



Published by
Faculty of
Biotechnology and
Food Sciences

IDENTIFICATION OF MICROBIAL COMMUNITY COLONIZING THE GUT OF *DYSDERCUS CINGULATUS* FABRICIUS (HEMIPTERA: PYRRHOCORIDAE)

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doi: [10.15414/jmbfs.2019/20.9.3.496-501](https://doi.org/10.15414/jmbfs.2019/20.9.3.496-501)

ARTICLE INFO

Received 18. 10. 2018
Revised 10. 5. 2019
Accepted 12. 5. 2019
Published 1. 12. 2019

Regular article



ABSTRACT

Red cotton stainer *Dysdercus cingulatus* sucks the sap of cotton plant and hence considered as one of the serious pests of cotton across the globe. Gut microbial community of this pest was studied using 16srRNA variable regions (V3 & V4) using Illumina MiSeq technology. Totally 11, 0,797 reads were obtained which were processed using QIIME pipeline. This study resulted in the identification of gut microbiota of *D. cingulatus* categorized into 34 different phyla, 88 classes, 132 classes, 206 families and 336 genera. Phylum level taxonomic classification identifies bacteria predominantly from Proteobacteria (46.7%), Actinobacteria (25.7%) and Firmicutes (18%). Species from the genus *Coriobacterium*, *Bifidobacterium*, *Corynebacterium*, *Klebsiella* & *Pseudomonas* are most abundant in the gut of *D. cingulatus*. Insights into the gut can help us to understand the role of microorganism which dismantles the plant.

Keywords: Cotton Pest, *Dysdercus cingulatus*, 16s rRNA, Gut Microbiome, Bioinformatics, NGS MiSeq

INTRODUCTION

Dysdercus cingulatus (Hemiptera: Lygaeidae) is one among 340 species of Pyrrhocoridae family. It is a sucking pest and a common cotton stainer feeding on flower buds sand seeds; which leads to early shedding of lower buds and reduces the quality of seeds (Singh, 1924). *D. cingulatus* is highly mobile and while searching food it easily gets adapted to different hosts; thereby it is more challenging to control the pest when it is abundant and mobile (Maxwell-Lefroy, 1906). Inhabitations in different ecological niches flourish insects with a variety of microbes participating in Plant-Microbe-Insect (PMI) interaction. Transmission of pathogens to plants via insects (disease transmitting vector) is an example of PMI interaction (Bennett, 2013). Similarly, *D. cingulatus* acts as a vector or transmitting fungal pathogens to cotton plants. The insect carries fungal spores externally on the mouth parts and transmits through proboscis on the external surfaces of the cotton bolls. *D. cingulatus* transmits *Nematospora gossypii* that causes internal boll disease in cotton plants (Ahmad & Schaefer, 1987). Another member of this genus *Nematospora coryli* associated with dry cultivated citrus fruit has been isolated and identified from Australia (Shivas et al., 2005).

Identification of species across different life forms using molecular approaches has been a handy tool. This is facilitated by various universal barcodes such as 16s rRNA (Bacteria) (Janda & Abbott, 2007) and Cytochrome Oxidase subunit I, COI (Eukaryotes) (Kress & Erickson, 2008). The 16S rRNA sequence has been a universal code in bacteria and an efficient method to identify microbial species from different environments without direct culturing of the bacterial sample. There are nine hyper variable regions in 16S rRNA sequence (Srinivasan et al., 2015) that are subsequently used for bacterial identification (Huse et al., 2008).

Characterization of the gut microbial community of *Pyrrhocoris apterus* and *Dysdercus fasciatus* revealed the presence of majorly unusual nutrition mutualist Actinobacteria of genera *Coriobacterium* and *Gordonibacter* (Hassan Salem, Kreuter, Sudakaran, & Kaltenpoth, 2012). Firmicutes such as *Clostridium* and several other Gamma Proteobacteria also harbor the gut. In Pyrrhocoridae, Actinobacteria provides nutritional support by supplementing vitamins that are unavailable to the insect through malvace food source. Similar studies conducted on *Halyomorpha halys* (Hemiptera: Pentatomidae) to find the relationship of gut bacteria to the host health, development and fecundity also indicate negative impact on the insect if deprived of gut bacteria (Taylor, Coffey, DeLay, &

Dively, 2014). The purpose of this study was to identify gut bacteria of *Dysdercus cingulatus*, a cotton stainer using 16S rRNA V3-V4 regions by Illumina MiSeq NGS technology. An insight into the microbial world residing in the gut of *D. cingulatus* can be explored for pest control management.

MATERIALS AND METHODS

Insect sampling and gut extraction

Insects collected in the month of September, 2015 from cotton field of Tindivanam, Tamil Nadu, India, were reared on cotton bolls under laboratory conditions. Identification and characterization of the sample to be *D. cingulatus* based on morphological features was done by an expert entomologist from The Department of Zoology, Guru Nanak College, Chennai – India. Prior to dissection, outer surface of insect was sterilized with 70% ethanol and rinsed twice in 1X Phosphate Buffer Saline (PBS). Midguts dissected from five *D. cingulatus* was homogenized in 180 µl PBS buffer using a micropesle under a sterile environment (Xiang et al., 2012).

16S rRNA gene sequencing, clustering and sequencing

The genomic DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen) following manufacturer's protocol with few modifications. Insect homogenate-proteinase K buffer mix was incubated at 37° C for 12hrs for better yield. To check the integrity and purity, extracted DNA was loaded on to 0.8% agarose gel and 1 µl of the sample was loaded on NanoDrop 8000 spectrophotometer for determining A260/280 ratio respectively. The DNA was quantified using Qubit® 2.0 Fluorometer.

Amplicon libraries were prepared using Nextera XT index Kit (Illumina Inc.) as per the 16S Metagenomics Sequencing Library preparation protocol Universal primers were used for the amplifying V3-V4 region of 16S rDNA gene (Table 1) using following reaction conditions: initial denaturation at 94° C for 3 minutes, followed by 30 cycles of denaturation at 94° C for 1 minute, annealing at 52° C for 1 minute and elongation at 72° for 1 minute and followed by final elongation at 72° for 10mins for identification of bacteria and (Takahashi, Tomita, Nishioka, Hisada, & Nishijima, 2014).

Table 1 List of Primers used for the amplification of V3-V4 hyper variable region

Primer Name	Primer Sequence (5' to 3')	Length of primer	Product size (Approx.)
ProkaryoteV3-Forward	CCTACGGGNBGCASCAG	17	460 bps
ProkaryoteV4-Reverse	GACTACNVGGGTATCTAATCC	21	

To generate the cluster as per the standard protocol of Illumina amplicons with the adaptors were amplified by using i5 and i7 primers which add multiplexing index sequences as well as common adapters for the process. The amplicon libraries were then purified by 1X AM pure XP beads and checked on Agilent High Sensitivity (HS) chip Bioanalyzer 2100 and quantified for library and mean peak size on fluorometer by Qubit dsDNA HS Assay kit (Life Technologies).

The library was loaded onto Illumina MiSeq platform at appropriate concentration (10-20pM) for cluster generation and sequencing. The kit reagents were used in binding of samples to complementary adapter oligos on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment (Bartram, Lynch, Stearns, Moreno-Hagelsieb, & Neufeld, 2011).

Data analysis

Forward and reverse read files were merged using Fast Length Adjustment of Short reads (FLASH) (Magoč & Salzberg, 2011) to form a single FASTQ file. This file was analysed using Quantitative Insights into Microbial Ecology (QIIME), for microbial community analysis (Caporaso et al., 2010). Operational taxonomical Units (OTUs) were picked based on Uclust_ref method, followed by selection of representative sequences for all OTUs. These representative sequences were aligned using Python Nearest Alignment Space Termination (PyNAST) and Ribosomal Database Project RDP classifier and

Greengenes version 13.8 databases were used to provide taxonomical identity to OTUs.

RESULTS

The Illumina MiSeq run of the gut *D. cingulatus* 16s rRNA amplicon yielded 1, 10,797 reads. The paired end reads were coupled to generate a single file using the FLASH software. Raw data was submitted to Sequence Read Archive (SRA), NCBI under the Accession ID SRR3355341.

