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Isolation of Cellulolytic Lactic-Acid Bacteria from Mentok (*Anas moschata*) Gastro-Intestinal Tract

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ABSTRACT

Mentok, **a local Indonesian name's of muscovy duck (***Anas moschata***), has been known as a duck with the capability to consume poor quality feed, high with non-starch carbohydrate (NSP) content. This capacity occurs because of the presence of microbial fermentation activity in the gastro intestinal tract (GIT) of** *mentok***. However, the information about the identification and characterization of the cellulolytic microbes involved is limited. This study was expected to provide scientific contributions about gastrointestinal microbes, especially Lactic Acid Bacteria (LAB), with cellulolytic activities. The experiment was conducted to select LAB with cellulolytic activity from the GIT of** *mentok***. Twenty six of selected LABs were isolated from the duodenum, cecum, and colon regions after microbiological characterization, i.e., morphology, catalase test, gas production, Gram staining, and motility test. Characterization for cellulolytic activity was analyzed by measuring the clearing zone on Carboxymethylcellulose (CMC) media, cell growth analysis on 1% CMC as a carbon source, and CMCase value.** *Pediococcus acidilactici* **MK 20 isolate from colon region was selected LAB with the highest cellulolytic activity with the clearing zone diameter, and the CMCase value of 2.33 mm and 0.0153 U/mL, respectively. Molecular identification using 16S rRNA gene sequences analysis revealed that** *P. acidilactici* **MK 20 isolate has 99% similarity with** *P. acidilactici* **strain ZW001. It was concluded that** *P. acidilactici* **MK 20 isolated from the colon part of the gastrointestinal tract of** *mentok***, the Indonesian muscovy duck (***A. moschata***) had cellulolytic activity.**

Keywords: lactic acid bacteria, mentok, muscovy duck (Anas moschata), cellulolytic bacteria

INTRODUCTION

The existence of Non-Starch polysaccharide (NSP) on feed diet of monogastric animals especially on poultry ration tends to be a factor that produce an antinutritive-value effect on feed digestion system at the gastrointestinal tract (GIT). The physicochemical properties of NSP contribute to the binding impact of water constituent with the material in the gut that produces a higher viscosity of the digesta decreasing the rate of digestibility (Bedford, 2000). In addition, the coating processes of materials on the surface of the feed could inhibit the enzymatic reaction produced in the GIT (Căpriţă *et al*., 2010). Cellulose has been classified as one of the NSP group (Bedford, 2000; Căpriţă *et al*., 2010) known as a polysaccharide constructed from glucose monomer binding with a 1,4 β glycoside bond. The monogastric animal does not have a specific site on their intestinal-tract organs to accommodate the massive microflora ecosystem that leads to the degradation of the cellulose (Fonty & Gouet, 1989). Some research groups have shown the degradation of cellulose by enzymatic action on poultry diets. The addition of cellulase from *Trichoderma reesei* and xylanase from a hyperproducing transgenic microorganism could decrease the viscosity of the digesta, increase sugar reduction level, and decrease pH (Boros *et al*., 1998). The addition of 1,3-1,4 β -glucanase 16A, and 1,4- β glucanase 8A showed the decrease in the viscosity of the purified 1,3-1,4 β -glucan solution and increase in the nutritive value of barley-based poultry diet (Fernandes *et al*., 2016). Meantime, the addition of exogenous NSP enzyme on broiler-chicken pasture during winter season improved the broiler performance since the chicken could use the NSP forage grown at that season as energy sources (Buchanan *et al*., 2005).

Mentok, as an Indonesian name's of muscovy duck (*Anas moschata*), has been studied for many years by some research groups to capture the information about their performances abilities as a meat producing animal (Tugiyanti *et al*., 2013; Ali *et al*., 2014; Suci *et al*., 2017). In Indonesia, muscovy duck is well known as one of three local ducks breeds that produces meat (CIVAS, 2006). For the duck, some carbohydrate fractions, such

as dietary fibre (DF) was used as one composition in feed. Józefiak *et al*. (2004) states that some carbohydrate fractions are not hydrolyzed by avian gastrointestinal enzymes but are fermented by the resident anaerobic microflora. Apajalahti (2015) reports that fermentation activities of bacteria at the GIT of the monogastric animals are concentrated in the cecum and colon. This condition contributes for the higher accumulation of short-chain fatty acids (SCFA) in the cecum compared with the other areas of the avian gastrointestinal tract (Józefiak *et al*., 2004). In the other studies, several specieses of lactic acid bacteria with probiotic properties were isolated from intestinal content of muscovy duck in Fujian Province, China (Xie *et al*., 2015) and in our previous study, meat quality and poultry performance were improved with LAB administration as probiotic (Istiqomah *et al*., 2013; Sofyan *et al*., 2012).

