APJAI
Asian Pacific Journal of
Allergy and Immunology

ORIGINAL ARTICLE

Characterization of gut microbiome profile in children with confirmed wheat allergy

Watcharoot Kanchongkittiphon,[1] Saifon Nopnipa,[2] Rubwad Mathuranyanon,[2] Nutthawan Nonthabenjawan,[3] Suphanich Sritournok,[4] Wiparat Manuyakorn,[1] Pagakrong Wanapaisan[4,]*

Abstract

Background: Food allergies pose serious health risks, including life-threatening anaphylactic reactions, increased morbidity, and reduced quality of life. Wheat allergy is a common concern in Asia. There is growing interest in understanding the potential association between dysregulation of the gut microbiome and the development of food allergies.

Objective: This study aimed to explore the gut microbiome of Thai children with wheat allergy and its potential association with allergic responses.

Methods: Microbial abundance was assessed using Quantitative Insights into Microbial Ecology 2 (QIIME2) microbiome analysis based on 16S rDNA data. The correlation between microbial richness and relevant parameters was evaluated using the Spearman correlation analysis. Additionally, the microbial community functions were predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2).

Results: The gut microbiome analysis revealed significant differences between the two groups at the phylum and genus levels. *Firmicutes* ($p = 0.012$) and *Verrucomicrobia* ($p < 0.001$) were enriched in wheat-allergic children, whereas specific gut microbes such as *Megamonas* ($p = 0.04$), *Romboutsia* ($p < 0.001$), *Fusobacterium* ($p < 0.001$), *Clostridium senso stricto1* ($p < 0.001$), and *Turicibacter* ($p < 0.001$) were more abundant in healthy children. *Anaerostripes* ($p = 0.011$), *Erysipelatoclostridium* ($p < 0.001$), *Prevotella* 2 ($p < 0.001$), *Ruminiclostridium 5* ($p < 0.001$), and *Clostridium innnocuum* ($p < 0.001$) were enriched in children with a confirmed wheat allergy. Functional analysis indicated disparities in the pathways related to arginine and polyamine biosynthesis.

Conclusion: These findings offer valuable insights into the gut microbiome of children with wheat allergy and its potential impact on symptom severity, laying the groundwork for further research and interventions aimed at addressing this health concern.

Key words: wheat allergy, gut microbiome, children, urticaria, anaphylaxis

Citation:
Kanchongkittiphon, W., Nopnipa, S., Mathuranyanon, R., Nonthabenjawan, N., Sritournok, S., Manuyakorn, W., Wanapaisan, P. (0000) Characterization of gut microbiome profile in children with confirmed wheat allergy. *Asian Pac J Allergy Immunol*, 00(0), 000-000. https://doi.org/10.12932/ap-080623-1626

Affiliations:
[1] Department of Pediatrics, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[2] Food Research Unit, CPF Food Research and Development Center, Phranakhon Si Ayutthaya, Thailand
[3] Feed Technology Department, Charoen Pokphand Group, Bangkok, Thailand
[4] Department of Microbiology, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

Corresponding author:
Pagakrong Wanapaisan
Department of Microbiology, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand
E-mail: pagakrong.wan@mahidol.ac.th

Introduction

Food allergies can lead to severe anaphylactic reactions, posing a significant threat to life and considerably affecting morbidity and quality of life. The prevalence of food allergies, particularly in children, is increasing, affecting up to 10% of the population, a trend likely attributable to modernization and lifestyle changes.[1] IgE-mediated food allergies are widely recognized as the best-known and most extensively studied type of food allergy. It has the highest prevalence in the Western world, particularly among children below the age of three, with incidence rates ranging from 6% to 8%.[2] Globally, cow milk and eggs are predominant allergens in children.[3] In Japan, Taiwan, and Thailand, wheat allergy are more prevalent and rank higher than shellfish and nut allergies. Wheat is a primary cause of food-induced anaphylaxis in South Korea and Japan.[4] Data from the last decade indicate a 4.85% prevalence of wheat allergy in children, with a report of seven children with anaphylaxis in 2005 in Thailand.[5] A recent study of food allergies in northern Thailand revealed that 6.1% of preschool children reported having wheat allergy.[6]

Microbial communities are implicated in allergic disorders, and their dysregulation may increase the risk of developing food allergies. Researchers are increasingly exploring the association between the gut microbiota and food allergy risk.[7] Gut microbiome diversity and specific taxa are presumed to protect against the development of allergic diseases, including asthma and food allergies. The microbiota may influence the function of the intestinal barrier and modulate mucosal immune functions. Firmicutes taxa, such as Clostridia, are enriched in the gut of milk-allergic infants whose allergies are resolved by age eight.[8] *Lachnospiraceae*, *Streptococcaceae*, and *Leuconostocaceae* are abundant in children with egg allergies.[9] *Blautia*, *Erysipelatoclostridium*, *Lachnospiraceae*, and *Akkermansia* are increased in patients with wheat-dependent exercise-induced anaphylaxis.[10] Clostridial colonization induces IL-22 production by innate lymphoid cells to promote barrier function with depletion of allergen exposure.[11] In mouse models, specific *Clostridium* strains promote colonic T regulatory cell populations, which are essential for inducing immune tolerance. *Bifidobacteria* and *Lactobacilli* are vital beneficial gut bacteria, particularly in breastfed infants. In murine models, they exhibit immunomodulatory effects and protect against atopy and allergy. Their absence may cause dysbiosis and affect gut microbiome function. Reduced *Bifidobacterium* levels in infants with cow milk allergies emphasize their crucial role in the development of cow milk allergies.[12]

This study represents a pioneering endeavor, as it is the first to explore the gut microbiomes of children with confirmed IgE-mediated wheat allergy, while previous research primarily concentrated on gut microbiomes in patients afflicted by allergies to foods such as milk and eggs.[8,9,13,14] The main aim of this groundbreaking study was to examine the gut microbiome of Thai children with wheat allergy and its potential connection with allergy severity. This study represents a pioneering endeavor, as it is the first to explore the gut microbiomes of children with confirmed IgE-mediated wheat allergy, while previous research primarily concentrated on gut microbiomes in patients afflicted by allergies to foods such as milk and eggs.[8,9,13,14] The main aim of this groundbreaking study was to examine the gut microbiome of Thai children with wheat allergy and its potential connection with allergy severity. Additionally, this study explored the correlation between participant characteristics and gut microbiome composition. To achieve these objectives, this study employed 16S rDNA microbiome analysis to assess the diversity and relative abundance of the gut microbiota in children with wheat allergy presenting as urticaria and anaphylaxis, as well as in healthy controls without food allergies. Furthermore, functional prediction of microbial communities was carried out considering host-related factors, such as dietary profiles, that could affect gut microbiota composition. These findings underscore the importance of tailored investigations into the role of the gut microbiome in the pathogenesis of wheat allergy, with possible implications for therapeutic interventions.

