Antibiotic-treatment induced changes of the allergenicity of cockroach extract

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Directed by Professor Tai-Soon Yong

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Seogwon Lee

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This certifies that the Doctoral Dissertation of Seogwon Lee is approved.

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The Graduate School
Yonsei University

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ABSTRACT

Antibiotic-treatment induced changes of the allergenicity of cockroach extract

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The microbiome of the cockroach can be influenced by diet and environmental factors, and it can vary between species. I conducted 16S rDNA-targeted high-throughput sequencing to evaluate the overall bacterial composition of the microbiomes of four cockroach species: *Periplaneta americana, P. japonica, P. fuliginosa*, and *Blattella germanica*, which were raised in a laboratory for several generations under the same conditions. Regarding the Shannon biodiversity index, *P. americana* had a higher microbial diversity than the other species. In phylogenetic indices, the *Periplaneta* species had a higher microbial diversity than *B. germanica*. Beta diversity analysis showed that *P. japonica* and *P. fuliginosa* had similar microbiome compositions, which differed from that of *P. americana* and *B. germanica*. Thus, although microbiome compositions may vary based on multiple conditions, it is possible to identify distinct microbiome compositions among different cockroach species, even when the individuals are reared under the same conditions. I decided to use the German cockroach in the next chapter because the microbiome according to the diet was large and there was an abundance of information on allergens.

This PhD thesis aimed to provide more comprehensive effects of antibiotics on

cockroach, and development of various extracts from them for future clinical trials. Allergens present in the frass of cockroaches can cause allergic sensitization in humans. The fecal and frass extracts have been previously investigated but have not yet been fully standardized. Antibiotics are known to affect the major allergens and total bacteria in German cockroaches. I treated German cockroaches with ampicillin to produce extracts with reduced amounts of total bacteria. Analysis of the microbiome revealed that the alpha diversity was lower in the ampicillin-treated group than in the control group. Beta diversity analysis indicated that ampicillin treatment altered the bacterial composition in the microbiome of cockroaches. Likewise, quantitative polymerase chain reaction revealed that almost all bacteria were removed by ampicillin-treatment. RNA-seq analysis revealed 1,236 differentially expressed genes (DEGs) in ampicillin-treated cockroaches. Unlike the bacterial composition, the DEGs varied between the two groups. Among major allergens, the expression of Bla g 2 decreased significantly by ampicillin-treatment. The reduced level of allergens observed in cockroaches may be related to lower amounts of total bacteria caused by antibiotic treatment. I conclude that it is plausible to produce an allergen extract with few bacteria for immunotherapy.

In chapter II, based on the results obtained in the previous experiment, I examined the altered capacity of antibiotic-treated German cockroaches to induce allergic airway inflammation in a mouse model. At first, I examined the effect of antibiotic treatment on lipopolysaccharide (LPS) and Bla g 1, 2, and 5 expressions in the German cockroach extracts. I then measured the ability of the German cockroach extract (with or without prior antibiotic exposure) to induce allergic inflammation of bronchial epithelial cells *in vitro*, and the *in vivo* induction of allergic airway inflammation in mice. A decrease in bacterial

16S rRNA and lipopolysaccharide levels from ampicillin-treatment was confirmed compared with that of the control group. Furthermore, Bla g 1, 2, and 5 expressions in ampicillin-treated cockroaches were decreased at both the protein and RNA levels. In human bronchial epithelial (BEAS-2B) cells exposed to ampicillin-treated German cockroach extract, IL-6 and IL-8 expressions were lower than that in the control group. The total cell and eosinophil counts in the bronchoalveolar lavage fluid were also lower in mice exposed to extracts from ampicillin-treated German cockroaches compared with that in the control group, which was exposed to normal cockroach extract. Mouse lung histopathology showed decreased immune cell infiltration and mucus production in the ampicillin group. Further, IL-4, IL-5, and IL-13 levels were decreased in the lung tissue and bronchoalveolar lavage fluid, and IgG1 and IgG2a levels were decreased in the serum of the ampicillin group. Overall, ampicillin treatment reduced the symbiont bacterial population and major allergen levels in German cockroaches, leading to reduced airway inflammation in exposed mice. These results will facilitate protein extract preparation for use in immunotherapy or diagnostics, as well as identify environmental issues associated with antibiotic release.

Key words: cockroaches, microbiome, *Blattella germanica, Periplaneta americana,* antibiotics, major allergen

CHAPTER I

Comparative microbiome analysis of four species of cockroaches and reduced major allergens in German cockroaches after antibiotic treatment

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I. INTRODUCTION

Insect microbiomes affect nutrient cycling, provide protection from parasites and pathogens, and modulate immune responses. Cockroach microbiomes consist of horizontally transmitted microbes and vertically transmitted symbionts. The diversity of these microbiomes can vary depending on developmental stage, diet, and rearing practices.[1] Cockroaches spread pathogenic bacteria through their feces while traveling between locations, such as homes, shops, and hospitals.[2] Their omnivorous nature enables them to survive under a wide variety of conditions. The German cockroach, *Blattella germanica*, and its microbiome have been extensively investigated.[3–6] In a recent study, laboratory-reared and field-collected *Blattella germanica* presented distinct microbiomes, although they shared the same core bacterial taxa, which appeared to differ depending on their location and diet.[3] However, no significant microbiome differences resulting from changes in diet have been observed in *Periplaneta americana*, although this species has been found to present microbiome differences due to environmental factors.[7]

Forty-seven species are included in the genus *Periplaneta*,[8] and four species of

the genus *Periplaneta* were maintained in the laboratory, i.e., *P. americana*, *P. japonica*, *P. fuliginosa*, and *B. germanica*. *P. americana* originated in Africa and is very common worldwide,[8] while *B. germanica* is distributed all over the world except Antarctica.[9]

As the features of each species differ within the genus *Periplaneta*, I expected that there would also be differences among the *Periplaneta* microbiomes. Hence, I conducted a study to establish a microbiome that minimized the aforementioned differences, which may have been because of diet and environmental factors. The laboratory-reared cockroaches used in this study were reared for many generations under the same laboratory conditions to minimize the differences resulting from diet and environmental factors.

Several studies have reported that antibiotics directly affect the bacterial composition in the cockroach microbiota.[5] One study showed that rifampicin altered the *B. germanica* microbiota and that the second generation of insects following antibiotic treatment underwent microbiota recovery through fecal intake.[5] Antibiotic-treated cockroaches showed changes in bacterial diversity and composition, including the removal of the endosymbiont *Blattabacterium*.[5] Another study reported difficulties in cockroach reproduction and growth following antibiotic treatment.[10] In *Riptortus pedestris*, the absence of an endosymbiont led to a decrease in hexamerin and vitellogenin, which affected egg production and insect development.[6]

Production of the major cockroach allergen Bla g 1 in female cockroaches is related to their reproductive cycle and is also affected by their food intake.[11] Previous studies have found an increase or decrease in the major cockroach allergen with reported pesticide use.[12,13] A higher concentration of Bla g 2 was confirmed in the fecal pellet of *B. germanica* exposed to boric acid.[12] On the other hand, it was confirmed that both Bla g 1 and Bla g 2 decreased when exposed to hydramethylnon.[13] Bla g 1 can bind various lipids,

suggesting that it has a digestive function related to the nonspecific transport of lipid molecules.[14] Similar to Bla g 1, Bla g 2 is present at high concentrations in the digestive organs of cockroaches (esophagus, gut, and proventriculus), suggesting that Bla g 2 functions as a digestive enzyme.[15] Bla g 2 is regarded as the most important *B. germanica* allergen, with the rate of sensitization being the highest among common cockroach allergens at 54–71%.[16]

Because the potency of the cockroach protein extract was different for each cohort depending on the allergen content of the extract—as recently demonstrated in several studies on allergen immunotherapy[17,18]—it was important to select a suitable protein extract for each patient.[17]

Despite these variables, no studies have been conducted to determine the effect of bacteria in the cockroach on allergen production before extracting the protein for immunotherapy. The extract of the cockroach not only contains allergens but also harbors various immunomodulatory molecules, such as LPS and bacterial DNA, from the microbiome, which are not easily removed by the filtration process.

Next, the cockroach microbiomes were analyzed using 16S rDNA-targeted high-throughput sequencing to compare the four cockroach species. This study aimed to obtain a protein extract of *B. germanica* with reduced levels of bacteria using ampicillin, a broad-spectrum antibiotic. In addition, the study attempted to investigate the size and composition of the microbiome of cockroaches treated with ampicillin, and whether the allergenicity of the cockroach extract was affected by the treatment.

II. MATERIALS AND METHODS

1. Collection of cockroaches and rearing conditions

Individuals belonging to three species of cockroaches (*P. americana*, *P. japonica*, and *P. fuliginosa*) were collected in Yongsan, Seoul, and Incheon, respectively. *Blattella germanica* was received from the National Institutes of Health. *P. americana* and *P. fuliginosa* individuals have been maintained in the laboratory of the Arthropods of Medical Importance Bank of Yonsei University College of Medicine in Seoul, Korea, since 1998. *P. japonica* individuals have been reared in laboratory since 2017. *B. germanica* individuals have been reared since 1991. All cockroaches used in this study were reared for many generations under the same laboratory conditions to minimize the potential influence of environmental factors and diet. In addition, the cockroaches used in this study were in the adult stage. A total of 40 cockroaches were reared separately in a plastic box (27×34×19 cm) maintained at 25˚C. The cockroaches were fed Purina Rat Chow (Nestle Purina PetCare, St Louis, MO, USA), containing crude protein, crude fat, crude fiber, crude ash, calcium, and phosphorus, and were supplied tap water *ad libitum*. Male adult cockroaches (not non-reproductive) were used 1 month after the last instar. Sampling was performed 2 days after starvation.

2. Experimental design

Newly hatched cockroaches (G1) were randomly divided into two groups (n = 5 from each group). Group Amp was offered ampicillin-treated water (autoclaved before the addition of 0.025% ampicillin), while group Ctrl (control) was offered untreated water. Twenty-one days after becoming adults, ampicillin-treated and untreated female

cockroaches were sampled for further analyses. *B. germanica* individuals were sacrificed with CO_2 then surface-sterilized using alcohol and flash-frozen in liquid nitrogen. They were then individually crushed using a mortar and pestle. The powder of the crushed body of each cockroach was used for DNA, RNA, and protein extraction. Three biological replicates were analyzed.

3. DNA extraction

Total DNA was extracted using the NucleoSpin DNA Insect Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. The DNA extracted from each sample was eluted in 20 μL of elution buffer. All processing and sequencing procedures were conducted at a clean bench, under a hood, and in a DNA-free room. DNA concentrations were quantified using an ND-1000 Nanodrop system (Thermo-Fisher Scientific, Waltham, MA, USA). The extracted DNA was stored at −80°C in a deep freezer.

4. Next-generation sequencing

The 16S rDNA V3–V4 region was amplified by PCR using 16S rDNA V3-V4_F and 16S rDNA V3-V4_R primers (Table I-1)[19,20] while utilizing an Illumina MiSeq V3 cartridge (San Diego, CA, USA) with 600 cycles. A limited-cycle amplification step was performed to add multiplexing indices and Illumina sequencing adapters. The libraries were normalized, pooled, and sequenced on the Illumina MiSeq V3 cartridge platform following the manufacturer's instructions.

5. Bioinformatics and statistics

Bioinformatics analyses were performed following the methods described in my

previous study.[19] The EzBioCloud database (https://www.ezbiocloud.net/)[20] was used for the taxonomic assignment using BLAST 2.2.22, and pairwise alignments were generated to calculate the similarity.[20] All the described analyses were performed using BIOiPLUG, a commercially available ChunLab bioinformatics cloud platform for microbiome research (https://www.bioiplug.com/).[19] The reads were normalized to 43,000 to perform the analyses. Determination of Shannon and phylogenetic indices, unweighted pair group method with arithmetic mean (UPGMA) clustering, principal coordinates analysis (PCoA), permutational multivariate analysis of variance (PERMANOVA), linear discriminant analysis (LDA), and effect size (LEfSe) analysis were performed according to the previous study.[19]

6. Two-dimensional electrophoresis (2DE) and ELISA using protein extraction

The total protein was extracted by first adding 2 mL of phosphate buffered saline (PBS) to each sample. The samples were then sonicated (QSonica Q500, Fullerton, CA, USA) and centrifuged at 10,000 ×*g* for 30 min at 4°C. The resulting supernatants were filtered using a 0.22 μm membrane filter (Millex®, Tullagreen, Carrigtwohill, Co. Cork, Ireland).

The protein concentration was determined by the Bradford method (Bio-Rad, Hercules, CA, USA). For 2DE analysis, pH 3-10 immobilized pH gradient (IPG) strips (GE Healthcare Life Sciences, Pittsburgh, PA, USA) were rehydrated in swelling buffer containing 7 M urea, 2 M thiourea, 2.5% (w/v) dithiothreitol (DTT), and 4% (w/v) 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS). The protein lysates (90 μg) were loaded into the rehydrated IPG strips using an IPGphor III (GE Healthcare Life Sciences, Pittsburgh, PA, USA) and the 2-D separation was performed on 10% sodium

dodecyl sulfate-polyacrylamide gels (SDS-PAGE). Following fixation of the gels for 1 h in a solution of 40% (v/v) methanol containing 5% (v/v) phosphoric acid, the gels were stained with Colloidal Coomassie Blue G-250 solution (ProteomeTech, Seoul, Korea). The gels were destained using deionized water and images were acquired with an image scanner (Bio-Rad, Hercules, CA, USA).

Protein bands from SDS-PAGE gels were excised and in-gel digested with trypsin according to established procedures.[21] In brief, protein bands were excised from stained gels and cut into pieces. The gel pieces were washed for 1 h at room temperature in 25 mM ammonium bicarbonate buffer (pH 7.8) containing 50 % (v/v) acetonitrile (ACN). Following the dehydration of the gel pieces in a centrifugal vacuum concentrator (Biotron, Inc., Incheon, Korea) for 10 min, gel pieces were rehydrated in 50 ng of sequencing grade trypsin solution (Promega, Madison, WI, USA). After incubation in 25 mM ammonium bicarbonate buffer (pH 7.8) at 37℃ overnight, the tryptic peptides were extracted with 5 μL of 0.5% formic acid (FA) containing 50% (v/v) ACN for 40 min with mild sonication. The extracted solution was concentrated using a centrifugal vacuum concentrator. Prior to mass spectrometric analysis, the peptide solution was subjected to a desalting process using a reversed-phase column.[22] In brief, after an equilibration step with 10 μL of 5% (v/v) FA, the peptide solution was loaded on the column and washed with 10 uL of 5% (v/v) FA. The bound peptides were eluted with 5 μL of 70% ACN and 5% (v/v) FA.

Liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis was performed through a nano ACQUITY UPLC and linear trap quadrupole (LTQ)-orbitrap-mass spectrometer (Thermo Electron, San Jose, CA, USA). The column used a bridged ethylene hybrid (BEH) C18 1.7 μm, 100 μm ×100 mm column (Waters, Milford, MA, USA). The mobile phase A for the LC separation was 0.1% FA in deionized water and

the mobile phase B was 0.1% FA in ACN. The chromatography gradient was set up to give a linear increase from 10% B to 40% B for 21 min, from 40% B to 95% B for 7 min, and from 90% B to 10% B for 10 min. The flow rate was 0.5 μL/min. For tandem mass spectrometry, mass spectra were acquired using data-dependent acquisition with a full mass scan (300-2000 m/z) followed by MS/MS scans. Each MS/MS scan acquired was an average of one microscan on the LTQ. The temperature of the ion transfer tube was controlled at 275℃ and the spray was 1.5-2.0 kV. The normalized collision energy was set at 35% for MS/MS. The individual spectra from MS/MS were processed using the SEQUEST software (Thermo Quest, San Jose, CA, USA) and the generated peak lists were used to query in the house database using the MASCOT program (Matrix Science Ltd., London, UK). I set the modifications of methionine, cysteine, arginine methylation, and serine, threonine, and tyrosine phosphorylation for the MS analysis, and the peptide mass tolerance was 10 ppm. The MS/MS ion mass tolerance was 0.8 Da, the allowance of missed cleavage was 2, and the charge states (+2, +3) were considered for data analysis. I took only significant hits as defined by the MASCOT probability analysis.

Cockroach protein extracts (2 mg/mL) were diluted 100-fold to measure the Bla g 1 and Bla g 2 levels, and 10-fold to measure the Bla g 5 level using the corresponding ELISA kits (Indoor Biotechnologies, Charlottesville, VA, USA) according to the manufacturer's instructions. In brief, the detection antibody and conjugate mix were used for the immunoassay, and color development was performed with the 3,3′,5,5′-tetramethylbenzidine substrate.

7. qPCR and RNA-seq analysis using RNA extraction

The total RNA was extracted by adding 1 mL of TRIZOL Reagent (GeneAll,

Seoul, Korea) to each sample. The TRIZOL supernatant was added to react with the sample and was mixed with isopropanol to obtain a pellet. The RNA extracted from each sample was eluted in 20 μL of the elution buffer. A master mix comprised of a 5× cDNA synthesis mix and 20× reverse transcriptase (10 units) was added to the mRNA samples in PCR tubes for cDNA synthesis.

Quantitative real-time PCR (qPCR) was performed to quantify *Bla g 1, Bla g 2, Bla g 5*, and bacterial 16S rRNA in whole cockroaches. Actin 5C (accession number AJ861721.1) was used as the internal control, and primers specific to this gene (ActinF and ActinR) were designed for this experiment (Table I-1).[5] All bacterial 16S rRNAs were amplified using the forward primer BACT1369 and the reverse primer PROK1492R (Table I-1) from XenoTech with the AMPIGENE qPCR Mix (Enzo Life Sciences, Inc., Farmingdale, NY, USA).[23] *Bla g 1* (accession number EF202179.1), *Bla g 2* (accession number EF203068.1), and *Bla g 5* (accession number EF202178.1) gene expression was used as a measurement of major allergen content. I designed the following primers for this experiment: Blag1F and Blag1R, Blag2F and Blag2R, and Blag5F and Blag5R (Table I-1). qPCR analyses were performed using the 2× SensiFAST™ SYBR® Hi-ROX kit (Bioline Meridian Bioscience, London, UK) with SYBR Green as the fluorescent reporter, H_2O, the corresponding primers, and either genomic or complementary DNA. At the end of each reaction, a melting curve was generated to check the specificity of amplification and to confirm the absence of primer dimers. All reactions, including negative controls (containing water instead of DNA), were run in duplicate in 96-well plates.

I used total RNA (n = 3 from each group) and the TruSeq Stranded mRNA LT Sample Prep Kit (San Diego, CA, USA) to construct cDNA libraries. The protocol consisted of polyA-selected RNA extraction, RNA fragmentation, random hexamer primed

reverse transcription, and 100 nt paired-end sequencing by the Illumina NovaSeq 6000 (San Diego, CA, USA). The libraries were quantified using qPCR according to the qPCR Quantification Protocol Guide and qualified using an Agilent Technologies 2100 Bioanalyzer.

Raw reads from the sequencer were preprocessed to remove low-quality and adapter sequences. The processed reads were aligned to the *B. germanica* genome using HISAT v2.1.0.[24] HISAT utilizes two types of indices for alignment (a global, whole-genome index and tens of thousands of small local indices). These index types are constructed using the same Burrows–Wheeler transform and graph Ferragina Manzini index as Bowtie 2. HISAT generates spliced alignments several times faster than the Burrows–Wheeler Aligner (BWA) and Bowtie because of how efficiently it utilizes these data structures and algorithms. The reference genome sequence of *B. germanica* and annotation data were downloaded from the National Center for Biotechnology Information (NCBI). Known transcripts were assembled using StringTie v1.3.4d,[25,26] and the results were used to calculate the expression abundance of transcripts and genes as read count or fragments per kilobase of exon per million fragments mapped (FPKM) value per sample. The expression profiles were used to further analyze differentially expressed genes (DEGs). DEGs or transcripts from groups with different conditions can be filtered through statistical hypothesis testing.

The relative abundances of gene expression were measured in the read count using StringTie. I performed statistical analyses to detect DEGs using the estimates of abundance for each gene in individual samples. Genes with more than one “zero” read count value were excluded. Filtered data were log2-transformed and subjected to trimmed mean of M-values normalization. The statistical significance of the fold change in expression (i.e.,

differential expression data) was determined using the exact test from edgeR,[27] wherein the null hypothesis was that no difference exists among groups. The false discovery rate (FDR) was controlled by adjusting the P-value using the Benjamini-Hochberg algorithm. For DEGs, hierarchical clustering analysis was performed using complete linkage and Euclidean distance as a measure of similarity. Gene-enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses for DEGs were also performed based on the Gene Ontology (http://geneontology.org/) and KEGG pathway (https://www.genome.jp/kegg/) databases, respectively. I used the multidimensional scaling (MDS) method to visualize the similarities among samples and applied the Euclidean distance as a measure of dissimilarity. Hierarchical clustering analysis was also performed using complete linkage and Euclidean distance as a measure of similarity to display the expression patterns of differentially expressed transcripts that satisfied a |fold change| ≥ 2 and a raw P-value <0.05.

