Screening of ammonia-degrading bacteria to reduce ammonia content in the manure of laying hens

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Abstract. This study aimed to screen probiotic candidates for the capacity to degrade ammonia content in the manure of laying hens. Seven bacteria previously isolated from broilers' intestine were assigned to a completely randomized design. A fresh culture of each bacterial isolate was cultured to reach optical density at OD_{600}:0.5. One ml suspension was inoculated into 100 ml samples (2g manure diluted in 100 ml of sterilized distilled water) and incubated for three days. Ammonia concentrations in each sample were measured daily using *Ammonium/Ammonia-Test sera ammonia kits*. The results showed that the ammonia concentration in all bacteria-treated samples was significantly lower than ammonia content in control, P<0.05). The average amount of ammonia in the control was 1.00±0.44 mg/l, while isolate I_1-treated sample was 0.03±0.00 mg/l, isolate I_2 0.02±0.00 mg/l, isolate I_3 0.02±0.00 mg/l, isolate I_4 0.04±0.01 mg/l, isolate I_5 0.04±0.01 mg/l, isolate I_6 0.02±0.01 mg/l and isolate I_7 0.05±0.00 mg/l. The best three ammonia degrading capacity among the seven isolates were I_2, I_3, and I_6. Based on the phenotypical characteristics, these bacteria were identified as *Nitrosomonas* sp. (I2), *Nitrosobolus* sp. (I3), and *Nitrosococcus* sp. (I6). Therefore, these three bacteria were recommended for probiotic candidates in laying hen.

1. Introduction

The problem with developing laying hens, especially in densely populated areas, is pollution. Manure produced by laying hens can be a source of water, air, and soil pollution for the community. The smell caused by laying chicken manure is more stinging compared to ruminant animal manure because there are still many nutrients contained in the feed that are not digested optimally. According to Pemungkas *et al.* [1], laying hens manure as livestock waste still has quite good nutrient content, especially protein.

The amount and composition of feces produced vary depending on the type of poultry, body weight, time of excreta collection, weather, type, and amount of feed. According to Abustan [2], an average daily chicken contributes 0.15 kg of manure, with total nitrogen contained ± 2.94%, where this amount can be a source of ammonia. For laying hens with a capacity of 1000 chickens at an average body weight of 2 kg during the 82-week maintenance period produces excreta of 1,091 kg [3].

Laying chicken manure has benefits as organic fertilizer. The quality of the fertilizer produced is quite high because there are still many nutrients contained in these impurities. According to Pangaribuan *et al.* [4], the percentage composition of nutrient content in manure produced by poultry is N 0.75%, P

0.50% K 0.45%, and water content 60%. According to Tomia *et al.* [5], with a relatively high N content, chicken manure is very good for use as fertilizer. However, not all layer farms pay attention to this.

According to *Syahminan et al.* [6], the impact of chicken farming on the surrounding environment is mainly in the form of odors generated during the maintenance process. Chicken manure will cause an unpleasant odor because it contains gases such as ammonia. Ammonia gas has an unpleasant odor when inhaled with a level of 5-20 ppm, which is considered to be high enough and endangering livestock and humans.

According to Syahminan *et al.* [7], the use of probiotics can reduce ammonia levels. It was further explained that *Bacillus amyloliquefaciens* is one of the bacteria as a probiotic that has the potential to reduce ammonia pollution in broiler cages. The use of probiotics in poultry is reported to reduce the activity of urease, an enzyme that works to hydrolyze urea to ammonia so that ammonia formation is reduced or even lost [8].

The results of previous studies indicate that several types of probiotic bacteria can reduce ammonia levels. Low ammonia concentration in the treatment of probiotics with a concentration of 0.8 ml / l can be influenced by the addition of several probiotic bacteria such as Bacillus sp., Saccharomyces sp., and *Lactobacillus* sp. These bacteria can increase the activity of the protease enzyme which functions to accelerate the hydrolysis reaction of proteins and cut peptide bonds [9].

