



# Comparison of Extracellularase-producing *Bacillus* Isolated from the Digestive Tract of Muscovy Ducks (*Cairina Moschata*) and Native Chicken (*Gallus Gallus Domesticus*) as Probiotic Candidates for Poultry

Zaid Al Gifari<sup>1</sup>, Khairunnisah<sup>2</sup>, Fatimah Azh Zhahro Bagis<sup>5</sup>, Fitri Andriani<sup>5</sup>, Khairil Anwar<sup>5</sup>, Muhammad Aidil Fitriyan Fadjar Suryadi<sup>2</sup>, Alimuddin<sup>3,5</sup>, Muhamad Amin<sup>4</sup>, Anwar Rosyidi<sup>5</sup>, I Wayan Wariata<sup>5</sup>, Made Sriasih<sup>5</sup>, and Muhamad Ali<sup>5,\*</sup>

<sup>1</sup> Laboratory of Animal Production, Faculty of Animal Sciences, University of Mataram, Mataram 83125, Indonesia

<sup>2</sup> Graduate Program of Animal Resources, Faculty of Animal Sciences, University of Mataram, Mataram 83125, Indonesia

<sup>3</sup> Faculty of Animal Sciences, University of Nahdlatul Wathan, Mataram 82137, Indonesia

<sup>4</sup> Fish Nutrition Research Group, Department of Aquaculture, Faculty of Fisheries and Marine, Airlangga University, Surabaya 60115, Indonesia

<sup>5</sup> Laboratory of Biotechnology and Animal Product Processing, Faculty of Animal Sciences, University of Mataram, Mataram 83125, Indonesia

\*m\_ali@unram.ac.id

**Abstract.** Probiotics have widely been used as an alternative feed supplement in poultry, contributing to enhanced productivity and the maintenance of livestock health. This study aimed to compare several extracellular cellulase-producing *Bacillus spp.* isolated from the digestive tract of muscovy ducks (*Cairina moschata*) and native chickens (*Gallus gallus domesticus*). A total of seven isolates were selected based on extracellular cellulase activity detected on carboxymethyl cellulose (CMC)-agar plates. Morphological, biochemical and molecular analyses were sequentially conducted to identify bacterial species. Three isolates exhibiting the best extracellular cellulase activity obtained from Muscovy Ducks (*Cairina moschata*) and native chickens (*Gallus gallus domesticus*) were selected and identified through 16S rRNA gene sequencing. The results showed that the A1 isolate was identified as *Bacillus cereus* strain SA275C, the A4 isolate as *Bacillus cereus* strain S8, and the A6 isolate as *Bacillus subtilis* strain IAM 12118. The three selected isolates showed different characteristics, including viability to digestive tract pH and antibacterial activity resulting in inhibition zones in isolates A1 and A4 of  $\pm 0.5$  mm, while isolate A6 was 1 mm against *Staphylococcus aureus*. In conclusion, the three selected isolates with the highest extracellular cellulase activity have potential as probiotic candidates for poultry.

**Keywords:** poultry, probiotics, Extracellular cellulase, *Bacillus sp.*, 16S rRNA gene sequencing

## 1 Introduction

Poultry is one of the livestock producing sources of animal protein that is very popular with the people of Indonesia because of its affordable price compared to meat from other livestock. Increasing the productivity of poultry can be done by improving the quality of livestock nutrition and maintaining livestock health. The provision of probiotics is expected to improve livestock health by manipulating the composition of bacteria in the digestive tract of livestock, as well as stimulating the immune response, so as to increase digestibility [1]. Probiotics are one of the competitive feed additives and exclusion agents that should be developed for disease control and increased livestock production.

The utilisation of cellulolytic bacteria as probiotics can help the degradation of substrates/feed sources that contain high levels of cellulose. These bacteria produce cellulase enzymes extracellularly, which are synthesised in the cell and moved out of the cell through transport pathways such as Sec or Tat. This process is called extracellular cellulase because the enzymes are expelled from the cell into the extracellular space, where they perform their function in substrate breakdown and help the bacteria obtain nutrients from the surrounding environment. These cellulase enzymes then hydrolyse cellulose into simpler glucose or oligosaccharides, helping the bacteria obtain nutrients from the surrounding environment and adapt to a fibre-rich environment. This can improve feed quality [2]. *Bacillus sp.* as a probiotic is able to produce antimicrobials such as bacteriocins, has immunity, and is able to compete with pathogenic bacteria in the adhesion process. Previous research [3, 4] related to the use of *Bacillus sp.* as a probiotic for chicks, was able to suppress the growth of *Escherichia coli*, *Salmonella enteritidis*, and *Clostridium perfringens*.

