R E S E A R C H   A R T I C L E

Lactiplantibacillus plantarum IS-10506 Enhances Tight Junction Integrity in Bronchial Epithelium: An Experimental Study

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Abstract

BACKGROUND: Airway epithelium constitutes the initial structural defense against inhaled allergens, with its integrity primarily maintained through the formation of tight junctions (TJ) consisting of claudins, occludins, and zonula occludens (ZO). Currently, the effects of probiotics on airway TJ remain unclear. Therefore, this study was conducted to explore the impact of *Lactiplantibacillus plantarum* IS-10506, a native probiotic from Indonesia, on bronchial epithelial TJ in a mouse model of allergic airway inflammation.

METHODS: Twenty-four BALB/c mice were randomly assigned to three groups: negative control, positive control subjected to *Dermatophagoides pteryonissinus* (Der p) sensitization and challenge, and treatment group exposed to Der p and administered *L. plantarum* IS-10506 since one week before sensitization up to three weeks after. The expression levels of claudin-18, occludin, and ZO-1 were investigated through immunohistochemistry analysis and quantified based on the immunoreactive score (IRS).

RESULTS: The results of this study demonstrated that *L. plantarum* IS-10506 significantly enhanced the expressions of claudin-18, occludin, and ZO-1 ($p<0.05$).

CONCLUSION: These findings underscore the probiotic's role in fortifying the integrity of airway TJ.

KEYWORDS: allergic inflammation, probiotic *Lactiplantibacillus plantarum*, immunology, respiratory system, tight junction, occludin, claudin, zonula occludens

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Introduction

Alarming trends indicate a global increase in allergic diseases, including rhinitis and asthma, affecting countries worldwide.(1,2) While some advanced industrial nations have seen a stabilization in their prevalence, developing countries, including Indonesia, continue to experience a rise in allergic diseases.(3-5) This rapid escalation highlights the significant impact of environmental changes. The

"hygiene hypothesis" suggests that reduced family size and lower infection rates in early life contribute to this trend.(6) Similarly, the biodiversity hypothesis proposes that diminished exposure to the natural environment and environmental microbiota stimulation lead to inadequate immune system regulation.(7) This theory underscores the positive role of microbial exposure, polarizing the immune response toward T-helper (Th)1 rather than the Th2 response, known to be involved in allergic diseases. Recent systematic reviews have revealed a connection between reduced bacterial diversity and the development of allergic diseases.(8) Additionally, the concept of the gut-lung axis, which explores the interaction between the gastrointestinal and respiratory tracts, presents opportunities for manipulating the gastrointestinal system to benefit the respiratory tract.(9) Although this concept holds promise, it requires further validation to confirm its validity. In light of these perspectives, probiotic supplementation emerges as a potential option for preventing allergic respiratory diseases. Probiotics are live microorganisms that, when provided in sufficient doses, enhance the host's health.(10) Certain probiotics, particularly those from the *Lactobacilli* and *Bifidobacteria* genera, have been extensively researched as alternative options for preventing and treating allergic diseases.(11)

Lactiplantibacillus plantarum IS-10506 is a native Indonesian probiotic strain isolated from *dadih*, traditional fermented buffalo milk from Sumatera Barat.(12-14) Previous research has demonstrated that administering *L. plantarum* IS-10506 significantly reduced allergic reactions in BALB/c mice sensitized with ovalbumin (OVA), as well as in pediatric atopic dermatitis patients, and adults with atopic dermatitis.(15-17) Another strain namely *L. plantarum* IS-20506 revealed to significantly restore the expressions of Galectin-4 and Myosin-1a in the intestinal of lipopolysaccharides (LPS)-induced rats, implying that it might assist in the repair of damaged intestinal brush borders.(18)

The airway epithelium serves as the primary structural barrier against inhalant allergens. This protective function is primarily maintained through the formation of tight junctions (TJ) composed of essential proteins such as claudins, occludin, and zonula occludens (ZO), as well as adherens junctions (AJ) consisting of E-cadherin, β-catenin, and α-catenin.(19,20) Changes in TJ proteins, evidenced by decreased TJP1 (ZO-1) and claudin-18 mRNA expression, have been observed in mice exposed to house dust mite extract, inducing allergic airway inflammation.(21) Claudin-18 deficiency leads to epithelial barrier

dysfunction, damage, and impaired alveolarization in mice.(22) This deficiency has also been identified in the airway cells of asthma patients.(23) These studies underscore the pivotal role of TJ proteins in the early stages of allergic asthma. Damage to the epithelial barrier can facilitate the entry of allergens into the airway epithelium, triggering the activation of the immune system and local inflammation, which are hallmark features of allergic diseases.(24) Consequently, the epithelial barrier represents a critical target in the prevention of allergic diseases.

Various published studies have primarily concentrated on the gastrointestinal tract when investigating the impact of probiotics on improving TJ. These studies include both *in vitro* experiments (25,26), *in vivo* tests with experimental animal models (27,28), as well as studies involving human subjects (29). Specifically, the effect of *L. plantarum* IS-10506 on gastrointestinal TJ repair was explored *in vivo* using Wistar rats induced by LPS from *Escherichia coli* serotype O55:B5. This study revealed an increased expression of occludin and ZO-1.(30) In contrast, research explaining the impact of probiotics on airway TJ remains quite limited. Currently, there is only one *in vitro* study where the stimulation of human bronchial epithelial cells Calu-3 with synthetic bacterial lipopeptide Pam3CysSK4 resulted in increased expression of claudin-1 and ZO-1.(31) Therefore, the precise role of probiotics on airway TJ remains unclear, emphasizing the need for further research in this area.

This study was conducted to investigate the impact of *L. plantarum* IS-10506 on the expression of constituting airway TJ proteins, specifically claudin-18, occludin, and ZO-1, aiming to prevent allergic airway inflammation. To model allergic airway inflammation, sensitization and exposure to the allergen *Dermatophagoides pteryonissinus* (Der p) were employed in this study.