Sitompo *et al.* [10] stated that the conversion of ammonia to nitrate takes place through two stages. The first step is the oxidation of ammonia to nitrite enzymatically, and the second step is oxidation of nitrite to nitrate enzymatically.

In this study, the use of several probiotic candidate bacteria to reduce the ammonia content in laying hens is tested. Some of these bacteria have been taken by Nurbaiti *et al.* [11] from the intestines of broiler chickens.

2. Materials and methods

2.1. Time and place of research

This research was conducted in January - June 2019, located at the Laboratory of Microbiology and Biotechnology, Faculty of Animal Husbandry, Mataram University.

2.2. Research materials

This research uses a microscope, hot, stirring plate, analytical scales, autoclave, shaker incubator, Ammonia / Ammonia-Test brand ammonia test kits (NH_4/NH_3) GmbH D 52518 Heinsberg, LB liquid and solid media, sterile aquades, gram staining substances, laying hens and sugar.

2.3. Bacterial isolation

Bacteria were isolated from bacteria collected by Nurbaiti *et al.* [11] in the intestines of broiler chickens. Bacterial isolation was carried out using the spread plate method. The spread plate method is done by stratified dilution using seven test tubes filled with nine ml of sterile aquades. The bacteria sample is inserted 1 ml into the first test tube. Bacterial dilution is carried out seven times (10^{-1} – 10^{-7} dilution series) by taking 1 ml of each dilution then transferred to the next dilution.

2.4. Identification of probiotic candidate bacteria

2.4.1. Catalase test

Bacteria that have been grown on solid LB media are taken with ose. The ose used first is burned off with a Bunsen lamp. Furthermore, the bacteria that have been taken are put on the sliding glass. After that, it is dripped with H_2O_2.

2.4.2. Gram staining

Bacterial smear preparations are made by taking a bacterial culture suspension and then flattened on the glass slide surface. If it is cold, then drop it with Gram A (substitute violet) paint, Gram B (lugol), Gram C (ethanol), and Gram D (water fuchsin). After the smear preparation is given coloring, each time the rinse is rinsed with running water. The smear preparation was observed under a 100 times magnification microscope.

2.4.3. Simple sugar media test

Bacterial isolates were taken from solid LB media and made on sugar media. After that, it was incubated using a shaker incubator for 24 hours at a speed of 120 rpm at 37^0C. The sugar media which will be used first is made by dissolving 20% of sugar into the Erlenmeyer tube from the total aquades used, sterilized using an autoclave.

2.5. Bacteria inoculation of probiotic candidates in manur laying chicken

The test sample was first made by taking 2 grams of layer chicken laying and dissolving it with 100 ml sterile distilled water. After that, the sample is filtered, and the solution is taken. Samples were made as many as eight samples, 1 sample as a control, and seven samples to inoculate bacteria. Furthermore, inoculate bacterial isolates of 1 ml into each test sample.

2.6. Ammonia measurement

The type of ammonia test kit used is Ammonium / Ammonia-Test (NH_4/NH_3) sera GmbH Heinsberg D 52518. The ammonia test kit has three test solutions or reagents used. The steps for measuring ammonia levels are (1) The measurement bottle is rinsed filled with 10 ml sample; and (2) Add 6 drops each of reagents 1, 2, and 3, then homogenized for 5 minutes.

2.7. Data analysis

This research uses a completely randomized design (CRD) and descriptive. Then analyzed statistically using ANOVA test with SPSS 21 software (IBM). If significant (significant) results are obtained, further tests use Duncan's Multiple Range Test

3. Results and discussion

3.1. Screening for ammonia-degrading bacteria

The results of the measurement of ammonia levels in laying hens can be seen in Table 1. Based on the results of the analysis of variance, the ammonia test results were significantly different ($P < 0.05$) between samples that had been inoculated by seven bacterial isolates with controls. This means that the seven bacterial isolates can reduce ammonia content in laying hens. Based on Duncan's test, there were no significant differences between the seven bacterial isolates between bacteria in reducing ammonia.

