Selection of Lactic Acid Bacteria as Probiotic Candidate for Chicken

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ABSTRACT

Lactic acid bacteria (LAB) regarded as safe microorganisms; they can naturally live in gastrointestinal tract, so appropriately used as a probiotic for chicken. This study aimed to select six isolates of LAB (E1223, E3, E4, E5, E7, and E8) to obtain the isolates potentially as probiotic candidate for chicken. The six isolates were derived from spontaneous fermented corn obtained from Laboratory of Animal Biotechnology and Biomedical, PPSHB, Bogor Agricultural University, Indonesia. LAB isolates were tested their susceptibility to antibiotics (bambermycin, erythromycin, chloramphenicol, and tetracycline) then were examined in vitro for their tolerance to gastrointestinal pH (2, 3, 4, and 7.2) and 0.5% bile salt condition, antimicrobial activity against Salmonella enteritidis and Enterococcus casseliflavus, and ability to adhere to chicken ileal cells. The results showed the isolates E5, E7, and E8 were sensitive to tetracycline and chloramphenicol, they could survive at pH 2, 3, 4, and 7.2, could survive at 0.5% bile salts, produced antimicrobial activity, and able to adhere to ileal cells (9.40±0.00 Log CFU/cm² of E8) and were significantly (P<0.05) higher than those of control (5.30±0.14 Log CFU/cm²). In conclusion, this study showed that isolate E8 had better potential compared to isolates E5 and E7 in most in vitro assays as a probiotic candidate for chicken. E5, E7, and E8 were closely related with Pediococcus pentosaceus based on 16S rRNA gene.

Key words: LAB, probiotic, chicken, in vitro

ABSTRAK

Bakteri asam laktat (BAL) dianggap sebagai mikroorganisme aman, dapat hidup di dalam saluran pencernaan, sehingga tepat digunakan sebagai mikroorganisme probiotik untuk ayam. Penelitian ini bertujuan menyeleksi secara in vitro enam isolat BAL (E1223, E3, E4, E5, E7, E8) untuk memperoleh BAL berpotensi sebagai kandidat probiotik ayam. Enam isolat berasal dari jagung fermentasi yang diperoleh dari Laboratorium Bioteknologi Hewan dan Biomedis, PPSHB, Institut Pertanian Bogor, Indonesia. Isolat BAL diuji sensitifitasnya terhadap antibiotik (bambermisin, eritromisin, kloramfenikol, dan tetrasiiklin) lalu diuji secara in vitro ketahanannya terhadap pH saluran pencernaan (2; 3; 4; dan 7,2) dan 0,5% garam empedu, aktivitas antimikroba terhadap Salmonella enteritidis dan Enterococcus casseliflavus, dan kemampuan menempel pada ileum ayam. Hasil penelitian menunjukkan bahwa isolat E5, E7, dan E8 sensitif terhadap tetrasiiklin dan kloramfenikol, toleran pada pH pencernaan dan garam empedu, menghasilkan aktivitas antimikroba, dan mampu menempel pada ileum ayam (sel isolat E8 yang menempel sebesar 9,40±0.00 Log CFU/cm²) signifikan (P<0,05) lebih tinggi dibandingkan dengan kontrol (5,30±0.14 Log CFU/cm²). Kesimpulan penelitian ini menunjukkan bahwa isolat E8 memiliki potensi lebih baik daripada isolat E5 dan E7 di sebagian besar uji in vitro sebagai kandidat probiotik ayam. Isolat E5, E7, dan E8 memiliki homologi 99% dengan Pediococcus pentosaceus berdasarkan gen 16S rRNA.

Kata kunci: BAL, probiotik, ayam, in vitro

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INTRODUCTION

Antibiotic growth promoters (AGPs) as feed additives have been used since 1940s to improve livestock productivity and disease prevention (Castanon, 2007). The excessive and continuous use of antibiotics can lead to antibiotic residues accumulation (Tao et al., 2012) and resistance of pathogenic bacteria (Vignaroli et al., 2011). Antibiotics use as feed additives has been restricted gradually since 1970s in Europe (Castanon, 2007). In Indonesia, a ban of the use of antibiotics as feed additives has been regulated in Law of the Ministry of Agriculture No. 18 in year 2009 of Article 22 Paragraph 4c about Animal Husbandry and Animal Health (Kementan, 2009). Screening of alternative materials as feed additives has been done by previous researchers using enzymes, probiotics, herbal plants, and organic acids (Huyghebaert et al., 2011; Gunal et al., 2006; Wiryawan et al., 2005).

