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Isolation of Cellulolytic Bacteria from Kalkun (*Meleagris gallopavo*) Gastro-Intestinal Tract as a Candidate Probiotics for Poultry

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Abstract: This study has been conducted to isolate and identify cellulolytic bacteria from the digestive tract of turkeys (Meleagris gallopavo) as probiotic candidates for poultry. This study has obtained 4 bacteria that have the potential as probiotic candidates through the morphological identification stage, the ability to produce cellulase enzymes and resistance to acidic pH conditions. The test found that RDL code bacteria have the highest ability to produce enzyme cellulase characterized by the formation of a clearing zone with a diameter of 11 mm, the second highest PVB2 code bacteria with a clearing zone diameter of 7 mm, DB1 4 mm, and RSL 2 mm and the results of bacterial viability tests isolated at low pH (2.0) showed the highest viability after incubating for 4 hours. RDL viability is 65%, RSL 48%, DB1 50%, and PVb2 29%. So based on the tests that have been carried out, it is estimated that the 4 bacteria are candidates for Lactobacillus sp. worthy to be used as a probiotic for poultry.

Keywords: Bactery; Cellulolytic; Cellulase; Probiotics.

Introduction

The poultry farming sub-sector is one of the sectors that is able to supply 65% of animal protein and is able to reduce unemployment by 10% (Ferlito & Respatiadi, 2018). Poultry farming is one of the promising businesses for the community in the success of one of the government's programs, namely opening up a wide range of job opportunities. This is evidenced by the increase in animal protein consumption of the community by 6.69 g / capita / year in 2016 increased to 7.52 g / capita / year in 2020 which means an increase of 12.4% (BPS, 2020).

Poultry rearing, which is one of the main determinants of success, is feed. Feed is one of the important factors that determine productivity, growth and survival. The high price of factory feed is one of the obstacles for farmers. So that the use of agricultural waste such as bran is used as an alternative to reduce feed costs. However, the use of agricultural waste has obstacles because it has a high fiber content causing the digestive tract of monogastric livestock does not have the ability to digest optimally.

The use of cellulolytic bacteria used as probiotics is a solution in overcoming high levels of fiber in feed. Cellulolytic bacteria are bacteria that are able to produce cellulase enzymes that can hydrolyze fiber levels in feed enzymatically. Some genus of bacteria that have cellulolytic ability are *Achromobacter, Angiococcus, Bacillus, Cellulomonas, Cytophaga, Clostridium, Cellivibrio, Flavobacterium, Pseudomonas, Poliangium, Sorangium, Sporocytophaga, Vibrio, Cellfalcicula, Klebsiella, Citrobacter, Serratia, Enterobacter, Aeromonas* and *Lactic acid bacteria* (LAB) (Herdian et al., 2018).

Cellulase enzyme is an enzyme that can hydrolyze β-1,4-glycosidic bonds in cellulose molecules to produce

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glucose (Afsahi et al., 2007). This enzyme is a protein consisting of 434 amino acid residues with several active sides located in several parts of the protein chain including glutamate 212, aspartate 214, glutamate 217, histidine 228, glutamate 295. Cellulase contains noncatalitics carbohydrate-binding modules (CBM s) located at the N- thermini or C atom end of its catalytic side (Zhang et al., 2006). Cellulase enzymes can be produced from cellulolytic microbes both molds and bacteria. Probiotics are living microorganisms which, when administered in sufficient amounts, confer health benefits to the host (Wang et al., 2022).

Probiotics are live microorganisms belonging mainly to the lactic acid bacteria. They modify the microflora of the host digestive system, especially the intestinal microflora. Prophylactic admin- istration of probiotics in the early stages of life (naturally in breast milk or milk substitute synthetic compounds) is very important because intestinal microflora plays a huge role in the development of the immune system (Zukiewicz-Sobczak et al., 2014).

In addition, the use of probiotics as a substitute for feed affixes such as antibiotic growth promoters (AGPs) is highly recommended. Probiotics have the ability to increase livestock immunity, productivity and feed efficiency. Since 2018, the use of AGPs has been banned in Indonesia, conveyed through Pasal 22 ayat 4c UU No 18/2009 juncto No 41/2014 about the prohibition of the use of AGPs as feed additives that have adverse consequences for the health of livestock and consumers. Because the use of AGPs continuously decreases in poultry can result in the presence of antibiotic residues in the body of livestock and livestock products which causes resistance of livestock and consumers to antibiotics (Afifah et al., 2021).

So, the study aims to isolate cellulolytic bacteria from turkeys (Meleagris gallopavo) as a candidate for poultry probiotics.