It appears in Table 1 that at three times, the measurements obtained varied results in the seven isolates in reducing the ammonia content. Some isolates also showed a constant decrease in ammonia content, as in isolates I_1, I_2, I_3, and I_7.

Based on the average value of the ammonia content, the control values were higher compared to the seven bacterial isolates. Each of these values were control 1.00 ± 0.44 mg/l, isolate I_1 0.03 ± 0.00 mg/l, isolate I_2 0.02 ± 0.00 mg/l, isolate I_3 0.02 ± 0.00 mg/l, isolate I_4 0.04 ± 0.01 mg/l , isolate I_5 0.04 ± 0.01 mg/l, isolate I_6 0.02 ± 0.01 mg/l and isolate I_7 0.05 ± 0.00 mg/l as shown in Table 1.

Table 1. ammonia concentration in the manure supernatant treated with either ammonia-degrading bacteria or without bacteria as the control

No	Bacterial Isolates	Ammonia Concentration (mg/l)			
		0h	24h	48h	72h
1.	Control	1.01	1.15	1.25	1.51
2.	I_1	1.01	0.03	0.03	0.03
3.	I_2	1.01	0.02	0.02	0.02
4.	I_3	1.01	0.02	0.02	0.02
5.	I_4	1.01	0.05	0.03	0.03
6.	I_5	1.01	0.05	0.03	0.03
7.	I_6	1.01	0.02	0.03	0.02
8.	I_7	1.01	0.05	0.05	0.05

different manuscripts indicate the average values of ammonia were significantly different

Statistically and Duncan's test results, there were no significant differences between the treatments of bacterial isolates. However, if seen from the average data, there are 3 bacterial isolates which are better than 4 other isolates in reducing ammonia. Isolates I_2, I_3 and I_6 have lower ammonia content of 0.02 ± 0.00 mg/l compared with isolates I_1, I_4, I_5 and I_7.

Ammonia levels are decreased after inoculation of bacterial isolates due to an overhaul process. The ammonia reshuffle into simple compounds occurs in two stages. The first stage is nitrification, in which this stage ammonia is oxidized to nitrite (nitritation) and nitrite is oxidized to nitrate (nitratation). The second step is denitrification. This stage is reformed nitrate into hydrogen gas (H_2). Following the ammonia reshuffle chemical process according to Andriani *et al* [9]:

Nitritation: $NH_4^+ + 1.5\ O_2 \rightarrow NO_2 + H_2O + 2H + 76$ kcal
Nitratation: $NO_2^- + 0.5\ O_2 \rightarrow NO_3^- + 24$ kcal

According to Umrah [12] states that the reduction in ammonia content can occur because of the oxidation process. Ammonia oxidation is carried out by nitrifying bacteria. Besides being carried out by nitrifying bacteria, according to Trisna *et al* [13] that probiotic microorganisms can also oxidize ammonia. The process of oxidation of ammonia to nitrite and oxidation of nitrite to nitrate, or oxidation of organic matter directly to nitrate is generally carried out by nitrifying bacteria, both autotrophic and heterotrophic in nature [14].
Ammonia overhaul is influenced by several factors according to Pujiyati [15], namely the environment that supports bacterial activity such as water content, temperature, and pH.

3.2. Characterization of bacterial isolates
Characterization is one of the processes carried out to observe isolated bacteria. Characterization can be done based on the nature of cytology (cell shape, motion, gram staining, and endospores), morphological and physiological properties. According to Kadir [16] states that one way to know the differences in bacterial colonies is to look at the morphological differences in the bacterial colony.

