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End-of-degree project
Degree in Biochemistry and Molecular Biology

Honey bee bacteriome in agricultural and pristine environments

Characterization of the bacteriome associated to honey bees (*Apis mellifera*) located in agricultural and pristine environments and possible implications in honey bee health

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Introduction and objectives

Honey bees (*Apis mellifera*) are the most important pollinator, being the main responsible for the pollination of natural ecosystems and a big part of the crop plants that feed us. Furthermore, they are of high economic interest since they produce propolis and honey for human consumption. However, in the last years honey bee populations have been declining at an unprecedented rate worldwide. Evidences have been mounting that there are many factors affecting honey bee health, such as climate change, pour nutrition and several pathogens and parasites, such as varroa mite (*Varroa destructor*) that affect them [1][2]. Additionally, honey bees used in agriculture are stressed by constant transport and crowding, and are exposed to a large number of herbicides, insecticides and other chemicals which damage their health and may modify their behaviour [3]. These factors do not only affect honey bees that are directly used for crop pollination, but also those that are close to agricultural areas indirectly.

Nowadays, the vast number of honey bees in apiaries is maintained mainly with the use of biocides to control parasites and supplemental feeding. It is known that social insects [4], like honey bees, have significantly fewer immune genes than expected compared to other insects, so recently work has begun to study the contribution of symbiotic microbes associated with honey bees to preserve their health, discovering that they play a key role in that issue [5]. These microbial communities are thought to aid honey bees in nutrition and defend them against pathogens, but the niche requirements and maintenance of beneficial honey bee symbionts remain largely unknown.

Honey bee gut core microbiome has already been characterized through culture-independent methods [6][7]. Depending on the article, bacterial group proportions in the gut differ among different studies or methodological approaches, but some bacteria appear consistently, even if in different abundance, in almost all the samples. For this reason, they have been considered the core of honey bee gut bacteriome. This is the case of *Lactobacillus* sp., *Gilliamella apicola*, *Snodgrassella alvi*, *Bifidobacterium* sp., *Frischella perrara* and Acetobacteriaceae family. Kakukamu *et al.* (2016) demonstrated that honey bee intestinal microbiome is altered by in-hive pesticide exposures [8]. In this study, when colonies were treated with chlorothalonil, the relative abundance of Lactobacillaceae was observed to decline, whereas Enterobacteriaceae and Caulobacteraceae families exhibited a relative increase.

Knowing that, in this project, samples from two opposite environments have been collected during June 2017 to characterize the bacterial community associated to the honey bees raised in each of them. One of the apiaries was located in Čeminac city, in the Osijek-Baranja region of Croatia, and the other one located in Unije island, about 40 km far from the mainland (a map showing the location of each sampling environment is given in the Supplementary Figure 1). The apiary in Čeminac is surrounded by crop fields and intensive agriculture, so honey bees are under the influence of human activity, pesticides, herbicides, etc. In addition, bees raised here are treated against varroa mite (*Varroa destructor*) with the acaricide *Checkmite*, since they are not naturally resistant to the parasite. For this experiment, half of the hives were kept untreated this year

to check the effect of the treatment over their microbiome. On the contrary, Unije is a small island with only 90 registered habitants, which makes it almost a wild place. Bees only travel an average of 4.5 km during their pollination flights [9], so the distance from the mainland makes sure that honey bees from Unije are isolated from both intensive crops and honey bees from the agricultural environment. Bees in this island show natural resistance to varroa, so even if the mite is present in their colonies, they survive without any treatment or human help. Apart from the bees original from the island, in this study we will also analyse bees from the agricultural apiary which had been translated to Unije at the beginning of June, two weeks prior to sampling.

The objective of this report was to make a comparative analysis of the bacterial communities present in each sample type (each of the micro-environments sampled from a hive) for each factor: treatment against varroa (treated and untreated samples), location (agricultural and pristine environments), and origin (within colonies located in pristine environment we will evaluate the bacteriome of colonies moved from Baranja plus colonies original from Unije). A better understanding of the effect that agriculture and beekeeping treatments have on the bacteriome of different parts of the hive may give us new insights into how to keep and improve honey bee health, possibly through the integrated management of honey bee microbial systems.

To date, studies have been mainly focused on the microbiome present in the gut of honey bees. A novelty of this work is that samples did not only include honey bees (gut), but also different environments inside the hive, such as pollen bread (or bee bread), brood, air from inside the colony and the material stuck to the entrance of the hive. Results from these sample types could provide new basis of knowledge. Understanding the hive as a superorganism, it is important to keep in mind the complex interactions between the different parts of it, so analysing the complete set of hive environments could be much more enriching and informative than just focusing on single honey bees. However, even if all the mentioned sample types were extracted and sequenced in this study, the bioinformatics analysis was done deeper for gut samples, since the big volume of data exceeded the characteristics of this work. The rest of sample types remains ready for posterior studies.

Materials and Methods

Sample collection

In June 2017, a total of 199 samples were collected from 22 colonies located in Baranja (11 treated against Varroa and 11 untreated) and 21 in Unije island (11 translated from Baranja and 10 original from Unije). Hereafter, Baranja will be referred to in this report as “agricultural environment” and Unije as “pristine environment”. The nomenclature of the samples was established as follows: B2-B12 (agricultural environment, treated), B13-B24 (agricultural environment, untreated), BU (pristine environment, original

from the agricultural environment), U (located in and original from the pristine environment). When it was possible, 5 sample types were collected from each colony, comprising pollen bread (a piece about 8 cm^2 in a zip bag), brood (a piece about 12 cm^2 in a zip bag), adult bee's gut (a 50 mL Falcon tube full of worker bees), filtered air from the colony (filters) and microorganisms stuck to entrance of the hive through swabs. The samples collected and the names given to them are illustrated in the Supplementary Table 1. To ensure sterility, all the material used was previously exposed to ultraviolet light and then cleaned with ethanol 100%. After collection, samples were put into dry ice and the cold chain (-20 °C) was kept until their arrival to the University of the Basque Country, where they were kept frozen until DNA extraction.

Processing of the samples and DNA extraction

Each sample from each colony was processed separately, avoiding contamination among them. For each gut (G) sample (N = 43), 10 guts from worker bees were extracted, put together in a 1.5 mL tube, homogenized in 600 µL PBS and centrifuged at 8000 rpm for 5 minutes. The supernatant was then put into a clean 1.5 mL tube (*clarifiat*). The pellet was resuspended in 400 µL PBS, homogenized, and centrifuged as in the previous step for recovering more supernatant. 200 µL of this *clarifiat* were used for DNA extraction with the QIAamp® DNA Mini Kit (Qiagen), following the protocol described by the manufacturer.

Regarding pollen bread (PB) samples (N = 43), for each pollen bread piece, the content of 3 or 4 cells was put into a 2 mL tube. 200 µL PBS and 400 µL AL buffer plus 40 µL Proteinase K from the QIAamp® DNA Mini Kit were added and the tubes were incubated at 56 °C for 1h to do the cell lysis. After a brief centrifugation the supernatant was put into a new tube and 600 µL Phenol:Chloroform:Isoamyl alcohol (25:24:1) were added. Then, the mixture was mixed and centrifuged at 14000 rpm for 15 min, and the supernatant was recovered and put into a new tube with 600 µL of chloroform. Afterwards, the tube was mixed and centrifuged at 14000 rpm for 5 min. The supernatant was put into a new tube with 400 µL of ethanol 100%, the tube was shaken and the liquid in it was applied to the columns provided in the DNA extraction kit. From this step on, the kit protocol instructions were followed.

In the case of Brood (B) samples (N = 38), two black eyed pupae were taken and their head was taken off due to the known inhibitors of PCR present in the eyes [10]. The two bodies without head were put into a precellys tube with 1 mL of PBS and homogenized. The homogenized was centrifuged at 8000g for 10 min at 4 °C and the resulting supernatant (*clarifiat*) was put into a new 1.5 mL tube. In addition, 100 mL of PBS were used to clean the hive cells where the larvae were taken and put together with the *clarifiat*, in order to recover all the microorganisms present in the brood cells environment. 200 µL of this *clarifiat* were used as input for QIAamp® DNA Mini Kit and the manufacturer instructions were followed.

Concerning DNA extraction of swabs (S) (N = 43), for each sample, the cotton parts of two swabs were put into a 2 mL tube with 400 µL of PBS, 20 µL of Proteinase K and 400 µL of AL Buffer. The tubes were vortexed and incubated at 56 °C for 90 min rotating at 900 rpm. The supernatant was recovered and put into

a new tube. This process was repeated twice with the same swabs to recover as many microorganisms as possible. 400 µL of ethanol were added to the tube containing the supernatant from the two steps, and it was vortexed. 700 µL of the mix were applied to the QIAamp® columns, following the manufacturer's instructions.

Finally, DNA from filters (F) (N = 32) was extracted using PowerSoil® DNA Isolation Kit, following MO BIO Laboratories' protocol (https://mobio.com/media/wysiwyg/pdfs/protocols/12888.pdf).

16S rRNA gene amplification and sequencing

A fragment of the V4 region of the 16S rRNA ribosomal gene was amplified using the primers 515F and 806R, which are included in the "Earth Microbiome Project" (http://www.earthmicrobiome.org/). A barcode sequence (12 bp) to identify each sample was bound to the forward primers and the Illumina sequencing adaptors were bound in both forward and reverse primers. The PCR conditions specified in Earth Microbiome Project to amplify this fragment of 16S rRNA gene with Illumina Amplicon protocol were followed. The PCR program was the following: an initial denaturing step at 95 °C for 4 min; and 35 cycles of 15 s at 95 °C, 30 s at 50 °C, 30 s at 72 °C, plus a final elongation of 2 min at 72 °C. In the case of Pollen Bread, additionally to the rest of PCR reagents, PNA clamps (called "blocking primers" in this report) were used to avoid the amplification of DNA from mitochondria and chloroplast [11]. For this purpose, 0.35 µL of PNA clamps (50 µM of both mPNA and pPNA) were added to the PCR mix, and PCR cycles were the denaturing step at 95 °C for 45 s; and 34 cycles of 15 s at 95 °C, 10 s at 78 °C, 30 s at 50 °C, plus 30 s at 72 °C. The resulting PCR products were checked in a 1.5% agarose gel stained with ethidium bromide.

The purification of PCR products, preparation of the libraries and sequencing were performed at the General Genomics Service Sequencing and Genotyping Unit of the University of the Basque Country. A paired-end sequencing of the samples pool was carried out on an Illumina MiSeq sequencer using the kit v2 PE 2 x 150 bp (300 cycles) and adding 10% of PhiX as the control of the sequencing process.

Bioinformatic analysis

Quality based trimming of the raw sequences was performed with the Sickle v1.33 program [12], using a threshold of Q20, and forward and reverse sequences were assembled with PEAR v0.9.10 [13], using an overlap of 15 bp. Non-existent barcodes were removed with the script *fastq-barcode.pl* and the Mapping File was corrected through *validate_mapping_file.py*. Afterwards, QIIME v1.9 [14] was used for demultiplexing the samples (*demultiplex_fasta.py*script), clustering the sequences in Operationa Taxonomic Units (OTUs), and assigning taxonomy to the OTUs (*pick_open_reference_otus.py)*. Since species concepts are difficult to apply to bacteria, <3% sequence divergence is considered standard for grouping bacteria into Operational Taxonomic Units (OUTs), so sequences were clustered at 97% similarity. The taxonomic assignment was performed against Silva 128 database, with an identity percentage of 97%. In order to

remove mitochondrial and chloroplast sequences, the script *filter_taxa_from_otu_table.py* was applied, and singletons were removed with the script *filter_otus_from_otu_table.py*, using n=10 as singleton threshold. In addition, samples with less than 6,000 sequences were excluded because they did not reach the minimum of sequences to have a representative sample of the present community, as observed in the rarefaction curves generated with the script *alpha_rarefaction.py* (not included in this report).

Thereafter, to assess species richness of each sample type, the complete table was normalized with *single_rarefaction.py* using a depth of 10,000 reads. The resulting table was used as input for calculating the alpha diversity (observed OTUs and Chao1 index) of the samples using R v3.3.3 (http://www.rstudio.com), *phyloseq* package. Alpha values were represented in a box plot grouped by sample type (Gut, Pollen Bread, Brood, Filters and Swabs). Beta diversity was also estimated with *phyloseq*, using the Bray Curtis metric, and a Principate Coordinate Analyses (PCoA plot) was generated. In this case the dataset was previously normalized with the CCS method in Qiime v1.9 (*normalize_table.py*). In addition, we evaluated if the bacterial community present in each sample type was significantly different with an ANOSIM test, through the script *compare_categories.py*.

