

The role of the bacterial community in the nutritional ecology of the bulb mite *Rhizoglyphus robini* (Acari: Astigmata: Acaridae)

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ABSTRACT The biology of many arthropods can only be understood when their associated microbiome is considered. The nutritional requirements of the bulb mite *Rhizoglyphus robini* Claparede (Acari: Astigmata: Acaridae) in the laboratory have been shown to be very easily satisfied, and in the field the mites prefer fungus-infected over uninfected plants. To test whether symbiotic bacteria facilitate the survival of *R. robini* on a temporarily nutritionally unbalanced diet, we investigated the composition of its microbiome. Using 454 pyrosequencing of 16S rRNA gene fragments, 3 genera were found to dominate the bacterial community: *Myroides* (41.4%), *Serratia* (11.4%), and *Alcaligenes* (4.5%); the latter 2 are known to include chitinase-producing species. Laboratory experiments demonstrated that mite fecundity is significantly higher (2 times) on fungus than on controls (sterilized potato dextrose agar and filter paper). Also, when mite homogenate was applied to a chitin layer, the halo produced through degradation was clearly visible, while the saline control did not produce a halo. We thus concluded that *R. robini* utilizes fungal chitin, at least to a certain extent, as a food source with the help of its associated bacteria. This information supports the general concept of multigenome organisms and the involvement of bacteria in the mite's nutritional ecology.—Zindel, R., Ofek, M., Minz, D., Palevsky, E., Zchori-Fein, E., Aebi, A. The role of the bacterial community in the nutritional ecology of the bulb mite *Rhizoglyphus robini* (Acari: Astigmata: Acaridae). *FASEB J.* 27, 000–000 (2013). www.fasebj.org

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Abbreviations: DGGE, denaturing gradient gel electrophoresis; FO, *Fusarium oxysporum*; GC, guanine-cytosine; LB, lysogeny broth; NB, nutrient broth; OTU, operational taxonomic unit; PCR, polymerase chain reaction; PDA, potato dextrose agar; rRNA, ribosomal ribonucleic acid; TAE, Tris base/acetic acid/EDTA; UPGMA, unweighted pair-group method with arithmetic mean

EXPLORATION OF THE MICROBIOME associated with many arthropods has proven to be very important to understanding their biology (see ref. 1 for examples). Among the many functions microbes can have in their hosts' biology, nutritional provision is of high importance (2). Specialized feeders, as well as omnivorous arthropods, may engage in obligate or facultative interactions with microbes. Arthropods with highly restricted diets may rely on bacterial symbionts for the provision of essential amino acids, vitamins, and digestive enzymes, as well as for the metabolism of fatty acids and nitrogen processing (3–5). However, omnivorous arthropods also maintain obligate and facultative bacterial associates, helping them use nutrients from different sources (6, 7). Overall, arthropods' highly divergent life strategies, from strict sap or blood feeding to omnivory, are facilitated by the association with microorganisms.

Rhizoglyphus robini Claparede (Acari: Astigmata: Acaridae), an important agricultural pest in Mediterranean countries, lives in the soil, and on the bulbs and tubers of its host plants (mainly ornamentals and crops such as lily, onion, and garlic) and is therefore commonly referred to as the bulb mite. Initially described as a primary pest directly affecting its host plant (8), the mite's biology is poorly understood. In the laboratory, it is usually kept on water-soaked peanuts but will also use roots, ferns, and decaying soil insects as a food source (9), and it should therefore be considered omnivorous. The extent to which this generalist lifestyle is supported by the activity of associated bacteria is unknown.

Field observations show that *R. robini* is almost exclusively found on plants that suffer from fungal infections (10). *R. robini* has been shown to choose fungus-

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infected over uninfected hosts in preference tests (10, 11) and to be attracted to alcohols extracted from cultures of *Fusarium oxysporum* (FO; ref. 12). In addition, *R. robini* exhibits high fecundity on fungal food sources (10, 13). Survival of *R. robini* on wet filter paper alone was explained by the mite's ability to derive nutrients from fungal mycelia developing on the cellulosic matrix (14). This evidence suggests that fungi make up a considerable proportion of the mite's diet. Fungal cell walls consist of 3 to 60% chitin (15, 16). While most animals do not possess the enzymes required to digest chitin, in most insects chitinases are found to be involved in molting processes (17). More important, for some pyroglyphid mite species, endogenous chitinases also have been reported. These chitinases are major allergens for dogs (18). However, fungus-feeding mites are thought to rely on chitinases provided by associated bacteria (19). The fact that *R. robini* readily feeds on fungal tissue—potentially with the help of bacteria—puts the pest status of *R. robini* into question.

Here we sought information on the nature of the *R. robini* microbiome and tested the hypothesis that symbiotic bacteria may be linked to *R. robini*'s ability to exploit fungi as a food source. The following approaches were used to describe the composition and role of the bacterial community associated with *R. robini*: denaturing gradient gel electrophoresis (DGGE) and subsequent fingerprinting methods to describe the bacterial community of individual mites, and 454 pyrosequencing to obtain an overview of the complete composition of the bacterial communities in the populations. The mite's ability to exploit fungal food sources and the consequent influence on its fecundity were tested in laboratory experiments, and chitinase activity of mite homogenate was examined in a bioassay. This combination of bioinformatic and ecological techniques revealed the importance of bacteria to understanding the biology of a major pest species.

MATERIALS AND METHODS

Mite origin and rearing

The mites used in this study stemmed from field populations originally collected on three different host plants [lily (*Lilium candidum*), *Ruscus* sp., and garlic (*Allium cepa*)] and isolated in separate containers since 2005. Mites were reared in Petri dishes containing wet filter paper and nonsterilized ground peanuts, and kept in a closed box in the dark. Petri dishes were stacked on a rack standing in soapy water to prevent escape of individual mites to other populations' boxes. Cultures were maintained by transferring 10 to 30 individual mites to a new Petri dish. Water and peanuts were added every 14 d.

Examination of bacterial community composition of individual mites

DGGE was used to describe the bacterial communities of mites stemming from different populations. Lysates from 18

individual mites (6 individuals from each of the 3 populations) were prepared as described previously (20) and used as template for amplification of bacterial 16S ribosomal ribonucleic acid (rRNA) gene fragments using the primer combination of 341F with a guanine-cytosine (GC) clamp (40-nt, GC-rich sequence) and 907R (21) as described by Gottlieb *et al.* (22).

The polymerase chain reaction (PCR)-amplified fragments were separated by DGGE using a 6% (w/v) acrylamide gel (acrylamide-*N,N'*-methylenebisacrylamide) prepared in 1× TAE buffer (2 M base, 1 M glacial acetic acid, 50 mM EDTA) with a denaturing gradient of 20 to 60% (100% denaturant corresponding to 7 M urea and 40% v/v formamide). Electrophoresis was performed at 200 V overnight in 1% (v/v) TAE buffer at 60°C in a Bio-Rad D-Code system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The gel was stained with ethidium bromide and photographed using a Polaroid camera on a UV table. The images were imported to Fingerprinting II software (Bio-Rad) and the DGGE patterns were aligned. Cluster analysis of the DGGE patterns was performed with Fingerprinting II using the unweighted pair-group method with arithmetic mean (UPGMA) algorithm based on 1-Pearson *r* distance matrix between patterns.