Methods

Animal Models

The experimental units in this study were 24 adult male BALB/c mice (*Mus musculus*) aged 6-8 weeks, weighing between 20-35 grams, retrieved from PT Bio Farma (Jakarta, Indonesia). BALB/c mice were chosen for this study due to their suitability for modeling atopic allergic diseases in animals. They exhibit immune responses that tend to lean towards Th2 responses. The immune responses observed in BALB/c mice closely resemble the immune responses seen in human atopic individuals. The protocol of this study

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was approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga (No. 2.KE.113.09.2021).

Study Design and Experimental Groups

This was a true experimental study conducted on experimental animals using a post-test only control group design. The design was structured to assess the effects of the treatment on the experimental units, with measurements of variables taken after the administration of the treatment (Figure 1). C0 represented the normal control group (negative control), consisting of mice subjected to sham sensitization and sham challenge, and administered a placebo from 7 days before sensitization until day 27 after sensitization. C1 represented the positive control group, consisting of mice sensitized intraperitoneally with 500 AU of house dust mite allergen Der p and exposed to 500 AU of house dust mite allergen Der p through inhalation (mice model of allergic airway inflammation). They were administered a placebo since 7 days before sensitization until day 27 after sensitization. Meanwhile, T represented the treatment group, comprising mice sensitized intraperitoneally with 500 AU of house dust mite allergen Der p and exposed to 500 AU of house dust mite allergen Der p through inhalation (mice model of allergic airway inflammation). They were administered *L. plantarum* IS-10506 since 7 days before sensitization until day 27 after sensitization.

The microencapsulated powder of *L. plantarum* IS-10506 (GenBank accession number DQ860148; deposited in culture collection of Turku University, Finland) was administered via a gastric gavage at a dose of 300 mg (viability: 2.8-5.6 x 10^9 CFU) dissolved in 0.6 mL of distilled water per mouse per day for 35 days for the T group. The sensitization with 500 AU of Der p allergen (ALK Laboratories catalog number STDP-14999V, Port Washington, NY, USA) intraperitoneally was precipitated in 2 mg of aluminum hydroxide and consisted of two types of treatments. First, sham sensitization where only a phosphate buffered saline (PBS) solution was administered to the C0 group on day 0 and day 14, and second, sensitization with 500 AU of Der p allergen, which was administered to both the C1 and T groups on day 0 and day 14. Additionally, the Der p allergen at a dosage of 500 AU was administered through inhalation/aerosol via spontaneous breathing using a jet nebulizer. This process involved two types of treatments: Sham challenge (in this treatment, a PBS aerosol solution was administered as a sham challenge to the C0 group from day 21 to day 27) and Der p allergen exposure (500 AU; The C1 and T groups were exposed to Der p allergen at a dosage of 500 AU from day 21 to day 27).

The allocation of experimental units into each group was carried out through simple randomization using a draw. Each group was distinguished by markings on different cages, and each experimental unit was assigned a specific
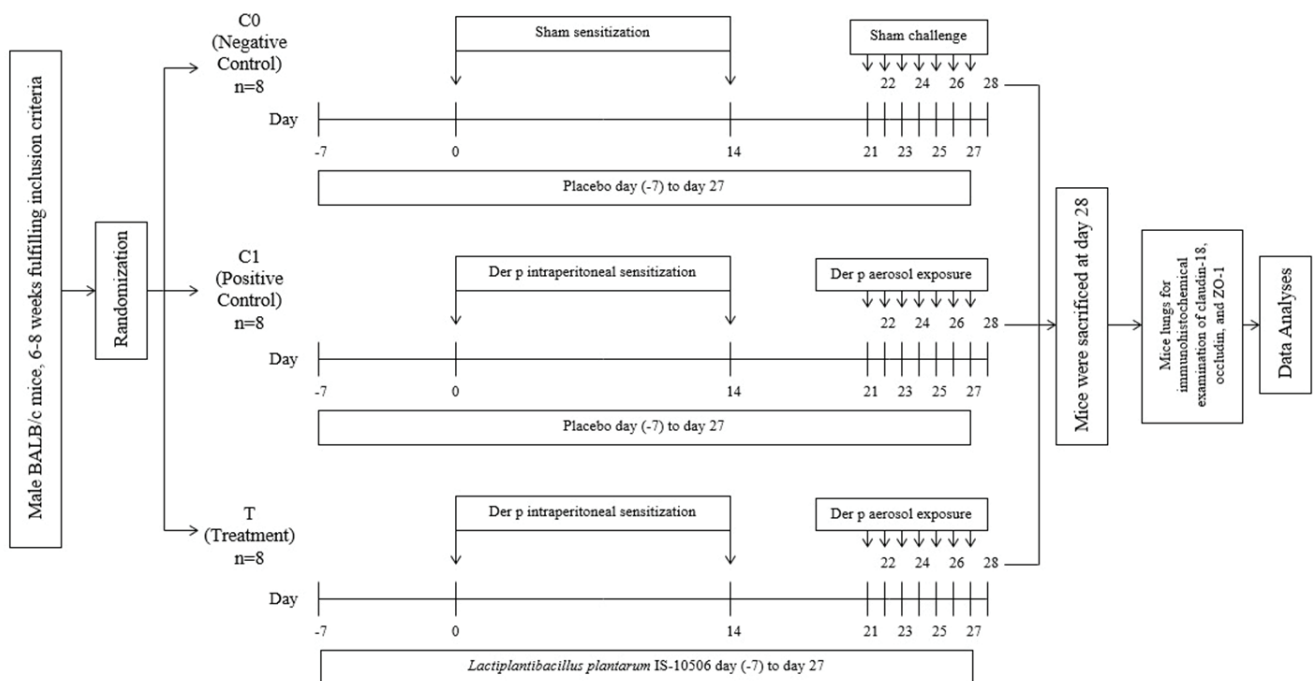

Figure 1. Flowchart of study design.

code indicated by coloring specific parts of the mice's bodies. Subsequently, they were placed into their respective groups, namely C0, C1, and T.