Taxonomic characterization of gut microbes

We analysed the 16S rRNA gene data of gut bacteria of *D. cingulatus* using QIIME and classified as 34 phyla, 88 classes, 132 orders, 200 families, and 345 genera and 171 species. At phylum level, gut bacteria were classified as Proteobacteria (47.10%), Actinobacteria (25.90%) and Firmicutes (18%), (Figure1) (Hassan Salem et al., 2012). Other abundant phyla identified in the gut were Bacteroidetes (4.20%), Cyanobacteria (1.40%), Fusobacteria (0.30%), Deferribacteres (0.20%), Tenericutes (0.20%), Acidobacteria (0.10%), and several unclassified bacteria (0.60%) (Table 2 & Figure 1).

Some of the Candidate division bacteria identified in this study were TM7 (Saccharibacteria), GN02 (from Guerrero Negro hyper saline microbial mat), OD1 (Parcubacteria), OP11 (Microgenomates), OP3 (Omnitrophica), SR1 (Abscondita bacteria), WS6 (from Wurtsmith contaminated aquifer), and WWE1 (Cloacimonetes).

Table 2 Percentage of species under phylum level classification

Percentage	Kingdom	Phylum	Percentage	Kingdom	Phylum
47.15	Bacteria	Proteobacteria	0.017	Bacteria	SR1
25.89	Bacteria	Actinobacteria	0.014	Bacteria	Elusimicrobia
17.96	Bacteria	Firmicutes	0.009	Bacteria	Nitrospirae
4.18	Bacteria	Bacteroidetes	0.009	Bacteria	Synergistetes
1.45	Bacteria	Cyanobacteria	0.007	Bacteria	Armatimonadetes
0.68	Bacteria	Other	0.006	Bacteria	WWE1
0.29	Bacteria	Fusobacteria	0.003	Bacteria	Chlorobi
0.20	Bacteria	Tenericutes	0.003	Bacteria	WS6
0.17	Bacteria	Deferribacteres	0.002	Bacteria	BRC1
0.13	Bacteria	Acidobacteria	0.002	Bacteria	GN02
0.13	Bacteria	TM7	0.002	Bacteria	Lentisphaerae
0.12	Bacteria	Verrucomicrobia	0.002	Bacteria	TM6
0.10	Bacteria	Chloroflexi	0.002	Bacteria	Thermotogae
0.09	Bacteria	Thermi	0.002	Bacteria	WS3
0.07	Bacteria	Planctomycetes	0.001	Bacteria	Fibrobacteres
0.04	Bacteria	Gemmatimonadetes	0.001	Bacteria	OP11
0.02	Bacteria	OD1	0.001	Bacteria	OP3
0.02	Bacteria	Spirochaetes			

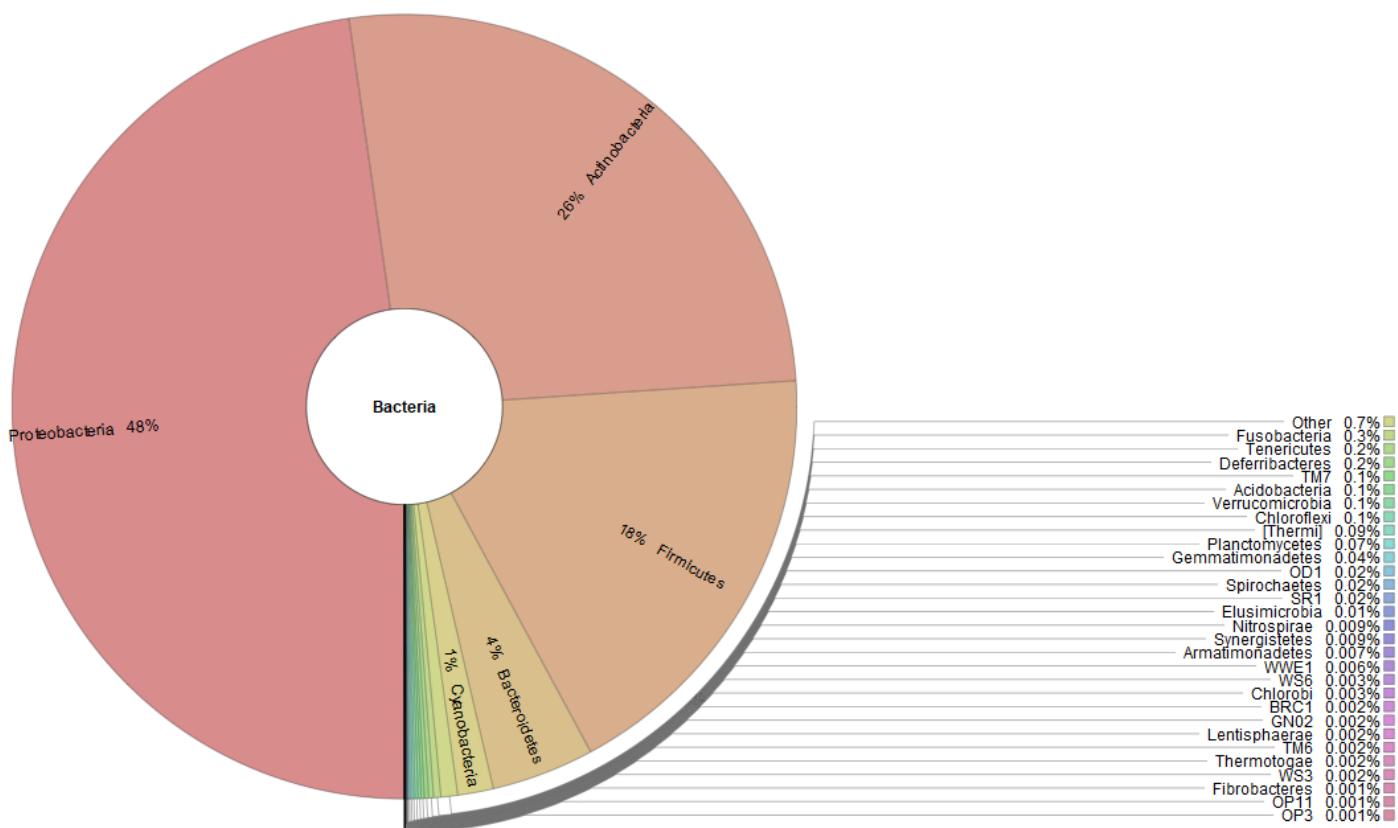
At the class level, Proteobacteria consisted of γ -Proteobacteria (23.13%), α -Proteobacteria (22.36%), β -Proteobacteria (0.77%), δ -Proteobacteria (0.37%), and ϵ -Proteobacteria (0.36%). Actinobacteria consisted of class Actinobacteria (15.56%) and Coriobacteria (10.24%) and minor classes such as Acidimicrobia, Thermoleophilia, Rubrobacteria and Nitriliruptoria. Firmicutes were categorized into class Clostridia (10.86%), Bacilli (6.91%), and Erysipelotrichia (0.068%) and an unidentified class. Bacteroidetes equals to 4.20% of total reads and comprised of classes Bacteroidia (3.6%), Flavobacteria (0.3%), Cytophaga (0.07%), Sphingobacteria (0.04%), and unclassified classes. Similarly, Fusobacteria, Deferribacteres, Tenericutes, comprised of classes Fusobacterium (0.3%), Deferribacteres (0.2%), and Mollicutes (0.2%), respectively (Table 3 & Figure 2).

Most abundant families of Alpha-Proteobacteria were Bartonellaceae (16.5%), Rhodobacteraceae (2.2%), Rhodospirillaceae (0.43%) and Rhizobiaceae (0.1%). Major families identified in Gamma-Proteobacteria were Enterobacteriaceae (17.6%), Pseudomonadaceae (4.4%), and Xanthomonadaceae (0.3%). Coriobacteriaceae (10.2%), Bifidobacteriaceae (8.2%), Corynebacteriaceae (3.7%), Micrococcaceae (2%), Propionibacteriaceae (0.5%) and other minor families from the phylum Actinobacteria (Table 4 & Figure 3). Gut of *D. cingulatus* also exhibited ample accumulation of Lachnospiraceae (4.8%),

Tissierellaceae (2.8%), Streptococcaceae (2.5%), Bacillaceae (1.5%), Ruminococcaceae (0.7%), Staphylococcaceae (1.1%), and Enterococcaceae (0.2%) from phylum Firmicutes.