Later, the complete dataset was split by sample type (*split_otu_table.py*), generating the following sub-datasets: gut (G), pollen bread (PB), brood (B), filters (F) and swabs (S). The following procedures were applied to the gut samples sub-dataset separately, while the rest of sub-datasets were kept for future analyses. On the one hand, the gut sub-dataset was normalized by single rarefaction (with a depth of 32,107 reads) and alpha diversity box plots were generated in R, grouping the samples by location. On the other hand, the sub-dataset was also normalized with DESeq2 method (*normalize_table.py* script in Qiime v1.9), which is recommended for groups with N<50, and beta diversity matrix was calculated. After, PCoA plots (in R) and a UPGMA tree (with Qiime) were performed in basis of that matrix. To check if the bacterial community present in the honey bees' gut is significantly different in those that have risen in different locations (pristine and agricultural), an ANOSIM test was conducted. Then, the OTUs were summarized by taxonomic levels using the script *summarize_taxa.py* and a Kruskal-Wallis test was performed to know the bacterial families that were more relevant in the bacteriome differences between the two locations. The rest of bacterial families were filtered out with the command *filter_taxa_from_otu_table.py* and the relative abundances of these relevant families were studied and represented in bars plots using the script *summarize_taxa_through_plots.py*. Within relevant families, and focusing the attention on the genera with abundance higher or equal to 0.1%, another Kruskal-Wallis test was conducted to evaluate what OTUs (strains) had different relative abundance in the samples from different locations. The OTUs that had significant values in this test were represented in a heatmap, using the *pheatmap* package of R, and clustering the samples and OTUs according to a distance matrix (Manhattan) based on their abundance.

The gut sub-set was further split according to location, creating 2 other sub-sets: pristine and agricultural location. Within each location, other parameters were studied. In agricultural samples we evaluated the bacteriome differences between treated (B2-B12) and untreated (B13-B24) samples, whereas

in pristine samples we evaluated the bacteriome differences between those samples original from Unije (pristine origin, U) and those which had been translated from Baranja (agricultural origin, BU). The alpha and beta diversities were drawn exactly as it has been described above and the significance of these parameters was evaluated with the previously mentioned ANOSIM test.

Results

From the 199 samples extracted, 192 were amplified and sequenced successfully (the fraction of samples sequenced per samples collected for each sample category is represented in the Supplementary Table 1).

Our efforts produced a total of 9,633,456 reads, after quality filtering, assembling and removing singletons (N<10), and chloroplast and mitochondrial sequences. Gut samples contained a total of 4,113,625 reads (mean=95,665.70 reads/sample); Pollen Bread samples, 958,333 reads (mean=23,373.98 reads/sample); Brood samples, 1,956,816 reads (mean=57,553.41 reads/sample); Filters, 388,095 reads (mean=12,127.97 reads/sample) and, finally, Swabs, 2,216,587 reads (mean=52,775.88 reads/sample).

The complete dataset alpha diversity box plot [Figure 1] showed that G samples had the lowest alpha diversity and S, the highest. These two sample types were also the ones whose samples had the greatest variance among the samples, as it is reflected in the boxes range. PB, B and F had all a similar alpha diversity, ranging between G and S medians. PCoA plot based on the beta diversity matrix [Figure 2] showed gut samples clearly separated, suggesting that they have the most different bacterial community respect to the others, with the 20.5% of diversity explained by the first axis. Among the rest of samples, the S and B samples seemed to have a particular composition despite being close to PB and F. These last two were more difficult

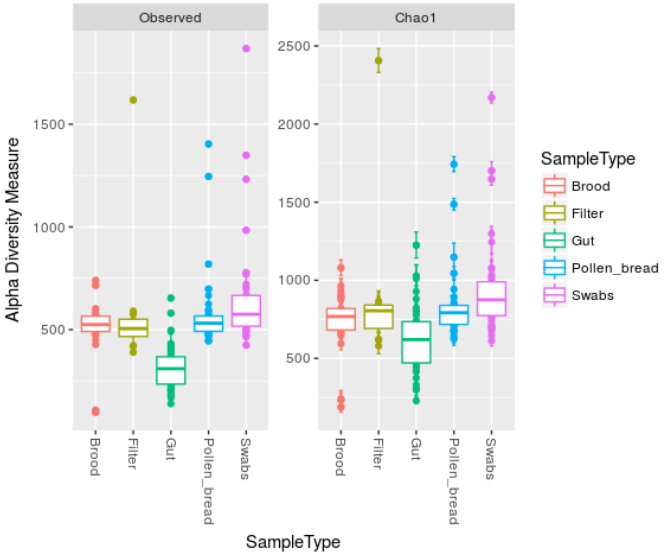

Figure 1. Alpha diversity box plot for each sample type. Bacterial richness was calculated with the "Observed OTUs" and "Chao1" metrics.

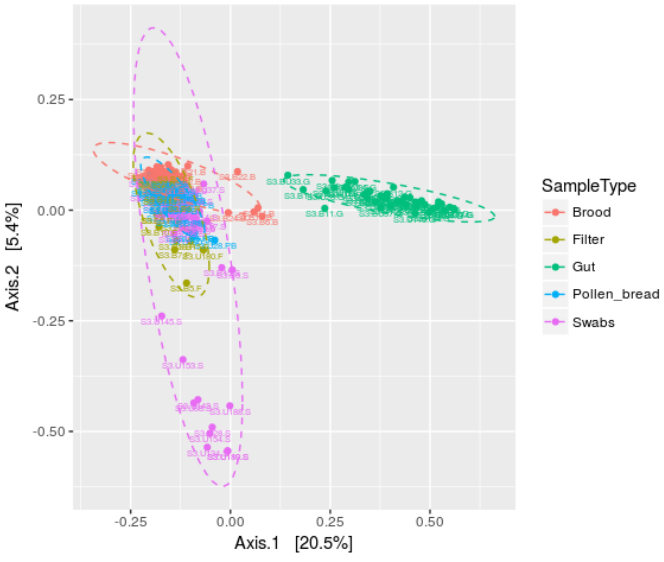

Figure 2. PCoA plot based on beta diversity divided in Sample Types. Beta diversity matrix was calculated using the Bray Curtis dissimilarity statistic.

to separate with naked eye. However, through the significant ANOSIM test (P = 0.001; R = 0.64267670698334833) it was statistically corroborated that the bacterial community structure was different for each sample type.

Focusing on gut samples, alpha diversity measures plotted by location [Figure 3] revealed that gut bacteriome of honey bees located in an agricultural environment had a much higher alpha diversity than those located in a pristine environment. Furthermore, amongst those bees of agricultural location, the ones that had received the treatment against Varroa had a higher bacteriome richness than those that had not, whose alpha diversity is closer to that of pristine location honey bees [Figure 3B]. Amongst the bees of pristine location, those that were translated from Baranja (agricultural origin) had a slightly lower bacterial richness than those original from the pristine environment [Figure 3A]. The graphs based on the beta diversity matrix, PCoA [Figure 4] and UPGMA [Figure 5], showed two different groups, one formed by pristine location samples (BU and U) and the other by agricultural location samples (B). The first axis in the PCoA explained 31.7% of bacteriome variation. This clear difference in the bacteriome from both locations resulted in a highly significant ANOSIM test for the location (P = 0.001; R = 0.84913272668374706), confirming the differences between the guts' bacteriome communities statistically.

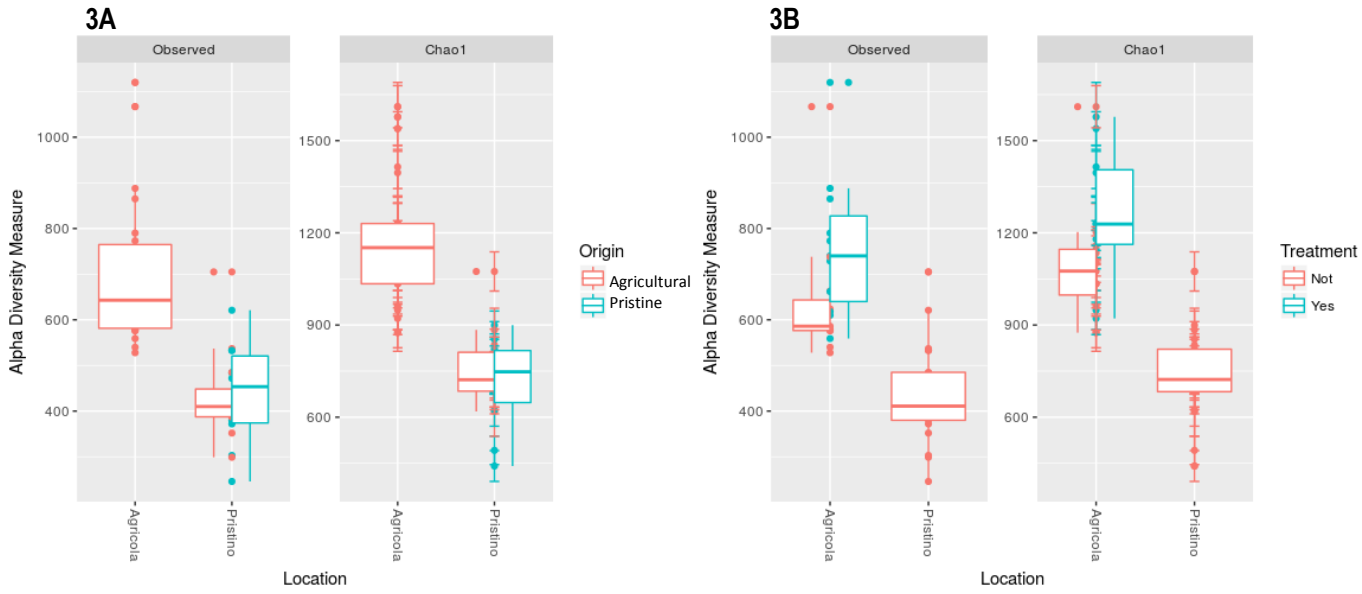

Figure 3. Alpha diversity box plots for honey bee gut bacteriome in pristine and agricultural locations. 3A: Pristine samples are split according to their origin. 3B: Agricultural samples are split according to whether the honey bees received the *Checkmite* treatment or not.

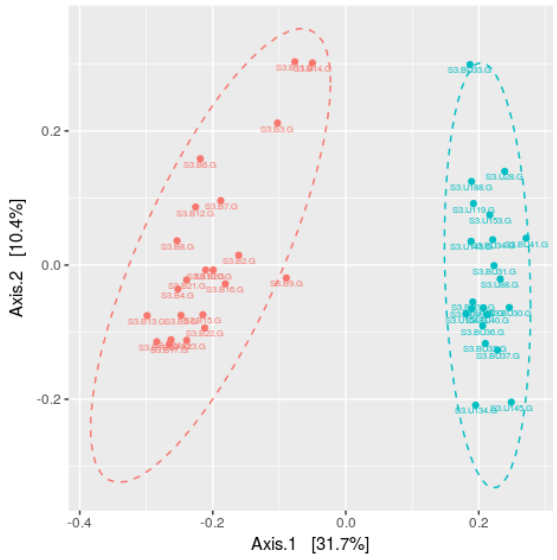

Figure 4. PCoA plot of the bacteriome of gut samples, by location. The PCoA is based on beta diversity distance matrixes calculated with the Bray Curtis metrics.