Table I-1. Primers used in this study

Primer Name	Primer Sequence (5' → 3')
16S rDNA V3–V4_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG
16S rDNA V3–V4_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC
ActinF	CACATACAACTCCATTATGAAGTGCGA
ActinR	TGTCGGCAATTCCGGTACATG
BACT1369	CGGTGAATACGTTCYCGG
PROK1492R	GGWTACCTTGTTACGACTT
Blag1F	CTATATGACGCCATCCGTTCTC
Blag1R	CACATCAACTCCCTTGTCCTT
Blag2F	TGATGGGAATGTACAGGTGAA A
Blag2R	TGTTGAGATGTCGTGAGGTTAG
Blag5F	GATTGATGGGAAGCAAACACAC
Blag5R	CGATCTCCAAGTTCTCCCAATC

III. RESULTS

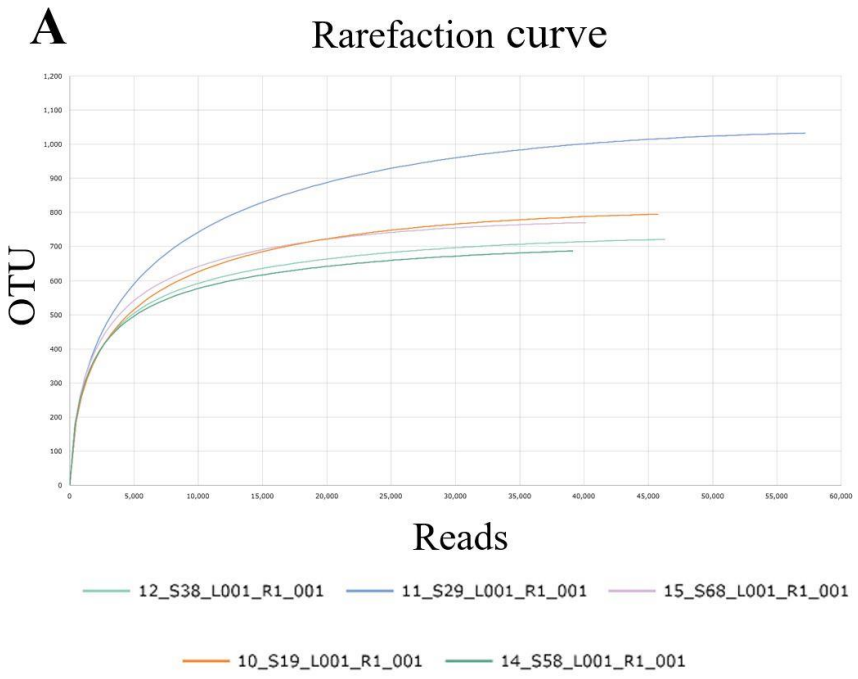
1. Bacterial composition of each cockroach species

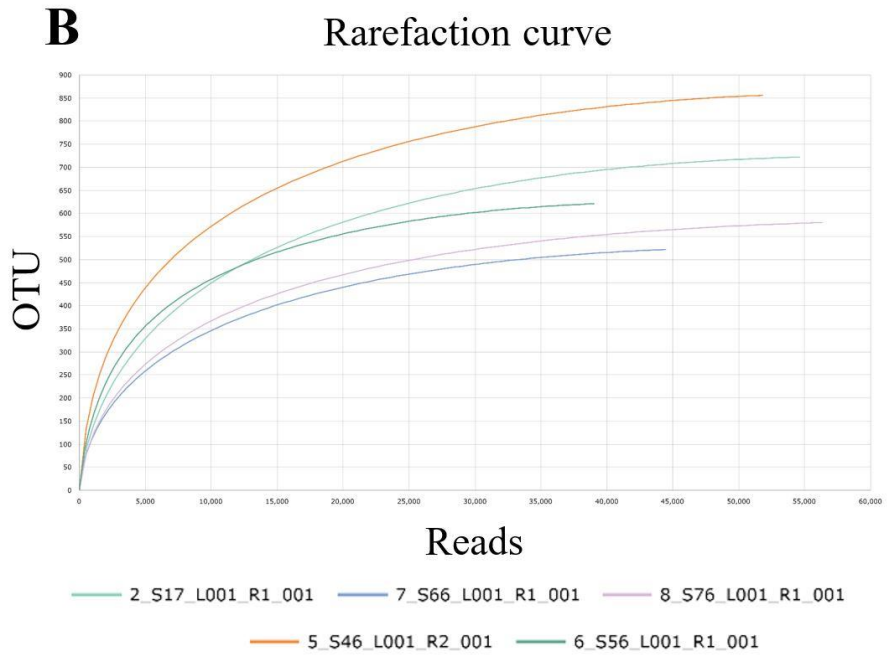
The average numbers of read counts assigned to *P. americana, P. japonica, P. fuliginosa,* and *B. germanica* were 45,713 reads corresponding to 801 operational taxonomic units (OTUs), 49,275 reads corresponding to 660 OTUs, 51,680 reads corresponding to 709 OTUs, and 65,400 reads corresponding to 469 OTUs, respectively. The rarefaction curve of all the samples formed a plateau (Figure I–1).

With respect to the bacterial taxa present in the four cockroach species, less than 1% of the OTUs in *P. americana* accounted for 53.54% of all microbial species present in the species. The endosymbiont *Blattabacterium* CP001429_s accounted for 65.99% and 56.04% of all OTUs in *P. japonica* and *P. fuliginosa*, respectively (Figure I–2A). *Blattabacterium* CP001487_s, an endosymbiont in *B. germanica*, accounted for 27.5%. In *P. japonica*, the endosymbiont *Blattabacterium*_uc was also present. However, *P. americana* lacked *Blattabacterium*. *P. japonica* and *P. fuliginosa* had many OTUs in common and presented similar microbial species compositions (Figure I–2A).

An LEfSe analysis was performed to identify significant differences in bacterial abundance among the cockroach genus. The genus showed the most difference from other German cockroaches. The taxa with high LDA scores in *P. americana* were *Porphyromonadaceae*_JN680567_g_uc and the *Serratia marcescens* group. In *P. japonica*, *Blattabacterium*_CP001429_s presented a high LDA score. *P. fuliginosa* was not shown in the LEfSe because it was similar to *P. japonica* and had a higher proportion of endosymbionts. Finally, *Blattabacterium*_CP001487_s and the *Fusobacterium varium* group were the species with the highest LDA scores in *B. germanica* (Figure I–2B). Three

Periplaneta species were compared separately. The taxa with high LDA scores in *P. americana* were *Desulfovibrio*_g2_uc, *Dysgonomonas*_JN680577_s, and *Serratia marcescens*. In *P. japonica*, *Blattabacterium*_CP001429_s and *Enterococcus faecium* presented high LDA scores. Finally, *Pediococcus*_uc was the species with the highest LDA score in *P. fuliginosa* (Figure I–2C). When the bacterial communities in *P. japonica* and *P. fuliginosa* were compared without *P. americana*, *Blattabacterium*_uc and *Lactobacillus*_uc were found to be highly abundant in *P. japonica*, while *Parabacteroides*_uc and *Enterobacillus tribolii* were highly abundant in *P. fuliginosa* and were the species with the highest LDA scores (Figure I–2D).





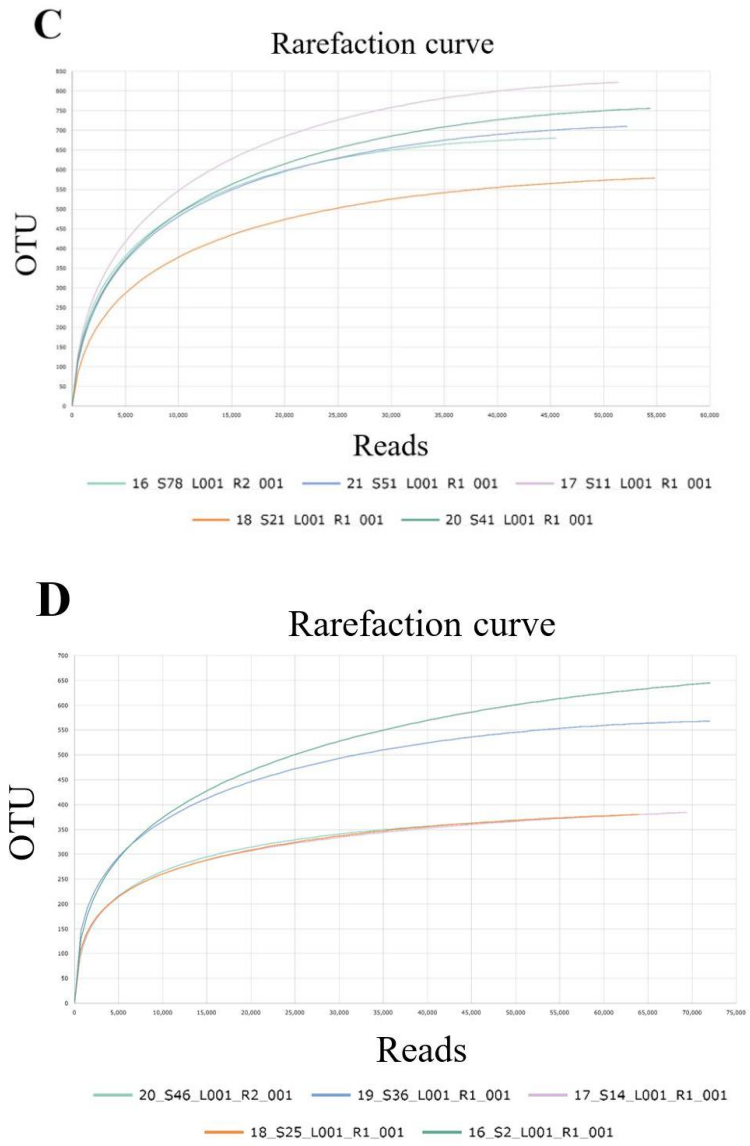
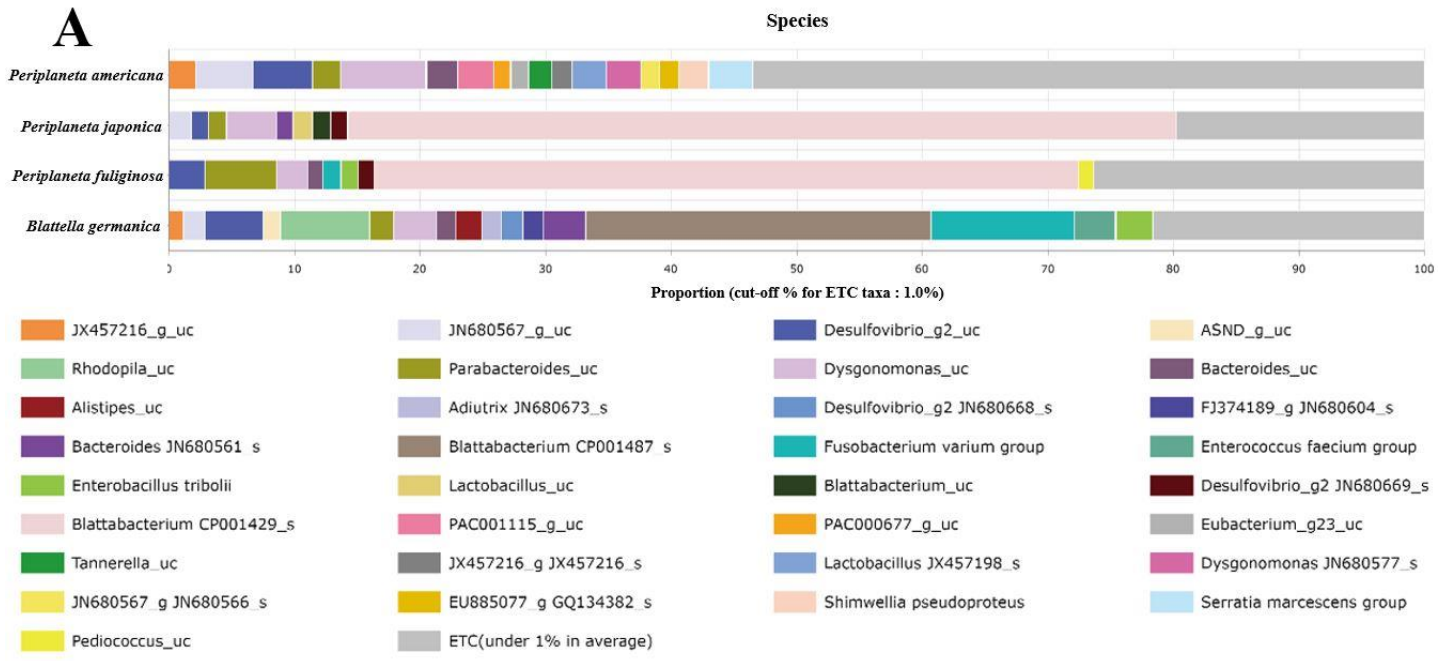
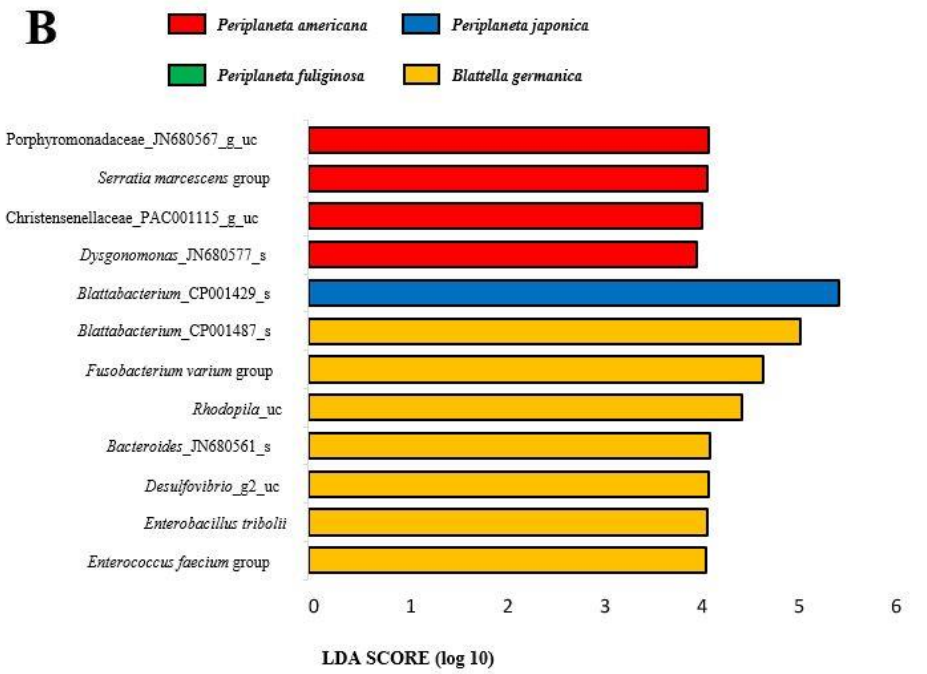
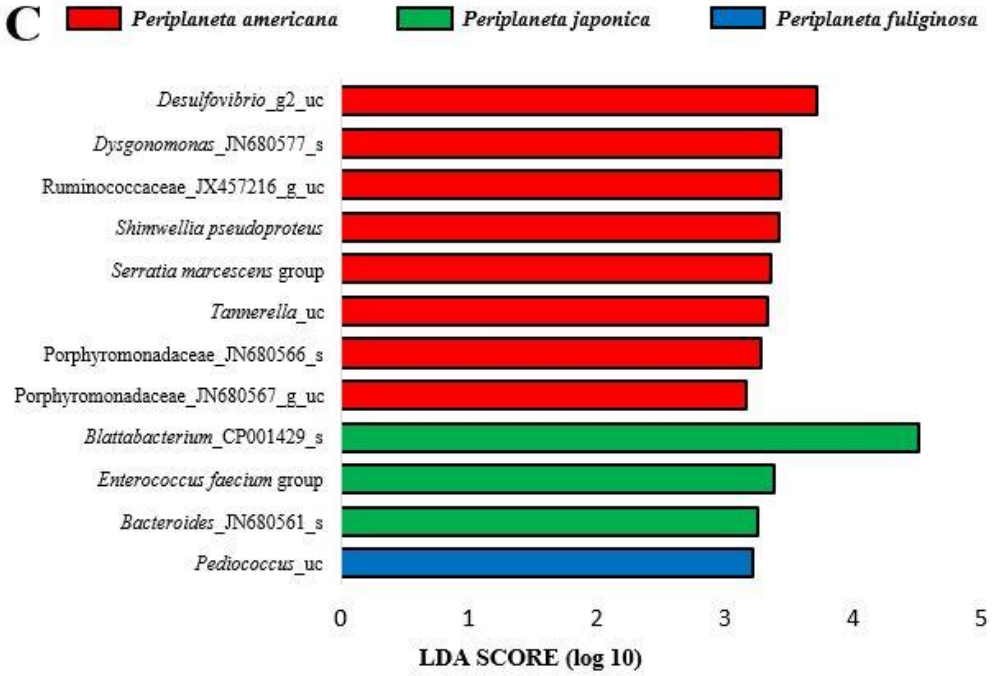


Figure I–1. Rarefaction curves for the number of operational taxonomic units (OTUs) of the four cockroach species. (A) *Periplaneta americana*. (B) *P. japonica*. (C) *P. fuliginosa*. (D) *Blattella germanica*.


A
Species
Periplaneta americana
Periplaneta japonica
Periplaneta fuliginosa
Blattella germanica
0
10
20
30
40
50
60
70
80
90
100
Proportion (cut-off % for ETC taxa : 1.0%)
JX457216_g_uc
JN680567_g_uc
Desulfovibrio_g2_uc
ASND_g_uc
Rhodopila_uc
Parabacteroides_uc
Dysgonomonas_uc
Bacteroides_uc
Alistipes_uc
Adiutrix JN680673_s
Desulfovibrio_g2 JN680668_s
FJ374189_g JN680604_s
Bacteroides JN680561_s
Blattabacterium CP001487_s
Fusobacterium varium group
Enterococcus faecium group
Enterobacillus tribolii
Lactobacillus_uc
Blattabacterium_uc
Desulfovibrio_g2 JN680669_s
Blattabacterium CP001429_s
PAC001115_g_uc
PAC000677_g_uc
Eubacterium_g23_uc
Tannerella_uc
JX457216_g JX457216_s
Lactobacillus JX457198_s
Dysgonomonas JN680577_s
JN680567_g JN680566_s
EU885077_g GQ134382_s
Shimwellia pseudoproteus
Serratia marcescens group
Pediococcus_uc
ETC(under 1% in average)





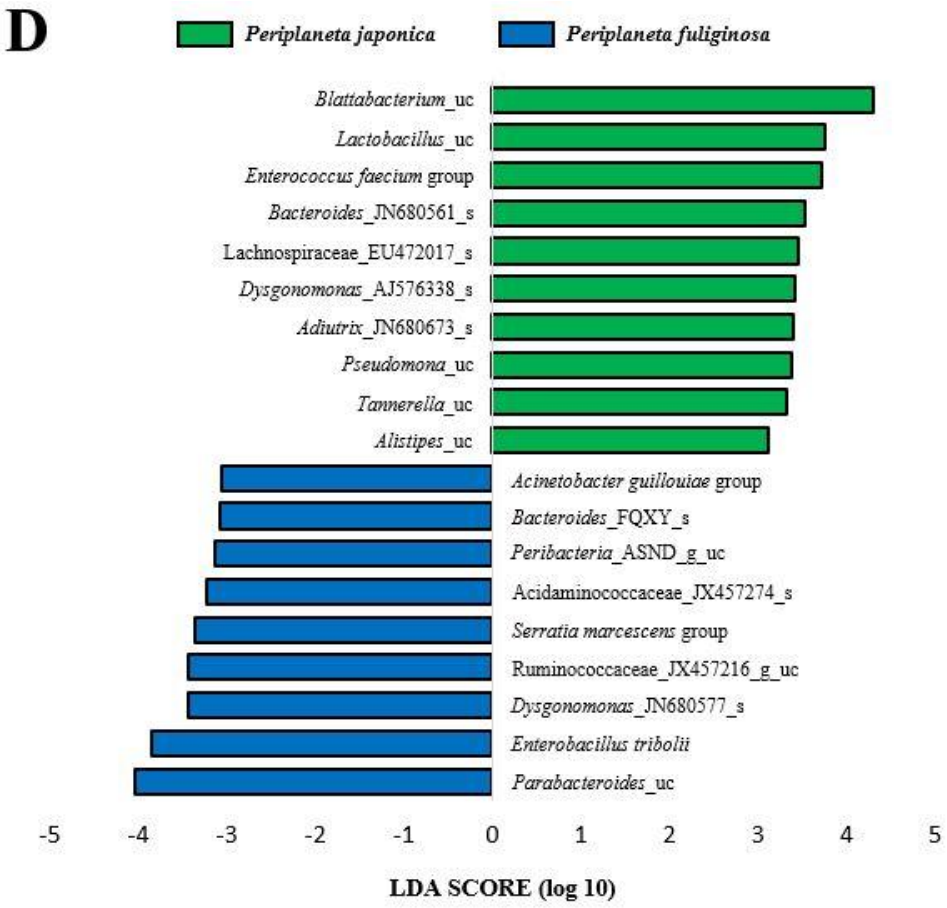
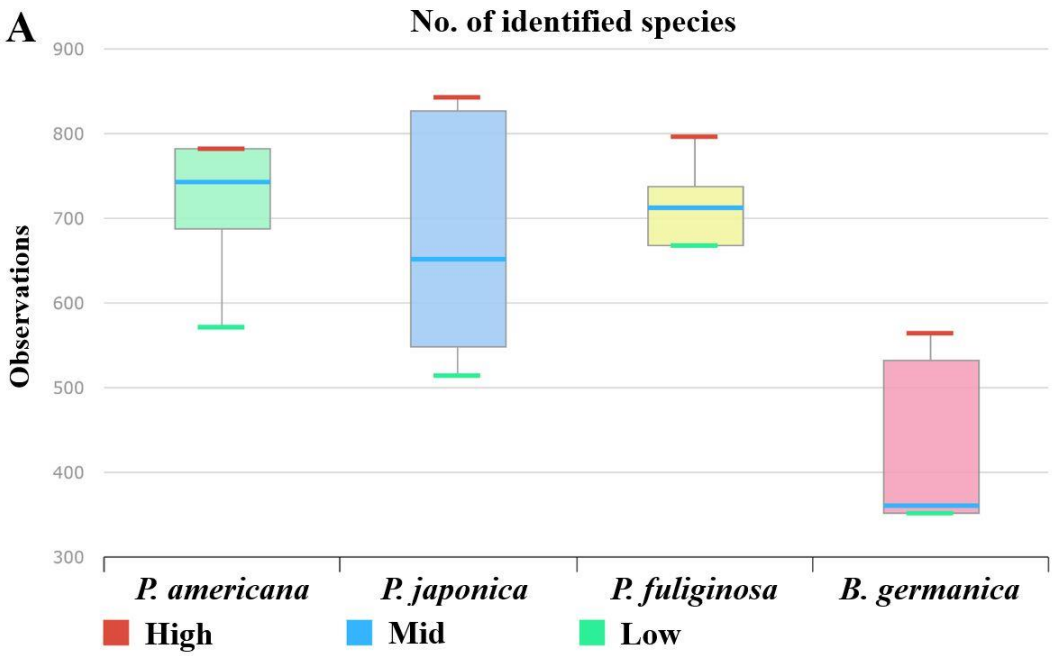
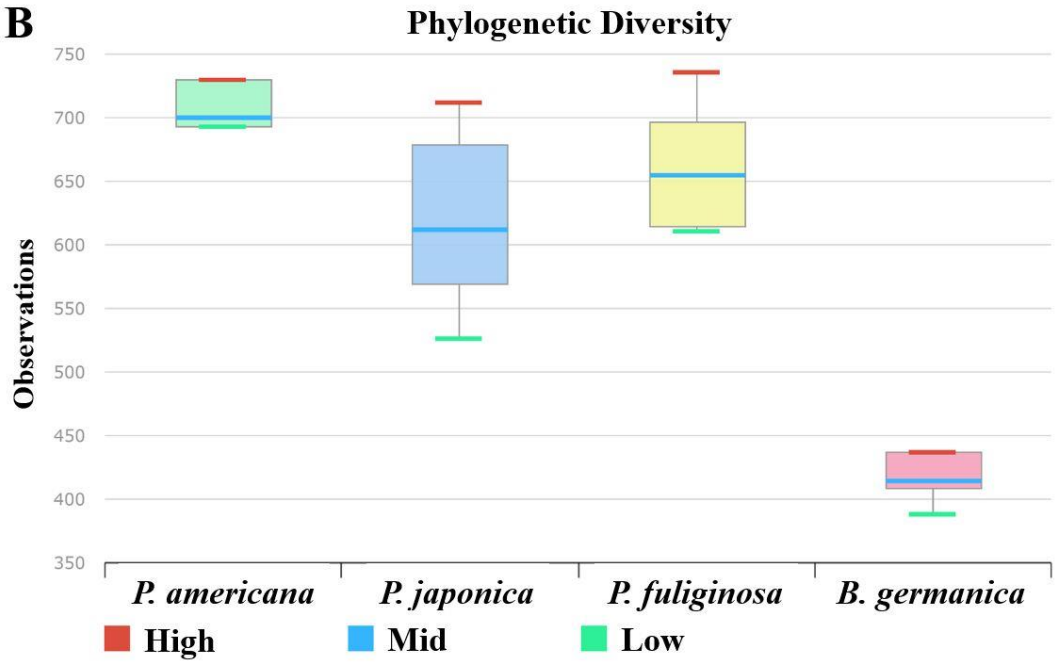


Figure I–2. (A) Distribution of bacterial taxa in the four cockroach species: *Periplaneta americana, P. japonica, P. fuliginosa,* and *Blattella germanica*. Each bar depicts the mean relative abundance value of the independent replicates (n = 6). (B) LEfSe analysis of differentially abundant bacterial taxa among the four cockroach species. Only taxa meeting a significant LDA threshold (>4) are shown. Bacterial communities were compared between (C) *P. Americana*, *P. japonica*, and *P. fuliginosa*, and between (D) *P. japonica* and *P. fuliginosa*. Only taxa meeting a significant LDA threshold (>3) are shown.