Methods

Study design and subjects

This is a cross-sectional study. Participants aged 1–18 years were recruited from an outpatient pediatric clinic at Ramathibodi Hospital. The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of Ramathibodi Hospital, Mahidol University (Approval ID: MURA2020/3 on 12 December 2019). After obtaining informed consent, patients underwent an initial assessment. Baseline demographic data including age, weight, height, mode of delivery, allergic diseases, tobacco smoke exposure, parental history of allergic diseases, and dietary history were recorded. Patients had wheat allergy if they had (i) either a positive skin prick test to the wheat allergen (ALK-Abello Pharm., Inc., New York, USA) or wheat-specific IgE level ≥ 0.35 kU/L by ImmunoCap (BiomedDiagnostics, Thailand) and (ii) a positive physician-supervised graded oral food challenge to wheat flour/bread or a history of physician-diagnosed anaphylaxis due to wheat ingestion within six months. Control participants had no known food allergies. The Mann-Whitney U test was used to compare differences between the two independent groups.

Skin prick test

Skin prick tests were performed using a blood lancet (Feather Safety Razor, Osaka, Japan) on the volar aspect of the forearm or upper back using 0.1% histamine (1 mg/mL) as the positive control solution, normal saline as the negative control, and commercial extracts of the wheat allergen (ALK-Abello Pharm., Inc.). After 15 min, the wheal size was measured and the mean of the longest diameter plus the perpendicular diameter, designated as the mean wheal diameter, was recorded. A wheal size 3 mm greater than that of the negative control was interpreted as positive.

Stool sample collection and nucleic acid extraction

Stool samples were collected from the participants during a study visit or from their parents at home using a stool nucleic acid collection kit (Norgen BioTek Corp, Ontario, Canada). DNA was extracted from all samples using the QIAamp PowerFecal Pro DNA Kit (Qiagen, Maryland, United States), following the manufacturer's protocol. The purity of the DNA samples was determined using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Massachusetts, United States).

16S rDNA sequencing and library preparation

The 16S rDNA gene was amplified using specific 16S V3 and V4 amplicon primers (338F:5′-ACTCCTACGGGA GGCAGCA-3′ and 806R:5′-GGACTACHVGGGTWTCTAA T-3′). Amplicon libraries were prepared according to the manufacturer's protocol. After indexing, all the samples were analyzed using a fragment analyzer (Agilent Technologies, United States). The expected size was approximately 550 bp, and the concentration from the fragment analysis was used for normalization. The final concentration of each sample was 4 nM, and it was pooled for sequencing. Pooled samples were sequenced using an Illumina MiSeq instrument with a v3 reagent kit (500 cycles).

Microbial community and differential abundance statistical analysis

The 16S rDNA sequences were cleaned and processed using Quantitative Insights into Microbial Ecology 2 (QIIME2).[15] Amplicon quality-filtered paired-end reads were combined into contigs using the DADA2 algorithm.[16] Human genomic DNA was removed from microbial reads. The 16S rDNA nucleotide reads were mapped to the human genome GRCh38 (hg38) using a short-read aligner by running Bowtie2 mapping.[17] No human DNA was detected in the microbial samples. The sequences were then aligned using the Ribosomal Database Project Naive Bayes classifier with a 95% confidence limit.[18] Sequences with ≥ 97% similarity were clustered into operational taxonomic units (OTUs). OTUs with < 0.05% of the total sequence reads were filtered from the dataset to account for sequencing errors. All OTUs were aligned using the SILVA 16S rDNA reference alignment database.[19]

Alpha diversity included the observed OTUs and Faith's phylogenetic diversity. Evenness and Shannon index between the two participant groups were calculated using normalized OTUs (35,000 reads) from random subsampling. Beta diversities were computed using normalized OTUs via Jaccard in QIIME2.[15] Kruskal-Wallis (pairwise) test measured at a 0.05 significance level were used to determine differences in alpha diversity between the groups using QIIME2.[15] To detect statistical differences in the beta diversity metrics between the groups, permutational multivariate analysis of variance (PERMANOVA) in QIIME2 was used. Differential abundances of taxa between the allergy and control groups were determined at the OTU level using the DESeq2 package in R. Correlations between relative abundance and individual parameters were analyzed using the Spearman correlation (R package).[19]

Functional prediction of microbial communities

Functional predictions were assessed utilizing Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) version 2.4.1, based on the Kyoto Encyclopedia of Genes (KEGG) pathway database.[20,21] To identify variations in the abundance of predicted metabolic functions among the different groups, the Statistical Analysis for Metagenomic Profile (STAMP) software was employed.[22] This analysis involved White's non-parametric t-test and was corrected for multiple-testing using the Benjamini-Hochberg false discovery rate (FDR).[23] Significance was determined by corrected *p*-values below < 0.05, indicating noteworthy statistical distinctions.

Results

Characterization of the study participants

Sixty children were recruited; 30 were diagnosed with wheat allergy and the other 30 were healthy controls with no food allergies. The baseline demographic characteristics are shown in **Table 1**. The mean age of the children was 44.9 months; 38/60 (63.3%) were male and 49/60 (81.7%) were born via cesarean section. The two groups did not differ in age, sex, birth weight, mode of delivery, or other allergic diseases (asthma, allergic rhinitis, and atopic dermatitis). Compared to healthy controls, wheat-allergic children had a more significant family history of food allergies (34.5% vs. 3.3%; $p < 0.001$; **Table 1**). Of the 30 children with wheat allergy, six had allergies to wheat, along with other foods. Two people were allergic to wheat and cow milk, and one to wheat, cow milk, egg white, and seafood. Additionally, one person was allergic to wheat, cow milk, soy, egg white, apple, and millet. Regarding wheat allergy symptoms, 15/30 wheat-allergic children (50%) had a history of anaphylactic reactions, while the other 15 had only urticarial rashes with wheat ingestion. Wheat-specific IgE levels were significantly higher in children with a history of anaphylaxis than in those who presented with urticarial rashes only (**Table 2**).

Children with wheat allergy consumed less bread, desert, and eggs than those without food allergies. Surprisingly, children with wheat allergy had a higher intake of vegetables than the healthy controls. However, there were no differences in the intakes of fruits, juices, rice, sparkling drinks, or proteins between the groups (**Table 1**). Additionally, our analysis did not reveal any statistically significant differences in the consumption of various types of milk, dairy products, or probiotics between the groups.

Table 1. Baseline characteristics and dietary intake frequency of the participants.