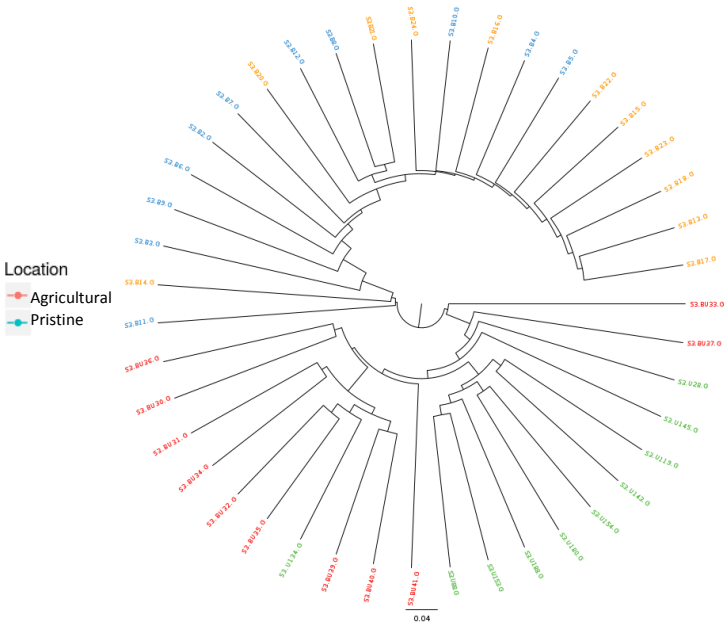

Figure 5. UPGMA tree of the bacteriome of gut samples. Colour code indicates sample category: BU (red), U (green), B treated (blue) and B untreated (orange).

In the UPGMA tree, the separation between BU samples and U samples (located in pristine environment but with different origin) was quite obvious, except for the sample S3.U134.G, which was grouped together with BU samples, and S3.BU33.G and S3.BU37.G, which appeared in branches separated from the rest of pristine samples [Figure 5]. In the PCoA plot generated with only pristine samples [Figure 6], the differences between samples original from Baranja (BU) and those original from Unije (U) was also observed, resulting in a significant but not high ANOSIM test (P = 0.001; R = 0.32690909090909093), meaning that the bacteriome present in the gut of samples coming from different origins was significantly different. However, among the samples located in the agricultural environment, the division was not clear in the UPGMA, appearing mixed treated (B2-B12) and untreated (B13-B24) samples. Accordingly, bacteriome structure differences for these samples were not clear in the PCoA plot (data not shown) although the ANOSIM test for treatment was weakly

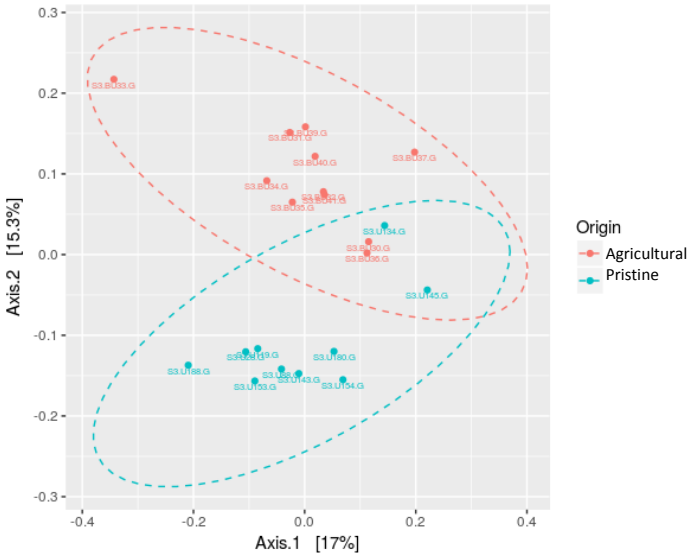

Figure 6. PCoA plot of the bacteriome of gut samples of honey bees located in pristine environment grouped according to the origin. The PCoA is based on beta diversity distance matrixes calculated with the Bray Curtis metrics.

significant (P = 0.01; R = 0.11374906085649886), meaning that both groups had weak differences respect to their gut bacteriome.

Due to the big differences between samples from different locations, a Kruskal-Wallis test was performed and it showed that 27 families were the ones that contributed the most to the differences in bacteriome structure between gut samples from honey bees located in pristine and agricultural locations [relative abundances shown in Figure 7]. The mean of these relative abundances and SD for each of these families for each sample category are given in the Supplementary Table 2. The biggest differences were for Enterobacteriaceae family, which was much more abundant in agricultural gut samples, where it had a relative abundance mean of 45.83%, and just 4.23% in pristine gut samples. On the contrary, Lactobacillaceae, Bartonellaceae, Neisseriaceae and Sphingomonadaceae families were more abundant in pristine gut samples.

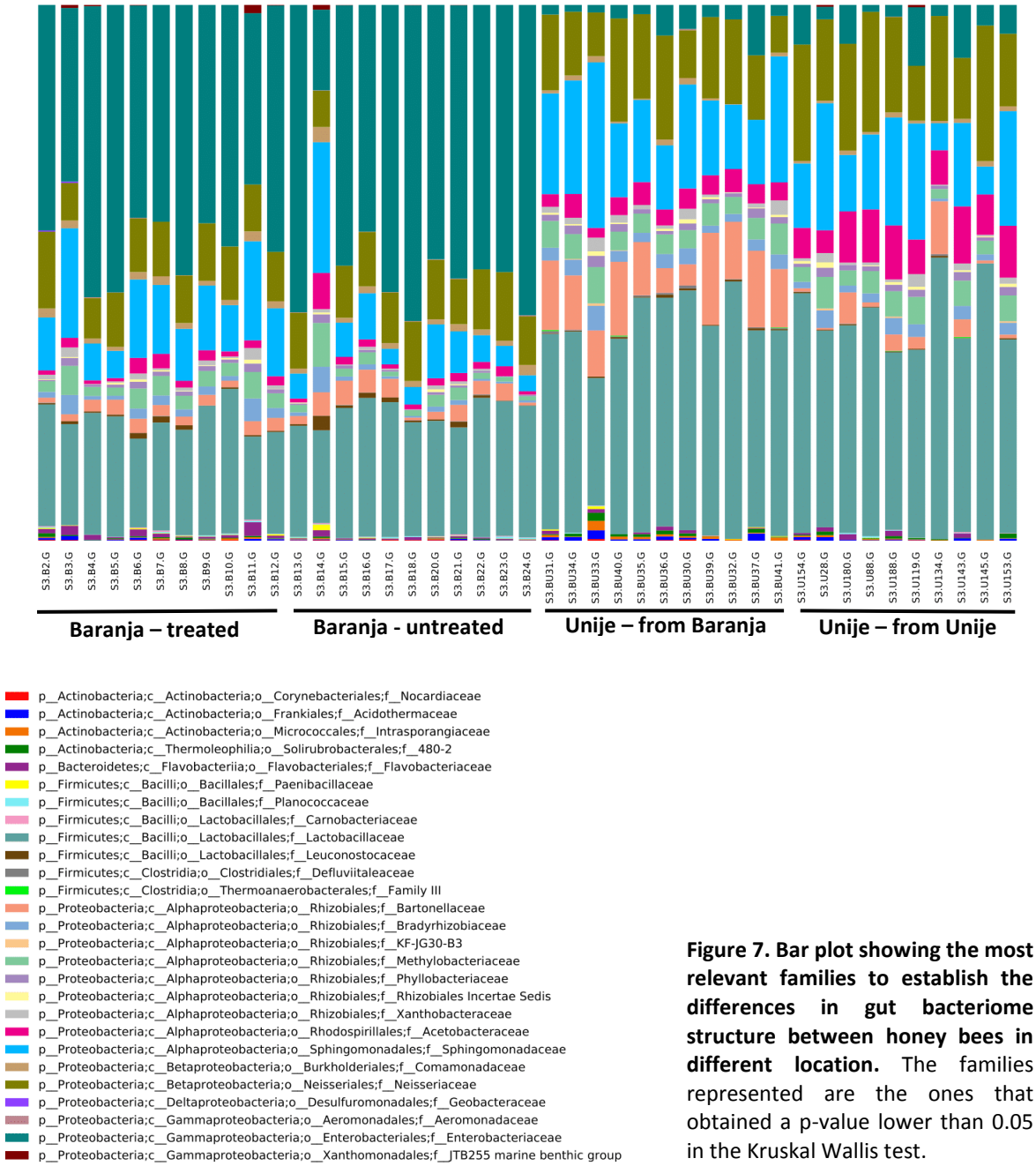

Figure 7. Bar plot showing the most relevant families to establish the differences in gut bacteriome structure between honey bees in different location. The families represented are the ones that obtained a p-value lower than 0.05 in the Kruskal Wallis test.

Regarding the genera inside these 27 families, 45 genera were in a relative abundance equal or superior to 0.1% on average for all gut dataset (Supplementary Table 3). The greatest differences between the two locations were the relative abundances of *Lactobacillus*, *Sphingomonas* and *Snodgrasella*, which were much more abundant in pristine gut samples, and *Enterobacter* and *Pantoea* (both belonging to the Enterobacteriaceae family), which had a greater relative abundance in honey bee guts from agricultural location. Some others, even if they had a lower presence in the samples, were also more abundant in one of the locations than in the other one. This is the case of *Citrobacter* and *Escherichia-Shigella*, more abundant in agricultural gut samples, and *Methylobacterium*, *Bartonella* and *Commensalibacter*, more abundant in pristine guts. It is also remarkable that *Bartonella* was much more abundant in BU samples (original from Baranja and translated to Unije) than in U samples (original from Unije).

The Kruskal-Wallis test performed when retaining only those 45 genera resulted in 560 OTUs (strains) relevant to establish the bacteriome differences between the two locations. The heatmap created with these OTUs [Figure 8] revealed a division between gut samples located in Unije and Baranja, whereas the division between treated and untreated (inside agricultural gut samples) and between pristine origin and agricultural origin (inside gut samples located in the pristine environment) was not so clear. The clusters that were more abundant in gut samples with pristine location included specific strains (OTUs) of just *Lactobacillus* (cluster 28), *Lactobacillus* plus specific strains of *Sphingomonas* and *Gluconobacter* (cluster 29), or specific strains of different genera belonging to Enterobacteraceae family (clusters 20, 24, 48, etc). In contrast, the clusters more abundant in agricultural samples were those mostly formed by Enterobacteriaceae, such as specific strains from the genera *Pantoea* (cluster 38), *Yersinia* (cluster 44), *Enterobacter* and *Pantoea* (cluster 18), which are clearly less abundant in pristine samples. So, differences were not only observed in family or genera abundances, but also at strains level, since some specific strains from some genera were more

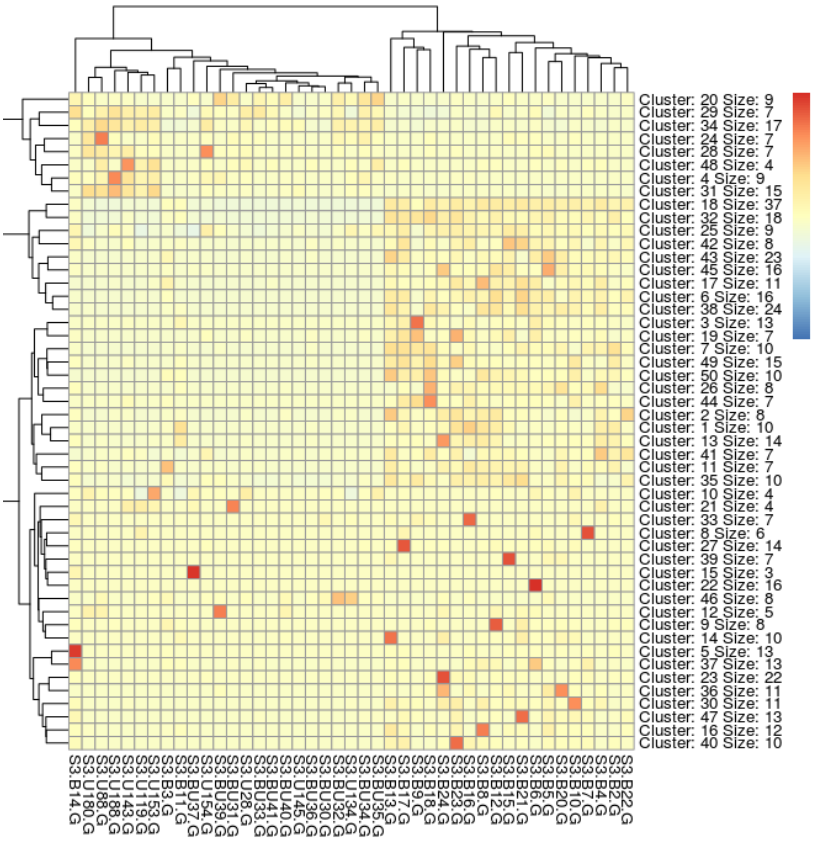

Figure 8. Heat map. The heat map was generated with the most relevant OTUs to establish bacteriome structure differences between each of the locations, belonging to the genera equal to or more abundant than 0.1%, from the most relevant families. OTUs were clustered according to their abundance based on the Manhattan distance matrix.

present in one of the environments than in the other one. The whole description of the taxonomy present in each cluster is given in the Supplementary Table 4, due to its big size.

