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Effectivity of Plantaricin from Indonesian Lactobacillus plantarum As Antimicrobial Substances against Escherichia coli

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ABSTRACT

Indonesian indigenous strains of Lactobacillus plantarum IIA-1A5 was isolated from Indonesian local beef, and identified by 16S rRNA sequencing. L. plantarum IIA-1A5 are able to produce plantaricin, termed plantaricin IIA-1A5. The aim of this research was to evaluate effectivity of plantaricin IIA-1A5 as antimicrobial substances against Escherichia coli. Parameters analyzed were the effect of the presence of lipoteichoic acid (LTA) on plantaricin IIA-1A5 adsorption, analysis of N-acetyl-glucosamine, bacterial cell leakage, and changes in bacterial cell morphology by scanning electron microscope. The result showed that LTA significantly improve the absorption of plantaricin to cell wall of E. coli 3-fold higher than in the absence of LTA. It is acceptable that the addition of LTA might facilitate the binding of plantaricin to the bacterium. Plantaricin IIA-1A5 could disrupt the N-acetyl-glucosamine (NAG) on peptidoglican of cell wall of E.coli. Microscopic morphology by Scanning Electron Microscope of E.coli treated with plantaricin IIA-1A5 was remarkably different compared to that of the untreated cells. This is hypothesized may be due to ability of plantaricin IIA-1A5 to disrupt cell membrane and promote cell leakage of E.coli.

Keywords: Lactobacillus plantarum, plantaricin, Escherichia coli

1. INTRODUCTION

Bacteriocins of lactic acid bacteria are potent food biopreservative agents and can be effective in controlling the incidences of food poisoning outbreaks. Bacteriocins are considered to be safe natural biopreservatives, because it is assumed that they are degraded by the proteases in the gastrointestinal tract and may be useful as a primary hurdle for controlling food-borne pathogens (Cleveland et al., 2001). Lactobacillus plantarum is lactic acid bacteria which produces bacteriocin, called plantaricin. Plantaricin A, Plantaricin EF and Plantaricin JK are included into Class II bacteriocin (Diep et al., 2009). Whereas Plantaricin W from Lactobacillus plantarum LMG 2379 belongs to a new family of two-peptide bacteriocins (Holo et al., 2001).

Our previous research showed that Indonesian indigenous strains of Lactobacillus plantarum was isolated from Indonesian beef e.g., L. plantarum IIA-1A5, L. plantarum IIA-1B1, L. plantarum IIA-1C4. They were identified species and strains by molecular technique using PCR and 16S rRNA sequencing, and have high similar identity as L. plantarum JDM 1 (97%) by phylogenetic tree analysis using Kimura model. The exploration about their functions have been done, such as antibacterial activities against pathogenic bacteria (Escherichia coli, Staphylococcus aureus and Salmonella Typhimurium). It was reported that all strains produces antibacterial substances that could inhibit growth of pathogenic bacteria. L. plantarum IIA-1A5 have better antimicrobial activities than others (Arief, 2011). It is being important to explore it completely. Purification the plantaricin as bacteriocin from indigenous L. plantarum IIA-1A5 is very important to describe their function as antimicrobial substances. Escherichia coli is Gram negative bacteria that commonly contaminate foods. Purification and biochemical characterization of plantaricin is essential to evaluate its potential for antimicrobial agents against E. coli.

2. MATERIALS AND METHODS

Bacterial strains and growth conditions

Lactobacillus plantarum IIA-1A5 was grown in MRS broth and agar media. Escherichia coli ATCC 25922 was grown in Nutrient Broth and Nutrient agar as slab cultured stock. Stock cultures are stored in media at -20°C, subculture twice for 24 hrs at 37°C in the same media and incubate at suitable temperature before use.

Purification of plantaricin

This experiment was carried out according to Tiwari and Srivastava (2008); Hata et al. (2010) modified by Arieff et al (2013). L. plantarum IIA-1A5 was grown in deMan Rogosa Sharpe (MRS) broth, supplemented with 3% yeast extract and incubated at 37°C without agitation for 20 hours for the production of bacteriocin. The cell are removed by centrifugation (10,000 rpm for 20 min, 4°C), followed by filter-sterilized (0.2 μm membrane). The plantaricin was purified from the cell free supernatant by ammonium sulfate precipitation and cation-exchange chromatography. In order to determine the molecular mass, purified plantaricin was subjected to SDS-page electrophoresis.

