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Anti-adhesion bioactivity of tempeh against enterotoxigenic *Escherichia coli* can be improved through the addition of *Lactobacillus plantarum* culture during fermentation

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Abstract

Diarrhoea is prevalently widespread in developing countries and can lead to death. This disease could be caused by a prerequisite attachment of Enterotoxigenic *Escherichia coli* (ETEC) on human intestinal epithelial cells and followed by toxin production. Several antibiotics have been developed to reduce diarrhoeal severity. However, the use of antibiotics could also disrupt the balance of gut microbiota. This research found that tempeh fermented with the addition of *Lactobacillus plantarum* culture could decrease the adhesion of ETEC to the intestinal epithelial cells. This study measured the ability of *L. plantarum* to produce exopolysaccharide (EPS) and examined the effect of *L. plantarum* addition to tempeh, particularly its effect on anti-adhesion activity against ETEC. By using yeast agglutination assay to observe anti-adhesion activity, the data showed that soybean tempeh fermented with the addition of *L. plantarum* at OD_{600} of 0.5 had an increase of anti-adhesion bioactivity against ETEC nearly three times compared to tempeh without the addition of *L. plantarum* culture. Moreover, the exclusion test showed that EPS from *L. plantarum* could act as a preventive agent against ETEC adhesion to eukaryotic cells. Our finding indicated that the addition of lactic acid bacteria culture can be considered to augment the anti-adhesion bioactivity in soybean tempeh against ETEC.

1. Introduction

Diarrhoea is one of the world's health problems found in many developing countries, including Indonesia. According to the Indonesian Ministry of Health (2018), about 25.2% of children died each year because of diarrhoea. If handled inappropriately, diarrhoea can cause abdominal pain and malnutrition to the sufferers. This disease can be caused by food contaminated with bacteria, such as *Escherichia coli* (Okhuysen and DuPont, 2010).

Human gut microbiota is vastly diverse, and one of the bacteria that can be found is *E. coli*. Although generally harmless, there is also pathogenic *E. coli,* such as the Enterotoxigenic *E. coli* (ETEC) group. ETEC is found in contaminated food and can cause diarrhoea. This is due to its capability to adhere to intestinal epithelial cells (Bin *et al.*, 2018). ETEC infection can be deducted using antibiotics but the long-term use of antibiotics will disrupt the balance of gut microbiota (Pop *et al.*, 2016).

One of the food products that can maintain the condition of the intestinal microbiota is tempeh, which is an Indonesian fermented food made from soybeans (Stephanie *et al.*, 2017). The fermentation process of tempeh involves the mould *Rhizopus* spp. which can increase the protein content in the product. In addition, tempeh has high soluble protein content, which makes the nutrients in tempeh more bioavailable in the human body, thereby reducing the potential for malnutrition (Nout and Kiers, 2005).

Tempeh is also known to have anti-adhesion activity against ETEC, and its extract is reported to be capable of coating the surface of ETEC cells thus inhibiting ETEC from adhering to intestinal epithelial cells. This capability is derived from the presence of bioactive carbohydrates, which contain arabinose residues, that are released during tempeh fermentation. The bioactive carbohydrate can bind to the receptor site of ETEC, particularly on the fimbriae. Due to this mechanism, ETEC could not adhere to the intestinal epithelial cells and they are transformed into aggregates of bacteria that can be easily removed from the gastrointestinal system (Roubos-van den Hil *et al.*, 2009; Roubos-van den Hil *et*

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al., 2010).

Microorganisms that can be found in tempeh include the mould *Rhizopus* spp. and also bacteria such as lactic acid bacteria (LAB) and Enterobacteriaceae. LAB can act as a probiotic but, in tempeh, it functions more as a paraprobiotic, which are dead cells that have a good influence on the immune system (Karunaratne, 2018). Moreover, LAB can produce antibacterial compounds, such as nisin and lacticin, which have an inhibitory effect towards pathogens (Arqués *et al.*, 2015). LAB can also produce exopolysaccharide (EPS), which has been reported to exhibit anti-adhesion properties against ETEC (Caggianiello *et al.*, 2016). As of the time of this writing, there has been no report discussing the addition of LAB in tempeh to increase anti-adhesion activity against ETEC.

This research aimed to study the effect of *L. plantarum* cultures in addition to tempeh and to measure the ability of *L. plantarum* to produce EPS. This research focused on *L. plantarum* because the species has been categorized as food-grade, which is safe to consume and widely found in fermented foods. After tempeh was fermented, its effect on anti-adhesion activity against ETEC was observed. Yeast agglutination assay was used in this experiment to study the anti-adhesion bioactivity of the product, in which the yeast *Saccharomyces cerevisiae* acted as a substitute to epithelial cells to model ETEC adhesion to eukaryotic cells. The results of this study are expected to support the development of tempeh as a functional food product that can reduce the severity of diarrhoea.