TJ Proteins Expression Analyses Using Immunohistochemistry Approach

The main transmembrane proteins constituting the TJ were measured by counting the number of cells showing positive reactions to monoclonal antibodies against claudin-18 (Claudin 18 Recombinant Rabbit Monoclonal Antibody (34H14L15); Cat. No. 700178; Lot No. 2327296; Thermo Fisher Scientific, Waltham, MA, USA), occludin (Occludin Mouse Monoclonal Antibody (E-5) labeled with HRP (Horseradish Peroxidase); Cat. No. sc-133256 HRP; Lot No. L2017; Santa Cruz Biotechnology, Paso Robles, CA, USA) and ZO-1 (ZO-1 Rat Monoclonal Antibody (R40.76) labeled with HRP (Horseradish Peroxidase); Cat. No. sc-33725 HRP; Lot No. D1321; Santa Cruz Biotechnology) using the immunohistochemical examination technique on post-treatment mouse lung specimens.

Initially, the tissue sections were heated at 65°C for an hour before deparaffinization. The primary antibodies were diluted as required: Claudin 18 Recombinant Rabbit Monoclonal Antibody at a dilution of 1:400, Occludin Mouse Monoclonal Antibody (E-5) at a dilution of 1:400, and ZO-1 Rat Monoclonal Antibody (R40.76) HRP at a dilution of 1:400. A diaminobenzidine (DAB) solution was prepared by mixing one drop of DAB with 1 mL of substrate buffer.

The lung tissue samples were fixated in a 10% neutral buffered formaldehyde solution (formaldehyde-PBS), followed by a comprehensive dehydration process using a graded alcohol series (30%, 50%, 70%, 80%, 96%, and absolute alcohol) for 60 minutes at each concentration level. The samples were then cleared with xylene in two 60-minute steps before being embedded in paraffin for 24 hours. Serial sections of 4-6 µm thickness were then cut from the paraffin blocks using a microtome, and the best slices were selected for further processing. These sections were placed on polylysine-coated glass slides to ensure adherence. Deparaffinization was achieved through three successive xylene treatments, each lasting 5 minutes. The tissue sections were then rehydrated in a descending alcohol series (absolute, 96%, 80%, and 70% alcohol), with each step lasting 4 minutes. Following rehydration, the sections are washed to remove any residual alcohol. Endogenous peroxidase activity was blocked by gently dabbing away excess water around the tissue sections, applying a peroxidase blocking reagent for 5 minutes, and then washing off the excess peroxidase with distilled water, gently agitating the staining jar holder.

Antigen retrieval was performed by placing the slides in a citrate buffer solution (pH 6) within a decloaking chamber, adjusting the temperature and incubation time as necessary, and allowing the slides to cool to room temperature. Background blocking was conducted by applying a protein block serum for 5 minutes. Primary staining involves applying one of the diluted primary antibodies (claudin 18, occludin, or ZO-1) to the marked areas on the slide, incubating overnight at 4°C washing with PBS, applying a secondary antibody for room temperature incubation for 10 minutes, followed by another PBS wash. The DAB solution was then applied for color development for 5 minutes, followed by a final wash in running water. Finally, counterstaining was performed by immersing the slides in Mayer's hematoxylin for 10 minutes at room temperature, followed by a wash in running water. The sections were then dehydrated through a series of alcohol baths (80%, 90%, and 96%), cleared in xylene, and finally mounted with a cover glass to preserve the staining.

The results were read semi-quantitatively using either the modified Remmele scale or the immunoreactive score (IRS), which is the product of the percentage score of immunoreactive cells and the color intensity score in these cells. The data for each sample represented the average IRS value observed in five different fields of view at 400x magnification. The semi-quantitative IRS scale was calculated as the product of the positive cell percentage score (A) and the color intensity score (B), where $IRS = A \times B$. The positive cell percentage score (A) included: Score 0: No positive cells; Score 1: Positive cells $\leq 10\%$; Score 2: Positive cells 11-50%; Score 3: Positive cells 51-80%; Score 4: Positive cells > 80%. The color intensity reaction score (B) included: Score 0: No color reaction; Score 1: Low color intensity; Score 2: Moderate color intensity; Score 3: Strong color intensity.

Data Analysis

The research findings were presented in various forms: tables, graphs, text, or written explanations to describe the tables and graphs. To ensure consistency between observer 1 and observer 2 in interpreting immunohistochemical examination results, the study used Kappa statistics. Normality tests were conducted to determine whether the data followed a normal distribution. The Shapiro-Wilk test was utilized for this purpose, where a $p > 0.05$ indicates a normal distribution. Homogeneity tests were performed to assess variations between groups. Levene's test was used for

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homogeneity testing, with a $p>0.05$ indicating homogeneous variances. To analyze the differences in effects among the three groups, several methods were employed based on the data's distribution and homogeneity: First, if the data were normally distributed and homogenous, one-way ANOVA was conducted, followed by *post hoc* LSD analysis if $p<0.05$. Second, if the data were normally distributed but not homogenous, Brown-Forsythe analysis was performed, followed by Games-Howell *post hoc* analysis if $p<0.05$. Third, if the data were not normally distributed, Kruskall-Wallis test was carried out, followed by Mann-Whitney post hoc analysis if $p<0.05$. *Post hoc* analyses were conducted to identify specific group differences. A $p<0.05$ indicated a statistically significant difference between groups.

Results

L. plantarum IS-10506 Increased Claudin-18 Expression in Mice with Allergic Airway Inflammation

The expression of claudin-18, a major transmembrane protein forming TJ in bronchial epithelium, was measured using immunohistochemical examination techniques. The results were quantified in IRS and assessed by two expert anatomical pathology specialists using a blinding method. A Kappa test was conducted to evaluate the observations of the two anatomical pathology experts, resulting in a Kappa value of 0.571, indicating weak consistency in observations. The IRS scores obtained were the average of the readings by both experts to enhance internal validity and reduce reading bias. The Shapiro-Wilk normality test for claudin-18 expression in C0, C1, and T groups showed $p=0.718$, $p=0.046$, and $p=0.014$, respectively, indicating non-normal data distribution in C1 and T groups (considered normal if $p>0.05$). Subsequently, a Kruskall-Wallis test was performed, revealing significant differences among the groups with a $p=0.021$ ($p<0.05$), as depicted in Table 1A.