Probiotics as live microorganisms which when administered in adequate amounts confer a health benefit to the host (FAO/WHO, 2002). LAB microorganisms are often used as probiotics and could be isolated from the digestive tract, fermentation of milk, and grain crops (Jannah et al., 2014; Rosyidah et al., 2013; Bao et al., 2010). Jannah et al. (2014) and Gunal et al. (2006) reported that interaction of LAB with intestine could reduce the population of intestinal pathogens. According to FAO/WHO (2002) probiotics should have minimum criteria as probiotics to exert their beneficial effect to the host: 1). Probiotic strains should identified as having phenotypic and genotypic specifications of characteristics, 2). In in vitro assays, probiotic strains should be able to survive in stomach acid, bile salts, adhere to the mucus or intestinal epithelial cells, able to produce antimicrobial activity, 3). Do not produce toxins, not resistant to antibiotics, and are not pathogenic bacteria (EFSA 2012). Exploration of LAB strains as probiotic has been carried out in previous studies by isolation and screening using in vitro methods (Babet et al., 2014; Jannah et al., 2014; Lee et al., 2014). In vitro assays are very important to assess the safety of probiotic strains, beside that the in vitro data can be information about the advantages of strains as a probiotic candidate (FAO/WHO, 2002). This study aimed to select six isolates of LAB (E1223, E3, E4, E5, E7, and E8) derived from spontaneous fermented corn (Rosyidah et al., 2013) to obtain the isolates potentially as probiotic candidate for chicken. Selection of candidate probiotic was derived from the fermented corn that may facilitate the initial selection to obtain isolates which are not resistant to antibiotics.

MATERIALS AND METHODS

Microorganisms and Culture Conditions

Six LAB isolates (E1223, E3, E4, E5, E7, and E8) used in the present study were derived from spontaneous fermented corn that were obtained from Laboratory of Animal Biotechnology and Biomedical, PPSHB, Bogor Agricultural University, Indonesia. Pathogenic bacteria indicator (Salmonella enteritidis and Enterococcus casseliflavus) were obtained from Laboratory of Animal Diseases and Veterinary Public Health, Faculty of Veterinary Biomedicine, Bogor Agricultural University, Indonesia. LAB isolates and E. casseliflavus were grown and propagated in MRS broth (HiMedia) and incubated in anaerobic jar incubator with GasPak (Merck, Germany) at 37°C. S. enteritidis was grown and propagated in Nutrient Broth (Difco) in shaker incubator at 37°C.

Susceptibility to Antibiotics

Antibiotics susceptibility assay were performed according to the method described by Babot et al. (2014) and modified by well diffusion agar method. Culture of 14 h of LAB isolates was poured (10^9–10^10 CFU/mL) into MRS agar plate. The wells were made by sterilized pipette tip with diameter size of 7 mm. Respectively, each well was filled with 50 µL antibiotics test (25 ppm bambermycin, 15 µg erythromycin, 30 µg tetracycline, and 30 µg chloramphenicol). Agar plates were incubated for 24 h at 37°C. The diameter of inhibition zone was measured by rule (mm). The susceptibility of isolates was classified as sensitive, intermediate, and resistant according to the interpretative standards described by Walker (2006) and Swenson et al. (1990). The sensitive isolates or not resistant to antibiotics tested were selected as candidate to be tested in subsequent assays.

Tolerance to Gastrointestinal pH and Bile Salt

Acid and bile tolerance assays were performed according to the method described by Messaoudi et al. (2012) and Bao et al. 2010. Culture of 14 h of LAB isolates were inoculated (10^5–10^9 CFU/mL) into MRS broth (adjusted to pH 2, 3, 4, and 7.2 with HCl 1N and NaOH 1N) and incubated for 3 h at 37°C. Beside that, bile salt tolerance assay was conducted by using MRS broth containing 0.5% w/v bile salt (HiMedia) and incubated for 5 h 37°C. The viable cell was enumerated by total plate count method using MRS agar plates. The parameter of survival ability was determined by calculating the decrease in cell viability after treatment (Log CFU/mL) and survival rate (%). The decrease in cell viability was determined by calculating:

\[ \text{Log}_{\text{before treatment}, \text{CFU/mL}} - \text{Log}_{\text{after treatment}, \text{CFU/mL}} \]