Over the last decade, the investigation on the identification, characterization, and utilization of microbes isolated from GIT of chicken increased rapidly. Some of them reported the use of lactic acid bacteria as a probiotic agent for chicken (Musikasang *et al*., 2009; Damayanti *et al*., 2014; Jannah *et al*., 2017; Hamida *et al*., 2015; Yeh *et al*., 2017; Julendra *et al*., 2017). Meanwhile, the information about the lactic acid bacteria isolated from the GIT of Indonesian ducks for poultry probiotic agent is still limited.

Regarding the concern of cellulolytic activity in poultry-animal digestive system, a study was conducted to isolate and characterize cellulolytic activity of Lactic Acid Bacteria isolated from GIT of *mentok*, Indonesian muscovy duck (*Anas moschata*). This study was expected to provide scientific contributions in the field of gastrointestinal microbes, especially BAL with cellulolytic activity.

MATERIALS AND METHODS

Isolation and Identification of Lactic Acid Bacteria

Lactic acid bacteria (LAB) were isolated from *mentok* (muscovy duck) by using the protocol used by Torshizi *et al*. (2008). Five grams of lumen samples from intestine, cecum, and colon were suspended in 0.85% NaCl solution (Merck) and made up to 10^{10} serial dilutions. One hundred µL of each serial dilution was spred on a plate covered with de Man Rogosa Sharpe (MRS) agar medium (Oxoid) supplemented with $CaCO_3$ (0.2%, v/v) in duplicate then incubated at 37°C for 24 h in the anaerobic chamber with Anaerocult CO_2 generator (Merck). The LAB colonies were detected by the existence of clearing zone. The parameters measured to identify Lactic acid bacteria consisted of morphology, catalase test, gas production, Gram staining, and motility test (Krieg *et al*., 2010).

Cellulolytic Activity Assay of The Selected Lactic Acid Bacteria

Preliminary screening of LAB isolates with cellulolytic activity was conducted by inoculating 10 µL of LAB on paper disk on Agar plate sterile medium containing 1% Carboxymethylcellulose (CMC). The composition of agar plate medium consisted of 1 g of CMC, 0.02 g of $MgSO_4$ $7H_2O$, 0.075 g of KNO, 0.05 g of K_2HPO_4, 0.002 g of $FeSO_4$ $7H_2O$, 0.004 g of $CaCl_2$ $2H_2O$, 0.2 g of yeast extract, 1.5 g of agar, and 0.1 g of glucose diluted in 100 mL of distilled water. The experiment used three replicates paper disks for each LAB isolate and incubated at 37°C for 48 h in anaerobic condition. After incubation, the plates were flooded with Congo red (1%, v/w) for 20 min then followed by destaining with 1 M NaCl for 20 min. The LAB isolates strain with the largest clearing zone indicating the highest cellulolytic activity. Cellulolytic activity was measured by determining the cellulolytic index which was the ratio between the diameters of a clearing zone with a diameter of colonies (Meryandini *et al*., 2009; Setyati & Subagiyo, 2012).

Cell Density Analysis

The analysis of cell density of LAB was performed to determine the ability of the LAB to consume cellulose by using Optical density (OD) as a growth parameter. The method of measuring referred to the procedure used by Rahayu *et al.* (2014) with a minor modification. One percent of the selected LAB culture (10^8 CFU/mL) was inoculated into 20 mL of sterile CMC Broth medium (consisted of 1 g of CMC, 0.02 g of $MgSO_4$ $7H_2O$, 0.075 g of KNO; 0.05 g of K_2HPO4, 0.002 g of $FeSO_4$ $7H_2O$, 0.004 g of $CaCl_2$ $2H_2O$, 0.2 g of yeast extract, and 0.1 g of glucose and diluted in 100 mL of distilled water) and then incubated at 30°C for 24 h. The treatments were performed using three replicates. Two hundred mL of sample from each treatment was placed on microplate consisted of 96 wells. The OD was measured at 620 nm of wavelength using microplate reader at 0, 24, and 48 h of incubation.