Superior probiotics for poultry can be isolated from the digestive tract of poultry [5]. Microbial strains isolated from the same type of animal as their intended use will make it easier for microbes to colonise the gut because they are in accordance with the initial ecosystem. This study aimed to compare the ability of *Bacillus sp.* isolated from two different sources, namely muscovy ducks (*Cairina moschata*) and native chickens (*Gallus gallus domesticus*). *Bacillus sp.* isolates with the best ability were sequenced to determine their species and potential to be used as probiotics.

## 2 Materials and Methods

### 2.1 Tools and Materials

This study used test tube, Erlenmeyer, beaker glass, petri dish, measuring cup, analytical balance, micro pipette, volume pipette, micro tip, pH meter, 200 µL micro tip, 20 µL micro tip, 10 µL micro tip, 2 µL micro tip, vortex, hot plate stirrer, autoclave, oven, glass object, cover glass, bunsen lamp, ose needle, spreader, laminar air flow, microscope, incubator, centrifuge, spectrophotometry, microcentrifuge tube 1.5 mL, microtube 0.5mL, PCR tube 0.2 mL, PCR machine, electroporator, UV transilluminator and freezer -80<sup>0</sup> C.

The materials used were bacterial isolates isolated from the digestive tract of ducks and bacterial isolates isolated from the digestive tract of native chickens sourced from the proventriculus and cecum, LB (luria bertani) liquid media, LB (luria bertani) solid, sterile distilled water, clean water, cotton, gauze, aluminium foil, label paper, plastic wrap, 70% alcohol, gram paint (carbol gentian violet, lugol, ethanol, fuchsin water or safranin) immersion oil, catalase test (3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)), SIM (sulfideindole motility) media, cellulase test media (NaCl, yeast, tripton, agar, CMC, red congo), paper discs, glycerol, FavorPrep brand DNA extraction kit™ Tissue Genomic DNA Extraction Mini Kit, Gotaq® Green brand PCR kit, one pair of 16S rRNA primers, 1x TAE buffer, DNA loading dye and 1 kb DNA ladder (marker).

## 2.2 Isolation, Identification, Characterisation

This study began with the isolation of bacteria from the digestive tract of muscovy ducks (*Cairina moschata*) and native chickens (*Gallus gallus domesticus*) then carried out several examinations in sequence, namely examination of colony morphology, gram painting.

## 2.3 Qualitative Test of Cellulolytic Bacteria

The ability of cellulolytic bacteria was tested by looking at cellulase enzyme activity on media containing carboxymethyl cellulose (CMC) [6]. Bacterial isolates as much as 20 µl were suspended on a paper disc that had been placed on the surface of the media. Furthermore, the media was incubated for 24 hours at a temperature of 350 C. After the incubation process, the cellulose testing media was stained using 0.1% red congo solution for 15 minutes and then rinsed with 1 M NaCl solution. Observations were performed by measuring the clear zone on the surface of CMC-agar plates indicating the presence of cellulase enzyme activity [7].

## 2.4 Molecular Identification

PCR technique was performed for 16S rRNA gene amplification with universal primers designed for V1-V5 region. Primer 27F (forward: 5'-AGAGTTTGATCCTGGCTCAG-3') and primer 907R (reverse: 5'-CTTGTGCGGGCCCGTCAATTC-3') were used. These PCR primers can amplify 16S rRNA with an amplicon size of 931 bp containing 50-65% GC content. DNA sequencing results were edited using BioEdit Sequence Alignment Editor 2013 software to produce consensus sequences. BLAST-N (Basic Local Alignment Search Tool - Nucleotide) on NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) as a place to process the data of 16S rRNA gene consensus sequence results were collected and aligned with the 16S rRNA database on the website. The data obtained were tabulated using Microsoft Excel and presented in descriptive form.