Survival rate (%) was calculated according to the following equation:

\[ (\text{Log}_{\text{after treatment}, \text{CFU/mL}}/\text{Log}_{\text{before treatment}, \text{CFU/mL}}) \times 100\% \]

Antimicrobial Activity of LAB Isolates

Antimicrobial activity was analyzed by using well diffusion agar method described by Klose et al. (2010). Cell-free supernatant of LAB isolates were obtained from overnight culture centrifugation (IEC Clinical Centrifuge 215, US) at 7000 rpm for 10 min. Supernatants were divided into 3 portions: 1) untreated supernatant (pH ±4.36) as positive control; 2) neutralized supernatant with 1N NaOH (pH 6.5) to evaluate organic acid activity; 3) neutralized supernatant (pH 6.5)
that was added with 1 mg/mL K-Protease (Nacalai Tesque, Inc.) to evaluate bacteriocin-like inhibitory substances activity, the mixture was incubated for 2 h at 30 °C. Enzymatic protease activities were inactivated by incubation at 100 °C for 5 min. All of the supernatants were sterilized by membrane filter with pore size 0.22 µm. Indicator bacteria S. enteritidis and E. casseliflavus (were cultured overnight with concentration 10^7-10^8 CFU/mL) were poured into Nutrient agar plates. The wells were made by sterilized pipette tip diameter 7 mm. Each supernatant (50 µL) was placed into different wells and assayed for inhibitory effects on agar plates. The plates were incubated for 24 h at 37 °C. The diameter of zone inhibition was measured by rule (mm).

**In Vitro Ability of LAB to Adhere to Chicken Ileal Cell Assay**

The method described by Mayra-Makinen et al. (1983) was used for the preparation of the intestinal cells. Segment of ileum from broiler were cut by 1x1 cm² size. The ileum was opened and washed twice with sterilized phosphate-buffer saline (PBS pH 7.2). Respectively, each piece of ileum tissue was held in PBS at 4 °C for 30 min to remove the surface mucus and then washed three times with PBS. The overnight broth culture of LAB were centrifuged at 7000 rpm for 10 min and resuspended in PBS. The suspension of LAB contained ±10^7 sel/mL. An ileal cell held in LAB suspension was incubated for 30 min at 37 °C. Meanwhile, the same preparation was performed without cell of LAB as control. After incubation, ileal cell was washed three times in PBS, extracted in 1 mL PBS, and vortexed with high speed for 2 min to obtain adhesive bacterial cells. Bacterial adherence was assessed by enumeration of the numbers of bacterial cell (from ileum extraction) on GYP+0.5% CaCO₃ agar plates. Agar plates were incubated for 24 h at 37 °C. The adhesion (%) was calculated according to the following equation:

\[
\text{Adhesion %} = \left( \frac{\log_{10} N_1 - \log_{10} N_0}{\log_{10} N_0} \right) \times 100\%
\]

N₀= cell numbers before treatment, N₁= cell numbers from ileum extraction after 30 min incubation.

**Molecular Identification of 16S rRNA Gene of LAB Isolates**

The early stage for molecular identification of 16S rRNA gene is isolation of genomic DNAs. Isolation of genomic DNAs of LAB isolates was done by using Genomic DNA Mini Kit Blood/Cultured Cell (Geneaid) according to the manufacturer’s protocol. The DNAs isolate from each LAB isolate were prepared for PCR reaction mixture. A PCR reaction mixture for each sample consisted of 20 µL GoTag Green Master Mix (Promega, USA), 2.5 µL of each primer (10 pmol), 10 µL nuclease free water, and 5 µL DNA template. The amplification reaction with forward primer 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and reverse primer 1387R (5'-GGG CGG WGT GTA CAA GGC-3') (Marchesi et al., 1998) using an Applied Biosystems Thermal Cycler 2720 (Life Tech). The temperature gradient of PCR reaction was set up at 94 °C for 5 min (an initial denaturation), 94 °C for 30 s (denaturation), 55 °C for 45 s (annealing), 72 °C for 1 min (elongation), these reaction (denaturation, annealing and elongation) were followed by 30 cycles, and then final elongation at 72 °C for 7 min. PCR products of each sample were confirmed by running an electrophoresis using 1% agarose gel in 1x TAE buffer for ±45 min on constanta 80 V and 33 mA. The DNA bands formed from electrophoresis process was visualized on UV transiluminator with ethidium bromide staining. The sequencing of genomic DNAs was conducted by sending the PCR products to a company providing sequences services. The DNA sequences were compared with other data sequences 16S rRNA gene in GenBank applied by using the BLASTN tools through the NCBI. The alignment of sequences used Clustal X program and was edited by MEGA 5.05 program. The construction of phylogenetic tree used neighbor-joining method by 1000 x bootstrap in MEGA 5.05 program (Felsenstein, 1985).