	Control (n = 30)	Wheat allergy group (n = 30)	*p*-value
Baseline characteristics			
Age, months, mean (SD)	47.2 (27.0)	42.6 (27.3)	0.403
Male, n (%)	19 (63.3)	19 (63.3)	1.000
Birth weight, g, mean (SD)	3002.1 (498.8)	3075.0 (340.6)	0.403
Family member with food allergy, n (%)	1 (3.3)	10 (34.5)	< 0.001**
Mode of delivery, C-section, n (%)	24 (80.0)	25 (83.3)	0.739
Allergic diseases, n (%)	0 (0)	4 (13.3)	0.112
- Asthma	0 (0)	2 (0.07)	
- Allergic rhinitis	0 (0)	1 (0.03)	
- Atopic dermatitis	0 (0)	1 (0.03)	
SPT to wheat, size in mm, median (IQR)	0 (0)	6.0 (2.1–8.5)	< 0.001**
sIgE to wheat, median (IQR)	0 (0)	12.3 (1.2–36.9)	< 0.001**
Average dietary intake frequency, times/months (SD)			
Dietary fiber			
- Fruit	24.6 (17.6)	35.1 (24.0)	0.154
- Juice	6.9 (8.5)	10.4 (11.1)	0.279
- Vegetable	26.2 (26.6)	41.4 (32.3)	0.041*
High-carbohydrate diet			
- Bread	3.7 (8.0)	0 (0)	0.011*
- Dessert	16.2 (12.6)	7.3 (10.3)	0.002**
- Rice	71.5 (23.9)	77.9 (17.0)	0.262
- Sparkling drink	3.8 (6.7)	5.1 (9.6)	> 0.900
High-protein diet			
- Egg	29.1 (17.0)	22.1 (19.7)	0.039*
- Fish	17.7 (14.8)	22.8 (22.4)	0.608
- Meat	52.4 (27.8)	53.9 (30.5)	0.769
- Tofu	7.0 (7.6)	5.2 (15.5)	0.064
Milk and dairy products			
- Breast milk	10.3 (23.5)	15.6 (35.0)	0.841
- Cow milk	45.5 (34.4)	51.1 (44.6)	0.870
- Yoghurt	2.9 (5.9)	2.5 (5.8)	0.187
- Yoghurt with probiotics	3.5 (4.8)	7.4 (9.1)	0.502

SD: Standard deviation, IQR: interquartile range, SPT: skin prick test
Statistically significant differences are indicated by *($P < 0.05$), **($P < 0.01$).

Table 2. Baseline characteristics of wheat allergy children with urticaria and anaphylaxis.

	Anaphylaxis (n = 15)	Urticaria (n = 15)	*p*-value
Age, months, mean (SD)	39.0 (17.0–54.0)	36.0 (19.0–60.0)	0.884
Male, n (%)	9 (60)	10 (66.67)	0.705
Birth weight, g, mean (SD)	3102.3 (348.9)	3047.7 (342.1)	0.606
Family member with food allergy, n (%)	7 (46.67)	3 (20)	0.121
Mode of delivery, C-section, n (%)	11 (73.33)	14 (93.33)	0.142
SPT to wheat, size in mm, median (IQR)	6 (2.25–7.5)	0 (0–3.5)	0.016*
sIgE to wheat, median (IQR)	34.9 (21.3–100.0)	1.5 (0.3–6.7)	< 0.001**

SD: Standard deviation, IQR: interquartile range, SPT: skin prick test
Statistically significant differences are indicated by *($P < 0.05$), **($P < 0.01$).

Microbiome analysis

We identified 5.76 million sequence reads from 60 stool samples (mean ± standard deviation: 95,937 ± 32,687 reads/sample), which were clustered into 16,141 OTUs with ±97% sequence identity. The baseline microbiota alpha diversity values, computed via observed OTUs ($p = 0.135$) and the Shannon index ($p = 0.188$), did not differ significantly between the children with wheat allergy and the controls (**Figure 1A**). Beta diversity, assessed using Jaccard (or PERMANOVA), demonstrated significant differences in the overall bacterial communities between children with wheat allergy and the control group ($p = 0.002$) (**Figure 1B**). Nineteen bacterial genera exhibited significant differences in the OTU distribution between the gut microbiota of the two groups, as determined by DESEq2 ($p < 0.05$). At the phylum level, Firmicutes ($p = 0.012$) and Verrucomicrobia ($p < 0.001$) were significantly enriched in wheat-allergic children compared to the controls (**Figure 1C**). At the genus level, *Megamonas* ($p = 0.004$), *Romboutsia* ($p < 0.001$), *Fusobacterium* ($p < 0.001$), *Clostridium* sensu stricto1 ($p < 0.001$), and *Turicibacter* ($p < 0.001$) exhibited higher relative abundances in the control group compared to wheat-allergic group. Conversely, *Anaerostipes* ($p = 0.011$), *Erysipelatoclostridium* ($p < 0.001$), *Prevotella* 2 ($p < 0.001$), *Ruminiclostridium 5* ($p < 0.001$), and *Clostridium innocuum* group ($p < 0.001$) were more abundant in children with wheat allergy than in the controls (**Figure 1D**).

Wheat-allergic children were further categorized into the urticaria (15/30) and anaphylaxis (15/30) groups based on their symptoms. No statistically significant differences were observed in alpha diversity measures, including observed OTUs ($p = 0.194$) and Shannon index ($p = 0.144$), between the control and symptom groups (**Figure 2A**). However, significant differences in beta diversity were found between the control group and both the urticaria ($p = 0.013$) and anaphylactic groups ($p = 0.002$). Moreover, the beta-diversity analysis between the urticaria and anaphylactic groups was not significantly different ($p = 0.104$) (**Figure 2B**). At the phylum level, significant differences were observed between Firmicutes ($p = 0.008$) and Fusobacteria ($p < 0.001$) in the control and urticaria groups. However, no significant differences were found between the control and anaphylactic groups (**Figure 2C**).

In the context of wheat allergy, urticaria, and anaphylaxis, the abundance of *Megamonas* progressively declined with significant differences among these conditions (**Figure 2D**). The control group was dominated by *Fusobacterium* and *Clostridium* sensu stricto1 compared to both the urticaria and anaphylactic groups. Notably, *Romboutsia* and *Turicibacter* were significantly more abundant in the control group than in the urticaria group but not in the anaphylactic group. Conversely, *Prevotella* 2 displayed an increased abundance in children with wheat allergy who presented with anaphylaxis compared with those who presented with urticaria and the control group (**Figure 2D**). Moreover, the genera *Anaerostripes*, *Erysipelatoclostridium*, and *Clostridium innocuum* were significantly more abundant in both the urticaria and anaphylactic groups than in the control group, with no significant differences between the urticaria and anaphylactic groups. Additionally, *Ruminiclostridium 5* dominated the urticaria group compared to the control and anaphylactic groups (**Figure 2D**).

