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The Potential of Indigenous Lactic Acid Bacteria Against *Salmonella* sp.

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Abstract. Lactic acid bacteria are a group of bacteria capable of producing lactic acid, H₂O₂, diacetyl and bacteriocins. Isolation purpose of lactic acid bacteria is to produce antimicrobial that will be used to inhibit the growth of pathogenic bacteria in the digestive tract. The study was conducted to isolate indigenous lactic acid bacteria from health broiler intestine and to investigate the ability to inhibit the growth of *Salmonella* sp. This study used 144 DOC (Day Old Chicken) final stock "Arbor acres CP 707". Lactic acid bacteria 10⁹ cell · mL⁻¹ administered through drinking water daily and twice per week for 5 wk. The research results showed that the potential as indigenous lactic acid bacteria were *Lactobacillus* spp., *Streptococcus* spp., and *Bifidobacterium bifidum*. The administration of lactic acid bacteria with a frequency of daily was effective to decrease the population of *Salmonella* sp. in the intestines of broiler chickens.

Keywords: broiler, lactic acid bacteria, *Salmonella* sp.

INTRODUCTION

Lactic acid bacteria are a group of bacteria capable of producing lactic acid, H₂O₂, diacetyl and bacteriocins. Isolation of lactic acid bacteria is to produce antimicrobial that will be used to inhibit the growth of pathogenic bacteria in the digestive tract. Therefore, it can be used as probiotics. Probiotics are microbial feed supplements that can improve the balance of microbes in the digestive tract of the host animal [1]. *Lactobacillus* is a genus of lactic acid bacteria are most often found in the gastrointestinal tract of cattle and can be used as probiotics in livestock that works to increase the productivity of livestock.

Probiotics as single or mixed cultures of live microorganisms which when administered to humans or animals, influence to decrease pathogenic bacteria in the intestine [1]. In another sense, a probiotic is a food containing live bacteria that are resistant physical barriers and chemical passes through the digestive tract. The bacteria are deposited in this food then actively multiply, forming colonies that line the intestine.

According to Holt [2], probiotics are live cultures of a microorganism mixing on cattle rations to ensure the availability of a population of organisms in the gut. The cultures contain specific bacteria, resistant to dry situations and certain environmental temperature, and produce the optimum response within a certain dosage. Amongst the cultures, probiotics include the class of *Lactobacillus*, *Bifidobacteria*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Pediococcus* and *Saccharomyces*. Most common bacteria which are used as probiotics are *Lactobacilli* and *Difidobacteria* [4].

MATERIAL AND METHODS

In this study, lactic acid bacteria were isolated from the intestines of healthy chickens. Chicken intestine samples were taken and inserted into the vial bottle, then added with 1 mL per g 0.9 % physiological NaCl for each gram of intestine. The intestine and NaCl buffer were mixed into homogeneous forming bacterial suspension, then the dilution was inserted into a petri dish sterile containing medium MRS normal pH and MRS acidic pH (this

procedure aimed to select bacteria that can survive in acidic environments) in the incubation. The growth of the bacteria was followed by macroscopic observation of colonies and bacterial identification.

Test animals used were one-day-old chicks (DOC) 300 “final stock 707” obtained from the “Poultry Shop Asco” maintained for 5 wk. The chickens were placed randomly in a cage consisting of 12 units. Each unit was filled with 25 chicks with a weight range of 35 g to 40 g with a maximum coefficient of variation of 10 %.

The study was conducted with experimental methods in the laboratory. Bacterial isolation and identification of bacteria were conducted with descriptive method. Chickens were maintained in the field with completely randomized design. There is treatment administration of lactic acid bacteria with a frequency of daily and twice a week and control. Before treatment, bacterial isolates was mixed into the drinking water of broiler given during maintenance, which is five weeks. At the end of the maintenance, the intestinal organs were taken for examination of broiler population numbers of *Salmonella* sp.

RESULT AND DISCUSSION

Isolation of Lactic Acid Bacteria

TABLE 1. Bacteria identification on normal MRS pH 6.8

Morphology	Sample Code		
	WA.A.1 (<i>Bacillus</i> sp.)	WA.A.2 (<i>Lactobacillus</i> sp.)	WA.A.3 (<i>Lactobacillus plantarum</i>)
Macroscopy			
Colony form	Circle (Facultative Anaerobic)	Circle (Facultative Anaerobic)	Long
Colony color	White milk	White milk	White milk
Perypher	Unflat	Flat	Unflat
Grow time	24 h	24 h	24 h
Microscopy			
Gram (+/-)	+	+	+
Spore	+ terminal	-	-
Form	Bacil	Bacil	Bacil
Catalase test	-	-	-

TABLE 2. Bacteria Identification on MRS acid with pH 4

Morphology	Sample Code		
	WA.B.1 (<i>Lactobacillus delbrueckii</i> spp. <i>delbrueckii</i>)	WA.A.2 (<i>Streptococcus</i> sp.)	WA.A.3 (<i>Bacillus licheniformis</i>)
Macroscopy			
Colony form	Star (Facultative Anaerobic)	Circle (Facultative Anaerobic)	Circle
Colony color	White milk	White milk	White milk
Perypher	Unflat	Flat	Flat
Grow time	48 h	48 h	48 h
Microscopy			
Gram (+/-)	+	+	+
Spore	-	-	+
Form	Bacil	Coccus	Bacil
Catalase test	-	-	-

Based on Table 1 and Table 2, the results of the identification carried out using a guidebook *Bergey's Manual of Determinative Bacteriology*. Six samples of chicken intestinal bacterial origin bacterial isolates obtained two approaching the characteristics of the bacteria *Bacillus* sp.; three samples of bacteria approached *Lactobacillus* sp.; one sample bacteria approaching *Streptococcus* sp.