Population of the species from the genus *Bartonella* (*Bartonellaceae*) was highest with (17.6%) of the total diversity in the gut of *D. cingulatus*. Interestingly, an unidentified genus under the family of *Enterobacteriaceae* was the next most abundant genus at 1.8%. Gut of *D. cingulatus* is nourished with genus *Klebsiella* (3.5%), *Pseudomonas* (3.5%), *Paracoccus* (2.1%), *Anaerococcus* (2.01%), *Erwinia*, *Acinetobacter*, and *Enterobacter* from *Proteobacteria*; and *Coriobacterium* (8.23%), *Bifidobacterium* (8.21%), *Corynebacterium* (3.9%), *Micrococcus* (1.1%), *Rothia*, and *Propionibacterium* from *Actinobacteria* (Table 5 & Figure 4).

Alpha diversity calculated for the annotated species was 57.354. Assessment at the base species level, the gut of *D. cingulatus* was rich in *Bartonella bacilliformis*, *Bifidobacterium asteroides*, *Corynebacterium mucificaciens*, *Bacillus subtilis* *Gardnerella vaginalis*, *Rothia mucilaginosa*, and *Corynebacterium matruchotii*.

**Figure 1** Pie chart showing the distribution of species under phylum level classification**Table 3** Percentage of species under Class level classification

Percentage	Phylum	Class	Percentage	Phylum	Class
23.14	Proteobacteria	Gammaproteobacteria	0.01	Acidobacteria	Sva0725
22.37	Proteobacteria	Alphaproteobacteria	0.01	Acidobacteria	iii1-8
15.57	Actinobacteria	Actinobacteria	0.01	Actinobacteria	Other
10.86	Firmicutes	Clostridia	0.01	Chloroflexi	TK10
10.24	Actinobacteria	Coriobacteriia	0.01	Chloroflexi	Other
6.91	Firmicutes	Bacilli	0.01	Cyanobacteria	Oscillatoriophycideae
3.60	Bacteroidetes	Bacteroidia	0.01	Gemmatimonadetes	Gemm-5
1.41	Cyanobacteria	Chloroplast	0.01	WWE1	Cloacamonae
0.78	Proteobacteria	Betaproteobacteria	0.01	Actinobacteria	MB-A2-108
0.68	Other	Other	0.01	OD1	-
0.37	Proteobacteria	Deltaproteobacteria	0.01	TM7	TM7-1
0.36	Proteobacteria	Epsilonproteobacteria	0.0041	Acidobacteria	Acidobacteriia
0.31	Bacteroidetes	Flavobacteriia	0.0041	Acidobacteria	TM1
0.29	Fusobacteria	Fusobacteriia	0.0041	Verrucomicrobia	Other
0.20	Tenericutes	Mollicutes	0.0031	Armatimonadetes	0319-6E2
0.17	Deferribacteres	Deferribacteres	0.0031	Bacteroidetes	Rhodothermi
0.13	Proteobacteria	Other	0.0031	Chloroflexi	TK17
0.13	Bacteroidetes	Other	0.0031	Cyanobacteria	-
0.12	Firmicutes	Other	0.0031	Spirochaetes	Brachyspirae
0.12	TM7	TM7-3	0.0031	WS6	SC72
0.09	Thermi	Deinococci	0.0021	Acidobacteria	S035
0.07	Bacteroidetes	Cytophagia	0.0021	Armatimonadetes	Chthonomonadetes
0.07	Verrucomicrobia	Opitutae	0.0021	BRC1	PRR-11
0.07	Firmicutes	Erysipelotrichi	0.0021	Chloroflexi	Ktedonobacteria
0.05	Acidobacteria	Acidobacteria-6	0.0021	Gemmatimonadetes	-
0.04	Planctomycetes	Planctomycetia	0.0021	Lentisphaerae	Lentisphaeria
0.04	Bacteroidetes	Sphingobacteriia	0.0021	Planctomycetes	OM190
0.04	Chloroflexi	Thermomicrobia	0.0021	TM6	SJA-4
0.04	Actinobacteria	Acidimicrobii	0.0021	TM7	-
0.03	Bacteroidetes	Saprospirae	0.0021	Thermotogae	Thermotogae
0.03	Actinobacteria	Thermoleophilia	0.0021	WS3	PRR-12
0.02	Chloroflexi	Anaerolineae	0.0010	Acidobacteria	Other

0.02	<i>Planctomycetes</i>	<i>Phycisphaerae</i>	0.0010	<i>Acidobacteria</i>	AT-s54
0.02	<i>Acidobacteria</i>	<i>Chloracidobacteria</i>	0.0010	<i>Acidobacteria</i>	Acidobacteria-5
0.02	<i>Acidobacteria</i>	<i>Solibacteres</i>	0.0010	<i>Acidobacteria</i>	EC1113
0.02	<i>Spirochaetes</i>	<i>Spirochaetes</i>	0.0010	<i>Actinobacteria</i>	Nitriliruptoria
0.02	<i>Verrucomicrobia</i>	<i>Spartobacteria</i>	0.0010	<i>Armatimonadetes</i>	Armatimonadia
0.02	<i>Gemmatimonadetes</i>	Gemm-3	0.0010	<i>Armatimonadetes</i>	Fimbriimonadia
0.02	<i>Gemmatimonadetes</i>	<i>Gemmatimonadetes</i>	0.0010	<i>Chlorobi</i>	-
0.02	<i>SR1</i>	-	0.0010	<i>Chlorobi</i>	OPB56
0.02	<i>Cyanobacteria</i>	Other	0.0010	<i>Chlorobi</i>	SJA-28
0.02	<i>Cyanobacteria</i>	4C0d-2	0.0010	<i>Chloroflexi</i>	SAR202
0.02	<i>OD1</i>	ZB2	0.0010	<i>Fibrobacteres</i>	Fibrobacteria
0.02	<i>Verrucomicrobia</i>	<i>Pedosphaerae</i>	0.0010	<i>GN02</i>	BD1-5
0.01	<i>Elusimicrobia</i>	<i>Elusimicrobia</i>	0.0010	<i>GN02</i>	GKS2-174
0.01	<i>Verrucomicrobia</i>	<i>Verrucomicrobiae</i>	0.0010	<i>OD1</i>	ABY1
0.01	<i>Actinobacteria</i>	<i>Rubrobacteria</i>	0.0010	<i>OD1</i>	SM2F11
0.01	<i>Acidobacteria</i>	BPC102	0.0010	<i>OP11</i>	WCHB1-64
0.01	<i>Chloroflexi</i>	<i>Chloroflexi</i>	0.0010	<i>OP3</i>	koll11
0.01	<i>Nitrospira</i>	<i>Nitrospira</i>	0.0010	<i>Planctomycetes</i>	vadinHA49
0.01	<i>Synergistetes</i>	<i>Synergistia</i>	0.0010	<i>Proteobacteria</i>	TA18
0.01	<i>Chloroflexi</i>	Ellin6529	0.0010	<i>TM7</i>	Other

Legend: '-' Classification data not available in the database.