The study also analyzed children ≥ 3 years old with persistent wheat allergy (n = 17) and found no statistically significant differences in the alpha diversity, as represented by observed OTUs ($p = 0.064$) and the Shannon index ($p = 0.508$), compared to the controls (n = 16) (**Figure 3A**). However, significant differences were observed in the beta diversity between healthy children and those with wheat allergy ($p = 0.015$) (**Figure 3B**). At the phylum level, significant differences were observed in *Firmicutes* ($p = 0.035$), *Fusobacteria* ($p < 0.001$), and *Verrucomicrobia* ($p = 0.006$) (**Figure 3C**). Upon closer examination, the top five genera exhibiting higher abundance in children with persistent wheat allergy were identified as *Anaerostipes* ($p = 0.007$), *Prevotella* 2 ($p < 0.001$), *Erysipelatoclostridium* ($p < 0.001$), *Ruminiclostridium* ($p < 0.001$), and *Rumicoccus gauvreauii* ($p = 0.001$) (**Figure 3D**). Conversely, *Megamonas* ($p < 0.001$), *Fusobacterium* ($p < 0.001$), *Agathobacter* ($p < 0.001$), *Megasphaera* ($p < 0.001$), and *Erysipalotrichaceae* UCG-003 ($p < 0.001$) were most abundant in the control group.

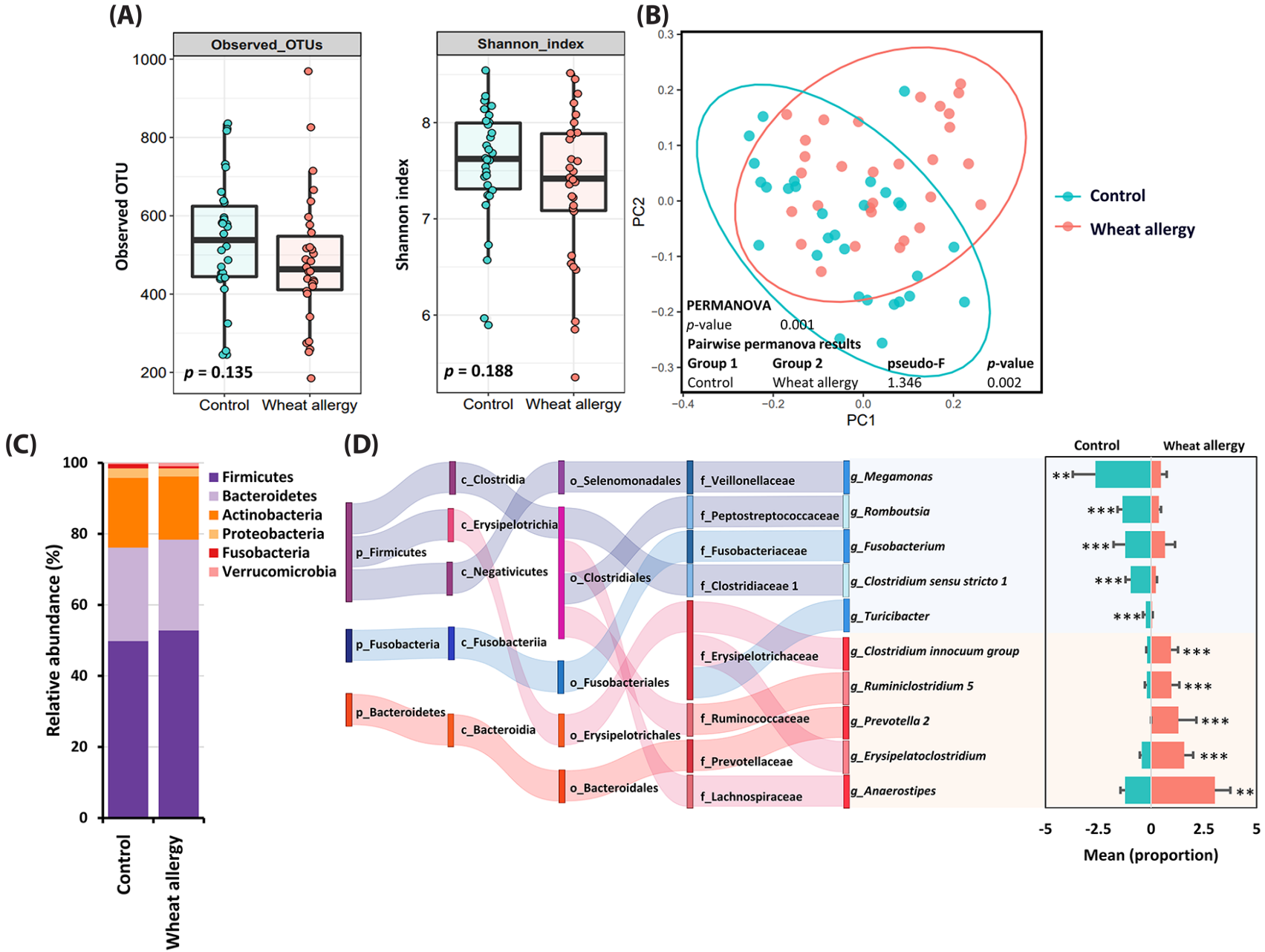

Figure 1. Gut microbiome composition in children with wheat allergy and their healthy counterparts (controls).
(A) Boxplots representing the alpha diversity by Observed OTU and Shannon indexes in children with wheat allergy and controls; (B) Separation of the bacterial populations computed by Jaccard. Differential abundances of microbes between children with wheat allergy and controls. (C) Relative abundance at the phylum level; (D) Mean proportion at the genus level. Statistically significant differences are plotted (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