Tag-encoded pyrosequencing of 16S rRNA gene fragments and data analysis

To further investigate the bacterial community at the mite population level, DNA was extracted from 3 replicates of ~250 pooled individuals from each of the 3 populations (garlic, lily, and *Ruscus*). The collected mites were surface sterilized with 70% ethanol and stored in 100% ethanol until use. Immediately before the extraction, individuals were washed with sterile saline solution (0.85% NaCl) to remove traces of ethanol. The PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) was used for DNA extraction according to the manufacturer's instructions. DNA concentrations were measured using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The extracted DNA was subjected to high-throughput sequencing. 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing was performed by the Research and Testing Laboratory (Lubbock, TX, USA) as described by Dowd *et al.* (23). The resulting sequences were analyzed using MOTHR (24). Low-quality sequences (quality score average of 30 over a 31-base window) along with fragments shorter than 200 bp (~15% of the sequences) were eliminated before further analysis was performed using the trim.seq module of MOTHR. Sequences were aligned using a Silva-compatible alignment database. The Chimera.slayer module of MOTHR was used to detect chimeras, which were subsequently removed. A 97% sequence-similarity threshold was used to group the sequences into operational taxonomic units (OTUs). Representatives of each OTU were selected using the MOTHR get.oturep module and classified with the MOTHR classify.seqs module using a Silva-compatible taxonomy database. The affiliation of each OTU representative was verified by ARB (<http://www.arb-home.de>) and by U.S. National Center for Biotechnology Information (NCBI; Bethesda MD, USA) Basic Local Alignment Search Tool (BLAST) analyses. The sequences of representatives of the most abundant OTUs were imported into MEGA 5.05 (25), along with sequences of closest relatives according to ARB and NCBI database searches. Sequences were aligned with the Muscle algorithm, and a neighbor-joining tree was calculated, based on Tamura's 3-parameter model using complete deletion for treatment of gaps and missing data. Hence, the tree was based on 200 nt, the minimal length threshold used for

initial selection of sequence reads. Support of tree topology was examined by bootstrap analysis with 1000 replicates.

After classification of the sequences into OTUs, OTU counts were used for comparison of the bacterial community compositions. Sample coverage was estimated by calculating the Good coverage estimate (26). For each sample, the Chao1 richness estimate (27) and the dominance and Shannon indexes of diversity were calculated in Excel (Microsoft, Redmond, WA, USA). To compare the relative abundances of different bacterial taxa, nonparametric Kruskal-Wallis ANOVA was performed with the Statistica 7.1 software package (StatSoft Inc., Tulsa, OK, USA). Pyrosequencing reads were deposited in the GenBank Short Read Archive under accession no. SRA 049893.

Comparison of mite fecundity on different diets

Fecundity of same-age females was examined under 4 different dietary conditions: no food; standard peanut diet; potato dextrose agar (PDA); and PDA with FO mycelia. To obtain young females for fecundity measurements, mites were placed in a Petri dish with peanut food and left for 2 d for oviposition before adults were removed. After ~12 d, adults with a maximal age difference of 2 d had developed from eggs. To avoid any possible influence of bacteria adhering to the mite cuticle, mites were starved for 1 d, then washed with a 5% commercial bleach solution (2 min) and rinsed with 75% ethanol before each experiment. A polyspore isolate of the phytopathogenic fungus FO (FO 070-041), originally isolated from wheat, was obtained from the local spore base [Agroscope Reckenholz-Tänikon (ART), Zürich, Switzerland] and cultivated on PDA at room temperature in the dark. The mites were divided into groups of 60 (40 females and 20 males) and placed on individual Petri dishes containing a wet filter paper with 4 different treatments: no food (negative control); nonsterilized ground peanuts; a 1-cm² piece of PDA; or a 1-cm² piece of PDA with FO mycelia. Peanuts were used as a positive control, representing the diet on which laboratory populations are reared. Each treatment was replicated 3 times. After 2 d, 25 females from each treatment were individually placed in 25-mm-diameter Petri dishes containing only a wet filter paper and were allowed to oviposit for 24 h. If a female was dead at that time, the measurement was excluded. The entire experiment was repeated 3 times. Data were analyzed using the Statistica 7.1 software package, applying 1-way ANOVA.

Chitinase activity of mite homogenates

Chitin-degrading ability of the mite holobiont was examined. Crabshell chitin (Sigma, St. Louis, MO, USA) was washed (28, 29), spread in a thin layer (3 ml/plate), and allowed to dry on minimal medium containing 10 ml of 5 mg/ml filter-sterilized nystatin solution per liter of medium, according to Faramarzi *et al.* (30). Mites were grown with the 4 different feeding treatments described above. Mites were starved and surface sterilized, and sterility was examined by rolling the mites on the surface of lysogeny broth (LB) agar plates. The plates were inspected 48 h later for the development of bacteria or fungi. Mite homogenates were prepared by grinding ~100 individual adult mites in 40 μ l sterile saline solution. Of the homogenates, 4 μ l (per replicate) in 5 serial dilutions of 5 to 100% of the stock solution was pipetted in droplets of 1 μ l on chitin agar medium. *Escherichia coli* and sterile saline solution (0.85% NaCl) served as negative controls, and a chitinase-producing *Bacillus subtilis* strain was used as a positive control. Plates were incubated at 30°C and inspected after 12, 24, and 36 h for the presence of haloes.

Bacteria were picked from the center and edge of the haloes with a wooden toothpick, transferred to liquid LB and nutrient broth (NB) medium, incubated at 28°C overnight, and then repipetted onto chitin plates to confirm the bacterial origin of the chitinases.

RESULTS

Bacterial community composition of individual mites

The bacterial community composition of *R. robini* was examined and compared among laboratory-reared populations. Complex DGGE profiles were found within all populations (Fig. 1). Most of the individuals shared dominant bands, but variations in banding pattern among individuals from different treatments was significant (Fig. 1). Similarity was higher within than among populations (Fig. 1).

Tag-encoded pyrosequencing of 16S rRNA gene fragments and data analysis

To further describe the bacterial community associated with *R. robini*, mass sequencing of 16S rRNA gene fragments was performed using 454-pyrosequencing technology. In total, 64,241 high-quality sequences were obtained. Sequences were classified into 709 OTUs using a 97% sequence-similarity threshold and were taxonomically affiliated with 113 different genera. **Table 1** presents the summary of sequence numbers and OTU classification for each population. A comparison of coverage values and diversity indices (Shannon H') and the derived evenness measure between the different populations revealed no significant differences. Relative abundances of most OTUs were below

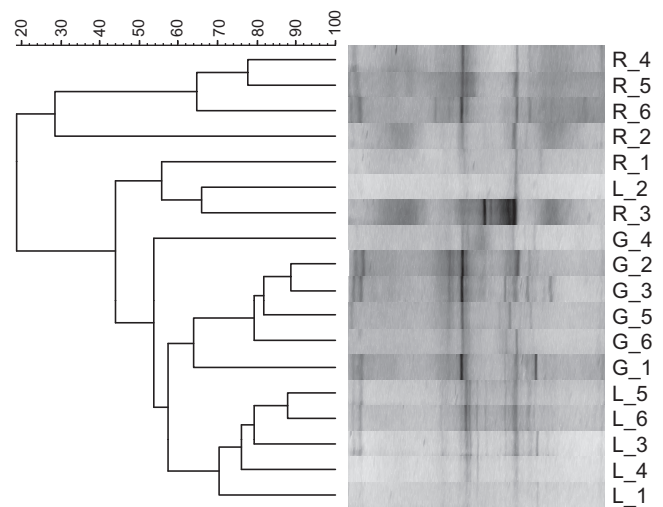


Figure 1. Comparison of bacterial community compositions of individual laboratory-reared *R. robini* from 3 populations: garlic (G), lily (L), and *Ruscus* (R). Compositions of bacterial communities were determined by PCR-DGGE using general bacterial primers. PCR-DGGE patterns were aligned using Fingerprinting II software, and UPGMA cluster analysis was performed based on cosine similarity matrix.

