Cutaneous Microflora from Geographically Isolated Groups of Bradysia agrestis, an Insect Vector of Diverse Plant Pathogens

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Abstract Larvae of Bradysia agrestis, an insect vector that transports plant pathogens, were sampled from geographically isolated regions in Korea to identify their cutaneous fungal and bacterial flora. Sampled areas were chosen within the distribution range of B. agrestis; each site was more than 91 km apart to ensure geographical segregation. We isolated 76 microbial (fungi and bacteria) strains (site 1, 29; site 2, 29; site 3, 18 strains) that were identified on the basis of morphological differences. Species identification was molecularly confirmed by determination of universal fungal internal transcribed spacer and bacterial 16S rRNA gene sequences in comparison to sequences in the EzTaxon database and the NCBI GenBank database, and their phylogenetic relationships were determined. The fungal isolates belonged to 2 phyla, 5 classes, and 7 genera; bacterial species belonged to 23 genera and 32 species. Microbial diversity differed significantly among the geographical groups with respect to Margalef’s richness (3.9, 3.6, and 4.5), Menhinick’s index (2.65, 2.46, and 3.30), Simpson’s index (0.06, 0.12, and 0.01), and Shannon’s index (2.50, 2.17, and 2.58). Although the microbial genera distribution or diversity values clearly varied among geographical groups, common genera were identified in all groups, including the fungal genus Cladosporium, and the bacterial genera Bacillus and Rhodococcus. According to classic principles of co-evolutionary relationship, these genera might have a closer association with their host insect vector B. agrestis than other genera identified. Some cutaneous bacterial genera (e.g., Pseudomonas) displaying weak interdependency with insect vectors may be hazardous to agricultural environments via mechanical transmission via B. agrestis. This study provides comprehensive information regarding the cutaneous microflora of B. agrestis, which can help in the control of such pests for crop management.

Keywords Biodiversity, Bradysia agrestis, Insect microflora, Insect vector

Global warming has inevitably led to problems in agricultural environments, including an increase in crop diseases transmitted by insect vectors, resulting in severe economic problems akin to the challenges of human vector-borne diseases [1-7]. Environmentally friendly agricultural practices have increased over time owing to rising consumer awareness of national and global food security requirements. However, given the concentration of agricultural environment in East Asia, it is difficult to find solutions for pest control that do not involve agricultural pesticides. Thus, implementation of environmentally friendly agricultural practices has become extremely challenging because of the emerging insect vector crisis.

The insect vector Bradysia agrestis represents an important emerging agricultural concern in East Asia given that it is responsible for the spread of significant infective fungal...
genera such as Fusarium, Phoma, Pythium, and Verticillium among commercial crops [2]. B. agrestis causes the most substantial damage during the larval stage. Larvae of the insect vector flourish in the rhizosphere, and their caterpillars feed on organic matter, decayed plant debris, and the colonies of fungi such as oomycetes, ascomycetes, and basidiomycetes [8, 9]. Larvae of this species can uniquely transmit several fungal diseases such as Fusarium blight via the release of fungal colonies from the inner tissues of the plant [8, 10]. In other words, they are capable of inducing both mechanical damage and secondary infection [10]. However, the entire cutaneous microflora of this vector, which could be mechanically transferred by disrupting the plant surface barrier, has not been well studied.

To date, most studies have only identified the transfer of classically well-known fungal strains. This strategy represents a type of post-facto management, which occurs following serious outbreaks. However, revealing the entire microbiota that could be transferred by the host vector would be a pro-active management strategy to minimize agricultural emergencies. Furthermore, the entire microflora is closely associated with and greatly affects the physiology, life cycle, reproduction, and immunity of host vectors. Therefore, this information is essential for combatting emerging vectors. In particular, comparing the microbial flora of geographically segregated vector populations may provide important information for controlling insect vectors and preventing possible fungal disease outbreaks [3, 4, 6, 11-14].

Our research team has performed comprehensive investigations of the gut fungal flora that induces biological transmission in B. agrestis, with special focus of Korean populations [10, 15]. In the present study, we aimed to identify the major cutaneous fungal and bacterial flora associated with mechanical transmission or secondary infection via mechanical damage, as part of the national public project to address the severe B. agrestis blight in Korea. The community structures, geological distribution, and microbial diversity of cutaneous fungal and bacterial flora of B. agrestis were investigated. Furthermore, the interdependency between the associated species and their host vectors, as it relates to the potential impact on agriculture, was assessed.

**MATERIALS AND METHODS**

**Collection and treatment.** Three regional groups (sites 1 to 3) (Table 1, Fig. 1) were sampled from September 16 to November 5 of 2016, which corresponds to the outbreak season for B. agrestis [10]. Each geographical group was geographically segregated (greater than 91 km apart in distance), and the possibility for ecological interaction was minimal [16]. A standard dipping technique was applied for larval sample collection using a 350-mL dipper [7]. The dippers were sterilized using ethanol (EtOH), and ultraviolet (UV) light was used to control microbial contamination [7]. Sixty larval samples were collected from each region, and B. agrestis specimens were identified by morpho-taxonomic methods [17]. Larvae samples (180 from each site) were transferred to the laboratory and immobilized by incubating

| Geographical group | Vegetation | Isolate counting |
|--------------------|------------|------------------|
| Site 1 Sendo-myeon, Buyeo-gun, Chungcheongnam-do, Republic of Korea | Tomato and lily | Fungi (5)/bacteria (24) |
| Site 2 Ssangchaek-myeon, Hapcheon-gun, Gyeongsangnam-do, Republic of Korea | Strawberry | Fungi (5)/bacteria (26) |
| Site 3 Iseo-myeon, Wanju-gun, Jeollabuk-do, Republic of Korea | Tomato and lily | Fungi (5)/bacteria (13) |

**Fig. 1.** Location of sampled sites in each geographical vector group in Korea. Site 1, Sendo-myeon, Buyeo-gun, and Chungcheongnam-do, Republic of Korea; Site 2, Ssangchaek-myeon, Hapcheon-gun, Gyeongsangnam-do; Site 3, Iseo-myeon, Wanju-gun, Jeollabuk-do.
at −20°C for 10 min. For isolation of cutaneous microbes, the samples were first sprayed with a cold sterile insect saline solution (9.32 g NaCl, 0.77 g KCl, 0.5 g CaCl₂, 0.18 g NaHCO₃, 0.01 g NaH₂PO₄, pH 7.4 in 1 L of distilled water) to eliminate foreign substances. They were then affixed to paraffin-filled dishes covered with insect saline solution. The skin of larvae was isolated from the body using a dissecting microscope. Isolated skin samples were homogenized with a mortar in a 2-mL Eppendorf tube with 150 µL insect saline.