Table 2. Morphology of isolated bacterial colonies

Code Isolate	Morphology of Bacterial Colonies				
	Color	**Margin**	**Surface**	**Elevation**	**Form**
I_1	Milky white	Round	Umbonate	Uneven	Smooth
I_2	White	Round	Mountain	Shiny	Smooth
I_3	Milky white	Round	Mountain	Shiny	Smooth
I_4	Milky white	Round	Flat	Uneven	Jagged
I_5	White	Round	Mountain	Shiny	Smooth
I_6	Milky white	Round	Arise	Shiny	Smooth
I_7	Milky white	Round	Umbonat	Uneven	Smooth

Morphology based on colony color, on isolates I_1, I_3, I_4, I_6 and I_7 are milky white, different from isolates I_2 and I_5 which have white colony color. In the form of the colony, the seven bacterial isolates have the same shape that is round, whereas in the bacterial colony elevation, there are different forms of elevation, namely in isolates I_1 and I_7 are uneven or umbonate. Isolates I_2, I_3, and I_5 have the shape of a colony protruding like a mountain. Whereas isolates I_4 and I_6 were flat and arising. The shape of the protrusion of the colony can be observed from the top of the colony.

Furthermore, if the bacterial colony is seen from its surface, there are two differences, namely a shiny surface and an uneven surface. The shiny surface was found in isolates I_2, I_3, and I_6, whereas the uneven surface of the colony was found in isolates I_1, I_4, and I_7. Morphology is based on margins or the shape of the colony from the edge when viewed from the edge, only in isolates I_4 that is different from the serrated margins while in isolates I_1, I_2, I_3, I_5, and I_6 have smooth margins.

Furthermore, bacterial characterization results are obtained as shown in Table 3. This characterization is based on biochemical tests on the biological characteristics and cell structure. Some bacterial characterizations carried out are based on gram staining, catalase test, and bacterial isolate growth test on simple sugar media.

Biochemical tests for gram staining are effective criteria for bacterial classification. The results of the staining will show the basic and complex differences in bacterial cells (cell wall structure), so that they can divide bacteria into two groups, namely Gram-positive bacteria and Gram-negative bacteria.

Table 3. Results of Characterization of Bacterial Isolates

No	Isolat code	Gram	Cell shape	Catalase	Test of sugar media
1	I_1	+	Basil	+	+
2	I_2	+	*Coccus*	-	-
3	I_3	+	*Coccus*	+	+
4	I_4	+	Basil	-	+
5	I_5	+	*Coccus*	+	+
6	I_6	-	*Coccus*	+	+
7	I_7	+	Basil	-	+

According to Rostinawati [17], based on the shape and effect of Gram staining, bacteria are grouped into Gram-positive (Gram) coccus (round), Gram-positive bacillus (stem) and Gram-negative. Based on the results in Table 3, it was shown that six bacterial isolates were gram-positive except for isolate I_6 which was gram-negative. The cell shape of the seven isolates was divided into two types namely bacilli and coccus. Isolates in the form of bacilli cells are I_1, I_4, and I_7. While isolates in the form of coccus cells include I_2, I_3, I_5, and I_6.

The gram difference, as explained by Holderman *et al.* [18] that in gram staining, gram-positive bacteria will give a purple color because it has a layer of peptidoglycan as thick as 20-80 nm. Whereas gram-negative bacteria have a thin layer of peptidoglycan, which is 5-10 nm with the main composition: lipoprotein, outer membrane, and polysaccharides.

In addition, Table 3 illustrates the ability of bacteria to degrade hydrogen peroxide (H_2O_2) by catalase testing. Bacteria that can form bubbles after adding H_2O_2 are isolates I_1, I_3, I_5, and I_6. Determination of the presence of catalase was tested with a solution of H_2O_2 where air bubbles formed which are oxygen gas (O_2) around the colony if the tests carried out gave positive results. This is consistent with the opinion of Riandi *et al.* [19]. The positive catalase shown by isolates indicates that the isolate has the enzyme catalase which is able to convert H_2O_2 in the form of lethal products from the byproducts of aerobic metabolism into molecules of oxygen and water. Whereas isolates I_2, I_4, and I_7 showed negative catalase test results because they did not form bubbles.

Utami **[20]** states that the catalase test is a test to determine the ability of bacteria to degrade hydrogen peroxide by producing catalase enzymes. Catalase is one of the enzymes used by microorganisms to decompose hydrogen peroxide.

The next test is a simple sugar media test. Sugar media is one of the media or a place to live for bacteria. Making sugar media is very simple by dissolving 20% sugar with distilled water. The purpose of this test is to find out what types of bacteria can grow in the sugar medium. In addition, to find out the sugar media as an alternative medium for bacteria to live, so it does not need expensive media.

