
ISOLATION OF LACTIC ACID BACTERIA FROM THE INTESTINAL INTESTINAL OF LAYING CHICKENS AS PROBIOTIC CANDIDATES FOR POULTRY

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Abstrak

Penggunaan antibiotik sebagai peningkat pertumbuhan pada ternak telah dilarang oleh pemerintah karena kekhawatiran tentang residu dalam produk unggas dan dampaknya terhadap kesehatan manusia. Larangan ini mendorong pencarian alternatif untuk menjaga unggas tetap sehat dan produktif tanpa antibiotik. Probiotik adalah salah satu alternatif yang menjanjikan untuk antibiotik. Bakteri Asam Laktat (BAL) adalah kelompok probiotik yang dapat menjaga keseimbangan mikroflora usus, meningkatkan daya cerna pakan, menekan mikroba patogen, dan mendukung sistem kekebalan tubuh unggas. Penelitian ini bertujuan untuk mengisolasi BAL dari usus ayam petelur yang dibuang, yaitu bagian jejunum, ileum, dan sekum sebagai kandidat probiotik unggas. Metode isolasi dilakukan dengan pengenceran serial (10⁻¹ - 10⁻⁵) isi usus dan implantasi pada media de Man Rogosa Sharpe Agar (MRSa), diinkubasi secara anaerob selama 48 jam pada suhu 37°C. Isolat yang dikultur diidentifikasi berdasarkan morfologi koloni, pewarnaan Gram, dan uji katalase. Hasil penelitian menunjukkan bahwa isolat yang diperoleh adalah bakteri Gram positif berbentuk batang (basil) dengan uji katalase negatif. Sifat-sifat ini sesuai dengan karakteristik BAL pada unggas, yaitu Gram positif, berbentuk basil, dan katalase negatif. Isolat BAL dari usus ayam petelur berpotensi untuk diaplikasikan sebagai probiotik unggas.

Kata Kunci: Bakteri Asam Laktat, Probiotik, Unggas, Mikroflora Usus.

Abstract

The use of antibiotics as growth boosters in livestock has been banned by the government due to concerns about residues in poultry products and their impact on human health. This ban encourages the search for alternatives to keep poultry healthy and productive without antibiotics. Probiotics are one of the promising alternatives to antibiotics. Lactic Acid Bacteria (BAL) are a group of probiotics that can maintain the balance of the intestinal microflora, improve feed digestibility, suppress pathogenic microbes, and support the immune system of poultry. This study aims to isolate BAL from the intestines of discarded laying hens, namely the jejunum, ileum, and

secum parts as poultry probiotic candidates. The isolation method was carried out by serial dilution (10⁻¹ - 10⁻⁵) of the intestinal contents and implantation in the medium de Man Rogosa Sharpe Agar (MRSa), incubated anaerobic for 48 hours at 37°C. Cultured isolates were identified based on colony morphology, Gram staining, and catalase assays. The results of the study showed that the isolate obtained was a Gram positive bacteria in the form of a rod (bacilli) with a negative catalase test. These properties are in accordance with the characteristics of BAL in poultry, namely Gram-positive, bacilli-shaped, and catalase-negative. BAL isolate from the intestines of laying hens has the potential to be applied as a poultry probiotic.

Keywords: *Lactic Acid Bacteria, Probiotics, Poultry, Intestinal Microflora.*

INTRODUCTION

The rapid development of science and technology has raised public awareness of the importance of health, leading to a more selective approach to the food they consume. Poultry products, such as eggs and meat, are highly sought after by all segments of society. In poultry farming, the government has banned the use of antibiotics to increase livestock productivity due to concerns that these antibiotics could leave residues in poultry products and result in health problems for those who consume them. The use of antibiotics in feed has been prohibited by the Minister of Agriculture Regulation No. 14/PERMETAN/PK.350/5/2017 concerning the Classification of Animal Drugs. This ban on antibiotics necessitates the search for alternatives to ensure chickens continue to grow healthily and productively.