**Fungal isolation and identification.**

For fungal isolation, serially diluted insect saline was spread onto potato dextrose broth (PDB; BD Difco, Franklin Lanes, NJ, USA) agar medium containing 80 mg/L streptomycin (Sigma-Aldrich, St. Louis, MO, USA). All media were incubated at 25°C for 14 days in the dark [18]. Serial subcultures were performed to obtain pure isolates. For partial molecular identification of all fungal isolates from the cutaneous microflora of *B. agrestis*, the internal transcribed spacer (ITS) rDNA sequences were analyzed. The isolates were inoculated onto the PDB (Difco, Detroit, MI, USA) agar plates and incubated at 25°C for 7 days on a rotary shaker at 120 rpm. Filtered mycobionts were lyophilized for 2 days, and then genomic DNA was extracted from the lyophilized mycobionts using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA). Primers targeting the ITS regions ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) were used for amplification [19]. The PCR cycling conditions were as follows: pre-denaturation (94°C, 4 min); denaturation (94°C, 1 min), annealing (55–58°C, 1 min), and extension (72°C, 2 min) for a total of 35 cycles; and final extension (72°C, 2 min) [20]. PCR products were confirmed by electrophoresis (1.5% agarose gel, stained with ethidium bromide [EtBr]), and the resulting bands were visualized using a UV transilluminator. The AccuPrep PCR & Gel Extraction Kit (Bioneer, Daejeon, Korea) was used to purify PCR products, and an ABI 3730XL DNA analyzer (Applied Biosystems, Carlsbad, CA, USA) was used for analysis [18]. To determine the taxonomic position of the isolates, the ITS sequences were compared with similar sequences from other fungal species in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) using the BLASTn tool [20]. The NCBI GenBank accession numbers for the fungal sequences are KY929272–KY929284 (Supplementary Table 1).

**Table 2.** Diversity index formulae used in this study

| Diversity indices          | Formula                                   | Description                                      |
|----------------------------|-------------------------------------------|--------------------------------------------------|
| Shannon’s diversity index (H) | $H' = \sum_{i=1}^{K} p_i \cdot \ln p_i$    | ni, the number of clones in the ith OUT          |
| Simpson’s index of diversity (1-D) | $D = \frac{1}{N} \sum_{i=1}^{K} n_i(n_i - 1)/N(N - 1)$ | N, total number of the individuals in each sample |
| Menhinick’s index (Dmn)     | $D_{mn} = S_i/n_i^{1/2}$                  | pi, ni over N                                    |
| Margalef’s index (Dmg)      | $D_{mg} = (S - 1)/\ln(N)$                 | S, the number of different genera in a sample    |

**Bacterial isolation and identification.**

For bacterial isolation and identification, the diluted supernatant (10⁻⁴–10⁻⁴) of the homogenized skin was spread onto 1/10 diluted tryptic soy broth (TSB; Difco) and nutrient broth (NB; Difco) agar plates. Each plate was incubated at 25°C for 72 hr. Thereafter, isolates from the 1/10 TSB and NB agar were streaked onto non-diluted TSB agar plates to exclude morphologically identical isolates. Isolates from the 1/10 TSB or NB agar medium were routinely cultured in non-diluted TSB. For molecular identification of the isolates, the cultures were grown in 50 mL TSB liquid media, with shaking at 150 rpm. Bacterial cells were collected by centrifugation at 10,000 xg for 10 min. To obtain genomic DNA, a boiling method with Chelex Resin (Bio-Rad, Hercules, CA, USA) was applied, and 16S rRNA gene sequences were amplified using the primer pair 27-F (forward primer 5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492-R (reverse primer 5′-GTT TAC CTT GTT TAC GAC ACT T-3′). For sequential PCR, an initial denaturation step (95°C, 15 min) was performed, followed by denaturation (95°C, 20 sec), annealing (50–58°C, 40 sec), and elongation (72°C, 90 sec) steps (30 cycles), and a final elongation step (72°C, 5 min). Amplified PCR products were precipitated in EtOH (65%) and sodium acetate (3 M, pH 4.6) for 15 min, and centrifuged at 2,000 xg for 45 min. The supernatants were discarded, and the samples were centrifuged in 70% EtOH (10,000 xg, 10 min). This process was repeated 7 or 8 times. The PCR products were purified using the Gel and PCR Extraction System-UB, and sequence analysis was performed using an ABI-3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA) [21, 22]. The acquired final partial 16S rDNA gene sequences were compared with sequences previously deposited in the Ez-Taxon database (http://eztaxon-e.ezbiocloud.net). BLAST was used to identify sequence similarities between strains [21-23]. The NCBI GenBank accession numbers for the bacterial sequences are KU711995–KU712065 (Supplementary Table 1).

**Phylogenetic analysis.**

To determine the phylogenetic relationship of the isolates, partial fungal ITS sequences or bacterial 16S rRNA genes were aligned using the Clustal X program [24], and arrayed by the BioEdit sequence alignment editor [25]. Phylogenetic trees were constructed based on the Kimura 2-parameter model via the MEGA 6.0 maximum likelihood algorithm [26, 27]. The stability of relationships
was evaluated by bootstrap analysis, with resampling 1,000 times [27]. To construct the fungal phylogenetic trees, *Kluyveromyces lactis* NRRL Y-8279 NR_131273 was used as the outgroup [27]. To construct the bacterial phylogenetic trees, *Staphylothermus hellenicus* strain DSM 12710^T^ (NR074532) was used as the outgroup.

**Biodiversity assessment.** Microbial diversity of insect vector skin from each geographical group was determined using various metrics (Table 2). Margalef’s richness (Dmg) index was used to examine the fungal and bacterial biota from each geographical group [28]. Diversity at the genus level was revealed using Margalef’s richness index [29], Menhinick’s index [30], Shannon diversity (H’) [31], and Simpson’s index (1-D) [31].

### RESULTS AND DISCUSSION

**Fungal and bacterial identification and distribution.** We isolated 13 fungal and 63 bacterial morphologically distinct strains from pure cultures obtained from the cutaneous surface of the host insect *B. agrestis* (Table 1, Supplementary Table 1). The fungal isolates belonged to 2 phyla, 5 classes, and 7 genera (Table 3, Fig. 2A and 2B);