## 2.5 Physiological Test

1. **Catalase test**, the catalase test is carried out by dripping 3% hydrogen peroxide ( $H_2O_2$ ) on the bacterial isolate that has been dripped on the preparation glass. Observations are made by looking at the changes that occur whether there are air bubbles or not.
2. SIM (sulfide, indole and motility) media test. Each bacterial isolate was taken using an ose needle and then inserted on SIM media, then incubated at  $37^0$  C for 24 hours. Observations were made by looking at the production of  $H_2$  S gas characterised by the presence of a black precipitate at the bottom of the tube due to the reaction between  $H_2S$  with Ferrous ammonium sulphate. Indole formation can be observed by dripping a solution of kovacs reagent and observing the formation of a red colour ring which indicates indole positivity. Bacterial motility is indicated by the growth of bacteria outside the ose puncture marks or the spread of bacterial growth in SIM media.
3. Sugar fermentation test (glucose, arabinose, sucrose, maltose, lactose and ramnose). Each bacterial isolate was taken using a previously heated ose needle and inserted into a test tube containing sugar media, and incubated at  $37^0$  C for 24 hours. Observations were made by looking at the colour change from the original colour of the media, which was purple, to yellow and brown. Durham tube was inserted into the sugar media to determine the formation of gas from the fermentation of sugar.

## 2.6 Test for Bacterial Viability in The Digestive Tract

A total of 1 ml of bacterial suspension was put into 5 ml of LB media at pH 7.0 and pH 2.0 (pH adjustment using 2 N HCl and 1 N NaOH solution) [8]. Then incubate at  $37^0$  C for 0 hours and 3 hours. The number of bacterial cells was counted using the Total plate count (TPC) method on PCA (plate count agar) media.

## 2.7 Antagonistic or Antimicrobial Test

The media used for the antagonistic test is MHA (Mueller Hinton Agar) media. The control bacteria used were *S. aureus* pathogenic bacteria culture. A total of 100  $\mu$ l of pathogenic bacteria suspension (0.5 McFarland) was levelled using a sprider into the MHA media. After that, a paper disc was attached to the MHA media that had been suspended with pathogenic bacteria. A total of 25  $\mu$ l of *Bacillus* bacteria suspension was dripped on the paper disc, then incubated for 6-24 hours at  $37^0$  C. Bacterial activity was characterised by the presence of a clear zone around the bacterial colonies and then each was measured at the diameter of the resulting clear zone.

### 3 Results and Discussion

The term ‘probiotic’ is derived from two Greek words, ‘pro’ meaning ‘for’ and ‘bios’ meaning ‘life’, thus meaning ‘for life’. The first concept of probiotics was most likely introduced in 1907 by Mechnikov [9], who observed that bacteria can have a positive impact on the natural microflora in the gut. Probiotics are live microorganisms that, when administered in sufficient amounts, can provide health benefits to their host [10]. Probiotics have been widely used to prevent and treat various diseases in humans [11] as well as in animals [12] by enhancing their immune response [13].

#### 3.1 Isolation and Identification of Bacteria

Morphological identification of bacteria in the digestive tract of Muscovy Ducks and Native Chicken consists of 2 observations, namely macroscopic and microscopic observations. Macroscopic observations included colony shape, margin, size, surface and colour. A total of 7 pure isolates were obtained from the digestive tract of Muscovy Ducks and Native Chicken, consisting of 5 isolates from the digestive tract of Muscovy ducks and 2 isolates from the digestive tract of Native Chicken. The following presents the results of morphological identification of bacteria in Table 1.

Morphology based on colony colour, the isolate is milky white. The colony shape of the seven bacterial isolates has the same shape which is round. Microscopic identification is done by Gram staining. The purpose of Gram staining is to distinguish bacteria based on Gram properties. *Bacillus* group bacteria are bacteria with Gram-positive properties characterised by purple cells. Based on the results of Gram staining, all test isolates are Gram- positive bacteria in the form of rods (*bacils*). *Bacillus sp.* bacteria have morphological characteristics with rod- shaped bacterial cells and are gram-positive (purple in colour) [14].