2. Materials and methods

2.1 Lactobacillus plantarum culture preparation

Lactobacillus plantarum preparation was carried out based on the method of Starzyńska-Janiszewska *et al.* (2015) with modifications. *Lactobacillus plantarum* Lp-115 cultures were acquired from the culture collection of the Food Microbiology Laboratory, Atma Jaya Catholic University of Indonesia and grown anaerobically in 5 mL of de Man, Rogosa, and Sharpe (MRS) broth (HiMedia, India) and incubated at 37˚C for 24 hrs. A total of 100 µL of culture were inoculated into the new MRS liquid medium and incubated at 37˚C for 24 hrs. The cells were washed twice with phosphate buffer saline (PBS) pH 7.0 (Ambion, USA) and the cell density was measured at OD_{600}. The OD_{600} of the culture was adjusted accordingly to 0.5 and 1.0 by dilution using PBS pH 7.0.

2.2 Identification of Lactobacillus plantarum capability to produce exopolysaccharide

The ability of *L. plantarum* to produce EPS was based on the method used by Kim *et al.* (2008). *Lactobacillus plantarum* Lp-115 culture was inoculated in skim milk medium with the addition of sucrose 10% w/v and incubated at 37˚C for 48 hrs. Skim milk medium without sucrose addition was used as a control. The presence of EPS was indicated by solidified skim milk and clear liquid formed on its surface. ETEC was also grown in skim milk medium that contains sucrose 10% w/v as a control for the bacterial culture that does not produce EPS.

2.3 Exopolysaccharide extraction from Lactobacillus plantarum culture

The extraction of EPS from *L. plantarum* Lp-115 was carried out based on the method of Mostefaoui *et al.* (2014) with modifications. A total of 100 µL of bacterial culture that had been grown in 10 mL of MRS broth augmented with sucrose 10% w/v were incubated at 37˚C for 24 hrs. Then, the bacterial cell density was adjusted to OD_{600} of 1.0 and the suspension was heated in boiling water for 15 mins to inactivate enzymes in the culture and afterwards cooled to room temperature. The suspension was centrifuged for 30 mins, 7000×*g* to remove coagulated cells and protein and both the supernatant and pellets were collected. EPS was precipitated from the supernatant by adding 3× the volume of chilled ethanol 96% and the mixture was incubated at -20˚C overnight. The mixture was centrifuged for 30 mins, 7000×*g*, 4˚C and the obtained precipitates were resuspended in hot water and dialyzed for 48 h. The dialyzed EPS suspension was frozen at -80˚C and freeze-dried for 96 hrs. The freeze-dried EPS powder was stored at 4^{o}C for further analysis.

2.4 Tempeh fermentation

The process of making tempeh was done based on the method of Kustyawati (2009) with modifications. All tempeh samples were made with the same type of yellow -seeded elongated soybean. A total of 500 g of yellow soybeans were washed and then immersed in sterile water at room temperature overnight. Soybeans were boiled for 30 mins. Then, the soybeans were separated from the skin and boiled for 30 mins. The soybeans were cooled until room temperature and dried. Cooled and dried soybean seeds were inoculated with Raprima (PT. Aneka Fermentasi Industri, Bandung, Indonesia) starter 0.2% w/w of the total initial soybean weight and 1 mL of *L. plantarum* Lp-115 suspension with an OD_{600} of 0.5 or 1.0, then stirred until homogenous. Tempeh without the addition of *L. plantarum* Lp-115 and cooked soybeans were used as controls. The soybeans were wrapped in perforated plastics and then incubated at 30˚C for 48 h. The resulting tempeh was stored at 4^{o}C for further analysis.

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2.5 Extraction of bioactive carbohydrates from soybean tempeh

The extraction of the bioactive fractions was carried out based on the extraction method described by Roubos-van den Hil *et al.* (2009) with modifications. Diced tempeh was spread evenly on a tray and freeze-dried for 96 hrs. The freeze-dried results were ground until they became powder and stored in the refrigerator. A total of 7.5 g of sample powder was suspended in 100 mL of distilled water. The suspension was stirred for 1 hr and the pH was adjusted to 8.0 every 30 mins using 2M NaOH solution. The suspension was centrifuged at 7000×*g* for 30 mins and the supernatant was collected and filtered using Whatman Grade 1 filter paper (Merck, USA). The eluent was collected and then freeze-dried for 96 hrs. The dried fraction was stored in the freezer for further analysis.