2. Comparison of alpha diversity among four species

The number of OTUs was not significantly different among the three *Periplaneta* species. Conversely, *B. germanica* had a significantly lower OTU amount than that of the three *Periplaneta* species (Figure I–3A, P = 0.009, 0.047, 0.016, respectively). The phylogenetic diversity was significantly higher in *P. americana* than in other species (P = 0.047, 0.016, 0.009, respectively), and significantly lower in *B. germanica* than that of *P. fuliginosa* (P = 0.028). In Shannon diversity, *P. americana* was also significantly higher than the other species (P = 0.009), but *B. germanica* was higher than *P. japonica* (Figure I–4B, C, P = 0.028).





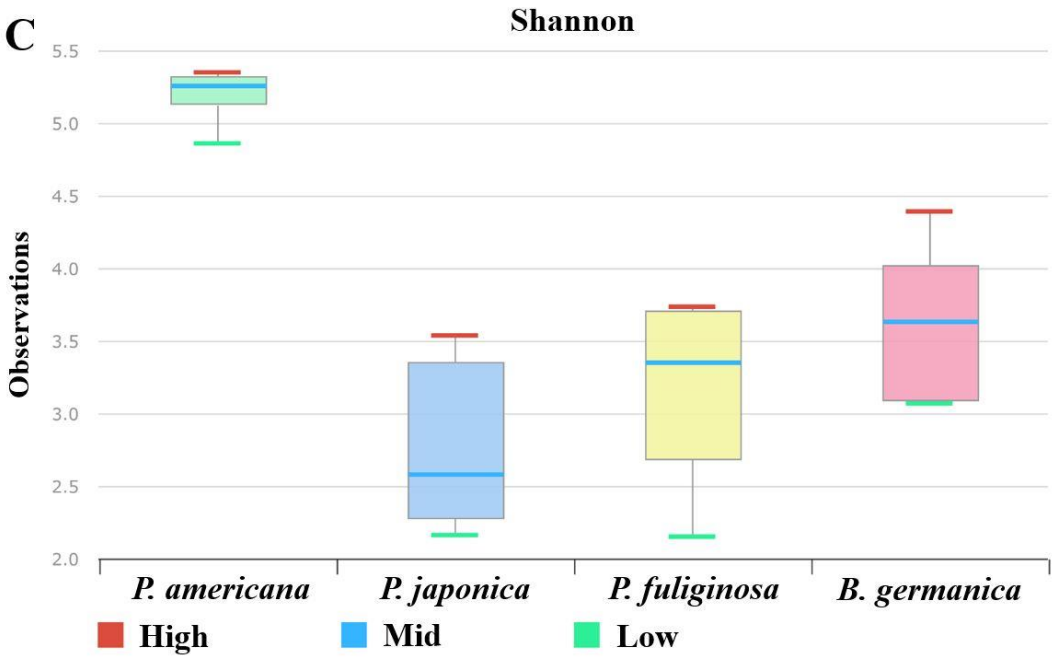
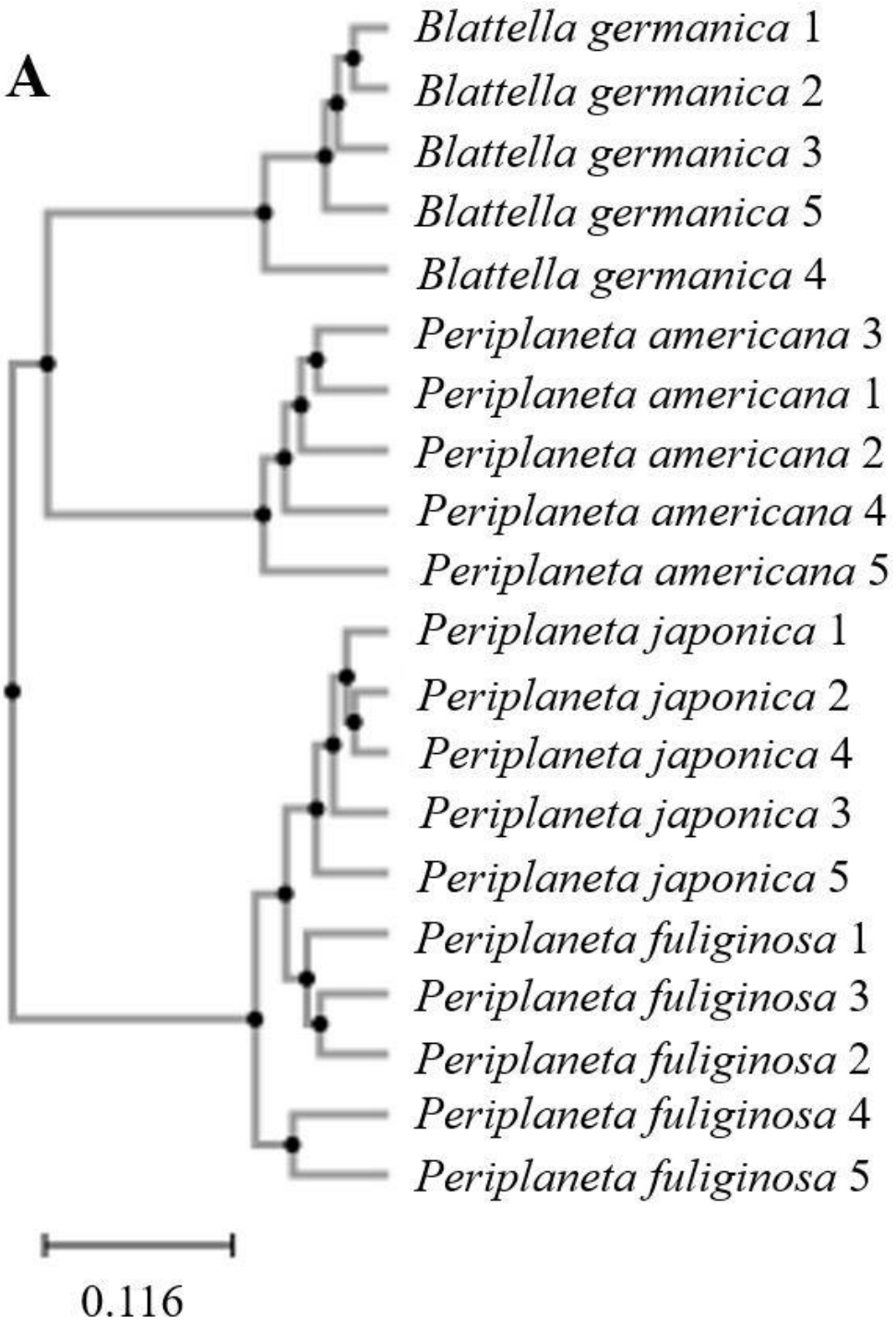


Figure I–3. Box plots showing alpha diversity (measurement of species richness, abundance, and equity within a habitat unit) of *Periplaneta americana, P. japonica, P. Fuliginosa,* and *Blattella germanica*. (A) The number of operational taxonomic units (OTUs). (B) Phylogenetic diversity. (C) Shannon diversity. The bar indicates median, and the hinge represents lower and upper quartiles. *Kruskal-Wallis test, $P < 0.01$.

3. Comparison of beta diversity among four species

UPGMA cluster analysis showed that the cockroaches were organized according to species. *P. americana* and *B. germanica* had good clustering; on the other hand, clustering was mixed in *P. japonica* and *P. fuliginosa* (Figure I–4A). The results of the PCoA showed that although all four groups clustered together, *P. americana* samples were more tightly clustered than the other three samples (P = 0.001) (Figure I–4B). Although clustering seemed to overlap in UPGMA, it was confirmed that it was statistically significant in PCoA. Moreover, a significant difference among the four cockroach species with respect to microbiome composition was detected using PERMANOVA [14].



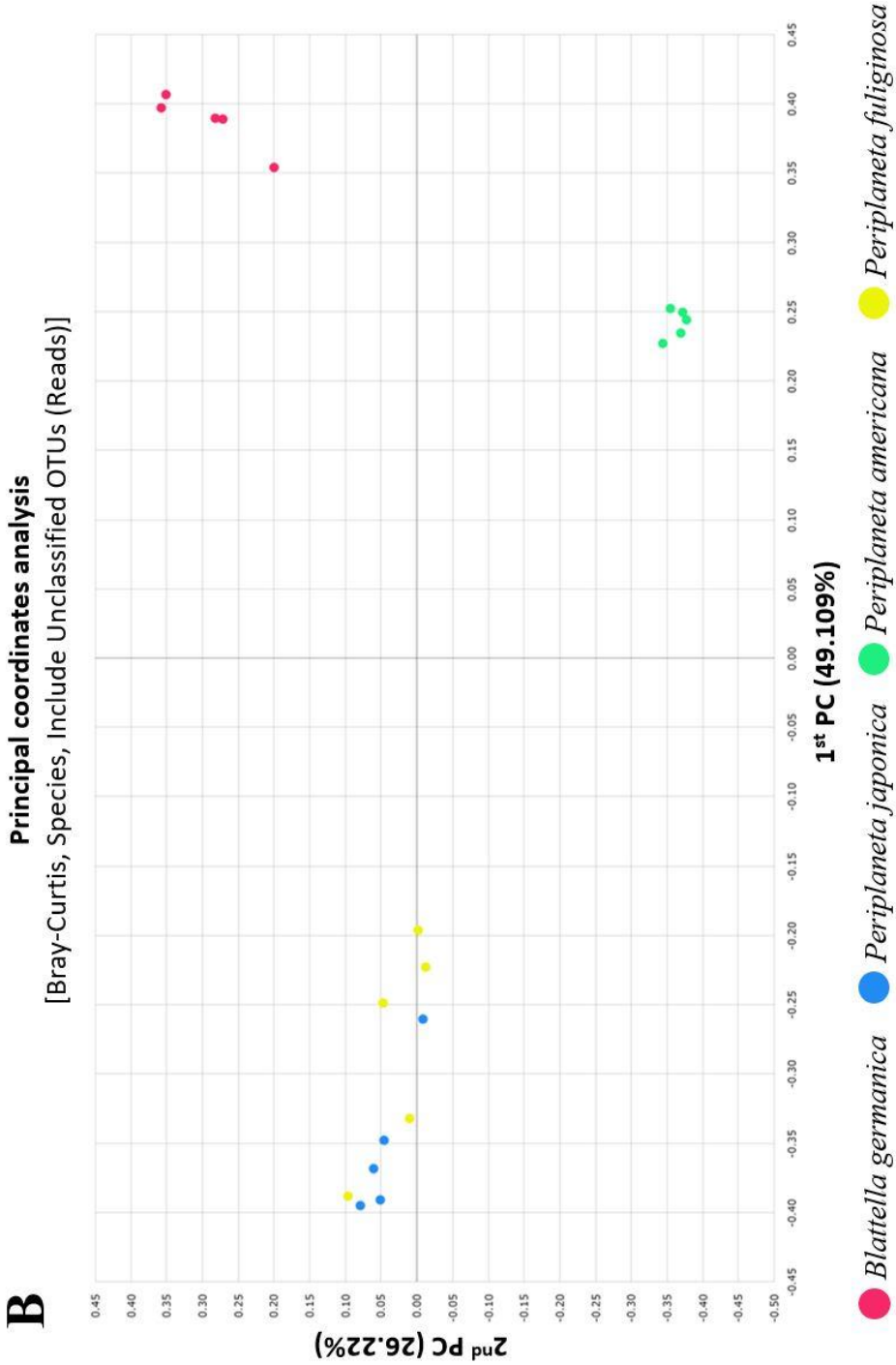


Figure I–4. Graphs showing beta diversity. (A) Unweighted pair group method with arithmetic mean clustering. (B) Principal coordinates analysis depicting differences in the taxonomic compositions of the bacterial communities among *P. americana*, *P. japonica*, *P. fuliginosa*, and *Blattella germanica*.

4. Reduction of total bacteria in cockroaches due to antibiotics

First-generation cockroaches reached the adult stage and were kept for an additional 21 days before being sacrificed for further analysis (Figure I–5). qPCR analysis showed that the number of total bacteria in the cockroaches was 2,000 times higher in the control group than that in the ampicillin-treated group (Figure I–6).

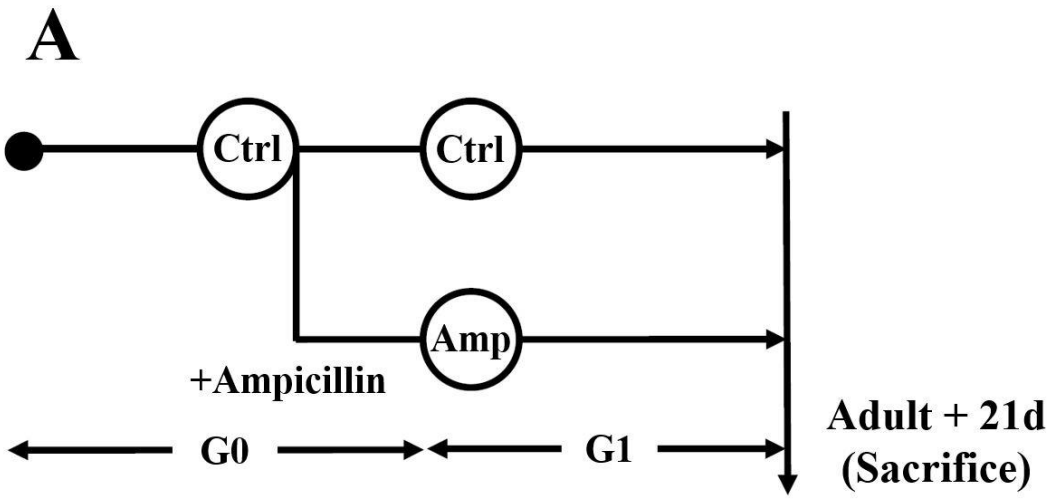


Figure I–5. Experimental design depicting ampicillin treatment of *Blattella germanica*.

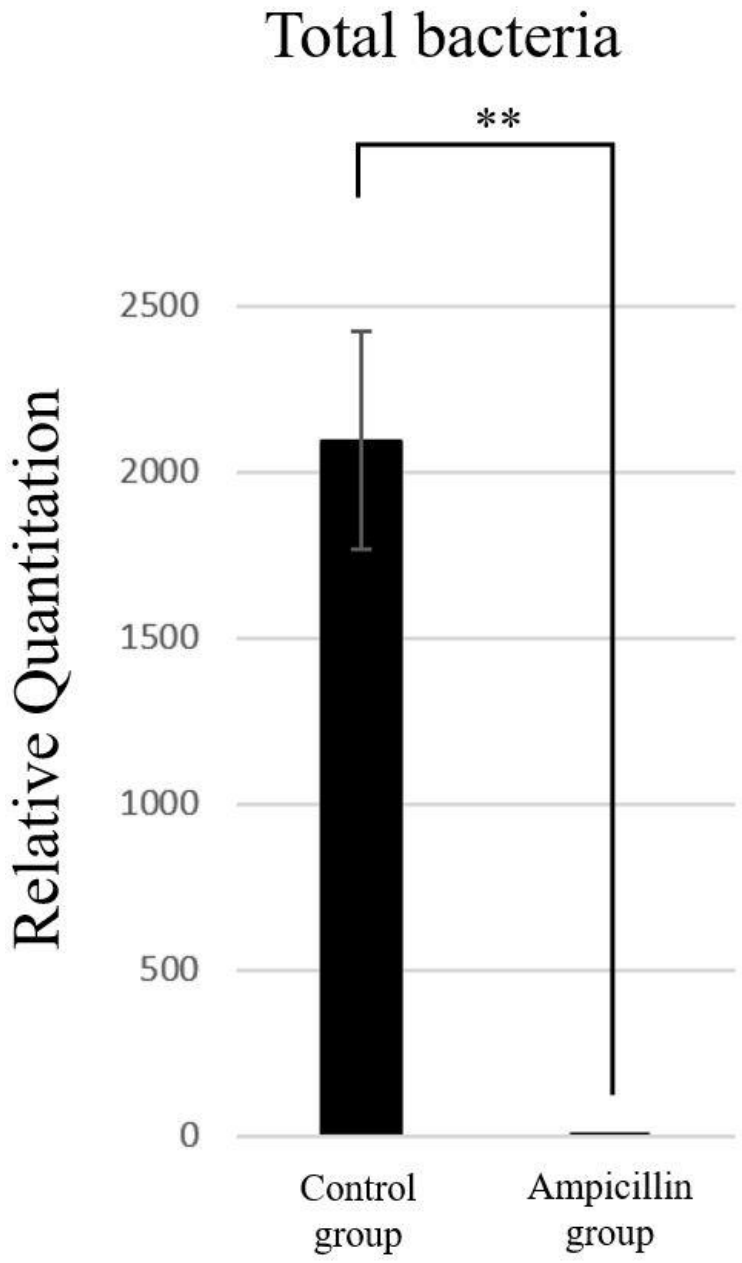


Figure I–6. Relative quantification of the total bacterial population in the ampicillin-treated and untreated cockroach groups.

5. Changes in bacterial composition and elimination of symbionts by ampicillin

In the control group, a comparison of the microbial composition data showed the endosymbiont *Blattabacterium* CP001487_s was the most abundant (27.43%), followed by *Fusobacterium varium*. By contrast, *Desulfovibrio*_g2 was the most abundant (64.39%) in the ampicillin-treated group (Figure I–7A). LEfSe analysis of the sampled cockroaches showed that, in the control group, *Blattabacterium* CP001487_s had the greatest difference in composition, followed by *F. varium*, *Rhodopila*_uc, and *Dysgonomonas*_uc (Figure I–7B). In the ampicillin-treated group, *Desulfovibrio*_g2 and *Planctomycetes* were the bacteria with the greatest differences in abundance (Figure I–7B). Analysis of alpha diversity revealed a significantly lower number of OTUs (P = 0.009) in the ampicillin-treated group (Figure I–8A). Although not statistically significant, the phylogenetic diversity tended to be low in the ampicillin-treated group (P = 0.076) (Figure I–8B), indicating a low overall abundance. A significant difference was noted in the Shannon diversity index, reflecting richness and equity simultaneously (P = 0.009) (Figure I–8C). Analysis of diversity using UPGMA clustering showed that the samples from the control and ampicillin-treated groups were clustered separately (Figure I–9A). Similarly, PCoA showed that both groups were clustered separately, with samples from the ampicillin-treated and control groups located on the left and right sides of the plot, respectively (Figure I–9B).