TABLE 1. Structure of *R. robini*-associated bacterial communities based on mass sequencing of 16S rRNA gene fragments

Parameter	Σ sequences	97% OTUs	S_1	Rare (%)	Genera	Coverage	Shannon	Evenness
Population								
<i>Alium</i>	20,247	556	113	96.6	63	0.966	3.49	0.636
<i>Lilium</i>	18,592	551	89	95.1	66	0.979	3.64	0.647
<i>Ruscus</i>	25,402	564	98	96.1	82	0.984	3.38	0.567
Neuman-Keuls critical range					33	0.014	0.27	0.129

Σ sequences: total number of sequences; 97% OTUs: number of OTUs using a 97% sequence similarity threshold for classification (total number of OTUs found for all replicates of the specified population); S_1 : number of singletons (OTUs represented by only one sequence); Rare: percentage of OTUs for which relative abundance was $<0.5\%$ of total number of sequences; Coverage: sample coverage estimate calculated after Good ($C_{\text{Good}} = 1 - F_1/N$, where F_1 is number of singletons, and N is number of sequences; based on 97% OTU classification; ref. 26); Shannon: average Shannon index of diversity [$H' = -\sum p_i \cdot \ln(p_i)$, where p_i is relative abundance of the i th OTU]; Evenness: average evenness ($E = H'/H_{\text{max}}$, where $H_{\text{max}} = H'/\ln S$ and S is total OTUs in sample); Neuman-Keuls critical range: Chao1, dominance, and Shannon indices were compared by nonparametric ANOVA, and the critical range was determined.

0.5% of the total sequences, and between 16 and 20% of the OTUs were represented by a single sequence (Table 1).

The bacterial community of laboratory-reared *R. robini* was dominated by bacteria belonging to 2 phyla, Bacteroidetes and Proteobacteria, together accounting for 92% of the sequences (Fig. 2). Moreover, sequences related to the Flavobacteriaceae (Bacteroidetes) and Enterobacteriaceae (γ -proteobacteria) comprised 56 and 15.9% of the total sequences, respectively. Only 24 OTUs had relative abundances of $>0.5\%$ and included 74.9% of the total sequences pooled from all populations. Closely related sequences of these 24 OTUs (according to ARB-Silva and NCBI databases) were retrieved previously from the gut of insects and other arthropods, the rhizosphere and endosphere of plants, compost, sludge, and wastewater (Figs. 3 and 4).

The single most abundant OTU, comprising 22.9% of the total sequences, was classified as *Myroides* (Flavobacteria) and was closely related ($>99\%$) to *Myroides odoratimimus* (represented by sequence A1_CF20C in Fig. 3). Two other OTUs were affiliated with the genus *Myroides*, which altogether accounted for 33.2% of the total sequences. The second most abundant OTU (9.8% of the total sequences) was affiliated with the genus *Serratia* (Enterobacteriaceae) and was closely related ($>99\%$) to *Serratia marcescens* (represented by sequence L2_BF92D in Fig. 4). Together with a second

Serratia-affiliated OTU (Fig. 4), 11.4% of the total bacterial sequences were explained.

Differing bacterial community profiles were found among the three populations examined (Table 2). These populations shared 22 of the 24 dominant OTUs, but in some the relative abundance differed significantly. However, differences in composition occurred. OTUs related to an unclassified Pseudomonadaceae and an unclassified Bacillaceae were among the dominant OTUs in the garlic population but were close to or below the detection limit in the lily and *Ruscus* mite populations (Table 2).

Comparison of mite fecundity on different diets

Monitoring the fecundity of females fed on 4 different food sources, FO-fed females and peanut-fed females were found to have similar fecundity levels (Fig. 5), which were approximately twice those of the negative control and PDA treatments. These effects of food source were highly significant (ANOVA, $n=60/\text{treatment}$; 3 degrees of freedom, $P<0.01$).

Chitinase activity of mite homogenates

To demonstrate the chitinolytic activity of the bacterial community associated with *R. robini*, we applied different concentrations of mite homogenate, prepared with surface-sterilized mites, on plates overlaid with a layer of chitin. The homogenate of all tested mites (from all 4 treatments) was able to clear chitin around the droplet (Fig. 6), whereas neither saline solution alone nor *E. coli* bacterial cells produced a halo. However, we did not quantify the effect of food source on presence or size of the cleared zones. Bacteria isolated from the center and outer circle of the halo showed the same strong chitinase activity when reapplied as colonies to a chitin-covered Petri dish (data not shown).

DISCUSSION

The rationale for this study was the acknowledged importance of arthropod host-microbiome interactions, particularly with respect to host nutrition. While

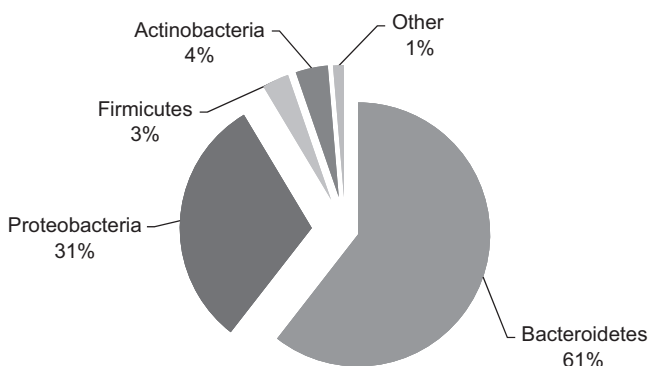


Figure 2. Composition of bacterial community associated with *R. robini*, based on 64,241 partial sequences of 16S rRNA genes pooled from the three laboratory-reared populations.