Analysis of mode of action plantaricin IIA-1A5 against E.coli

Effects of detergents, organic solvents and plantaricin IIA-1A5 on the cell leakage of E. coli. To test the effect of organic solvents on subsequent adsorption of plantaricin IIA-1A5, cells were suspended in methanol, ethanol, hexan and chloroform. Suspensions obtained after detergent or organic solvent treatments were mixed with plantaricin IIA-1A5 and incubated at 30°C for 30 min. After centrifugation, residual plantaricin IIA-1A5 was assayed in supernatant (Arieff et al., 2001).

Effect of the presence of lipoteichoic acid on plantaricin IIA-1A5 adsorption.

Purified lipoteichoic acid (2 mg) prepared from Staphylococcus aureus (Sigma) was added to 2.6 ml of E. coli cell suspension (10⁶ cfu mL⁻¹). Plantaricin is added at a final concentration of 160 AU mL⁻¹ and the mixture was incubated for 40 min at 30°C. Following centrifugation (15,000X g, 10 min), the supernatant were measured for residuals non-adsorbed plantaricin. Controls were cells mixed with plantaricin or lipoteichoic acid mixed with plantaricin (Arieff et al., 2001).

Analysis of N-acetyl-glucosamine.

The experiment was conducted to determine the effect of plantaricin IIA-1A5 of the bacterial cell wall by measuring the levels of N-acetyl-glucosamine as a precursor mukopeptida cell wall constituent.
Analysis of bacterial cell leakage. Observations leaks was carried out to study how plantaricin IIA-1A5 interfere with cell membrane permeability. The mechanism of destruction of the cell membrane is one sign of abnormal cells after treatment of plantaricin. Analysis of cell leakage spectrograms were performed using an UV-VIS Spectrophotometer at a wavelength of 280 nm and 260 nm (Atri et al., 2001).

Analysis of changes in bacterial cell morphology by Scanning Electron Microscope. Analysis of cell morphology due to the treatment of plantaricin IIA-1A5 include damage to cell morphology, structure of bacteria, as well as damage to the cell wall.

Data Analyzes
Data was collected and analyzed by Analysis of variance (ANOVA). If there were significantly different, the Duncan test was used for further analysis (Steel and Torrie, 1995).

3. RESULTS AND DISCUSSION

Effects of detergents, organics solvents and plantaricin IIA-1A5 on the cell leakage of E. coli
Cell leakage was investigated to study how plantaricin IIA-1A5 interferes with cell membrane permeability. The mechanism of destruction of the cell membrane is a common feature of the plantaricin-treated cells. Analysis of cell leakage spectrograms were performed using an UV-VIS Spectrophotometer at a wavelength of 260 nm and 280 nm to detect the presence of cellular proteins and genetic material (DNA or RNA), respectively. Higher absorbance value at these wavelengths indicates that more cellular content (protein or genetic materials) released from the cell due to more severe membrane cell disruption. The effectivity of plantaricin IIA-1A5 was similar with SDS and better than ethanol, hexane and methanol (Table 1).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Absorbance (280 nm)</th>
<th>Absorbance (260 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic solvent</td>
<td>80% Ethanol</td>
<td>1.41 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>80% Chloroform</td>
<td>7.20 ± 1.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>80% Hexane</td>
<td>2.18 ± 0.80&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>80% Methanol</td>
<td>0.52 ± 0.39&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Detergen</td>
<td>1% SDS</td>
<td>4.81 ± 1.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2% Triton X-100</td>
<td>12.27 ± 3.51</td>
</tr>
<tr>
<td>Plantaricin</td>
<td>20 AU/ml</td>
<td>5.33 ± 1.23</td>
</tr>
<tr>
<td>IIA-1A5</td>
<td></td>
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</tbody>
</table>

Value represents mean ± sd (n=6). Means in the same column with different superscript indicates significance differences (p<0.05).