Method

This research was conducted by isolating cellulolytic bacteria from the digestive tract of turkeys (Meleagris gallopavo) that were more than 1 year old at the Microbiology and Biotechnology Laboratory, Faculty of Animal Husbandry, University of Mataram. Bacterial isolates that have been single colony obtained are then identified macroscopically and microscopically.

After these observations, biochemical profile tests were then carried out before testing the ability of bacteria to produce cellulase. After these observations, biochemical profile tests were then carried out before testing the ability of bacteria to produce cellulase. Bacterial candidates who have the ability to produce the best enzymes are selected and characterized through viability tests such as testing the ability of live bacteria at pH 2.0 and 7.0 with the aim of knowing the viability of cellulolytic bacteria in acidic media or pH conditions of the digestive tract of poultry (Ehrmann et al. 2002; Bao et al. 2012).

Result and Discussion

Bacterial isolation is the process of transferring bacteria from nature to artificial media with the aim of obtaining a bacterium. Bacterial isolation in this study used solid carboxymethly cellulose (CMC) selective media to obtain cellulolytic bacteria. The bacterium was isolated from the gastrointestinal tract of turkeys (Meleagris gallopavo). The following are the results of isolation of cellulolytic bacteria in Figure 1.

Figure 1. Culture of the mixture taken from a dilution 10^{-4}.

Bacterial isolation has obtained 6 pure bacterial isolates obtained grown in CMC liquid media for the purpose of being stored as further test material. After obtaining pure isolate, then tested the ability to produce cellulase enzymes as shown in figure 2. Cellulase enzyme activity test was performed by taking 20 ul of bacteria (OD600: 0,25± 0,05) dripped over solid CMC medium incubated for 72 hours at 34 °C based on (Nadelman et al., 2020).

The result of cellulase enzyme production tests from 6 isolates found that RDL code bacteria have the highest ability to produce enzyme cellulase characterized by the formation of a clearing zone with a diameter of 11 mm, the second highest PVB2 code bacteria with a clearing zone diameter of 7 mm, DB1 4 mm, and RSL 2 mm. This proves that of the 6 isolates only 4 isolates have the ability to produce cellulase enzymes.

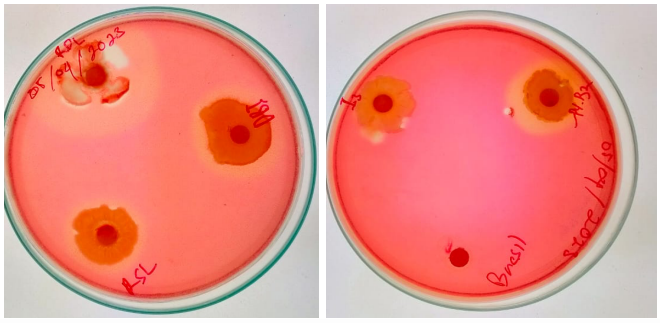

Figure 2. Cellulase enzyme production test

Batubara *et al*., (2021) reported, cellulolytic colonies that produce extracellular cellulase will form a clear zone with a red background as a non-degradable area. The addition of about 0.1% Congo red solution in the cellulose degradation test to detect the clear zone, that can be degraded by cellulolytic bacteria. Congo red 0.1% solution is a dye that will diffuse into the agar medium and was absorbed by long chains of polysaccharides. Long-chain polysaccharides have D-Glucan bonds resulting from the cellulolytic activity. The process of administering 0.1% Congo red solution was carried out when the test bacterial colonies had grown with an incubation period of 48 hours at 37 °C and neutral pH. The ability of bacteria to grow on CMC agar medium showed that these bacteria were able to utilize cellulose as a source of nutrition, especially as a carbon source. The halo zone is an early indication to determine the ability of bacteria to decompose cellulose. Qualitatively, the wider the halo zone formed, the greater the potential for cellulolytic bacteria (Hajiabadi et al., 2020).

Cellulase enzyme is an enzyme that can accelerate the hydrolysis process of feed polysaccharides to produce simpler molecules so that they are easily absorbed by the digestive tract of poultry (Nadelman et al., 2020). The clearing zone arises because bacterial isolates RDL, RSL, DB1 and PVB2 utilize cellulose as the only source of carbon by producing extracellular enzymes that are able to convert cellulase into monomers by breaking β-1,4 glycosidic bonds in CMC media (Raharjo & Isnawati, 2022).

Identification of bacterial morphology

Bacteria identified as capable of producing cellulase enzymes were then morphologically identified. The following are the results of morphology identification in Table 1.