| Sampling sites | Phylum          | Class                    | Genera                  | Genera ratio (%) |
|----------------|-----------------|--------------------------|-------------------------|------------------|
| Site 1         | Fungal species  | Ascomycota (5)           | Eurotiomycetes (3)      | Aspergillus (1)  |
|                |                 |                          |                         | 3.4              |
|                |                 |                          | Penicillium (2)         |                  |
|                |                 |                          |                         | 6.9              |
|                |                 | Dothideomycetes (2)      | Cladosporium (2)        |                  |
|                |                 |                          |                         | 6.9              |
|                | Bacterial species| Proteobacteria (9)       | Gamma proteobacteria (5) | Stenotrophomonas (1) |
|                |                 |                          |                         | 3.4              |
|                |                 |                          | Kosakonia (1)           |                  |
|                |                 |                          |                         | 3.4              |
|                |                 |                          | Enterobacter (3)        |                  |
|                |                 |                          |                         | 10.3             |
|                |                 | Beta Proteobacteria (2)  | Achromobacter (1)       |                  |
|                |                 |                          |                         | 3.4              |
|                |                 | Alpha Proteobacteria (2) | Ochrobactrum (2)        |                  |
|                |                 |                          |                         | 6.9              |
|                |                 | Actinobacteria (12)      | Rhodococcus (4)         |                  |
|                |                 |                          |                         | 13.8             |
|                |                 |                          | Microbacterium (3)      |                  |
|                |                 |                          |                         | 10.3             |
|                |                 |                          | Gordonia (1)            |                  |
|                |                 |                          |                         | 3.4              |
|                |                 |                          | Arthrobacter (4)        |                  |
|                |                 |                          |                         | 13.8             |
|                | Bacterial species| Proteobacteria (8)       | Gamma proteobacteria (6) | Pseudomonas (5)  |
|                |                 |                          |                         | 17.2             |
|                |                 |                          | Leclercia (1)           |                  |
|                |                 |                          |                         | 3.4              |
|                |                 | Alpha proteobacteria (1) | Rhizobium (1)           |                  |
|                |                 |                          |                         | 3.4              |
|                |                 | Beta Proteobacteria (1)  | Variovorax (1)          |                  |
|                |                 |                          |                         | 3.4              |
|                |                 | Actinobacteria (5)       | Microbacterium (2)      |                  |
|                |                 |                          |                         | 6.9              |
|                |                 |                          | Cellulosimicrobium (1)  |                  |
|                |                 |                          |                         | 3.4              |
|                |                 |                          | Arthrobacter (1)        |                  |
|                |                 |                          |                         | 3.4              |
|                |                 |                          | Rhodococcus (1)         |                  |
|                |                 |                          |                         | 3.4              |
|                | Fungal species  | Ascomycota (3)           | Leotiomycetes (1)       | Acremonium (1)   |
|                |                 |                          |                         | 3.4              |
|                |                 | Eurotiomycetes (1)       | Aspergillus (1)         |                  |
|                |                 | Sordariomycetes (2)      | Penicillium (2)         |                  |
|                |                 | Dothideomycetes (1)      | Cladosporium (2)        |                  |
|                |                 |                          |                         | 6.9              |
|                | Bacterial species| Proteobacteria (6)       | Doctorum (1)            |                  |
|                |                 |                          |                         | 3.4              |
|                |                 | Gamma proteobacteria (4) | Stenotrophomonas (1)    |                  |
|                |                 |                          |                         | 3.4              |
|                |                 | Bethe Proteobacteria (1) | Variovorax (1)          |                  |
|                |                 |                          |                         | 3.4              |
|                |                 | Actinobacteria (3)       | Microbacterium (2)      |                  |
|                |                 |                          |                         | 6.9              |
|                |                 |                          | Cellulosimicrobium (1)  |                  |
|                |                 |                          |                         | 3.4              |
|                |                 |                          | Arthrobacter (1)        |                  |
|                |                 |                          |                         | 3.4              |
|                |                 |                          | Rhodococcus (1)         |                  |
|                |                 |                          |                         | 3.4              |
|                | Bacterial species| Proteobacteria (1)       | Serratia (2)            |                  |
|                |                 |                          |                         | 11.1             |
|                |                 | Basidiomycota (1)        | Enterobacter (1)        |                  |
|                |                 |                          |                         | 5.6              |
|                |                 | Tremellomycetes (1)      | Ochrobactrum (2)        |                  |
|                |                 | Sordariomycetes (2)      |                         | 11.1             |
|                |                 | Dothideomycetes (2)      | Cladosporium (2)        |                  |
|                |                 |                          |                         | 11.1             |
|                | Site 3          | Fungal species  | Ascomycota (4)           | Ilyonectria (1)   |
|                |                 | Dothideomycetes (2)      | Penicillium (2)         |                  |
|                |                 | Sordariomycetes (2)      |                         | 5.6              |
|                |                 | Basidiomycota (1)        | Tolytocladium (1)       |                  |
|                |                 | Tremellomycetes (1)      |                         | 5.6              |
|                |                 | Proteobacteria (6)       | Trichosporon (1)        |                  |
|                |                 | Gamma proteobacteria (4) | Acinetobacter (1)       |                  |
|                |                 | Bethe Proteobacteria (1) | Serratia (2)            |                  |
|                |                 | Actinobacteria (3)       |                         | 11.1             |
|                |                 |                          | Enterobacter (1)        |                  |
|                |                 |                          |                         | 5.6              |
|                |                 | Actinobacteria (3)       | Ochrobactrum (2)        |                  |
|                |                 |                          |                         | 11.1             |
|                | Fungal species  | Ascomycota (4)           | Cladosporium (2)        |                  |
|                |                 | Dothideomycetes (2)      |                         | 11.1             |
|                |                 | Sordariomycetes (2)      | Ilyonectria (1)         |                  |
|                |                 | Basidiomycota (1)        | Tolytocladium (1)       |                  |
|                |                 | Tremellomycetes (1)      | Trichosporon (1)        |                  |
|                |                 | Proteobacteria (6)       | Acinetobacter (1)       |                  |
|                |                 | Gamma proteobacteria (4) | Serratia (2)            |                  |
|                |                 | Bethe Proteobacteria (1) | Enterobacter (1)        |                  |
|                |                 | Actinobacteria (3)       | Ochrobactrum (2)        |                  |
|                |                 |                          |                         | 11.1             |
|                |                 | Actinobacteria (3)       | Novosphingobium (1)     |                  |
|                |                 |                          |                         | 5.6              |
|                |                 | Firmicutes (2)           | Bacilli (2)             |                  |
|                |                 |                          |                         | 11.1             |
bacterial species belonged to 23 genera and 32 species (Table 3, Fig. 3A and 3B). On the basis of identification and phylogenetic analysis of the fungal strains (Fig. 3A–3C), most of the isolates belonged to the phylum Ascomycota, excluding a single fungal isolate that belongs to Basidomycetes (genus *Trichosporon* in geographical region 3). The most prevalent fungal genus was *Cladosporium* (38.5%), which was identified in all three geographical vector populations. According to bacterial identification and phylogeny (Fig. 4A–4C), the isolates were determined to belong to four phyla. The predominant group was Proteobacteria (39.7%), followed by Actinobacteria (31.7%), Firmicutes (19.1%), and Bacteroidetes (9.5%) (Table 3). In the proteobacterial group, Gammaproteobacteria was the predominant class (60.0%), and the most prevalent genera were *Bacillus* (17.5%), *Chryseobacterium* (9.5%), *Microbacterium* (9.5%), *Rhodococcus* (9.5%), *Pseudomonas* (7.9%), and *Arthrobacter* (7.9%). More than 20% of total isolates showed the potential to be novel bacterial species based on a sequence similarity less than 98.5% to existing strain types found in the EzTaxon or BLAST database [23].