Discussion

As it was expected, the bacteriome from each sample type was statistically different, which means each of the analysed micro-environments inside the hive are niche to specific bacteria and/or bacteria in specific abundances. Among all sample types, gut samples were the ones with the lowest alpha diversity, which can be understood since gut is a restrictive environment that only allows the growth of specific bacteria, as it has already been extensively described in bibliography. Gut and swabs showed the greatest variance between samples in the alpha diversity, which could mean that the greatest differences between samples of different categories (location, treatment and origin) could be found in these two sample types.

Swabs were not separately analysed in this report, but gut bacteriome showed big differences between pristine and agricultural locations in both, alpha and beta diversities. Alpha diversity was higher in guts of agricultural location samples, possibly because the combination of pesticides, herbicides, treatments, etc. kills some microorganisms from the gut of honey bees, leaving spaces that other opportunistic or transitory microorganisms occupy and, this way, raising the alpha diversity. In the pristine environment, instead, intestinal microbiota is less affected by external factors, so core groups are settled in a more stable way, and new strains cannot easily colonise their niche.

All the bacteria described as core in honey bee gut [6][7] were found in our samples in the following proportions: 21.2% *Lactobacillus*, 8.7% *Snodgrasella*, 2.4% Acetobacteraceae family, 4.7% *Gilliamella*, 3.5% *Bifidobacterium* and 2.3% *Frischella*.

Amongst pristine gut samples, those translated from Baranja (BU) and those original from Unije (U) had similar alpha diversity [Figure 3A] but different beta diversity [Figure 6]. In other words, they had a similar bacterial richness but the structure of the bacteriome (presence and abundance of specific bacterial taxa) was different among the samples. When they were translated to Unije, BU honey bees were set aside from the anthropic pressure related to agricultural environments or beekeeping (*Checkmite*, pesticides, agricultural by-products, etc.) and this allowed changes to happen within its bacteriome. Likely, they started to naturally recover strains that had been decreased because of agricultural substances and treatments, as well as acquiring them directly from the pollination environment [6]. However, since they had just spent two weeks in Unije island at the time of sampling, their gut's bacteriome structure was not completely changed, so this could explain the different aggrupation in the PCoA plot with respect to the gut samples from honey bees original from Unije.

In contrast, the bacteriome differences between the guts of honey bees treated and untreated in the agricultural location were weakly significant (very low R value in the ANOSIM test), so the differences between groups should be taken with caution and more experiments are needed to get robust conclusions about the effect of *Checkmite* treatments in agricultural areas. Considering this, we can elucidate that the major responsible for the changes in bacteriome structure was not mainly the treatment by itself, but some other factor or combination of factors present in the agricultural location (continuous transport, products derived from human activity, herbicides or insecticides used in crop fields, etc.).

When comparing the differences in abundances of concrete bacterial taxa in each location, it can be seen that lactic acid bacteria (LAB) are clearly more abundant in pristine location gut samples. It has previously been reported that these bacteria inhibit the growth of the pathogen *Paenibacillus larvae* [16] as well as *Melissococcus plutonius* [15], so a greater abundance of them in the gut of honey bees could have beneficial effects in both individual bee health and colony health, preventing the infections of emerging larvae. More concretely, *Lactobacillus* spp. are almost twice as abundant in the pristine location guts than in the agricultural location ones. Negative correlations have been observed between the abundance of the *Lactobacillus* genus in honey bee guts and the degree of infection by some pathogens, such as the Sacbrood virus (*Morator aetatulas*) [17], *Paenibacillus larvae* [18] and *Melisococcus plutonius* [19]. Furthermore, *Lactobacillus* spp. are thought to be involved in nectar processing, carbohydrate metabolism and immunomodulation, apart from the already mentioned pathogen interference [15]. The pathogenic potential of commensal hive fungi is largely unknown, but may be mediated in part by lactic acid bacteria, as it is the case of *Lactobacillus kunkeei*. *Sacchromyces* spp. and similar yeasts are direct competitors for the niche occupied by *L. kunkeei*, so a decrease in the abundance of this bacterium could lead to fungal infections [20], being in our case more susceptible the colonies in agricultural location.

Seemingly, the abundance of Neisseriaceae family and, in concrete, *Snodgrasella* genus, is higher in pristine samples, and amongst those, higher in U than BU. Probably, if BU honey bees had stayed longer in Unije, this abundance would be higher, approaching that of U samples. *Snodgrasella alvis* has been reported to inhibit the growth of the honey bee pathogen *Lotmaria passim* [21]. Also, Kwong *et al.* proved that when honey bees were injected with *E.* coli, those that had been fed with *S. alvi* had increased survival rates, apparently due to the up-regulation of antimicrobial peptides apidaecin and hymenoptaecin induced by *S. alvi* [22]. Moreover, exactly as for *Lactobacillus* spp., a negative correlation was observed between the presence of *S. alvi* and Sacbrood virus [17]. For these reasons above, the decrease of *Snodgrasella* in honeybees from Baranja could result in higher infectivity and, thus, reduced viability.

Oppositely, Enterobacteriaceae family was 10 times more abundant in samples located in Baranja than in samples located in Unije. All *Yersinia*, *Hafnia*, *Enterobacter*, *Klebsiella* and *Serratia* genera, belonging to this family, had a much greater presence in B samples than in BU and U. This is surely related with the products derived from human activity. *Hafnia alvi*, and species

of *Enterobacter*, *Klebsiella* and *Serratia* genera likely represent opportunistic organisms with pathogenic potential if their growth gets out of control, so, when some other bacteria decrease due to the environment, the niches left by them seem to be colonised by these Enterobacteriaceae species, greatly increasing their relative abundance. More precisely, *Serratia marcescens* can be pathogenic, causing sepsis and death [23] and some strains isolated from hives can cause mortality when administered orally to workers in the laboratory [24]. Potentially, these Enterobacteriaceae pathogens are under-recognized as causes of bee mortality, since infected bees usually leave the hive to die [23]. Considering this, the vastly increased abundance of Enterobacteriaceae in agricultural location gut samples could be somehow correlated with the higher mortality of bees from this location.

To summarize, as it has been discussed above, it could be possible that the differences in specific bacterial taxas' structure were one of the reasons why honey bees located in Unije show natural resistance to varroa mite and the infections caused by it, whereas honey bees in Baranja do not. As it has already been demonstrated by Kwong *et al*. [22], the administration of native gut microbiota could be a short-term solution to possibly improve the health of honey bees affected by agricultural practices. However, in the long term, it will be necessary to focus the attention on preserving honey bee's natural associated microbiota, introducing changes in agricultural and beekeeping practices and reducing those factors that can affect the microbiome of honey bees in a negative way.

Conclusion

In this work, through the amplification of the 16S rRNA using next generation sequencing, it has been possible to characterize the bacterial communities present in honey bee colonies (focusing on gut samples) located in pristine and agricultural environments, and also evaluate in each location the effect of the treatment against varroa (in agricultural location) and the influence of moving the honey bees from agricultural areas to pristine environments (here called "origin" factor). Differences in bacterial richness and bacteriome structure between sample categories were discovered, showing disparities in the abundance of bacterial taxa described as either honey bee symbionts or potential pathogens. Although it is not yet possible to affirm that the differences in bacteriome composition are the only responsible for the higher mortality of honey bees in the agricultural location or the resistance to varroa of honey bees in the pristine location, here we have seen that the differences found may imply important consequences in honey bee health. The therapeutic modulation of the gut microbiota could be one possible approach to improve honey bee health. Indeed, it may become necessary in the short term to supplement the compromised microbial community with beneficial bacteria (probiotics) or treatments that promote the growth of it (prebiotics). However, a sustainable management will only be possible if attention is focused not only on preserving honey bees, but also their associated microbiota.

In this study 16S rRNA from gut, pollen bread, brood, filters and swabs was extracted and sequenced, but only gut samples were extensively analysed. Nevertheless, regarding the hive as a superorganism, it is essential to characterize the microbial community of all these micro-environments of the hive in order to properly understand the interactions between them and the effects of the environment on the honey bee colony. Therefore, future studies should include the analysis of the rest of sample types, which are already extracted and sequenced. In addition, for future projects, it would also be interesting to perform transcriptome sequencing, in order to see which microbial or host genes are expressed in response to changes in the environment and microbial community composition.

Finally, in order to know the possible changes of our results in other seasons and the stability of the bacteriome over time, this study could be extended for more than one year, sampling in at least two different seasons. Later, other collaborative projects could be interesting to perform the same experiments in other locations and with other honey bee subspecies in order to confirm that our results can be extrapolated to any agricultural area and any honey bee. It should be noted that the research described in this report is the baseline (or time 0) of a bigger study that will be analysing honey bees in Baranja and Unije for longer than a year, hopefully obtaining interesting results about the concerning issue.

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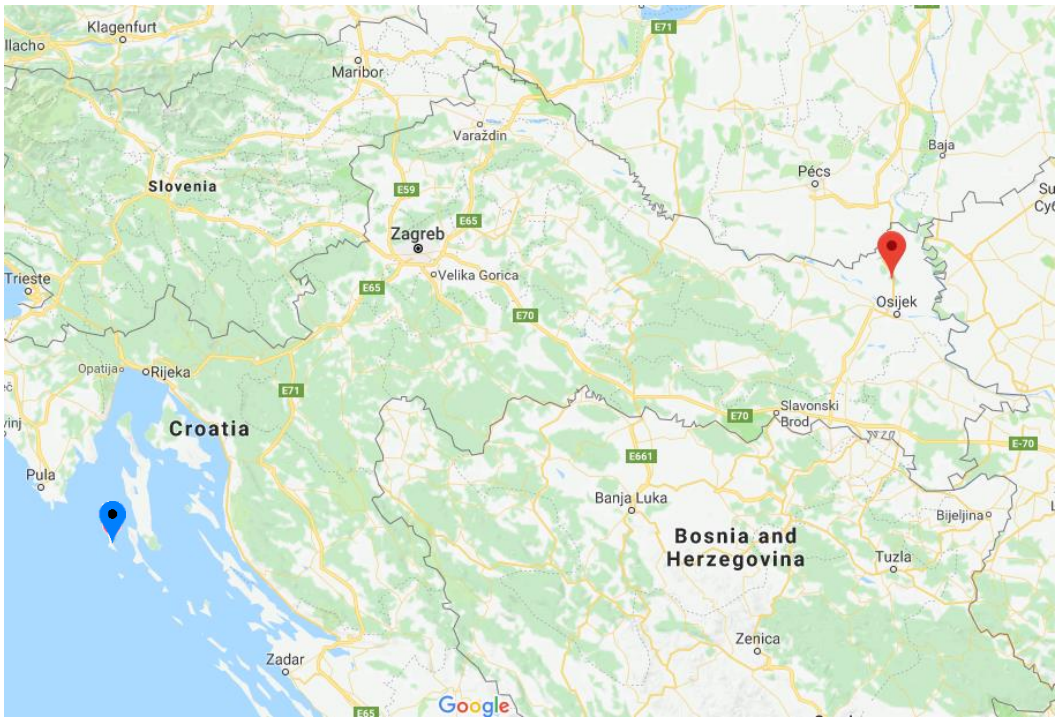
APPENDIX



Supplementary Figure 1. Sampling locations. The red pin indicates Čeminac city, Baranja (agricultural location) and the blue pin, Unije island (pristine location).

Supplementary Table 1. Summary of the samples collected. Samples from Baranja are divided according to whether they have been treated with the acaricide *Checkmite* or not, and samples from Unije according to whether they are original from Unije or moved from Baranja. The fraction in brackets represents the number of samples sequenced over the total number of samples collected for each sample type and category.

