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Research Article
Prebiotic Effect of Inulin Extract from Dahlia Tubers (*Dahlia Pinnata* L.) On the Growth Performance of Intestinal-origin *Lactobacillus Casei* AP.

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Abstract

Background and Objective: A prebiotic is a non-digestible food component that supports the growth of probiotics in the gastrointestinal tract, thus providing beneficial effects to the host. One commercially available prebiotic is inulin, produced from Jerusalem artichokes and chicory. A previous study reported that the inulin from dahlia tubers is of better quality due to the presence of soluble and insoluble fibres. Due to its potential, inulin from dahlia tubers has been increasingly tested for its capability as a prebiotic. The aim of this study was to investigate the prebiotic effects of inulin and inulin extract from dahlia tubers on the viability of *Lactobacillus casei* AP and representative Enterobacteriaceae and to measure the prebiotic index and production of short-chain fatty acids. **Methodology:** This study focused on the combined effect of a prebiotic (inulin or inulin extract from dahlia tubers) and a probiotic, *L. casei* AP, which originated in a human gastrointestinal tract. This combination resulted in the growth suppression of pathogenic bacteria, the growth promotion of the probiotic and a high prebiotic index. The combination of *L. casei* AP and inulin/inulin extract, which was defined as a synbiotic, was also evaluated for the growth suppression of *H. pylori* and *S. dysenteriae*. **Results:** The *L. casei* AP had a higher growth rate in the presence of inulin extract or inulin than MRS did. Co-culturing *E. coli* and *L. casei* AP in media containing inulin extract suppressed the growth of *E. coli* and simultaneously resulted in the highest prebiotic index, at 4.70. The synbiotic effect of inulin extract together with *L. casei* AP was stronger in suppressing the growth of *S. dysenteriae* than of *H. pylori*. This inhibition was concomitant with the production of short-chain fatty acids, lactate, propionate, propionate and butyrate, which contributed to lowering the pH in the media. During fermentation, lactate was produced at the highest concentration (33.83-35.53%), followed by acetate (9.83-10.35%), propionate (0.05-0.45%) and butyrate (0.03-0.49%). **Conclusion:** Inulin extract from dahlia tubers acts very well as a prebiotic by supporting the growth of probiotic *L. casei* AP and suppressing the growth of the pathogens *S. dysenteriae* and *H. pylori*, resulting in a high prebiotic effect.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inulin is a storage carbohydrate that is found in more than 36.000 plant species[1]. Three main plant species [Jerusalem artichoke (*Helianthus tuberosus* L.), dahlia (*Dahlia pinnata* L.) and chicory (*Cichorium intybus*)] are currently employed to produce inulin. Inulin produced from Jerusalem artichoke and chicory is the main source for the inulin available on the market, whereas inulin produced from dahlia tubers has not yet been developed or made available on the market. A previous study reported that the inulin from dahlia tubers is of better quality due to the presence of soluble and insoluble fibres[2]. Kusmiyati *et al.*[3] previously reported a selective method, ultrasonic- and microwave-assisted extraction (UMAE), that yielded high concentrations of inulin from dahlia tubers with a degree of polymerization of 19-20 and a molecular weight of 3.173-3.316.

Inulin fermentation *in vivo* contributes significantly to the increase of certain metabolites. This prebiotic can be selectively fermented by certain probiotics and other beneficial gastrointestinal microbes into short-chain fatty acids (SFCAs), mainly lactate, acetate, propionate and butyrate. The production of these metabolites eventually decreases the luminal pH and exerts an antagonistic activity on a large number of enteric pathogenic bacteria[4] apart from competition for nutrients or for niche establishment[5]. In this study, the inulin extracted from dahlia tubers, together with commercial inulin, was assessed for its capacity to support the growth of probiotics and suppress pathogens and was measured for its prebiotic index (PI). *Lactobacillus casei* AP was used to measure PI due to its capability as a probiotic[6]. Widodo *et al.*[6] reported that *Lactobacillus casei* AP, isolated from an Indonesian's gastrointestinal tract, was capable of utilizing inulin as a carbon source. This present study investigated the capabilities of the probiotic *L. casei* AP and inulin extract from dahlia tubers to synergistically control the growth of *S. dysenteriae* ATCC 11835 and *H. pylori* ATCC 26695. The objectives of this study were to investigate the prebiotic effects of inulin and inulin extract from dahlia tubers and to measure the prebiotic index and production of short chain fatty acids.