2.6 Culture preparation for anti-adhesion assay

Yeast and ETEC culture preparations were carried out based on the method of Mirelman *et al.* (1980) with modifications. *Saccharomyces cerevisiae* and ETEC cultures were grown respectively in potato dextrose broth (HiMedia) at 30°C overnight with shaking at 120 rpm and Luria agar (HiMedia) at 37°C overnight without shaking. The cultures were transferred to 1 mL vial tubes each and centrifuged at 3000×*g* for 10 mins. The supernatant was removed and the pellets were washed twice with 500 µL PBS pH 7.0 and centrifuged again. The pellets were resuspended in 1 mL of PBS pH 7.0. The cell density was measured at OD_{600}, and was adjusted with PBS to 1.0 and 0.5 for *S. cerevisiae* and ETEC respectively.

2.7 Anti-adhesion bioactivity assay

Anti-adhesion bioactivity of the samples was determined using yeast agglutination assay as described by Mirelman *et al.* (1980) with modifications. The same amount of tempeh extract suspension and ETEC culture were mixed in a 96-well microplate and incubated for 10 mins at 300 rpm agitation. Then, the mixture of tempeh extract suspension and ETEC was added with yeast culture in the same amount and incubated for 30 mins at 300 rpm agitation. The suspension was transferred to the concave slides and observed under a light microscope (Nikon Eclipse E100; Tokyo, Japan). The number of yeast cells in agglutinates was calculated from 12 points at 400× magnification using the DinoCapture 2.0 application (Dino-Lite, Torrance, CA, USA). A suspension of ETEC and yeast with PBS pH 7.0 was used as a negative control. Meanwhile, mannose 1% w/v (Sigma Aldrich, Darmstadt, Germany) was used as a positive control. An Anti-adhesion assay was also carried out on *L. plantarum* Lp-115 EPS extracts from both the supernatant and pellet. The anti-adhesion percentage of the samples was determined by comparing the number of cells that form agglutinates in suspension treated with the sample to the negative control. All experiments were done in triplicates.

2.8 Exclusion and displacement test

An exclusion test was carried out based on the method of Zhu *et al.* (2019) with modifications to obtain more information on the mechanism of anti-adhesion bioactivity of the EPS extract. In the exclusion test, the same volume of EPS suspension and yeast culture were mixed in a 96-well microplate and was incubated for 10 mins, 300 rpm, and then was added with ETEC culture in the same amount and incubated for 30 mins, 300 rpm. The displacement test had a similar procedure but EPS suspension was added to a mixture of yeast and ETEC instead. The suspension was transferred to concave slides and anti-adhesion bioactivity was determined using the same approach as the one in the previous part. All experiments were done in triplicates.

3. Results

3.1 Exopolysaccharide-producing capability of Lactobacillus plantarum

Figure 1 shows the difference between the *L. plantarum* grown in skim milk media with and without the addition of sucrose 10% w/v. It can be seen that *L. plantarum* culture grown in skim milk without sucrose did not undergo any alteration in the appearance of the medium which looks just like regular skim milk (Figure 1A). On the other hand, EPS was formed in *L. plantarum* culture grown in skim milk augmented with sucrose 10% w/v as indicated by the presence of clear liquid while skim milk coagulated as shown in Figure 1B. ETEC was used in this experiment due to its inability to produce EPS indicated by the absence of skim milk coagulation when it was grown in skim milk and sucrose 10% w/v medium (Figure 1C).

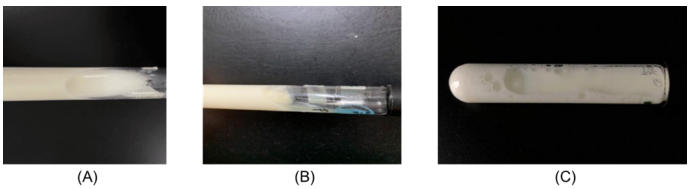

Figure 1. EPS-producing capability of *L. plantarum* shown as a comparison between A) *L. plantarum* grown in skim milk medium, B) *L. plantarum* grown in skim milk medium added with sucrose 10% w/v and C) ETEC grown in skim milk medium added with sucrose 10% w/v. Coagulation of skim milk was observed only in *L. plantarum* culture grown in skim milk medium added with sucrose 10% w/v indicating EPS production. Cultures were incubated at 37˚C for 48 hrs.