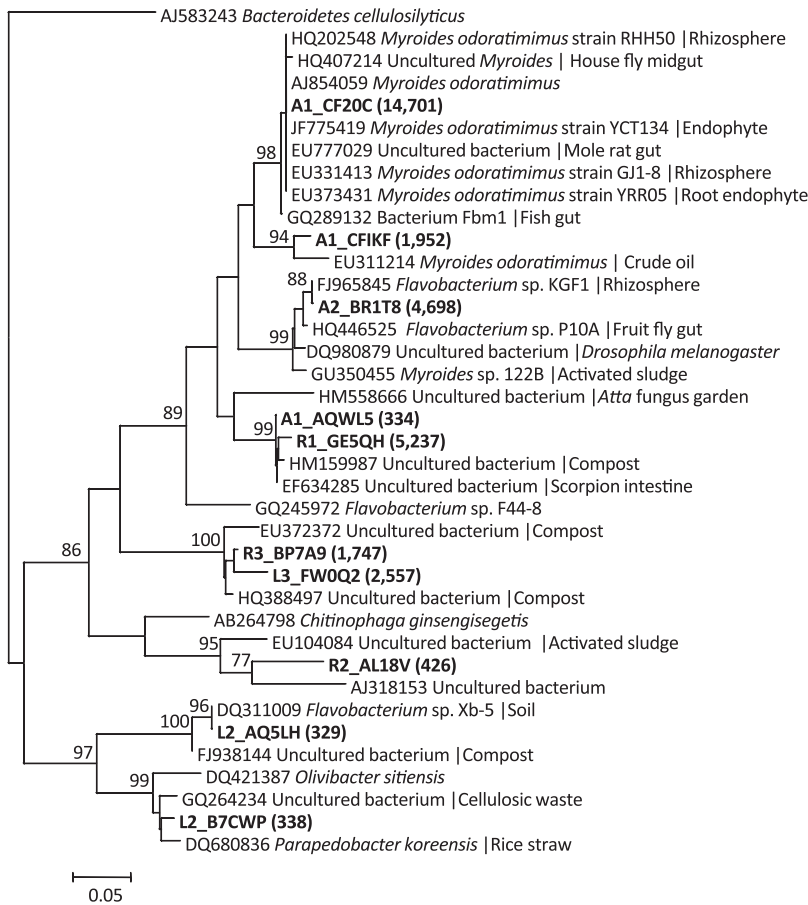


Figure 3. Phylogenetic tree of sequences representing the most abundant Bacteroidetes OTU 16S rRNA gene fragments obtained by mass sequencing and related sequences. A neighbor-joining tree was constructed using the ARB software package (31). Scale bar = 0.05 substitutions/nucleotide position. Sequences recovered from this study are in boldface. Numbers in brackets indicate the number of sequences (out of 64,241 in total) included in the specific OTU. The origin of the sample from which related sequences were recovered is indicated after the | symbol.

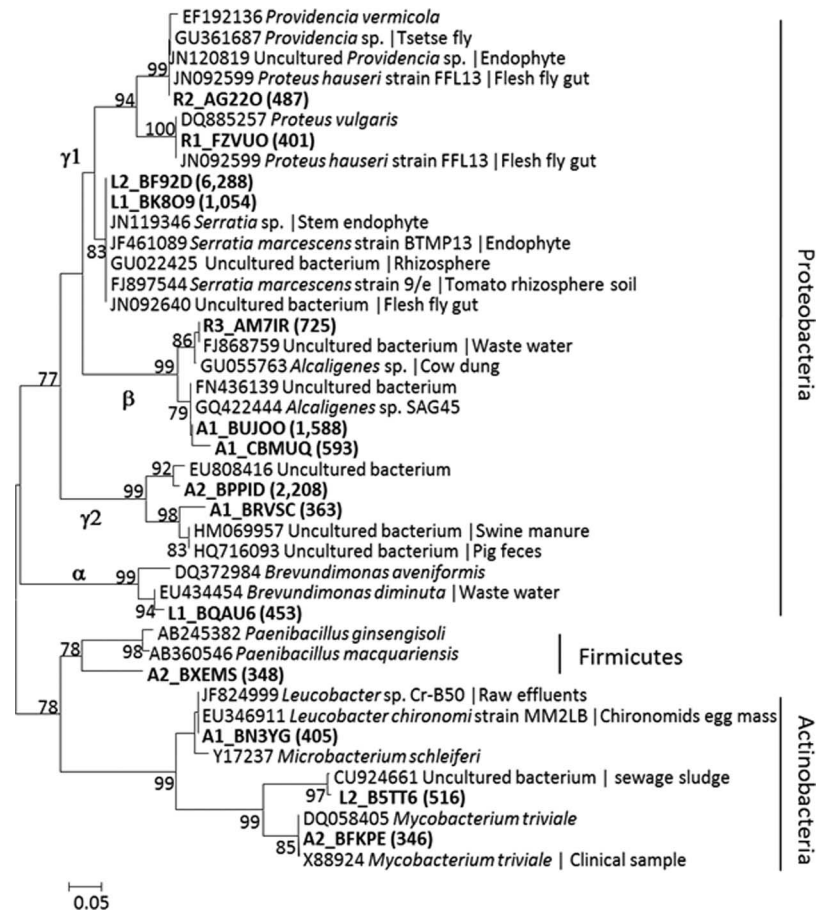
some studies have examined the ability of *R. robini* to transmit fungal and bacterial plant pathogens (32), bacterial symbionts of this plant-pest mite have never been studied. More important, the combination of culture-independent characterization of the associated microbiome with *in vivo* tests provides a unique view on the interactions between bacteria and their mite host.

A complex bacterial community was described in *R. robini* using DGGE. The analyzed mites were taken from 3 laboratory-reared populations, originally derived from infected material of 3 plant species. Initial comparison of the mites' bacterial profiles revealed shared dominant populations (Fig. 1), indicating the presence of an autochthonous core community. In contrast, several bands were only present in a subset of the analyzed individuals, suggesting a species-rich, facultative interaction. Due to the laboratory background of the populations used for this study, differences between populations are difficult to interpret in an evolutionary context. In other generalist feeders, a similarly complex DGGE profile has been observed (33), which lead us to the idea that arthropods with diverse diets might harbor rich microbial communities to assist them in overcoming the nutritional challenges to which they are exposed. Gerson *et al.* (9) describe *R. robini* as a generalist, feeding on different types of decaying material and fungi, consistent with the observed complex bacterial profile. In a preliminary attempt to look at field-collected populations, we detected an even greater diversity in bacterial community composition (pre-

liminary data, not shown) possibly reflecting a greater diversity of food sources found under natural conditions.

A metagenomic approach was used to further describe the bacterial diversity associated with the examined populations. Such an approach has been recently used for comprehensive elucidation of bacterial community compositions and structures in different arthropods, including fleas (34), honeybees (35), and ticks (36). High-throughput sequencing of bacterial 16S rRNA gene fragments revealed high taxonomic richness in the *R. robini* bacterial community (Table 1). Indeed, >700 OTUs, belonging to 113 different genera, were identified. However, for each of the populations examined, <5% of the OTUs had relative abundance values > 0.5% (Table 1), meaning that very few species represented the majority of bacterial cells in the community, while the rest were present in low relative abundance. The importance of rare species within this system cannot be neglected, as this genetic pool may become significant to the host following variations in environmental conditions (37). However, rare species may be nonresident (allochthonous) populations, ingested with the food and passing through the mite's elementary tract. Wong *et al.* (38) have stressed that allochthonous populations artificially inflate diversity in assessments of individual hosts as well as host populations. Due to the permissive feeding habits of *R. robini*, such a scenario is possible, and we would expect this to

Figure 4. Phylogenetic tree of sequences representing the most abundant Actinobacteria, Firmicutes, and Proteobacteria OTU 16S rRNA gene fragments obtained by mass sequencing and related sequences. A neighbor-joining tree was constructed using the ARB software package (31). Scale bar = 0.05 substitutions/nucleotide position. Sequences recovered from this study are in boldface. Numbers in brackets indicate the number of sequences (out of 64,241 in total) included in the specific OTU. The origin of the sample from which related sequences were recovered is indicated after the | symbol.



be even more pronounced in field-collected populations.