Carboxymethyl Cellulase Activity Assay

Carboxymethylcellulose activity (CMCase) was measured using Dinitrosalisic acid reagent (DNS) based on the estimated amount of reducing sugar released from 1% CMC diluted in 0.05 M citric buffer at a pH of 4.8. A reaction medium containing 0.5 mL of CMC substrate dissolved in citrate buffer (acidity of 4.8) and 0.5 mL of enzymatic crude extract from cell-free supernatant of LAB culture and incubated at 50ºC for 30 min. The reaction was terminated by adding 1.5 mL of the DNS reagent and boiled in the water bath at 100°C for 5 min, cooled at room temperature, and then added 10 mL of distilled water. For each LAB isolate, three replicates were performed in this experiment. The quantity of sugar released was determined by measuring at a wavelength of 540 nm and Critical enzyme concentration (IU) = 0.185 x CMC (sugar reduction) (mg) (Ghose, 1987).

Molecular Identification of Lactic Acid Bacteria

Lactic acid bacterial identification was performed molecularly by amplifying the 16S ribosomal DNA (16S rDNA) sequence region. Amplification of 16S rDNA

area of bacteria was performed using PCR colony method. PCR 16S rDNA amplification per reaction of 30 µL using primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3 ') and 1492R primer (5'-GGTTACCTTGTTACGACTT-3') (Gong *et al*., 2007). The composition of the PCR reaction consisted of 15 µL of Go Tag Green Master Mix 2x (Promega, 2016), 2.4 µL of primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3 ') 10 µM, 2.4 µL of primary 1492R (5' -GGTTACCTTGTTACGACTT-3 ') 10 µM, 1 µL of DNA template, and 9.2 µL of ultrapure water DNA/RNase free. The PCR reaction used a PCR (Eppendorf German) machine with a first pre-denaturation at 94°C for 90 s, followed by 30 cycles consisted of denaturation at 95°C for 30 s, primer adherence at 50°C for 30 s, and extension at 72°C for 90 s. After 30 cycles were completed, the processed was continued with elongation phase at 72°C for 5 min and cooling at 4°C for 20 min (Promega, 2016).

The PCR product was further purified and cycled sequenced with the same primer Sequence analysis conducted in the laboratory of First Base (Malaysia). The next sequenced data were trimmed and assembled with BioEdit program and then converted in FASTA format. The DNA sequencing results in the following FASTA format at Basic Local Alignment Search Tool – Nuclotide (BLASTN) to locate the homology online at the DNA database center at National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). Phylogenetic analysis was performed by Neighbor-Joining tree method using ClustalX2 and MEGA5 programs. Grouping stability was calculated using 1000 bootstrap value (Cobos *et al*., 2011).

Scanning Electron Microscope Analysis

Morphological characteristics of strain *P. acidilactici* MK20 were observed using scanning electron microscope (SEM) after incubation at 37°C for 18 h on MRSB medium. After incubation, one loop of LAB single colony was added evenly on the surface of the carbon-tape covered stub. The sample was air dried and coated with Au (using Au ion sputter-Hitachi MC1000) with a setting of 10 mA for 60 s and observed using SEM Hitachi SU3500. The SEM setting of high vacuum mode, accelerating voltage of 5 kV, spot intensity of 30%, and magnification of 10,000x.

Statistical Method

The results of OD and glucose concentration measurement at 48 h observation was analyzed by one-way ANOVA with completely randomized design followed by Least Significant Difference method for testing the difference between treatments (Gomez & Gomez, 1984). The statistical-analyses process was carried out by CoStat version 6.400 Program from CoHort Software with the one-way completely randomized and LSD for Means Test procedures. Graphic presentation was made by Microsoft Excel version 2010 from Microsoft Software.

RESULTS

Isolation and Identification of Lactic Acid Bacteria

There were 26 of LAB samples isolated from *mentok* GIT i.e., 7 taken from the duodenum, 6 from cecum, and 13 from colon region. The shapes of the LAB isolates appeared as cocci and rod morphologies. All of the LAB isolates were observed as negative catalase, nonmotile, Grampositive, and non-gas producer. The characterizations of the five selected LAB isolates with the highest cellulolytic activity are presented in Table 1.