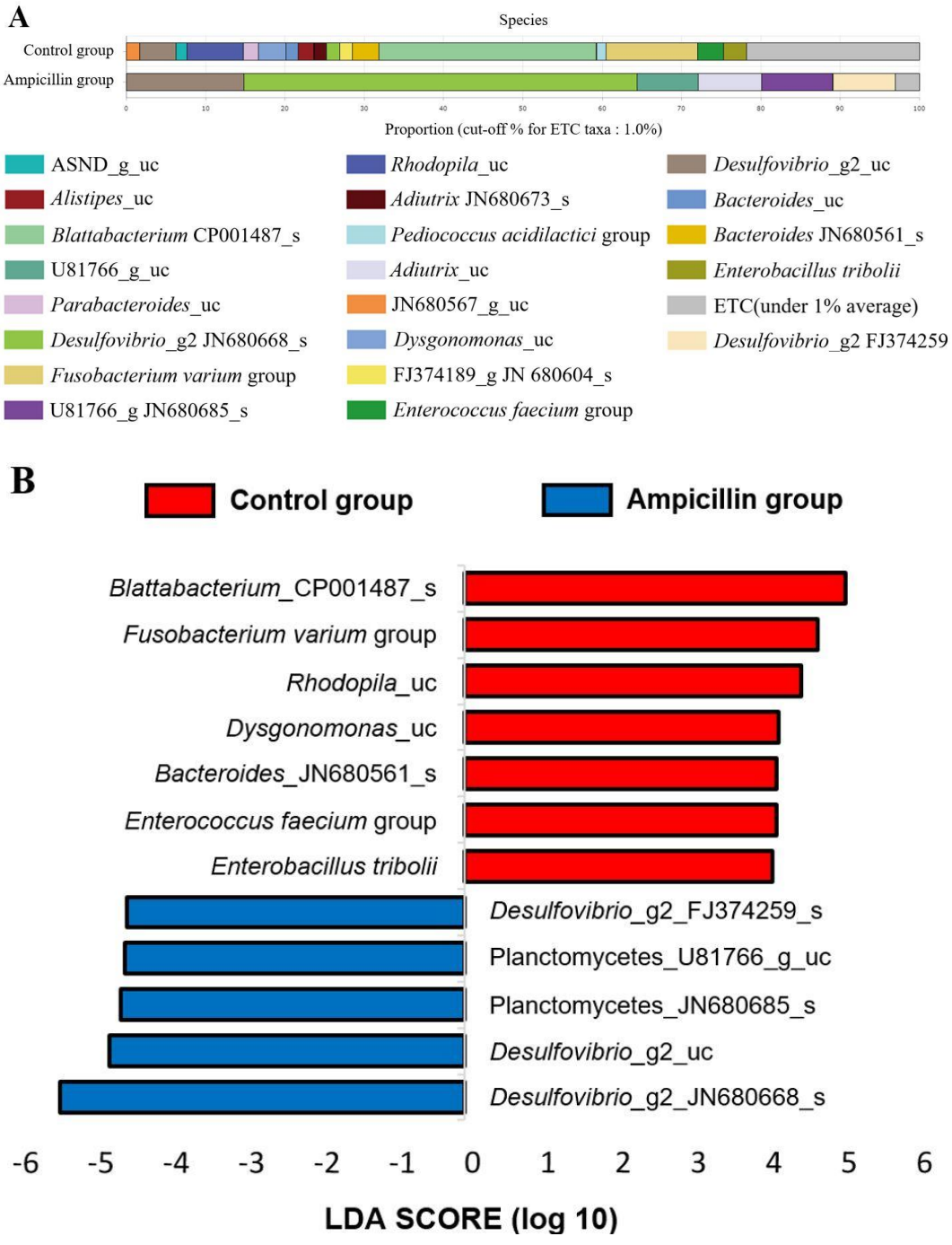
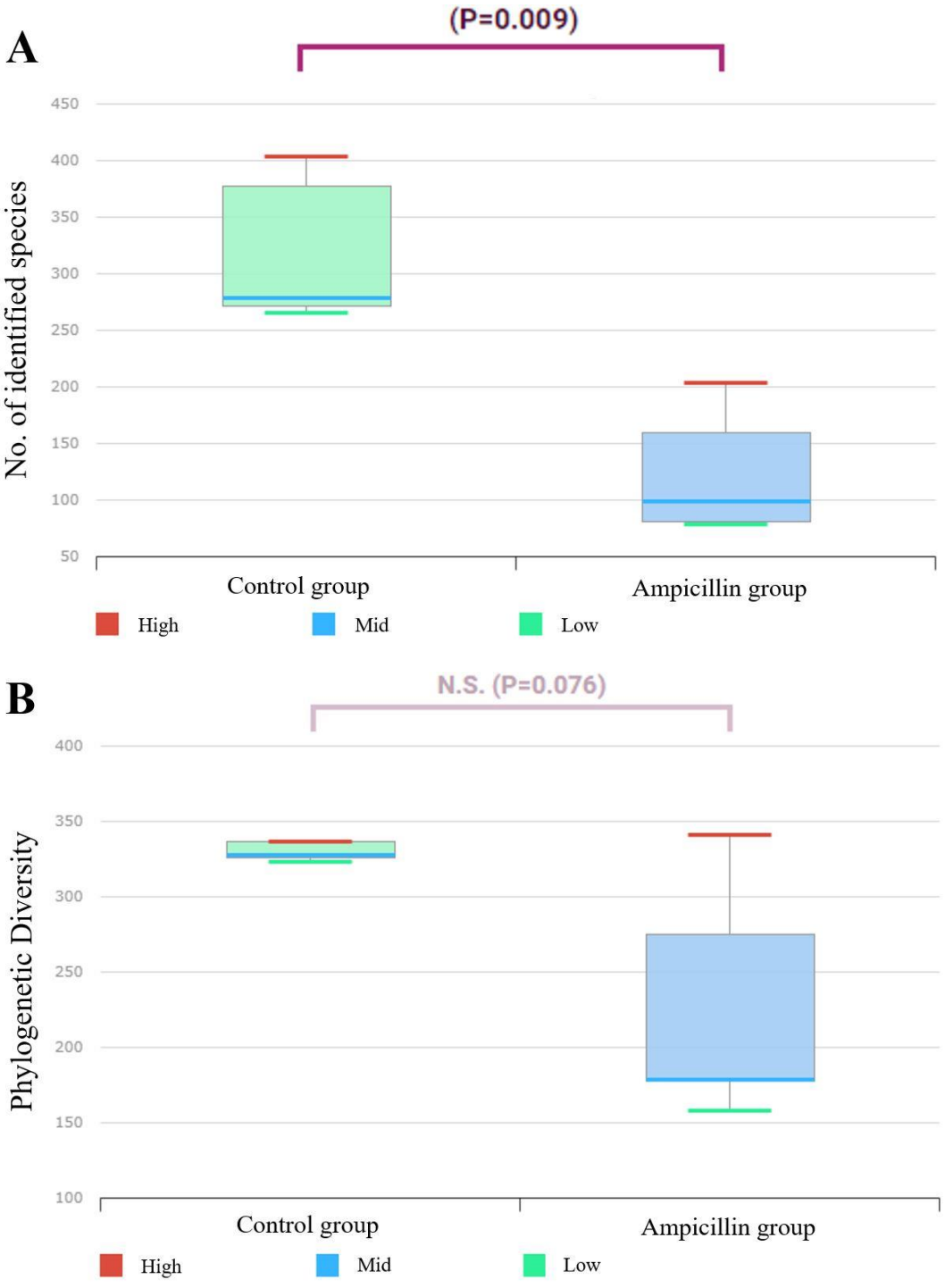


Figure I–7. Bacterial composition at the genus level in the control and ampicillin-treated groups. (A) Microbiome composition of each group (n = 5). (B) Linear discriminant analysis of differentially abundant bacterial taxa among the two groups. Only taxa meeting a significant linear discriminant analysis threshold (>4) are shown.



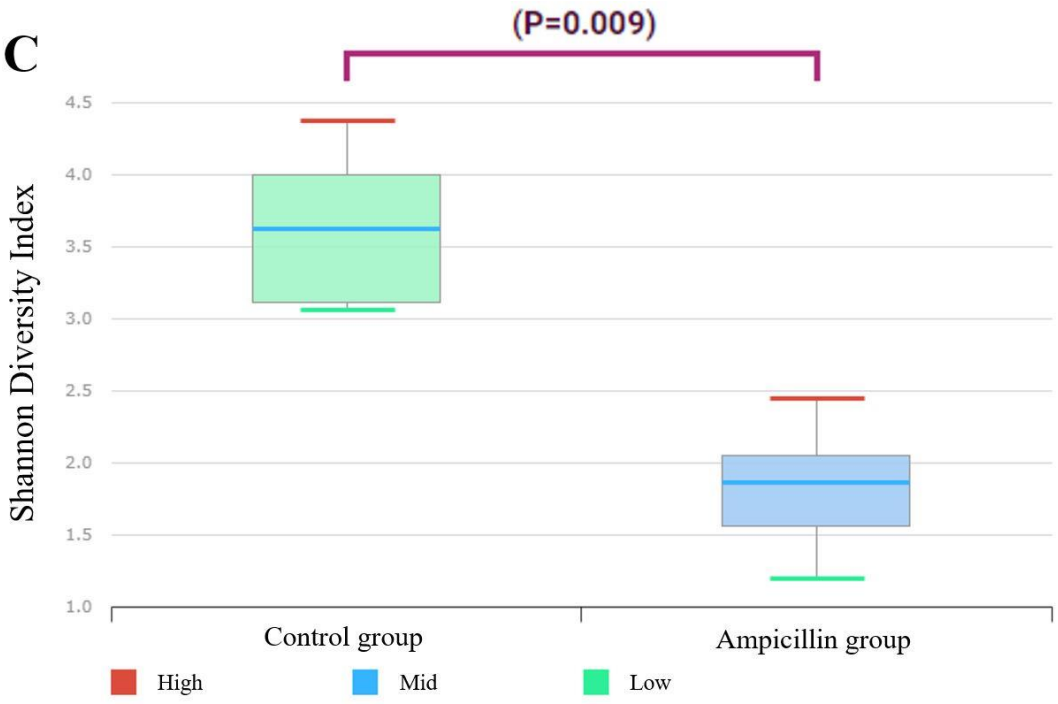
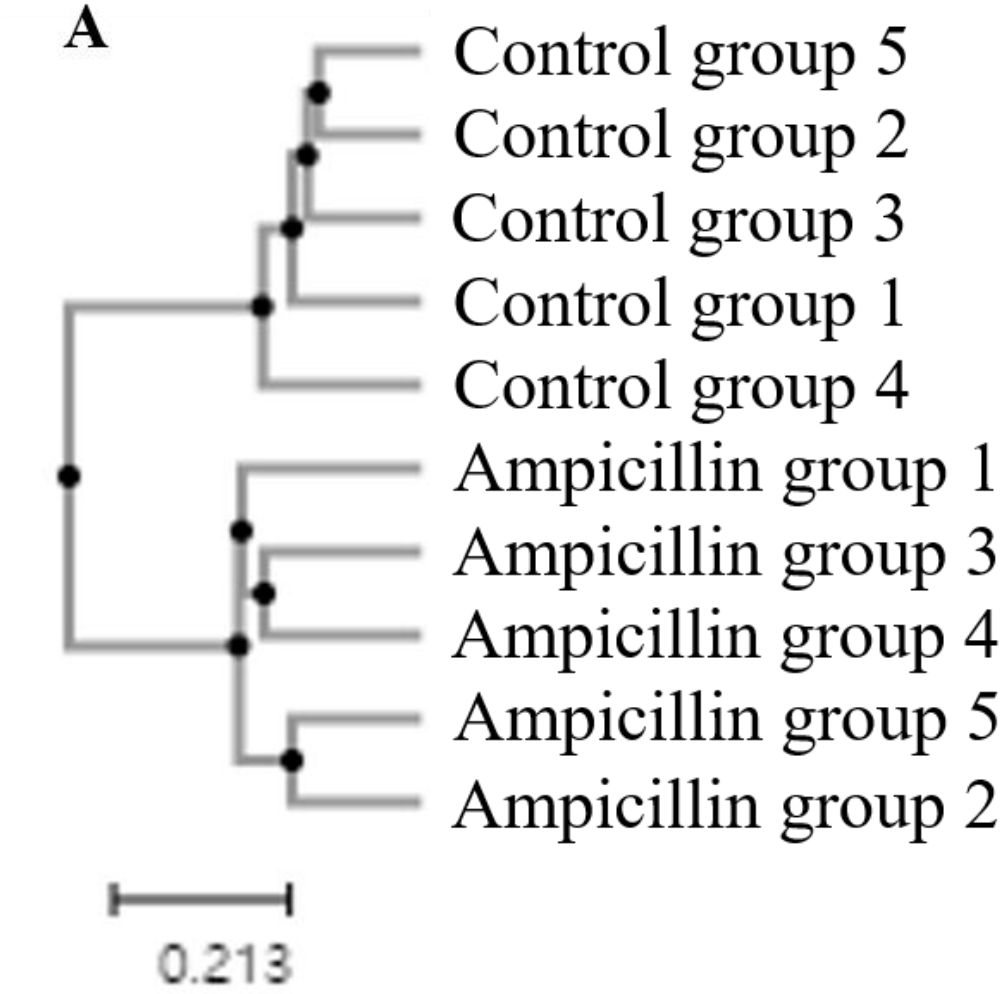


Figure I–8. Box plots showing alpha diversity in the control and ampicillin-treated groups. (A) The number of operational taxonomic units. (B) Phylogenetic diversity (abundance). (C) Shannon diversity index (measuring richness and equity in the distribution of the species).



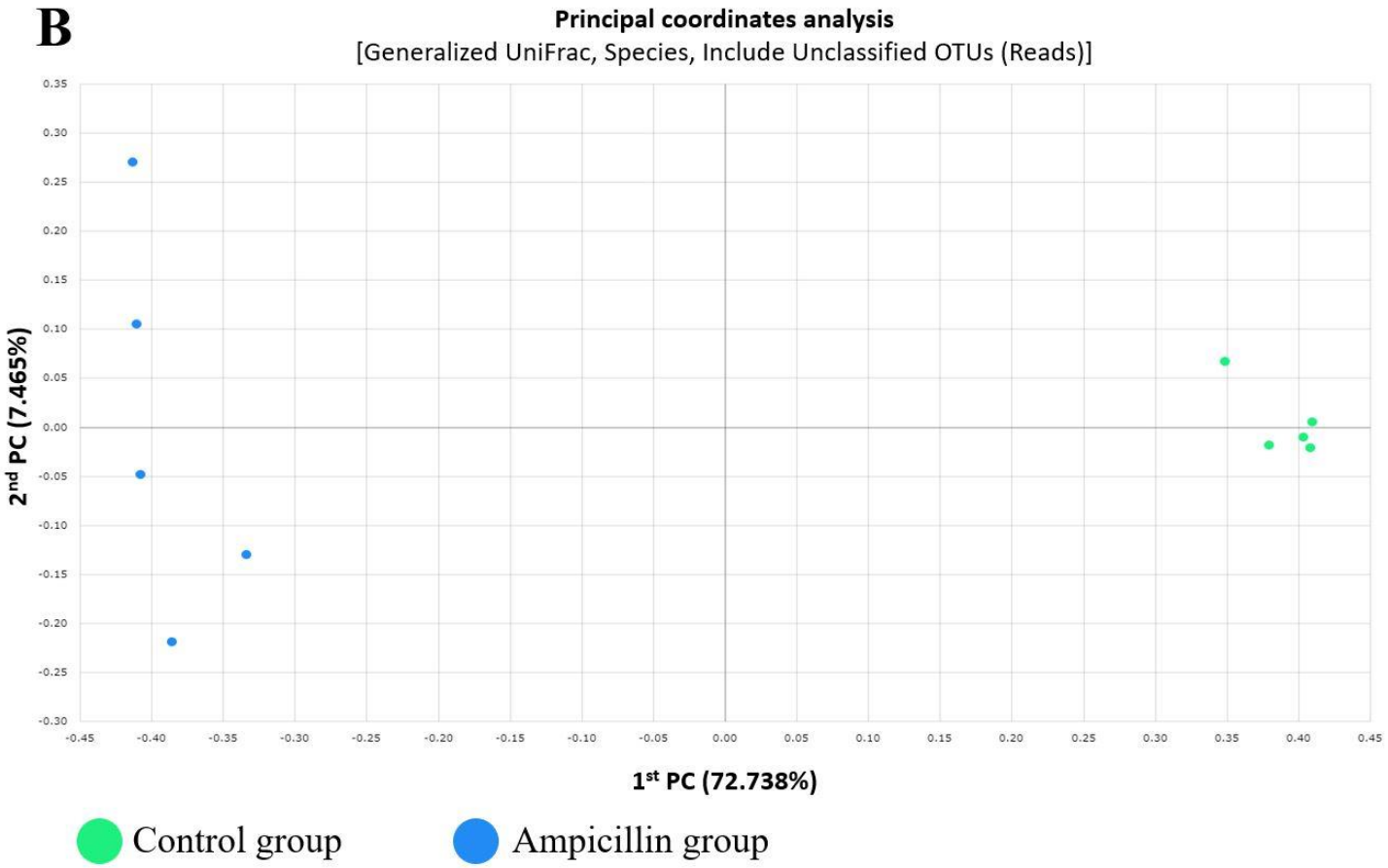
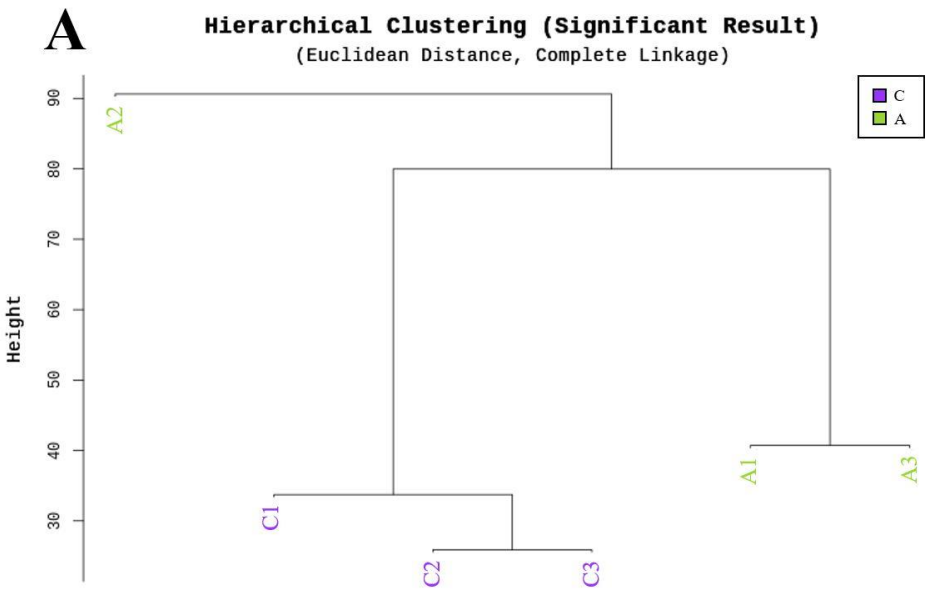
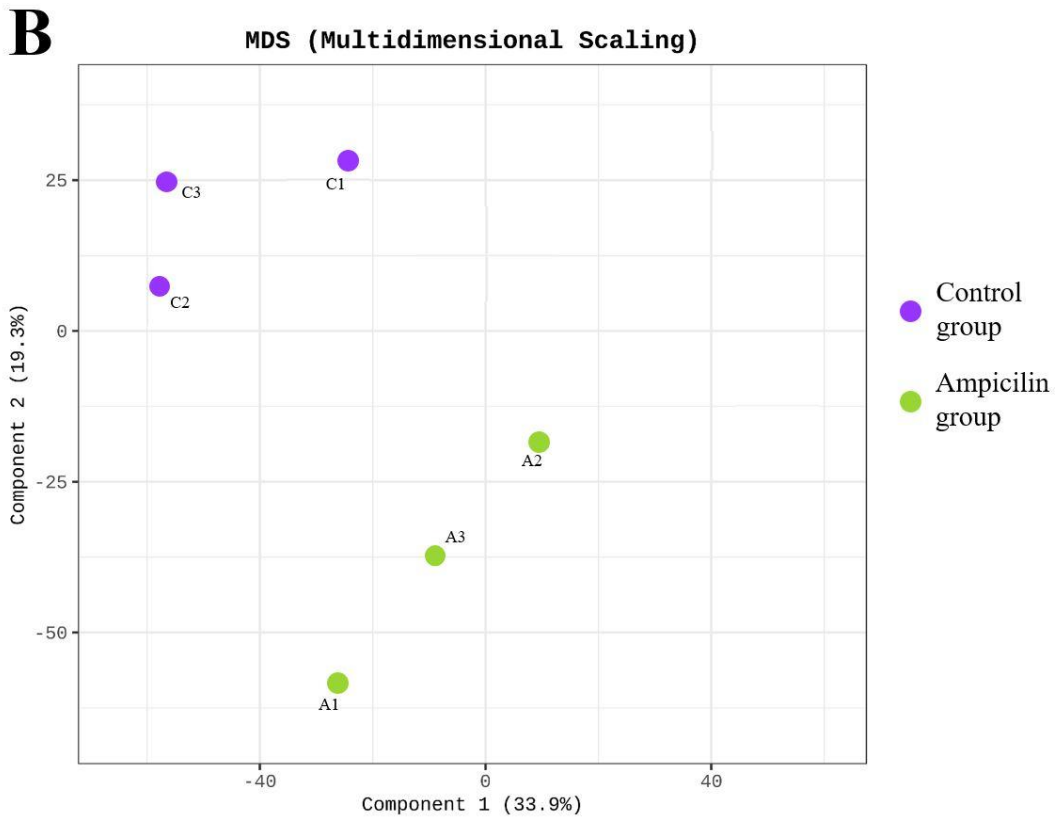

B
Principal coordinates analysis
[Generalized UniFrac, Species, Include Unclassified OTUs (Reads)]
2nd PC (7.465%)
1st PC (72.738%)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
-0.45
-0.40
-0.35
-0.30
-0.25
-0.20
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
Control group
Ampicillin group

Figure I–9. Beta diversity in the control and ampicillin-treated groups. (A) UPGMA (unweighted pair group method with arithmetic mean) clustering. (B) Principal coordinates analysis depicting differences in the taxonomic compositions of the bacterial communities among the two groups. OTUs, operational taxonomic units.

6. Changes in gene expression due to the effect of ampicillin

RNA-sequencing was performed to explore the effect of ampicillin on gene expression in cockroaches. UPGMA clustering results showed that the control group was grouped together, but that one ampicillin-treated sample was clustered separately (Figure I–10A). Principal components analysis (PCA) confirmed that separation was achieved between the control and ampicillin-treated groups (Figure I–10B). Hierarchical clustering analysis between the control and ampicillin-treated groups generated a heat map of 1,236 DEGs for both groups (Figure I–10C). Gene Ontology (GO) functional classification analysis revealed that these 1,236 DEGs were divided among three main categories (biological process, cellular component, and molecular function) where differential expression more than doubled in 28, 16, and 13 items, respectively, between the control and ampicillin-treated groups (Figure I–11).