Bacteroidetes and Proteobacteria together represented 92% of the bacterial community (Fig. 2). Thus, the core community of resident bacteria of the mite pest consists of a restricted number of phyla and is shared by all 3 examined populations. Indeed, variation between populations was related to differences in relative abundance of the core populations, while differences in composition were uncommon (Table 2). Furthermore, the 24 most abundant OTUs, belonging to 14 genera, represented 74.9% of the bacterial community. A comparable magnitude of diversity has been found for adult blood-feeding fleas (34). In that study, the 23 most abundant OTUs represented ~50% of the bacterial community and included well-studied maternally transmitted endosymbionts belonging to the order Rickettsiales, or the genera *Cardinium* and *Bartonella*. In the survey of laboratory-reared *R. robini* populations presented here, at least 3 bacterial genera previously reported as intracellular endosymbionts were detected: *Serratia* sp. (39), *Flavobacterium* sp. (40), and *Alcaligenes* sp. (41). However, PCR assays using species-specific primers did not reveal the presence of the insect-endosymbionts *Flavobacterium*, *Wolbachia*, *Cardinium*, *Rickettsia*, or *Spiroplasma* in individual mites (unpublished results). Further studies are required to explore the localization (gut lumen or within the host, intra- or extracellular) and maternal transmission of

the bacteria found in order to characterize their individual associations with the mite. Maternal transmission as well as specialized structures such as bacteriocytes within the host would indicate a very close association and an important role of the symbiotic bacterium for the host.

The genera *Myroides*, *Serratia*, *Flavobacterium*, *Alcaligenes*, *Providencia*, *Proteus*, and *Brevundimonas* comprised the core bacterial community of the laboratory-reared *R. robini* populations tested (Figs. 4 and 5). These genera have been reported to share habitats, such as the soil and rhizosphere of plants (42–46) and, most relevant to this study, the gut of arthropods (47–51). *Serratia* sp. have previously been found associated with mites feeding on fungi (18). Some of the above mentioned genera include species and subspecies of bacteria that are pathogenic to insects and other arthropods (e.g., *Serratia* sp.; ref. 52). Also, we find many species with a generalist lifestyle among them, which could suggest a rather loose association with the host, and possibly exchange with the surrounding soil and plant environment. Such an exchange could make a major contribution to the mite's ability to become established and grow on its host plant. As demonstrated by Okabe and Amano (11), *R. robini* will preferentially attack fungus-infected or injured hosts, where its population establishment is considerably improved. While bulb injury physically facilitates the mite's movement through the plant tissue, exchange of bacteria with the

TABLE 2. Comparison of relative abundances of the most dominant bacterial OTUs between 3 laboratory-reared populations of *R. robini*

Phylogenetic affiliation	OTU representative	Relative abundance (%)		
		<i>Allium</i>	<i>Lilium</i>	<i>Ruscus</i>
Bacteroidetes				
Flavobacteria				
<i>Myroides</i>	A1_CF20C	19.36 ^b	9.95 ^b	35.65 ^a
	A2_BR1T8	12.65 ^a	7.98 ^{a,b}	4.14 ^b
	A1_CFIKF	2.85 ^{a,b}	1.33 ^b	4.97 ^a
<i>Flavobacterium</i>	R1_GE5QH	8.13	10.02	8.46
	L2_AQ5LH	0.22	0.76	0.64
Flavobacteriaceae	L3_FW0Q2	1.14 ^c	7.82 ^a	3.40 ^b
	R3_BP7A9	0.81	4.68	1.94
	A1_AQWL5	0.38	0.51	0.54
Sphingobacteria				
<i>Parapedobacter</i>	L2_B7CWP	0.03 ^b	1.40 ^a	0.51 ^{a,b}
Unclassified	R2_AL18V	0.02 ^b	0.32 ^b	1.46 ^a
γ -Proteobacteria				
<i>Serratia</i>	L2_BF92D	7.78	14.74	1.38
	L1_BK8O9	1.49	2.54	0.18
<i>Providencia</i>	R2_AG22O	0.06 ^b	0.54 ^{a,b}	1.51 ^a
<i>Proteus</i>	R1_FZVUO	0.08 ^b	0.48 ^b	1.21 ^a
Alcanivoracaceae	A2_BPPID	6.22 ^a	2.26 ^b	1.86 ^b
Pseudomonadaceae	A1_BRVSC	2.38 ^a	0.03 ^b	0 ^b
β -Proteobacteria				
<i>Alcaligenes</i>	A1_BUJOO	3.43	1.85	2.48
	R3_AM7IR	2.21	0.69	1.19
	A1_CBMUQ	1.95 ^a	0.23 ^b	0.97 ^{a,b}
α -Proteobacteria				
<i>Brevundimonas</i>	L1_BQAU6	0.15	1.25	1.04
Firmicutes				
Bacillaceae	A2_BXEMS	2.09 ^a	0.004 ^b	0 ^b
Actinobacteria				
<i>Mycobacterium</i>	A2_BFKPE	0.47	0.19	0.69
<i>Leucobacter</i>	A1_BN3YG	0.87	1.6	0.21
Actinomycetales	L2_B5TT6	0.35 ^b	1.74 ^a	0.43 ^b
Σ		75.12	72.91	74.86

Values are averages ($n=3$) of relative abundance for each OTU. OTU representative: sequence representing the OTU classified at 97% sequence similarity threshold. See Figs. 3 and 4. ^{a,b,c} Values with different superscript letters differ significantly ($P<0.05$).

host may support the mite's nutrition, particularly if the bacteria can efficiently predigest the fungal phytopathogen, the plant material, or both. Due to its high protein content (53, 54), a fungus-based diet may be favored by the mite. Besides the chitin, which requires the presence of chitinases, also other components from the cell content of the hyphae, such as trehalose, can be used by the mites (55).

The main taxonomic groups of the bacterial community associated with *R. robini* may differ in their chitinolytic activities. Chitinolytic activity of *Myroides* spp. has not been reported to date. However, the closest relative of the most dominant *Myroides*-related OTU in our dataset (Fig. 3) exhibits *in vitro* antagonism toward FO as well as *Rhizoctonia solani* (56). This finding suggests that chitinolytic activity for these members is possible. In contrast to *Myroides*, members of the genus *Serratia* (Enterobacteriaceae), several *Alcaligenes* species (Alcaligenaceae), and *Flavobacterium* species are known for their chitinase activity (57–64). *Serratia* species are among the most effective chitin-degrading bacteria and

the dominant species found here, *S. marcescens*, is able to produce at ≥ 4 different chitinase enzymes, as well as a chitin-binding protein (58). This set of biochemical capabilities has been shown to enable growth of this bacterium on fungal mycelium as a sole source of nutrition (64). Smrz and Catska (19) published a list of chitin-degrading bacteria found in association with mycophagous mites. This list comprises two *Serratia* sp., *S. liquefaciens*, and *S. marcescens*, isolated from *Tyrophagus putrescentiae*, another acarid mite to *R. robini*.