Cellulolytic Activity of Lactic Acid Bacteria

Preliminary results of the screening cellulolytic assay showed that all of LAB isolates produced clear zones on CMC agar plate media after Congo red staining around the colony with diameter ranged from 0.23 to 3.22 cm (Table 1). Five isolates with the highest clear zone were MU 4 (2.31 cm), MS 14 (2.40 cm), MK 20 (2.33 cm), MK 27 (1.67 cm), and MK 44 (3.22 cm) and these isolates were selected to proceed with growth assay and glucose concentration productivity assays. The observations of cell densities on 1% of CMC medium at 37°C for 48 h incubation were shown in Figure 1. After a 48-h incubation period, the results of the measurements of OD, glucose concentration, and CMCase were shown in Table 2. The highest values for OD and CMCase were 0.3398, and 0.0153 U/mL, respectively, that were obtained from MK 20 isolates derived from the *mentok's* colon.

Table 1. Characterization of lactic acid bacteria isolated from Muscovy duck gastrointestinal tract (GIT) and cellulolytic activity on 1% CMC agar plate with Congo-red staining

Isolate code	Morphology	Assays				
		Gram Staining	Catalase	Motility	Gas production	Clearing zone diameter (cm)
MU 4	Long rod	+	-	-	-	2.31
MS 14	Cocci	+	-	-	-	2.40
MK 20	Cocci	+	-	-	-	2.33
MK 27	Cocci	+	-	-	-	1.67
MK 44	Cocci	+	-	-	-	3.22

Note: "+" (positive) = observed; "-" (negative) = not observed; MU = LAB isolated from duodenum; MS = LAB isolated from cecum; MK= LAB isolated from colon of *Mentok.*

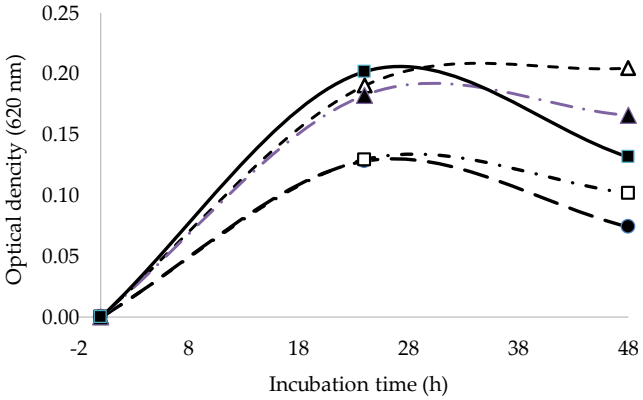

Figure 1. Cell density of lactic acid bacteria isolated from Muscovy duck gastrointestinal tract on 1% CMC medium at 37°C for 48 h incubation. MU 4 (-•-) = LAB isolated from duodenum; MS 14 (-□-) = LAB isolated from cecum; and MK 20 (-Δ-), MK 27 (-▲-), MK 44 (-■-) = LAB isolated from colon.

Molecular Identification of Selected Lactic Acid Bacteria

The MK 20 isolate was identified as *Pediococcus acidilactici*. Homology BLAST sequence 16S rDNA MK 20 isolate with a length of 1428 bp in DNA database (https://blast.ncbi.nlm.nih.gov/Blast.cgi, was accesed on on 12 March 2018) had max identity of 99%, max score of 2627, total score of 2627, 100% of query coverage, E value of 0,0 against the nearby bacterial taxon *P. acidilactici* strain ZW001 (JN255181). Classification of the bacteria is Kingdom: Bacteria, Division: Firmicutes, Class: Bacilli, Order: Lactobacillales, Family: Lactobacillaceae, Genus: Pediococcus, Species: *P. acidilactici*. Phylogenetic tree of the MK 20 isolate was presented in Figure 2. Morphological characteristics of *P. acidilactici* MK20 using Scanning Electron Microscope (SEM) were shown in Figure 3.