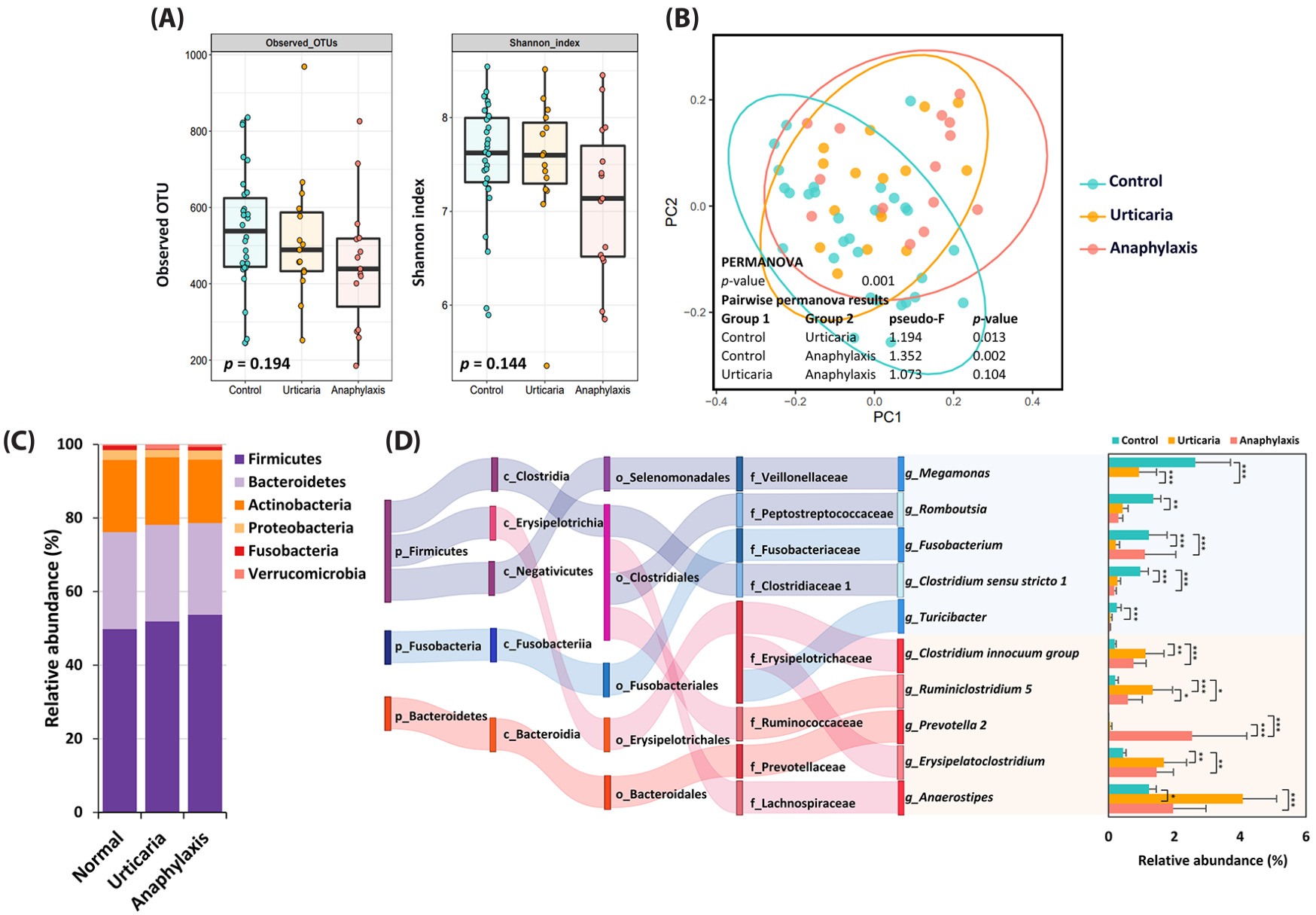

Figure 2. Gut microbiome composition in children with wheat allergy presenting with urticaria rash and anaphylaxis.
(A) Boxplots representing the alpha diversity by Observed OTU and Shannon indexes in children with wheat allergy and controls; (B) Separation of the bacterial populations computed by Jaccard. Differential abundances of microbes between children with wheat allergy presenting with urticaria rash and anaphylaxis. (C) Relative abundance at the phylum level; (D) Relative abundance at the genus level. Statistically significant differences are plotted (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