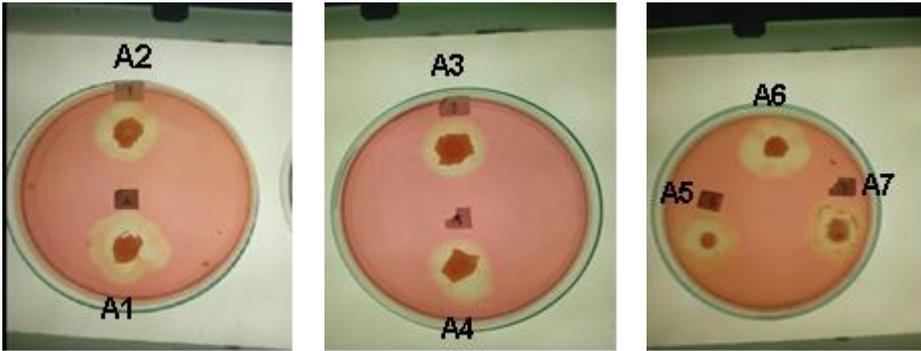
**Table 1.** Morphological identification results of the digestive tract of Egret and Kampung Chicken

No.	Code	Colony Morphology				Cell Morphology		
		Shape	Margin	Size	Surface	Colour	Gram	Shape
1	A1	Round	Serrated	Medium	Umbonate	Creamy white	+	Trunk
2	A2	Round	Smooth	Medium	Convex	Creamy white	+	Trunk
3	A3	Round	Smooth	Small	Raised	Creamy white	+	Trunk
4	A4	Round	Smooth	Medium	Convex	Creamy white	+	Trunk
5	A5	Round	Smooth	Medium	Raised	Creamy white	+	Trunk
6	A6	Round	Embossed	Medium	Raised	Creamy white	+	Trunk
7	A7	Round	Smooth	Medium	Umbonate	Creamy white	+	Trunk

Description: A1; A2; A3; A4 Entok (Proventriculus), A5; Entok (Cecum), A6; Ayam Kampung (Cecum), A7; Ayam Kampung (Cecum).

### 3.2 Cellulase Enzyme Activity Test

Some bacteria that belong to cellulolytic bacteria include; *Bacillus subtilis*, *Pseudomonas diminuta*, *Micrococcus luteus*, and *Plesiomonas shigelloides* [15]. The results of isloat test in producing cellulase enzyme can be seen in **Figure 1**.



**Fig. 1.** Cellulase enzyme activity test results, A1; A2; A3; A4 Entok (proventriculus), A5; Entok (cecum), A6; Ayam Kampung (cecum) and A7; Ayam Kampung (cecum).

The test results showed that all bacterial isolates produced cellulase enzymes as indicated by the formation of a clear zone around the disc after adding 0.1% red congo solution. According to [16], the presence of cellulase enzyme-producing bacteria in the digestive tract of egrets is most likely influenced by the high frequency of consuming feed containing fibre. The results of the measurement of the clear zone (Table 2) after being stained with red congo and have different clear zone sizes. The clear zone formed indicates that the isolate is able to produce extracellular enzymes [17].

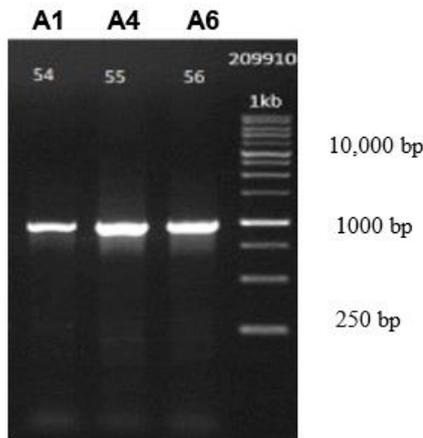
**Table 2.** Results of extracellular enzyme activity of *Bacillus sp.*

No.	Isolate Code	Area of clear zone
1	A1	7 mm
2	A2	4 mm
3	A3	3 mm
4	A4	6 mm
5	A5	5 mm
6	A6	8 mm
7	A7	4 mm

### 3.3 Molecular Identification

A total of 3 *Bacillus* sp. bacterial isolates with the ability to produce the best extracellular cellulase enzyme (A1, A4, and A6) were rejuvenated for molecular identification. Amplification of 16S rRNA gene was performed using PCR technique with primer 27F (forward: 5'-AGAGTTTGATCCTGGCTCAG-3') and primer 907R (reverse: 5'-CTTGTGCGGGCCCGTCAATTC-3'). The results of PCR amplification of the 16S rRNA gene of *Bacillus* sp. bacterial isolates have been visualised by performing electrophoresis, with amplicons of  $\pm 931$  DNA bands observed as presented in **Figure 2**.