	BARANJA		UNIJE		
	Treated	Untreated	From Baranja	From Unije	TOTAL
GUT	B2-B12 (11/11)	B13-B18, B20-B24 (11/11)	BU30-BU37, BU39-BU41 (11/11)	U28, U88, U119, U134, U143, U145, U153, U154, U180, U188 (10/10)	(43/43)
PB	B2-B12 (10/11)	B13-B18, B20-B24 (10/11)	BU30-BU37, BU39-BU41 (11/11)	U28, U88, U119, U134, U143, U145, U153, U154, U180, U188 (10/10)	(41/43)
BROOD	B2-B12 (11/11)	B13-B18, B20-B24 (11/11)	BU30-BU32; BU34-BU36, BU39 (7/7)	U28, U119, U134, U143, U145, U153, U154, U180, U188 (5/9)	(34/38)
FILTER	B2-B12 (11/11)	B13-B18, B20-B24 (11/11)		U28, U88, U119, U134, U143, U145, U153, U154, U180, U188 (10/10)	(32/32)
SWABS	B2-B12 (10/11)	B13-B18, B20-B24 (11/11)	BU30-BU37, BU39-BU41 (11/11)	U28, U88, U119, U134, U143, U145, U153, U154, U180, U188 (10/10)	(42/43)
TOTAL	(53/55)	(54/55)	(40/40)	(45/49)	(192/199)

Supplementary Table 2. Relative abundance of the families that are significant to establish bacteriome structure differences between agricultural and pristine location samples. The abundances are given in percentages and the standard deviation was also calculated. These families are the ones illustrated in the bar plot in Figure 7.

SAMPLE CATEGORY	B TREATED (B2-B12)		B UNTREATED (B13-B24)		BU		U	
FAMILY	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lactobacillaceae	0.2081	0.0339	0.2290	0.0287	0.3891	0.0635	0.4076	0.0691
Enterobacteriaceae	0.4334	0.0752	0.4832	0.1212	0.0321	0.0246	0.0526	0.0346
Sphingomonadaceae	0.1194	0.0466	0.0725	0.0618	0.1754	0.0597	0.1493	0.0678
Neisseriaceae	0.0972	0.0192	0.1002	0.0162	0.1341	0.0425	0.1772	0.0499
Bartonellaceae	0.0197	0.0063	0.0266	0.0149	0.1064	0.0397	0.0272	0.0306
Acetobacteraceae	0.0153	0.0077	0.0169	0.0172	0.0344	0.0082	0.0802	0.0223
Methylobacteriaceae	0.0307	0.0127	0.0206	0.0211	0.0434	0.0112	0.0395	0.0135
Bradyrhizobiaceae	0.0178	0.0115	0.0099	0.0128	0.0245	0.0091	0.0191	0.0091
Comamonadaceae	0.0127	0.0037	0.0132	0.0059	0.0061	0.0044	0.0068	0.0023
Phyllobacteriaceae	0.0085	0.0043	0.0044	0.0041	0.0122	0.0047	0.0112	0.0043
Xanthobacteraceae	0.0084	0.0065	0.0031	0.0026	0.0124	0.0084	0.0090	0.0071
Flavobacteriaceae	0.0088	0.0072	0.0032	0.0031	0.0029	0.0032	0.0040	0.0047
Leuconostocaceae	0.0048	0.0037	0.0070	0.0075	0.0028	0.0015	0.0017	0.0009
Acidothermaceae	0.0024	0.0023	0.0009	0.0007	0.0055	0.0052	0.0025	0.0023
Rhizobiales Incertae Sedis	0.0024	0.0021	0.0012	0.0017	0.0036	0.0026	0.0038	0.0032
480-2	0.0022	0.0017	0.0009	0.0009	0.0048	0.0042	0.0029	0.0029
Intrasporangiaceae	0.0013	0.0012	0.0007	0.0008	0.0049	0.0045	0.0015	0.0012
JTB255 marine benthic group	0.0023	0.0046	0.0009	0.0026	0.0000	0.0000	0.0008	0.0017
Planococcaceae	0.0012	0.0009	0.0015	0.0010	0.0002	0.0005	0.0008	0.0011
Paenibacillaceae	0.0010	0.0009	0.0014	0.0030	0.0010	0.0016	0.0001	0.0003
Defluviitaleaceae	0.0002	0.0004	0.0004	0.0005	0.0021	0.0018	0.0005	0.0011
Nocardiaceae	0.0008	0.0005	0.0007	0.0005	0.0007	0.0011	0.0003	0.0005
Carnobacteriaceae	0.0008	0.0009	0.0006	0.0007	0.0001	0.0003	0.0003	0.0005
KF-JG30-B3	0.0000	0.0000	0.0000	0.0000	0.0006	0.0014	0.0009	0.0015
Aeromonadaceae	0.0002	0.0004	0.0007	0.0006	0.0002	0.0007	0.0001	0.0002
Family III	0.0000	0.0000	0.0000	0.0000	0.0004	0.0008	0.0003	0.0006
Geobacteraceae	0.0005	0.0010	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Supplementary Table 3. Relative abundance of the genera with total relative abundance equal or superior to 0.1% on average (belonging to the 27 families relevant for location) for each sample category. The abundances are given in percentages and the standard deviation was also calculated.

SAMPLE CATEGORY	B TREATED (B2-B12)		B UNTREATED (B13-B24)		BU		U	
GENERA	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rhodococcus	0.0545	0.0522	0.0636	0.0505	0.0364	0.0674	0.0200	0.0422
Acidothermus	0.2545	0.2382	0.0636	0.0674	0.5455	0.5126	0.2500	0.2121

Oryzihumus	0.0636	0.1027	0.0182	0.0405	0.2000	0.2324	0.0300	0.0675
Actibacter	0.0636	0.1206	0.0273	0.0905	0.0636	0.1120	0.0800	0.1476
Chryseobacterium	0.1909	0.1973	0.1818	0.0751	0.0273	0.0647	-	-
Paenibacillus	0.1000	0.0775	0.1273	0.2611	0.1000	0.1549	0.0100	0.0316
Planomicrobium	0.1091	0.0944	0.1455	0.1293	0.0091	0.0302	0.0800	0.1033
Lactobacillus	20.8182	3.3802	22.9000	2.8896	38.9091	6.3581	40.7600	6.9229
Fructobacillus	0.2273	0.1191	0.3818	0.2926	0.2273	0.1618	0.1600	0.0843
Oenococcus	0.1455	0.2339	0.1636	0.3641	0.0273	0.0647	-	-
Weissella	0.0818	0.1471	0.1273	0.3289	0.0364	0.1206	-	-
Incertae Sedis	0.0182	0.0405	0.0364	0.0674	0.2182	0.1940	0.0500	0.0972
Bartonella	1.9727	0.6467	2.6545	1.4895	10.6545	3.9690	2.7300	3.0623
Bradyrhizobium	1.6182	1.0028	0.9273	1.1118	2.2364	0.7865	1.7600	0.8195
Methylobacterium	3.0727	1.2962	2.0545	2.0854	4.3455	1.1255	3.9200	1.3231
Mesorhizobium	0.1455	0.1572	0.0545	0.0820	0.1636	0.1859	0.1900	0.1370
Phyllobacterium	0.6182	0.3341	0.3727	0.3259	1.0182	0.3894	0.8700	0.2946
Rhizomicrobium	0.2182	0.1991	0.1091	0.1814	0.3455	0.2622	0.3500	0.2635
Pseudolabrys	0.1000	0.2049	0.0091	0.0302	0.1000	0.1612	0.0600	0.0966
Commensalibacter	0.8182	0.3710	0.9364	0.4739	2.9455	0.8490	7.5500	2.3467
Gluconobacter	0.1636	0.2461	0.1909	0.4277	0.0545	0.1214	0.0100	0.0316
Saccharibacter	0.0273	0.0647	0.0455	0.0820	0.0364	0.0674	0.2300	0.2406
Novosphingobium	0.3727	0.1794	0.1909	0.2773	0.2818	0.2442	0.3400	0.1897
Sphingomonas	11.4273	4.4708	6.9636	5.8076	17.1545	5.6950	14.4000	6.5713
Acidovorax	0.1909	0.0944	0.1636	0.1362	0.0545	0.0820	0.0800	0.1033
Comamonas	0.6000	0.0894	0.6545	0.1809	0.0273	0.0467	0.2800	0.1874
Variovorax	0.1909	0.1514	0.1455	0.1753	0.1182	0.0874	0.0800	0.1033
Neisseria	0.1545	0.0934	0.1545	0.0688	-	-	0.0400	0.0516

Snodgrassella	9.5273	1.8815	9.8273	1.5634	13.4000	4.2528	17.6100	5.0019
Arsenophonus	0.5364	0.2203	0.6182	0.2750	0.5727	0.3744	0.4400	0.2675
Citrobacter	1.2455	0.9994	1.7273	1.0845	0.1273	0.2370	0.1600	0.2366
Cronobacter	0.1273	0.0905	0.1636	0.0674	0.0182	0.0603	0.0400	0.0699
Enterobacter	13.2455	2.7182	14.4818	3.8230	0.5455	0.5733	1.8300	1.2798
Erwinia	0.2273	0.0786	0.2091	0.0831	-	-	-	-
Escherichia-Shigella	1.5727	0.3379	1.8545	0.8847	0.1818	0.2089	0.2900	0.2885
Hafnia	0.4364	0.2730	0.3818	0.2442	0.1273	0.3003	0.0200	0.0422
Klebsiella	0.7000	0.3286	0.8545	0.3012	0.0182	0.0405	0.0400	0.0699
Morganella	0.0636	0.1286	0.2909	0.6156	-	-	-	-
Pantoea	8.0545	2.9163	8.4455	2.9602	0.8000	0.9220	1.1300	1.3149
Pectobacterium	0.3545	0.1440	0.3636	0.1120	0.1455	0.2734	-	-
Proteus	0.0273	0.0647	0.1909	0.3562	0.0455	0.1508	0.0100	0.0316
Providencia	0.4909	0.1375	0.5364	0.1963	0.0455	0.1214	0.0300	0.0675
Rahnella	0.1000	0.1183	0.0909	0.1136	0.0182	0.0603	-	-
Serratia	0.7909	0.2982	0.9364	0.4456	0.0455	0.0820	0.0800	0.1476
Yersinia	0.2000	0.1673	0.3000	0.1549	0.1182	0.3920	0.0200	0.0422

Supplementary Table 4. List of the OTUs grouped in each cluster of the heatmap (Figure 8) and the taxonomy corresponding to them. The number on the left column indicates the cluster. The taxonomy of each OTU is given in family, genus and species level, when it was resolved.