The Kruskal-Wallis test yielded significant results; therefore, *post hoc* analysis using Mann-Whitney tests was conducted to determine the specific differences between groups, as shown in Table 1B. Based on the *post hoc* analysis results in Table 1B, a significant difference was observed between C1 and T groups, although no significant differences were found between C0 and C1 groups, as well as between C0 and T groups. Thus, it can be concluded that there was a significant increase in claudin-18 expression in the group of mice with allergic airway inflammation model receiving *L. plantarum* IS-10506 compared to the group of mice with allergic airway inflammation model without *L. plantarum*

IS-10506 supplementation. This indicated the influence of *L. plantarum* IS-10506 administration on claudin-18 expression. The immunohistochemical examination results of bronchial epithelium expressing claudin-18 can be seen in Figure 2.

L. plantarum IS-10506 Increased Occludin Expression in Mice with Allergic Airway Inflammation

The expression of occludin, a transmembrane protein composing TJ in bronchial epithelium, was measured using immunohistochemical examination techniques. The results were quantified in IRS and assessed by two expert anatomical pathology specialists using a blinding method. A Kappa test was conducted to evaluate the observations of the two anatomical pathology experts, resulting in a Kappa value of 0.639, indicating moderate consistency in observations. The IRS scores obtained were the average of the readings by both experts to enhance internal validity and reduce reading bias. The Shapiro-Wilk normality test results for occludin expression in C0, C1, and T groups showed $p=0.157$, $p=0.369$, and $p=0.185$, respectively, indicating normal data distribution for all three groups (considered normal if $p>0.05$). The homogeneity of variance was confirmed through Levene's test with a $p=0.541$ ($p>0.05$), indicating homogeneous data variance among groups. Subsequently, a one-way ANOVA was performed, revealing significant differences among the groups with a $p=0.004$ ($p<0.05$), as shown in Table 2A.

The significant result from the one-way ANOVA test led to further *post hoc* analysis using LSD to determine specific differences between groups, as illustrated in Table 2B. Based on the *post hoc* analysis results in Table 2B, significant differences were found between C0 and T groups, as well as between C1 and T groups.

There was a significant increase in the expression of occludin in the group of mice with allergic airway inflammation model receiving *L. plantarum* IS-10506 compared to the group of mice with allergic airway inflammation model without *L. plantarum* IS-10506 administration and the normal control mice group. This indicates an influence of *L. plantarum* IS-10506 administration on the expression of occludin. The results of immunohistochemical examination of bronchial epithelium expressing occludin can be observed in Figure 3.

L. plantarum IS-10506 Increased ZO-1 Expression in Mice with Allergic Airway Inflammation

ZO-1 is a scaffolding protein that regulates the stability of TJ in bronchial epithelium. In this study, it was measured

Table 1. The difference in claudin-18 expression in bronchial epithelium.

	IRS Score of Claudin-18			Post Hoc Analysis	
Group	Mean±SD	Median (min–max)	p-value	Group	p-value
C0	6.500±3.162[a]	7.000 (2.00–12.00)	0.021*	C0 versus C1	0.220
C1	4.875±1.553	4.000 (3.00–7.00)[b]		C0 versus T	0.253
T	8.000±1.773	8.000 (6.00–12.00)[b]		C1 versus T	0.003*

[a]Normal distribution; [b]Non-normal distribution; *Significanct if $p<0.05$. Number of mice in each group n=8.

using immunohistochemical techniques. The results were interpreted in terms of IRS by two expert anatomical pathology readers employing a blinding method. Kappa statistics were employed to assess the consistency between the observations of the two anatomical pathology experts, yielding a Kappa value of 0.601, indicating moderate consistency in their observations. The IRS scores were derived as the average of readings from both experts to enhance internal validity and minimize reading bias. The Shapiro-Wilk normality test for ZO-1 expression in C0, C1, and T groups yielded $p=0.122$, $p=0.005$, and $p=0.110$, respectively. It was concluded that there was a non-normal distribution of data in group C1 (considered normal if $p>0.05$). Subsequently, a Kruskall-Wallis test was conducted, revealing a significant difference among the groups with a $p=0.001$ ($p<0.05$), as indicated in Table 3A.