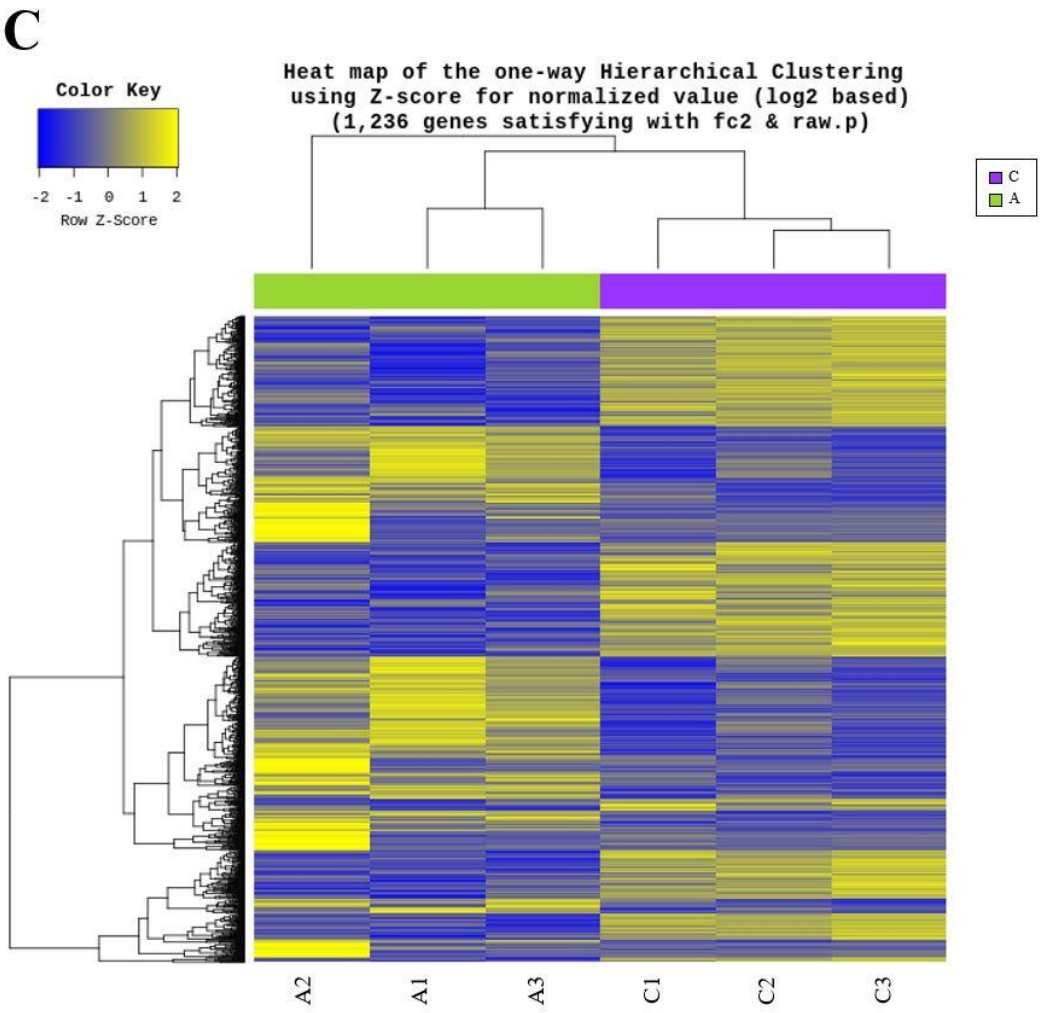
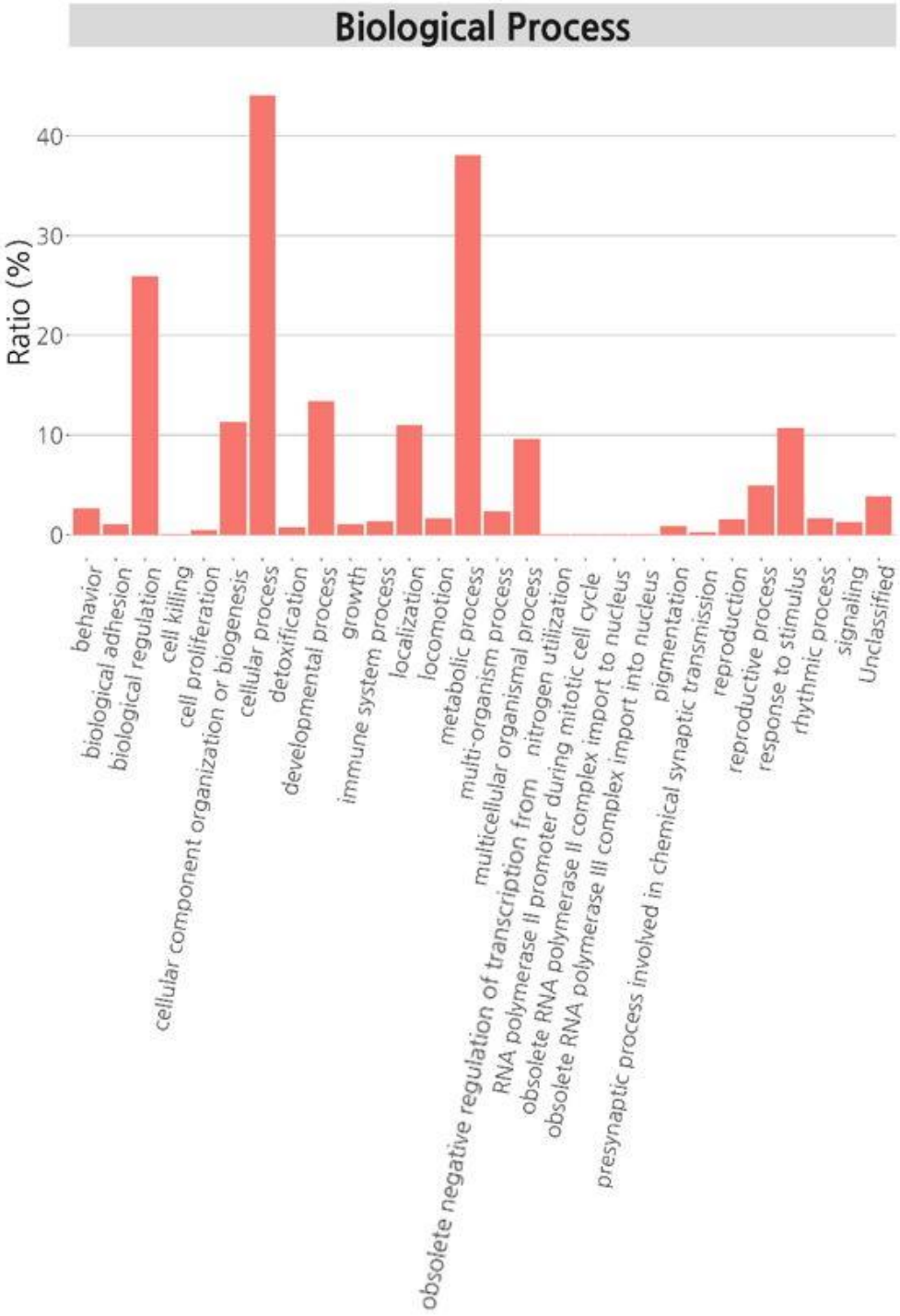


Figure I–10. Transcriptome analysis in ampicillin-treated and control cockroaches. (A) UPGMA (unweighted pair group method with arithmetic mean) clustering. (B) Principal components analysis depicting the differences in the differentially expressed genes (DEGs) between the control and ampicillin-treated groups. (C) Heat map of transcriptional expression patterns of the two groups displaying the expression profile of the top 1,236 DEGs for each sample in the RNA-seq dataset.



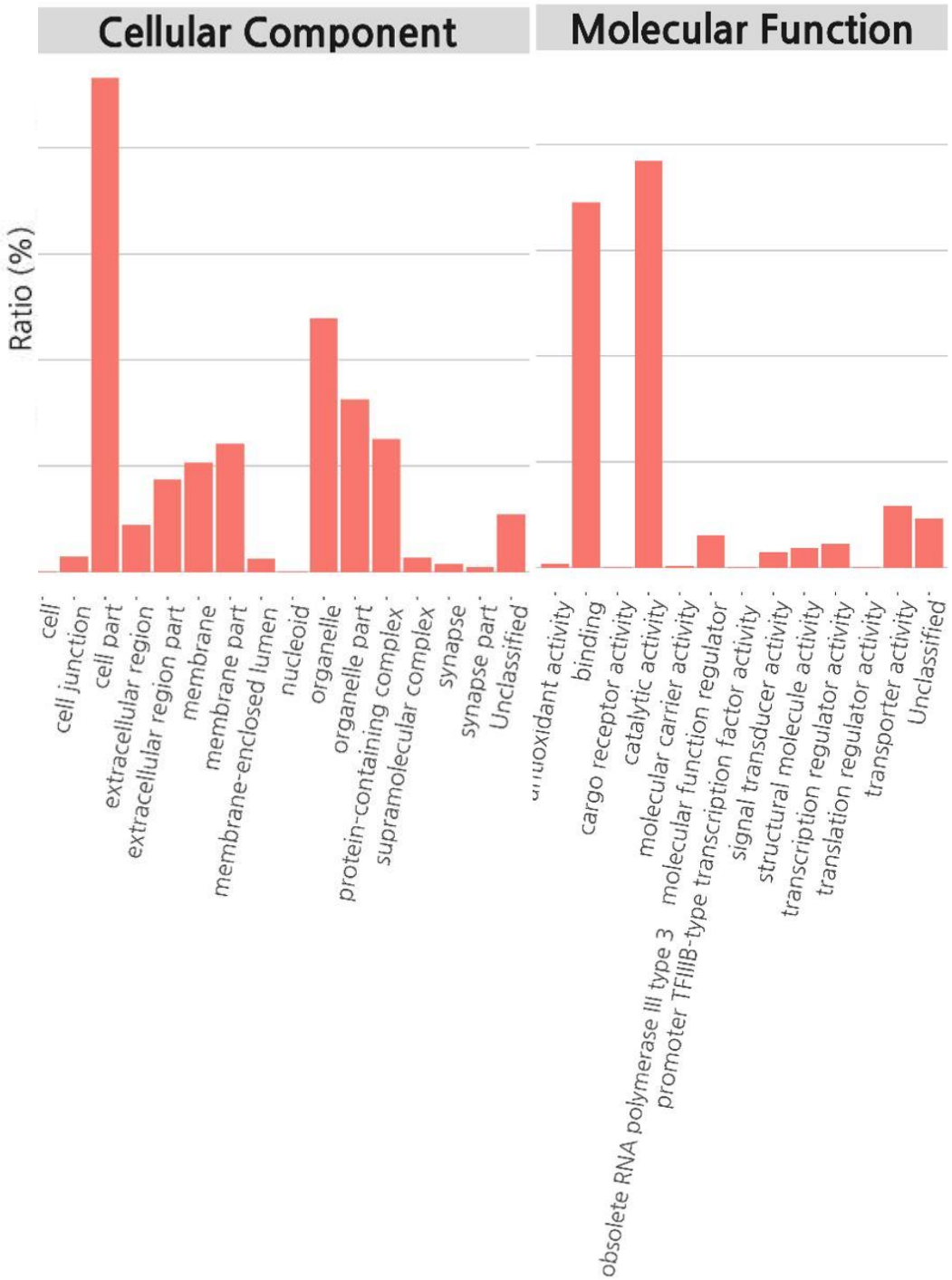


Figure I–11. Gene Ontology (GO) functional classification analysis of differentially expressed genes (DEGs) between the control and ampicillin-treated groups. Based on sequence homology, 1,236 DEGs were categorized into three main categories (biological process, cellular component, and molecular function) with 28, 16, and 13 functional groups, respectively.

7. Reduction of cockroach allergen gene expression levels by ampicillin

RNA-seq showed that the expression level of *Bla g 2* decreased by four times in the ampicillin-treated group. Subsequently, RNA levels of the genes encoding the three major allergens, *Bla g 1* (Figure I–12A), *Bla g 2* (Figure I–12B), and *Bla g 5* (Figure I–12C), were measured using qPCR. My findings confirmed that the expression levels of *Bla g 1* ($P < 0.001$) and *Bla g 2* ($P < 0.001$), but not *Bla g 5* ($P = 0.051$), were significantly decreased in the ampicillin-treated group compared to those in the control group. Additionally, I noted a larger decrease in the level of *Bla g 2* than that of *Bla g 1* (Figure I–12).

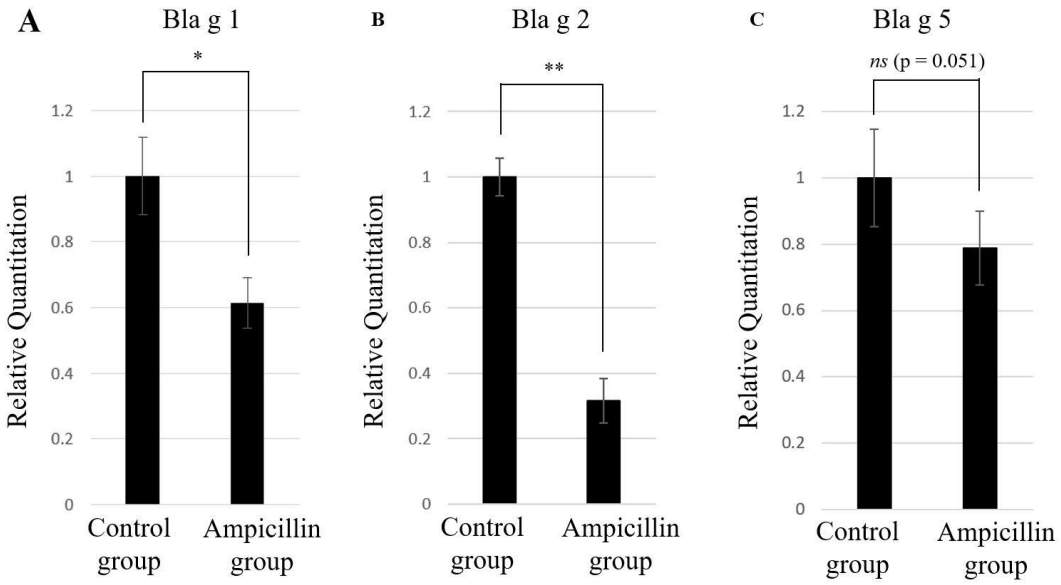


Figure I–12. Quantitative PCR (qPCR) analysis showing gene expression levels in cockroaches. (A) *Bla g 1*, (B) *Bla g 2*, and (C) *Bla g 5*.

8. Ampicillin-induced changes in protein patterns confirmed by 2DE analysis

In the previous GO functional classification analysis, the gene expression of the German cockroach was both increased and decreased due to ampicillin. Therefore, the protein expression pattern was confirmed with 2DE using German cockroach extract (Figure I–13A). Changes in 58 large and small spots were confirmed. In the ampicillin group, 31 increases and 27 decreases were confirmed (Figure I–13B). Proteins that decreased in the ampicillin group included Bla g 2, serine/threonine-protein kinase, vitellogenin, and Bla g 3; increased proteins included myosin heavy chain, heparan-alpha-glucosaminide N-acetyltransferase (HGSNAT), and others.

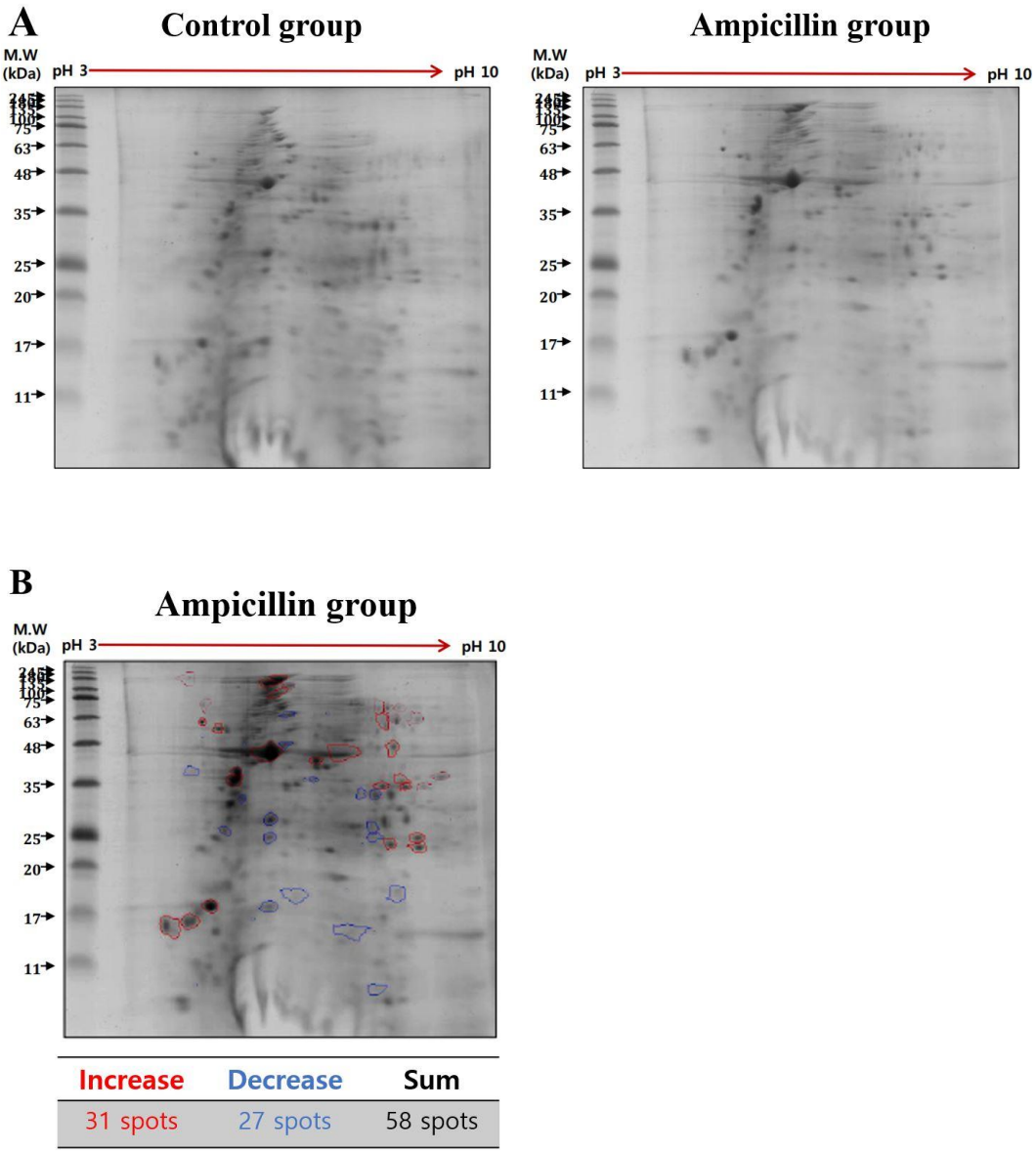


Figure I–13. Changes in protein patterns confirmed by 2DE analysis. (A) 2DE analysis of control and ampicillin groups. (B) The number of protein spots that increased and decreased in the ampicillin group.

9. Reduction of cockroach allergen expression in protein levels

At the protein level, I measured the amounts of Bla g 1 (Figure I–14A), Bla g 2 (Figure I–14B), and Bla g 5 (Figure I–14C). The results were similar to those obtained from transcriptomic analyses. No significant difference was detected in Bla g 5 ($P = 0.297$), whereas a significant decrease in the expression of Bla g 1 ($P < 0.001$) and Bla g 2 ($P < 0.001$) was observed in the ampicillin-treated group compared to that in the control group. In addition, Bla g 2 sustained a more notable decrease than Bla g 1 (Figure I–14).

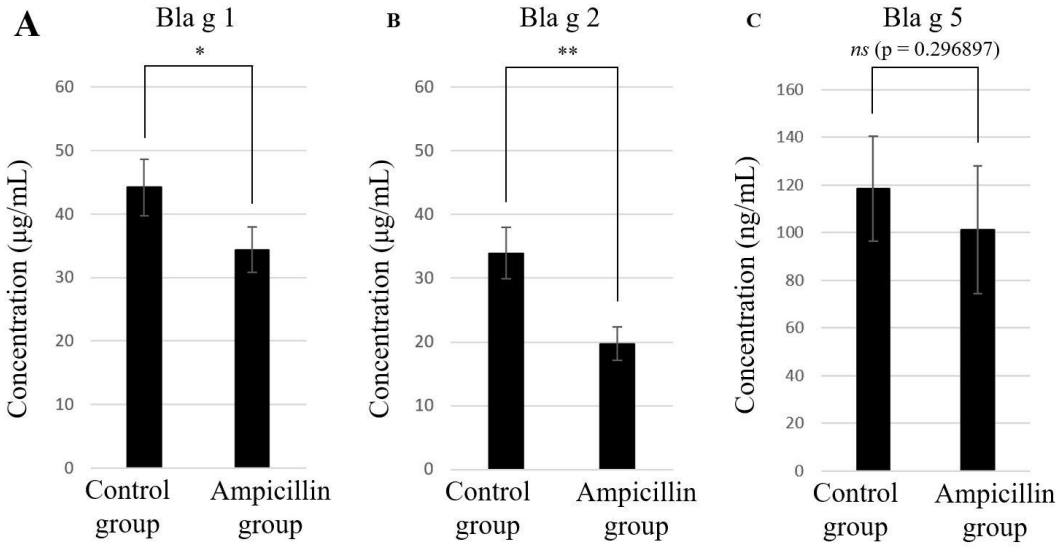


Figure I–14. Allergen levels in the extracts from the two cockroach groups. Concentrations of (A) Bla g 1, (B) Bla g 2, and (C) Bla g 5 in the extracts were measured using enzyme-linked immunosorbent assays.

IV. DISCUSSION

The cockroaches used in this study lived for many generations under the same conditions; therefore, it was assumed that other variables, such as diet, temperature, or humidity, would not strongly affect their microbiome.

I evaluated the microbiomes of the four cockroach species to determine whether there was any difference among their bacterial profiles. The results indicated that species richness did not differ between the *Periplaneta* cockroach species, but *Blattella germanica* was significantly lower than that of *Periplaneta*. Abundance was highest in *P. americana* and lowest in *B. germanica*. Specifically, abundance and equity were significantly higher in *P. americana*. This means that richness clearly differed according to the genus even with the same diet and environment, and that abundance and equity clearly differed within the genus.

A previous study showed that the microbiome of *P. americana* was resilient and stable when the cockroach underwent a dietary shift.[7] In addition, no significant phylum-level differences were reported in the observed microbiomes among the three *Periplaneta* groups (i.e., diet with six foods, mixed diet, and starvation diet). Furthermore, no differences were found among the three *Periplaneta* groups with respect to either alpha or beta diversity, although differences in the microbiome composition that were attributable to environmental factors were observed.[7] Similarly, in this study, assuming *P. americana* was stable with regard to dietary shifts, I observed differences in the microbial composition attributable to environmental factors at the phylum level (Figure I–3). With respect to the species composition, OTUs (over 1%) in *P. americana* accounted for the greatest diversity among the three *Periplaneta* cockroach species. Previous studies reported the presence of

Blattabacterium in *P. americana*, contrasting with the findings on my laboratory-reared cockroaches.[28] However, despite the lack of *Blattabacterium*, this strain showed no difficulties with reproduction or growth. In a previous study, wood-feeding dictyopteran insects significantly reduced the amount of *Blattabacterium*, resulting in nutrients gained by other means.[29] Therefore, it is possible that the microbiome of the *P. americana* strain reared in my laboratory may have adapted to the laboratory-related environmental conditions. In *P. japonica* and *P. fuliginosa*, *Blattabacterium* CP001429_s accounted for more than half of all OTUs. In addition, I observed that many OTUs were present in the microbiomes of *P. japonica* and *P. fuliginosa*. Nonetheless, the differences among the three cockroach species with respect to OTU composition were identified from the results of the UPGMA and PCoA clustering analyses. I confirmed that *P. japonica* and *P. fuliginosa* clustered before *P. americana*. In combination with the species composition results, these results suggest that *P. japonica* and *P. fuliginosa* have more similar bacterial compositions compared to that of *P. americana*. *B. germanica* also showed a varied species composition similar to that of *P americana*. The endosymbiont *Blattabacterium*_CP001487_s, which is a strain different from that found in *P. japonica* and *P. fuliginosa*, accounted for the most abundant bacterial species. *Serratia marcescens* has been found to be associated with hospital-acquired infections and is an opportunistic pathogen that is often involved in urinary tract and wound infections.[30] The *Dysgonomonas* species, which was present in *P. americana*, may cause gastroenteritis in immunocompromised humans.[31] This species is common in cockroaches,[32] and unclassified *Dysgonomonas* species were also present in *P. japonica*, *P. fuliginosa*, and *B. germanica*. Though *Blattabacterium*_CP001429_s was present in *P. fuliginosa*, it was more specific to *P. japonica*. Likewise, the endosymbiont *Blattabacterium*_CP001487_s was most significant in *B. germanica*. *Enterococcus*

faecium can live in the gastrointestinal tract of both humans and animals, and it can cause endocarditis.[33] *P. fuliginosa* had many OTUs in common with *P. japonica*, but *Pediococcus*_uc was more abundant in all three cockroach species. Nevertheless, the PCoA analyses showed different clustering results, and *P. fuliginosa* showed a substantial number of OTUs in common with *P. japonica*, although differences were found between the two.