	OTU ID (Accesion number)	Taxonomy
1	AB845274.1.1329	Enterobacteriaceae; g_Pantoea
	GQ260087.1.1456	Enterobacteriaceae; g_Klebsiella
	New.CleanUp.ReferenceOTU134438	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU145495	Comamonadaceae; g_Comamonas
	New.CleanUp.ReferenceOTU173932	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	New.CleanUp.ReferenceOTU194636	Neisseriaceae; g_Snodgrassella; s_Apis mellifera
	New.CleanUp.ReferenceOTU24912	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU28715	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU64333	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU76054	Enterobacteriaceae; g_Arsenophonus

2	AB740357.1.1462	Enterobacteriaceae; g_Pantoea
	HG800034.1.1424	Enterobacteriaceae; g_Erwinia; s_Erwinia mallotivora
	HM049693.1.1502	Enterobacteriaceae; g_Pantoea
	HM069215.1.1466	Enterobacteriaceae; g_Pantoea
	KJ670085.1.1438	Enterobacteriaceae; g_Pantoea
	KP297452.1.1273	Enterobacteriaceae; g_Enterobacter
3	HQ441178.1.1510	Enterobacteriaceae; g_Enterobacter
	JF825892.1.1478	Enterobacteriaceae; g_Enterobacter
	JN990102.1.1503	Enterobacteriaceae; g_Pantoea
	KF058023.1.1454	Enterobacteriaceae; g_Enterobacter
	KP704422.1.1484	Enterobacteriaceae; g_Serratia
	LN848745.1.1370	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU33992	Planococcaceae; g_Planomicrobium; s_uncultured bacterium
	New.CleanUp.ReferenceOTU39383	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU60901	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU77044	Lactobacillaceae; g_Lactobacillus
4	DQ645728.1.1450	Enterobacteriaceae; g_Enterobacter; s_Enterobacter aerogenes
	FJ906788.1.1415	Enterobacteriaceae; g_Pectobacterium; s_carotovorum
	FJ906793.1.1456	Enterobacteriaceae; g_Pantoea
	GU815113.1.1466	Enterobacteriaceae; g_Enterobacter
	HM475278.1.1381	Enterobacteriaceae; g_Enterobacter
	HM556352.1.1382	Enterobacteriaceae; g_Enterobacter
	HQ591433.1.1202	Enterobacteriaceae; g_Enterobacter
	JQ353817.1.1259	Enterobacteriaceae; g_Enterobacter
	JQ734774.1.1276	Enterobacteriaceae; g_Enterobacter
	JX202607.1.1495	Enterobacteriaceae; g_Enterobacter
	KU355542.1.1445	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU126753	Enterobacteriaceae; g_Serratia
	New.CleanUp.ReferenceOTU148454	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU186196	Neisseriaceae; g_Snodgrassella; s_Apis mellifera
	New.CleanUp.ReferenceOTU188481	Sphingomonadaceae; g_Sphingomonas
	New.CleanUp.ReferenceOTU95063	Acetobacteraceae; g_Commensalibacter
5	KF600124.1.1264	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU10182	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU103627	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU128596	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU145972	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU203174	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU20631	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU21822	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU27197	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU28709	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU33061	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU35806	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU3748	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.CleanUp.ReferenceOTU5531	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU61686	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU62105	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU78247	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.CleanUp.ReferenceOTU81569	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU94053	Acetobacteraceae; g_Commensalibacter
	New.ReferenceOTU341	Acetobacteraceae; g_Commensalibacter
	New.ReferenceOTU662	Lactobacillaceae; g_Lactobacillus
6	FJ686827.1.1447	Enterobacteriaceae; g_Enterobacter
	HM112780.1.1382	Leuconostocaceae; g_Fructobacillus; s_Fructobacillus fructosus
	JN695891.1.1560	Enterobacteriaceae; g_Enterobacter

	KJ094576.1.1474	Enterobacteriaceae; g_Enterobacter
	KM029958.1.1579	Enterobacteriaceae; g_Enterobacter
	KT029554.1.1464	Enterobacteriaceae; g_Arsenophonus
	New.CleanUp.ReferenceOTU170315	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU18153	Lactobacillaceae; g_Lactobacillus; s_Apis melli
	New.CleanUp.ReferenceOTU193646	Sphingomonadaceae; g_Sphingomonas
	New.CleanUp.ReferenceOTU55829	Lactobacillaceae; g_Lactobacillus; s_Apis melli
	New.CleanUp.ReferenceOTU78605	Leuconostocaceae; g_Fructobacillus; s_Fructobacillus fructorus
	New.CleanUp.ReferenceOTU95668	Enterobacteriaceae; g_Enterobacter
	New.ReferenceOTU290	Enterobacteriaceae; g_Arsenophonus
7	GQ884173.1.1469	Enterobacteriaceae; g_Citrobacter
	HE821227.1.1451	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	HM461170.1.1466	Enterobacteriaceae; g_Citrobacter; s_uncultured bacterium
	KF842561.1.1418	Enterobacteriaceae; g_Morganella
	New.CleanUp.ReferenceOTU138266	Enterobacteriaceae; g_Morganella
	New.CleanUp.ReferenceOTU152666	Enterobacteriaceae; g_Morganella; s_uncultured organism
	New.CleanUp.ReferenceOTU163433	Lactobacillaceae; g_Lactobacillus; s_Apis melli
	New.CleanUp.ReferenceOTU16640	Enterobacteriaceae; g_Morganella
	New.CleanUp.ReferenceOTU22963	Enterobacteriaceae; g_Morganella; s_uncultured organism
	New.CleanUp.ReferenceOTU87132	Enterobacteriaceae; g_Hafnia
	New.ReferenceOTU48	Enterobacteriaceae; g_Morganella; s_uncultured organism
8	AB246807.1.1484	Bartonellaceae; g_Bartonella; s_uncultured Rhizobiales bacterium
	COIL01000054.3400.4868	Bartonellaceae; g_Bartonella; s_uncultured Rhizobiales bacterium
	FJ477593.1.1426	Bartonellaceae; g_Bartonella; s_uncultured Rhizobiales bacterium
	GQ202672.1.1426	Bartonellaceae; g_Bartonella; s_uncultured Rhizobiales bacterium
	JF417799.1.1446	Bartonellaceae; g_Bartonella; s_uncultured Rhizobiales bacterium
	KC433740.1.1373	Bartonellaceae; g_Bartonella; s_uncultured Rhizobiales bacterium
	New.CleanUp.ReferenceOTU111204	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU174961	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU54527	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU99148	Bartonellaceae; g_Bartonella; s_uncultured Rhizobiales bacterium
9	HM112051.1.1397	Lactobacillaceae; g_Lactobacillus
	HM113359.1.1404	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	HM215048.1.1486	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU166462	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU42662	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.CleanUp.ReferenceOTU5176	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU58040	Lactobacillaceae; g_Lactobacillus
	New.ReferenceOTU555	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
10	HM461125.1.1469	Enterobacteriaceae; g_Pantoea
	HM998315.1.1484	Enterobacteriaceae; g_Pantoea
	KC593288.1.1472	Enterobacteriaceae; g_Serratia; s_Serratia marcescens
	KF731613.1.1459	Enterobacteriaceae; g_Enterobacter
	KM406401.1.1510	Enterobacteriaceae; g_Enterobacter
	KP322632.1.1520	Enterobacteriaceae; g_Pantoea; s_uncultured bacterium
	KU057012.1.1465	Enterobacteriaceae; g_Enterobacter
	LHTF01000009.1335.2930	Enterobacteriaceae; g_Providencia
	New.CleanUp.ReferenceOTU138886	Enterobacteriaceae; g_Rahnella
	New.CleanUp.ReferenceOTU21376	Planococcaceae; g_Planomicrobium; s_uncultured bacterium
	New.CleanUp.ReferenceOTU25943	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU70878	Enterobacteriaceae; g_Arsenophonus
	New.CleanUp.ReferenceOTU94553	Enterobacteriaceae; g_Enterobacter
11	KR261400.1.1398	Acetobacteraceae; g_Gluconobacter
	KT334808.1.1406	Enterobacteriaceae; g_Enterobacter
	LC068761.1.1580	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	New.ReferenceOTU190	Enterobacteriaceae; g_Pantoea

	New.ReferenceOTU439	Neisseriaceae; g_Snodgrassella; s_Apis mellifera
	New.ReferenceOTU443	Lactobacillaceae; g_Lactobacillus
12	AB920798.1.1415	Enterobacteriaceae; g_Erwinia; s_Erwinia mallotivora
	DQ372663.1.1392	Enterobacteriaceae; g_Enterobacter
	EF685273.1.1507	Enterobacteriaceae; g_Erwinia; s_Erwinia mallotivora
	GU451166.1.1380	Flavobacteriaceae; g_Chryseobacterium
	GU815107.1.1473	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	JF815403.1.1489	Enterobacteriaceae; g_Enterobacter
	JQ950496.1.1465	Enterobacteriaceae; g_Pantoea
	KC447389.1.1411	Enterobacteriaceae; g_Enterobacter
	KF199337.1.1335	Enterobacteriaceae; g_Providencia
	KP322636.1.1501	Enterobacteriaceae; g_Enterobacter
13	CP010423.419794.421347	Enterobacteriaceae; g_Arsenophonus
	DQ816137.1.1330	Enterobacteriaceae; g_Citrobacter; s_uncultured bacterium
	EF102863.1.1450	Enterobacteriaceae; g_Pantoea
	JF766456.1.1504	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	JN173078.1.1468	Enterobacteriaceae; g_Pantoea
	KU711903.1.1350	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU134067	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU143518	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU510	Neisseriaceae; g_Snodgrassella
	New.ReferenceOTU105	Lactobacillaceae; g_Lactobacillus
14	DQ816692.1.1470	Enterobacteriaceae; g_Citrobacter
	HM461143.1.1466	Enterobacteriaceae; g_Enterobacter
	JF901373.1.1471	Enterobacteriaceae; g_Enterobacter
	KF843127.1.1394	Enterobacteriaceae; g_Enterobacter
	KF920746.1.1416	Enterobacteriaceae; g_Enterobacter
	LN558630.1.1450	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	New.CleanUp.ReferenceOTU131377	Lactobacillaceae; g_Lactobacillus
15	AJ620950.1.1460	Enterobacteriaceae; g_Citrobacter
	DQ539030.1.1418	Enterobacteriaceae; g_Citrobacter; s_Citrobacterfreundii
	EU773842.1.1403	Enterobacteriaceae; g_Citrobacter; s_uncultured bacterium
	HM244941.1.1357	Enterobacteriaceae; g_Citrobacter
	HQ324431.1.1445	Enterobacteriaceae; g_Enterobacter
	JAJC01000079.72.1593	Enterobacteriaceae; g_Citrobacter
	JX526877.1.1432	Enterobacteriaceae; g_Citrobacter
	KF945103.1.1227	Enterobacteriaceae; g_Citrobacter
	KM252710.1.1464	Enterobacteriaceae; g_Citrobacter; s_uncultured bacterium
	KT582290.1.1443	Enterobacteriaceae; g_Citrobacter
	LIFX01000001.387538.389059	Enterobacteriaceae; g_Citrobacter
16	AB740386.1.1414	Enterobacteriaceae; g_Pantoea
	AB845265.1.1326	Enterobacteriaceae; g_Pantoea
	AY616170.1.1364	Enterobacteriaceae; g_Pantoea
	EF406662.1.1508	Enterobacteriaceae; g_Pantoea
	EF593083.1.1537	Enterobacteriaceae; g_Pantoea
	EU072497.1.1416	Enterobacteriaceae; g_Pantoea
	FJ477666.1.1463	Enterobacteriaceae; g_Enterobacter
	FJ655529.1.1396	Enterobacteriaceae; g_Enterobacter
	FM179752.1.1686	Enterobacteriaceae; g_Pantoea
	HM224395.1.1465	Enterobacteriaceae; g_Pantoea
	HM448993.1.1452	Enterobacteriaceae; g_Pantoea; s_Pantoea agglomerans
	JQ958825.1.1395	Enterobacteriaceae; g_Pantoea; s_Pantoea agglomerans
	JX020764.1.1477	Enterobacteriaceae; g_Pantoea
	KF085272.1.1367	Enterobacteriaceae; g_Pantoea
	KF842672.1.1427	Enterobacteriaceae; g_Enterobacter
	KF906832.1.1563	Enterobacteriaceae; g_Pantoea