Since the Kruskall-Wallis test yielded significant results, a *post hoc* Mann-Whitney analysis was conducted to determine which specific groups exhibited differences, as shown in Table 3B. Based on the *post hoc* analysis presented in Table 3B, significant differences were found between C0 and C1 groups, C0 and T groups, as well as C1 and T groups. Therefore, it can be concluded that there was a significant increase in ZO-1 expression in the group of mice with allergic airway inflammation model receiving *L. plantarum* IS-10506 compared to the group of mice with allergic airway inflammation model without *L. plantarum* IS-10506 administration and the normal control mice group. This indicates an influence of *L. plantarum* IS-10506 administration on ZO-1 expression. The results of immunohistochemical examination of bronchial epithelium expressing ZO-1 can be observed in Figure 4.
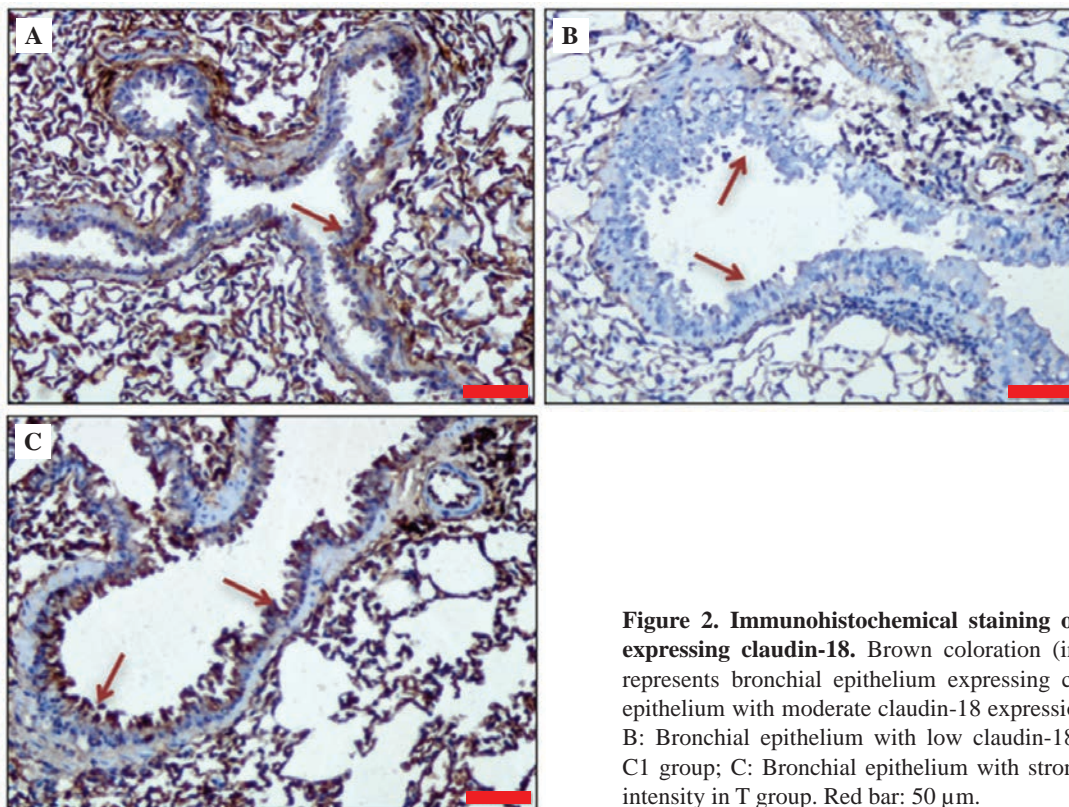

Figure 2. Immunohistochemical staining of bronchial epithelium expressing claudin-18. Brown coloration (indicated by red arrows) represents bronchial epithelium expressing claudin-18. A: Bronchial epithelium with moderate claudin-18 expression intensity in C0 group; B: Bronchial epithelium with low claudin-18 expression intensity in C1 group; C: Bronchial epithelium with strong claudin-18 expression intensity in T group. Red bar: 50 µm.

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Table 2. The difference in occludin expression in bronchial epithelium.

IRS Score of Occludin			Post Hoc Analysis			
Group	Mean±SD	p-value	Group	Average difference	p-value	Confidence Interval (CI) 95%
C0	5.938±2.008	0.004*	C0 versus C1	1.813	0.106	-0.417 – 4.043
C1	4.125±1.642		C0 versus T	2.313	0.043*	-4.543 – -0.082
T	8.250±2.659		C1 versus T	4.125	0.001*	-6.355 – -1.895

*Significanct if $p<0.05$. Number of mice in each group n=8.

Discussion

This study aimed to investigate the effect of *L. plantarum* IS-10506 on the expression of airway TJ proteins as a potential preventive measure against allergic inflammation in the respiratory tract. In this research, a murine model of allergic airway inflammation was utilized, specifically employing BALB/c mice due to their genetic predisposition towards Th2 immune responses.(32) BALB/c mice were intraperitoneally sensitized and exposed to aerosolized Der p allergen, the primary inhalant allergen associated with asthma and allergic rhinitis. Allergic inflammation serves as a pathological hallmark of inflammatory diseases, including asthma.(33) The results of our study demonstrate that *L. plantarum* IS-10506 enhances the expression of claudin-18, occludin, and ZO-1 proteins in the bronchial epithelium, as assessed through immunohistochemical methods. To the best of our knowledge, this study represents the first *in vivo* research on experimental animals to investigate the probiotic influence on the expression of TJ proteins in the respiratory tract epithelium.

The TJ proteins examined in this study within the bronchial epithelium include claudin-18, occludin, and ZO-1. Claudins, as transmembrane proteins, serve as the primary functional units in TJ, mediating epithelial barrier functions throughout the upper and lower respiratory tracts.(24) Among the non-classical claudins, claudin-18 is predominantly expressed in the lungs.(34) Classified as a barrier-forming claudin, claudin-18 restricts the movement
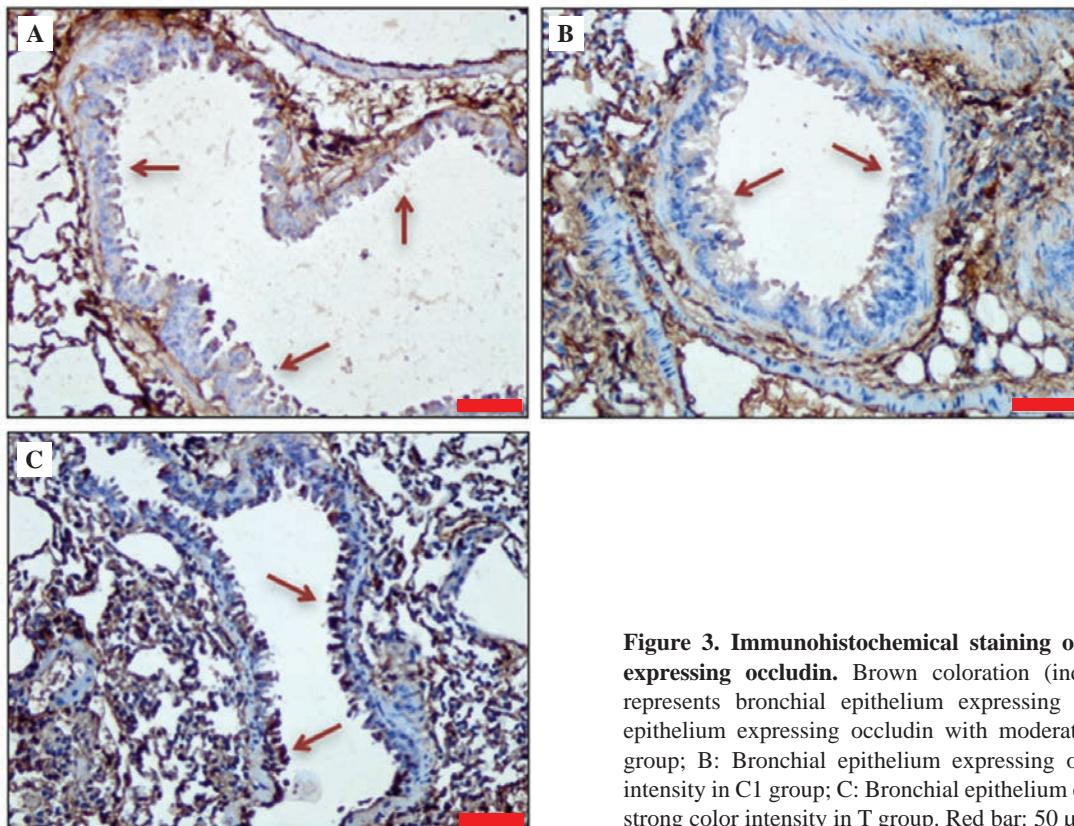