Live microorganisms, known as probiotics, can provide benefits to their hosts by maintaining the balance of microflora in the digestive tract., state that probiotics play a role in stabilizing intestinal microflora, improving feed digestibility, suppressing the growth of pathogenic microbes, and enhancing the immune system of poultry. Lactic acid bacteria (LAB) are microorganisms found in the digestive tract and act as probiotics. state that lactic acid bacteria (LAB) are microorganisms that function as probiotics. LAB, such as *Lactobacillus* spp., *Bifidobacterium* spp., and *Lactococcus* spp., are capable of producing organic acids and bacteriocins with antimicrobial properties, thereby inhibiting the colonization of pathogens such as *Escherichia coli* and *Salmonella* spp. in the digestive tract of poultry.

Lima Puluh Kota is a regency in West Sumatra Province, a center for laying hen farming. According to data from the West Sumatra Provincial Animal Husbandry and Animal Health Service, the population of laying hens in Lima Puluh Kota Regency in 2024 was 6,282,788. Culled

laying hens are those that are no longer producing eggs, and farmers typically sell them for meat consumption. Chicken intestines are waste from slaughterhouses because they have no economic value.

The intestine is one of the digestive organs in chickens that plays a role in the breakdown and absorption of food. Intestinal activity contains microorganisms, including bacteria, that provide benefits to livestock health. Research by [6], who isolated bacteria from the intestines of broiler chickens after slaughter at Narmada Market in West Lombok Regency, found the lactic acid bacteria *Lactobacillus* sp. These bacteria can be used as probiotics for poultry because they produce antibacterial compounds that effectively inhibit the growth of the pathogenic *E. coli*. stated that *Lactobacillus acidophilus* and *Lactobacillus fermentum* can act as probiotics, following the identification of bacteria in the digestive tract of native chickens raised on peatland.

This study isolated lactic acid bacteria from the intestines of culled laying hens as an effort to utilize waste that has the potential to act as a probiotic for poultry. The presence of lactic acid bacteria in the digestive tract of poultry should be utilized as a probiotic feed additive for poultry, as it facilitates the bacteria's adaptation to environmental conditions and the digestive system of poultry.

RESEARCH METHOD

This research was conducted in the Animal Health and Disease Laboratory of the Payakumbuh State Agricultural Polytechnic, using the digestive tract of discarded laying hens, consisting of the jejunum, ileum, and cecum.

The equipment required for this study included: test tubes, Erlenmeyer flasks, measuring cylinders, beakers, Petri dishes, colony counters, anaerobic jars, microscopes, refrigerators, analytical balances, spatulas, pH meters, micropipettes, vortex mixers, cell spreaders, Bunsen burners, hot plates, incubators, ovens, autoclaves, and laminar air flow.

The materials needed for this study were Nutrient agar, deMan Rogosa and Sharpe Agar (MRSA), MRS Broth, alcohol, spirits, aluminum foil, plastic wrapping, cotton, tissue, physiological saline solution, plastic bags, micropipette tips, and distilled water.

Isolation of Lactic Acid Bacteria

The chicken intestines, including the jejunum, ileum, and cecum, were cut into 2 cm lengths. A 1 gram sample was scraped and dissolved in 9 ml of physiological NaCl to obtain a 10⁻¹ dilution. Homogenization was then performed using a vortex to obtain 100 µL, and 900 µL of physiological NaCl was added to obtain a 10⁻² dilution. Serial dilutions were continued until a concentration of 10⁻⁵ was reached. Next, 100 µL of dilutions from 10⁻³ to 10⁻⁵ were transferred to a Petri dish, followed by solid MRS medium, and then shaken to mix the sample solution and medium thoroughly. The sample was then allowed to stand at room temperature and placed in an anaerobic jar containing an anaerobic sachet. Incubation was carried out at 37°C for 2 x 24 hours. The edge shape, shape, color, and elevation of the colonies were observed macroscopically.