	KJ934775.1.1503	Enterobacteriaceae; g_Pantoea
	KM116008.1.1667	Enterobacteriaceae; g_Serratia; s_Serratia marcescens
	KR261608.1.1396	Enterobacteriaceae; g_Pantoea
	KT005529.1.1393	Enterobacteriaceae; g_Pantoea
	KT248083.1.1269	Enterobacteriaceae; g_Pantoea; s_Pantoea agglomerans
	New.CleanUp.ReferenceOTU102857	Planococcaceae; g_Planomicrobium; s_uncultured bacterium
	New.CleanUp.ReferenceOTU154178	Neisseriaceae; g_Snodgrassella
	New.CleanUp.ReferenceOTU165501	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU20225	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU48439	Enterobacteriaceae; g_Pantoea; s_uncultured bacterium
	New.CleanUp.ReferenceOTU89009	Enterobacteriaceae; g_Pantoea; s_Pantoea agglomerans
17	EF187245.1.1409	Lactobacillaceae; g_Lactobacillus
	GAYR01026360.62.1864	Sphingomonadaceae; g_Sphingomonas
	HM111870.1.1397	Lactobacillaceae; g_Lactobacillus
	JQ659451.1.1348	Sphingomonadaceae; g_Sphingomonas
	New.CleanUp.ReferenceOTU137711	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU17294	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU24679	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU30939	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU50635	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU51455	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU81697	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU90817	Lactobacillaceae; g_Lactobacillus
18	AY131237.1.1436	Enterobacteriaceae; g_Pantoea
	GU936975.1.1477	Enterobacteriaceae; g_Enterobacter
	HE975029.1.1449	Enterobacteriaceae; g_Enterobacter
	HQ148916.1.1299	Enterobacteriaceae; g_Pantoea
	HQ757307.1.1206	Enterobacteriaceae; g_Pantoea
	JQ189164.1.1375	Enterobacteriaceae; g_Klebsiella
	JQ712521.1.1335	Enterobacteriaceae; g_Enterobacter
	KF464370.1.1319	Enterobacteriaceae; g_Pantoea
	KF928776.1.1515	Enterobacteriaceae; g_Enterobacter
	KM459025.1.1408	Enterobacteriaceae; g_Enterobacter
	KP189202.1.1394	Enterobacteriaceae; g_Enterobacter
	KT767956.1.1386	Enterobacteriaceae; g_Pantoea
	LN568376.1.1375	Enterobacteriaceae; g_Pantoea
19	HG917720.1.1538	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	HM556955.1.1375	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	JN969334.1.1526	Enterobacteriaceae; g_Klebsiella
	KF625190.1.1731	Enterobacteriaceae; g_Enterobacter
	KP704431.1.1475	Enterobacteriaceae; g_Cronobacter; s_Erwinia mallotivora
	New.CleanUp.ReferenceOTU153319	Enterobacteriaceae; g_Klebsiella
	New.CleanUp.ReferenceOTU203622	Lactobacillaceae; g_Lactobacillus
20	CP007799.4645197.4646665	Enterobacteriaceae; g_Enterobacter
	FN827311.1.1451	Comamonadaceae; g_Acidovorax
	JN698636.1.1422	Enterobacteriaceae; g_Escherichia-Shigella
	KF077490.1.1364	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	KF842067.1.1412	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	KF842153.1.1380	Enterobacteriaceae; g_Enterobacter
	KF842550.1.1395	Enterobacteriaceae; g_Escherichia-Shigella
	KF842626.1.1396	Enterobacteriaceae; g_Enterobacter
	KF842936.1.1408	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	KF843056.1.1419	Enterobacteriaceae; g_Enterobacter
	KP322628.1.1535	Enterobacteriaceae; g_Enterobacter
	LN558640.1.1436	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	New.CleanUp.ReferenceOTU147520	Lactobacillaceae; g_Lactobacillus

	New.CleanUp.ReferenceOTU7418	Planococcaceae; g_Planomicrobium; s_uncultured bacterium
21	AF130891.1.1244	Enterobacteriaceae; g_Serratia
	EF438210.1.1467	Enterobacteriaceae; g_Serratia
	FJ913065.1.1309	Enterobacteriaceae; g_Pantoea
	GQ144701.1.1205	Enterobacteriaceae; g_Citrobacter
	GQ360016.1.1504	Enterobacteriaceae; g_Cronobacter; s_Erwinia mallotivora
	HM159968.1.1503	Nocardiaceae; g_Rhodococcus
	HM368664.1.1488	Flavobacteriaceae; g_Chryseobacterium
	HM556602.1.1374	Enterobacteriaceae; g_Erwinia
	JQ734773.1.1248	Enterobacteriaceae; g_Enterobacter
	KF052592.1.1446	Enterobacteriaceae; g_Pantoea
	KF058018.1.1465	Enterobacteriaceae; g_Serratia
	KF842913.1.1420	Enterobacteriaceae; g_Citrobacter
	KT254303.1.1235	Enterobacteriaceae; g_Yersinia; s_Yersinia ruckeri
22	HM113202.1.1389	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	HP459621.147.1556	Acetobacteraceae; g_Commensalibacter
	JQ726788.1.1416	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU10420	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU108723	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.CleanUp.ReferenceOTU127398	Neisseriaceae; g_Snodgrassella
	New.CleanUp.ReferenceOTU132733	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU144482	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.CleanUp.ReferenceOTU170570	Neisseriaceae; g_Snodgrassella; s_Apis mellifera
	New.CleanUp.ReferenceOTU187914	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU188867	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.CleanUp.ReferenceOTU204062	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU87717	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU96214	Acetobacteraceae; g_Commensalibacter
	New.ReferenceOTU245	Neisseriaceae; g_Snodgrassella
	New.ReferenceOTU410	Acetobacteraceae; g_Commensalibacter
	New.ReferenceOTU761	Acetobacteraceae; g_Commensalibacter
23	New.CleanUp.ReferenceOTU191215	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU68609	Lactobacillaceae; g_Lactobacillus
	New.ReferenceOTU306	Neisseriaceae; g_Snodgrassella
	New.ReferenceOTU60	Bartonellaceae; g_Bartonella; s_uncultured Rhizobiales bacterium
24	JX138619.1.1446	Enterobacteriaceae; g_Pantoea
	KC431794.1.1408	Enterobacteriaceae; g_Enterobacter
	KF193261.1.1412	Enterobacteriaceae; g_Enterobacter
	KF842955.1.1391	Enterobacteriaceae; g_Enterobacter
	KP676111.1.1354	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU104373	Sphingomonadaceae; g_Sphingomonas
	New.CleanUp.ReferenceOTU129169	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU165607	Enterobacteriaceae; g_Arsenophonus
	New.CleanUp.ReferenceOTU185435	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU79025	Enterobacteriaceae; g_Pantoea
25	HM112474.1.1407	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU16531	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU80083	Neisseriaceae; g_Snodgrassella
	New.ReferenceOTU429	Lactobacillaceae; g_Lactobacillus
26	EU742143.1.1515	Enterobacteriaceae; g_Enterobacter
	FJ975788.1.1359	Enterobacteriaceae; g_Enterobacter
	HE590766.1.1518	Leuconostocaceae; g_Fructobacillus
	New.CleanUp.ReferenceOTU14040	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU23248	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.ReferenceOTU134	Comamonadaceae; g_Variovorax
27	AB696297.1.1439	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium

	DQ267701.1.1266	Enterobacteriaceae; g_Enterobacter
	FJ950694.1.1472	Enterobacteriaceae; g_Escherichia-Shigella
	FN185731.1.1513	Lactobacillaceae; g_Lactobacillus; s_uncultured bacterium
	GQ493993.1.1387	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	GU374067.1.1356	Enterobacteriaceae; g_Citrobacter; s_uncultured bacterium
	GX182404.8.1529	Enterobacteriaceae; g_Enterobacter
	HQ757459.1.1472	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	JN969318.1.1475	Enterobacteriaceae; g_Enterobacter
	JX526926.1.1443	Enterobacteriaceae; g_Citrobacter
	KC011147.1.1494	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	KC113042.1.1459	Enterobacteriaceae; g_Proteus
	KC333901.1.1225	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	KF842175.1.1377	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	KF842889.1.1425	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	KF843043.1.1407	Enterobacteriaceae; g_Klebsiella; s_uncultured Klebsiella sp.
	KF941208.1.1402	Enterobacteriaceae; g_Citrobacter
	KT215427.1.1399	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	KT372239.1.1449	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	KX090031.1.1467	Enterobacteriaceae; g_Serratia
	New.CleanUp.ReferenceOTU159247	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU33780	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
28	New.CleanUp.ReferenceOTU48386	Lactobacillaceae; g_Lactobacillus
	New.ReferenceOTU641	Lactobacillaceae; g_Lactobacillus
	New.ReferenceOTU651	Lactobacillaceae; g_Lactobacillus
29	AEKT01000037.108.1664	Leuconostocaceae; g_Weissella
	FJ948822.1.1302	Sphingomonadaceae; g_Sphingomonas; s_uncultured bacterium
	FN421907.1.1364	Sphingomonadaceae; g_Sphingomonas; s_uncultured bacterium
	GQ915087.1.1394	Flavobacteriaceae; g_Chryseobacterium; s_uncultured bacterium
	HM112612.1.1410	Leuconostocaceae; g_Weissella
	HM241048.1.1310	Sphingomonadaceae; g_Sphingomonas
	HQ898871.1.1335	Paenibacillaceae; g_Paenibacillus
	JQ080256.1.1369	Acetobacteraceae; g_Gluconobacter
	New.CleanUp.ReferenceOTU31235	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU53740	Bradyrhizobiaceae; g_Bradyrhizobium
	New.CleanUp.ReferenceOTU63293	Sphingomonadaceae; g_Sphingomonas
	New.CleanUp.ReferenceOTU7216	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU8919	Lactobacillaceae; g_Lactobacillus
30	AB703086.1.1464	Enterobacteriaceae; g_Pantoea
	HM534814.1.1439	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU120228	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU12866	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU89966	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
31	FJ937924.1.1398	Enterobacteriaceae; g_Serratia
	GQ103633.1.1347	Flavobacteriaceae; g_Chryseobacterium
	GQ497246.1.1596	Enterobacteriaceae; g_Enterobacter
	HQ774402.1.1443	Enterobacteriaceae; g_Enterobacter
	JN969317.1.1530	Enterobacteriaceae; g_Enterobacter
	KP322630.1.1479	Enterobacteriaceae; g_Citrobacter; s_uncultured bacterium
	New.CleanUp.ReferenceOTU13391	Enterobacteriaceae; g_Arsenophonus
	New.ReferenceOTU329	Methylobacteriaceae; g_Methylobacterium; s_uncultured bacterium
32	New.CleanUp.ReferenceOTU171743	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU59138	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU89116	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.CleanUp.ReferenceOTU97458	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.ReferenceOTU441	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.ReferenceOTU84	Neisseriaceae; g_Snodgrassella

33	KF058019.1.1429	Enterobacteriaceae; g_Yersinia; s_Yersinia ruckeri
	KT232080.1.1496	Bartonellaceae; g_Bartonella; s_uncultured Rhizobiales bacterium
	New.CleanUp.ReferenceOTU106247	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
34	ACSE01000027.13373.14916	Leuconostocaceae; g_Oenococcus
	FN421850.1.1371	Methylobacteriaceae; g_Methylobacterium; s_uncultured bacterium
	FN421989.1.1411	Comamonadaceae; g_Variovorax; s_Variovorax paradoxus
	FN421999.1.1369	Sphingomonadaceae; g_Sphingomonas
	HM556504.1.1386	Enterobacteriaceae; g_Pantoea
	HV513934.1.1477	Paenibacillaceae; g_Paenibacillus
	JQ410861.1.1532	Enterobacteriaceae; g_Pantoea
	JX989244.1.1447	Methylobacteriaceae; g_Methylobacterium
	New.CleanUp.ReferenceOTU146581	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU147929	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU187853	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU65046	Acetobacteraceae; g_Commensalibacter
	New.ReferenceOTU319	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
35	AB845267.1.1411	Enterobacteriaceae; g_Enterobacter
	EU531808.1.1271	Enterobacteriaceae; g_Klebsiella
	FJ494892.1.1240	Enterobacteriaceae; g_Enterobacter
	GQ114876.1.1365	Enterobacteriaceae; g_Enterobacter
	HM367734.1.1404	Enterobacteriaceae; g_Enterobacter
	HM557134.1.1342	Enterobacteriaceae; g_Enterobacter
	JX892866.1.1397	Enterobacteriaceae; g_Enterobacter
	KF543066.1.1445	Enterobacteriaceae; g_Escherichia-Shigella
	KF843257.1.1388	Enterobacteriaceae; g_Enterobacter
	KJ574410.1.1248	Enterobacteriaceae; g_Enterobacter
	KM459022.1.1396	Enterobacteriaceae; g_Enterobacter
	KT287074.1.1438	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU31972	Enterobacteriaceae; g_Pantoea
36	HM534796.1.1438	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU111516	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU120784	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU137487	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU141081	Bartonellaceae; g_Bartonella; s_uncultured Rhizobiales bacterium
	New.CleanUp.ReferenceOTU143932	Sphingomonadaceae; g_Sphingomonas
	New.CleanUp.ReferenceOTU167535	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU71734	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU8318	Acetobacteraceae; g_Commensalibacter
	New.ReferenceOTU525	Neisseriaceae; g_Snodgrassella
37	AJ010486.1.1532	Enterobacteriaceae; g_Pantoea
	EU771597.1.1411	Enterobacteriaceae; g_Pantoea
	GQ417674.1.1463	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	HF572835.1.1411	Enterobacteriaceae; g_Enterobacter
	HM582880.1.1446	Enterobacteriaceae; g_Pantoea
	JN244975.1.1447	Enterobacteriaceae; g_Enterobacter
	JQ901378.1.1433	Enterobacteriaceae; g_Klebsiella
	KC634087.1.1454	Enterobacteriaceae; g_Pantoea
	KJ160217.1.1469	Enterobacteriaceae; g_Klebsiella
	New.CleanUp.ReferenceOTU100600	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU73469	Lactobacillaceae; g_Lactobacillus
	New.ReferenceOTU24	Neisseriaceae; g_Snodgrassella
38	AB668076.1.1399	Enterobacteriaceae; g_Pantoea
	AF130903.1.1466	Enterobacteriaceae; g_Pantoea
	HK555801.1.1387	Enterobacteriaceae; g_Pantoea
	JQNE01000001.4249096.4250617	Enterobacteriaceae; g_Pantoea
	KM650212.1.1462	Enterobacteriaceae; g_Pantoea