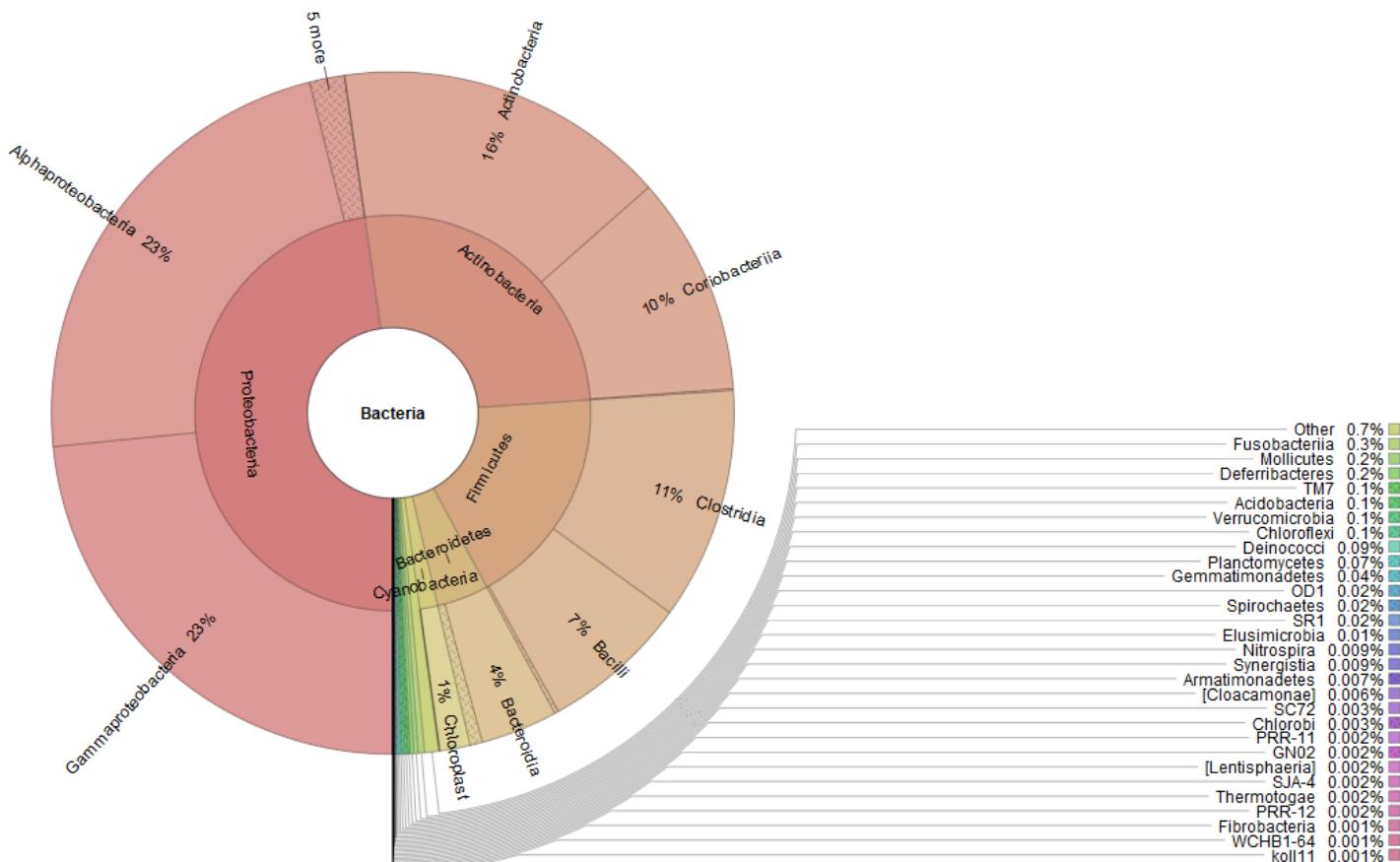


Figure 2 Pie chart showing the distribution of species under Class level classification

Table 4 Percentage of species under Family level classification

Percentage	Order	Family	Percentage	Order	Family
17.65	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	0.010	<i>Rickettsiales</i>	-
16.48	<i>Rhizobiales</i>	<i>Bartonellaceae</i>	0.010	<i>Bdellovibrionales</i>	<i>Bdellovibrionaceae</i>
10.24	<i>Coriobacteriales</i>	<i>Coriobacteriaceae</i>	0.009	<i>iii1-15</i>	<i>mb2424</i>
8.23	<i>Bifidobacteriales</i>	<i>Bifidobacteriaceae</i>	0.009	<i>Gaiellales</i>	<i>Gaiellaceae</i>
4.77	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	0.009	<i>Clostridiales</i>	<i>Acidaminobacteraceae</i>
3.71	<i>Actinomycetales</i>	<i>Corynebacteriaceae</i>	0.008	<i>Actinomycetales</i>	<i>Pseudonocardiaceae</i>
3.30	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	0.008	<i>Cytophagales</i>	<i>Flammeovirgaceae</i>
2.76	<i>Clostridiales</i>	<i>Tissierellaceae</i>	0.008	<i>Elusimicrobiales</i>	<i>Elusimicrobiaceae</i>
2.50	<i>Lactobillales</i>	<i>Streptococcaceae</i>	0.008	<i>Nitrospirales</i>	<i>0319-6A21</i>