Total Bacterial Colonies

The total number of bacterial colonies in the medium can be counted using a colony counter. The calculation is performed by multiplying the average number of colonies by the dilution factor used (cfu/ml). The total colony count must meet the International Commission on Microbiology and Food (ICMF) standard of between 30 and 300 colonies per Petri dish (Pelczar and Chan, 2008).

$$\text{Total bacterial colonies (CFU/mL)} = \text{Number of bacteria} \times \text{1 Dilution factor}$$

Microscopic Observations

Microscopic observations were performed on bacterial cultures that had been incubated for 24 hours for Gram staining. The Gram staining procedure was based on the method of Dwidjoseputro (1989). The slide was first dripped with physiological NaCl, then the bacterial culture was placed on the slide and spread evenly. It was then fixed over a Bunsen burner and stained with crystal violet for 2 minutes. Afterward, it was rinsed with running water, re-sprayed with iodine complex solution, and left for 1 minute. Next, rinse the slide again with running water so the dye can penetrate the bacteria. Use alcohol to drop the slide and wait 15 seconds. Rinse again with water. Next, add safranin dye to the slide and wait 1 minute, then rinse with running water. After adding the oil immersion drops, the slide is dried and examined under a microscope at 100x magnification. Gram-positive bacteria appear purple, indicating their ability to bind crystal violet dye.

Catalase Test

This test is performed by preparing a slide for bacterial examination and then adding 2-3 drops of H_2O_2 reagent to the slide until it covers the surface. Observations are made under a microscope. The presence of gas bubbles indicates a positive result, and vice versa.

Data Analysis

The results obtained are then analyzed descriptively, with the research results presented in tables and figures.

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria from the intestines of discarded laying hens

Lactic acid bacteria were obtained from the jejunum, ileum, and cecum of chicken intestines. Isolation began with sequential dilutions of the jejunum, ileum, and cecum. The resulting dilutions, from 10^{-1} to 10^{-5} , were grown on MRSA (De Mann Ragosa-Sharpe Agar) using the pour-over method and then incubated in an anaerobic jar for 48 hours at $37^\circ C$. [8] stated that MRSA is a suitable medium for growing, multiplying, and isolating lactic acid bacteria. After incubation, separate colonies were observed, producing clear zones around the isolates from the jejunum, ileum, and cecum at 10^{-5} dilutions. This can be seen in Figures 1A, 1B, and 1C.

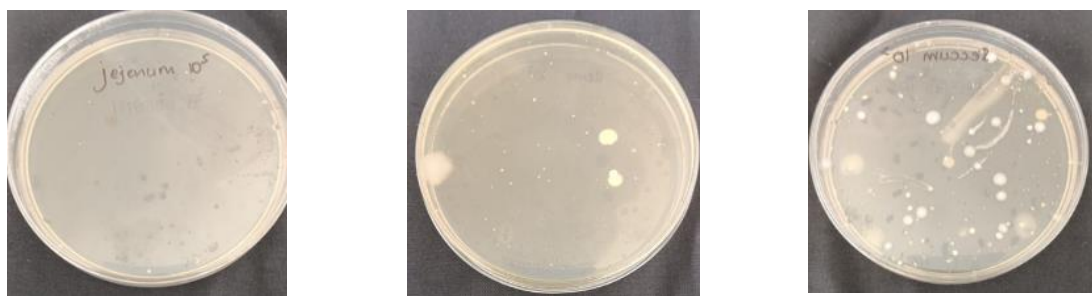


Figure 1. 1A. LAB isolate from jejunum 1B. LAB isolate from ileum 1C. LAB isolate from cecum

Figures 1A, 1B, and 1C show the isolates obtained from the 10^{-5} dilution. This is because lactic acid bacteria colonies have grown separately and are more clearly visible and can be counted compared to the 10^{-3} dilution. Figures 1A, 1B, and 1C show the presence of lactic acid bacteria. *Lactobacillus*, the dominant genus in the intestine, plays a role in promoting health for LAB due to

its contribution to the stability of the gut microbiota. Figure 1C, an isolate from the cecum, has a denser colony than isolates from the jejunum and ileum of laying hens. Table 1 shows the total number of bacterial colonies in the cecum was 146×10^5 , while in the jejunum and ileum, respectively, 101×10^5 and 94×10^5 . Research results indicate that lactic acid bacteria are predominantly found in the chicken small intestine, particularly in the jejunum and ileum, but are more abundant in the cecum. Bacterial densities can be significantly higher in the large intestine than in the small intestine, including the cecum, emphasizing its role as a nutrient-rich habitat for LAB. In the ceca, LAB populations significantly increase and dominate the microbiota, comprising over 95% of the specific microbial community, primarily from the phylum Firmicutes, including *Lactobacillus* and *Clostridium* species. Differences in LAB populations in these intestinal regions may also be related to pH and nutrient availability. The cecum operates at a lower pH due to carbohydrate fermentation, creating a favorable environment for LAB proliferation.

