ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM THE DIGESTIVE TRACT OF KAMPUNG CHICKEN (Gallus gallus domesticus)

by Widya P

Submission date: 12-Feb-2019 02:20PM (UTC+0800)

Submission ID: 1076882816

File name: ISOLATION AND IDENTIFICATION OF......pdf (1.97M)

Word count: 3881

Character count: 22106

RESEARCH NOTE

ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM THE DIGESTIVE TRACT OF KAMPUNG CHICKEN

(Gallus gallus domesticus)

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ABSTRACT

The aim of this study was to isolate and identify lactic acid bacteria (LAB) derived from the digestive tract of Kampung chicken or Indonesian native chicken (Gallus gallus domesticus). This study used ten 20-week old male native chicken with an average body weight of 1 kg, slaughtered and processed in accordance with Halal methods. Ten-centimeter sections of the esophagus, crop, proventriculus and ventriculus were obtained, stored in sterile bottles and placed in an icebox. LAB were isolated from the chickens' gastrointestinal tract. LAB identification was done through microscopic morphology, gram staining, catalase test and biochemical tests. Isolates were gram positive, negative on catalase test, non-motile and rod-shaped. Isolates YL 117, YL 217, and YL 317 can ferment glucose, sucrose and lactose; isolate YL 117 can ferment xylose, sorbitol, arabinose and raffinose; and isolate YL 317 can ferment malonate, arabinose and raffinose. This study suggests the presence of three LAB isolates from the gastrointestinal tract of Gallus gallus domesticus: Lactobacillus plantarum, L. acidophilus and L. casei.

Key words: Gallus gallus domesticus, Kampung chicken, lactic acid bacteria

Philipp. J. Vet. Med., 55(SI): 67-72, 2018

INTRODUCTION

The balance of non-beneficial beneficial bacteria in chicken gastrointestinal tract (GIT) is important. When a microbiota balance exists, maximum growth efficiency is likely to be attained. However, when animals are subjected to stressful conditions, the beneficial microbiota tend to decrease, and this can lead to high susceptibility to disease (Kabir, 2009). To maintain balance of beneficial microbiota, it is necessary to supplement feeding with lactic acid bacteria derived from livestock. The presence of Lactobacillus spp., a common microbiota in chicken broiler gastrointestinal tract, is essential to maintaining ecological balance in the

microbiota (Kokosharov, 2001). In the cecum of healthy broilers, the most commonly found bacterial species is *Lactobacillus salivarius* (Gusils *et al.*, 1999). According to Mitsuoka (2002), the dominant lactic acid microbiota in the GIT of broilers are *Lactobacillus reuteri*, *L. salivarius*, *L. agilis* and *L. acidophilus*.

Probiotics can help cultivate beneficial microflora population in the intestines and eliminate pathogenic bacteria. These beneficial bacteria also release several enzymes which aid in digestion of food (Fioramonti et al., 2003). The use of lactic acid bacteria (LAB) provides many benefits to humans and livestock. These microorganisms can balance the microflora in the digestive tract, improve health and provide protection against pathogenic bacteria, such as Eschericia coli and Salmonella typhimurium.

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Lactobacillus species known to have probiotic properties are L. casei, L. acidophilus and L. reuteri/ L. fermentum (Klein et al., 1998). The genus is one the most commonly observed lactic acid bacteria in human and animal stomachs. Lactobacillus can be as much as 10-19×10⁷ CFU/ml in the small intestine (Manin et al., 2010) and 10¹⁰ to 10¹¹ cell/g in the large intestine. Adding probiotics to feeds can maintain microflora balance in the digestive tract and inhibit pathogenic bacteria, increase digestive enzyme activity, decrease ammonia production, improve feed intake and digestion, neutralize enterotoxins and stimulate the immune system (Manin et al., 2010).

The microenvironment of GIT affects nutrition, feed conversion and host disease; thus, it is important to maintain a healthy gut microbiome (Guarner and Malagelada, 2003). When animals experience stress, illness, or antibiotic treatment, the gastrointestinal flora often changes, which tend to support the growth of harmful bacteria, causing diarrhea or loss of appetite (Cremonini et al., 2002; Harish and Varghese, 2006).