Figure 3. Immunohistochemical staining of bronchial epithelium expressing occludin. Brown coloration (indicated by red arrows) represents bronchial epithelium expressing occludin. A: Bronchial epithelium expressing occludin with moderate color intensity in C0 group; B: Bronchial epithelium expressing occludin with low color intensity in C1 group; C: Bronchial epithelium expressing occludin with strong color intensity in T group. Red bar: 50 μm.

Table 3. The difference in ZO-1 expression in bronchial epithelium.

	IRS Score of ZO-1			*Post Hoc* Analysis	
Group	Mean±SD	Median (min–max)	*p*-value	Group	*p*-value
C0	5.625±2.460[a]	6.500 (2.00–8.00)	0.001*	C0 versus C1	0.001*
C1	4.125±1.275	4.000 (2.50–7.00)[b]		C0 versus T	0.008*
T	9.187±1.888[a]	9.000 (7.00–12.00)		C1 versus T	0.001*

[a]Normal distribution; [b]Non-normal distribution; *Significant if $p<0.05$. Number of mice in each group n=8.

across the epithelial barrier, contributing to the integrity of the barrier and sealing properties.(34-36) Deficiency in claudin-18 results in epithelial barrier dysfunction, damage, and impaired alveolarization in mice.(22) Occludin, another transmembrane TJ protein, was the first identified and does not directly contribute to normal paracellular mechanical barrier function but plays a crucial role in regulating the barrier during inflammation. Several studies indicate that occludin serves as a significant regulator in epithelial barrier function across various tissues, including the respiratory tract.(24,37,38) ZO-1 acts as a scaffolding protein primarily binding to the actin cytoskeleton and aiding in anchoring TJ. ZO-1 is expressed in the epithelial cells lining the respiratory tract, predominantly localized at cell boundaries.(24) ZO-1 contains multiple domains capable of mediating protein-protein interactions with signaling molecules.(38)

The influence of *L. plantarum* IS-10506 on the improvement of TJ in the gastrointestinal tract has been studied in Wistar rats induced with LPS from *E. coli*, which found an increase in the expression of occludin and ZO-1. The mechanism underlying this improvement occurred through simultaneous activation of the ERK1/MAPK and JNK/MAPK signaling pathways.(30) A previous *in vitro* study involved the stimulation of human bronchial epithelial cells (Calu-3) with synthetic bacterial lipopeptide Pam3CysSK4, resulting in increased expression of claudin-1 and ZO-1, as examined using immunofluorescence methods. This study concluded that enhanced TJ barrier function was induced by toll-like receptor (TLR)2 stimulation, subsequently leading to atypical protein kinase C (PKC)ζ phosphorylation. This atypical PKCζ mediates TJ protein transcription through the activation of the nuclear factor (NF)-κB signaling pathway. (31,39)
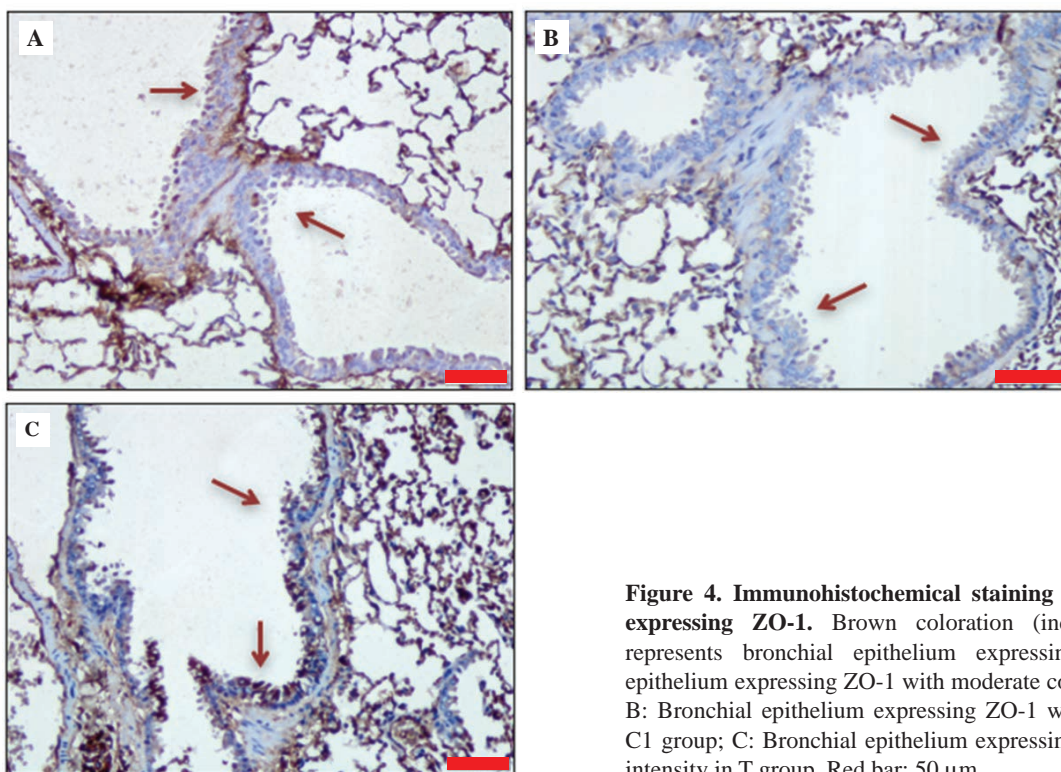