Characteristics of Lactic Acid Bacteria Isolates

The characteristics of lactic acid bacteria isolates can be observed macroscopically, with the naked eye, or under a microscope. In this section, the morphology of the bacterial isolates examined includes colony shape, colony color, margin shape, and elevation. Isolate characteristics were also measured microscopically using Gram staining and a catalase test using H₂O₂. The characteristics of lactic acid bacteria isolates from the intestines of discarded laying hens are shown in Table 1.

Table 1. Characterization Results of Isolates and Total Lactic Acid Bacteria Colonies from the Intestines of Culled Laying Hens

Characterization	Isolate Origin		
	Jejunum	ileum	Cecum
Morphology			
Elevation	Convex	Convex	Convex
Colony Shape	Round	Round	Round
Edge Shape	Average	Average	Average
Colony Color	White	White	White
Gram Staining	White	White	White
Microscopic Shape	Bacillus	Bacillus	Bacillus
Catalase Test	Negative	Negative	Negative
Total Bacterial Colonies	94×10^5	101×10^5	146×10^5



Figure 2. Bacterial Gram Staining Results (2A. LAB Isolate from Jejunum 2B. LAB Isolate from Ileum
2C. LAB Isolates from the Cecum

Based on the morphological characterization of lactic acid bacteria isolates from the intestines of discarded laying hens, lactic acid isolates from the jejunum, ileum, and cecum showed elevations in the three isolates, with convex colonies, round colonies, even edges, and white colonies. The next step after bacterial morphology testing was Gram staining to classify the bacteria as gram-positive or gram-negative. In this study, the Gram stain results for isolates from the jejunum, ileum, and cecum showed rod-shaped/bacillus-like bacteria, as seen under a microscope at 100x magnification. The Gram stain results for isolates from the jejunum, ileum, and cecum showed a purple color. Gram staining of lactic acid bacteria (LAB) is an important technique in characterizing these microorganisms. Lactic acid bacteria are generally classified as Gram-positive, meaning they have thick cell walls rich in peptidoglycan. In Gram staining, LAB shows a positive reaction by retaining the purple color of crystal violet, distinguishing it from Gram-negative bacteria which will appear red due to safranin staining. Gram-negative bacteria have thin cell walls that are unable to retain crystal violet and safranin dyes after washing with 95% alcohol, resulting in a red Gram stain. Meanwhile, Gram-positive bacteria have thick cells and contain peptidoglycan, thus retaining the blue-purple crystal violet dye. Lactic acid bacteria (LAB) colonies show variations in morphology, shape, and color that can be evaluated microscopically. Typically, LAB colonies are rod-shaped (bacillus) and small to medium in size. Observations using Gram staining often indicate that LAB are Gram-positive, which is characterized by deep purple staining under the microscope. Colony color can vary from white to cream or even yellowish, depending on the growth medium and substrate used during the fermentation process.

Based on the catalase test, isolates from the jejunum, ileum, and cecum showed negative results, indicated by the absence of air bubbles in bacteria dripped with H₂O₂. The catalase test is a biochemical method used to identify bacteria based on their ability to produce the catalase

enzyme, which functions to convert hydrogen peroxide (H₂O₂) into water and oxygen. The catalase test results on bacterial isolates from the jejunum, ileum, and cecum were negative, indicated by the absence of air bubbles, thus proving that the organism was a lactic acid bacteria. Lactic acid bacteria (LAB) are known as catalase-negative bacteria, which do not produce gas bubbles when tested with H₂O₂, as in the results of research [18] where isolates from broiler chickens showed positive results for Gram-positive properties and negative in the catalase test. The morphological characteristics of LAB are usually rod-shaped (bacilli) and catalase-negative.

CONCLUSION

Based on the results of research on the jejunum, ileum, and cecum of discarded laying hens, lactic acid bacteria were successfully isolated. The total number of bacterial colonies was found in the cecum. The isolates showed Gram-positive bacilli and catalase-negative characteristics, thus possessing LAB characteristics. LAB isolates from the intestines of discarded laying hens have the potential to be developed as poultry probiotics, thus becoming an alternative to antibiotics to improve livestock health and productivity.

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