Based on base sequence alignment analysis of the 16S rRNA gene sequence available at GenBank NCBI using the BLAST-N (Basic Local Alignment Search Tool-Nucleotide) programme. Bacterial isolates derived from the digestive tract of ducks have DNA similarities (Table 3), namely isolate A1 with *Bacillus cereus* strain SA275C1, isolate A4 with *Bacillus cereus* strain S8 and isolates derived from the digestive tract of native chickens isolate A6 has similarities with *Bacillus subtilis* strain IAM 12118.



**Fig. 2.** PCR results of bacterial rRNA genes of probiotic candidate isolates visualised by electrophoresis with 0.8% agarose gel. 1 kb: maeker, 1: isolates A1, A4 and A6.

**Table 3.** Comparison of 16S rRNA gene sequences with GenBank database

Isolat Code	Strain Name	Acc. No.	Query Coverage	Max. Identity
A1	<i>Bacillus cereus</i> strain SA275C1	ON795198.1	100 %	100.00%
A4	<i>Bacillus cereus</i> strain S8	MT611946.1	100 %	100.00%
A6	<i>Bacillus subtilis</i> strain IAM 12118	NR_112116.2	100%	100.00%

The 16S rRNA gene sequence results showed that the maximum identity value of all isolates from the digestive tract of muscovy ducks (*Cairina moschata*) and native

chickens (*Gallus gallus domesticus*) had the same value of 100%. Isolates that have 16S rRNA sequence similarities of more than 97% can be said to represent up to the same species level, 16S rRNA sequence similarities between 93-97% can represent up to the same genus level [18]. If below 93%, it is likely that the species is not the same and the sequence of nitrogenous bases has not been registered in Genbank.

One of the *Bacillus* genus that has potential as a probiotic is *Bacillus cereus*, because it can inhibit several pathogenic bacteria such as *Salmonella* and *Campylobacter* in the digestive tract and is also a heterotrophic bacterium that can degrade toxic organic matter in the environment [19, 20, 21]. *Bacillus cereus* is a probiotic bacterium that is still rarely applied as a useful product in biotechnology. In addition, [22] used probiotic *Bacillus subtilis*, and it was shown to have positive effects on productivity, stimulate immunity, and increase antioxidant capacity in poultry rearing. The absorption of enzymes produced by *Bacillus subtilis* bacteria affects egg quality, especially increasing the viscosity of the yolk, thereby increasing the yolk index value [23].

### 3.4 Physiological Identification

Isolate A1 (*Bacillus cereus* strain SA275C1), isolate A4 (*Bacillus cereus* strain S8) and A6 (*Bacillus subtilis* strain IAM 12118) were tested physiologically. Catalase test results showed all isolate codes produced positive catalase, and SIM test results showed negative indole (-), positive motility (+), negative H<sub>2</sub> S production (-). Spore staining results show that all are positive or have spores which are the main characteristics of *Bacillus* sp. Endospores have the ability to be resistant to chemicals found in nature, resistant to extreme heat, water deprivation, and radiation [24, 25]. The presence of endospores in *Bacillus* sp. then this group of bacteria has the ability to adapt to extreme environments. Further identification to see the ability of bacteria to ferment sugar using 6 types of sugar, namely glucose, sucrose, maltose, arabinose, lactose, and rannose. The data obtained are presented in **Table 4**.

**Table 4.** Biochemical test

Isolate Code	Sugar Fermentation Ability					
	Glucose	Lactose	Sucrose	Ramnose	Maltose	Arabinose
A1	+	-	+	-	+	+
A4	+	-	+	-	+	-
A6	+	-	+	-	+	+

Based on the results obtained, the 3 isolates sourced from the digestive tract of Entok and Kampung Chicken have almost the same ability to ferment sugar. According to [26], explaining in the sugar-sugar fermentation test, only glucose and maltose media experienced acid formation which was marked by a change in the colour of the media from blue to yellow, meaning that these bacteria formed acid from glucose fermentation.

### 3.5 OD (Optical Density) Measurement

In this study, the media used is LB (lysogeny Broth) which is a medium commonly used for bacterial growth [27]. The results of OD (Optical Density) measurements or the growth of extracellular enzyme-producing bacteria are presented in **Table 5**.