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3.2 Exopolysaccharide produced by Lactobacillus plantarum exhibited anti-adhesion bioactivity against ETEC

Anti-adhesion bioactivity of EPS was tested with yeast agglutination assay. *Lactobacillus plantarum* was grown in MRS broth augmented with sucrose 10% w/v to stimulate the production of bacterial EPS. After extraction with ethanol, EPS fraction was present in the supernatant which was collected and tested. Figure 2 shows that EPS from *L. plantarum* resulted in adhesion inhibition activity at 84.08±2.54%. This level of adhesion inhibition bioactivity was close to mannose which acted as a positive control. It indicates that EPS from *L. plantarum* does have the ability to prevent ETEC from adhering to the eukaryotic cells. The supernatant from ETEC culture grown in skim milk and sucrose was also subjected to yeast agglutination assay which yielded a negligible level of anti-adhesion bioactivity at 2.91±0.99%. Yeast agglutination assay of the medium showed that the sucrose content in the medium did not result in adhesion inhibition thus eliminating possible bias caused by the medium component.

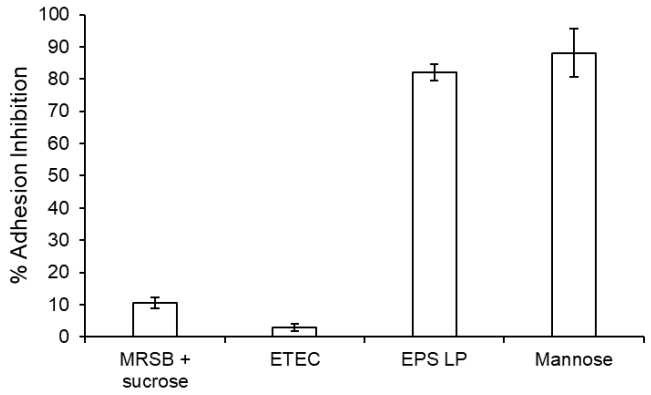

Figure 2. Adhesion inhibition bioactivity against ETEC adhesion to *S. cerevisiae* from MRSB + sucrose 10% w/v, supernatant from ETEC culture, *L. plantarum*'s exopolysaccharide (EPS LP), and mannose (positive control). Both ETEC and *L. plantarum* cultures were grown in MRS broth augmented with sucrose 10% w/v and incubated at 37˚C for 24 hrs. Bars indicate averaged value from three experiments. Vertical lines indicate standard error.

3.3 Exopolysaccharide from Lactobacillus plantarum showed both exclusion and displacement bioactivity against Enterotoxic Escherichia coli

The anti-adhesion bioactivity measured in the previous part illustrated the capability of EPS from *L. plantarum* in inhibiting ETEC adhesion to eukaryotic cells by pre-emptively binding to bacterial fimbriae. Other possible anti-adhesion mechanisms of the EPS were also explored by conducting exclusion and displacement tests. In the exclusion test, eukaryotic cells were exposed to the sample before exposure to ETEC. This sequence allowed us to assess the capability of the sample as a protective agent against ETEC adhesion by pre-emptively binding to binding sites on eukaryotic cells thus excluding ETEC from further interaction. In the displacement test, the sample was added to eukaryotic cells that have been previously exposed to ETEC in order to see its capability to displace adhering ETEC cells.

It is shown in Figure 3 that EPS from *L. plantarum* exhibited both exclusion and displacement bioactivity against ETEC adhesion to eukaryotic cells. However, the exclusion bioactivity of the sample was slightly stronger than its displacement bioactivity at 58.99±7.32% and 41.12±6.77% respectively. This indicated that EPS from *L. plantarum* tended to act more as a preventive agent rather than a displacing agent. The displacement test showed that EPS from *L. plantarum* can only weakly displace the attachments of ETEC to eukaryotic cells in a condition where ETEC has previously adhered to the cells. However, it should be noted that both the exclusion and the displacement bioactivities of EPS from *L. plantarum* were lower compared to its anti-adhesion bioactivity. This gave a strong indication that the optimum anti-adhesion bioactivity was achieved by initial exposure of ETEC to EPS.

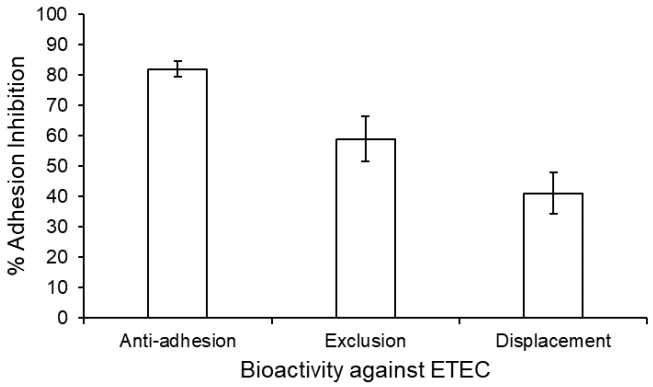

Figure 3. Adhesion inhibition of EPS from *L. plantarum* based on anti-adhesion, exclusion and displacement tests. Bars indicate averaged value from three experiments. Vertical lines indicate standard error.