Comparison of the results from each geographical group showed that the distribution or constituents of insect flora varied according to vector habitat (Table 3). At site 1, *Cladosporium* and *Penicillium* were the predominant fungal species, whereas *Rhodococcus*, *Arthrobacter*, *Microbacterium*, and *Enterobacter* were the predominant bacterial species. At site 2, however, *Bacillus* (28.0%), *Chryseobacterium*
Fig. 3. Phylogenetic trees of fungal flora from each geographic vector group. Trees were constructed using fungal internal transcribed spacer sequences. Trees of isolates from site 1 (A), site 2 (B), and site 3 (C) were obtained using the maximum-likelihood (ML) algorithm with the Kimura 2-parameters. To construct the fungal phylogenetic trees, *Kluyveromyces lactis* NRRL Y-8279 NR_131273 was used as the outgroup, and the stability of relationships was evaluated by bootstrap analysis, with resampling 1,000 times.
(24.0%), and Pseudomonas (20.0%) were the dominant bacterial species, but there was no specific dominant fungal species. At site 3, Cladosporium was the dominant fungal group, and Ochrobactrum, Bacillus, and Serratia were the dominating bacterial species. Fungal species from vector cuticles have often been regarded as useful resources for bio-control. Fungal species compatible with their host vector can survive in the environment for months as spores. Therefore, comparative analysis of cutaneous fungal flora will provide fundamental information [32] for developing
novel bio-control strategies aimed at combating the emerging *B. agrestis* problem.

Many fungal genera showing high homology with the isolates were reported to be crop pathogens based on their taxonomy. Some species of *Cladosporium* (identified at sites 1, 2, and 3), *Aspergillus* (identified at sites 1 and 2), and *Ilyonectria* (identified at site 3) are known as ubiquitous environmental saprobic or endophytic fungi, as well as major plant pathogens [33-36]. In contrast to these potentially harmful pathogens, some beneficial fungal species were also identified in this study. *Trichosporon* species showed the closest homology with isolates from site 3, suggesting their potential for antagonistic and antibiotic effects against pathogenic microorganisms of plants [37]. *Aspergillus terreus*, which showed high homology with fungal isolates in site 1, is a microorganism that is beneficial to its host plant, as a constituent of the endophytic fungal community, and is not a phytopathogen. Furthermore, *A. terreus* is a close symbiont of some other insects such as dragonflies [38]. This fungal taxon exhibits antifungal activity comparable to that of existing chemical antifungal agents against the growth of *Alternaria solani* and *Fusarium oxysporum* [38]. In general, confirmation of specific microbial taxa is not possible using fungal ITS profiling alone, and thus further taxonomic analysis is needed to secure candidates for controlling harmful insect vectors. Obtaining detailed taxonomic information on these fungal flora will provide fundamental information to develop effective bio-control strategies aimed at emerging problems such as *B. agrestis*. Meanwhile, *Kosakonia radicicincta* [39, 40], *Rhodococcus erythropolis* [41], and *Serratia fonticola* [42, 43] have been reported to have beneficial effects for crop plants or agricultural environments. Further studies will be required to determine the taxonomic relationships of these isolates. 

**Microbial diversity.** The Shannon’s diversity (H’) index of the three sites was similar (2.50, 2.17, and 2.58) (Table 4). Furthermore, Margalef’s richness index (Dmg) (3.90, 3.60, and 4.50) and Menhinick’s index (Dmn) (2.65, 2.46, and 3.30) showed differences between sites: site 3 had the highest values among all sites. Notably, site 3 had the lowest value (0.01) for Simpson’s index (D), which likely reflects species dominance in the community. These contrasting results may be due to the lower abundance of certain genera at site 3 compared to those at site 1 (Arthrobacter or Rhodococcus) or site 2 (Bacillus, Chryseobacterium, Pseudomonas), which may have been influenced by local habitat and environmental factors. For these genera, there were over four species identified for a given genus. However, no isolates at site 3 showed a particularly large abundance for a given genus, although the total genera numbers were similar to those at site 1 or site 2.

**Symbionts: Cladosporium, Bacillus, and Rhodococcus.** Regardless of the variance in the distributions of bacterial and fungal species among geographical vector groups, *Cladosporium, Bacillus,* and *Rhodococcus* were identified in all groups (Table 5). Identification of common microbial genera or species from different vector groups suggests close symbiotic relationships (symbiosis) with the host vector [44]. Therefore, comparison of insect microbial flora from geographically segregated (due to geographical barriers) groups provides important information regarding insect vector or fungal disease control. These results were based on the principles of co-evolutionary relationship that commonly identified microbial flora identified in a specific host from ecologically segregated regions can represent a relatively strong interdependency of a host-microbe relationship.

### Table 4. Microbial diversity of each geographical group

| Microbial taxon            | Site 1 | Site 2 | Site 3 |
|----------------------------|--------|--------|--------|
| Total isolates number      | 28     | 28     | 18     |
| S (number of genera)       | 14     | 13     | 14     |
| Shannon’s index (H’)       | 2.50   | 2.17   | 2.58   |
| Margalef’s richness (Dmg)  | 3.90   | 3.60   | 4.50   |
| Menhinick’s index (Dmn)    | 2.65   | 2.46   | 3.30   |
| Simpson’s index (D)        | 0.06   | 0.12   | 0.01   |

The numbers indicate the number of identified species belonging to each genus.

### Table 5. Microbial distribution per geographical group

| Genera             | Site 1 | Site 2 | Site 3 |
|--------------------|--------|--------|--------|
| Acremonium         | -      | 1      | -      |
| Aspergillus        | 1      | 1      | -      |
| Cladosporium       | 2      | 1      | 2      |
| Ilyonectria        | -      | -      | 1      |
| Penicillium        | 2      | -      | -      |
| Tolypocladium      | -      | -      | 1      |
| Trichosporon       | -      | -      | -      |
| Acinetobacter      | -      | -      | 1      |
| Arthrobacter       | 4      | 1      | -      |
| Bacillus           | 2      | 7      | 2      |
| Cellulosimicrobium | -      | 1      | -      |
| Chryseobacterium   | -      | 6      | -      |
| Delftia            | -      | -      | 1      |
| Enterobacter       | 3      | -      | 1      |
| Gordonia           | 1      | -      | -      |
| Kosakonia          | 1      | -      | -      |
| Leclercia          | -      | 1      | -      |
| Leifsonia          | -      | -      | 1      |
| Leucobacter        | -      | -      | 1      |
| Microbacterium     | 3      | 1      | -      |
| Ochrobacterium     | 2      | -      | 2      |
| Novosphingobium    | -      | -      | 1      |
| Paenibacillus      | 1      | -      | -      |
| Pseudomonas         | -      | 5      | -      |
| Rhizobium          | -      | 1      | -      |
| Rhodococcus        | 4      | 1      | 1      |
| Serratia           | -      | -      | 2      |
| Stenotrophomonas   | 1      | -      | -      |
| Varivorax          | 1      | 1      | -      |

The numbers indicate the number of identified species belonging to each genus.
interaction. For example, if certain species of Lactobacillus, as human gastrointestinal commensal flora, are identified in all human ethnic groups, including different races or geographically segregated populations (such as those residing on physically separated islands or mountains), it can be concluded that Lactobacillus species have a close evolutionary association with humans. Therefore, this species can be a good candidate to serve as a stable drug deliverer of effective molecules such as insulin genes, and for the production of insulin hormones given their ability for successful colonization and settlement in the gut of all human populations. By this same logic, identification of commonly identified microbial strains from geographically segregated hosts is an important indicator of interdependency with their host. By contrast, if a given microbial species is identified in only one vector population and not in another that is ecologically or physically separated, strong interdependencies between the host vector and microbe cannot be inferred. In the field of biological control, strategies for controlling specific insect vector (such as cockroach or fly) populations may not result in complete eradication of the associated microbe, as in the case of Salmonella or Shigella species, which do not show strong interdependency to their host vector, given that these bacteria can also be isolated from other diverse insect vectors.

