isolates on the presence of 0.1% bile salts decreased after 5 hours of incubation at 0.1% bile salt suspension (Figure 3). Iniguez-Palomares *et al.* (2007) reported that LAB isolates had no resistance to CPBS (conjugated porcine bile salts) at concentrations greater than 0.1% bile salts. Bile released in the small intestine can damage bacteria because of its effect in destroying the cell membrane. For some bacteria, such as lactic acid bacteria that have hydrolase enzymes (BSH = bile salt hydrolase) that work against bile salts, so that LAB has the ability to hydrolyze bile salts and reduce its solubility. To increase the viability of bacteria in order to pass through the upper gastrointestinal tract can be done by bacterial encapsulation method with alginate and skim milk (Chavarri *et al.* 2010).

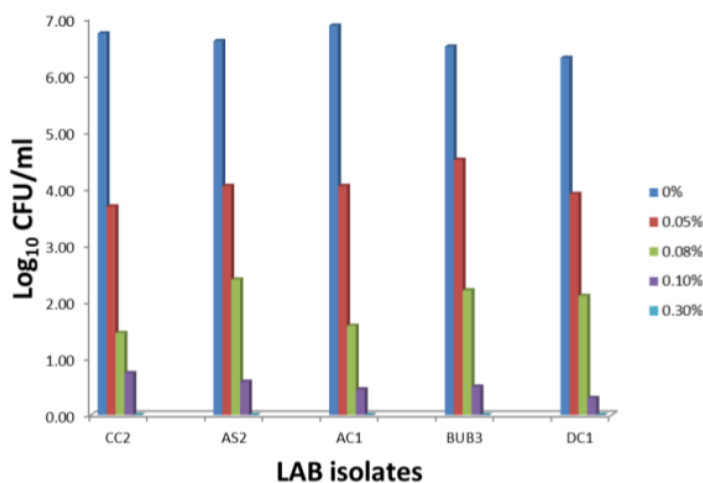


Figure 3. Bile resistance of 5 LAB isolates isolated from gastrointestinal tract

Acknowledgments

This study was supported by Indonesia Higher Education, Ministry of research Technology Higher Education and Diponegoro University for RPP research.