3.4 Addition of Lactobacillus plantarum culture during fermentation can increase the anti-adhesion bioactivity of soybean tempeh against Enterotoxic Escherichia coli

The anti-adhesion test was carried out on boiled soybeans, soybean tempeh with and without the addition of *L. plantarum* culture with OD_{600} of 0.5 and 1.0, and mannose as a positive control. The results of the adhesion inhibition activity test can be seen in Figure 4. The samples tested showed a varying level of anti-adhesion activities. The anti-adhesion activity of mannose was the highest at 88.04±7.45%. Boiled soybeans showed the lowest anti-adhesion activity

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compared to the other samples at 14.11±4.10% confirming that the anti-adhesion activity against ETEC is likely due to the fermentation process rather than being inherent in soybeans. Interestingly, it was found that tempeh with the addition of *L. plantarum* culture was better in preventing the attachment of ETEC to eukaryotic cells. The anti-adhesion activity of the extract of tempeh + *L. plantarum* OD_{600} 0.5 was 83.01±5.85%. This level of anti-adhesion bioactivity was close to that of the positive control. Surprisingly, the anti-adhesion bioactivity of tempeh + *L. plantarum* OD_{600} 1.0 was lower at 65.36%±12.04. This indicated that an increase in the amount of LAB culture that is added to tempeh fermentation might decrease the adhesion inhibition bioactivity of tempeh against ETEC. Nevertheless, the anti-adhesion bioactivity of both samples was higher compared to tempeh without the addition of *L. plantarum* culture (28.35±6.88). This experiment showed that, in general, the addition of LAB culture during tempeh fermentation can improve the anti-adhesion bioactivity of the product against ETEC.

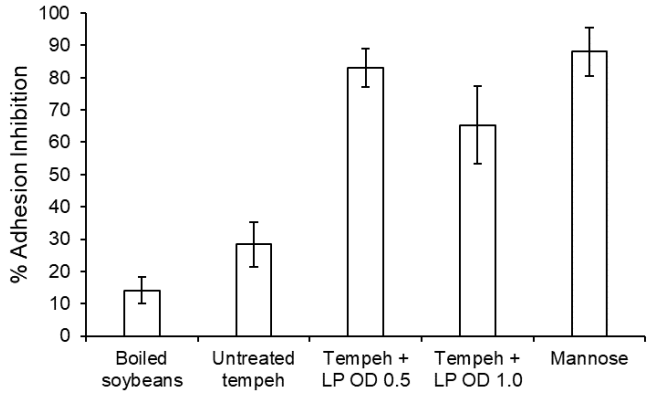

Figure 4. Adhesion inhibition bioactivity against ETEC adhesion to *S. cerevisiae* from the extract of boiled soybeans, untreated tempeh (tempeh without any *L. plantarum* grown culture addition), tempeh with the addition of *L. plantarum* OD_{600} 0.5 (Tempeh + LP OD 0.5), tempeh with the addition of *L. plantarum* OD_{600} 1.0 (Tempeh + LP OD 1.0), and mannose (positive control). Bars indicate averaged value from three experiments. Vertical lines indicate standard error.

4. Discussion

Diarrhoea in humans is commonly found in developing countries due to infection by ETEC that will cause several side effects, such as severe abdominal pain to watery discharge. ETEC infection is widespread through contaminated foods and drinking water (Qadri *et al.*, 2005). Tempeh modified with the addition of *L. plantarum* culture was tested for anti-adhesion bioactivity against ETEC and the result was a remarkable outcome. Tempeh is one of the Indonesian fermented products derived from soybeans and is known to have bioactive components that can profoundly reduce the severity of diarrhoea by preventing ETEC from adhering to human epithelial cells (Roubos-van den Hil *et al.*, 2009). In addition, our finding indicates that ETEC inhibition on the eukaryotic cell can be prevented by EPS from tempeh extract.

Before *L. plantarum* was added to tempeh fermentation, its EPS producing capability was observed on a skim milk media with 10% w/v sucrose addition, which resulted in the formation of EPS indicated as a clear liquid on the surface of the media. Moreover, the skim milk was clotted, indicating that *L. plantarum* contributed to the transformation of the texture of the media due to the release of EPS (Caggianiello *et al.*, 2016). *Lactobacillus plantarum* might also have used several carbon sources, such as glucose, galactose, fructose, lactose, and sucrose, which were present in skim milk. The presence of lactose and sucrose effectively enhanced the production of EPS during its exponential phase. Sucrose is added not only to increase EPS production but also to enhance the optical density of the bacteria (Kim *et al.*, 2008). Moreover, EPS produced by *L. plantarum* can also be derived from environmental stress factors, such as excessive nutrients added to the culture medium (Nguyen *et al.*, 2020). In addition, nutrition in the medium must be sufficient and the incubation temperature must be optimum (Tallon *et al.*, 2003), which in this study was conducted at 37˚C, in order to stimulate EPS production.