**Statistical Analysis**

All research data (except data of susceptibility to antibiotic and molecular identification) were shown as the mean ± standard deviation of three replicates of treatment. All quantitative data were calculated to ANOVA analysis by using IBM SPSS statistics 21.0. Duncan test was conducted as a further test to identify statistically significant differences in the experiment (α=0.05).

**RESULTS AND DISCUSSION**

**Susceptibility to Antibiotics**

Susceptibility of LAB isolates to antibiotics showed variations in results (Table 1). All isolates were resistant to bambermycin. Previous study by Jeong et al. (2009) reported that most species of LAB were found resistant to bambermycin and it was considered as an intrinsically resistant. The LAB intrinsically resistant allowed its use as probiotic (EFSA, 2012). Intrinsically resistant was caused by several natural physiological factors and non-transmissible (Pfaller, 2006). E3 isolate was resistant to erythromycin, chloramphenicol, and tetracycline. Similarly, E4 and E1223 isolates were resistant to erythromycin and tetracycline but intermediate to chloramphenicol. Thumu & Halami (2012) and Nawaz et al. (2011) reported that many LAB resistant to erythromycin, tetracycline, and chloramphenicol related to the presence of gene encoding resistant due to mutation or mobile genes. In contrast, Klare et al. (2007) reported that gram-positive bacteria are generally sensitive to antibiotics that inhibit the protein synthesis such as erythromycin and chloramphenicol. Isolates of E5, E7, and E8 did not resistant to erythromycin, chloramphenicol, and tetracycline. Some species of LAB showed phenotypic sensitive to tetracycline and chloramphenicol, then intermediate to erythromycin but the gene encoding of erythromycin resistance was not found (Ouoba et al., 2008). According to FAO/WHO (2002) and EFSA (2012) probiotics as feed additives should not resistant to anti-
biotics. Isolates E5, E7, and E8 were chosen to be tested to the next assay because they showed no resistant to antibiotics tested.

**Tolerance to Gastrointestinal pH and Bile Salt**

The result showed that each of LAB isolates had different survival abilities to different pH and bile salt conditions (Table 2 and Table 3). All LAB isolates had good survival ability at pH 7.2 condition. E8 isolate could survive better than E5 and E7 isolates at pH 4 condition. In contrast, E5 and E7 isolates had better survival ability than E8 isolate at pH 3 condition. Compared with previous study by Lin et al. (2007), the survival ability of E5, E7, and E8 isolates were better than L. fermentum which decreased about 2-3 Log CFU/mL at pH 3.2 condition after 3 h incubation. The LAB isolates that were able to survive at low pH could be due to the physiological mechanisms that regulate intracellular pH homeostasis (Guchte et al., 2002). The viable cell of LAB isolates decreased significantly at pH 2 conditions. This condition was similar with previous study by Anderson et al. (2010) reporting that L. plantarum DSM 2648 and L. rhamnosus HN001 were able to survive at pH 4 but both

**Table 1. Susceptibility of LAB isolates to antibiotics on MRS agar for 24 h**

<table>
<thead>
<tr>
<th>LAB isolates</th>
<th>Bambermycin (20 ppm)</th>
<th>Erythromycin (15 µg)</th>
<th>Chloramphenicol (30 µg)</th>
<th>Tetracycline (30 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1223</td>
<td>[R]</td>
<td>[R]</td>
<td>[I]</td>
<td>[R]</td>
</tr>
<tr>
<td>E3</td>
<td>[R]</td>
<td>[R]</td>
<td>[R]</td>
<td>[R]</td>
</tr>
<tr>
<td>E4</td>
<td>[R]</td>
<td>[I]</td>
<td>[I]</td>
<td>[R]</td>
</tr>
<tr>
<td>E5</td>
<td>[R]</td>
<td>[I]</td>
<td>[S]</td>
<td>[S]</td>
</tr>
<tr>
<td>E7</td>
<td>[R]</td>
<td>[I]</td>
<td>[S]</td>
<td>[S]</td>
</tr>
<tr>
<td>E8</td>
<td>[R]</td>
<td>[I]</td>
<td>[S]</td>
<td>[S]</td>
</tr>
</tbody>
</table>