Microbes interacting with their host vector could have positive or negative effects within the outbreak locale. If they are plant pathogens that form close symbiotic relationships with the vector, they could be a good control target to prevent fungal disease manifestation in agricultural lands. However, microbes that form positive symbiotic relationships with the agricultural environment could be developed or proposed as good biological control mediators [15, 45]. For example, Bacillus aryabhattai, which was identified as a common species in this study, may be a close symbiont of B. agrestis (Tables 5 and 6). Most species belonging to the Bacillus genus, especially B. aryabhattai, are not plant pathogens. Instead, they confer diverse beneficial effects on plant growth, such as inducing plant resistance to rhizosphere conditions and reducing disease incidence in agricultural soils [46-49]. Therefore, Bacillus, especially B. aryabhattai, may exert beneficial roles via the secretion of effector molecules such as anti-parasitic antibodies [15, 50-52].

### Table 6. Bacterial species distribution in each geographical group

| Genera            | Species                  | Site 1 | Site 2 | Site 3 |
|-------------------|--------------------------|--------|--------|--------|
| Acinetobacter     | guillouiae               | -      | -      | 1      |
| Arthrobacter      | pascens                  | 1      | -      | -      |
|                   | ureafaciens              | -      | 1      | -      |
| Bacillus          | acidiceler               | 1      | -      | -      |
|                   | aryabhattai              | 1      | 7      | 1      |
|                   | subtilis subsp. inaquosorum | -   | -      | 1      |
| Cellulosimicrobium| funkei                   | -      | 1      | -      |
| Chryseobacterium  | kwangjuense              | -      | 1      | -      |
|                   | lactis                   | -      | 4      | -      |
| Delftia           | acidovorans              | -      | -      | 1      |
| Enterobacter      | soli                     | 2      | -      | 1      |
| Gordonia          | effusa                   | -      | -      | -      |
| Kosakonia         | radicincians             | 1      | -      | -      |
| Leclercia         | adecarboxylata           | -      | 1      | -      |
| Leucobacter       | iarius                   | -      | -      | 1      |
| Microbacterium    | oxydans                  | 1      | 2      | -      |
|                   | paraoxydans              | 2      | -      | -      |
| Ochrobactrum      | pituitosum               | 1      | -      | -      |
|                   | rhizosphaerae            | 1      | -      | -      |
| Novosphingobium   | rosa                     | -      | -      | 1      |
| Pseudomonas       | hunanensis               | -      | 1      | -      |
|                   | monteili                 | -      | 1      | -      |
|                   | mosselii                 | -      | 1      | -      |
|                   | plecoglossicida          | -      | 1      | -      |
|                   | taiwanensis              | -      | 1      | -      |
| Rhizobium         | radiobacter              | -      | 1      | -      |
| Rhodococcus       | erythropolis             | 1      | -      | 1      |
|                   | jiujiangue               | 3      | -      | -      |
|                   | wratislaviensis          | -      | 1      | -      |
| Serratia          | fonticola                | -      | -      | 2      |
| Variovorax        | guangxiensis             | 1      | -      | -      |
|                   | soli                     | 1      | -      | -      |
species found in this study have been reported to have potential for plant pathogenicity [38, 53]. Therefore, these fungal species could be critical in controlling pathogenic outbreaks, and thus further pathogenicity studies (i.e., disease-causing assays) in Cladosporium are required. So far, these species have not been reported as being transmissible by the insect vector B. agrestis [47].

In contrast to Cladosporium or Bacillus species, the region-dependent fungal (Acremonium, Aspergillus, Ilyonectria, Penicillium, Tolypocladium, and Trichosporon) and bacterial (Pseudomonas) genera could be classified as loosely associated symbionts, which demonstrate weak interdependent relationships with host vectors (e.g., synergism, protocooperation, and amensalism) [54]. These genera are not significantly dependent on the specific properties of host insects, and could establish relationships with other host species (known as ‘host changes’). It may be interesting to note that the major determinants of the high Simpson index value (reflecting the abundance of specific genera) at site 2 were Chryseobacterium and Pseudomonas, which were not found in other sites. This suggests that strains that interact weakly with B. agrestis might be more strongly affected by environmental conditions than host vector characteristics.

Some Pseudomonas species (Table 6) have been reported to be representative plant pathogens [55-58]. In general, confirmation of disease-causing factors via 16S rDNA profiling is impossible. However, interactive relationships between the microflora and host vectors are determined by physiological interdependency [59], and species belonging to the same bacterial genera share comparatively similar physiological phenotypes. Therefore, if specific species are identified in the vector, another bacterial species in same genus could be part of the microflora in the same host vector [54].

Another possible issue worthy of consideration is the aforementioned phenomenon of host changes. This concept has been well-studied in the context of insect vectors that transmit human infectious disease. Causative agents such as *Flavivirus*, *Plasmadium*, and *Trypanosoma* are transferred only by specific insect vectors, including *Aedes*, *Anopheles*, and *Glossinidae* [4, 6, 52, 60, 61], and are known to exhibit strong host specificity. These host vectors provide optimal and physiological interdependency [59], and species belonging to the same bacterial genera share comparatively similar physiological phenotypes. Therefore, if specific species are identified in the vector, another bacterial species in same genus could be part of the microflora in the same host vector [54].

Changes in the hosts of potential plant pathogens.

**ELECTRONIC SUPPLEMENTARY MATERIAL**

Supplementary data including one table can be found with this article online at http://www.mycobiology.or.kr/src/sm/mb-45-160-s001.pdf.