	New.CleanUp.ReferenceOTU175177	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU193870	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU61944	Enterobacteriaceae; g_Pantoea
39	AB809070.1.1465	Enterobacteriaceae; g_Pantoea
	AY753196.1.1459	Enterobacteriaceae; g_Pantoea
	GQ379617.1.1220	Enterobacteriaceae; g_Pantoea
	HM756471.1.1305	Enterobacteriaceae; g_Pantoea
	KM650211.1.1464	Enterobacteriaceae; g_Pantoea
	KP322623.1.1499	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU36956	Enterobacteriaceae; g_Serratia; s_Serratia marcescens
	New.ReferenceOTU237	Neisseriaceae; g_Snodgrassella
	New.ReferenceOTU241	Lactobacillaceae; g_Lactobacillus
	New.ReferenceOTU594	Neisseriaceae; g_Snodgrassella; s_Apis mellifera
40	HM756468.1.1253	Enterobacteriaceae; g_Enterobacter
	HM854257.1.1450	Enterobacteriaceae; g_Enterobacter
	JF901360.1.1471	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	KF193247.1.1413	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU1072	Acetobacteraceae; g_Commensalibacter
	New.CleanUp.ReferenceOTU7711	Enterobacteriaceae; g_Serratia
	New.CleanUp.ReferenceOTU94828	Neisseriaceae; g_Snodgrassella
41	DQ517952.1.1496	Bradyrhizobiaceae; g_Bradyrhizobium
	GU124503.1.1504	Enterobacteriaceae; g_Pantoea
	GU942724.1.1475	Enterobacteriaceae; g_Enterobacter
	HQ222366.1.1260	Enterobacteriaceae; g_Enterobacter
	KU057010.1.1484	Enterobacteriaceae; g_Rahnella
	New.CleanUp.ReferenceOTU153065	Enterobacteriaceae; g_Rahnella
	New.CleanUp.ReferenceOTU76949	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.ReferenceOTU258	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.ReferenceOTU343	Enterobacteriaceae; g_Enterobacter
	New.ReferenceOTU541	Enterobacteriaceae; g_Pantoea
42	EU777652.1.1401	Enterobacteriaceae; g_Pectobacterium; s_ carotovorum
	EU828415.1.1454	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured soil bacterium
	FJ906789.1.1268	Enterobacteriaceae; g_Hafnia
	GU940797.1.1396	Enterobacteriaceae; g_Hafnia
	HQ220166.1.1429	Enterobacteriaceae; g_Yersinia; s_Yersinia ruckeri
	KF625187.1.1727	Enterobacteriaceae; g_Yersinia; s_Yersinia ruckeri
	KF941202.1.1463	Enterobacteriaceae; g_Klebsiella
	New.CleanUp.ReferenceOTU149933	Enterobacteriaceae; g_Hafnia
	New.CleanUp.ReferenceOTU28810	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU57456	Enterobacteriaceae; g_Hafnia
	New.ReferenceOTU323	Neisseriaceae; g_Neisseria; s_uncultured bacterium
43	AY370188.1.1455	Acetobacteraceae; g_Commensalibacter
	HM113281.1.1396	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.CleanUp.ReferenceOTU54533	Neisseriaceae; g_Snodgrassella
44	FJ976590.1.1390	Enterobacteriaceae; g_Enterobacter
	JQ918072.1.1462	Enterobacteriaceae; g_Enterobacter; s_Enterobacter asburiae
	KC800913.1.1433	Enterobacteriaceae; g_Pantoea
	KC834379.1.1333	Enterobacteriaceae; g_Enterobacter
	KP987884.1.1528	Bartonellaceae; g_Bartonella
	New.CleanUp.ReferenceOTU181769	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU21804	Enterobacteriaceae; g_Pantoea
45	New.CleanUp.ReferenceOTU121062	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.CleanUp.ReferenceOTU154805	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU158838	Lactobacillaceae; g_Lactobacillus
	New.CleanUp.ReferenceOTU67566	Acetobacteraceae; g_Commensalibacter

	New.CleanUp.ReferenceOTU90524	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.ReferenceOTU409	Lactobacillaceae; g_Lactobacillus
	New.ReferenceOTU415	Neisseriaceae; g_Snodgrassella; s_Apis mellifera
	New.ReferenceOTU691	Neisseriaceae; g_Snodgrassella
46	AB174835.1.1287	Enterobacteriaceae; g_Enterobacter
	AF010251.1.1373	Enterobacteriaceae; g_Enterobacter
	EF153301.1.1320	Enterobacteriaceae; g_Enterobacter
	FKEX01000004.14.1517	Enterobacteriaceae; g_Enterobacter
	GU594297.1.1474	Enterobacteriaceae; g_Enterobacter
	GU815104.1.1471	Enterobacteriaceae; g_Enterobacter
	GU826686.1.1256	Enterobacteriaceae; g_Enterobacter
	GU938599.1.1401	Enterobacteriaceae; g_Enterobacter
	HM156130.1.1405	Enterobacteriaceae; g_Enterobacter
	HM195201.2.1419	Enterobacteriaceae; g_Klebsiella
	HM445996.1.1494	Enterobacteriaceae; g_Enterobacter
	HM461124.1.1465	Enterobacteriaceae; g_Klebsiella; s_uncultured Klebsiella sp.
	HM556526.1.1379	Enterobacteriaceae; g_Enterobacter
	HM557461.1.1366	Enterobacteriaceae; g_Enterobacter
	JQ407547.1.1460	Enterobacteriaceae; g_Enterobacter
	JX872312.1.1449	Enterobacteriaceae; g_Enterobacter
	JX872314.1.1455	Enterobacteriaceae; g_Yersinia; s_Yersinia ruckeri
	KC431795.1.1203	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	KF146956.1.1518	Enterobacteriaceae; g_Enterobacter
	KJ160188.1.1489	Enterobacteriaceae; g_Enterobacter
	KR026980.1.1475	Enterobacteriaceae; g_Enterobacter
	KT308333.1.1496	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	New.CleanUp.ReferenceOTU81810	Enterobacteriaceae; g_Arsenophonus
47	JN867384.1.1518	Enterobacteriaceae; g_Pantoea
	JN975122.1.1371	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU130593	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU149771	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU156821	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU157121	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU178370	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU179788	Enterobacteriaceae; g_Enterobacter
	New.CleanUp.ReferenceOTU25624	Enterobacteriaceae; g_Enterobacter
	New.ReferenceOTU775	Sphingomonadaceae; g_Sphingomonas; s_uncultured bacterium
48	DQ816230.1.1461	Enterobacteriaceae; g_Providencia
	FJ984609.1.1451	Enterobacteriaceae; g_Providencia
	GQ264264.1.1403	Rhizobiales Incertae Sedis; g_Rhizomicrobium; s_uncultured bacterium
	HM461227.1.1469	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	HM556940.1.1327	Enterobacteriaceae; g_Enterobacter
	HM756480.1.1308	Enterobacteriaceae; g_Enterobacter
	JQ712533.1.1331	Enterobacteriaceae; g_Cronobacter; s_Erwinia mallotivora
	KC283053.1.1547	Enterobacteriaceae; g_Enterobacter
	KF599198.1.1363	Leuconostocaceae; g_Fructobacillus; s_Fructobacillus fructosus
	KT308759.1.1474	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU140249	Sphingomonadaceae; g_Sphingomonas
	New.ReferenceOTU194	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.ReferenceOTU728	Neisseriaceae; g_Snodgrassella; s_Apis mellifera
49	AB703089.1.1467	Enterobacteriaceae; g_Pantoea
	EF189920.1.1497	Enterobacteriaceae; g_Pantoea
	EF608520.1.1459	Enterobacteriaceae; g_Pantoea; s_Pantoea agglomerans
	EU471029.1.1408	Enterobacteriaceae; g_Pantoea
	FJ799754.1.1523	Enterobacteriaceae; g_Pantoea

	HM185888.1.1371	Enterobacteriaceae; g_Pantoea
	HM556769.1.1335	Enterobacteriaceae; g_Pantoea
	HM556888.1.1355	Enterobacteriaceae; g_Pantoea
	JX522468.1.1418	Enterobacteriaceae; g_Enterobacter
	KF193173.1.1418	Enterobacteriaceae; g_Pantoea
	KM598235.1.1456	Enterobacteriaceae; g_Pantoea
	KP409622.1.1215	Enterobacteriaceae; g_Pantoea
	KR019684.1.1490	Enterobacteriaceae; g_Pantoea
	KR106656.1.1344	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU171792	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU177187	Enterobacteriaceae; g_Enterobacter
50	AB845275.1.1413	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	AY345547.1.1503	Enterobacteriaceae; g_Enterobacter
	CJRA01000034.26673.28140	Enterobacteriaceae; g_Enterobacter
	CP014474.759683.761184	Enterobacteriaceae; g_Pantoea
	FJ947065.1.1274	Enterobacteriaceae; g_Enterobacter
	FJ976607.1.1390	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	FKZG01000038.3719.5200	Enterobacteriaceae; g_Enterobacter
	FN421601.1.1418	Enterobacteriaceae; g_Pantoea
	FR863613.1.1483	Enterobacteriaceae; g_Pantoea
	GQ264499.1.1490	Enterobacteriaceae; g_Pantoea
	GQ416875.1.1467	Enterobacteriaceae; g_Pantoea
	GQ927306.1.1467	Enterobacteriaceae; g_Enterobacter
	GU553044.1.1341	Enterobacteriaceae; g_Pantoea
	HE681389.1.1540	Enterobacteriaceae; g_Enterobacter
	HG799974.1.1694	Enterobacteriaceae; g_Enterobacter
	HM159971.1.1501	Comamonadaceae; g_Comamonas
	HM557327.1.1357	Enterobacteriaceae; g_Enterobacter
	HQ419280.1.1500	Enterobacteriaceae; g_Enterobacter
	JF766379.1.1511	Enterobacteriaceae; g_Enterobacter
	JN969353.1.1512	Enterobacteriaceae; g_Enterobacter
	JQ712525.1.1317	Enterobacteriaceae; g_Enterobacter
	JZBZ01000043.3774.5314	Enterobacteriaceae; g_Escherichia-Shigella; s_uncultured bacterium
	KC509582.1.1329	Enterobacteriaceae; g_Enterobacter
	KF842990.1.1401	Enterobacteriaceae; g_Enterobacter
	KM377658.1.1498	Enterobacteriaceae; g_Pantoea
	KR027006.1.1391	Enterobacteriaceae; g_Enterobacter
	KU711913.1.1414	Enterobacteriaceae; g_Enterobacter
	LGJY01000007.95396.97084	Enterobacteriaceae; g_Enterobacter; s_uncultured bacterium
	New.CleanUp.ReferenceOTU101303	Lactobacillaceae; g_Lactobacillus; s_uncultured Lactobacillus sp.
	New.CleanUp.ReferenceOTU131659	Neisseriaceae; g_Neisseria; s_uncultured bacterium
	New.CleanUp.ReferenceOTU137264	Lactobacillaceae; g_Lactobacillus; s_Apis mellifera
	New.CleanUp.ReferenceOTU174748	Enterobacteriaceae; g_Pantoea
	New.CleanUp.ReferenceOTU86952	Neisseriaceae; g_Snodgrassella; s_Apis mellifera
	New.ReferenceOTU113	Neisseriaceae; g_Snodgrassella
	New.ReferenceOTU177	Enterobacteriaceae; g_Escherichia-Shigella
	New.ReferenceOTU26	Enterobacteriaceae; g_Enterobacter
	New.ReferenceOTU527	Comamonadaceae; g_Comamonas