Note: [Interpretation susceptibility to antibiotics: R= resistant, I= Intermediate, S= Sensitive]. Determination of interpretation according to standards described by Swenson et al. (1990) and Walker (2006): erythromycin (R= diz ≤ 13 mm, I= 13 mm < diz < 23 mm, S= diz ≥ 23 mm); chloramphenicol (R= diz ≤ 12 mm, I= 12 mm < diz < 18 mm, S= diz ≥ 18 mm); tetracycline (R= diz ≤ 14 mm, I= 14 mm < diz < 18 mm S= diz ≥ 18 mm); bambermycin (R= diz ≤ 15 mm, I= 15 mm < diz < 20 mm, S= diz ≥ 20 mm).

**Table 2. The decrease in cell viability (Log CFU/mL) of LAB isolates in MRS broth with various pH (3 h incubation) and MRS broth+0.5% (w/v) bile salt (5 h incubation) at 37 °C (n=3)**

<table>
<thead>
<tr>
<th>LAB Isolates</th>
<th>pH 2</th>
<th>pH 3</th>
<th>pH 4</th>
<th>pH 7.2</th>
<th>0.5% Bile Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>E5</td>
<td>6.02±0.12a</td>
<td>1.23±0.08a</td>
<td>0.68±0.21ab</td>
<td>0.47±0.03a</td>
<td>0.47±0.09a</td>
</tr>
<tr>
<td>E7</td>
<td>6.69±0.10a</td>
<td>1.35±0.13a</td>
<td>1.04±0.25ab</td>
<td>0.33±0.37a</td>
<td>0.40±0.07a</td>
</tr>
<tr>
<td>E8</td>
<td>5.80±0.04a</td>
<td>1.67±0.08a</td>
<td>0.29±0.24ab</td>
<td>0.12±0.07a</td>
<td>0.64±0.07b</td>
</tr>
</tbody>
</table>

Note: Means in the same column with different superscripts differ significantly (P<0.05).
isolates decreased about 6-7 Log CFU/mL at pH 2 condition. The survival ability of E8 isolate was better than E5 and E7 isolates at pH 2 condition shown by survival rate was 41.15±0.19%. Compared with previous study by Pan et al. (2009), survival ability of E8 isolate was better than L. acidophilus NIT, the survival rate of L. acidophilus NIT was zero after 2 h incubation at pH 2 conditions. The decrease of viable cell of LAB isolates was caused by oxidizing cell by acid. Hydrochloric acid (HCl) was secreted by stomach that can lead to oxidize cell components, damaging the membrane electron transport, decreased activity of various enzymes that are sensitive to acid (van de Guchte et al., 2002).

The observation of survival ability of LAB isolates to bile salt showed that the survival rate of all LAB isolates were above 90% (Table 2 and 3). These results were better than previous study by Lee et al. (2014) that reported survival rate of P. pentosaceus F66 was 26.6% at 0.3% bile salt condition after 2 h incubation and Jannah et al. (2014) reported that CCM011, CSP004, and CVM002 isolates could not grow at 0.3% bile salt condition after 5 h incubation. The LAB isolates are resistant to bile salts because of their ability to detoxify the conjugated bile acids into deconjugated bile acid by the activities of bile salt hydrolases (BSH). The conjugated bile acids have bactericidal effect against sensitive microorganisms (Begley et al., 2006).