MATERIAL AND METHODS

Bacterial cultures and media preparation: This study was a laboratory-based experiment carried out at the Research Centre for Biotechnology, Universitas Gadjah Mada, Yogyakarta, Indonesia. Bacterial cultures used in this study were *Lactobacillus casei* AP[7], *Enteropathogenic Escherichia coli* (EPEC), *Shigella dysenteriae* ATCC 11835 and *Heliobacter pylori* ATCC 26695. The media used were inulin extract from dahlia tubers (*Dahlia pinnata* L.), inulin (Sigma-Aldrich, MO, USA), commercial de-Mann Rogosa Sharpe (MRS), synthetic de-Mann Rogosa Sharpe (glucosa-MRS), Cook Meat Medium (CMM) (Sigma-Aldrich, MO, USA), Plate Count Agar (PCA) (Oxoid, Hampshire, UK) and Trypticase Soy Broth/Agar (TSB/A) (Merck, Darmstadt, Germany).

Growth media for *L. casei* AP was MRS broth: The TSB was used for the propagation of *H. pylori* and *S. dysenteriae*. *L. casei* AP growth was microaerophilic/anaerobic (Oxoid, Hampshire, UK). The media used as the control and as the base for inulin media were sterilized by autoclaving at 121°C for 15 min. In the case of modified MRS broth, filter-sterilized L-cysteine hydrochloride was added to the autoclaved media. Filter-sterilized carbohydrates (inulin extract, inulin and glucose) were added to the modified MRS broth to final concentrations of 1.0% (w/v)[8].

Bacterial growth measurement in inulin-containing media: To investigate the effects of inulin and inulin extract on the growth of *L. casei* AP, the bacteria were cultivated overnight in the appropriate selective media at 37°C. The activated cultures were harvested for 15 min with 2.500 rpm at 4°C using a Hettich centrifuge (Fisher Scientific, NH, USA). Then, the precipitate was washed twice with phosphate-buffered saline (0.1 mol L^{-1} phosphate buffer pH = 7.4 and 0.9% saline) and the final pellet was suspended in phosphate-buffered saline and diluted to approximately 10^6 cells mL^{-1}. The ability of strains to utilize inulin as a carbon source was examined by growing them on media with inulin or inulin extract as the only carbon source, as a control, cells were grown in MRS. The growth of bacterial cells at 37°C was monitored for 24 h and measured using UV Spectrophotometry (Shimadzu, Kyoto, Japan) at an optical density (OD) of 620 nm. All measurements were repeated twice. The changes in pH were measured using an Inolab pH meter (Thomas Scientific, NJ, USA).

Prebiotic index (PI): The test was initiated by adding 1 mL inoculum separately into medium containing 1% inulin, 1% inulin extract or 1% sterile MRS. The media were then incubated at 37°C for 24 h. Total viable cells were measured by plating on specific media at 0, 12 and 24 h after bacterial growth using the pour plate method. For the enumeration of *L. casei*, the AP medium used was MRS agar and TSA was used for *E. coli*, the media were incubated for 48 h at 37°C. The

Prebiotic Index was calculated using the formula described by Sanz *et al.*[9]. The PI was calculated using formula 1:

$$PI = \frac{(Log_{10}\text{probiotic})_{tx-t0} - (Log_{10}\text{enterobacteriaceae})_{tx-t0}}{Log_{10}\text{total bacteria}} \quad (1)$$

Short-chain fatty acid analysis: Short-chain fatty acid analysis was performed using the GC-MS chromatogram (Shimadzu, Kyoto, Japan) with a modified method by Conti e Silva *et al.*[10]. The column used was SP 1200, GP 10, SP-1200/1% H_3PO_4 on 80/100 chromosorb W AW (Sigma-Aldrich, MO, USA), with a column length of 1.5 m and a diameter of 3 mm. The injector temperature was 240°C, the detector temperature was 240°C, the column temperature was 140°C, the gas pressure was $H_2 = 0.6$ kg cm^{-2} and the air pressure = 0.5 kg cm^{-2}. The carrier gas was N_2 gas at a pressure of $N_2 = 1.25$ kg cm^{-2}.

