

The PHILIPPINE JOURNAL OF

Veterinary Medicine

Volume 55

Special Issue

December 2018

**Published by the College of Veterinary Medicine
University of the Philippines Los Baños**

ISSN 0031-7705



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

The Philippine Journal of Veterinary Medicine

Volume 55

Special Issue

December 2018

The Philippine Journal of Veterinary Medicine is a peer-reviewed international journal of basic and applied research in veterinary medicine and science. It is published semi-annually, for the periods January-June and July-December each year, by the College of Veterinary Medicine, University of the Philippines Los Baños. All articles are subjected to double-blind review.

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This journal is Abstracted/Indexed by: SCOPUS, Biological Abstracts, Focus on: Veterinary Science & Medicine, Zoological Records, CAB Abstracts, Index Veterinarius, Veterinary Bulletin, Parasitology Database, Helminthological Abstracts, Protozoological Abstracts, Review of Medical and Veterinary Entomology, EBSCO, ASEAN Citation Index, Prescopus Russia, *i-journals* (www.ijournals.my), *i-focus* (www.ifocus.my), *i-future* (www.ifocus.my), Philippine E-Journals (<https://ejournals.ph>) and UPLB Journals Online (<http://journals.uplb.edu.ph/index.php/PJVM>).

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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 and 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 *Escherichia 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 $10^{19} \times 10^7$ CFU/ml in the small intestine (Manin *et al.*, 2010) and 10^{10} to 10^{11} 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 and 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 10^5 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 medium, then incubated at facultative 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 Microbact™ Identification Kits (Microbact™ 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, catalase-negative, 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.

	YL 117	YL 217	YL 317
Shape	Rod shape	Rod shape	Rod shape
Gram	Positive	Positive	Positive
Catalase	-	-	-
Lysine	-	-	-
Ornithine	+	-	-
H ₂ S	-	-	-
VP	-	-	-
ONPG	+	+	+
Indole	-	-	-
Urease	-	-	-
Mannitol	+	-	-
Arginin	-	-	-
Citrate	+	-	-
TDA	-	-	-
Gelatin	-	-	-
Malonate	+	-	+
Inositol	-	-	-
Adonitol	-	-	-
Sorbitol	+	-	-
Glucose	+	+	+
Xylose	+	-	-
Rhamnose	+	+	-
Sucrose	+	+	+
Lactose	+	+	+
Arabinose	+	-	+
Raffinose	+	-	+
Salisin	-	+	-

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

	<i>L. plantarum</i>	<i>L. acidophilus</i>	<i>L. casei</i>
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 Zlotorzycza, 1985; Saengkerdsub *et al.*, 2007). Protists are less commonly found and are usually regarded as pathogens (Okulewicz and Zlotorzycza, 1985). The gastrointestinal tract of an adult chicken can hold as much as 10^{13} 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 (H_2O_2) 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 mechanisms *i.e.*, competitive exclusion, 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
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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