Table 1. The results of morphology identification of probiotic candidate bacteria

Isolate Code	Colony Morphology					Gram	Cell
	Shape	Margin	Surface	Size	Color		
RDL	Round	Appear	Pulvinate	Small	White cream	+	Bassill
RSL	Round	Soft	Convex	Small	White cream	+	Bassill
DB1	Round	Soft	Convex	Small	White cream	+	Bassill
PVB2	Irregular	Jagged	Umbonat	Moderate	White cream	+	Bassill

Based on the results of morphological identification of 4 isolates that were positively identified as capable of producing cellulase enzymes, it was found that RDL bacteria have a round colony shape, appear margin, pulvinate surface, small size and whie cram color, RSL bacteria have a round colony shape, soft margin, convex surface, small size, white cream color, DB1 bacteria have a round colony shape, soft margin, convex surface, small size, white cream color, and PVB2 bacteria have irregular colony shapes, jagged margin, umbonat surface, moderate size, and white cream. All of isolate bacteria is gram positive and has bassill cell.

Bacterial Viability in the pH of the Gastrointestinal Tract

The test of the ability of bacteria to defend themselves in the pH conditions of the digestive tract of chickens is carried out in vivo with the aim of knowing the ability and adjusting to acidic conditions (Wang et al., 2022). Bacterial resistance to the pH conditions of the digestive tract is used as an important indicator as a candidate for probiotics. Bacteria that are resistant to low pH conditions can generally be used as probiotic candidates (Istiqomah et al., 2019).

Tabel 2. The result of pH test conditions of the digestive tract of chicken

Code Bacteria	Viability (Bacteria x 10^7)						pH 702 (%)	pH 200 (%)
	pH 7,2			pH 2,0				
	0 Jam	2 Jam	4 Jam	0 Jam	2 Jam	4 Jam		
RDL	14.50	11.20	10.40	13.92	10.67	9.06	71	65
RSL	17.90	14.50	11.00	16.70	14.45	8.09	62	48
DB1	13.30	11.20	9.00	11.70	9.15	6.07	68	50
PVB2	8.60	7.00	3.20	8.20	5.20	2.40	36	29

The results of bacterial viability tests isolated at low pH (2.0) showed the highest viability after incubating for 4 hours. RDL viability is 65%, RSL 48%, DB1 50%, and PVB2 29%. The bacterial isolate tested is suspected to be

lactic acid bacteria (BAL) because it grows well with high viability at low pH. Bacteria that do not produce lactic acid will experience a significant population decline. Acidity (pH) 2.5 to pH 4 is the optimal pH range for the growth of BAL type *Lactobacillus plantarum* (Khurorin, 2006).

Lactobacillus johnsonii and *Lactobacillus gasseri* viability 1-10% at pH 1.0 for 2 hours (Aiba et al., 2015). As many as 70% of *Lactobacillus reuteri* bacteria can survive at pH 2.0 for 4 hours, while *Lactobacillus salivarius* only 0.8% can survive at pH 2.0 for 4 hours (Ehrmann et al., 2002).

Wasis et al., (2019) also stated that 59.18% of BAL isolated from sauerkraut ground bamboo shoots were able to survive at pH 2.0 for 3 hours. Based on observations, bacterial viability is higher at normal pH than low pH. This indicates that microbial growth is affected by pH.

The ability of bacteria to grow at low pH is one of the criteria for these bacteria to be used as probiotics (Istiqomah et al., 2019) . Some previous studies revealed that probiotics from lactic acid bacteria are able to withstand acidic atmospheres such as Lactobacillus vebris, *Lactobacillus plantarum* and *Pediococcus cerevisiae* because these bacteria are able to remodel complex compounds into simpler compounds with lactic acid end results (Kurnia et al., 2020). Touw (2014) states that the most tolerant BAL at low pH exposure *is Lactobacillus fermentum* S21209, BAL with medium tolerance is *Lactobacillus plantarum* W22409 and *Pediococcus pentosaceus* W2SR.

After analysis using the Bergey's Manual of Determinative Bacteriology method (Holt, 1994), it was suspected that the candidate type of Lactobacillus sp. The candidate bacteria obtained are worthy to be used as probiotics.

Conclusion

The isolation of cellulolytic bacteria from the turkey (*Meleagris gallopavo*) gastro-intestinal tract obtained 4 bacteria (RDL, RSL, DB1, and PVB2) that have the potential to be used as probiotic candidates for poultry and are worthy of further testing sequencing using the 16S rRNA gene with the aim of knowing the species of each bacterium obtained.

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The conceptualization and investigation.; Muh. Aidil Fitriyan Fadjar Suryadi. Methodology and data curation.; Muh. Aidil Fitriyan Fadjar Suryadi. Writing-review and editing.; Muh. Aidil Fitriyan Fadjar Suryadi, Muhammad Hipzul Mursyid and Khairil Anwar, supervision.; Muhamad Ali, and Djoko Kisworo.

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Conflicts of Interest
The authors declare no conflict of interest.

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