Based on the sugar media test, six bacterial isolates are able to grow. Isolate I_2 does not grow in the sugar media; it can be seen from the absence of turbidity in the test tube that has been filled with sugar media. In addition, the growth of microbes in a liquid medium in this case a simple sugar media can be seen from the sediment (sediment). Sediment is a collection of cells that collect at the bottom of the tube and will spread again if the tube is moved.

The six bacterial isolates that grow on simple sugar media show that granulated sugar contains nutrients needed by microbes. According to Anisah and Triastuti [21], growth media must meet the nutritional requirements needed by a microorganism. According to Maryana [22], granulated sugar contains a lot of sucrose obtained from sugarcane processing. Sucrose or cane sugar is the sweetest disaccharide consisting of glucose and fructose.

4. Conclusions

Seven bacterial isolates showed a capacity to degrade ammonia content in laying-hen manure, and three of which (I_2, I_3, and I_6) showed the highest degrading capacity. Phenotypically, these bacteria were identified as *Nitrosomonas* sp., *Nitrosolobus* sp., *Nitrosococcus* sp. Further studies such as identification of the three bacteria using a molecular technique are required to have a more accurate bacterial identity. Also, in vivo assay by supplementation of bacteria via feed need to be done to investigate the capacity of the bacteria to reduce ammonia concentration on the excreted manure.

5. References

[1] Pemungkas G S, Sutarno and Edwi M 2012 *Biotech.* **9**, 26-34
[2] Abustan and A A 2018 *JPLB.* **2,** 42-54
[3] Kamaludin E 2011 Department of Animal Husbandry Production and Technology Faculty of Animal Husbandry Bogor Agricultural University (Bogor)
[4] Pangaribuan D H, Yasir M and Utami N K 2012 *J. Ind. Agron.* **40,** 204-210
[5] Tomia A 2012 *J. Agri. and Fish. Sci. (UMMU-Ternate agri.)* **5**
[6] Syahminan 2018 *Poor. J. Link.* **27**
[7] Riza H, Wizna Y, Rizal and Yusrizal 2018 *J. Ind. Ani. Husb.* **20,** 99-107
[8] Hartono M and Tintin K 2015 *J. of App. Agri. Res.* **15**, 214-219
[9] Andriani Y, Taufik I K and Iskandar I 2018 *J. of Aq., Coastal and Fish. Sci.* **7**, 209-217
[10] Sutomo 1989 *Oseana.* **16**, 19–26
[11] Nurbaiti 2016 *Thesis* Postgraduate Study Program Faculty of Animal Husbandry University of Mataram (Mataram)
[12] Umrah 2007 *J. of Aq. Resources* **1**, 15-20
[13] Trisna, D E, A D Sasanti and Muslim 2013 *J. Ind. Swamp Aq* **1**, 90–102
[14] Sitorus, H, Bambang W, Bibiana W L and Kadarwan S 2005 *Ind. J. of Aq. Sci. and Fish.* **12,** 59-67
[15] Pujiyati A 2004 Faculty of Animal Husbandry Bogor Agricultural Institute Bogor
[16] Kadir I R 2016 *J. of Ani. Husb. Faculty of Sci. and Tech.*
[17] Rostinawati T 2008 *Skirpsi* Independent research Faculty of Pharmacy Padjadjaran University Jatinangor (Bandung)
[18] Holderman, M V, Edwin Q and Sendy B R 2017 *J. of Scientific Sci.* **17**
[19] Riandi M I, Retno K and Sang K S 2017 *J. of Sym.* **5,** 58-63
[20] Utamy, S P 2016 *Essay* Chemistry Department. Faculty of Math and Science Halu Oleo University Kendari (Kendari)
[21] Anisah and Triastuti R 2015 *XII National Seminar of Biology Education FKIP* Semarang State University
[22] Maryana, D 2014 Faculty of Animal Husbandry Hasanuddin University Makassar (Makasar)

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