EPS from *L. plantarum* was characterized for its capability to inhibit ETEC adhesion to eukaryotic cells, which in this research was represented by *S. cerevisiae*. A crucial observation that can be made is that the anti-adhesion activity of EPS from *L. plantarum* was virtually on the same level as mannose (Figure 1), which was the positive control for the anti-adhesion assay. This shows that EPS from *L. plantarum* had a massive anti-adhesion effect on ETEC. It was presumably because EPS from *L. plantarum* contained receptors that can be recognized by ETEC, thus preventing the attachment of ETEC on eukaryotic cells. Carbohydrates such as glucose and mannose can also be present in EPS from *L. plantarum* (Liu *et al.*, 2017). These carbohydrates have a similar structure to adherence sites on ETEC, resulting in an attachment competition to eukaryotic cells (Pretzer *et al.*, 2005). Moreover, inhibition of ETEC adhesion to eukaryotic cells by EPS has been previously reported to be exhibited by other lactic acid bacteria, such as *Lactobacillus reuteri* (Wang *et al.*, 2010).

ETEC has been reported to adhere to human epithelial cells by its attachment to the mucosal surfaces to induce toxins secretion. Hence, it is crucial to observe whether EPS from *L. plantarum* can either prevent or relieve the adhesion of ETEC to the eukaryotic cell

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surface. This study demonstrated the inhibition activity of EPS using exclusion and displacement tests. The exclusion test was aimed to see the ability to block the cells' receptors, and the displacement test was to observe the ability of the sample to displace ETEC that are already attached to the cells (Zhu *et al.*, 2019). Both of these tests show distinct mechanisms of anti-adhesion activity.

In the exclusion test, EPS and yeast were pre-mixed together enabling EPS to adhere onto yeast, and when ETEC was then added to the mixture, anti-adhesion bioactivity at 58.99±7.32% was observed (see Figure 3), which was lower than the anti-adhesion activity of tempeh with added *L. plantarum* culture at 83.01±5.85% (see Figure 4). This indicates that EPS might have coated several parts of eukaryotic cells prior, implying that EPS can avert the adherence of ETEC to eukaryotic cells. However, EPS could not thoroughly act as a preventive substance towards ETEC compared to tempeh, which had a higher anti-adhesion capability. This might be due to the inadequate amount of EPS production by *L. plantarum* to inhibit ETEC attachment, and it was also possible that the specificity of EPS in adhering to yeast cells was constrained. This could be caused by various factors, such as the physicochemical and structural characteristics of the EPS and the environmental circumstances during fermentation (Zhu *et al.*, 2019).

Anti-adhesion activity from the exclusion test was higher than that of the displacement test. This shows that both EPS and ETEC have related structures of carbohydrate receptors that will recognize and attach to eukaryotic cells (Liu *et al.*, 2017). The more similar in carbohydrate structure between EPS and ETEC fimbriae receptors, the more potential they can compete and attach to the eukaryotic cells. Moreover, several factors can contribute to ETEC inhibition by EPS, such as aggregator compounds and receptor analogues (Wang *et al.*, 2010). On the contrary, in the case of the displacement test, pre-mixed ETEC and yeast were then added with EPS and the effect was lower than the exclusion test at 41.12±6.77% (see Figure 3). This result confirmed that when ETEC had attached to the eukaryotic cells mediated by type-1 fimbriae on ETEC, it will hardly move away due to the exact receptors of ETEC that had adhered to the eukaryotic cells. Thus, EPS was unable to compete with ETEC (Pretzer *et al.*, 2005). However, as shown in the exclusion test, yeast and EPS adhesion were stronger than ETEC and EPS adhesion. Hence, it indicated that EPS could displace some ETEC attachments from the eukaryotic cells.