**Table 5.** Bacterial growth measurement results (OD)<sub>600</sub>

No.	Isolate Code	Culture Time			
		3 hours	4 hours	5 hours	6 hours
1	A1	0.192	0.249	0.292	0.397
2	A4	0.105	0.204	0.220	0.318
3	A6	0.162	0.282	0.340	0.382

The results of this study indicate that the growth of test bacteria has good growth and varies every hour. Bacterial growth shows that the adaptation time of bacteria is relatively short to grow in the first 3 hours, in accordance with the opinion of Yuliana [28] that the short adaptation phase in bacteria is due to the same media at the time of refreshment, causing a short time of adjustment to the new environment. At a culture time of 3 hours to 4 hours, bacterial growth has entered the exponential phase which shows constant growth in bacterial cells.

### 3.6 Bacterial Activity at pH Conditions of the Gastrointestinal Tract

The resistance of probiotic bacteria to the pH conditions of the poultry digestive tract is an important indicator in the selection of probiotic candidate bacteria. Bacteria that are resistant to low pH are generally suitable for use as probiotics as long as they are not pathogenic and non-toxic. [29] stated that there was no significant difference in the viability of probiotic bacteria tested *in vivo* and *in vitro*. [30] stated that pH conditions and transit time in each digestive organ of poultry are quite varied including the cache (pH 5.5 for 50 minutes), proventriculus (pH 2.5-3.5 for 90 minutes), duodenum (pH 5.6 for 5-8 minutes), jejunum (pH 6.5-7 for 20-30 minutes), and ileum (pH 7-7.5 for 50-70 minutes). According to [31] pH affects the activity of cellulose degrading bacteria, where acidity can inhibit enzyme activity in bacteria. According to [32], the optimum pH of cellulolytic bacteria ranges from 5-8. The number of bacteria in this study was tested using PBS *in vitro* at pH 2.0 and pH 7.0 with incubation times of 0 hours and 3 hours presented in **Table 6**.

**Table 6.** Bacterial counts at pH 7.0 and 2.0 conditions

No.	Code	Bacterial viability (log CFU/ml)	
		pH 7.0 3 hours (10) <sup>-6</sup>	pH 2.0 3 hours (10) <sup>-4</sup>
1	A1	7.96	5.93
4	A4	7.62	5.61
6	A6	7.68	5.51

The results of this study showed that bacterial growth at pH 7.0 and 2.0 showed good growth. The ability of bacteria to survive at extreme pH can be used as a parameter for selecting probiotic bacteria and can be used as a benchmark for determining the dose of probiotics for poultry. Bacterial viability at low pH conditions is influenced by very specific homeostatic conditions. There are three mechanisms in the homeostatic process of bacteria including arginine deiminase (ADI) system, H<sup>+</sup>-ATPase proton pump and glutamate decarboxylase (GAD) system. A different mechanism is shown by probiotics from the genus *Bacillus spp.* which initiates endospore formation to defend its genome from unfavourable conditions. Each bacterial cell will produce one endospore cell which can then germinate to form vegetative cells when environmental conditions improve.

### 3.7 Antagonistic Properties of Bacteria Against Pathogenic Bacteria

Probiotics are defined as microorganisms that are able to modify the composition of bacteria in the digestive tract. Probiotics are live microorganisms that, when present in sufficient quantities, are beneficial for improving host health, especially in the digestive tract. Microbiological antagonism tests utilise microorganisms as test indicators where these microorganisms are used as determinants of the concentration of certain components in complex chemical mixtures.

The formation of inhibition zones in the antagonistic test is due to the content of compounds that are antimicrobial in nature produced by bacteria from the digestive tract of ducks and native chickens. The inhibition zone formed indicates that bacteria from the digestive tract of ducks and native chickens can inhibit the growth of pathogenic bacteria *S. aureus*. This is in accordance [33] that probiotics can provide a balance of microbiota in the digestive tract that has changed due to the entry of pathogens, so that pathogens can be suppressed in population through the influence of antimicrobials present in probiotics.

According to [34] microbial populations can release chemical substances that have bactericidal or bacteriostatic abilities that can affect other bacterial populations. The ability of a bacterium to affect other bacterial populations because this antibacterial agent is able to reduce pH to low so that pathogenic bacteria are difficult to survive. Antimicrobial activity against pathogenic bacteria is presented in **Table 7**.