2.25	Rhodobacterales	Rhodobacteraceae	0.008	Gemmatales	Isosphaeraceae
2.04	Actinomycetales	Micrococcaceae	0.007	Acidimicrobiales	C111
1.47	Bacillales	Bacillaceae	0.007	Actinomycetales	Geodermatophilaceae
1.10	Clostridiales	Ruminococcaceae	0.007	Actinomycetales	Micromonosporaceae
1.09	Bacillales	Staphylococcaceae	0.007	Flavobacteriales	Cryomorphaceae
1.09	Pseudomonadales	Moraxellaceae	0.007	Rhizobiales	Beijerinckiaceae
0.91	Bacteroidales	Prevotellaceae	0.007	Rhizobiales	Xanthobacteraceae
0.67	Bacteroidales	S24-7	0.007	Syntrophobacterales	Syntrophobacteraceae
0.49	Actinomycetales	Propionibacteriaceae	0.006	Solirubrobacterales	Solirubrobacteraceae
0.44	Clostridiales	Veillonellaceae	0.006	Caldilineales	Caldilineaceae
0.43	Rhodospirillales	Rhodospirillaceae	0.006	Gemmatales	Gemmataceae
0.40	Rickettsiales	mitochondria	0.006	Planctomycetales	Planctomycetaceae
0.40	Neisseriales	Neisseriaceae	0.006	Rickettsiales	Rickettsiaceae
0.34	Bacteroidales	Rikenellaceae	0.006	Rhodocyclales	Rhodocyclaceae
0.34	Bacteroidales	Porphyromonadaceae	0.005	Actinomycetales	Dermatophilaceae
0.34	Bacteroidales	Paraprevellaceae	0.005	Actinomycetales	Nocardiaceae
0.33	Bacteroidales	Bacteroidaceae	0.005	Bacteroidales	Barnesiellaceae
0.32	Campylobacterales	Helicobacteraceae	0.005	Cytophagales	Cyclobacteriaceae
0.31	Bacteroidales	Odoribacteraceae	0.005	Oscillatorioides	Phormidiaceae
0.31	Xanthomonadales	Xanthomonadaceae	0.005	Vibrionales	Pseudoalteromonadaceae
0.29	Desulfovibrionales	Desulfovibrionaceae	0.004	Acidobacteriales	Acidobacteriaceae
0.27	Lactobacillales	Aerococcaceae	0.004	Solibacterales	Solibacteraceae
0.23	Lactobacillales	Leuconostocaceae	0.004	Actinomycetales	Dermacoccaceae
0.22	Lactobacillales	Enterococcaceae	0.004	Bacteroidales	BA008
0.22	Flavobacteriales	Flavobacteriaceae	0.004	AKYG85	Dolo_23
0.19	Fusobacteriales	Leptotrichiaceae	0.004	Bacillales	Alicyclobacillaceae
0.19	Rhizobiales	Aurantimonadaceae	0.004	Rhodobacterales	Hyphomonadaceae
0.19	Vibrionales	Vibrionaceae	0.004	Oceanospirillales	Oceanospirillaceae
0.18	Gemellales	Gemellaceae	0.004	Salinisphaerales	Salinisphaeraceae
0.17	Deferribacterales	Deferribacteraceae	0.004	Synergistales	Dethiosulffovibrionaceae
0.17	Lactobacillales	Carnobacteriaceae	0.004	Pedosphaerales	R4-41B
0.16	Pasteurellales	Pasteurellaceae	0.003	Acidimicrobiales	AKIW874
0.15	Mycoplasmatales	Mycoplasmataceae	0.003	Actinomycetales	Glycomycetaceae
0.14	Rhodospirillales	Acetobacteraceae	0.003	Actinomycetales	Sporichthyaceae
0.14	Sphingomonadales	Sphingomonadaceae	0.003	Solirubrobacterales	Patulibacteraceae
0.12	Burkholderiales	Comamonadaceae	0.003	Rhodothermales	Balneolaceae
0.11	Actinomycetales	Intrasporangiaceae	0.003	Anaerolineales	Anaerolinaceae
0.11	Rhizobiales	Rhizobiaceae	0.003	Clostridiales	Christensenellaceae
0.10	Fusobacteriales	Fusobacteriaceae	0.003	Desulfovibrionales	Desulfomicrobiaceae
0.10	Actinomycetales	Actinomycetaceae	0.003	Myxococcales	Nannocystaceae
0.09	Lactobacillales	Lactobacillaceae	0.003	Syntrophobacterales	Syntrophaceae
0.09	Actinomycetales	Dietziaceae	0.003	Alteromonadales	211ds20
0.09	Clostridiales	Mogibacteriaceae	0.003	Thiotrichales	Piscirickettsiaceae
0.08	Actinomycetales	Nocardiooidaceae	0.003	Brachyspirales	Brachyspiraceae
0.08	Actinomycetales	Dermabacteraceae	0.003	Pedosphaerales	Ellin517
0.07	Methylophilales	Methylophilaceae	0.003	Cloacamonales	CW-1
0.07	Rhizobiales	Brucellaceae	0.003	Cloacamonales	Cloacamonaceae
0.07	Actinomycetales	Microbacteriaceae	0.002	Acidimicrobiales	koll13
0.07	Flavobacteriales	Weeksellaceae	0.002	Actinomycetales	Kineosporiaceae
0.07	Erysipelotrichales	Erysipelotrichaceae	0.002	Actinomycetales	Mycobacteriaceae
0.07	Clostridiales	Clostridiaceae	0.002	Actinomycetales	Promicromonosporaceae
0.07	Alteromonadales	Chromatiaceae	0.002	Gaiellales	AK1AB1_02E
0.07	Rhizobiales	Methylobacteriaceae	0.002	Bacteroidales	SB-1
0.06	Bacillales	Planococcaceae	0.002	Turicibacterales	Turicibacteraceae
0.06	Thermales	Thermaceae	0.002	Gemmatimonadales	Gemmatimonadaceae
0.06	Actinomycetales	Streptomycetaceae	0.002	Z20	R4-45B
0.06	Aeromonadales	Aeromonadaceae	0.002	Rhizobiales	Rhodobiaceae
0.05	Burkholderiales	Burkholderiaceae	0.002	Nitrosomonadales	Nitrosomonadaceae
0.05	Burkholderiales	Oxalobacteraceae	0.002	Desulfarculales	Desulfarculaceae
0.05	Caulobacterales	Caulobacteraceae	0.002	Myxococcales	Cystobacteraceae
0.04	Rhizobiales	Bradyrhizobiaceae	0.002	Myxococcales	Myxococcaceae
0.04	Anaeroplasmatales	Anaeroplasmataceae	0.002	Oceanospirillales	Hahellaceae

0.04	<i>Sphingomonadales</i>	<i>Erythrobacteraceae</i>	0.002	<i>Sphaerochaetales</i>	<i>Sphaerochaetaceae</i>
0.04	<i>Campylobacterales</i>	<i>Campylobacteraceae</i>	0.002	<i>Thermotogales</i>	<i>Thermotogaceae</i>
0.04	<i>Cytophagales</i>	<i>Cytophagaceae</i>	0.002	<i>Deinococcales</i>	<i>Trueperaceae</i>
0.04	<i>Sphingobacteriales</i>	<i>Sphingobacteriaceae</i>	0.001	<i>Solibacterales</i>	<i>Bryobacteraceae</i>
0.04	<i>Clostridiales</i>	<i>Peptostreptococcaceae</i>	0.001	<i>Acidimicrobiales</i>	<i>EB1017</i>
0.04	<i>Actinomycetales</i>	<i>Brevibacteriaceae</i>	0.001	<i>Actinomycetales</i>	<i>Actinopolysporaceae</i>
0.04	<i>Bacillales</i>	<i>Exiguobacteriaceae</i>	0.001	<i>Actinomycetales</i>	<i>Actinosynnemataceae</i>
0.03	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	0.001	<i>Actinomycetales</i>	<i>Bogoriellaceae</i>
0.03	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	0.001	<i>Actinomycetales</i>	<i>Thermomonosporaceae</i>
0.03	<i>Burkholderiales</i>	<i>Alcaligenaceae</i>	0.001	<i>Actinomycetales</i>	<i>Yaniellaceae</i>
0.03	<i>Saprospirales</i>	<i>Chitinophagaceae</i>	0.001	<i>Nitriliruptorales</i>	<i>Nitriliruptoraceae</i>
0.03	<i>Deinococcales</i>	<i>Deinococcaceae</i>	0.001	<i>Armatimonadales</i>	<i>Armatimonadaceae</i>
0.02	<i>Oceanospirillales</i>	<i>Halomonadaceae</i>	0.001	<i>Chthonomonadales</i>	<i>Chthonomonadaceae</i>
0.02	<i>Rhizobiales</i>	<i>Phyllobacteriaceae</i>	0.001	<i>Fimbriimonadales</i>	<i>Fimbriimonadaceae</i>
0.02	<i>Pirellulales</i>	<i>Pirellulaceae</i>	0.001	<i>Saprospirales</i>	<i>Saprospiraceae</i>
0.02	<i>Clostridiales</i>	<i>Peptococcaceae</i>	0.001	<i>Ardenscatenales</i>	<i>Ardenscatenaceae</i>
0.02	<i>Alteromonadales</i>	<i>Shewanellaceae</i>	0.001	<i>Thermogemmatisporales</i>	<i>Thermogemmatisporaceae</i>
0.02	<i>Chthoniobacterales</i>	<i>Chthoniobacteraceae</i>	0.001	<i>Clostridiales</i>	<i>EtOH8</i>
0.02	<i>Bacillales</i>	<i>Paenibacillaceae</i>	0.001	<i>Nitrosopirales</i>	<i>Nitrosopiraceae</i>
0.02	<i>Xanthomonadales</i>	<i>Sinobacteraceae</i>	0.001	<i>Phycisphaerales</i>	<i>Phycisphaeraceae</i>
0.02	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	0.001	<i>Kiloniellales</i>	<i>Kiloniellaceae</i>
0.01	<i>Legionellales</i>	<i>Legionellaceae</i>	0.001	<i>Rhizobiales</i>	<i>Methylocystaceae</i>
0.01	<i>Verrucomicrobiales</i>	<i>Verrucomicrobiaceae</i>	0.001	<i>Myxococcales</i>	<i>Polyangiaceae</i>
0.01	<i>RB41</i>	<i>Ellin6075</i>	0.001	<i>NB1-j</i>	<i>NB1-i</i>
0.01	<i>Rubrobacterales</i>	<i>Rubrobacteraceae</i>	0.001	<i>Alteromonadales</i>	<i>HTCC2188</i>
0.01	<i>Legionellales</i>	<i>Coxiellaceae</i>	0.001	<i>Alteromonadales</i>	<i>Idiomarinaceae</i>
0.01	<i>Opitutales</i>	<i>Opitutaceae</i>	0.001	<i>Cardiobacteriales</i>	<i>Cardiobacteriaceae</i>
0.01	<i>Actinomycetales</i>	<i>Cellulomonadaceae</i>	0.001	<i>I025</i>	<i>Rs-045</i>
0.01	<i>Actinomycetales</i>	<i>Gordoniacae</i>	0.001	<i>Pedosphaerales</i>	<i>Ellin515</i>

Legend: ‘-’ Classification data not available in the database.