Figure 4. Immunohistochemical staining of bronchial epithelium expressing ZO-1. Brown coloration (indicated by red arrows) represents bronchial epithelium expressing ZO-1. A: Bronchial epithelium expressing ZO-1 with moderate color intensity in C0 group; B: Bronchial epithelium expressing ZO-1 with low color intensity in C1 group; C: Bronchial epithelium expressing ZO-1 with strong color intensity in T group. Red bar: 50 µm.

Conclusion

The current study findings demonstrate that oral supplementation of *L. plantarum* IS-10506 enhances the expression of airway TJ proteins (claudin-18, occludin, and ZO-1) in bronchial epithelium, indicating its potential in preventing allergic inflammation in the respiratory tract through the concept of the gut-lung axis. Further in-depth studies are necessary to uncover the mechanisms underlying the damage prevention or repair of TJ proteins in the respiratory epithelium.

Acknowledgments

Authors Contribution

DF, GS, AE and CRSP were involved in concepting and planning the research, DF, GS, ISS, AE, AFA, YH, DFH, IKS, BU and CRSP contributed to the methodology, DF, GS and CRSP performed the data acquisition/collection, DF and BU calculated the experimental data and performed the analysis, DF, GS and CRSP aided in interpreting the results, DF and GS drafted the manuscript, DF designed the figures and tables. All authors took parts in giving critical revision of the manuscript.

References

1. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, *et al.* Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet. 2006; 368(9537): 733–43.

2. Brozek G, Lawson J, Szumilas D, Zejda J. Increasing prevalence of asthma, respiratory symptoms, and allergic diseases: Four repeated surveys from 1993-2014. Respir Med. 2015; 109(8): 982–90.

3. Lundbäck B, Backman H, Lötvall J, Rönmark E. Is asthma prevalence still increasing? Expert Rev Respir Med. 2016; 10(1): 39–51.

4. Hamid F, Wiria AE, Wammes LJ, Kaisar MM, Djuardi Y, Versteeg SA, *et al.* Risk factors associated with the development of atopic sensitization in Indonesia. PLoS ONE. 2013; 8(6): e67064. doi: 10.1371/journal.pone.0067064.

5. Soegiarto G, Abdullah MS, Damayanti LA, Suseno A, Effendi C. The prevalence of allergic diseases in school children of metropolitan city in Indonesia shows a similar pattern to that of developed countries. Asia Pac Allergy. 2019; 9(2): e17. doi: 10.5415/apallergy.2019.9.e17.

6. Strachan DP. Hay fever, hygiene, and household size. Br Med J. 1989; 299(6710): 1259.

7. Von Hertzen L, Hanski I, Haahtela T. Natural immunity: Biodiversity loss and inflammatory diseases are two global megatrends that might be related. EMBO Rep. 2011; 12(11): 1089–93.

8. Zimmermann P, Messina N, Mohn WW, Finlay BB, Curtis N. Association between the intestinal microbiota and allergic sensitization, eczema, and asthma: A systematic review. J Allergy Clin Immunol. 2019; 143(2): 467–85.

9. Enaud R, Prevel R, Ciarlo E, Beaufils F, Wieërs G, Guery B, *et al.* The gut-lung axis in health and respiratory diseases: A place for inter-organ and inter-kingdom crosstalks. Front Cell Infect Microbiol. 2020; 10: 9. doi: 10.3389/fcimb.2020.00009.

10. Araya M, Morelli L, Reid G, Sanders ME, Stanton C, Pineiro M, *et al.* Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada: Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food; 2002.

11. Cuello-Garcia CA, Brożek JL, Fiocchi A, Pawankar R, Yepes-Nuñez JJ, Terracciano L, *et al.* Probiotics for the prevention of allergy: A systematic review and meta-analysis of randomized controlled trials. J Allergy Clin Immunol 2015; 136(4): 952–61.

12. Collado MC, Surono I, Meriluoto J, Salminen S. Indigenous dadih lactic acid bacteria: Cell-surface properties and interactions with pathogens. J Food Sci. 2007; 72(3): M89–93.

13. Surono IS. In vitro probiotic properties of indigenous dadih lactic acid bacteria. Asian-Aust J Anim Sci. 2003; 16(5): 726–31.

14. Umanets A, Surono IS, Venema K. I am better than I look: Genome based safety assessment of the probiotic Lactiplantibacillus plantarum IS-10506. BMC Genomics. 2023; 24(1): 518. doi: 10.1186/s12864-023-09495-y.

15. Endaryanto A. Imunoregulasi Th1, Treg dan Th2 melalui TLR2 dan TLR4 oleh probiotik LGG dan Lactobacillus plantarum IS-10506 dalam penurunan reaksi alergi. Studi eksperimental pada mencit coba [Dissertation]. Surabaya: Universitas Airlangga; 2007.

16. Prakoeswa CRS, Herwanto N, Prameswari R, Astari L, Sawitri S, Hidayati AN, *et al.* Lactobacillus plantarum IS-10506 supplementation reduced SCORAD in children with atopic dermatitis. Benef Microbes. 2017; 8(5): 833–40.