DISCUSSION

Isolation and Identification of Lactic Acid Bacteria

In this result, we found that the cocci LAB was found dominant in isolates from cecum and colon. This result was different from that reported by Musikasang *et al.* (2009) that the proportion of rod and cocci of LAB isolated from the GIT of chicken were 85% and 15%, respectively. In the other study, Xie *et al*. (2015) founded 4 rod selected LAB among 6 LAB from intestinal content of muscovy duck. Based on cellulolytic activity assay on CMC agar plate media, the clear zone appearance indicated that LAB colonies had the ability to degrade cellulose. The highest clear zone diameter of a LAB in this study was revealed by MK 44 isolate from colon region (3.22 cm). This result was lower than *Bacillus pumilus*, *Klebsiella* spp. (5.0–7.0 cm) but it was higher than *Stenotrophomonas* spp., *Enterococcus casseliflavus* and *Microbacterium* spp. (1.5–2.5 cm) as a cellulolytic bacteria from the intestine of *Diatraea saccharalis* larvae (Dantur *et al.*, 2015) and cellulolytic *Enterobacter cloacae* (2.5 cm) isolated from the rumen of Aceh cattle (Sari *et al.*, 2017). All of the five potential LAB isolates produced higher clear zone diameters compared to those produced from cellulolytic bacterial isolated from the gut of Worker *Macrotermes gilvus*, i.e. 0.05 to 0,145 cm (Ferbiyanto *et al.*, 2015). This result also relatively higher than cellulolytic bacteria such as *Burkholderia*, *Bacillus*, *Chryseobacterium*, *Citrobacter*, and *Dyella* which were isolated from different natural reserves of the subtropical region in China (Liang *et al.*, 2014).

Table 2. Optical density (OD) of cell density, glucose concentration, and CMCase activity of selected lactic acid bacteria (LAB) isolated from *Mentok* gastrointestinal tract on 1% carboxymethilcellulose (CMC) medium at 37°C for 48 h incubation

LAB Isolate	OD Value (λ 620 nm)	Glucose concentration (ppm)	CMCase (U/mL)
MU 4	0.1744±0.005 ^{b}	44.52± 3.39 ^{c}	0.008
MS 14	0.1709±0.002 ^{b}	42.30± 2.57 ^{c}	0.008
MK 20	0.3398±0.026 ^{a}	82.67± 3.34 ^{a}	0.015
MK 27	0.2307±0.031 ^{b}	56.37±11.40 ^{b}	0.010
MK 44	0.2944±0.063 ^{a}	76.37± 8.04 ^{a}	0.014

Nmte: Means in the same column with different superscript differ significantly (P<0.05), MK (20,27,44)= LAB isolated from colon; MS 14= LAB isolated from cecum, MU 4= isolated from duodenum.

The other studies reported that cellulolytic bacteria isolated from horse GIT and identified as genus Lactobacilllus such as *L. mucosae*, *L. delbrueckii*, and *L. salivarius* (Al Jassim *et al.*, 2005), *Bacillus subtilis* BY-2 isolated from pig intestine (Yang *et al.*, 2014), *Bacteroides succinogenes* and *Ruminococcus flavifaciens* isolated from rat GIT (Macy *et al.*, 1982), *Klebsiella*, *Stenotrophomonas*, *Microbacterium*, *Bacillus* and *Enterococcus* isolated from *Diatraea saccharalis* larvae (Dantur *et al.*, 2015), cellulolytic bacteria B-6 isolated from the rumen of Buffalo (Bidura *et al.*, 2014), and *Enterobacter cloacae* isolated from the rumen of Aceh cattle (Sari *et al.*, 2017).

To identify the cell growth on liquid medium with CMC as a carbon source, the five selected cellulolytic LAB isolates were grown on medium containing 1% CMC for 48 h of incubation. Figure 1 illustrated that three cellulolytic LAB isolates (MK 20, MK 44 and MK 27) from colon region had the highest cell growth than MS 14 from caecum region and MU 4 from duodenum region of muscovy duck. The colon-derived LAB tend to be more adaptive in media containing cellulose substrates like CMC than those derived from the front part of the intestine. This difference was possibly related to the digestive process of feed in the colon of duck. Apajalahti (2005) stated that caecum and colon were parts of the GIT received dietary compounds that escape host digestion and absorption in the small intestine. In these regions, endogenous bacteria ferment the entering substrates. Józefiak *et al.* (2004) also concluded that dietary fiber (DF) consisted of a complex mixture of polymers which associated with non-carbohydrate components that was fermented in the avian GIT. The DF is predominantly found in plant cell walls and consists of NSP together with non-carbohydrate compounds in-