Various microbiome of the digestive tract can have different interactions, such as competition, cooperation and antagonism (Pan and Yu, 2014). GIT in poultry is an ideal habitat for microorganisms, but it does not provide unlimited support for microbial growth or proliferation, since bacteria compete for limited nutrients and attachment sites – a common phenomenon in gastrointestinal ecosystems (Soler et al., 2010).

To cause infection in chickens, pathogenic bacteria must first be attached to and penetrate the intestinal epithelial barrier. In healthy birds, the community of commensal bacteria in the GIT colonizes the intestinal mucosa and forms a layer of bacteria that covers the mucosal surface. By occupying a different row of adjacent niches along the GIT, layers composed of dense and complex microbial communities can effectively inhibit the attachment and colonization of pathogenic bacteria that attack the gastrointestinal tract (Lan et al., 2005; Lawley and Walker, 2013).

Indonesian native chickens have inherently low productivity level due to genetic factors, so that reaching their optimal weight takes a long time. Likewise, feeds given are of poor quality, contributing to their slow growth. This study then intends to address the need to supplement feeds with probiotics derived from natural bacteria of local chicken GIT to boost their productivity. However, the use of probiotics for chickens is still inefficient. This is because probiotics used are not derived from indigenous bacteria capable of surviving in the GIT. In fact, the probiotic bacteria are eliminated before they even reach the small intestine. Thus, as a countermeasure, isolation and identification of lactic acid bacteria will be obtained from the chicken's upper digestive tract, since it is more tolerant to low pH and bile salts.

This study seeks to find new strains of lactic acid bacteria which can be used as probiotics to improve chicken productivity. Indonesian native chickens are deemed to be a potent source of lactic acid bacteria. In line with this, the present study aims to isolate and identify lactic acid bacteria from the upper digestive tract of Kampung or Indonesian native chickens (Gallus gallus domesticus), with the assumption that they are free-range, and their microflora are more diverse than broiler or laying chicken breeds whose feeds are often supplemented with antibiotics (Harimurti et al., 2007).

MATERIALS AND METHODS

Animals

Ten 20-week old male Kampung chickens, with average body weight of ±1 kg, and are typically not given antibiotic-supplemented feeds, were obtained from Tembok Dukuh Surabaya traditional market. The digestive tract of chickens, specifically the esophagus, crop, proventriculus and ventriculus were collected and processed.

Isolation and identification of lactic acid bacteria

Lactic acid bacteria were collected from 10 samples of digestive tract of Kampung chicken (Gallus gallus domesticus) based on a modified method adopted from Torshizi

et al. (2008). Ten-centimeter section of esophagus, 🔞 crop, proventriculus ventriculus wastes samples were placed in sterile polythene bags immediately after the chickens were slaughtered and placed in a an icebox A mucosal scraping of 1 g each from the esophagus, crop, proventriculus and ventriculus was aseptically removed and taken for bacteriological examinations. Samples were serially homogenized in diluted 0.85% NaCl solution (Merck, Germany) up to 10⁵ dilutions and plated onto de Man Rogosa and Sharpe (MRS) agar and incubated for 72 h at 37°C. After incubation, isolates were subcultured in MRS broth (Merck, Germany) at 37°C for 18-24 h under microaerophilic conditions. Samples in enrichment medium showing rod-shaped bacteria (from microscopic observation) were scratched on MRS agar then incubated at facultative medium. anaerobic conditions at 37°C for 48 h for each serial dilution. The colonies of all morphologies were taken and purified until a single colony was obtained using the same medium as the subculture. Isolate considered as presumptive LAB is gram-positive and catalase-negative. The pure LAB isolates were then stored in 15% glycerol at -80°C. For all subsequent tests, isolates were activated in the same medium at 37°C for 48 h and sub-subcultured under the same conditions. These colonies were separated and further purification and identification were done to determine the species. LAB characterization procedures involved morphology, gram staining and catalase assay (Blajman et al., 2015) with some modifications.