Antimicrobial Activity of LAB against Pathogenic Bacteria

One of the major probiotic’s properties is their inhibitory effect against the growth of pathogenic bacteria. In this study, untreated supernatants (positive control) from all LAB isolates showed antimicrobial activity against S. enteritidis and E. casseliflavus (Table 4). The isolate of E8 showed the highest inhibitory effect against E. casseliflavus. These inhibitory effects could be due to the activity of organic acids (Makras & De Vuyst, 2006), bacteriocin or bacteriocin-like inhibitory substances and hydrogen peroxide (Klose et al., 2010) produced by LAB. The neutralized supernatants (pH 6.5) from all LAB isolates have no effects against S. enteritidis. This result indicated that the inhibitory activity was related to the organic acids produced by LAB. Untreatment supernatants pH 4.36 in this study. Organic acids produced by LAB lead to bactericidal effect against Gram-positive and Gram-negative (Tejero-Sarinena et al., 2012). In contrast, inhibitory activity was non-activated against E. casseliflavus when the neutralized supernatants were added by proteinase. This result indicated that the organic acid is not the only inhibitory factor of E. casseliflavus growth but is partly due to the inhibitory activities of bacteriocin or bacteriocin-like inhibitory substances. Based on these results, we can conclude that bacteriocin or bacteriocin-like inhibitory substances produced by LAB isolates were not effective in inhibiting S. enteritidis growth. Todorov & Dicks (2009) reported that bacteriocins from LAB species did not affect Gram-negative bacteria except few strains, however, effective to most of species closely related phylogenetic to the bacteriocin producer. Isolate of E8 had inhibitory activity of bacteriocin or bacteriocin-like inhibitory substances better than E5 and E7 isolates. Bacteriocin binds to non-specific receptors or specific receptor on the cell surface of Gram-positive sensitive, probably lipoteichoic acid layer. Ionic reaction between bacteriocins and cell membranes lead to membrane leakage and the release of the membrane components (Todorov & Dicks, 2009).

Ability of LAB Isolates to Adhere to Chicken Ileal Cell

All LAB isolates could adhere to the ileal cells. These results were exhibited by the number of viable cell of LAB isolates were significantly (P<0.05) higher than control after 30 min incubation (Table 5). These

<table>
<thead>
<tr>
<th>LAB isolates</th>
<th>Untreatment supernatant (positive control pH 2±3.6)</th>
<th>Neutralized supernatant (pH 6.5)</th>
<th>Neutralized supernatant (pH 6.5) added by K-Proteinase 1 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. enteritidis</td>
<td>E. casseliflavus</td>
<td>S. enteritidis</td>
</tr>
<tr>
<td>E5</td>
<td>12.7±0.50b</td>
<td>18.7±0.50b</td>
<td>-</td>
</tr>
<tr>
<td>E7</td>
<td>16.0±0.82b</td>
<td>16.5±0.57b</td>
<td>-</td>
</tr>
<tr>
<td>E8</td>
<td>17.7±0.93b</td>
<td>19.7±0.93b</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Means in the same column with different superscripts differ significantly (P<0.05). (- ) = no inhibition zone.
results also exhibited that each isolates had different adhesion abilities. The adhesion ability of E8 isolate was better than those of E5 and E7 isolates, which was indicated by the number of viable bacteria was 9.40±0.00 log CFU/cm² with adhesion cell was 94.77±0.09%, while E5 isolate had adhesion ability that was too low i.e., 81.81±1.46%. Babot et al. (2014) reported that two strains isolated from broiler’s intestine had different adhesion abilities, L. reuteri LET 206 was able to adhere to chicken ileal cell as much as 43.3% while L. reuteri LET 211 was able to adhere to ileal cell as much as 37%. The differences in adhesion abilities of strains are specific and depend on physiology of cell and composition of cell wall (Ranadheera et al., 2012). Adhesive activity was caused by the interaction between the multiple components of glycoproteins, lipid anchored proteins of the bacterial cell surface layer with specific receptor of molecules carbohydrate extracellular matrix layer of epithelial cells and intestinal mucus layer (Schillinger et al., 2005).

**Molecular Identification of 16S rRNA Gene of LAB Isolates**

Electrophoresis visualization showed that the DNA fragments of 16S rRNA gene amplification products of LAB isolates E5, E7, and E8 were ± 1500 bp (Fig 1a). The BLAST-N result showed that sequences alignment of three isolates of LAB (isolate E5, E7, and E8) had 99% similarity with *Pediococcus pentosaceus* ATCC 25745 strain. The phylogenetic dendrogram showed that isolates E5, E7, and E8 were clustered in a group and they were closely related with *P. pentosaceus* (Fig 1b).

**CONCLUSION**

LAB isolates E5, E7, and E8 were sensitive to antibiotics, able to survive to gastrointestinal pH and 0.5% bile salt, able to produce antimicrobial activity such as organic acids and bacteriocin-like inhibitory substances, and able to adhere to chicken ileal cell in *in vitro*. E8 isolate had a better potential compared to E5 and E7 isolates in most in *vitro* assay as a candidate probiotics. E5, E7, and E8 isolates had 99% similarity with *Pediococcus pentosaceus* ATCC 25745 strain.

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**REFERENCES**