During fermentation, *L. plantarum* culture was grown to the OD_{600} of 0.5 and 1.0 and was added to soybeans. The bioactive components from this modified tempeh were extracted, freeze-dried, measured, and examined for anti-adhesion bioactivity. Research conducted by Roubos-van den Hil *et al.* (2010) indicated that bioactive arabinose in tempeh was found in the fraction with a molecular weight of >30kDa explaining the presence of bioactive components in untreated tempeh. Based on the yeast agglutination assay, tempeh with added *L. plantarum* culture at OD_{600} 1.0 had lower anti-adhesion activity. This could be due to the abundant *L. plantarum* cells added during fermentation that caused a high demand for carbohydrates to grow and survive. However, as there was only a limited amount of carbohydrates, *L. plantarum* consumed bioactive carbohydrates in tempeh resulting in lower anti-adhesion activity. To some extent, further research using in vivo testing is necessary to consider contributing nutrients uptake and metabolism of eukaryotic cells to ensure the efficacy of tempeh with *L. plantarum* addition in both preventing and reducing ETEC adhesion in humans.

5. Conclusion

By using distinctive concentrations of *L. plantarum* added during tempeh fermentation, it is possible to enhance the anti-adhesion activity of tempeh against ETEC. Tempeh added with *L. plantarum* OD_{600} 0.5 appears as the most effective to increase its anti-adhesion activity possibly due to the capability of *L. plantarum* in producing bioactive EPS during fermentation. Furthermore, the EPS from *L. plantarum* was proven from the exclusion test to be capable of acting as a preventive measure against ETEC adhesion to eukaryotic cells.

Conflict of interest

The authors declare no conflict of interest.

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References

Arqués, J.L., Rodríguez, E., Langa, S., Landete, J.M. and Medina, M. (2015). Antimicrobial Activity of Lactic Acid Bacteria in Dairy Products and Gut: Effect on Pathogens. *BioMed Research International*, 2015, e584183. https://doi.org/10.1155/2015/584183

Bin, P., Tang, Z., Liu, S., Chen, S., Xia, Y., Liu, J., Wu, H. and Zhu, G. (2018). Intestinal microbiota mediates Enterotoxigenic *Escherichia coli*-induced

diarrhea in piglets. *BMC Veterinary Research*, 14(1), 385. https://doi.org/10.1186/s12917-018-1704-9

Caggianiello, G., Kleerebezem, M. and Spano, G. (2016). Exopolysaccharides produced by lactic acid bacteria: From health-promoting benefits to stress tolerance mechanisms. *Applied Microbiology and Biotechnology*, 100(9), 3877–3886. https://doi.org/10.1007/s00253-016-7471-2

Indonesian Ministry of Health. (2018). Laporan Hasil Riset Kesehatan Dasar (Riskesdas). Retrieved on March 18, 2021 from Badan Litbangkes Kementerian Kesehatan RI: https://www.litbang.kemkes.go.id/laporan-riset-kesehatan-dasar-riskesdas/ [In Bahasa Indonesia].

Karunaratne, A.M. (2018). Probiotic foods: Benefits to the cereal based Sri Lankan diet. *Ceylon Journal of Science*, 47(2), 105–123. https://doi.org/10.4038/cjs.v47i2.7506

Kim, M.J., Seo, H.N., Hwang, T.S., Lee, S.H. and Park, D.H. (2008). Characterization of exopolysaccharide (EPS) produced by *Weissella hellenica* SKkimchi3 isolated from kimchi. *Journal of Microbiology (Seoul, Korea)*, 46(5), 535–541. https://doi.org/10.1007/s12275-008-0134-y

Kustyawati, M. (2009). Kajian Peran Yeast dalam Pembuatan Tempe. *Agritech*, 29(2), 64–70.

Liu, Z., Zhang, Z., Qiu, L., Zhang, F., Xu, X., Wei, H. and Tao, X. (2017). Characterization and bioactivities of the exopolysaccharide from a probiotic strain of *Lactobacillus plantarum* WLPL04. *Journal of Dairy Science*, 100(9), 6895–6905.

Mirelman, D., Altmann, G. and Eshdat, Y. (1980). Screening of bacterial isolates for mannose-specific lectin activity by agglutination of yeasts. *Journal of Clinical Microbiology*, 11(4), 328–331. https://doi.org/10.1128/jcm.11.4.328-331.1980

Mostefaoui, A., Hakem, A., Yabrir, B., Boutaiba, S. and Badis, A. (2014). Screening for exopolysaccharide-producing strains of thermophilic lactic acid bacteria isolated from Algerian raw camel milk. *African Journal of Microbiology Research*, 8(22), 2208–2214. https://doi.org/10.5897/AJMR2014.6759

Nguyen, P.-T., Nguyen, T.-T., Bui, D.-C., Hong, P.-T., Hoang, Q.-K. and Nguyen, H.-T. (2020). Exopolysaccharide production by lactic acid bacteria: The manipulation of environmental stresses for industrial applications. *AIMS Microbiology*, 6(4), 451–469. https://doi.org/10.3934/microbiol.2020027