Biochemical test was performed with MicrobactTM Identification Kits (MicrobactTM GNB 12 A/B, 24 E | Oxoid, Thermo Fisher Scientific, UK), following the manufacturer's protocol. The Microbact is a substrate system which is capable of identifying the majority of commonly encountered routine laboratory isolates to at least the genus level. Organism identification is based on pH change and substrate utilization (Onwenefah and Adedeji, 2013). These systems consist of dehydrated substrates contained in a microtitre tray to which saline suspensions of organisms to be tested are added. After the purity of the

cultures was established, an inoculum was prepared for each system as recommended by the manufacturer. Each system was prepared, inoculated, overlaid with oil where necessary, and incubated as stated. Characteristics of isolates obtained were analyzed and adjusted based on Oxoid instruction manual and Bergey's Manual of Systematic Bacteriology. The results of the biochemical test are shown in Table 1.

RESULTS AND DISCUSSION

Lactobacillus and Bifidobacterium, among the many microorganisms used as probiotics, are the most commonly exploited. Probiotics are living microorganisms thought to have beneficial effects on host organisms. According to the definition now adopted by FAO/WHO, probiotics are living microorganisms, which, when administered in sufficient quantities, provide health benefits to the host. In addition, nonpathogenic species belonging to Saccharomyces, Streptococcus and Lactococcus are also used as probiotics (Fioramonti et al., 2003). In the case of chickens, probiotics from potential bacteria can bring about balance in GIT microflora, so that normal microflora can thrive. Endurance against pathogenic bacterial attacks in chickens is higher when there is a balance in microbial populations (Harimurti et al., 2007). Results of isolation and identification of lactic acid bacteria in Kampung chicken are shown in Table 2.

This study was able to identify three Lactobacillus isolates from the gastrointestinal tract (esophagus, crop, proventriculus and ventriculus) of Gallus gallus domesticus. Isolated LABs were gram-positive, catalasenegative, non-motile and rod-shaped. Isolates YL 117, YL 217, and YL 317 can ferment glucose, sucrose and lactose. Isolate YL 117 can ferment xylose, sorbitol, arabinose and raffinose, and isolate YL 317 can ferment malonate, arabinose and raffinose.

Based on biochemical tests, LAB isolates from the esophagus were *L. plantarum*, *L. acidophilus* and *L. casei* from the proventriculus; *L. plantarum* and *L. casei* from the crop; and *L. plantarum*, *L. acidophilus* and

Table 1. Biochemical test results of lactic acid bacteria isolated from the gastrotestinal tract of Kampung chicken.

	m YL~117	m YL~217	${ m YL}317$
Shape	Rod shape	Rod shape	Rod shape
Gram	Positive	Positive	Positive
Catalase	<u> </u>	•	
Lysine		27.	
Ornithine	+		*
H_2S	÷	33#0	₩.
VP	2		2
ONPG	+	+	+
Indole		3 5 0	-
Urease			•
Mannitol	+		•
Arginin	S2	7.40	20
Citrate	+	0. <u>4</u> 4	
TDA		: -	
Gelatin		21 23	-
Malonate	+		+
Inositol		3 - 5	¥3
Adonitol	2	8.4	S
Sorbitol	+	-	-
Glucose	+	+	+
Xylose	+	3(*)	#3
Rhamnose	+	+	-
Sucrose	+	+	+
Lactose	+	+	+
Arabinose	+	3 - 0	+
Raffinose	+		+
Salisin	: -	+	-

Table 2. Identification of lactic acid bacteria from the gastrointestinal tract of Kampung chicken.

	L. plantarum	L. acidophilus	L. casei
Esophagus	+	\$ = \$	=
Crop	+	+	+
Proventriculus	+	+	+
Ventriculus	+		+

L. casei from the ventriculus. These findings concur with other studies. Lactobacilli settle in the crop after a few days of feeding. Depending on the length of the stationary feed in the crop, lactobacilli may have an effect on the fermentation process (Barnes, 1979). The path from the crop to the small intestine involves

a drastic change in the luminal environment. Proventriculus of birds plays an important role as a chemical barrier against pathogens through variations in pH and enzymatic actions. In fact, *E. coli* and *Campylobacter* are found in higher numbers in crop than in gizzards (Smith and Berrang, 2006).