**Table 7.** Antimicrobial activity test results against pathogenic bacteria

NO	Isolate Code	Antibacterial Test
		<i>S. Aureus</i>
1	A1	± (0.5 mm)
4	A4	± (0.5 mm)
6	A6	+ (1 mm)

This study aims to determine the ability of each isolate in inhibiting pathogenic bacteria. The pathogen used as a control is *S. aureus*. The results showed that the 3 isolates were able to inhibit *S. aureus* bacteria. [35] in his research mentioned that the way antibacterial compounds work against pathogenic bacteria is to disrupt the constituent components of the cell wall so that there is a decrease in cell permeability which causes the loss of cell constituent components and the destruction of genetic material.

[36] reported that the category of antimicrobial inhibition based on its diameter is divided into 3, namely, if the diameter is 0-3 mm the growth inhibition response is weak, according to this study that all isolates have weak inhibition. Antimicrobial *Bacillus* is produced in the stationary phase, which is when it produces secondary metabolism [37]. In this phase physiological changes occur, the number of dead cells increases, and the number of living cells is constant [38]. In this phase, bacteria do not multiply cells due to decreased oxygen levels, depleted nutrients, and produce many antimicrobials [39]. Antimicrobial production is governed by overall regulatory controls that play a role in growth rate and specific regulatory effects on each metabolic pathway, genetic factors, and media composition. *Bacillus sp.* bacteria produce immunity and antimicrobials such as bacteriocins [39]. The ability of *Bacillus* bacteria to inhibit the growth of pathogenic bacteria is also influenced by the cell wall structure of a bacterium.

## 4 Conclusion

This study successfully isolated four bacterial isolates of the genus *Bacillus* from the digestive tract of ducks (*Cairina moschata*) and native chickens (*Gallus gallus domesticus*), with three isolates showing the ability to produce cellulase enzymes, namely A1 (*Bacillus cereus* strain SA275C), A4 (*Bacillus cereus* strain S8), and A6 (*Bacillus subtilis* strain IAM 12118). These three isolates have cellulase activity indicated by different clear zones, namely A1 (7 mm), A4 (6 mm), and A6 (8 mm). Physiological tests showed that all three were positive for catalase, positive motility, and negative for indole and H<sub>2</sub>S production. However, all three isolates showed a weak ability to inhibit the growth of *Staphylococcus aureus*, with inhibition zone diameters of about ±0.5 mm for A1 and A4, and 1 mm for A6. Thus, these *Bacillus* isolates have potential as probiotic candidates for poultry, although their antimicrobial effectiveness is still limited.

**Disclosure of Interests.** The authors have no competing interests to declare that are relevant to the content of this article.