Because the results showed that *R. robini*'s microbiome abounds in chitinolytic bacteria, we investigated the mite's fecundity on a fungal diet as well as the holobiont's chitinase activity. The standard peanut diet and the FO mycelium diet are similar in that they are both rich in fats and protein. However, chitinase activity is required for efficient utilization of FO mycelia. Transferring the mites from the peanut diet to the FO diet did not change their fecundity (Fig. 5). This shows that *R. robini* readily feeds on fungus and apparently also possesses the ability to use it as a food source. The

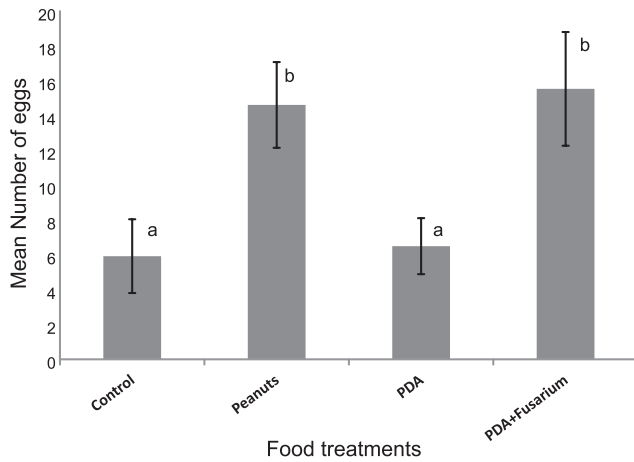


Figure 5. Number of eggs laid by same-age *R. robini* females after feeding on 4 different food sources for 2 d: control, no food; peanuts, nonsterilized ground peanuts; PDA; and PDA + FO (an ~7-d-old culture of FO on PDA) (ANOVA, $n=60$ /treatment; 3 degrees of freedom, $P<0.01$). All containers contained wet filter paper for moisture supply. ^{a,b}Bars with different letters differ significantly ($P<0.05$).

chitinase activity of mites associated with their bacterial community was evaluated by application of mite homogenate on a thin chitin layer, where enzymatic decomposition of the purified carbohydrate could be observed directly. The fact that homogenate of surface-sterile mites was able to produce a clear halo in the chitin layer can be interpreted as the presence of chitinases in either the mite's body itself or its microbiome (Fig. 6). Potential *R. robini* endogenous chitinases as well as present lysozyme, which can to a certain extent degrade chitin as well (65), cannot be distinguished from bacterial chitinases at this point. In mites, endogenous chitinases have to date only been isolated from Dermatophagoidae (66), where they were described as allergens to dogs and humans. However, bacteria picked from the haloes also grew on chitin as sole food source in subsequent cultivation and were able to produce a halo again. These results confirm that at least some of chitinases are of bacterial origin. The chitinolytic activity was obvious in all treatments, excluding the possibility that the food is the source of the activity. Moreover, this result shows that the potential to feed on fungi is maintained during periods of starvation (mites were starved for 2 d before being transferred to the different diets) or during periods of suboptimal nutrition (represented here by the no food control and PDA diets). This corresponds to the findings of Woody and Fashing (14) that the subsistence of *R. robini* on filter paper alone is dependent on the progressive development of cellulolytic fungi. The thriving of mites feeding on peanuts in the rearings as well as in this experiment could be explained by the fact that the peanuts fed to the mites are not sterilized, but kept dry and frozen. Prior to feeding, it only takes a few hours for fungi, probably vectored by the mites themselves, to start growing and serve as a food source for the mites. Also, *R. robini* has been shown to possess

cellulose to digest plant material (67). The results of the experiment further support the hypothesis that *R. robini* is a secondary pest, feeding at least to a certain extent, on the fungi growing on their host plant rather than on the host plant itself (unpublished results). Of course, it is highly likely that the mite actually feeds on a combination of fungal tissue and (predecomposed) plant material. Chitinase (along with trehalase and cellulase) activity has been used to classify the species of oribatid mites in feeding guilds as fungivorous grazers or herbofungivorous grazers (55). Based on the results of this study and those of Bowman and Childs (67), *R. robini* should probably be classified in the latter feeding category. However, trehalase activity would still have to be confirmed and further studies would be needed to determine the relative importance of the different food components in the mite's nutrition. Moreover, both of the above-mentioned studies did not distinguish between endogenous enzymes and enzymes of potential bacterial origins. Also, in our system, we cannot exclude the presence of additional endogenous chitinases in the mite, besides the enzymatic activity we could assign to culturable bacteria. Finally, we would like to mention the possibility of some of the associated bacteria themselves being a food source to the mite, as shown and discussed in Erban and Hubert (68).

We show here that the diverse bacteriome associated with an arthropod can be responsible for the host's ability to use a wide range of food sources, including fungi, which are otherwise mostly unavailable. The data support a growing number of studies stressing the

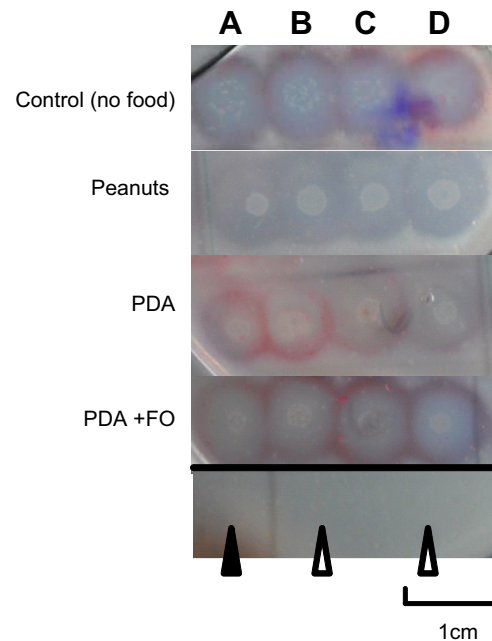


Figure 6. Demonstration of chitin degradation (halos) around 1- μ l droplets of mite homogenate diluted from 1:20 (A) to 1:4 (D). Mites were previously fed on different food sources: control, no food; peanuts, nonsterilized ground peanuts; PDA + FO (~7 d old culture of FO on PDA). The photo was taken after 24 h of incubation. *E. coli* (open arrowheads) and pure saline solution (solid arrowhead) were used as negative controls.

importance of considering the multigenome organism (arthropod with its microbiome) when trying to unravel the complex life strategy of certain arthropods. Expanding our knowledge of this neglected aspect of arthropod biology and detailing the different species in the microbiome might open up possibilities for various manipulations of these biological systems toward, for example, eliminating essential bacteria, disrupting the community's composition and function, or controlling fungal infection, to achieve effective pest or disease-vector control. Also, the number of studies investigating bacterial communities associated with arthropods and other organisms is quickly increasing and will help with the general interpretation of diversity and composition and give a stronger base for extrapolation in general patterns. **FJ**