Chicken GITs are inhabited by various bacteria (Qu et al., 2008), methanogenic archaea and fungi (Okulewicz and Zlotorzycka, 1985; Saengkerdsub et al., 2007). Protists are less commonly found and are usually regarded as pathogens (Okulewicz and Zlotorzycka, 1985). The gastrointestinal tract of an adult chicken can hold as much as 10¹³ bacteria (Apajalahti and Kettunen, 2006).

(Another study showed that L. johnsonii F-6 and L. crispatus F-59 were isolated from broiler chickens (Kim et al., 2015). According to Taheri et al. (2009), lactic acid bacteria in the gastrointestinal tract can reduce the number of microbial pathogens through the production of organic acids, hydrogen peroxide (H₂O₂) and bacteriocins.

Microbiota in the GIT of chickens have extensive metabolic potential that affects the nutrition and health of the host. These microbial communities, collectively termed as microbiomass, play an important role in the growth and development of GIT, including production of energy-rich short-chain fatty acids; increased villus morphology of GIT; use of nutrients; reduction of luminal viscosity, deconstruction of feed polysaccharides, absorption of nutrients; and detoxification (Apajalahti and Kettunen, 2006; Yeoman et al., 2012).

GIT microbiota are important because they provide resistance against non-beneficial enteric pathogens through some known competitive exclusion. mechanisms i.e., bacterial antagonism, barrier effect and bacterial interference or colonization resistance. Specifically, the mechanisms by which indigenous intestinal bacteria inhibit pathogens include competition for nutrients, competition for colonization sites, production of toxic compounds, or stimulation of the immune system (Patterson and Burkholder, 2003).

The composition of the gastrointestinal microbiome reflects the co-evolution among host microbial, genetic, immune, and host metabolic interactions, and environmental influences. Microbes can be found along the gastrointestinal tract, and their populations show spatial variation in biogeographic composition between luminal and mucosal

populations (Gong et al., 2007).

This study isolated and identified three lactic acid bacteria (*Lactobacillus plantarum*, *L. acidophilus* and *L. casei*) from the gastrointestinal tract (esophagus, crop, proventriculus, and ventriculus) of Kampung chickens (*Gallus gallus domesticus*). These findings necessitate further research to determine the potential of the isolates in improving livestock productivity through tests on acidity, bile salt, and antimicrobial activity.

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ACKNOWLEDGMENTS

- The editorial staff wishes to thank the following who served as evaluators on an ad-hoc capacity for their critical review of manuscripts submitted to the journal:
- Dr. Marietta C. Amatorio, College of Veterinary Medicine, Benguet State University, Philippines
- Dr. Edwin C. Atabay, Philippine Carabao Center at Central Luzon State University (CLSU), Philippines
- Dr. Ayasan, Çukurova Agricultural Research Institute, Turkey
- Dr. Vasudevan Bakthavatchalu, Division of Comparative Medicine, Massachusets Institute of Technology, USA
- Dr. Jose Arceo N. Bautista, Animal and Dairy Sciences Cluster, College of Agriculture, UPLB
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- Dr. Carmencita D. Mateo, College of Agricultural and Food Science, UPLB
- Dr. Claro T. Mingala, Philippine Carabao Center National Headquarters and Gene Pool, Philippines
- Dr. Noraine P. Medina, College of Veterinary Science and Medicine, CLSU
- Dr. Anantharaman Muthuswamy, Wisconsin National Primate Research Center, Wisconsin, USA
- Dr. Mildred A. Padilla, College of Veterinary Medicine, UPLB
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- Dr. Alessandra Pelagalli, Facoltà di Medicina Veterinaria, Università degli Studi di Napoli "Federico II", Napoli, Italy
- Dr. Antonio A. Rayos, Animal and Dairy Sciences Cluster, College of Agriculture, UPLB
- Dr. Frances C. Recuenco, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan
- Dr. Cesar C. Sevilla, College of Agricultural and Food Science, UPLB
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- Dr. Guangliang (Johnny) Wang, John Hopkins University School of Medicine, Baltimore, USA

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The Philippine Journal of Veterinary Medicine

Volume 55 Special Issue December 2018



The 2nd Veterinary Medicine International Conference Surabaya, Indonesia 4-5 July 2018

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