References

- [1] Barrow PA., MB. Huggins MA. Lovell, and Simpson JM. 1987. Observation on the pathogenicity of experimental *Salmonella typhimurium* infection in **chickens**. Res. Vet. Sci. 42:194–199.
- [2] Bjerrum L, Engberg RM, Leser TD, Jensen BB, Finster K, and Pedersen K. 2006. Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and cellular-based techniques. *Poultry Sci.* 85: 1151 –1164.
- [3] Chavarri M, Marano I, Ares R, Ibanez FC, Marzo F, and Villaran MC. 2010. Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in stimulated gastro-intestinal conditions. *Int J Food Microbiol.* 142: 185-189.
- [4] Dumonceaux TJ, Hill JE, Hemmingsen SM, Van Kessel AG. 2006. Characterization of intestinal microbiota and response to dietary virginiamycin supplementation in the broiler chicken. *Appl Environ Microbiol*, 72:2815-2823.
- [5] FAO/WHO – Food and Agriculture organization of the United Nations/World Health Organization (2002): Guidelines for evaluation of probiotics in food. Available at: http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf
- [6] Jannah SN, Dinoto A, Wiryawan KG and Rusmana I. 2014. Characteristics of Lactic Acid bacteria Isolated from Gastrointestinal Tract of Cemani Chicken and Their Potensial use as Probiotics. *Jurnal Media Peternakan, Journal of Animal Science and Technology.* 37:3
- [7] Heravi RM, Kermanshahi H, Sankian M, Nassiri MR, Moussavi AH, Nasiraii LR, and Varasteh AR. 2011. Screening of lactobacilli bacteria isolated from gastrointestinal tract of broiler chickens for their use as probiotic. *African J. of Microbiol. Res.* 5:1858-1868.
- [8] Iniguez-Palomares C, Perez-Morales R, and Acedo-Felix E. 2007. Evaluation of probiotic properties in Lactobacillus isolated from samll intestine of piglets. *Microbiologia:* 49:46-54.
- [9] Konings WN, Lolkema JS, Bolhuis H, Van Veen HW, Poolman B, and Driessen AJM. 1997. The role of transport processes in survival of lactic acid bacteria. *Antonie v leeuwenhoek* 71:117-128.
- [10] Mojgani N, Torshizi MAK, and Rahimi S. Screening of locally isolated lactic acid bacteria for use as probiotics in poultry in Iran. *J of Poultry Sci.* 44: 357-365. 2007.
- [11] Patterson JA, Burkholder KM. 2003. Application of prebiotics and probiotics in poultry production. *Poultry Science.* 82: 627-631.
- [12] Salminen S and Wright AV. 1993. Lactic Acid Bacteria. Marcel Dekker, Inc. New York.
- [13] Taheri HR, Moravej H, Tabandeh F, Zaghari M, and Shivazad M. 2009. Screening of lactic acid bacteria toward their selection as a source of chicken probiotic. *Poult. Sci.* 88 :1586–1593.
- [14] Torshizi MAK, Rahimi SH, Mogjani N, Esmailkhanian S, and Grimes JL. 2008. Screening of indigenous strains of lactic acid bacteria for development of a probiotic for poultry. *Asian-Australian J Anim sci*, 21:1495-1500.
- [15] Vargas-Rodriguez LM, Duran-Melendez LA, Garcia-Masias JA, Arcos-Garcia JL, Joaquin-Torres BM and Ruelas-Inzunza MG. Effect of probiotic and population density on the growth performance and carcass characteristics in broiler chickens. *Int J of Poultry Sci.* 12: 390-395. 2013.

Antibacterial Activity of Lactic Acid Bacteria Isolated from Gastrointestinal Tract of ayam Kampung

ORIGINALITY REPORT

7%

SIMILARITY INDEX

2%

INTERNET SOURCES

7%

PUBLICATIONS

4%

STUDENT PAPERS

PRIMARY SOURCES

- 1 Reis, Nayara Alves, Margarete Alice Fontes Saraiva, Elizabeth Amélia Alves Duarte, Elaine Araújo de Carvalho, Brenda Borges Vieira, and Norma Suely Evangelista-Barreto. "Probiotic properties of lactic acid bacteria isolated from human milk", *Journal of Applied Microbiology*, 2016.
Publication 2%
- 2 Ghazal Aziz, Hafiz Fakhar, Sajjad ur Rahman, Muhammad Tariq, Arsalan Zaidi. "An assessment of the aggregation and probiotic characteristics of *Lactobacillus* species isolated from native (desi) chicken gut", *The Journal of Applied Poultry Research*, 2019
Publication 1%
- 3 Submitted to Asian Institute of Technology
Student Paper 1%
- 4 Nguyen Thi My Le, Nguyen Thi Huong. "EVALUATION OF PROBITOIC POTENTIAL OF LACTOBACILLUS STRAINS FROM 1%

FERMENTED FOODS AND FEACES OF INFANTS", Vietnam Journal of Science and Technology, 2016

Publication

5

Submitted to Philippine Science High School Central Luzon Campus

Student Paper

1%

6

Mahdieh Iranmanesh, Hamid Ezzatpanah, Naheed Mojgani. "Antibacterial activity and cholesterol assimilation of lactic acid bacteria isolated from traditional Iranian dairy products", LWT - Food Science and Technology, 2014

Publication

1%

7

Submitted to University of Central Lancashire

Student Paper

1%

8

repository.unika.ac.id

Internet Source

1%

Exclude quotes

Off

Exclude matches

< 1%

Exclude bibliography

On

Antibacterial Activity of Lactic Acid Bacteria Isolated from Gastrointestinal Tract of ayam Kampung

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8