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**REFERENCES**

1. Dennis DJ. Observations of fungus gnat damage to glasshouse cucurbits. N Z J Exp Agric 1978:6:83-4.
2. Ludvig SW, Oetting RD. Evaluation of medium treatments for management of Frankliniella occidentalis (Thripidae: Thysanoptera) and Bradysia coprophila (Diptera: Sciaridae). Pest Manag Sci 2001:57:1114-8.
3. Akbari S, Oshaghi MA, Hashemi-Aghdam SS, Hajikhani S, Oshaghi G, Shirazi MH. Aerobic bacterial community of American cockroach Periplaneta americana, a step toward finding suitable paratransgenesis candidates. J Arthropod Borne Dis 2014:9:35-48.
4. Beard CB, Cordon-Rosales C, Durvasala RV. Bacterial symbionts of the triatominae and their potential use in control of Chagas disease transmission. Annu Rev Entomol 2002:47:123-41.
5. Chavshin AR, Oshaghi MA, Vatandoost H, Pourmand MR, Raeisi AA, Enayati AA, Mardani N, Ghoorchian S. Identification of bacterial microflora in the midgut of the larvae and adult of wild caught Anopheles stephensi: a step toward finding suitable paratransgenesis candidates. Acta Trop 2012:121:129-34.
6. Chavshin AR, Oshaghi MA, Vatandoost H, Pourmand MR, Raeisi A, Terenius O. Isolation and identification of culturable bacteria from wild Anopheles culicifacies, a first step in a paratransgenesis approach. Parasit Vectors 2014:7:419.
7. Liu HM, Yang PP, Cheng P, Wang HF, Liu LJ, Huang X, Zhao YQ, Wang HW, Zhang CX, Gong MQ. Resistance level of mosquito species (Diptera: Culicidae) from Shandong province, China. Int J Insect Sci 2015:19:47-52.
8. Gardiner RB, Jarvis WR, Shipp JL. Ingestion of Pythium spp. by larvae of the fungus gnat Bradysia impatiens (Diptera: Sciaridae). Ann Appl Biol 1990:116:205-12.
9. Evans MR, Smith JN, Cloyd RA. Fungus gnat population development in coconut coir and Sphagnum peat-based substrates. HortTechnology 1998:8:406-9.
10. Kim HH, Jeon HY, Yang CY, Kang TJ, Han YK. Transmission of Fusarium oxysporum by the fungus gnat, Bradysia dfferens (Diptera: Sciaridae). Res Plant Dis 2009:15:262-5.
11. Dillon RJ, Dillon VM. The gut bacteria of insects: nonpathogenic interactions. Annu Rev Entomol 2004:49:71-92.
12. Gaio Ade O, Gusmão DS, Santos AV, Ber bert-Molina MA,
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Pimenta PF, Lemos FJ. Contribution of midgut bacteria to blood digestion and egg production in aedes aegypti (Diptera: Culicidae) (L.). Parasit Vectors 2011;4:105.

Hillesland H, Read A, Subhadra B, Hurwitz I, McKelvey R, Ghosh K, Das P, Durvasula R. Identification of aerobic gut bacteria from the kala azar vector, Phlebotomus argentipes: a platform for potential paratransgenic manipulation of sand flies. Am J Trop Med Hyg 2008;79:881-6.

Valiente Moro C, Tran FH, Rahimalalana FN, Ravelonandro P, Mavingui P. Diversity of culturable bacteria including Pantoea in wild mosquito Aedes albopictus. BMC Microbiol 2013;13:70.

You YH, Park JM, Yi PH, Back CG, Park MJ, Han KS, Yoon JB, Kim HH, Park JH. Microflora of a phytopathogen transferring Bradysia agrestis, a step toward finding ideal candidates for paratransgenesis. Symbiosis 2017;71:35-46.

Maleki-Ravanas N, Oshagi MA, Afshar D, Arandian MH, Hajkhani S, Akhavan AA, Yakhchali B, Shirazi MH, Rassi Y, Jafari R, et al. Aerobic bacterial flora of biotic and abiotic compartments of a hyperendemic zoonotic cutaneous leishmaniasis (ZCL) focus. Parasit Vectors 2015;8:63.

Barnard PC. The royal entomological Society Book of British Insects. Hoboken (NJ): Wiley-Blackwell; 2011. p. 191-201.

You YH, Park JM, Park JH, Kim JG. Endophyte distribution and comparative analysis of diversity in wetlands showing contrasting geomorphic conditions. Symbiosis 2016;69:21-36.

Park JM, You YH, Back CG, Kim HH Ghim SY, Park JH. Fungal load in Bradysia agrestis, a phytopathogen-transmitting insect vector. Symbiosis 2017 May 19 [Epub]. https://doi.org/10.1007/s13199-017-0494-3.

White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego (CA): Academic Press; 1990. p. 315-22.

Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 2006;103:626-31.

Margalef DR. Information theory in ecology. Gen Syst 1958;3:36-71.

Whittaker RH. Evolution of species diversity in land communities. In: Hecht MK, Steere BW, editors. Evolutionary biology. Vol. 10. New York: Plenum Press; 1972. p. 1-67.

Lambshad PJ, Platt HM, Shaw KM. The detection of differences among assemblages of marine benthic species based on an assessment of dominance and diversity. J Nat Hist 1983;17:859-74.

van Emden HF, Service MW. Pest and vector control. Cambridge: Cambridge University Press; 2004. p. 102-3.

De Lucca AJ. Harmful fungi in both agriculture and medicine. Rev Iberoam Micol 2007;24:3-13.

Farh ME, Kim YJ, Singh P, Yang DC. Cross interaction between Ilyonectria mor-sanicas isolates infecting Korean ginseng and ginseng saponins in correlation with their pathogenicity. Phytopathology 2017;107:561-9.

Torres DE, Rojas-Martinez RI, Zavala-Mejia E, Guevara-Fefer P, Martinez-Guzmán GJ, Pérez-Martinez C. Cladosporium cladosporioides and Cladosporium pseudocladosporioides as potential new fungal antagonists of Puccinia horiana Henn., the causal agent of chrysanthemum white rust. PLoS One 2017;12:e0170782.

Walker C, Muniz MF, Rolim JM, Martins RR, Rosenthal VC, Maçeli CG, Mezzomo R, Reiniger LR. Morphological and molecular characterization of Cladosporium cladosporioides species complex causing pecan tree leaf spot. Genet Mol Res 2016;15:gmor.15038714.

Ladjal S, Harzallah D, Dahamna S, Bouamra D, Bouharati S, Khennouf S. Endophytic fungi isolated from Pinus halepensis needles in M’sila (Algeria) region and their bioactivities. Commun Agric Appl Biol Sci 2013;78:625-31.

Lu YH, Jin LP, Kong LC, Zhang YL. Phytotoxic, antifungal and immunosuppressive metabolites from Aspergillus terreus QT122 isolated from the gut of dragonfly. Curr Microbiol 2017;74:84-9.

Berger B, Baldermann S, Ruppel S. The plant growth-promoting bacterium Kosakonia radiicinctans improves fruit yield and quality of Solanum lycopersicum. J Sci Food Agric 2017 Apr 6 [Epub]. https://doi.org/10.1002/jsfa.8357.

Bergottini VM, Filippidou S, Junier T, Johnson S, Chain PS, Otegui MB, Zapata PD, Junier P. Genome sequence of Kosakonia radiicinctans strain YD4, a plant growth-promoting rhizobacterium isolated from Yerba Mate (Ilex paraguariensis St. Hila). Genome Announce 2015;3:e00239-15.

Trivedi P, Pandey A, Sa T. Chromate reducing and plant growth promoting activities of psychrotrophic Rhodococcus erythropolis MtCC 7905. J Basic Microbiol 2007;47:513-7.