Growth inhibition of pathogenic strains: Growth inhibition of pathogenic strains in response to synbiotic products was evaluated by using the agar diffusion method[11]. Briefly, the pathogenic strains *S. dysenteriae* and *H. pylori* were inoculated on 5 mL of TSA at a final concentration of 10^7 cfu mL^{-1}. Then, the synbiotic products were inserted into a well in the TSA. The plates were incubated in conditions suitable for each pathogen for 24-48 h. All of the petri dishes (Fisher Scientific, NH, USA) were duplexed. A clear zone around the wells was measured using calipers.

RESULT AND DISCUSSION

Effects of inulin extract on bacterial populations: *In vitro* experiments on three different growth media showed that both inulin (commercial standard) and the inulin extract from dahlia tubers supported the growth of *L. casei* AP (Fig. 1). The ability of *L. casei* AP to grow in inulin media was consistent with previous findings of Widodo *et al.*[6], who demonstrated the ability of *L. casei* AP to utilize inulin as a carbon source. The growth pattern of *L. casei* AP in inulin extract was similar to that observed in inulin-containing and MRS media, in which all bacteria grew rapidly after approximately 6 h and reached the maximum growth at approximately 24 h. The optical density of the media containing inulin extract was significantly higher (p<0.05) than that of media containing inulin, which indicated that inulin extract was more effective at supporting the growth of *L. casei* AP than were inulin-containing or MRS media. Supplementation with inulin extract was found to have a significantly better effect on the viability of bacteria compared to media without inulin extract
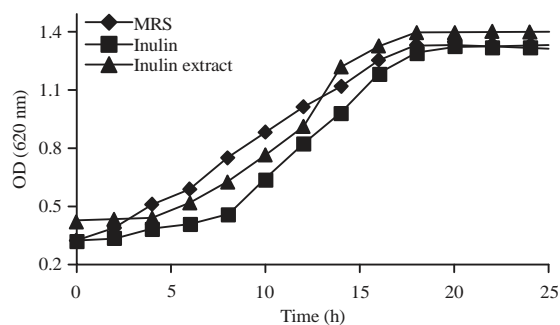

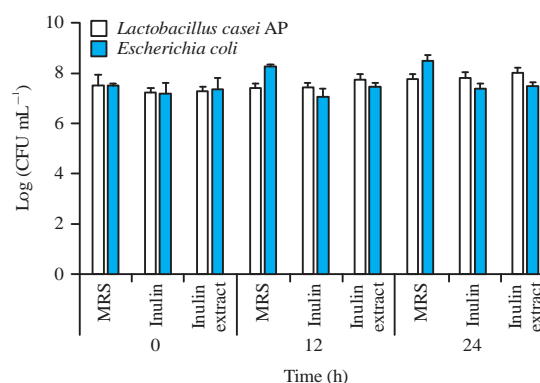Fig. 1: Growth of *L. casei* AP in three different media

Fig. 2: Bacterial populations at 0, 12 and 24 h in MRS, inulin and inulin extract as media

(p<0.05). Watson *et al.*[12] demonstrated that lactulose, maltodextrin, fructooligosaccharides, galactooligosaccharides and inulin stimulate the growth performance of twelve different *Lactobacillus* species.

Co-culturing *L. casei* AP and *E. coli* in different growth media: To evaluate the effects of different media on the growth of *L. casei* AP and *E. coli*, both bacteria were co-cultured in various media (MRS, inulin, inulin extract) for 24 h. The growth of *L. casei* AP and *E. coli* was measured based on the enumeration of viable bacterial cells by means of total plate count (Fig. 2). After being grown in MRS as the growth medium for 12 and 24 h, *E. coli* had a faster growth rate than *L. casei* AP did. Meanwhile, in inulin- and inulin extract-containing media, *E. coli* had a slower growth rate than *L. casei* AP did (Fig. 2). Several studies have reported that fructooligosaccharides can support the growth of *E. coli*, Enterobacter and Salmonella[13]. In contrast, other studies have indicated that *E. coli* is unable to utilize fructooligosaccharides[14]. Van Laere *et al.*[15] reported that arabino-oligosaccharides could support the growth of *E. coli* but that fructooligosaccharides could not do the same.