## References

1. Shah, N. P, J. Dairy Science. Vol. 84(4):894-907. (2000).
2. Mudita, I M, Penapisan dan Pemanfaatan Bakteri Lignoselulolitik Cairan Rumen Sapi Bali dan Rayap Sebagai Inokulan dalam Optimalisasi Limbah Pertanian Sebagai Pakan Sapi Bali. (Disertasi. Program Studi Doktor Ilmu Peternakan Fakultas Peternakan Universitas Udayana, Denpasar, 2019).
3. La Ragione, R.M., G. Casula, S.M. Cutting, dan M.J. Woodward. *Dokter hewan*.79:133–142. 31. (2001)
4. La Ragione, RM, dan MJ Woodward, *Dokter hewan*. 94:245–256. (2003)
5. Yulianto, A, B., dan Lokapirnasari, W. P, *The Philippine Journal of Veterinary Medicine*. 55, (1), 73-78. (2018)
6. Rudiansyah D, Rahmawati, dan Rafdinal, *Jurnal Protobiont* Vol. 6 (3). ISSN: 255-262. (2017)
7. Murtianingsih H dan Hazmi M, *Jurnal Agritop* Vol. 15 (2). ISSN: 1693-2877. E ISSN: 2502-0455. (2017)
8. Lin, W. H, C. F. Hwang, L. W. Chen and H.Y. Tsen. *Food Microbiology*, 23: 74-81. (2006)
9. Miecznikow E. *O Naturze Ludzkiej – Zarys Filozofii Optymistycznej* (translation F. Wermiński). Wydawnictwo Biblioteka Naukowa. Warszawa; 1907.
10. Hamilton. Miller, J. *Int J Antimicrob Agents*. 22:360–6. (2003)
11. Dawood MA, Abo-Al-Ela HG, Hasan MT. *Fish Shellfish Immunol*. 97:268–82. (2020)
12. Nayak, S, K. *Fish Shellfish Immunol*. 29:2–14. (2010)
13. Nayak, S, K. *Rev Aquacult*. 13:862–906. (2021)
14. Sumardi, Farisi, S, Ekowati, C. N, Diana, M. S, 2004. *Jurnal Riset Akuakultur*, 14(3), 193-199. (2004)
15. A. Meryandini, W. Widosari, B. Maranatha, T. C. Sunarti, N. Rachmania, and H. Satria, *Jurnal Sains*, 13(1):33–38. (2009)
16. H. Herdian, L. Istiqomah, E. Dharmayanti, A. E. Suryani, A. S. Anggraeni, N. Rosyada and A. Susilowati. *Tropical Animal Science Journal*. 41(3): 200-206. (2018)
17. A. Kusumaningrum, I. B. W. Gunam, dan I. M. M. Wijaya, *Jurnal Rekayasa dan Manajemen Agroindustri* Vol. 7 (2): 243-253. (2019)
18. Dewa, G. A. W. I. D. M. Sukrama, dan I. W. Suardana, *JS*. 33 (2): 228-233 (2015)
19. A. H. Nainggolan, Uji metabolit Bakteri Heterotrofik dari Muara Sungai Siak Terhadap Bakteri Patogen. (Skripsi. Pekanbaru. Fakultas Perikanan dan Kelautan Universitas Riau, 2019)
20. I. A. Siregar, I. A. B (Skripsi. Pekanbaru. Fakultas Perikanan dan Kelautan Universitas Riau, 2019)
21. B. Vilà, A. Fontgibell, I. Badiola, E. E. Garcia, G. Jiménez, M. Castillo, and J. Brufau, *Poultry Science*, 88(55): 975-979. (2009)
22. K. W. Lee, G. Li, H. S. Lillehoj, S. H. Lee, S. I. Jang, U. S. Babu, E. P. Lillehoj, A. P. Neumann, G. R. Siragusa, *Research in Veterinary Science*, 91(3), 87-91.
23. I. P. Kopian, *Jurnal pengembangan inovasi pertanian*, 2(3),177-191. (2009)
24. R. P. Astuti RP, *Rhizobacteria Bacillus sp. asal tanah rizosfer kedelai yang berpotensi memicu pertumbuhan tanaman* (Tesis. Sekolah Pascasarjana IPB. Bogor, 2008).

25. S. T. Pratiwi, Mikrobiologi Farmasi (Penerbit Airlangga, Jakarta, 2008)
26. Fardiaz, Analisis Mikrobiologi Pangan (PT. Raja Grafindo Persada, Jakarta, 1993)
27. R. Michael, The Journal of heredity. 98. 97–98. (2007)
28. Yuliana, Jurnal Teknologi Industri dan Hasil Pertanian.73:2. (2008)
29. G. Priadi, F. Setiyoningrum, F. Afiati, R. Irzaldi, and P. Lisdiyanti, Teknol. dan Industri Pangan Vol . 31(1): 21-28 (2020)
30. L. S. Surono, Probiotik Susu Fermentasi dan Kesehatan, (Tri Cipta Karya, Jakarta, 2004)
31. E. Khairiah, S. Khotimah, and A. Mulyadi, Jurnal Protobiont Vol. 2 (2): 87-95. (2013)
32. D. Wahyuni, S. Khotimah, and R. Linda, 2015. Jurnal Protobiont, Vol. 4 (1): 69-76. (2015)
33. H. Ritonga, Majalah Ayam dan Telur. 73: 24 – 26. (1992)
34. L. Verschuere, G. Rombaut, P. Sorgeloos and W. Verstraete. Mol. Biol. Rev, 64 (4) : 665. (2000)
35. J. L. Balcazar, Microbiology. 114:173-186. (2006)
36. X. F. Pan, T. Chen, H. Wu, Tang, and Z. Zhou, Food Control. 20 (6): 598-602. (2009)
37. K. Todar, Antimicrobial Agents Used in the Treatment of Infectious Disease (University of Wisconsin-Madison, 1-11, 2002)
38. T. Purwoko, Fisiologi Mikroba (PT Bumi Aksara. Jakarta, 2007)
39. J. A. Patterson, dan K. M Burkholder, Journal of Poultry Science 82 (2) : 627- 631 (2003)

**Open Access** This chapter is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits any noncommercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