Jung BK, Khan AR, Hong SJ, Park GS, Park YJ, Park CE, Jeon HJ, Lee SE, Shin JH. Genomic and phenotypic analyses of Serratia fonticola strain GS2: a rhizobacterium isolated from sesame rhizosphere that promotes plant growth and produces N-acyl homoserine lactone. J Biotechnol 2017;241:158-62.
43. Lim YL, Yong D, Ee R, Krishnan T, Tee KK, Yin WF, Chan KG. Complete genome sequence of *Serratia fonticola* DSM 4576 T, a potential plant growth promoting bacterium. J Biotechnol 2015;214:43-4.

44. Wilke AB, Marrelli MT. Paratransgenesis: a promising new strategy for mosquito vector control. Parasit Vectors 2015;8:342.

45. Walters D. Disease control in crops: biological and environmentally-friendly approaches. Chichester: Wiley-Blackwell; 2009.

46. Ambardar S, Vakhu J. Plant growth promoting bacteria from *Crocus sativus* rhizosphere. World J Microbiol Biotechnol 2013;29:2271-9.

47. Lee S, Ka JO, Song HG. Growth promotion of *Xanthium italicum* by application of rhizobacterial isolates of *Bacillus aryabhattai* in microcosm soil. J Microbiol 2012;50:45-9.

48. Mesa J, Mateos-Naranjo E, Caviedes MA, Redondo-Gómez S, Pajuelo E, Rodriguez-Llorente ID. Scouting contaminated estuaries: heavy metal resistant and plant growth promoting rhizobacteria in the native metal rhizoaccumulator *Spartina maritima*. Mar Pollut Bull 2015;90:150-9.

49. Siddikee MA, Chauhan PS, Anandham R, Han GH, Sa T. Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. J Microbiol Biotechnol 2010;20:1577-84.

50. Madigan MT, Martinko J. Brock biology of microorganisms. 12th ed. New York: Pearson Education Asia; 2011.

51. Noirot P, Noirot-Gros MF. Protein interaction networks in bacteria. Curr Opin Microbiol 2004;7:505-12.

52. Yadav KK, Bora A, Datta S, Chandel K, Gogoi HK, Prasad GB, Veer V. Molecular characterization of midgut microbiota of *Aedes albopictus* and *Aedes aegypti* from Arunachal Pradesh, India. Parasit Vectors 2015;8:641.

53. Nam MH, Park MS, Kim HS, Kim TI, Kim HG. *Cladosporium cladosporioides* and *C. tenuissimum* cause blossom blight in strawberry in Korea. Mycobiology 2015;43:354-9.

54. Barton LL, Northup DE. Microbial ecology. Hoboken (NJ): Wiley-Blackwell; 2011.

55. Cha JY, Lee JS, Oh JI, Choi JW, Baik HS. Functional analysis of the role of Fur in the virulence of *Pseudomonas syringae* pv. tabaci 11528: Fur controls expression of genes involved in quorum-sensing. Biochim Biophys Res Commun 2008;366: 281-7.

56. Chen WJ, Kuo TY, Hsieh FC, Chen PY, Wang CS, Shih YL, Lai YM, Liu JR, Yang YL, Shih MC. Involvement of type VI secretion system in secretion of iron chelator pyoverdine in *Pseudomonas taiwanensis*. Sci Rep 2016;6:32950.

57. Song YR, Choi MS, Choi GW, Park IK, Oh CS. Antibacterial activity of cinnamaldehyde and estragole extracted from plant essential oils against *Pseudomonas syringae* pv. *actinidiae* causing bacterial canker disease in kiwifruit. Plant Pathol J 2016;32:363-70.

58. Soto-Rodriguez SA, Cabanillas-Ramos J, Alcaraz U, Gomez-Gil B, Romanle JL. Identification and virulence of *Aeromonas dhakensis*, *Pseudomonas mosselii* and *Microbacterium paraoxydans* isolated from Nile tilapia, *Oreochromis niloticus*, cultivated in Mexico. J Appl Microbiol 2013;115:654-62.

59. Tannock GW. Normal microflora: an introduction to microbes inhabiting the human body. Alphen aan den Rijn: Springer Netherlands; 1994.

60. Barzon L, Trevisan M, Sinigaglia A, Lavezzo E, Palù G. Zika virus: from pathogenesis to disease control. FEMS Microbiol Lett 2016;363:fsu202.

61. Leon G. Epidemiology. New York: Elsevier Science Health Science; 2004.

62. Olafson PU, Lohmeyer KH, Edrington TS, Loneragan GH. Survival and fate of *Salmonella enterica* serovar Montevideo in adult horn flies (Diptera: Muscidae). J Med Entomol 2014; 51:993-1001.

63. Hail D, Lauzìere I, Dowd SE, Bextine B. Culture independent survey of the microbiota of the glassy-winged sharpshooter (*Homalodisca vitripennis*) using 454 pyrosequencing. Environ Entomol 2011;40:23-9.

64. Chaivong T, Srivoramas T, Sueabsamran P, Sukontason K, Sanford MR, Sukontason KL. The blow fly, *Chrysomya megacephala*, and the house fly, *Musca domestica*, as mechanical vectors of pathogenic bacteria in Northeast Thailand. Trop Biomed 2014;31:336-46.
**Supplementary Table 1.** Microbial flora from each regional group