I treated cockroaches with ampicillin to obtain a protein extract containing a minimal number of bacteria for immunotherapy. Analysis of *B. germanica* following treatment revealed several changes. First, the total bacterial population was notably affected. Compared with the control group, the total bacteria in cockroaches from the ampicillin-treated group disappeared almost completely, perhaps because ampicillin eliminated both gram-positive and gram-negative bacteria. One of the objectives of the study was to produce a protein extract from cockroach with a reduced bacteria content. When rearing cockroaches for clinical use, such as for allergy diagnosis and immunotherapy, strict control of the bacteria using measures such as ampicillin treatment is recommended.

The microbiome study revealed marked differences at the species level. A 'super-resistant' taxon was previously identified in *B. germanica* treated with rifampicin instead of ampicillin.[5] The *Desulfovibrio* and *Planctomycetes* genera occurred in lower numbers in control cockroaches but comprised most of the microbiota of ampicillin-treated cockroaches. This finding is supported by the fact that all *Planctomycetes* are resistant to β-lactam antibiotics,[34] including ampicillin, which belongs to the penicillin group of antibiotics. Similarly, *Desulfovibrio* and *Adiutrix* are resistant to ampicillin, which eliminated other bacterial species. Among the bacterial species found in the control group, *Blattabacterium* is the most important. In a previous study, treatment with rifampicin failed

to eliminate *Blattabacterium* from the first generation of cockroaches; however, with continued treatment, the bacteria were eliminated from second-generation specimens.[5] My data showed that treatment with ampicillin immediately eliminated *Blattabacterium* from first-generation adults. *Blattabacterium* is an endosymbiont of *B. germanica*, where it is involved in the synthesis of essential amino acids and various vitamins, as well as nitrogen recycling.[28] A previous study showed that tetracycline removed the endosymbiont of *Riptortus pedestris*, and that the expression of genes encoding hexamerin and vitellogenin was reduced. Consequently, these findings confirmed the factors that affected egg production and development.[6] Here, I expected that the absence of an endosymbiont would lead to several changes. Essentially, the reported decrease in bacterial composition produced differences in alpha and beta diversity. Because ampicillin reduced the bacterial load, OTUs were significantly lower in the ampicillin-treated group than in the control group. The phylogenetic diversity (indicating abundance) was not significantly different but tended to be lower in the ampicillin-treated group. In addition, the Shannon diversity index significantly decreased, reducing both richness and equity. Analysis of beta diversity using UPGMA and PCoA showed a clear difference in clustering between the two groups.

RNA-seq was performed to identify ampicillin-induced changes in gene expression at the RNA level. Results similar to those from the microbiome clustering analysis were confirmed at the RNA level. Hierarchical clustering and heat map analyses showed that one of the ampicillin-treated samples was clustered separately, but samples in the control group clustered well. PCA confirmed that each group was well clustered. Most of the ampicillin-treated *B. germanica* showed decreased levels of DNA; however, gene levels were either substantially increased or decreased at the RNA level. In addition, DEGs were enriched in biological, metabolic, and cellular processes. Differential expression of

various genes from the two groups was also noted in developmental processes and growth, as well as in cellular components, with several differences noted between the cells and the organelles. The RNA-seq data showed that the expression level of the *Bla g 2* gene (encoding aspartic protease) was reduced by more than four times following ampicillin treatment. Therefore, gene expression levels for the major allergens *Bla g 1*, *Bla g 2*, and *Bla g 5* were further measured via qPCR, and the protein production level was also measured. As with the bacteria, some proteins decreased while others increased. This suggests that antibiotics did not decrease the total amount of protein, but instead caused a change. The serine/threonine-protein kinase, which was reduced in the ampicillin group, is responsible for cell proliferation, programmed cell death, and cell differentiation.[35] It has also been demonstrated that inhibitors of serine/threonine-protein kinase are beneficial in ovarian cancer.[35] Vitellogenin, which was also largely absent from the ampicillin group, plays a role in providing a major yolk protein that is a source of nutrients in the early development of animals.[36] It was also correlated with the results of RNA-sequencing, which showed that the reproduction of the ampicillin group was reduced. Similar patterns of Bla g 1, Bla g 2, and Bla g 5 expression changes were observed at both the mRNA and protein levels. There was no significant difference in Bla g 5 expression between the ampicillin-treated cockroaches and control group, but its abundance tended to be lower in the ampicillin-treated group than in the control group. By contrast, Bla g 1 and Bla g 2 showed a significant decrease in abundance in the ampicillin-treated group compared to the control group.

The exact mechanism of allergen production in German cockroaches is unknown. However, clear differences in Bla g 1 production were observed based on the insect's level of starvation or its stage in the gonadotropic cycle.[11] Therefore, I expected to observe

changes caused by several factors in the present study. The bacteria that were removed by ampicillin treatment likely included species that promoted the growth of cockroaches, thus accounting for the difference in total bacteria. Moreover, the inhibition of bacterial growth may have affected allergen production, as *Blattabacterium* is potentially the most influential member of the cockroach microbiota. In other insects, *Blattabacterium* reduces the expression of genes involved in reproduction and growth inhibition. Likewise, German cockroaches may also experience changes in reproduction and growth due to the removal of *Blattabacterium*. Similar growth rates were observed between *R. pedestris* originally without the endosymbiont and that treated with antibiotics to remove the bacterium.[6] RNA-seq highlighted numerous changes in gene expression. Therefore, I suggest that ampicillin may have influenced the production of allergens. Although antibiotics primarily affect bacteria, they may also indirectly affect allergens through their effects on bacterial gene expression.

In this study, treatment with ampicillin eliminated *Blattabacterium*, an endosymbiont of *B. germanica*, and reduced the production of several allergens. This suggests that *Blattabacterium* may play a key role in allergen production in cockroaches either directly or indirectly.

In future studies, differences between *P. japonica* and *P. fuliginosa* regarding diet and environmental shifts should also be assessed to elucidate which species characteristics are determined by bacteria. Furthermore, this information will be beneficial for identifying species-specific cockroach features.

Ampicillin treatment reduced the total amount of bacteria associated with cockroaches. As a result, I suggest that a reduced number of bacteria may have influenced the production of allergens. Future studies should investigate the effect of bacteria on the

therapeutic efficacy of immunotherapy using protein extracts obtained from the German cockroach. In addition, further research is needed to confirm whether a reduced allergen content in the cockroach protein extract after ampicillin treatment induces immune tolerance in immunotherapy recipients.

V. CONCLUSION

In conclusion, I compared the microbiomes of three *Periplaneta* species and *B. germanica* and found differences in the bacterial composition of their microbiomes despite being reared under the same conditions for many generations. Additionally, protein extraction from ampicillin-treated *B. germanica* was optimized to obtain an extract containing a small amount of Bla g 2 (compared to Bla g 1 and Bla g 5) and very few bacteria.

CHAPTER II

Reduced airway inflammation caused by German cockroaches exposed to ampicillin: analyses in lung cells and a mouse model of asthma

I. INTRODUCTION

Cockroach infestations are associated with allergic diseases, such as asthma, in humans. When German cockroaches were treated with antibiotics, their microbiome changed.[5] Further, expression of Bla g 1, a digestive protein in German cockroaches, and the composition of the microbiome changed upon dietary modifications.[37] Bla g 1 can bind various lipids and has a digestive function related to the nonspecific transport of lipid molecules.[14] Similar to Bla g 1, Bla g 2 is present at high concentrations in the digestive organs of German cockroaches and functions as a digestive enzyme.[15] Bla g 5 is a sigma class glutathione *S*-transferase (GST) and is one of the major allergens among all German cockroach allergens, as it induces a high IgE response.[38] Although Bla g 5 is present at a concentration 100 times lower than those of Bla g 1 and Bla g 2, some patients respond predominantly to Bla g 5.[17]

In cockroaches, the microbiome and allergen levels change depending on the environment or food.[5] Cockroaches spread pathogenic bacteria through their feces or frass in various locations, including homes, shops, and hospitals.[39] Thus, cockroaches can cause asthma in humans through the allergens in their feces or frass.[32]

Active ingredients are substances that exert beneficial health effects on consumers.[40] The active ingredient of a pharmaceutical drug is called an active pharmaceutical ingredient (API).[40] APIs are released into the natural environment during their production, use, and disposal.[41] Moreover, exposure to APIs causes problems, such as the generation of antibiotic-resistant bacteria and feminization in fish.[41,42] As cockroaches tend to prefer warm and humid areas,[43] they are highly likely to be exposed to APIs

discharged into rivers or catchments. During this process, the microbiome or allergen in the cockroaches might change upon exposure to normal food or water.

The cockroach extract used for immunotherapy or diagnostics has not been standardized. The composition of the extracts varies greatly depending on the breeding environment of the cockroach and extraction method.[17] Further, the effect of cockroach extracts on T cell potency or IgE reactivity varies according to a patient's allergen profile.[17] Therefore, suitable methods are needed to develop a standardized extract, as well as diverse extracts for various patients.

In a previous study, ampicillin-treated German cockroaches contained few bacteria, confirming the altered microbiome composition compared to that of the control German cockroaches.[39] Further, cockroach gene expression was altered, and levels of *Bla g 1* and *Bla g 2* decreased.[39] However, the ability of these ampicillin-treated German cockroaches to cause allergic inflammation remained unclear.

A recent study showed that birch allergen Bet v 1 alone could not cause allergic sensitization.[44] On the other hand, Bet v 1-depleted birch pollen extract caused Th2 polarization. [44] As the results show, the influence of other substances and allergens cannot be ignored as factors that cause allergic sensitization. Therefore, in this study, I examined the changes in airway inflammation caused by treating bronchial cells and mice with ampicillin-treated German cockroach extracts.

II. MATERIALS AND METHODS

1. Rearing conditions

German cockroaches (*Blattella germanica*) were reared for several generations under the same laboratory conditions to minimize the potential influence of environmental factors and diet. All cockroaches were reared in plastic boxes (27 cm × 34 cm × 19 cm) incubated at 25°C and 50% relative humidity. *B. germanica* was fed sterilized fish food and provided sterilized untreated or ampicillin-containing (autoclaved before the addition of 0.025% ampicillin) tap water *ad libitum* (Fig 1A).

2. DNA extraction

Total DNA was extracted using the NucleoSpin DNA Insect Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The DNA extracted from each sample was eluted in 20 μL elution buffer. All procedures were conducted on a clean bench under a sterilized hood and in a DNA-free room. The DNA concentration was quantified using an ND-1000 NanoDrop system (Thermo Fisher Scientific, Waltham, MA, USA).

3. Protein extraction

Total protein was extracted by adding 2 mL PBS to each sample. The samples were then sonicated (QSonica Q500, Fullerton, CA, USA) and centrifuged at 10,000 × *g* for 30 min at 4°C. The resulting supernatants were filtered using a 0.22 μm membrane filter (Millex®; Tullagreen, Carrigtwohill, Co. Cork, Ireland).

4. ELISA

Cockroach protein extracts (2 mg/mL) were diluted 100-fold to measure Bla g 1 and Bla g 2 levels and 10-fold to measure Bla g 5 levels using the corresponding ELISA kits (Indoor Biotechnologies, Charlottesville, VA, USA) according to the manufacturer's instructions. Briefly, the detection antibody and conjugate mix were used for the immunoassay, and color development was obtained using the 3,3′,5,5′-tetramethylbenzidine (TMB) substrate.

5. RNA extraction and cDNA synthesis

Total RNA was extracted by adding 1 mL TRIZOL reagent (GeneAll, Seoul, Korea) to each sample. The TRIZOL supernatant was added to react with the sample and mixed with isopropanol to obtain a pellet. RNA extracted from each sample was eluted in 20 μL elution buffer. A master-mix comprised of a 5× cDNA synthesis mix and 20× reverse transcriptase was added to the mRNA samples in PCR tubes for cDNA synthesis.

6. qPCR

qPCR was performed to quantify *Bla g 1*, *Bla g 2*, *Bla g 5*, and bacterial 16S rRNA levels in cockroaches. Actin 5C was used as an internal control, and primers specific to this gene (ActinF and ActinR) were designed for this experiment (Table I-1).[5] All bacterial 16S rRNAs were amplified using the forward primer BACT1369 and the reverse primer PROK1492R (Table I-1) from XenoTech with the AMPIGENE qPCR Mix (Enzo Life Sciences, Inc., Farmingdale, NY, USA).[5] *Bla g 1*, *Bla g 2*, and *Bla g 5* gene expression was used to measure the major allergen content. qPCR analyses were performed using the 2× SensiFAST™ SYBR® Hi-ROX kit (Bioline Meridian Bioscience, London, UK) with

SYBR Green as the fluorescent reporter, H_2O, the corresponding primers, and either genomic or complementary DNA. At the end of each reaction, a melting curve was generated to check the specificity of amplification and confirm the absence of primer dimers. All reactions, including the negative controls (containing water instead of DNA), were run in duplicate in 96-well plates.

7. Cell culture and exposure to extracts

Human bronchial epithelial cells (BEAS-2B cells) were maintained in DMEM/F40 medium at 37°C with 5% CO_2. Cells were seeded at a concentration of 1×10^6 cells/well in 6-well plates (SPL Life Sciences, Gyeonggi, South Korea) and then treated with each German cockroach extract (GCE) sample. Cells were sampled 24 h after a single exposure to 100 μg/mL of each GCE and compared with the PBS-treated controls. Four independent samples were examined for each GCE extract and PBS control.

8. Cytokine measurement

The concentrations of cytokines secreted from BEAS-2B cells were measured from the supernatants using the DuoSet human IL-6 and IL-8 ELISA (R&D Systems, Minneapolis, MN, USA).

9. Mouse model of allergic airway inflammation

Wild-type BALB/c mice (6–8 weeks old) were purchased from Orient Bio 13 (Seongnam, South Korea). All experiments were approved by the Institutional Review Board of Yonsei University College of Medicine (IACUC no. 2021-0319). A mouse model of allergic airway inflammation was used to investigate the effect of allergen-reduced

German cockroaches on allergic inflammation. GCE from each group (120 μg) was administered intranasally twice a week for 3 weeks.[45] There were three groups: PBS treatment (PBS group), extract of normal German cockroaches (Control group), and extract of ampicillin-treated German cockroaches (Ampicillin group). Mice were sacrificed on day 21, and bronchoalveolar lavage (BAL) was performed with 1 mL of PBS. Blood from the cardiac tissue was stored at 4°C. Lungs were dissected and frozen or fixed in formalin.

10. Bronchoalveolar lavage fluid (BALF) cells

The procedure for BALF cell collection was identical to that described previously.[45] After elimination of red blood cells, BALF cells were counted using a hemocytometer. All slides were subjected to Diff Quik staining (Sysmex Corporation, Wakinohama-Kaigandori, Japan). Eosinophil, macrophage, lymphocyte, and neutrophil cell counts were determined in 400 BALF cells.

11. Lung histology in the mouse model of asthma

Lung tissue samples were sectioned (5 μm) and subjected to hematoxylin and eosin (H&E) as well as periodic acid-Schiff (PAS) staining using standard histological protocols to detect mucus-containing cells.[45] The pathological change index of H&E-stained slides was assigned numerical values based on inflammatory cell infiltration and thickness around the airway and blood vessels (0, normal or no cells; 1, ≤ 3 cell diameter thickness; 2, 4–6 cell thickness; 3, 7–9 cell thickness; and 4, ≥ 10 cell thickness). Similarly, numerical values were assigned according to the proportion of airways and blood vessels in each section surrounded by inflammatory cells (0, normal or no airways or blood vessels; 1, < 25% of the airways or blood vessels; 2, 25–50%; 3, 51–75%; and 4, ≥ 75%). The

exponent was calculated by multiplying severity with range, with a maximum possible score of 9. The number of mucus-containing cells/mm^2 in the basement membrane and the intensity of bronchial and perivascular inflammation were also measured. Furthermore, airway epithelial cells were scored on the degree of goblet cell hyperplasia on a % scale of PAS+ cytoplasm. PAS+ cells in the epithelial region were counted six times per section in two tissue sections per mouse (n = 8 mice/group).[46,47] Each value is expressed as the mean ± standard deviation (SD).

12. Lung lysate analysis

The lungs were harvested after BAL fluid collection and homogenized using the T-PER Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, MA, USA). After homogenization, the suspensions were incubated at 4°C for 30 min and centrifuged at 2500 rpm for 10 min. The supernatants were filtered through a 0.45 μm filter to analyze the cytokine levels.[45]

13. Quantification of cytokines in BALF and lung homogenates

ELISAs for IL-4, IL-5, IL-13, and IFN-γ were performed using the respective commercial kits (Pepro Tech, Rocky Hill, NJ, USA) according to the manufacturer's instructions.

14. Quantification of German cockroach-specific IgE, IgG1, and IgG2a in serum

German cockroach-specific IgE, IgG1, and IgG2a levels in mouse sera were assessed using antigen-capture ELISA. Briefly, 96-well plates were coated with 20 μg GCE in 100 μL coating buffer and incubated overnight at 4°C. The plates were blocked with 200

μL/well of the assay diluent. Diluted serum samples (1:10 dilution) were then added to each well and incubated. Next, the wells were washed, and biotin-anti-mouse IgG1, IgG2a, or IgE (BioLegend, San Diego, CA, USA) was added and incubated with the samples for 2 h. Afterward, this was followed by 30 min of incubation with avidin-goat peroxidase (BioLegend, San Diego, CA, USA). The TMB substrate solution (100 μL) was added to each well and incubated in the dark for 20 min. The reaction was then stopped using 2 N sulfuric acid (H_2SO_4). Optical densities were measured at 450 nm using a spectrophotometer.

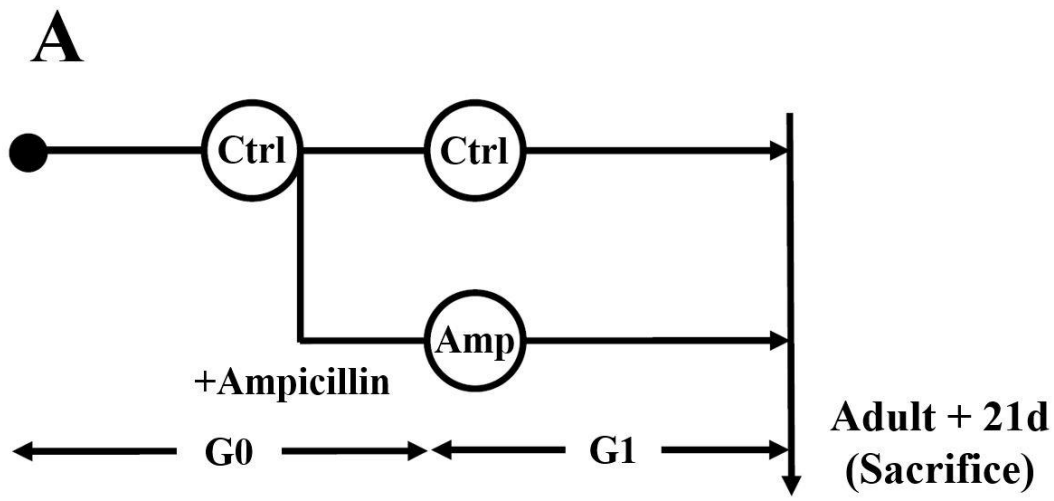
15. Statistical analysis

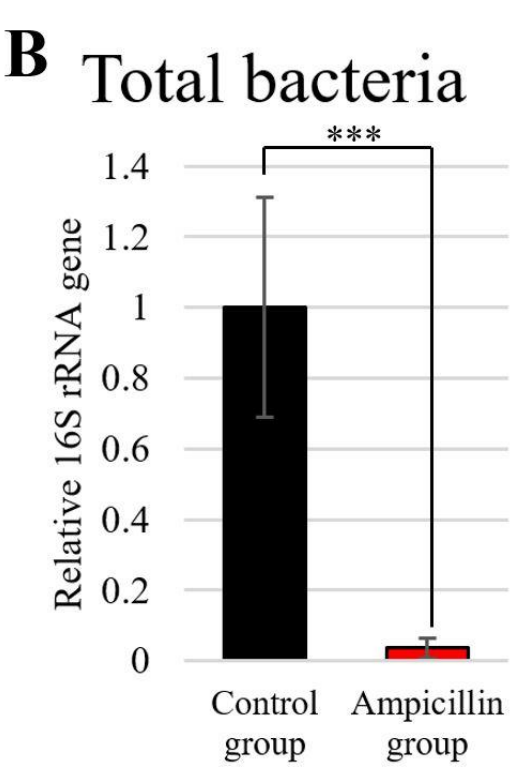
Student's *t*-test and analysis of variance (ANOVA) with Bonferroni correction as a post-hoc analysis were used to analyze the data. Differences with P-values of 0.05 or less were considered statistically significant.