Table 1: Concentration of SCFA in media

Analysis	MRS (%)	Inulin (%)	Inulin extract (%)
Lactic acid	33.83±2.11[c]	33.83±1.74[c]	35.53±1.63[c]
Acetic acid	9.83±1.53[b]	12.42±0.56[b]	10.35±0.79[b]
Propionic acid	0.05±0.17[a]	0.36±0.16[a]	0.43±0.10[a]
Butyric acid	0.03±0.41[a]	0.28±0.11[a]	0.49±0.28[a]

Different letters show significant differences, according to the BNT test (p<0.05)

Prebiotic index: The prebiotic indices (PIs) of inulin and inulin extracts were higher than that of MRS (Fig. 3). When grown in MRS media, the PI was 1.1. In contrast, when grown in inulin-containing and inulin extract media, the PI was 4.3 and 4.7, respectively (Fig. 3). The high prebiotic index indicated a larger probiotic population of *L. casei* AP than that of enterobacteriaceae/pathogen (*E. coli*) when co-cultured in the same growth medium, as seen in Fig. 2, suggesting that *L. casei* AP has a higher capability to consume inulin than *E. coli* does. Studies have reported differences in the PIs due to the differences in the prebiotics and probiotic strains used. It was reported that each bacterium has an unequal bacterial activity score. The PI value of dahlia tubers has not been reported previously but that of galactooligosaccharide was reported to be 0.18[16]. The PI score from static batch culture fermentation of FOS was 2.78[17]. In this study, inulin and inulin extracts were able to provide optimal carbon source requirements for the growth of *L. casei* AP by suppressing the growth of *E. coli*.

Synbiotic effect on the growth of *S. dysenteriae* and *H. pylori*: The data on the size of the inhibition zone when *H. pylori* and *S. dysenteriae* were grown in the presence of probiotic *L. casei* AP and prebiotic inulin or inulin extract are presented in Fig. 4. The synbiotic interaction between *L. casei* AP and inulin or inulin extract inhibited the growth of *H. pylori* and *S. dysenteriae*. The highest growth inhibition of *S. dysenteriae* by *L. casei* AP was obtained when both bacterial species were grown in inulin extract as the sole carbon source. *S. dysenteriae* and *H. pylori* experienced the same level of growth inhibition by *L. casei* AP when both were grown in inulin-containing media. Meanwhile, the lowest growth inhibition of *S. dysenteriae* and *H. pylori* was obtained when either species was grown with *L. casei* AP in MRS media (Fig. 4). Figure 4 also shows that *S. dysenteriae* was more easily inhibited by the synbiotic interaction between *L. casei* AP and inulin extract than *H. pylori* was (p<0.05). Kabir *et al.*[18] reported that *L. casei* and *L. acidophilus* were capable of producing large amounts of lactic acid, thus completely inhibiting the growth of *H. pylori* in a mixed culture. Findings
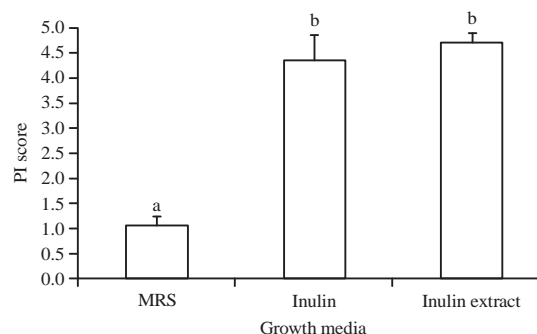

Fig. 3: Prebiotic index in three different media. Different letters show significant differences (p<0.05)

of the current study revealed that the survival of *S. dysenteriae* and *H. pylori* when co-cultured with probiotic *L. casei* AP depended on the media utilized. Widodo *et al.*[6] demonstrated the ability of *L. casei* AP to inhibit the growth of *Shigella flexnerii*, *E. coli*, *Staphylococcus aureus* and *Enterococcus faecalis*.