| Sampling sites | Microorganisms | Strain name | Closest related microbial species | Similarity (%) | Accession No. |
|---------------|----------------|-------------|------------------------------------|----------------|---------------|
| Site 1        | Fungal species | NIHHS501    | Penicillium cf. phoeniceum (AY742694) | 100            | KY929272      |
|               |                | NIHHS502    | Penicillium cf. brevicompactum (KP942939) | 100            | KY929273      |
|               |                | NIHHS503    | Cladosporium cf. gossypicola (AF393702) | 100            | KY929274      |
|               |                | NIHHS504    | Cladosporium cf. conioides (AF393695) | 100            | KY929275      |
|               |                | NIHHS505    | Aspergillus cf. tereus (K610363) | 100            | KY929276      |
|               | Bacterial species | NIHHS100 | Ochrobactrum rhizosphaerae (AM490632) | 98.93          | KU711995      |
|               |                | NIHHS101    | Bacillus acidiceler (DQ374637) | 98.54          | KU711996      |
|               |                | NIHHS102    | Variorax soli (DQ432053) | 98.96          | KU711997      |
|               |                | NIHHS103    | Kosakonia radicincitans (AY563134) | 99.03          | KU711998      |
|               |                | NIHHS104    | Gordonia sp. (AB162799) | 97.87          | KU711999      |
|               |                | NIHHS105    | Arthrobacter sp. (BAEG010000072) | 96.85          | KU712000      |
|               |                | NIHHS106    | Microbacterium oxydans (Y17227) | 98.80          | KU712001      |
|               |                | NIHHS107    | Ochrobactrum pituitosum (AM490609) | 99.46          | KU712002      |
|               |                | NIHHS108    | Rhodococcus jialingiae (DQ185597) | 98.95          | KU712003      |
|               |                | NIHHS109    | Stenotrophomonas sp. (JALY01000036) | 98.38          | KU712004      |
|               |                | NIHHS110    | Rhodococcus jialingiae (DQ185597) | 98.96          | KU712005      |
|               |                | NIHHS111    | Arthrobacter sp. (X80740) | 96.93          | KU712006      |
|               |                | NIHHS112    | Microbacterium oxydans (AJ491806) | 98.88          | KU712007      |
|               |                | NIHHS113    | Rhodococcus jialingiae (DQ185597) | 99.40          | KU712008      |
|               |                | NIHHS114    | Arthrobacter sp. (BAEG010000072) | 97.46          | KU712009      |
|               |                | NIHHS115    | Arthrobacter sp. (BAEG010000072) | 97.43          | KU712010      |
|               |                | NIHHS116    | Microbacterium oxydans (AJ491806) | 99.32          | KU712011      |
|               |                | NIHHS117    | Bacillus aryabhattai (EF114313) | 99.11          | KU712012      |
|               |                | NIHHS118    | Rhodococcus erythropolis (X79289) | 99.02          | KU712013      |
|               |                | NIHHS119    | Paenibacillus sp. (AF391124) | 97.88          | KU712014      |
|               |                | NIHHS120    | Enterobacter sp. (CP003026) | 98.44          | KU712015      |
|               |                | NIHHS121    | Achromobacter sp. (HE613446) | 96.06          | KU712016      |
|               |                | NIHHS122    | Enterobacter soli (CP003026) | 98.81          | KU712017      |
|               |                | NIHHS123    | Enterobacter soli (CP003026) | 98.66          | KU712018      |
| Site 2        | Fungal species | NIHHS506    | Acremonium cf. sclerotigenum (KJ194115) | 100            | KY929277      |
|               |                | NIHHS507    | Cladosporium cf. cladosporioides (HM148014) | 100            | KY929278      |
|               |                | NIHHS508    | Aspergillus cf. rugulosus (KU666681) | 99             | KY929279      |
|               | Bacterial species | NIHHS124 | Chryseobacterium sp. (JX100821) | 98.00          | KU712019      |
|               |                | NIHHS125    | Chryseobacterium lactis (JX100821) | 98.51          | KU712020      |
|               |                | NIHHS126    | Chryseobacterium kwanjusense (AY514021) | 99.56          | KU712021      |
|               |                | NIHHS127    | Microbacterium oxydans (Y17227) | 99.41          | KU712022      |
|               |                | NIHHS128    | Bacillus aryabhattai (EF114313) | 99.49          | KU712023      |
|               |                | NIHHS129    | Rhodococcus wratislaviensis (BAWF01000105) | 98.79        | KU712024      |
|               |                | NIHHS130    | Pseudomonas masseli (AF072688) | 99.40          | KU712025      |
|               |                | NIHHS131    | Bacillus aryabhattai (EF114313) | 99.42          | KU712026      |
|               |                | NIHHS132    | Bacillus aryabhattai (EF114313) | 98.98          | KU712027      |
|               |                | NIHHS133    | Bacillus aryabhattai (EF114313) | 98.76          | KU712028      |
|               |                | NIHHS134    | Bacillus aryabhattai (EF114313) | 99.42          | KU712029      |
|               |                | NIHHS135    | Microbacterium oxydans (Y17227) | 99.33          | KU712030      |
|               |                | NIHHS136    | Pseudomonas monteilii (BBIS01000088) | 99.33          | KU712031      |
|               |                | NIHHS137    | Pseudomonas hunanensis (JX545210) | 99.12          | KU712032      |
|               |                | NIHHS138    | Leclercia adecarboxylata (AB273740) | 99.03          | KU712033      |
|               |                | NIHHS139    | Bacillus aryabhattai (EF114313) | 99.40          | KU712034      |
|               |                | NIHHS140    | Arthrobacter ureafaciens (X80744) | 98.95          | KU712035      |
|               |                | NIHHS141    | Bacillus aryabhattai (EF114313) | 99.26          | KU712036      |
|               |                | NIHHS142    | Pseudomonas taiwanensis (EU103629) | 99.18          | KU712037      |
|               |                | NIHHS143    | Chryseobacterium lactis (JX100821) | 98.85          | KU712038      |
|               |                | NIHHS144    | Cellulosimicrobium funkei (AY51364) | 98.87          | KU712039      |
|               |                | NIHHS145    | Chryseobacterium lactis (JX100821) | 98.94          | KU712040      |
|               |                | NIHHS146    | Chryseobacterium lactis (JX100821) | 98.95          | KU712041      |
|               |                | NIHHS147    | Rhizobium radiobacter (AJ389904) | 99.07          | KU712042      |
|               |                | NIHHS148    | Pseudomonas plecglossicida (BBIV01000080) | 98.90         | KU712043      |
### Supplementary Table 1. Continued

| Sampling sites | Microorganisms | Strain name | Closest related microbial species | Similarity (%) | Accession No. |
|----------------|----------------|-------------|-----------------------------------|----------------|---------------|
| Site 3         | Fungal species | NIHHS149    | Variovorax guangxiensis (JF495126) | 98.50           | KU712044      |
|                |                | NIHHS509    | Cladosporium cf. sphaerospermum (KP701965) | 100              | KY929280      |
|                |                | NIHHS510    | Cladosporium cf. sphaerospermum (KP701965) | 100              | KY929281      |
|                |                | NIHHS511    | Cladosporium cf. sphaerospermum (KP701965) | 100              | KY929282      |
|                |                | NIHHS512    | Tolyphomadum sp. (KU556539) | 95              | KY929283      |
|                |                | NIHHS513    | Ilyonectria cf. robusta (JF735265) | 99              | KY929284      |
|                | Bacterial species | NIHHS150    | Serratia fonticola (AVAH01000293) | 99.18           | KU712045      |
|                |                | NIHHS151    | Acinetobacter guillouiae (APOS01000028) | 99.33           | KU712046      |
|                |                | NIHHS152    | Enterobacter soli (CP003026) | 99.33           | KU712047      |
|                |                | NIHHS153    | Leifsonia sp. (DQ232614) | 98.28           | KU712048      |
|                |                | NIHHS154    | Leucobacter iarius (AM040493) | 99.17           | KU712049      |
|                |                | NIHHS155    | Serratia fonticola (AVAH01000293) | 99.41           | KU712050      |
|                |                | NIHHS156    | Bacillus subtilis subsp. inaquosorum (AMXN01000021) | 99.27           | KU712051      |
|                |                | NIHHS157    | Bacillus aryabhattai (EF114313) | 99.41           | KU712052      |
|                |                | NIHHS158    | Delfta acidovorans (JOUB010000065) | 98.90           | KU712053      |
|                |                | NIHHS159    | Rhodococcus eurythropolis (X79289) | 99.10           | KU712054      |
|                |                | NIHHS160    | Ochrobactrum sp. (AM422370) | 98.31           | KU712055      |
|                |                | NIHHS161    | Ochrobactrum sp. (AM422370) | 97.98           | KU712056      |
|                |                | NIHHS162    | Novosphingobium rosa (D13945) | 98.70           | KU712057      |