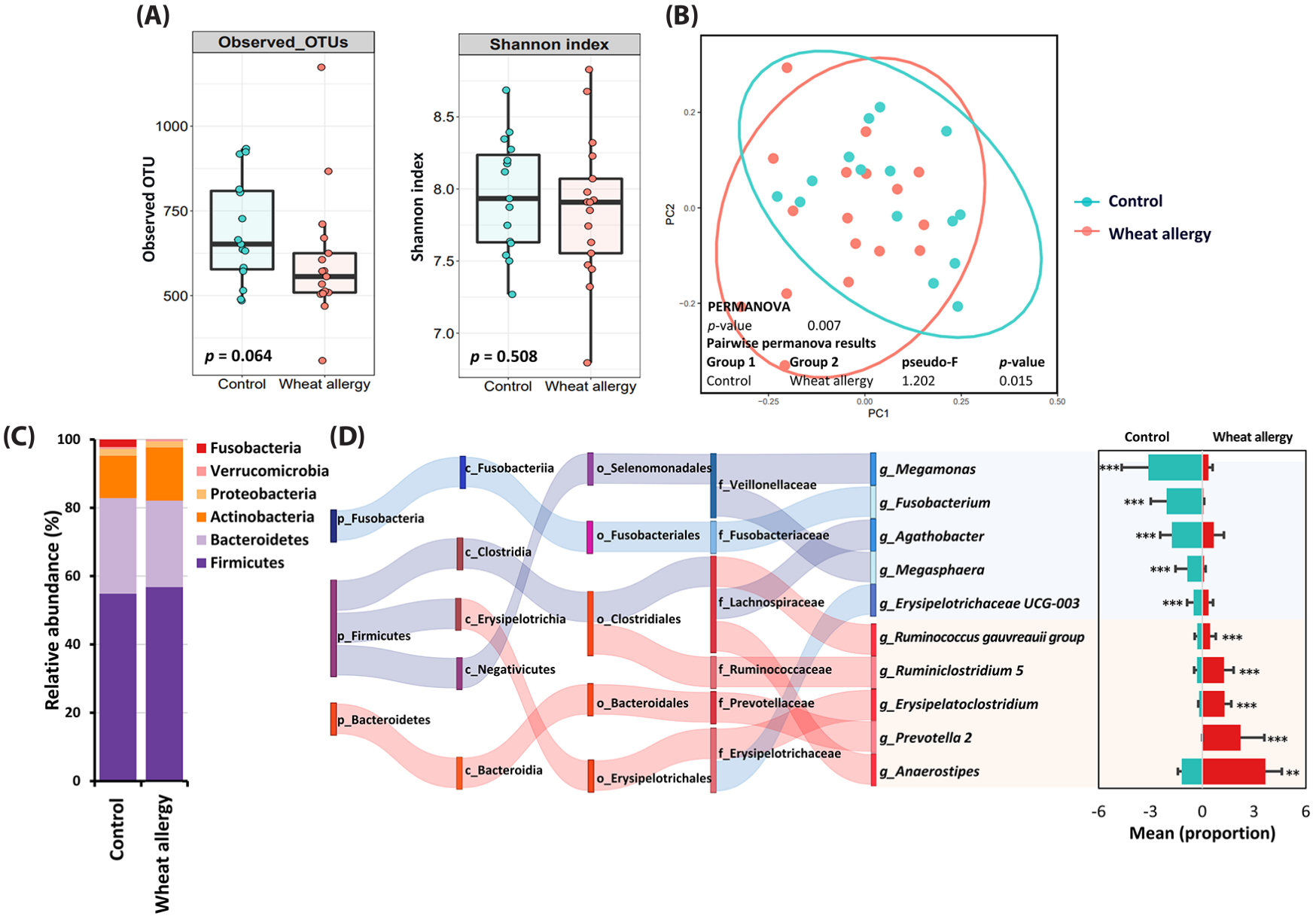

Figure 3. Gut microbiome composition in children with persistent wheat allergy and controls.
(A) Boxplots representing the alpha diversity by Observed OTU and Shannon indexes in children with wheat allergy and controls; (B) Separation of the bacterial populations computed by Jaccard. Differential abundances of microbes between children with persistent wheat allergy and controls. (C) Relative abundance at the phylum level; (D) Relative abundance at the genus level. Statistically significant differences are plotted (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Functional analysis of the bacterial communities in both the control and symptom groups was conducted using PICRUSt2. Four KEGG pathways, specifically tropane, piperidine, and pyridine alkaloid biosynthesis (p = 0.043); terpenoid backbone synthesis (p = 0.043); cell cycle (p = 0.035); and protein export pathway (p = 0.047), were significantly different between the two groups. Furthermore, enzymatic analysis revealed polyamine biosynthesis I (p = 0.045) and II (p = 0.048), arginine and polyamine biosynthesis (p = 0.028), and biosynthesis of cob(II)yrinate c-diamide (p = 0.036) in the control group, along with enzymes involved in l-histidine degradation (p = 0.030) in the wheat allergy group (**supplementary data**).

Correlations between the gut microbiota, host factors, and diet

Host factors and dietary habits can significantly influence gut microbiota. Therefore, we examined the correlations between the gut microbiota and allergy symptoms, as well as host factors such as age, birth weight, specific immunoglobulin E (sIgE) to wheat, and dietary habits (**Figure 4**). We observed significant negative correlations between wheat-specific IgE levels and bacterial abundance in the control group, including *Megamonas*, *Romboutsia*, and *Clostridium* sensu stricto 1. Conversely, wheat-specific IgE levels positively correlated with *Erysipelatoclostridium* and *Clostridium innocuum* levels. Participant age was positively

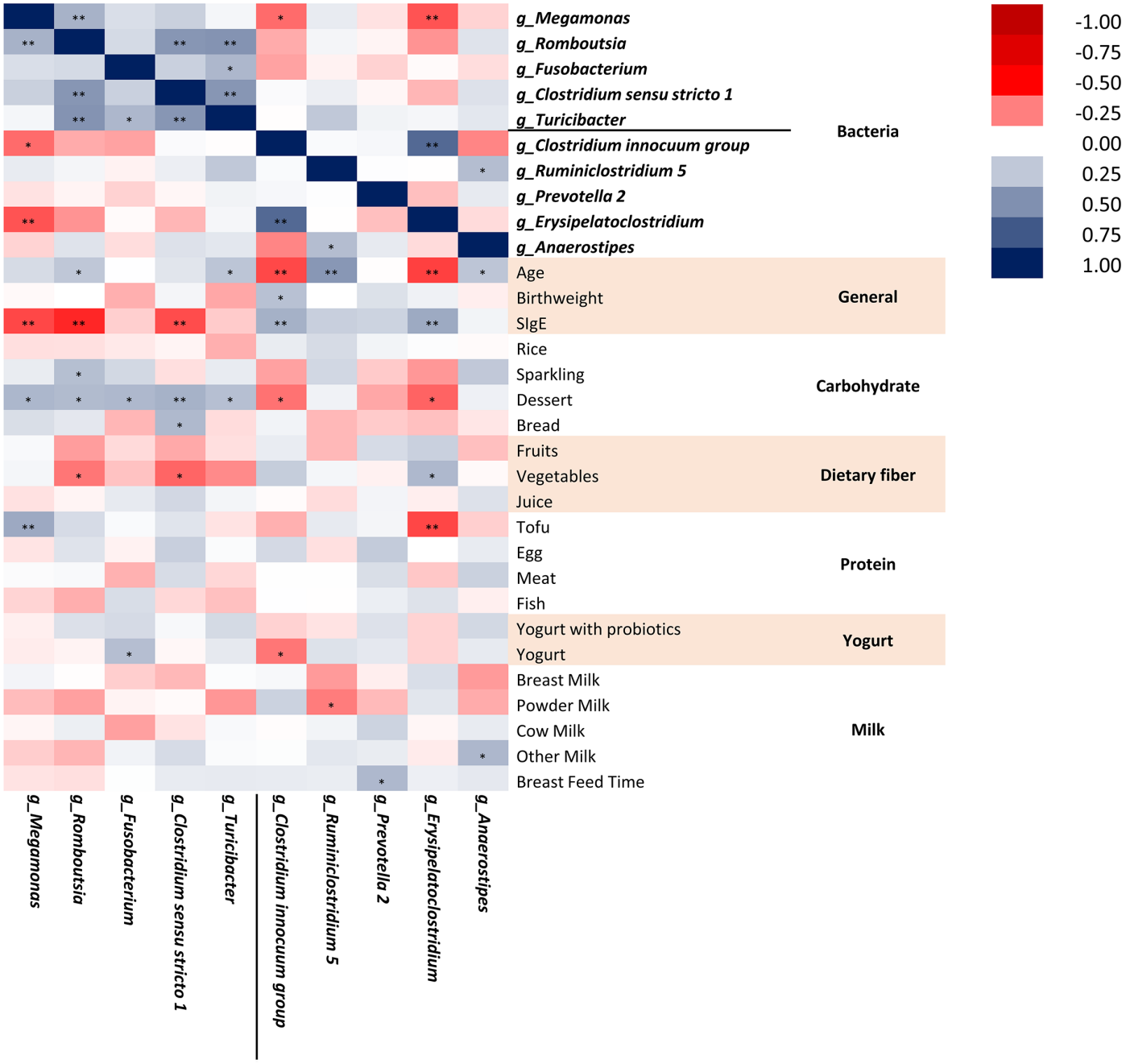

Figure 4. Spearman correlations between bacterial genera and others.
Blue and red represent positive and negative correlations, respectively. Statistically significant correlations are indicated (*P < 0.05; **P < 0.01).

correlated with *Romboutsia*, *Turicibacter*, *Ruminiclostridium*, and *Anaerostripes*, and negatively correlated with *Erysipelatoclostridium* and *Clostridium innocuum*. Birth weight was positively correlated with *Clostridium innocuum*, and breastfeeding duration was positively correlated with *Prevotella* 2.