Production of short-chain fatty acids and pH changes: The ability of *L. casei* AP to produce short-chain fatty acids (SCFAs) was analysed by measuring the concentrations of lactate, acetate, propionate and butyrate. The concentrations of these SCFAs, as percentages (%) are presented in Table 1. The data show that differences in the growth media (MRS, inulin and inulin extract) did not affect the SCFA concentrations. Lactate was present at the highest concentration (33.83-33.53%), followed by acetate (9.83-12.42%), propionate (0.05-0.43%) and butyrate (0.03-0.49%). Lactate and acetate were already known to be the main metabolites produced by lactic acid bacteria[13].

The high production of lactate and acetate consequently decreased the pH of the media (Fig. 4). A longer fermentation time resulted in a greater reduction of pH and the lowest pH was observed in growth media containing the inulin extract, followed by inulin-containing media and MRS (Fig. 5). This decreased pH was related to the increased activity of *L. casei* AP in producing short chain fatty acids. Decreased pH was likely due to the ionization of potential cytotoxic components,
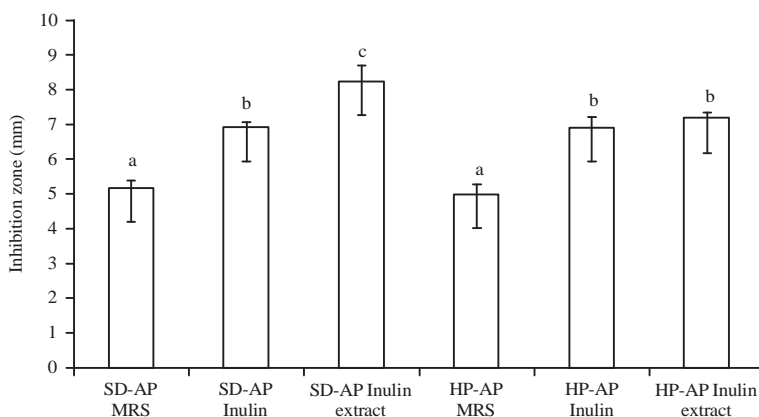
Fig. 4: Competition between pathogens and a symbiotic *L. casei* AP with inulin/inulin extract (SD: *S. dysenteriae* ATCC 11835, HP: *H. pylori* ATCC 26695, AP: *L. casei* AP). Different letters show statistically significant differences (p<0.05)
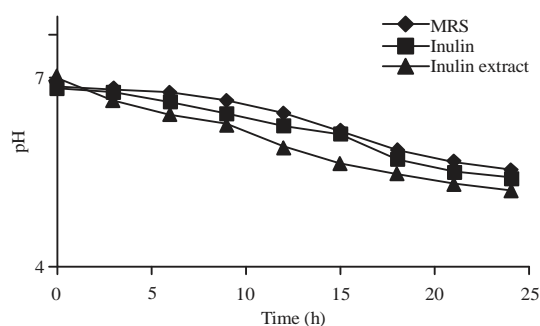

Fig. 5: Changes in pH of three different media after inoculation with *L. casei* AP

such as biogenic amines and ammonia[19]. Lactic acid bacteria, particularly of the genus Lactobacillus are acid-producing bacteria and tolerate acidic conditions in the medium, with an optimum pH (5.5-6.4). In addition to the activity of *L. casei* AP in producing SCFA, it was proposed that *E. coli* was able to produce acids. Muza-Moons *et al.*[20] reported that enteropathogenic *E. coli* was also able to produce acids, thus lowering the pH of the media.

This study reveals the ability of inulin extracts obtained from dahlia tubers to support the growth of probiotics and, accordingly, suppress pathogenic bacteria.

CONCLUSION

It is concluded that inulin extracts obtained from dahlia tubers can act as prebiotics that support the growth of probiotics *L. casei* AP.

The implication of these findings supports the utilization of dahlia tubers as inulin sources and will thus aid in the development of inulin-based food industries, particularly in tropical countries.

SIGNIFICANCE STATEMENT

This study discovers the prebiotic effect of inulin extract produced from local dahlia tubers, which can be beneficial for supporting probiotics in a host's gastrointestinal tract. This study will support the utilization of dahlia tubers as inulin sources, thus aiding the development of inulin-based food industries, particularly in tropical countries.

ACKNOWLEDGMENT

REFERENCES

1. Nair, K.K., S. Kharb and D.K. Thompkinson, 2010. Inulin dietary fiber with functional and health attributes-A review. Food Rev. Int., 26: 189-203.