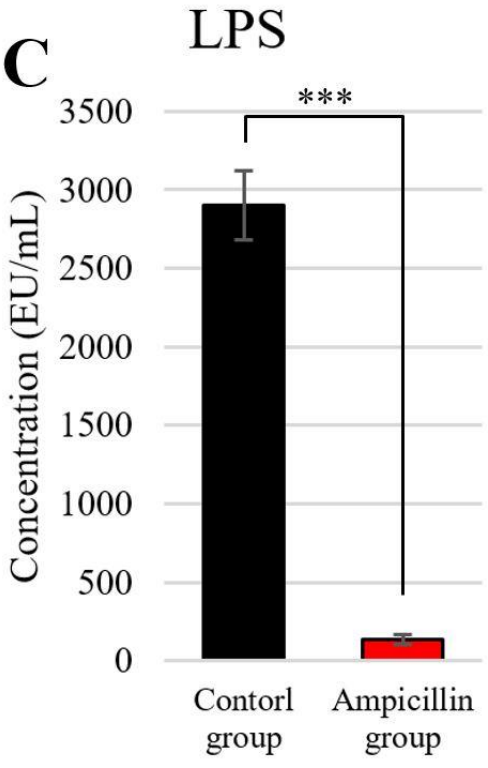
III. RESULTS

1. Reduction of total bacteria and allergens in German cockroaches due to antibiotics

First, I checked whether the total bacterial count of cockroaches was reduced by antibiotic treatment when rearing cockroaches. qPCR analysis showed that the total number of bacteria in the cockroaches was 20 times higher in the control group than in the ampicillin-treated group (Figure II–1B). Additionally, a significant decrease in LPS, a bacteria-derived substance, was observed in the ampicillin-treated group (Figure II–1C). The gene expression levels of the three major allergens *Bla g 1*, *Bla g 2*, and *Bla g 5* were measured using qPCR; the results showed that the expression level of these allergens significantly decreased in the ampicillin-treated group compared with those in the control group (Figure II–1D). I also measured the protein levels of Bla g 1, Bla g 2, and Bla g 5 (Figure II–1E); the gene expression level was reduced by nearly half, but the protein level decreased by only 20%. The results were similar to those obtained from the gene expression analyses.







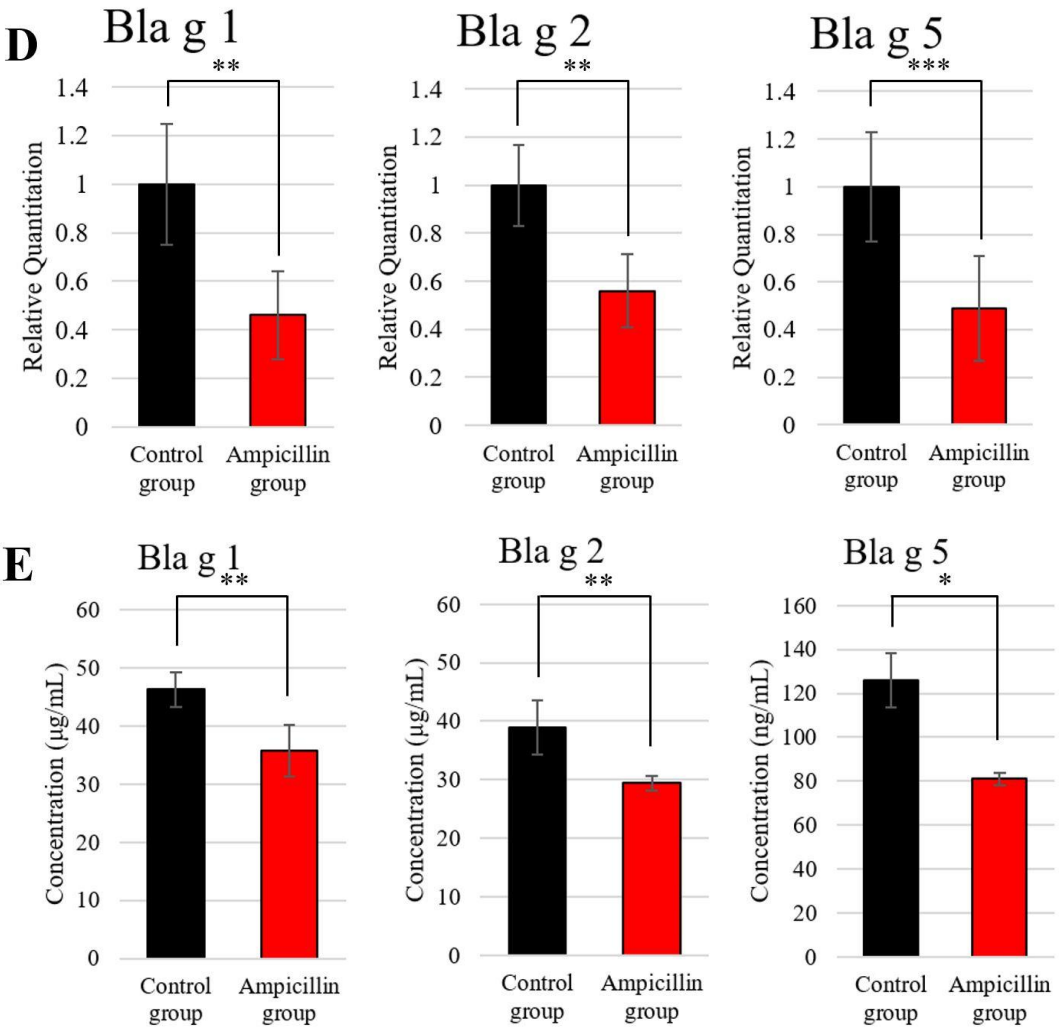


Figure II–1. Decreased total bacteria and major allergens in the extract of German cockroaches treated with ampicillin. (A) Experimental design depicting ampicillin treatment of *B. germanica*. The cockroaches were divided into two groups, and individuals were either treated with ampicillin (Ampicillin-treated group: Amp) or left untreated as control specimens (Control group: Ctrl). Ampicillin was administered to cockroaches from the G1 (i.e., offspring from G0) generation 21 days after they reached the adult stage. (B)

Relative levels of the bacterial 16S rRNA gene in the normal German cockroach (Control group) and ampicillin-treated German cockroach groups (Ampicillin group). (C) Concentration of lipopolysaccharide (LPS) in 1 mg/mL *of B. germanica* extract. (D) Quantitative PCR (qPCR) analysis showing *Bla g 1*, *Bla g 2*, and *Bla g 5* gene expression levels in German cockroaches. (E) Allergen levels in extracts from the two German cockroach groups. *P < 0.05, **P < 0.01, ***P < 0.001.

2. Changes in IL-6 and IL-8 in human bronchial epithelial cells

Next, to examine the effect of extracts from antibiotic-treated cockroaches on airway inflammation, cytokine secretion was measured by treating airway epithelial cells with the cockroach extract. When human bronchial epithelial cells (BEAS-2B) were treated with an extract of ampicillin-treated cockroaches, the secretion of the pro-inflammatory cytokines IL-6 and IL-8 was significantly lower compared with that in the control extract group (Figure II–2A, B).

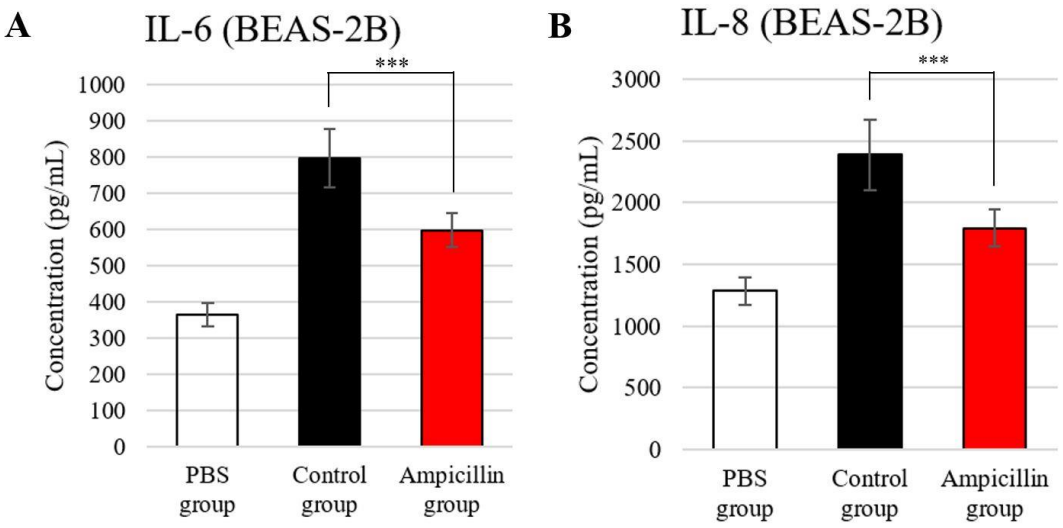
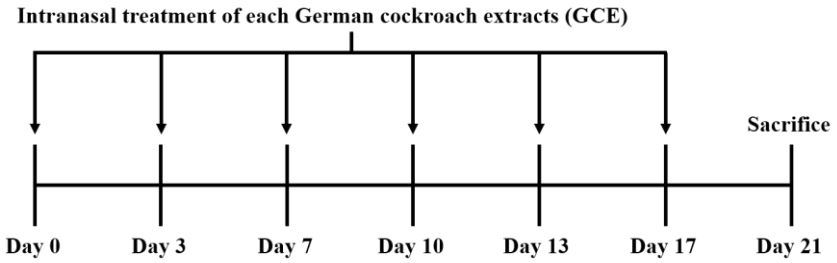


Figure II–2. Effect of the antibiotic-treated extract exposure on cytokine expression in bronchial epithelium *in vitro*. Concentrations of (A) IL-6 and (B) IL-8 secreted from human bronchial epithelial cells (BEAS-2B) exposed to the extract of German cockroaches treated with ampicillin (pg/mL). ***P < 0.001.

3. Reduction of airway inflammation in a mouse asthma model

Based on the effect of BEAS-2B cell exposure to the antibiotic-treated extract, a mouse asthma model was established using GCEs (Figure II–3A). In the BAL, eosinophil levels were found to be lower in the ampicillin-treated cockroach extract group compared with those in the normal cockroach extract group (Figure II–3B). Lung histopathology showed less immune cell infiltration in the ampicillin group than in the positive group (Figure II–3C–E). The ampicillin group also had lower immune cell infiltration and inflammation scores than those of the positive group (Figure II–3D, E). Further, the concentrations of IL-4, IL-5, and IL-13 in the BAL fluid and lung tissue of the ampicillin group were lower than those in the positive group (Figure II–3F, G). However, there was no statistically significant difference in IFN-γ levels between the two groups (Figure II–3F, G). Serum immunoglobulin (Ig) levels were also measured. *B. germanica*-specific IgE levels increased in both asthma groups but were not significantly different between the two (Figure II–3H). Contrarily, serum IgG1 and IgG2a levels were lower in the ampicillin group than in the positive group (Figure II–3I, J).

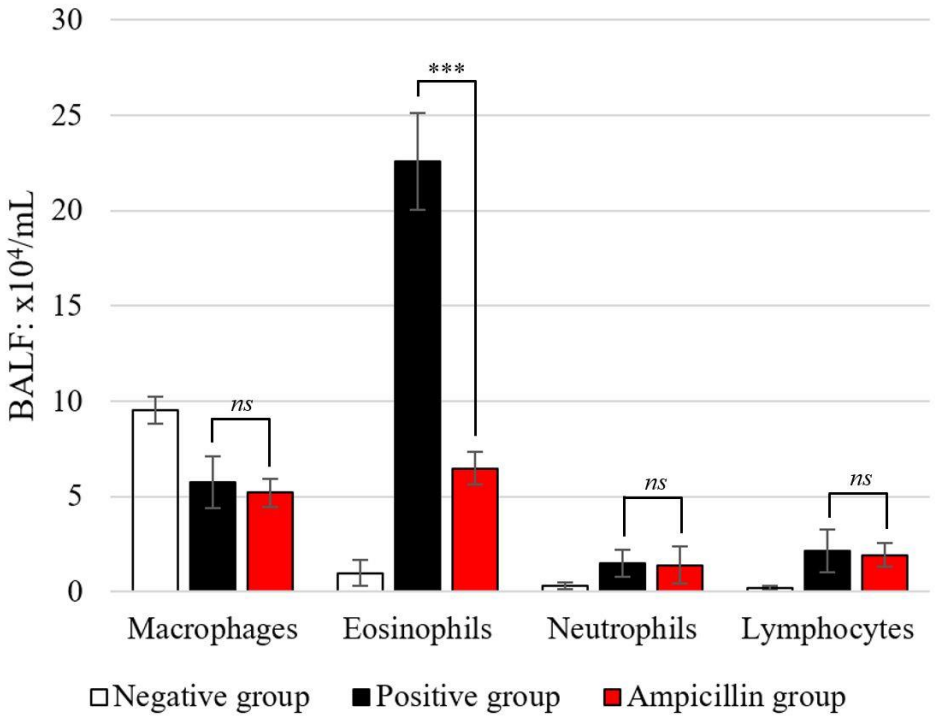
A



Groups

- **PBS (Negative group)**
- **Extract of normal *B. germanica* (Positive group)**
- **Extract of Ampicillin-treated *B. germanica* (Ampicillin group)**

B



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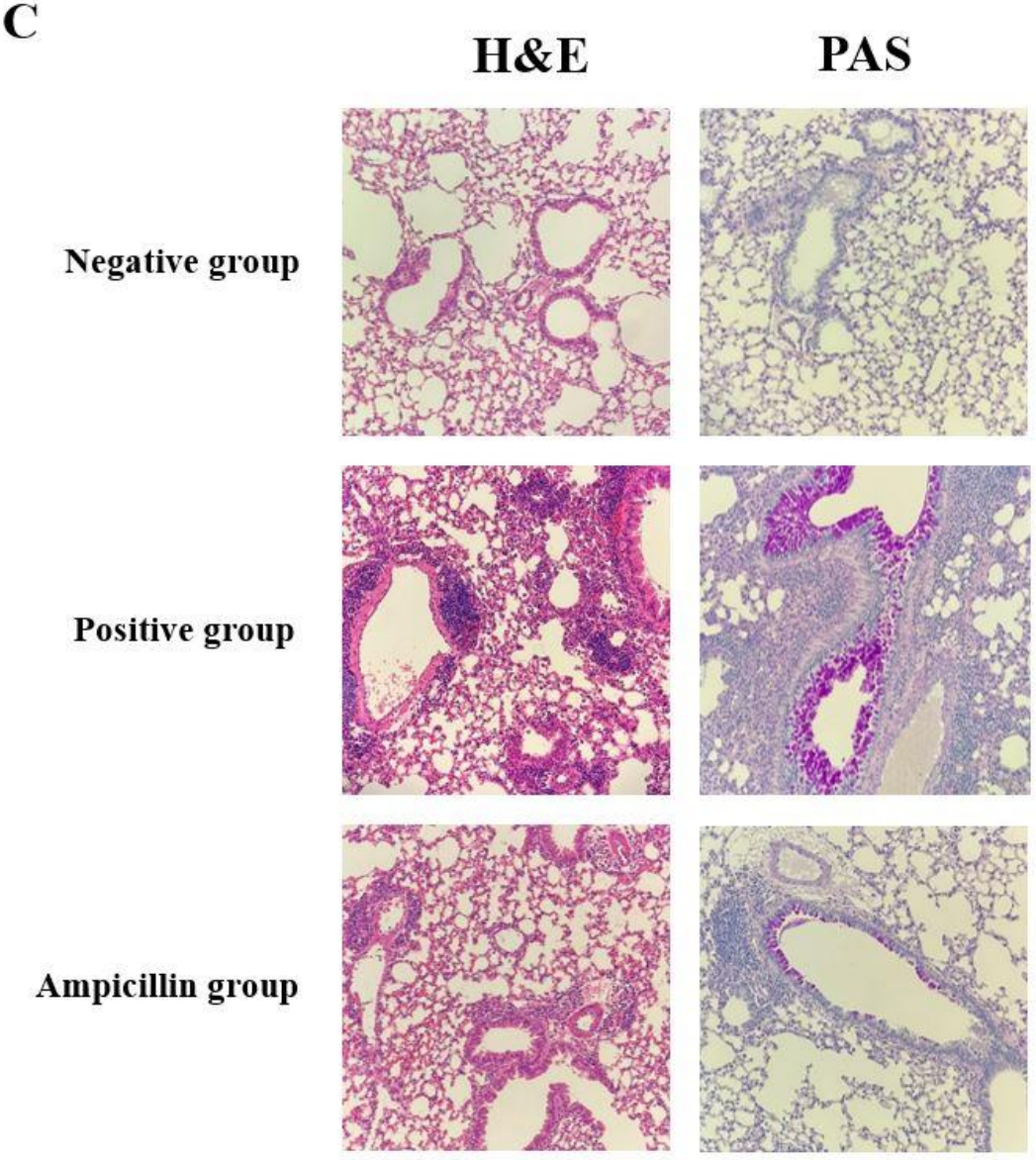

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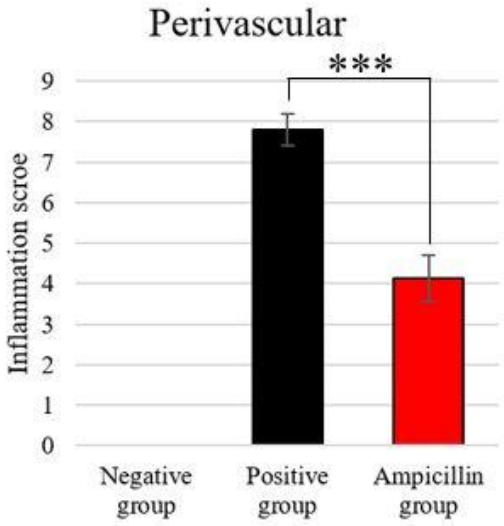

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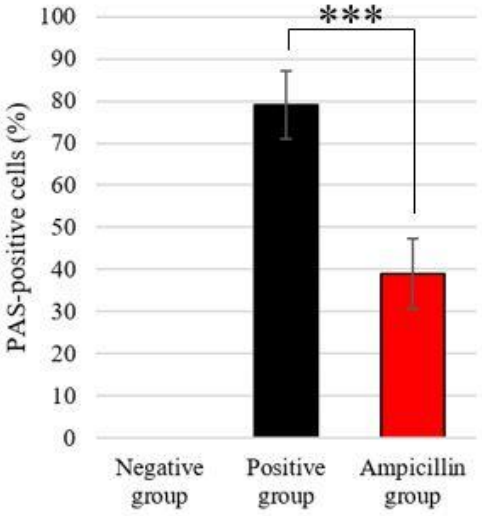
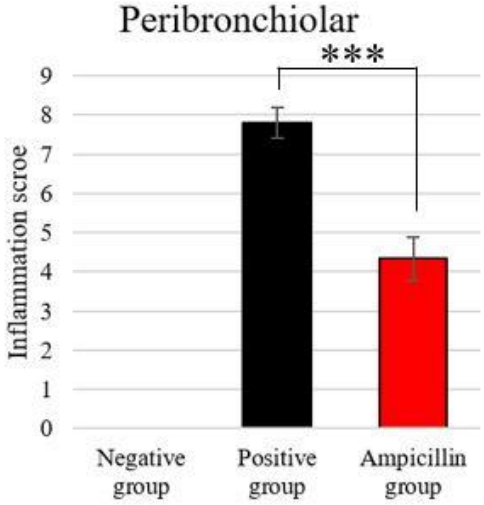
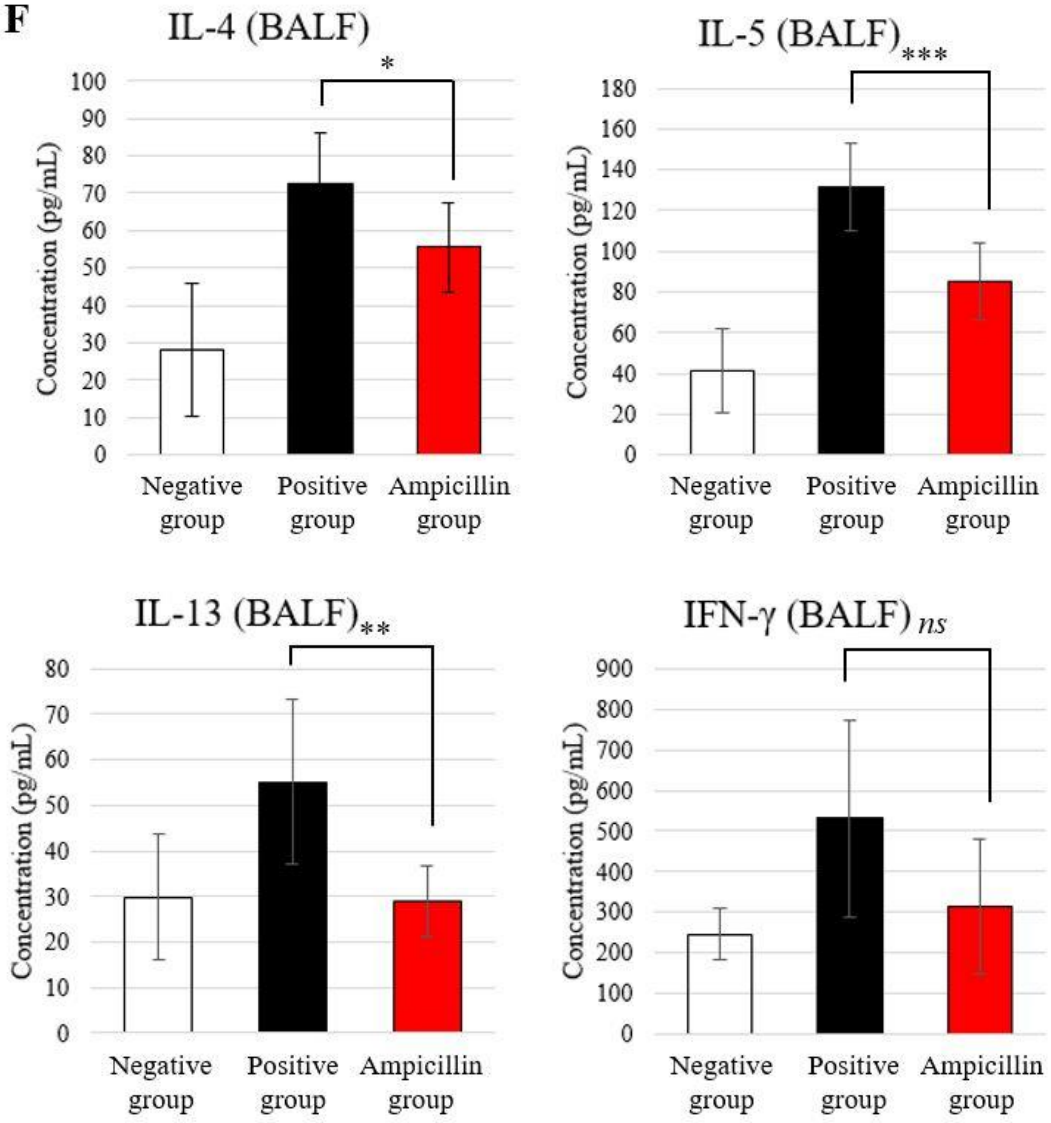
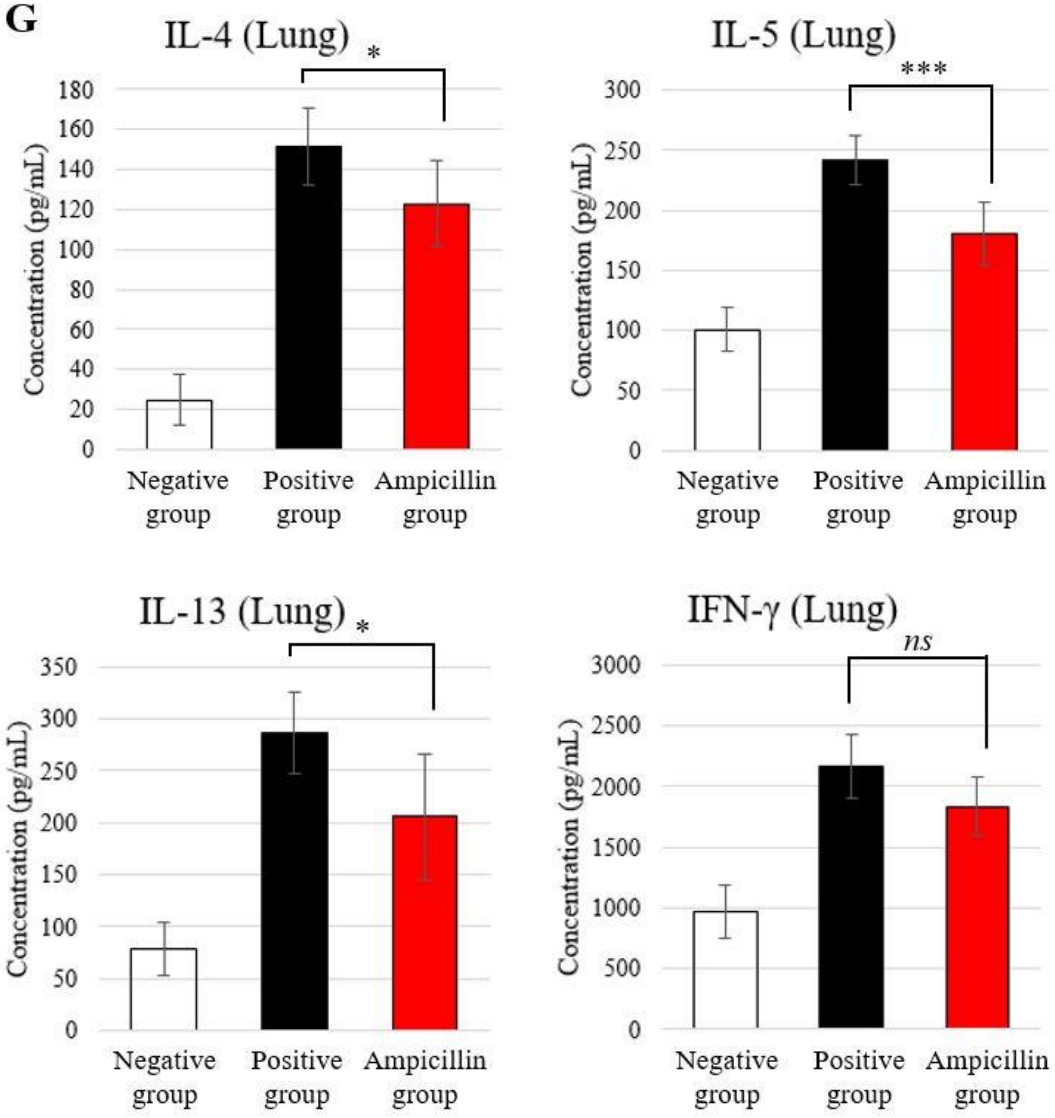