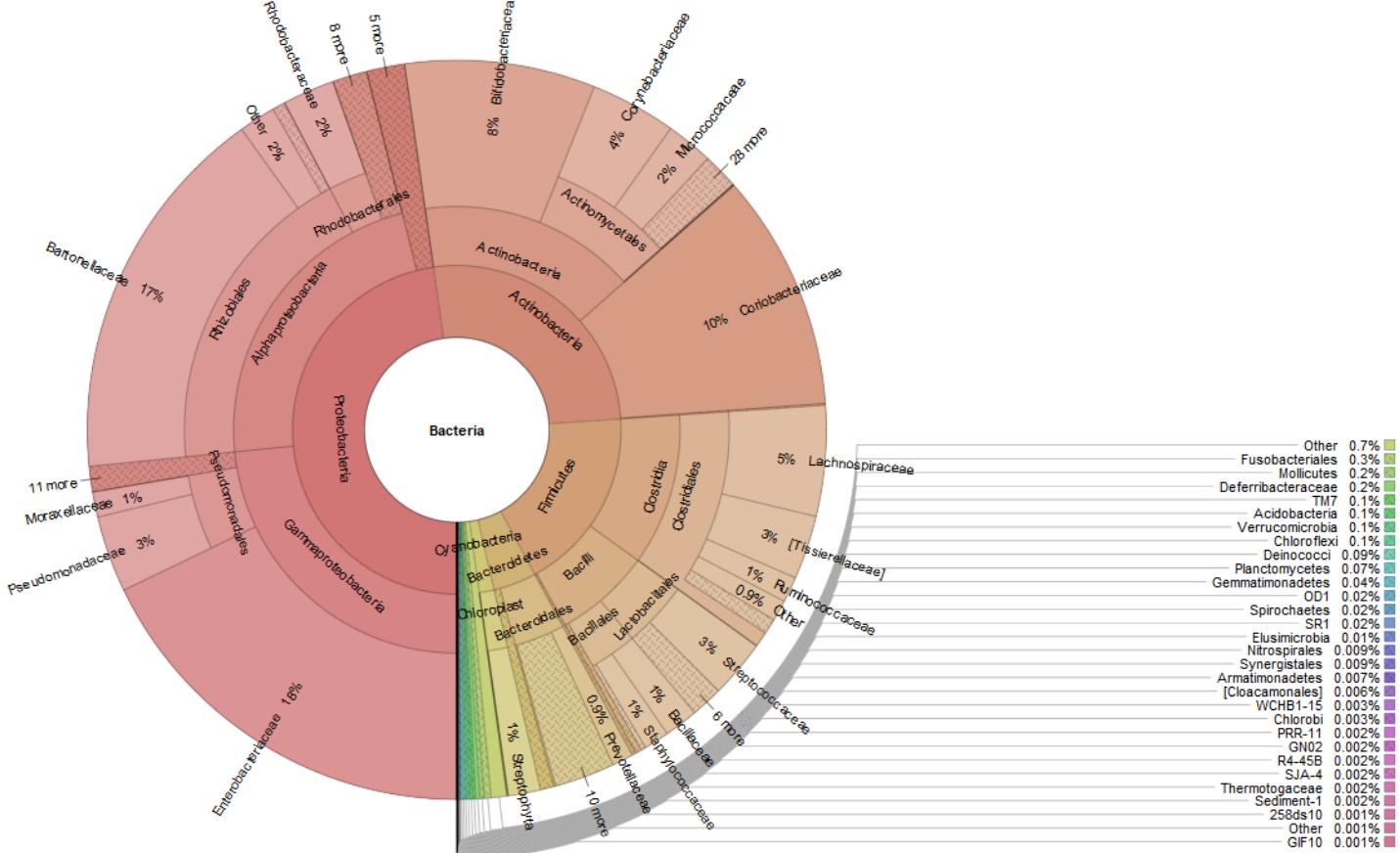


Figure 3 Pie chart showing the distribution of bacteria at Family level classification