Nout, M.J.R. and Kiers, J.L. (2005). Tempe fermentation, innovation and functionality: Update into the third millenium. *Journal of Applied Microbiology*, 98(4), 789–805. https://doi.org/10.1111/j.1365-2672.2004.02471.x

Okhuysen, P.C. and DuPont, H.L. (2010). Enteroaggregative *Escherichia coli* (EAEC): A Cause of Acute and Persistent Diarrhea of Worldwide Importance. *The Journal of Infectious Diseases*, 202(4), 503–505. https://doi.org/10.1086/654895

Pop, M., Paulson, J.N., Chakraborty, S., Astrovskaya, I., Lindsay, B.R., Li, S., Bravo, H.C., Harro, C., Parkhill, J., Walker, A.W., Walker, R.I., Sack, D.A. and Stine, O.C. (2016). Individual-specific changes in the human gut microbiota after challenge with enterotoxigenic *Escherichia coli* and subsequent ciprofloxacin treatment. *BMC Genomics*, 17(1), 440. https://doi.org/10.1186/s12864-016-2777-0

Pretzer, G., Snel, J., Molenaar, D., Wiersma, A., Bron, P.A., Lambert, J., de Vos, W.M., van der Meer, R., Smits, M.A. and Kleerebezem, M. (2005). Biodiversity-Based Identification and Functional Characterization of the Mannose-Specific Adhesin of *Lactobacillus plantarum*. *Journal of Bacteriology*, 187(17), 6128–6136. https://doi.org/10.1128/JB.187.17.6128-6136.2005

Qadri, F., Svennerholm, A.-M., Faruque, A.S.G. and Sack, R.B. (2005). Enterotoxigenic *Escherichia coli* in Developing Countries: Epidemiology, Microbiology, Clinical Features, Treatment, and Prevention. *Clinical Microbiology Reviews*, 18(3), 465–483. https://doi.org/10.1128/CMR.18.3.465-483.2005

Roubos-van den Hil, P.J., Nout, M.J.R., Beumer, R.R., van der Meulen, J. and Zwietering, M.H. (2009). Fermented soya bean (tempe) extracts reduce adhesion of enterotoxigenic *Escherichia coli* to intestinal epithelial cells. *Journal of Applied Microbiology*, 106(3), 1013–1021. https://doi.org/10.1111/j.1365-2672.2008.04068.x

Roubos-van den Hil, P.J., Schols, H.A., Nout, M.J.R., Zwietering, M.H. and Gruppen, H. (2010). First Characterization of Bioactive Components in Soybean Tempe That Protect Human and Animal Intestinal Cells against Enterotoxigenic *Escherichia coli* (ETEC) Infection. *Journal of Agricultural and Food Chemistry*, 58(13), 7649–7656. https://doi.org/10.1021/jf101379y

Starzyńska-Janiszewska, A., Stodolak, B. and Wikiera, A. (2015). Antioxidant Potential and α-galactosides Content of Unhulled Seeds of Dark Common Beans Subjected to Tempe-type Fermentation with *Rhizopus microsporus* var. *Chinensis* and *Lactobacillus plantarum*. *Food Science and Technology Research*, 21(6), 765–770. https://

doi.org/10.3136/fstr.21.765

Stephanie, S., Ratih, N.K., Soka, S. and Suwanto, A. (2017). Effect of Tempeh Supplementation on the Profiles of Human Intestinal Immune System and Gut Microbiota. *Microbiology Indonesia*, 11(1), 2. https://doi.org/10.5454/mi.11.1.2

Tallon, R., Bressollier, P. and Urdaci, M.C. (2003). Isolation and characterization of two exopolysaccharides produced by *Lactobacillus plantarum* EP56. *Research in Microbiology*, 154 (10), 705–712. https://doi.org/10.1016/ j.resmic.2003.09.006

Wang, Y., Gänzle, M.G. and Schwab, C. (2010). Exopolysaccharide Synthesized by *Lactobacillus reuteri* Decreases the Ability of Enterotoxigenic *Escherichia coli* To Bind to Porcine Erythrocytes. *Applied and Environmental Microbiology*, 76(14), 4863–4866. https://doi.org/10.1128/AEM.03137-09

Zhu, Y., González-Ortiz, G., Benítez-Cabello, A., Calero -Delgado, B., Jiménez-Díaz, R. and Martín-Orúe, S.M. (2019). The use of starter cultures in the table olive fermentation can modulate the antiadhesive properties of brine exopolysaccharides against enterotoxigenic *Escherichia coli*. *Food and Function*, 10(6), 3738–3747. https://doi.org/10.1039/ C9FO00425D