In terms of dietary intake, the five dominant genera in the control group showed a significant positive correlation with dessert consumption, whereas the genera *Erysipelatoclostridium* and *Clostridium innocuum*, prevalent in the symptom group, exhibited a negative correlation with dessert consumption. Regarding vegetable intake, distinct patterns were observed for certain microbial genera. *Erysipelatoclostridium* showed a positive correlation with vegetable consumption, whereas *Romboutsia* and *Clostridium* senso stricto 1 showed negative associations. In addition, *Clostridium* senso stricto 1 positively correlated with bread consumption. Regarding other food items, *Megamonas* showed a positive correlation, while *Erysipelatoclostridium* exhibits a negative correlation with tofu consumption. *Fusobacterium* showed a positive correlation, while *Clostridium innocuum* group exhibits a negative correlation with yogurt consumption. Moreover, *Anaerostripe* were positively correlated with other types of milk consumption, whereas *Ruminiclostridium 5* was positively correlated with powdered milk consumption. These findings indicate potential associations between specific microbial genera and dietary preferences in the study groups.

Discussion

In our study, no statistically significant differences in the alpha diversity were observed between children with wheat allergy and their healthy counterparts. Although microbial diversity tended to be higher in the healthy controls, the difference was not significant. These findings are consistent with previous studies by Savage et al. and Marri et al. who reported similar results in individuals with food allergies and asthma, respectively.[24,25] Significant differences in the gut microbiome composition at the phylum level were observed between children with and without wheat allergy, with *Firmicutes* and *Verrucomicrobia* being more predominant in wheat-allergic children, consistent with previous findings on egg allergy.[9]

The microbiome undergoes significant changes influenced by genetics, diet, lifestyle, and environment, developing in infants from birth and maturing to an adult-like state by 36 months.[26] Assessing age-related dynamics of the microbiome is crucial to understanding its influence on health and disease across various age groups. The study conducted two gut microbiome analyses, one involving participants of all ages and the other specifically focusing on children aged three years and older, considering the previous evidence of wheat allergy persistence beyond this age.[2] *Megamonas* and *Fusobacterium* were consistently more abundant in the control group, suggesting a potential protective or regulatory role against wheat allergy, both in the all-age and age group of more than three years old. In contrast, *Agathobacter*, *Megasphaera*, and *Erysipalotrichaceae* UCG-003 were more abundant in the control group of children aged more than three years old, whereas *Romboutsia*, *Clostridium* sensu stricto1, and *Turicibacter* were observed across all age groups. *Anaerostipes*, *Prevotella* 2, *Erysipelatoclostridium*, and *Ruminiclostridium* consistently showed higher abundances in children with persistent wheat allergy and in the general analysis across all ages, indicating their potential as diagnostic biomarkers for early identification and intervention in individuals at risk of developing wheat allergy. Additionally, *Ruminococcus gauvreauii* was exclusively associated with the persistent form of wheat allergy, while *Clostridium innocuum* was found in the general analysis across all ages, suggesting that specific bacterial genera may have significant implications in the development of wheat allergy, whereas others could potentially offer protective effects.

Our study found significant differences in *Megamonas* and *Prevotella* 2 abundances in relation to wheat allergy severity. Megamonas was highly abundant in the control group, whereas an increased abundance of *Prevotella* was observed in children with wheat allergy who presented with anaphylaxis. These findings highlight the potential role of gut microbiota in the pathophysiology of these allergic responses The higher abundance of *Megamonas* in children without food allergies aligns with previous studies linking reduced *Megamonas* levels to chronic spontaneous urticaria.[27] Furthermore, our results are in line with those of previous studies showing *Megamonas* and *Fusobacterium* dominance in healthy gut microbiomes. In contrast, *Prevotella* 2 and *Ruminiclostridium* were more prevalent in adults with allergic rhinitis.[28] Although the specific mechanism of action of *Megamonas* in relation to food allergies remains unexplored, it is known to ferment glucose into health-promoting short-chain fatty acids (SCFAs), including acetate and propionate.[29] Notably, propionate and acetate have previously been implicated in the activation of the p38 and ERK MAPK pathways via GPR41 and GPR43 receptors in colonic epithelial cells. These receptors are also present on immune cells including dendritic cells (DCs) and regulatory T cells (Tregs). The expression GPR109a in DCs promotes Treg proliferation and contributes to tolerogenic response in the gut.[30]

Dynamic fluctuations in the abundance of *Clostridium* sensu stricto 1 and *Erysipelatoclostridium* have been previously observed in allergic infants and healthy controls of various ages, particularly during the first year of life, in southwestern China.[31] *Clostridium* sensu stricto 1, a gut commensal, crucially prevents and reduces food allergen sensitization.[32] The association between food allergies and *Clostridium* has been the subject of investigation in various studies involving different allergens such as cow milk, eggs, and peanuts.[33] *Clostridium* has been shown to promote the accumulation of T regulatory cells and induce IL-22 production, which may enhance epithelial barrier function as an innate mechanism.[11,34] *Romboutsia*, a *Clostridium* member, produces butyrate, an SCFA known for its anti-inflammatory effects through inhibiting NF-kB activation and reducing proinflammatory cytokine

expression.[35] Conversely, *Anaerostipes* enrichment in the wheat allergy group and the *Clostridium innocuum* group in patients with allergic rhinitis imply potentially contrasting roles in allergic responses.[36] *Anaerostipes*, belonging to the *Lachnospiraceae* family, have been identified as mucin degraders and can exhibit either pro- or anti-inflammatory effects depending on the gut microbiota composition and dietary intake.[37] For example, *Anaerostipes caccae* has been identified as a protective bacterium against allergic responses in infants allergic to cow milk.[38] Indeed, in the context of wheat-dependent exercise-induced anaphylaxis in adults, the genera *Blautia*, *Erysipelatoclostridium*, and *Akkermansia* were enriched.[10] Nevertheless, the precise mechanisms underlying these enrichments are yet to be elucidated. This study highlights several candidate genera, such as *Megamonas*, *Romboutsia*, *Fusobacterium*, and *Clostridium* sensu stricto 1, as potential therapeutic targets and *Anaerostipes*, *Prevotella* 2, *Erysipelatoclostridium*, and *Ruminococcus* as diagnostic markers for allergic conditions. These genera show promise for modulating immune responses and inflammation in allergies, advancing personalized treatments, and precise diagnostics. However, further research is needed to establish causal relationships and underlying mechanisms for effective management through gut microbiota modulation.