F



G



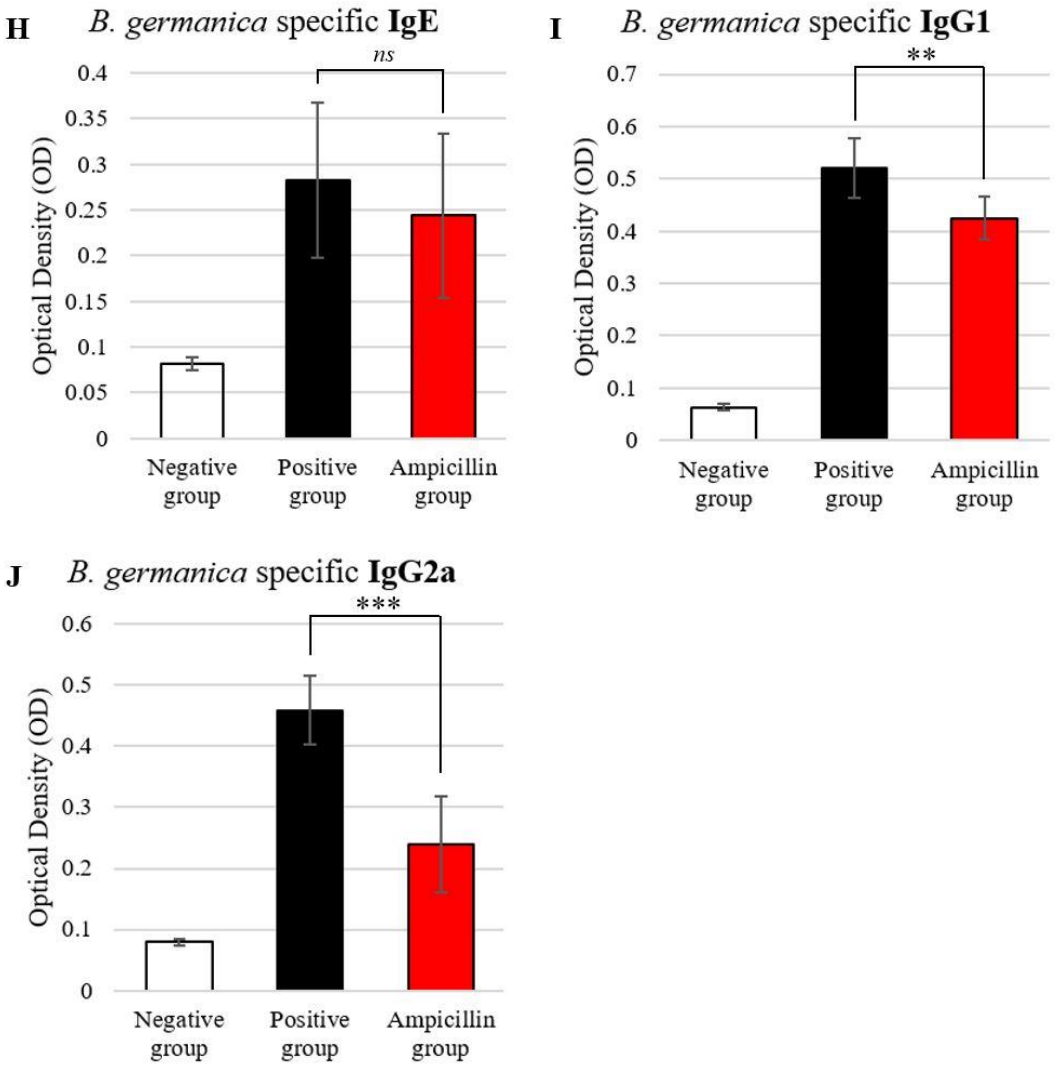


Figure II–3. Effect of the antibiotic-treated extracts on the induction of airway inflammation in a mouse model of asthma. (A) Intranasal treatment with each German cockroach extract (GCE). The mice were sacrificed 4 days after the last challenge. (B) The numbers of macrophages, eosinophils, neutrophils, and lymphocytes in the bronchoalveolar lavage (BAL) fluid of asthma model mice. (C–E) Lung histologic findings in the mouse model of allergic airway inflammation. (C) Histologic findings with

hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining of lung tissues. (D, E) Immune cell infiltration and inflammation scores, including mucus production scores. Data are reported as mean ± standard error (SE) (n = 8/group). (F, G) Supernatants of BAL fluid were collected after centrifugation, and the production of IL-4, IL-5, IL-13, and IFN-γ was measured. The concentrations of IL-4, IL-5, IL-13, and IFN-γ in lung tissues were analyzed using the respective ELISA kits. Comparison of serum *B. germanica*-specific (H) IgE, (I) IgG1, and (J) IgG2a levels among *B. germanica*-induced asthmatic mice groups. The optical density (OD) measured during ELISA is presented. Data are reported as mean ± SE (n = 8/group). One-way analysis of variance (ANOVA) was conducted, and Bonferroni-corrected P-values are presented. Negative group: PBS treated, Positive group: exposure to normal German cockroach extract, Ampicillin group: exposure to ampicillin-treated German cockroach extract. *P < 0.05, **P < 0.01, ***P < 0.001.

IV. DISCUSSION

My previous study confirmed a reduction in bacterial abundance and diversity in German cockroaches treated with ampicillin.[39] Similarly, I found that cockroaches reared under ampicillin treatment showed reduced amounts of total bacteria in their bodies. Further, they showed decreased amounts of LPS, an immunomodulatory molecule derived from bacteria. Although not measured in this study, I expect that several microbe-associated molecules other than LPS decreased in the extract of cockroaches reared under ampicillin treatment. Similar to a previous study, the levels of the major allergens Bla g 1, Bla g 2, and Bla g 5 significantly decreased at both the protein and mRNA levels. Exposure to Bla g 1 is a strong risk factor for sensitization.[48] Bla g 2 is the most important allergen in German cockroaches and generally has the highest sensitization rate at 54–71%.[16] In a previous study, among 32 sera from a cockroach-sensitized cohort, 12 (37.5%) showed positive IgE reactivity against recombinant Bla g 5.[38] As mentioned previously, I confirmed the reduction in total bacteria caused by antibiotics and changes in gene expression, and expected that this would result in the reduction of allergen levels.[39]

IL-6 and IL-8 levels were decreased in airway epithelial cells (BEAS-2B) when treated with the ampicillin-treated cockroach extract. IL-6 is a major proinflammatory cytokine responsible for immune response activation.[49] IL-8 is also a proinflammatory cytokine with proangiogenic, proliferative, and promotility activities.[50]

The mechanism underlying cockroach allergen-induced sensitization is that cockroach-derived proteases may disturb airway epithelial cell integrity and increase cockroach allergen penetration. Moreover, proteases have been demonstrated to modulate airway epithelial cell responses. Cockroach-derived proteases, as well as others, can induce airway epithelial cell responses through G protein-coupled, proteinase-activated receptors

(PARs).[51] Stimulation of PAR2 has been demonstrated to induce the release of granulocyte-macrophage colony-stimulating factor (GM-CSF), a neutrophil and eosinophil chemoattractant and a survival factor, from airway epithelial cells,[52] as well as the upregulation of PAR2 in the respiratory epithelium of patients with asthma.[53] In my previous study, I reported that house dust mite (HDM) group 1 allergens and LPS were recognized by PAR2 and toll-like receptor 4 (TLR4) and stimulated the secretion of IL-6 and IL-8 in airway epithelial cells. In addition, LPS inhibitor treatment reduced the amount of IL-6 and IL-8 released from BEAS-2B cells treated with HDM extract. This indicates that bacterial components, such as LPS, act synergistically on airway epithelial cells to induce an inflammatory response.[54] Similar to these findings, decreased LPS levels in GCE owing to antibiotic usage are expected to be associated with a decrease in allergic airway inflammation in bronchial epithelial cells.

My asthma model was confirmed to be sensitized to intranasal injections with cockroach extract. In the BALF, the eosinophil count was confirmed to have decreased in the ampicillin group. Eosinophilic airway inflammation, a key feature of allergic airway diseases, such as allergic asthma, was significantly reduced in the ampicillin group. Similarly, in lung sections, perivascular and peribronchiolar immune cell infiltration scores were significantly reduced. Further, PAS staining showed that the number of mucus-producing cells was greatly reduced. In the serum, IgE levels showed a decreasing trend compared with that in the positive group, although this was not statistically significant. Further, IgG1 and IgG2a levels were significantly lower in the ampicillin group than those in the positive group. IgG1 and IgG2a are immunoglobulins associated with the Th2 and Th1 responses, respectively. Levels of the cytokines IL-4, IL-5, IL-13, and IFN-γ were measured in both BALF and lung homogenates. Although the IFN-γ level was not

significantly different between the ampicillin and positive groups, IL-4, IL-5, and IL-13 levels decreased significantly in the ampicillin group compared with those in the positive group. I confirmed a decrease in the Th2 response similar to the immunoglobulin levels, and thus, confirmed that the ampicillin group showed reduced airway inflammation.

Reduction in airway inflammation was confirmed in BEAS-2B cells and in a mouse asthma model induced by ampicillin-treated cockroaches. As the total bacteria in the cockroaches was greatly reduced by the ampicillin treatment, it is expected that their extract contained less LPS and other bacteria-derived substances. During protein extraction, bacteria are removed using a filter, but bacteria-derived substances cannot be filtered out. Further, extracts from ampicillin-treated German cockroaches showed lower levels of the major allergens Bla g 1, 2, and 5. Thus, I expected that the reduced airway inflammation was caused by a decrease in major allergen levels and the absence of bacteria-derived substances owing to a decrease in total bacteria.

The GCE has not been standardized for immunotherapy or diagnosis. A previous study reported that the potency of the allergic response in the patient varies according to the allergen level present in German cockroaches.[18] Further, it is important to prepare several extracts because the T cell potency of each extract differs depending on the most dominant allergen for the patient.[17] Although the levels of major allergens were lower in the protein extract from cockroaches treated with ampicillin, the levels of all proteins did not decrease; however, the expression pattern changed. Therefore, the use of extracts from ampicillin-treated German cockroaches is considered a suitable approach for preparing various extracts for immunotherapy and diagnosis.

The amount of LPS or major allergens in the extract differs depending on the extraction method or rearing environment of the cockroaches.[17] From an environmental

perspective, large amounts of antibiotics are discharged into rivers and lakes.[55] As cockroaches are omnivores and inhabit sewers, they are expected to consume water containing antibiotics. In this experiment, I only used ampicillin-treated German cockroaches to assess allergen production and the potency of inducing airway inflammation; however, wild cockroaches consume various antibiotics. Therefore, they are expected to show varying allergen production and cause varying airway inflammation in humans. Thus, further research is needed to determine the changes in the ability of cockroaches to cause allergies in different countries or regions, owing to exposure to APIs.

In conclusion, major allergens were reduced by ampicillin, but most of the LPS was eliminated. This product is different from crude extract, which contains LPS that is difficult to remove. German cockroaches treated with ampicillin caused reduced airway inflammation in human epithelial cells and mice. Therefore, I developed an approach to create a safer cockroach extract (CRE) without LPS. It is expected that this extract can serve as a basis for the development of various extracts in future clinical trials.

V. CONCLUSION

Taken together, these results suggest that the role of antibiotics on German cockroaches not only affects bacteria, but also allergens and their ability to cause airway inflammation.

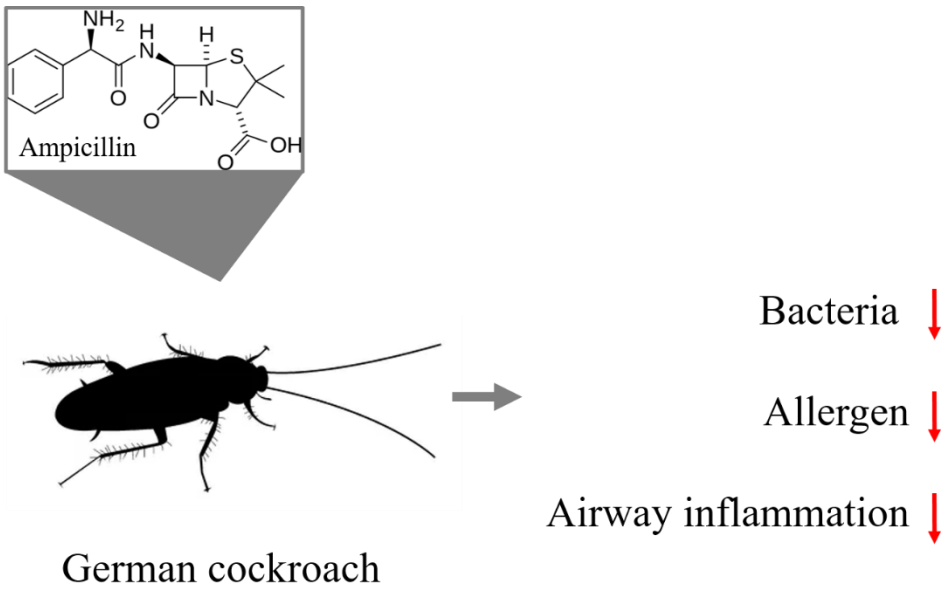


Figure II-4. Effect of ampicillin on the German cockroach.

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ABSTRACT(IN KOREAN)

항생제 처리로 인한 바퀴벌레 추출물의 알레르기유발성 변화

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이 석 원

바퀴벌레는 마이크로바이옴에 영향을 미치는 다양한 서식지에 서식한다. 마이크로바이옴은 식단과 환경적 요인의 영향을 받을 수 있지만 바퀴벌레 종마다 다를 수 있다. 따라서, 16S rDNA-targeted high-throughput sequencing을 통해서 동일 한 조건에서 여러 세대에 걸쳐 실험실에서 사육된 네 가지 바퀴벌레 종 (*Periplaneta americana, P. japonica, P. fuliginosa* 그리고 *Blattella germanica*)의 마이크로바이옴의 전체적인 박테리아 구성을 평가했다. Shannon 및 Phylogenetic 지표와 관련하여 *B. germanica*와 *P. americana, P. japonica*, *P. fuliginosa*와의 큰 차이가 나타났다. 베타 다양성 분석은 추가로 *P. japonica*와 *P. fuliginosa*가 유사한 마이크로바이옴 구성을 가짐을 보여주었고, *P. americana*, *B. germanica*와 큰 차이를 나타냈다. 따라서 마이크로바이옴 구성은 여러 조건에 따라 다를 수 있지만 동일한 조건에서 사육되는 경우에도 서로 다른 바퀴벌레 종 사이에서 고유한 마이크로바이옴 구성을 식별하는 것이 가능하다. 독일바퀴벌레가 식단에 따라 마이크로바이옴의 차이가 크고, 알레르겐에 관한 많은 연구가 진행되어 있기 때문에 독일바퀴벌레를 다음 실험에 사용하기로 결정하

였다.

바퀴벌레의 분변이나 가루 부스러기에 존재하는 알레르겐은 인간에게 알레르기 감작을 일으킬 수 있다. 면역 요법을 위한 분변 및 가루 부스러기 추출물의 사용은 이전에 조사되었지만 아직 완전히 표준화되지 않았다. 또한 바퀴벌레는 강과 하수구로 배출되는 항생제에 노출된다. 항생제는 독일바퀴(*Blattella germanica*)의 주요 알레르겐 및 전체적인 박테리아에 영향을 미치는 것으로 알려져 있다. 우리는 독일바퀴에 암피실린을 처리하여 총 박테리아의 양이 감소된 추출물을 생산했다. 마이크로바이옴 분석 결과 알파 다양성이 대조군보다 암피실린 처리군에서 더 낮은 것으로 나타났다. 베타 다양성 분석은 암피실린 처리가 바퀴벌레의 마이크로바이옴에서 박테리아의 구성을 변경했음을 나타낸다. qPCR은 암피실린이 처리된 바퀴벌레에서 거의 모든 박테리아가 제거되었음을 보여주었다. RNA-seq 분석은 암피실린 처리된 바퀴벌레에서 1,236개의 DEG를 나타냈다. 박테리아 구성과 달리 DEG는 두 그룹 간에 다양했다. 주요 알레르겐 중 Bla g 2의 발현은 암피실린 처리된 바퀴벌레에서 유의미하게 감소하였다. 본 연구에서 바퀴벌레에서 관찰된 알레르겐의 감소는 항생제 투여로 인한 총 박테리아의 감소와 관련이 있을 수 있다. 면역 요법에 사용하기 위한 굉장히 적은 수의 박테리아가 포함된 바퀴벌레 단백질 추출물의 추출이 가능했다.

챕터 II에서는, 앞의 결과를 기반으로, 우리는 알레르기성 기도 염증을 유도하기 위해 항생제로 처리된 독일바퀴벌레의 바뀐 능력을 조사했다. 우리는 독일바퀴벌레에서 lipopolysaccharide와 Bla g 1, 2, 5 발현에 대한 항생제 투여

의 효과를 조사했다. 그런 다음 독일 바퀴벌레 추출물이 인간 기관지 상피 세포와 알레르기성 기도 염증의 마우스 모델에서의 알레르기성 염증을 유도하는 능력을 측정했다. 암피실린을 처리한 바퀴벌레는 대조군에 비해 세균성 16S rRNA와 lipopolysaccharide 수치의 감소를 확인하였다. 암피실린으로 처리된 바퀴벌레에서 Bla g 1, 2, 5 발현은 단백질과 RNA 단계 모두에서 감소했다. 암피실린으로 처리된 독일바퀴벌레 추출물에 노출된 BEAS-2B 세포에서 IL-6 및 IL-8 발현은 대조군보다 낮았다. 기관지 폐포 세척액의 총 세포 수와 호산구 수는 일반적인 바퀴벌레 추출물에 노출된 대조군에 비해 암피실린으로 처리 독일바퀴벌레 추출물에 노출된 마우스에서 더 낮았다. 마우스 폐 조직 병리학 결과는 암피실린 그룹에서 감소된 면역 세포 침윤 및 점액 생성을 보여주었다. 또한, IL-4, IL-5, IL-13은 폐 조직과 기관지 폐포 세척액에서 감소하였고, IgG1과 IgG2a는 암피실린을 처리한 그룹의 혈청에서 감소하였다. 전반적으로 암피실린 처리는 독일 바퀴벌레의 공생 박테리아 수와 주요 알레르겐 발현 양을 감소시켜 추출물에 노출된 마우스의 기도 염증을 감소시켰다. 이러한 결과는 면역 요법 또는 진단에 사용하기 위한 단백질 추출물을 위한 준비를 용이하게 할 뿐만 아니라 강 호수 등에서의 항생제 방출과 관련된 환경 문제를 식별하는 데 도움이 된다.

핵심되는 말 : 바퀴벌레, 마이크로바이옴, 환경, 마우스, 기도 염증, 항생제, 알레르겐

PUBLICATION LIST

– **Lee S**, Kim JY, Yi MH, Lee IY, Lee WJ, Moon HS et al. Comparative Microbiome Analysis of Three Species of Laboratory-Reared *Periplaneta* Cockroaches. Korean J Parasitol. 2020;58:537-542.

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