17. Prakoeswa CRS, Bonita L, Karim A, Herwanto N, Umborowati MA, Setyaningrum T, *et al.* Beneficial effect of Lactobacillus plantarum IS-10506 supplementation in adults with atopic dermatitis: A randomized controlled trial. J Dermatolog Treat. 2022; 33(3): 1491–8.

18. Ranuh RG, Darma A, Riawan W, Surono IS, Sandra F, Sudarmo SM. Lactobacillus plantarum IS-20506 probiotic restores galectin-4 and myosin-1a expressions in duodenum, jejunum and ileum of lipopolysaccharide-induced rats. Indones Biomed J. 2020; 12(3): 283–7.

19. Holgate ST. The airway epithelium is central to the pathogenesis of asthma. Allergol Int. 2008; 57(1): 1–10. doi: 10.2332/allergolint.R-07-154.

20. Post S, Nawijn MC, Hackett TL, Baranowska M, Gras R, Van Oosterhout AJM, *et al.* The composition of house dust mite is critical for mucosal barrier dysfunction and allergic sensitisation. Thorax. 2012; 67(6): 488–95.

21. Tan HT, Hagner S, Ruchti F, Radzikowska U, Tan G, Altunbulakli C, *et al.* Tight junction, mucin, and inflammasome-related molecules are differentially expressed in eosinophilic, mixed, and neutrophilic experimental asthma in mice. Allergy. 2019; 74(2): 294–307.

22. LaFemina MJ, Sutherland KM, Bentley T, Gonzales LW, Allen L, Chapin CJ, *et al.* Claudin-18 deficiency results in alveolar barrier dysfunction and impaired alveologenesis in mice. Am J Respir Cell Mol Biol. 2014; 51(4): 550–8.

23. Sweerus K, Lachowicz-Scroggins M, Gordon E, LaFemina M, Huang X, Parikh M, *et al.* Claudin-18 deficiency is associated with airway epithelial barrier dysfunction and asthma. J Allergy Clin Immunol. 2017; 139(1): 72–81.

24. Smith P, Koval M, Levy JM. Mucosal barrier defects: What have we learned from atopic dermatitis, asthma, and allergic rhinitis? Curr Otorhinolaryngol Rep. 2020; 8(1): 19–23.

25. Wang J, Ji H, Wang S, Liu H, Zhang W, Zhang D, *et al.* Probiotic Lactobacillus plantarum promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. Front Microbiol. 2018; 9: 1953. doi: 10.3389/fmicb.2018.01953.

26. Rhayat L, Maresca M, Nicoletti C, Perrier J, Brinch KS, Christian S, *et al.* Effect of Bacillus subtilis strains on intestinal barrier function and inflammatory response. Front Immunol. 2019; 10: 564. doi: 10.3389/fimmu.2019.00564.

27. Laval L, Martin R, Natividad J, Chain F, Miquel S, De Maredsous CD, *et al.* Lactobacillus rhamnosus CNCM I-3690 and the commensal bacterium Faecalibacterium prausnitzii A2-165 exhibit similar protective effects to induced barrier hyper-permeability in mice. Gut Microbes. 2015; 6(1): 1–9. doi: 10.4161/19490976.2014.990784.

28. Tulyeu J, Kumagai H, Jimbo E, Watanabe S, Yokoyama K, Cui L, *et al*. Probiotics prevents sensitization to oral antigen and subsequent increases in intestinal tight junction permeability in juvenile–young adult rats. Microorganisms. 2019; 7(10): 463. doi: 10.3390/microorganisms7100463.

29. Karczewski J, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer RJM, *et al.* Regulation of human epithelial tight junction proteins by Lactobacillus plantarum in vivo and protective effects on the epithelial barrier. Am J Physiol Gastrointest Liver Physiol. 2010; 298(6): G851–9.

30. Ranuh IRG. Ekspresi protein galectin-4, myosin-1a, occludin dan ZO-1 sebagai efek pemberian probiotik Lactobacillus plantarum IS pada perbaikan kerusakan brush border usus akibat lypopolysaccharida E. coli [Dissertation]. Surabaya: Universitas Airlangga; 2008.

31. Ragupathy S, Esmaeili F, Paschoud S, Sublet E, Citi S, Borchard G. Toll-like receptor 2 regulates the barrier function of human bronchial epithelial monolayers through atypical protein kinase C zeta, and an increase in expression of claudin-1. Tissue Barriers. 2014; 2(2): e29166. doi: 10.4161/tisb.29166.

32. Herz U, Renz H, Wiedermann U. Animal models of type I allergy using recombinant allergens. Methods. 2004; 32(3): 271–80.

33. Nakayama T. Introduction to "allergic inflammation". Immunol Rev. 2017; 278(1): 5–7.

34. Schlingmann B, Molina SA, Koval M. Claudins: Gatekeepers of lung epithelial function. Semin Cell Dev Biol. 2015; 42: 47–57.

35. Steelant B, Seys SF, Boeckxstaens G, Akdis CA, Ceuppens JL, Hellings PW. Restoring airway epithelial barrier dysfunction: A new therapeutic challenge in allergic airway disease. Rhinology. 2016; 54(3): 195–205.

36. Steelant B, Farré R, Wawrzyniak P, Belmans J, Dekimpe E, Vanheel H, *et al.* Impaired barrier function in patients with house dust mite–induced allergic rhinitis is accompanied by decreased occludin and zonula occludens-1 expression. J Allergy Clin Immunol. 2016; 137(4): 1043–53.

37. Feldman GJ, Mullin JM, Ryan MP. Occludin: Structure, function and regulation. Adv Drug Deliv Rev. 2005; 57(6): 883–917.

38. Georas SN, Rezaee F. Epithelial barrier function: At the front line of asthma immunology and allergic airway inflammation. J Allergy Clinical Immunol. 2014; 134(3): 509–20.

39. Teusch N, Lombardo E, Eddleston J, Knaus UG. The low molecular weight GTPase RhoA and atypical protein kinase Cζ are required for TLR2-mediated gene transcription. J Immunol. 2004; 173(1): 507–14.