Concomitant with alterations in the gut microbiota composition, functional analysis revealed significant differences in pathways associated with arginine and polyamine biosynthesis. Previous studies have demonstrated the beneficial effects of arginine and polyamines in bolstering gut immunity by facilitating the maturation of Tregs in murine models.[39] Notably, the intestinal microbiota can metabolize arginine into immunomodulatory polyamine derivatives.[40] *Bacteroides* spp. and *Fusobacterium* spp. are the primary sources of polyamines in the intestinal microbiota.[41]

Differences in the gut microbiota between wheat allergy and healthy control groups may be influenced by nutritional factors. Breastfed infants harbor beneficial species such as *Bifidobacterium* and *Lactobacillus*, known for immunomodulation and allergy protection, which persist for up to 12 months and lead to delayed adult-like gut microbiome development.[12] Breast milk differs from cow milk in that processed powdered milk is a distinct form. Non-cow alternatives, such as soy, almond, rice, and oat milk, offer unique nutritional profiles. Our analysis revealed intriguing correlations between specific microbial genera and milk preference. *Anaerostripe* positively correlated with other milk types, whereas *Ruminiclostridium* 5 positively correlated with powdered milk consumption. These findings emphasize the potential interactions between milk choice and gut microbiome.

Children with wheat allergy, due to their inability to consume wheat-containing deserts or bread, tended to consume more vegetables, while the control group consumed more bread, deserts, and eggs, potentially affecting the gut microbiota composition. Excessive sugar intake in westernized diets disrupts gut microbiota, reduces diversity, and promotes gut inflammation.[42] We observed a correlation between gut microbiota and the consumption of deserts and sparkling beverages. The dominant genera in the healthy control group were positively associated with desert consumption, whereas the two genera enriched in the wheat allergy group showed a significant negative association with desert consumption. A *Lachnospiraceae* member that metabolizes polysaccharides into beneficial short-chain fatty acids[37] showed distinct patterns between children with and without wheat allergy in our study. *Agathobacter* was predominant in children without allergies, whereas *Anaerostipes* and *Ruminococcus gauvreauii* were predominant in children with wheat allergy. Further research is needed to understand the precise role of *Lachnospiraceae* in wheat allergy and its potential therapeutic implications.

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Probiotic administration has been shown to be significant in the prevention and treatment of allergies.[43] However, probiotic yogurt intake did not show statistically significant differences between the control and wheat-allergic groups. Further studies are needed to explore the link between dietary habits, including probiotic consumption, and the gut microbiome in the context of wheat allergy. Understanding this relationship may lead to novel therapeutic and dietary interventions that mitigate allergic responses.

Our study had several strengths, including a confirmed diagnosis of wheat allergy in children through oral challenge and history. However, it also had limitations, such as a small sample size and cross-sectional design, which limits the ability to establish causation. Larger prospective studies are needed to explore the association between specific gut microbes and wheat allergy. Despite these limitations, our findings shed light on the complex interactions between the gut microbiota and wheat allergy. However, further research is required to fully understand these mechanisms and to establish causality. Therapeutic approaches involving the modulation of the gut microbiota to manage or prevent wheat allergy are promising and deserve further investigation.

Conclusion

Distinct bacterial genera have been associated with wheat allergy in healthy controls, suggesting their potential roles in disease pathogenesis. Gut microbes are promising candidates for the management and prevention of wheat allergy. Probiotics that increase the abundance of *Megamonas*, *Romboutsia*, *Fusobacterium*, and *Clostridium* sensu stricto 1 may promote gut health and reduce the risk of wheat allergy. Additionally, specific gut microbial genera, such as *Anaerostipes*, *Prevotella* 2, *Erysipelatoclostridium*, and *Ruminiclostridium* 5, could serve as diagnostic biomarkers for early identification and management of wheat allergy. Dietary interventions with prebiotics and probiotics have the potential to modulate gut microbiota, supporting immune regulation and gut health. Although these clinical applications offer personalized therapeutic strategies for wheat allergy management, further research and clinical trials are essential to validate their efficacy, safety, and the underlying mechanisms of action.

Acknowledgments

We thank Piyawut Kreetapirom for assisting with participant recruitment. This research was funded by the Research Fund, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, CPF Food Research and Development Center, and the Charoen Pokphand Group.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

- Conceptualization: W.K., W.M.,: N.N. and P.W.;
- Software: W.K., S.N., S.S., R.M.,: W.K., W.M., and P.W.;
- Data curation: P.W.;
- Writing—original draft preparation:, review, and editing: W.M., W.K., and P.W.;
- Visualization: S.X.;
- Supervision: W.K., W.M., and P.W.;
- Project administration: R.M.;
- Funding acquisition: W.K., W.M.

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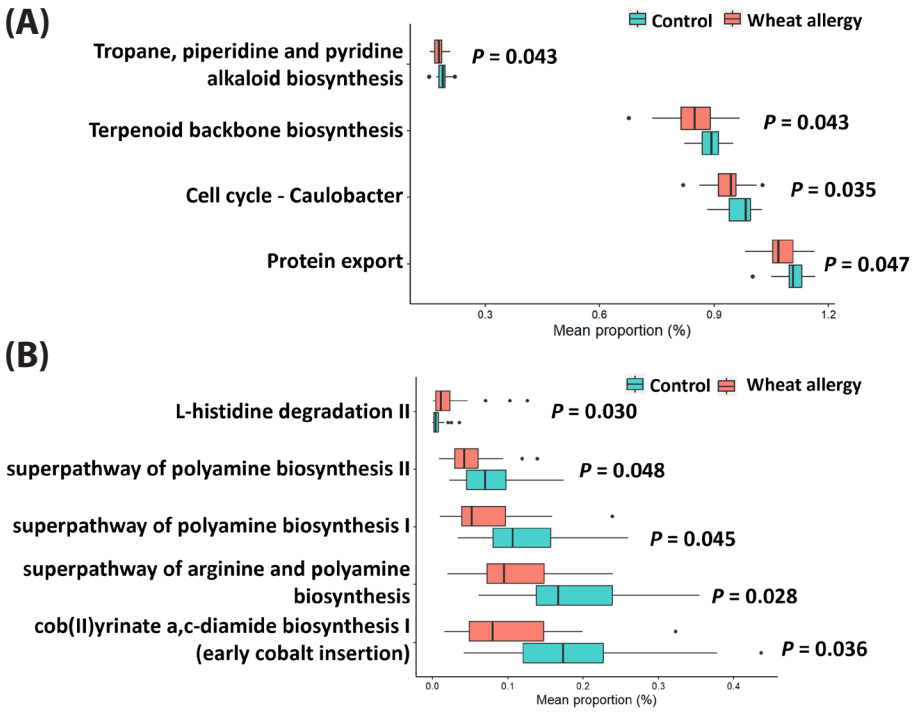
Supplementary data



Differential PICRUSt-predicted KEGG pathways and enzymes were analyzed using the STAMP software to compare the control group with the wheat allergy group.

(A) Variations in KEGG pathways were observed between the control and wheat-allergy groups. (B) Distinct enzymes differed between the control and wheat allergy groups. The mean proportions of the predicted values are graphically depicted and the corresponding p-values are reported.