Table 5 Percentage of species under phylum Genus classification

Percentage	Family	Genus	Percentage	Family	Genus
8.305552	<i>Coriobacteriaceae</i>	<i>Coriobacterium</i>	0.00612	<i>Alteromonadaceae</i>	<i>Marinobacter</i>
3.734496	<i>Bifidobacteriaceae</i>	<i>Bifidobacterium</i>	0.005177	<i>Dermatophilaceae</i>	<i>Piscicoccus</i>
3.708612	<i>Corynebacteriaceae</i>	<i>Corynebacterium</i>	0.005177	<i>Nocardiaceae</i>	<i>Rhodococcus</i>
3.004576	<i>Pseudomonadaceae</i>	<i>Pseudomonas</i>	0.005177	<i>Bacillaceae</i>	<i>Lentibacillus</i>
2.49415	<i>Streptococcaceae</i>	<i>Streptococcus</i>	0.005177	<i>Peptostreptococcaceae</i>	<i>Filifactor</i>
2.094506	<i>Rhodobacteraceae</i>	<i>Paracoccus</i>	0.005177	<i>Veillonellaceae</i>	<i>Mitsuokella</i>
1.977512	<i>Tissierellaceae</i>	<i>Anaerococcus</i>	0.005177	<i>Alcaligenaceae</i>	<i>Achromobacter</i>
1.29936	<i>Bacillaceae</i>	<i>Bacillus</i>	0.005177	<i>Alcaligenaceae</i>	<i>Sutterella</i>
1.16373	<i>Lachnospiraceae</i>	<i>Clostridium</i>	0.005177	<i>Neisseriaceae</i>	<i>Vogesella</i>
1.018781	<i>Micrococcaceae</i>	<i>Micrococcus</i>	0.005177	<i>Desulfovibrionaceae</i>	<i>Bilophila</i>
0.909034	<i>Prevotellaceae</i>	<i>Prevotella</i>	0.005177	<i>Moraxellaceae</i>	<i>Perlucidibaca</i>
0.8055	<i>Moraxellaceae</i>	<i>Acinetobacter</i>	0.005177	<i>Verrucomicrobiaceae</i>	<i>Prosthecobacter</i>
0.803429	<i>Staphylococcaceae</i>	<i>Staphylococcus</i>	0.004141	<i>Dermacoccaceae</i>	<i>Dermacoccus</i>
0.680223	<i>Micrococcaceae</i>	<i>Rothia</i>	0.004141	<i>Microbacteriaceae</i>	<i>Pseudoclavibacter</i>
0.680223	<i>Ruminococcaceae</i>	<i>Oscillospira</i>	0.004141	<i>Micrococcaceae</i>	<i>Nesterenkonia</i>
0.658481	<i>Enterobacteriaceae</i>	<i>Citrobacter</i>	0.004141	<i>Nocardoidaceae</i>	<i>Aeromicrobium</i>
0.473153	<i>Propionibacteriaceae</i>	<i>Propionibacterium</i>	0.004141	<i>Coriobacteriaceae</i>	<i>Eggerthella</i>
0.399644	<i>Tissierellaceae</i>	<i>GW-34</i>	0.004141	<i>Bacteroidaceae</i>	<i>5-7N15</i>
0.323028	<i>Bacteroidaceae</i>	<i>Bacteroides</i>	0.004141	<i>Porphyromonadaceae</i>	<i>Tannerella</i>
0.310604	<i>Odoribacteraceae</i>	<i>Odoribacter</i>	0.004141	<i>Cytophagaceae</i>	<i>Rhodocytophaga</i>
0.29818	<i>Neisseriaceae</i>	<i>Neisseria</i>	0.004141	<i>Phormidiaceae</i>	<i>Phormidium</i>
0.272296	<i>Paraprevotellaceae</i>	<i>Prevotella</i>	0.004141	<i>Alicyclobacillaceae</i>	<i>Alicyclobacillus</i>
0.261943	<i>Rhodospirillaceae</i>	<i>Rhodospirillum</i>	0.004141	<i>Bacillaceae</i>	<i>Marinibacillus</i>
0.235024	<i>Porphyromonadaceae</i>	<i>Porphyromonas</i>	0.004141	<i>Planococcaceae</i>	<i>Planomicrobium</i>
0.218458	<i>Leuconostocaceae</i>	<i>Weissella</i>	0.004141	<i>Planococcaceae</i>	<i>Rummeliibacillus</i>
0.218458	<i>Xanthomonadaceae</i>	<i>Stenotrophomonas</i>	0.004141	<i>Enterococcaceae</i>	<i>Enterococcus</i>
0.197751	<i>Ruminococcaceae</i>	<i>Ruminococcus</i>	0.004141	<i>Ruminococcaceae</i>	<i>Faecalibacterium</i>
0.184292	<i>Helicobacteraceae</i>	<i>Helicobacter</i>	0.004141	<i>Aurantimonadaceae</i>	<i>Aurantimonas</i>
0.174974	<i>Vibriionaceae</i>	<i>Photobacterium</i>	0.004141	<i>Phyllobacteriaceae</i>	<i>Chelatavorans</i>
0.172903	<i>Micrococcaceae</i>	<i>Kocuria</i>	0.004141	<i>Acetobacteraceae</i>	<i>Roseococcus</i>
0.169797	<i>Deferribacteraceae</i>	<i>Mucispirillum</i>	0.004141	<i>Acetobacteraceae</i>	<i>Roseomonas</i>
0.160479	<i>Moraxellaceae</i>	<i>Enhydrobacter</i>	0.004141	<i>Comamonadaceae</i>	<i>Limnohabitans</i>
0.154267	<i>Leptotrichiaceae</i>	<i>Leptotrichia</i>	0.004141	<i>Salinisphaeraceae</i>	<i>Salinisphaera</i>
0.145984	<i>Enterobacteriaceae</i>	<i>Escherichia</i>	0.004141	<i>Pseudoalteromonadaceae</i>	<i>Vibrio</i>
0.128383	<i>Tissierellaceae</i>	<i>Peptoniphilus</i>	0.004141	<i>Xanthomonadaceae</i>	<i>Xanthomonas</i>
0.127348	<i>Veillonellaceae</i>	<i>Veillonella</i>	0.004141	<i>Dethiosulfovibrionaceae</i>	<i>Pyramidobacter</i>
0.122171	<i>Aerococcaceae</i>	<i>Alloiococcus</i>	0.003106	<i>Actinomycetaceae</i>	<i>Arcanobacterium</i>
0.094217	<i>Actinomycetaceae</i>	<i>Actinomyces</i>	0.003106	<i>Cellulomonadaceae</i>	<i>Cellulomonas</i>
0.084898	<i>Flavobacteriaceae</i>	<i>Capnocytophaga</i>	0.003106	<i>Geodermatophilaceae</i>	<i>Geodermatophilus</i>
0.081792	<i>Porphyromonadaceae</i>	<i>Parabacteroides</i>	0.003106	<i>Glycomycetaceae</i>	<i>Glycomyces</i>
0.079722	<i>Fusobacteriaceae</i>	<i>Fusobacterium</i>	0.003106	<i>Microbacteriaceae</i>	<i>Candidatus Aquiluna</i>
0.076616	<i>Pasteurellaceae</i>	<i>Haemophilus</i>	0.003106	<i>Patulibacteraceae</i>	<i>Patulibacter</i>
0.071439	<i>Brucellaceae</i>	<i>Ochrobactrum</i>	0.003106	<i>Odoribacteraceae</i>	<i>Butyricimonas</i>
0.069368	<i>Dermabacteraceae</i>	<i>Brachybacterium</i>	0.003106	<i>Cytophagaceae</i>	<i>Leadbetterella</i>
0.067298	<i>Tissierellaceae</i>	<i>WAL_1855D</i>	0.003106	<i>Flavobacteriaceae</i>	<i>Gillisia</i>
0.065227	<i>Methylophilaceae</i>	<i>Methylotenera</i>	0.003106	<i>Anaerolinaceae</i>	<i>Anaerolinea</i>
0.062121	<i>Tissierellaceae</i>	<i>1-64</i>	0.003106	<i>Planococcaceae</i>	<i>Lysinibacillus</i>
0.062121	<i>Moraxellaceae</i>	<i>Moraxella</i>	0.003106	<i>Carnobacteriaceae</i>	<i>Desemzia</i>
0.06005	<i>Dietziaceae</i>	<i>Dietzia</i>	0.003106	<i>Streptococcaceae</i>	<i>Lactococcus</i>
0.059015	<i>Sphingomonadaceae</i>	<i>Sphingomonas</i>	0.003106	<i>Mogibacteriaceae</i>	<i>Mogibacterium</i>
0.059015	<i>Helicobacteraceae</i>	<i>Flexispira</i>	0.003106	<i>Erysipelotrichaceae</i>	<i>Asteroleplasma</i>
0.057979	<i>Streptomycetaceae</i>	<i>Streptomyces</i>	0.003106	<i>Fusobacteriaceae</i>	<i>Cetobacterium</i>
0.056944	<i>Aerococcaceae</i>	<i>Aerococcus</i>	0.003106	<i>Hypomicrobiaceae</i>	<i>Rhodoplanes</i>
0.054873	<i>Paraprevotellaceae</i>	<i>YRC22</i>	0.003106	<i>Oxalobacteraceae</i>	<i>Massilia</i>
0.051767	<i>Clostridiaceae</i>	<i>Clostridium</i>	0.003106	<i>Neisseriaceae</i>	<i>Conchiformibius</i>
0.051767	<i>Erysipelotrichaceae</i>	<i>Bulleidia</i>	0.003106	<i>Rhodocyclaceae</i>	<i>Dechloromonas</i>
0.051767	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i>	0.003106	<i>Desulfomicrobiaceae</i>	<i>Desulfomicrobium</i>
0.049697	<i>Intrasporangiaceae</i>	<i>Serinicoccus</i>	0.003106	<i>Enterobacteriaceae</i>	<i>Erwinia</i>
0.047626	<i>Rikenellaceae</i>	<i>Alistipes</i>	0.003106	<i>Oceanospirillaceae</i>	<i>Oleibacter</i>
0.047626	<i>Tissierellaceae</i>	<i>Parvimonas</i>	0.003106	<i>Moraxellaceae</i>	<i>Alkanindiges</i>
0.045555	<i>Aerococcaceae</i>	<i>Facklamia</i>	0.003106	<i>Xanthomonadaceae</i>	<i>Thermomonas</i>