Effect of the presence of lipoteichoic acid on plantaricin IIA-1A5 adsorption
Plantaricin to Gram negative absorption was smaller (25%) compared with Gram positive (40%) (Table 2). Plantaricin has low absorption due to Gram negative bacteria because Gram negative bacteria do not have the LTA on the cell wall peptidoglycan layer. The role of LTA is apparently to mediate interaction (attachment) bacteriocin to the cell wall. LTA located in peptidoglycan in large quantities on Gram positive bacteria. These results were in line with the inhibition of plantaricin to E. coli was smaller than S. aureus, and N-acetyl-glucosamine (NAG) that regardless of the E. coli was much less than the S. aureus. (Figure 1).

LTA was reported to improve the absorption of plantaricin to the cell wall of Gram positive and Gram negative bacteria. In this result, LTA was able to improve the effectiveness of absorption plantaricin to E. coli as 208%, while the addition of LTA to S. aureus only increase absorption of plantaricin by 25% (Table 2). Increased absorption of plantaricin in S. aureus was less than the E. coli because S. aureus has a lot LTA on the cell wall. LTA increase attachment of the plantaricin to Gram negative bacteria. For improvement purpose, plantaricin engineering might be required to create better plantaricin enable to kill Gram negative bacteria stronger, which can be done by the addition of those ways and hydrophobic groups on the sugar molecule plantaricin (LTA hydrophobic nature and had a sugar moiety).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>S. aureus (%)</th>
<th>E. coli (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plantaricin adsorbed with LTA</td>
<td>50</td>
<td>77</td>
</tr>
<tr>
<td>Plantaricin adsorbed without LTA</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Effectivity of LTA (%)</td>
<td>25</td>
<td>208</td>
</tr>
</tbody>
</table>

Analysis of N-acetyl-glucosamine
The inhibition of plantaricin to E.coli was smaller than S. aureus, and N-acetyl-glucosamine (NAG) regardless of the E. coli was much less than the S. aureus (Figure 1). Peptidoglycan on Gram positive bacteria is considerably thicker than Gram negative bacteria. Figure 1 showed that plantaricin can disrupt the N-acetyl-glycosamine on peptidoglycan of cell wall bacteria. These results were in line with diametere zone of inhibition from plantaricin.
Analysis of changes in bacterial cell morphology by Scanning Electron Microscopy (SEM)

Microscopic morphology of *S. aureus* treated with plantaricin IIA-1A5 was remarkably different compared to that of the untreated cells (Figure 2). This was hypothesized due to ability of plantaricin IIA-1A5 to disrupt cell membrane and promote cell leakage of *E. coli*. Untreated *E. coli* cell looks normal, with bacil-shaped cells clearly visible. However, after treatment with plantaricin IIA-1A5, cell rupture was visible which were smaller in size and show pores-like structures in the surface with cell material leaking from the cells. The result was similar with pediocin PD-1 acts on the cytoplasmic membrane of *Dinemococcus oeni*, and its activity may be due to the generation of pores in the cell membrane (Bauer et al., 2005).

![Image A](image1.png)

![Image B](image2.png)

Figure 2. Microscopic analysis of morphology *E.coli* treated by plantaricin IIA-1A5 (B) in comparison to control, in the absence of plantaricin IIA-1A5 (A). The bars in each figure correspond to 1 μm in size, as reference.

5. CONCLUSION

Plantaricin IIA-1A5 shows ability to promotes leakage of *E.coli*. Cell leakage is caused by cell membrane disruption by plantaricin IIA-1A5 to the release of proteinoac and genetic material, and *N-acetyl-glucosamine*. Lipoteichoic acid can increase the plantaricin adsorption. SEM analysis showed that plantaricin IIA-1A5 formed pores in the surface of *E.coli* cells. Mode of action plantaricin IIA-1A5 against *E. coli* seemed to be bacteriocidal rather than bacteriostatic.

6. ACKNOWLEDGEMENT

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7. REFERENCES


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