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REFERENCES

- Zindel, R., Gottlieb, Y., and Aebi, A. (2011) Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. *J. Appl. Ecol.* **48**, 864–872
- Dillon, R. J., and Dillon, V. M. (2004) The gut bacteria of insects: Nonpathogenic interactions. *Ann. Rev. Entomol.* **49**, 71–92
- Guenduez, E. A., and Douglas, A. E. (2009) Symbiotic bacteria enable insect to use a nutritionally inadequate diet. *Proc. R. Soc. Lond. B Biol. Sci.* **276**, 987–991
- Grünwald, S., Pilhofer, M., and Höll, W. (2010) Microbial associations in gut systems of wood- and bark-inhabiting long-horned beetles [Coleoptera: Cerambycidae]. *Syst. Appl. Microbiol.* **33**, 25–34
- Snyder, A. K., Deberry, J. W., Runyen-Janecky, L., and Rio, R. V. M. (2010) Nutrient provisioning facilitates homeostasis between tsetse fly (Diptera: Glossinidae) symbionts. *Proc. R. Soc. Lond. B Biol. Sci.* **277**, 2389–2397
- Feldhaar, H., and Gross, R. (2009) Insects as hosts for mutualistic bacteria. *Int. J. Med. Microbiol.* **299**, 1–8
- Sabree, A. L., Kambampati, S., and Moran, N. A. (2009) Nitrogen recycling and nutritional provisioning by *Blattabacterium*, the cockroach endosymbiont. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 19521–19526
- Manson, D. C. M. (1972) A contribution to the study of the genus *Rhizoglyphus* Claparède, 1869 (Acarina: Acaridae). *Acarologia* **13**, 621–650
- Gerson, U., Cohen, E., and Capua, S. (1991) Bulb mite, *Rhizoglyphus robini* (Astigmata Acaridae) as an experimental animal. *Exp. Appl. Acarol.* **12**, 103–110
- Hanuny, T., Inbar, M., Tsrur, L., and Palevsky, E. (2008) Complex interactions between *Rhizoglyphus robini* and *Fusarium oxysporum*: implications on onion pest management. *IOBC Proceedings of Integrated Control of Protected Crops, Temperate Climate. IOBC/WPRS Bulletin* **32**, 71–74
- Okabe, K., and Amano, H. (1991) Penetration and pop growth of the robine bulb mite, *Rhizoglyphus robini* Claparède on healthy and *Fusarium*-infested rakkyo bulbs. *Appl. Entomol. Zool.* **26**, 129–136
- Okabe, K., and Amano, H. (1990) Attractancy of alcohols isolated from culture filtrates of *Fusarium* fungi for the robine bulb mite *Rhizoglyphus robini* Claparède (Acari, Acaridae) in sand. *Appl. Entomol. Zool.* **25**, 397–404
- Okabe, K., and Oconnor, B. M. (2001) A method for both mass and individual rearing of fungivorous astigmatid mites (Acari). *Exp. Appl. Acarol.* **25**, 493–504
- Wooddy, M. W., and Fashing, N. J. (1993) The ability of *Rhizoglyphus robini* Claparède (Astigmata: Acaridae) to subsist solely on a diet of filter paper. *Int. J. Acarol.* **19**, 345–348
- Hudson, H. J. (1986) *Fungal Biology*. Edward Arnold, London
- Smrz, J., and Soukalova, H. (2008) Mycophagous mites (Acari: Oribatida and Acaridida) and their cooperation with chitinolytic bacteria. In *Integrative Acarology*. Proceedings of the 6th European Congress (Bertrand, M., Kreiter, S., McCoy, K. D., Migeon, A., Navajas, M., Tixier, M.-S., and Vial, L., eds.) pp. 374–377, EURAAC, Montpellier, France
- Arakane, Y., and Muthukrishnan, S. (2010) Insect chitinase and chitinase-like proteins. *Cell. Mol. Life Sci.* **67**, 201–216
- McCall, C., Hunter, S., Stedman, K., Weber, E., Hillier, A., Bozic, C., Rivoire, B., and Olivry, T. (2001) Characterization and cloning of a major high molecular weight house dust mite allergen (Der f 15) for dogs. *Vet. Immunol. Immunopathol.* **78**, 231–247
- Smrz, J., and Catska, V. (2010) Mycophagous mites and their internal associated bacteria cooperate to digest chitin in soil. *Symbiosis* **52**, 33–40
- Frohlich, D. R., Torres-Jerez, I., Bedford, I. D., Markham, P. G., and Brown, J. K. (1999) A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Mol. Ecol.* **8**, 1683–1691
- Muyzer, G., Dewaal, E. C., and Uitterlinden, A. G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S ribosomal RNA. *Appl. Environ. Microbiol.* **59**, 695–700
- Gottlieb, Y., Ghanim, M., Chiel, E., Gerling, D., Portnoy, V., Steinberg, S., Tzuri, G., Horowitz, A. R., Belausov, E., Mozes-Daube, N., Kotsedalov, S., Gershon, M., Gal, S., Katzir, N., and Zchori-Fein, E. (2006) Identification and localization of a *Rickettsia* sp. in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Appl. Environ. Microbiol.* **72**, 3646–3652
- Dowd, S. E., Callaway, T. R., Wolcott, R. D., Sun, Y., McKeehan, T., Hagevoort, R. G., and Edrington, T. S. (2008) Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiol.* **8**, 125
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., and Weber, C. F. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**, 7537–7541
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739
- Good, I. J. (1953) The population frequencies of species and the estimation of the population parameters. *Biometrika* **40**, 237–264
- Chao, A. (1984) Non-parametric estimation of the number of classes in a population. *Scand. J. Stat.* **11**, 265–270
- McBride, M. J., and Braun, T. F. (2004) GldI is a lipoprotein that is required for *Flavobacterium johnsoniae* gliding motility and chitin utilization. *J. Bacteriol.* **186**, 2295–2302
- Reichenbach, H. (2006) The genus *Lysobacter*. In *The Prokaryotes* (Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K. H., and Stackebrandt, E., eds) pp 939–957, Springer, New York
- Faramarzi, M. A., Fazeli, M., Tabatabaei Yazdi, M., Adrang, S., Jami Al Ahmadi, K., Tasharrofi, N., and Aziz Mohseni, F. (2009) Optimization of cultural conditions for production of chitinase by a soil isolate of *Massilia timonae*. *BioTechnology* **8**, 93–99
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Forster, W., Brettske, I., Gerber, S., Ginhart, A.W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lussmann, R., May,