Lactobacillus belongs to Lactic Acid Bacteria (LAB). These bacteria are not pathogenic and safe for the health of host act like a normal flora in the digestive system. Its function to maintain the balance of acids and bases so that a constant pH in the colon. Probiotic bacteria maintain intestinal health, help the absorption of food, produce vitamins and prevent the growth of pathogenic bacteria. Moreover, it can improve the function of the immune system [5].

According to Holt [3], *Bacillus* sp. is Gram-positive and usually motile by peritrichous flagella. Endospores are oval, sometimes round or cylindrical and very resistant to unfavorable conditions. There are no more than a single spore per cell and sporulation cannot stand in the open air. These bacteria are aerobic or facultatively anaerobic. *Bacillus* sp. has diverse physiology and very sensitive to heat, pH and salinity. The bacteria are also chemoorganotroph with fermentation metabolism or respiration. *Bacillus* sp. is usually catalase-positive and oxidase-positive. Many species of *Bacillus* sp. are widespread in habitat, but few species are also pathogenic to vertebrates or invertebrates.

Streptococcus is a genus of Gram-positive bacteria, which owns a round of the phylum Firmicutes. *Streptococcus* is a group of lactic acid bacteria. These bacteria grow in the form of a chain or pairs. The name *Streptococcus* comes from Greek, *streptos*, meaning easily bent or twisted, like a chain (twisted chain). *Streptococcus* bacteria are oxidase-positive and catalase-negative. Many of them are facultative anaerobic.

Based on the results, the six types of bacteria found from chicken intestine samples are *Lactobacillus plantarum*, *Lactobacillus delbruckii*, *Bacillus licheniformis*, *Lactobacillus* sp., *Bacillus* sp. and *Streptococcus* sp. Three genus of them are probiotic bacteria, consisting *Lactobacillus* sp., *Bacillus* sp. and *Streptococcus* sp. They have potential as a profitable and viable association as normal flora in the organism both inside and outside the body.

The Effect of Lactic Acid Bacteria on the Population of *Salmonella* sp.

The data of population of *Salmonella* in broiler chickens are shown in Fig. 1.

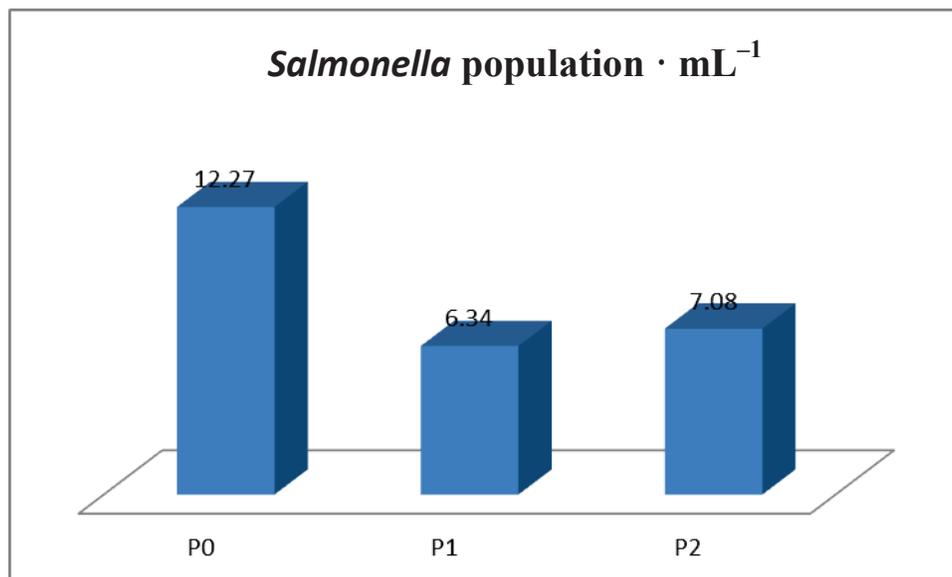


FIGURE 1. Population *Salmonella* sp.; P0 = without probiotics, P1 = probiotic 10⁹ cell · mL⁻¹ (daily), P2 = probiotics 10⁹ cell · mL⁻¹ (twice per week)

Administration of lactic acid bacteria (*Lactobacillus* sp., *Bacillus* sp. and *Streptococcus* sp.) decreased the number of population of *Salmonella* by 48.32 % in the carrying out of probiotics once a day, demonstrating that lactic acid bacteria can inhibit the growth of pathogenic bacteria. Mechanisms of probiotics in reducing microbial pathogens is the process of translocation of probiotics on pathogenic microbes to produce a toxin that inhibits the growth of microbial pathogens. Toxins produced are antibiotics for microbial pathogens. Provision of microbial cultures improves the ecosystem and the population of microflora in the digestive tract [6].

CONCLUSION

There is one beneficial pathogenic microorganism isolated from the chicken intestine and a group of selected lactic acid bacteria for use as probiotics in reducing microbial pathogens *Salmonella* and improving the immune system of chicken. Supplementation of lactic acid bacteria with the frequency of daily results reduces *Salmonella* populations.

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