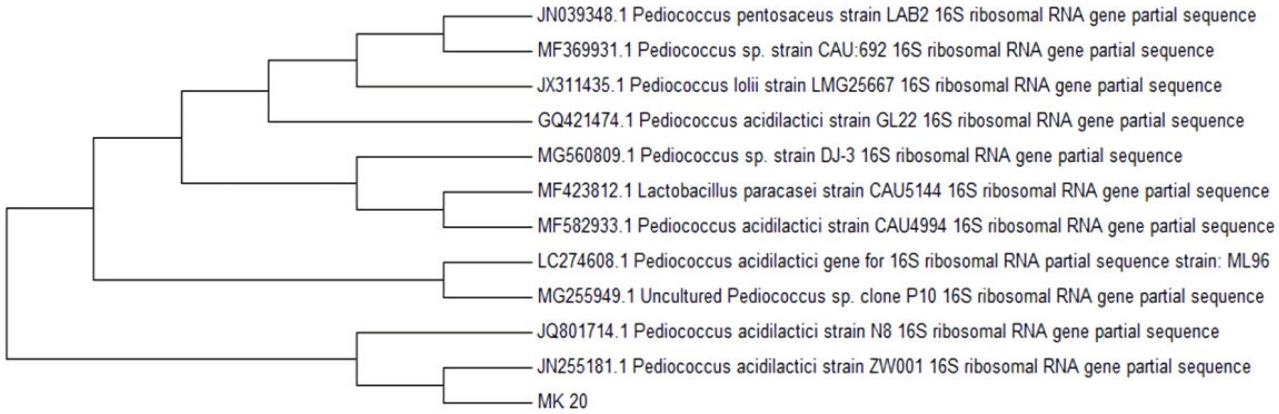

Figure 2. Phylogenetic tree of MK 20 lactic acid bacteria isolated from muscovy duck colon using the 16S rDNA gene for sequence homology studies. Sequences of reference strains obtained from DNA databases with accession numbers. Bootstrap values are based on 1,000 replicates.

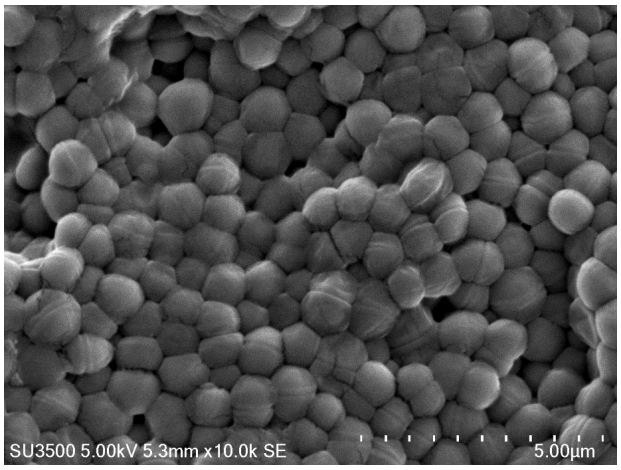

Figure 3. Scanning electron micrograph of *Pediococcus acidilactici* MK20 growth on MRSB medium, 37°C, 18 h (magnification 10,000x)

cluding lignin, protein, fatty acids, and waxes. Insoluble polysaccharides such as cellulose also fermented in avian GIT. NSP fractions have an anti-nutritive role in avian metabolism, but it was possible that some beneficial properties may be associated with the end-products of fermentation.

The five potential cellulolytic LAB were further assayed for CMCase activity in the liquid medium. The progress of carboxymethylcellulose (CMCase) was determined by measuring the release of reducing sugars from CMC (Yang *et al*., 2014). Screening the cellulolytic microorganism according to its cellulose degradation process could be done by Congo red, but this process could not represent the enzyme productivity by a microorganism (Liang *et al*., 2014).

In this study, the highest activity of CMCase in liquid medium was shown by MK 20 isolate. This result was contrary to cellulolytic assay on CMC media agar that MK 44 isolate showed the highest cellulolytic activity though both isolates were isolated from the same colon region. This result similar to those reported by Liang *et al.* (2014) that some strains presented large and clear hydrolyzing zones, but the activities of CMCase were undetectable in various liquid media containing CMC. This condition indicated that the concentration of the enzyme produced by the strains was difficult to be detected after cultivation in liquid medium or the ability of the strains to secrete CMCase was weak.