- M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., and Bode, A. (2004) ARB: a software environment for sequence data. *Nucleic Acids Res.* **32**, 1363–1371
32. Díaz, A., Okabe, K., Eckenrode, C. J., Villani, M. G., and Oconnor, B. M. (2000) Biology, ecology, and management of the bulb mites of the genus *Rhizoglyphus* (Acari: Acaridae). *Exp. Appl. Acarol.* **24**, 85–113
33. Mrázek, J., Štrosová, L., Fliegerová, K., Kott, T., and Kopečný, J. (2008) Diversity of insect intestinal microflora. *Folia Microbiol.* **53**, 229–233
34. Jones, R. T., Knight, R., and Martin, A. P. (2010) Bacterial communities of disease vectors sampled across time, space, and species. *ISME J.* **4**, 223–231
35. Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., Quan, P. L., Briese, T., Hornig, M., Geiser, D. M., Martinson, V., vanEngelsdorp, D., Kalkstein, A. L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S. K., Simons, J. F., Egholm, M., Pettis, J. S., and Lipkin, W. I. (2007) A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* **318**, 283–287
36. Andreotti, R., de León, A. A. P., Dowd, S. E., Guerrero, F. D., Bendele, K. G., and Scoles, G. A. (2011) Assessment of bacterial diversity in the cattle tick *Rhipicephalus (Boophilus) microplus* through tag-encoded pyrosequencing. *BMC Microbiol.* **11**, 6
37. Zilber-Rosenberg, I., and Rosenberg, E. (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.* **32**, 723–735
38. Wong, C. N. A., Ng, P., and Douglas, A. E. (2011) Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environ. Microbiol.* **13**, 1889–1900
39. Oliver, K. M., Moran, N. A., and Hunter, M. S. (2006) Costs and benefits of a superinfection of facultative symbionts in aphids. *Proc. R. Soc. Lond. B Biol. Sci.* **273**, 1273–1280
40. Bandi, C., Damiani, G., Magrassi, L., Grigolo, A., Fani, R., and Sacchi, L. (1994) *Flavobacteria* as intracellular symbionts in cockroaches. *Proc. R. Soc. Lond. B Biol. Sci.* **257**, 43–48
41. Bextine, B., Lauzon, C., Potter, S., Lampe, D., and Miller, T. A. (2004) Delivery of a genetically marked *Alcaligenes* sp. to the glassy-winged sharpshooter for use in a paratransgenic control strategy. *Curr. Microbiol.* **48**, 327–331
42. Tripathi, A. K., Verma, S. C., and Ron, E. Z. (2002) Molecular characterization of a salt-tolerant bacterial community in the rice rhizosphere. *Res. Microbiol.* **153**, 579–584
43. Berg, G., Krechel, A., Ditz, M., Sikora, R. A., Ulrich, A., and Hallmann, J. (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiol. Ecol.* **51**, 215–229
44. Harichová, J., Karellová, E., Chovanová, K., Stojnev, T., Prokšová, M., Brindza, J., Brindza, P., Tóth, D., Pangallo, D., and Ferienc, P. (2006) Comparison of culturable gram-negative bacterial community structures in the rhizosphere of three fruit plants. *Biologia* **61**, 663–670
45. Teixeira, L., Peixoto, R. S., Cury, J. C., Sul, W. J., Pellizari, V. H., Tiedje, J., and Rosado, A. S. (2010) Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. *ISME J.* **4**, 989–1001
46. Jin, F., Ding, Y., Ding, W., Reddy, M. S., Fernando, D. W. G., and Du, B. (2011) Genetic diversity and phylogeny of antagonistic bacteria against *Phytophthora nicotianae* isolated from tobacco rhizosphere. *Int. J. Mol. Sci.* **12**, 3055–3071
47. Spiteller, D., Dettner, K., and Boland, W. (2000) Gut bacteria may be involved in interactions between plants, herbivores and their predators: Microbial biosynthesis of N-acylglutamine surfactants as elicitors of plant volatiles. *Biol. Chem.* **381**, 755–762
48. Jaffe, K., Caetano, F. H., Sanchez, P., Hernandez, J., Caraballo, L., Vitelli-Flores, J., Monsalve, W., Dorta, B., and Lemoine, V. R. (2001) Sensitivity of ant (*Cephalotes*) colonies and individuals to antibiotics implies feeding symbiosis with gut microorganisms. *Can. J. Zool.* **79**, 1120–1124
49. Dharne, M. S., Gupta, A. K., Rangrez, A. Y., Ghate, H. V., Patole, M. S., and Shouche, Y. S. (2008) Antibacterial activities of multi drug resistant *Myroides odoratimimus* bacteria isolated from adult flesh flies (Diptera: Sarcophagidae) are independent of metallo beta-lactamase gene. *Braz. J. Microbiol.* **39**, 397–404
50. Zaspel, J. M., and Hoy, M. A. (2008) Microbial diversity associated with the fruit-piercing and blood-feeding moth *Calyptra thalictri* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* **101**, 1050–1055
51. Wang, H., Jin, L., and Zhang, H. (2011) Comparison of the diversity of the bacterial communities in the intestinal tract of adult *Bactrocera dorsalis* from three different populations. *J. Appl. Microbiol.* **110**, 1390–1401
52. Kleespies, R. G., Huger, A. M., and Zimmermann, G. (2008) Diseases of insects and other arthropods: results of diagnostic research over 55 years. *Biocontrol. Sci. Technol.* **18**, 439–482
53. Christias, C., Couvarki, C., Georgopoulos, S. G., Macris, B., and Vomvovanni, V. (1975) Protein content and amino acid composition of certain fungi evaluated for microbial protein production. *Appl. Microbiol.* **29**, 250–254
54. Srivastava, S., Pathak, A., and N. Srivastava, P. (2011) Identification of limiting factors for the optimum growth of *Fusarium oxysporum* in liquid medium. *Toxicol. Int.* **18**, 111–116
55. Siepel, H., and de Ruiter-Dijkman, E. M. (1993) Feeding guilds of oribatid mites based on their carbohydrase activities. *Soil Biol. Biochem.* **25**, 1491–1497
56. Seo, W. T., Lim, W. J., Kim, E. J., Yun, H. D., Lee, Y. H., and Cho, K. M. (2010) Endophytic bacterial diversity in the young radish and their antimicrobial activity against pathogens. *J. Korean. Soc. Appl. Biol. Chem.* **53**, 493–503
57. Monreal, J., and Reese, E. T. (1969) The chitinase of *Serratia marcescens*. *Can. J. Microbiol.* **15**, 689–696
58. Brurberg, M. B., Synstad, B., Klemsdal, S. S., VanAalten, D. M. F., Sundheim, L., and Eijsink, V. G. H. (2001) Chitinases from *Serratia marcescens*. *Recent Res. Dev. Microbiol.* **5**, 187–204
59. Vaidya, R., Roy, S., Macmil, S., Gandhi, S., Vyas, P., and Chhatpar, H. S. (2003) Purification and characterization of chitinase from *Alcaligenes xylosoxydans*. *Biotechnol. Lett.* **25**, 715–717
60. McBride, M. J., Xie, G., Martens, E. C., Lapidus, A., Henrissat, B., Rhodes, R. G., Goltsman, E., Wang, W., Xu, J., Hunnicutt, D. W., Staroscik, A. M., Hoover, T. R., Cheng, Y. Q., and Stein, J. L. (2009) Novel features of the polysaccharide-digesting gliding bacterium *Flavobacterium johnsoniae* as revealed by genome sequence analysis. *Appl. Environ. Microbiol.* **75**, 6864–6875
61. Annamalai, N., Rajeswari, M. V., Vijayalakshmi, S., and Balasubramanian, T. (2011) Purification and characterization of chitinase from *Alcaligenes faecalis* AU02 by utilizing marine wastes and its antioxidant activity. *Ann. Microbiol.* **61**, 801–807
62. Hariprasad, P., Divakara, S. T., and Niranjana, S. R. (2011) Isolation and characterization of chitinolytic rhizobacteria for the management of *Fusarium* wilt in tomato. *Crop Protect.* **30**, 1606–1612
63. Someya, N., Ikeda, S., Morohoshi, T., Tsujimoto, M. N., Yoshida, T., Sawada, H., Ikeda, T., and Tsuchiya, K. (2011) Diversity of culturable chitinolytic bacteria from rhizospheres of agronomic plants in Japan. *Microbes Environ.* **26**, 7–14
64. Ordentlich, A., Elad, Y., and Chet, I. (1988) The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. *Phytopathology* **78**, 84–88
65. Childs, M., and Bowman, C. E. (1981) Lysozyme activity in 6 species of economically important astigmatid mites. *Comp. Biochem. Phys. B Biochem. Mol. Biol.* **70**, 615–617
66. O'Neil, S. E., Heinrich, T. K., Hales, B. J., Hazell, L. A., Holt, D. C., Fischer, K., and Thomas, W. R. (2006) The chitinase allergens Der p 15 and Der p 18 from *Dermatophagoides pteronyssinus*. *Clin. Exp. Allergy* **36**, 831–839
67. Bowman, C. E., and Childs, M. (1982) Polysaccharidases in astigmatid mites (Arthropoda, Acari). *Comp. Biochem. Phys. B* **72**, 551–557
68. Erban, T., and Hubert, J. (2008) Digestive function of lysozyme in synanthropic acaridid mites enables utilization of bacteria as a food source. *Exp. Appl. Acarol.* **44**, 199–212