2. Sardesai, V.M., 2003. Introduction to Clinical Nutrition. 2nd Edn., Marcel Dekker Inc., USA.

3. Kusmiyati, N., T.D. Wahyuningsih and Widodo, 2018. Extraction and identification of inulin-type fructo-oligosaccharides from *Dahlia pinnata* L. Asian J. Chem., 30: 355-358.

4. Liong, M.T., 2008. Roles of probiotics and prebiotics in colon cancer prevention: Postulated mechanisms and *in-vivo* evidence. Int. J. Mol. Sci., 9: 854-863.

5. Nakakuki, T., 2002. Present status and future of functional oligosaccharide development in Japan. Pure Applied Chem., 74: 1245-1251.

6. Widodo, N.S. Anindita, T.T. Taufiq and T.D. Wahyuningsih, 2014. Evaluation of two *Lactobacillus* strains as probiotics with emphasis in utilizing prebiotic inulin as energy source. Int. Res. J. Microbiol., 5: 33-40.

7. Widodo, T.T. Taufiq, E. Aryati, A. Kurniawati and W. Asmara, 2012. Human origin *Lactobacillus casei* isolated from Indonesian infants demonstrating potential characteristics as probiotics *in vitro*. Indonesian J. Biotechnol., 17: 79-89.

8. Bridson, E.Y., 1998. The Oxoid Manual. 8th Edn., Oxoid Ltd., Hampshire, UK.

9. Sanz, M.L., G.R. Gibson and R.A. Rastall, 2005. Influence of disaccharide structure on prebiotic selectivity *in vitro*. J. Agric. Food Chem., 53: 5192-5199.

10. Conti e Silva, A.C., D.H.M. Bastos, J.A.G. Areas, R. Facanali and M.O.M. Marques, 2008. Volatile compounds in the thermoplastic extrusion of bovine rumen. Quim. Nova, 31: 1990-1993.

11. Satirapathkul, C. and T. Leela, 2011. Growth inhibition of pathogenic bacteria by extract of *Quercus infectoria* galls. Int. J. Biosci. Biochem. Bioinform., 1: 26-31.

12. Watson, D., M.O. Motherway, M.H.C. Schoterman, R.J.J. van Neerven, A. Nauta and D. van Sinderen, 2013. Selective carbohydrate utilization by lactobacilli and bifidobacteria. J. Applied Microbiol., 114: 1132-1146.

13. Roberfroid, M., 2004. Inulin-Type Fructans: Functional Food Ingredients. CRC Press, Boca Raton, pp: 88-95.

14. Holt, S.M., C.M. Miller Fosmore and G.L. Cote, 2005. Growth of various intestinal bacteria on alternansucrase-derived oligosaccharides. Lett. Applied Microbiol., 40: 385-390.

15. Van Laere, K.M., R. Hartemink, M. Bosveld, H.A. Schols and A.G.J. Voragen, 2000. Fermentation of plant cell wall derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria. J. Agric. Food Chem., 48: 1644-1652.

16. Rycroft, C.E., M.R. Jones, G.R. Gibson and R.A. Rastall, 2001. A comparative *in vitro* evaluation of the fermentation properties of prebiotic oligosaccharides. J. Applied Microbiol., 91: 878-887.

17. Depeint, F., G. Tzortzis, J. Vulevic, K. I'Anson and G.R. Gibson, 2008. Prebiotic evaluation of a novel galactooligosaccharide mixture produced by the enzymatic activity of *Bifidobacterium bifidum* NCIMB 41171, in healthy humans: A randomized, double-blind, crossover, placebo-controlled intervention study. Am. J. Clin. Nutr., 87: 785-791.

18. Kabir, A.M., Y. Aiba, A. Takagi, S. Kamiya, T. Miwa and Y. Koga, 1997. Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. Gut, 41: 49-55.

19. Topping, D., 2007. Cereal complex carbohydrates and their contribution to human health. J. Cereal Sci., 46: 220-229.

20. Muza-Moons, M.M., E.E. Schneeberger and G.A. Hecht, 2004. Enteropathogenic *Escherichia coli* infection leads to appearance of aberrant tight junctions strands in the lateral membrane of intestinal epithelial cells. Cell. Microbiol., 6: 783-793.