The highest CMCase activity from the supernatant of MK 20 isolates in this study was 0.0153 U/mL at 48 h of incubation on 1% CMC substrate (Table 2). The CMCase values obtained from MK 20 was lower than the CMCase values of the cellulolytic bacteria (*Streptomyces variabilis, Kocuria rosea,* and *Stenotrophomonas maltophilia*) isolated from the Persian Gulf bay with a mean value of 0.088 U/mL (Samira *et al*., 2011), *Paenibacillus terrae* ME27-1 isolated from subtropical region in China (0.18 U/mL) (Liang *et al*., 2014), and *Bacillus subtilis* BY-2 isolated from pig's intestine (1.5 U/mL) at 24 h incubation (Yang *et al*., 2014), but higher than those of *Enterococcus casseliflavus* and *Stenotrophomonas maltophilia* (<0.01 U/mL) isolated from the intestine of *Diatraea saccharalis* larvae (Dantur *et al*., 2015).

Endo- 1,4-β-glucanase (CM-cellulase, endoglucanase, endocellulase) enzymes was known as an enzyme that breaks down the CMC substrate into sugars (Wood & Bhat, 1988). First endo-glucanases brake cellulose into termini of cellulose chain, followed by exo-glucanases with the action to release the crystalline structure of the microfibril (Dantur *et al*., 2015).

The supplementation of the cellulolytic LAB as poultry probiotic was a potential strategy for increasing the digestibility of feed with cellulose content. The highest cellulolytic LAB of muscovy duck GIT in this study was identified as *P. acidilactici* MK 20 isolated from the colon region. Several studies had found genus *Pediococcus* LAB from the digestive tract of poultry such as *P. acidilactici* R01, and *P. acidilactici* R02 were isolated from the proventriculus of broilier chicken which has resistance on bile salt and acid condition (Damayanti *et al*., 2014), *P. pentosaceus* Db9 was isolated from duodenum of broiler chicken (Damayanti *et al*., 2012), *P. pentosaceus* KT3CE27 from Thai endogenous chicken (Musikasang *et al*., 2009), *Pediococcus* from intestinal content of duck (Kurzak *et al*., 1998).

P. acidilactici MK 20 from *mentok* GIT in this study was different from LAB species isolated from intestinal of Muscovy duck which reported in other studies such as *Lactobacillus rhamnosus*, *Lactococcus lactis* subsp *lactis*, *L. salivarius*, *Streptococcus lactis*, *Enterococcus columbae* and *L. murinus* from the intestine of Muscovy duck (Xie *et al*., 2015), *Streptococcus, Enterococcus, Weissella,* and *Lactobacillus from intestinal content of duck* (Kurzak *et al*., 1998), *L. delbrueckii* subsp. *bulgaricus* and *L. reuteri* F275T isolated from ducks and geese in Thailand (Kimprasit, 2013) and *L. salivarius* I11 from the Pengging duck's intestine (Sumarsih *et al*., 2014).

Pediococcus spp. are widely described as probiotics and characterized as coccus-shaped bacteria (arranged in tetrads), Gram-positive, non-motile, non-spore forming, catalase negative, and facultative anaerobes. *Pediococcus* strains that produce pediocin, an effective bacteriocin (Porto *et al*., 2017). In this study it was shown that *P. acidilactici* which was grown on MRSB medium at 37°C during 18 h arranged in single cocci or diplococci morphology (Figure 4). *P. acidilactici* species have *Generally Regarded as Safe* (GRAS) status from Food and Drug Administration of United States (FDA USA) and *Qualified Presumption of Safety* (QPS) status from European Food Safety Authority (EFSA) (Gaggia *et al*., 2010). The further research was needed to explore the potential of *P. acidilactici* MK 20 with CMCase activity as a probiotic for poultry which has beneficial properties to increase digestibility of NSP as anti-nutrient on feed.

CONCLUSION

Pediococcus acidilactici MK 20 isolated from the colon part of the gastrointestinal tract of *mentok*, the Indonesian muscovy duck (*Anas moschata*) had cellulolytic activity. Further research was needed for exploring and studying the probiotic properties of this LAB as poultry probiotic to improve feed digestibility.

CONFLICT OF INTEREST

We declare that there is no conflict of interest of the manuscript material with any financial, personal, or other relationship of other people or organization.

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