0.04452	Rhodospirillaceae	Azospirillum	0.003106	Brachyspiraceae	Brachyspira
0.043485	Lactobacillaceae	Lactobacillus	0.003106	Mycoplasmataceae	Mycoplasma
0.043485	Methylobacteriaceae	Methylobacterium	0.003106	Chthoniobacteraceae	heteroC45_4W
0.042449	Tissierellaceae	ph2	0.003106	Cloacamonaceae	W22
0.041414	Lachnospiraceae	Oribacterium	0.002071	Solibacteraceae	Candidatus Solibacter
0.041414	mitochondria	Lupinus	0.002071	Dermabacteraceae	Dermabacter
0.041414	Anaeroplasmataceae	Anaeroplasma	0.002071	Mycobacteriaceae	Clavibacter
0.041414	Thermaceae	Thermus	0.002071	Mycobacteriaceae	Mycobacterium
0.036237	Brevibacteriaceae	Brevibacterium	0.002071	Promicromonosporaceae	Promicromonospora
0.036237	Staphylococcaceae	Jeotgalicoccus	0.002071	Propionibacteriaceae	Brooklawnia
0.034166	Staphylococcaceae	Macrococcus	0.002071	Propionibacteriaceae	Tessaracoccus
0.034166	Veillonellaceae	Dialister	0.002071	Pseudonocardiaceae	Actinomycetospora
0.034166	Burkholderiaceae	Burkholderia	0.002071	Bifidobacteriaceae	Allocardovia
0.033131	Lachnospiraceae	Ruminococcus	0.002071	Coriobacteriaceae	Collinsella
0.032096	Sphingomonadaceae	Novosphingobium	0.002071	Coriobacteriaceae	Slackia
0.032096	Enterobacteriaceae	Klebsiella	0.002071	Porphyromonadaceae	Dysgonomonas
0.03106	Micrococcaceae	Microbispora	0.002071	Cyclobacteriaceae	Algiphagus
0.03106	Chromatiaceae	Rheinheimera	0.002071	Cytophagaceae	Adhaeribacter
0.03106	Pasteurellaceae	Actinobacillus	0.002071	Weeksellaceae	Cloacibacterium
0.030025	Staphylococcaceae	Salinicoccus	0.002071	Bacillaceae	Virgibacillus
0.030025	Campylobacteraceae	Campylobacter	0.002071	Turicibacteraceae	Turicibacter
0.02899	Deinococcaceae	Deinococcus	0.002071	Lachnospiraceae	Butyrivibrio
0.027954	Veillonellaceae	Megasphaera	0.002071	Lachnospiraceae	Defluvitalea
0.025884	Veillonellaceae	Selenomonas	0.002071	Lachnospiraceae	Dorea
0.025884	Enterobacteriaceae	Serratia	0.002071	Ruminococcaceae	Clostridium
0.024848	Coriobacteriaceae	Atopobium	0.002071	Veillonellaceae	Schwartzia
0.024848	Peptostreptococcaceae	Peptostreptococcus	0.002071	Erysipelotrichaceae	Catenibacterium
0.023813	Flavobacteriaceae	Flavobacterium	0.002071	Erysipelotrichaceae	RFN20
0.023813	Hyphomicrobiaceae	Devosia	0.002071	Gemmimonadaceae	Gemmimonas
0.022778	Caulobacteraceae	Brevundimonas	0.002071	Gemmataceae	Gemmata
0.022778	Rhodospirillaceae	Skermanella	0.002071	Pirellulaceae	A17
0.020707	Microbacteriaceae	Micromonas	0.002071	Caulobacteraceae	Caulobacter
0.020707	Weeksellaceae	Chryseobacterium	0.002071	Caulobacteraceae	Mycoplana
0.019672	Shewanellaceae	Shewanella	0.002071	Rhizobiaceae	Rhizobium
0.019672	Pasteurellaceae	Aggregatibacter	0.002071	Rhodospirillaceae	Inquilinus
0.018636	Bradyrhizobiaceae	Balneimonas	0.002071	Neisseriaceae	Eikenella
0.018636	Chromatiaceae	Alishewanella	0.002071	Nitrosomonadaceae	Nitrosovibrio
0.017601	Cytophagaceae	Pontibacter	0.002071	Rhodocyclaceae	C39
0.017601	Sphingobacteriaceae	Sphingobacterium	0.002071	Myxococcaceae	Myxococcus
0.017601	Lachnospiraceae	Coprococcus	0.002071	Nannocystaceae	Nannocystis
0.016566	Chitinophagaceae	Flavisolibacter	0.002071	Alteromonadaceae	Microbulbifer
0.016566	Peptococcaceae	Peptococcus	0.002071	Enterobacteriaceae	Proteus
0.016566	Rhizobiaceae	Shinella	0.002071	Coxiellaceae	Aquicella
0.016566	Rhodobacteraceae	Rhodobacter	0.002071	Coxiellaceae	Rickettsiella
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0.016566	Alteromonadaceae	Cellvibrio	0.002071	Xanthomonadaceae	Dokdonella
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0.01553	Carnobacteriaceae	Granulicatella	0.002071	Thermotogaceae	AUTHM297
0.01553	Sphingomonadaceae	Kaistobacter	0.002071	Trueperaceae	B-42
0.01553	Spirochaetaceae	Treponema	0.001035	Actinomycetaceae	Mobiluncus
0.014495	Enterobacteriaceae	Providencia	0.001035	Actinopolysporaceae	Actinopolyspora
0.014495	Enterobacteriaceae	Salmonella	0.001035	Bogoriellaceae	Georgenia
0.014495	Xanthomonadaceae	Lysobacter	0.001035	Kineosporiaceae	Kineococcus
0.01346	Rhodobacteraceae	Rubellimicrobium	0.001035	Micrombacteriaceae	Cryocola
0.01346	Burkholderiaceae	Lautropia	0.001035	Micromonosporaceae	Actinoplanes
0.01346	Halomonadaceae	Halomonas	0.001035	Micromonosporaceae	Catellatospora
0.01346	Xanthomonadaceae	Luteimonas	0.001035	Micromonosporaceae	Virgisporangium
0.012424	Lachnospiraceae	Catonella	0.001035	Thermomonosporaceae	Actinomadura
0.012424	Comamonadaceae	Variovorax	0.001035	Yaniellaceae	Yaniella
0.011389	Rubrobacteraceae	Rubrobacter	0.001035	Fimbriimonadaceae	Fimbriimonas
0.011389	Porphyromonadaceae	Paludibacter	0.001035	Cytophagaceae	Emticicia

0.011389	<i>Flavobacteriaceae</i>	<i>Gramella</i>	0.001035	<i>Cytophagaceae</i>	<i>Larkinella</i>
0.011389	<i>Weeksellaceae</i>	<i>Wautersiella</i>	0.001035	<i>Cytophagaceae</i>	<i>Sporocytophaga</i>
0.011389	<i>Paenibacillaceae</i>	<i>Paenibacillus</i>	0.001035	<i>Weeksellaceae</i>	<i>Elizabethkingia</i>
0.011389	<i>Exiguobacteraceae</i>	<i>Exiguobacterium</i>	0.001035	<i>Sphingobacteriaceae</i>	<i>Muciluginibacter</i>
0.011389	<i>Bradyrhizobiaceae</i>	<i>Bosea</i>	0.001035	<i>Sphingobacteriaceae</i>	<i>Pedobacter</i>
0.011389	<i>Sinobacteraceae</i>	<i>Steroidobacter</i>	0.001035	<i>Balneolaceae</i>	<i>Balneola</i>
0.010353	<i>Gordoniaceae</i>	<i>Gordonia</i>	0.001035	<i>Balneolaceae</i>	<i>KSA1</i>
0.010353	<i>Micrococcaceae</i>	<i>Arthrobacter</i>	0.001035	<i>Ardenscatenaceae</i>	<i>Ardenscatena</i>
0.010353	<i>Nocardoidaceae</i>	<i>Nocardoides</i>	0.001035	<i>Phormidiaceae</i>	<i>Planktothrix</i>
0.010353	<i>Planococcaceae</i>	<i>Staphylococcus</i>	0.001035	<i>Bacillaceae</i>	<i>Marinococcus</i>
0.010353	<i>Clostridiaceae</i>	<i>SMB53</i>	0.001035	<i>Paenibacillaceae</i>	<i>Cohnella</i>
0.010353	<i>Lachnospiraceae</i>	<i>Shuttleworthia</i>	0.001035	<i>Planococcaceae</i>	<i>Bacillus</i>
0.010353	<i>Bdellovibrionaceae</i>	<i>Bdellovibrio</i>	0.001035	<i>Planococcaceae</i>	<i>Planococcus</i>
0.010353	<i>Xanthomonadaceae</i>	<i>Pseudoxanthomonas</i>	0.001035	<i>Aerococcaceae</i>	<i>Granulicatella</i>
0.009318	<i>Rikenellaceae</i>	<i>Rikenella</i>	0.001035	<i>Aerococcaceae</i>	<i>Marinilactibacillus</i>
0.009318	<i>Paraprevotellaceae</i>	<i>CF231</i>	0.001035	<i>Clostridiaceae</i>	<i>Proteiniclasticum</i>
0.009318	<i>Ruminococcaceae</i>	<i>Anaerotruncus</i>	0.001035	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>
0.009318	<i>Tissierellaceae</i>	<i>Finegoldia</i>	0.001035	<i>Peptostreptococcaceae</i>	<i>Clostridium</i>
0.009318	<i>Tissierellaceae</i>	<i>Gallicola</i>	0.001035	<i>Veillonellaceae</i>	<i>Acidaminococcus</i>
0.009318	<i>Rhizobiaceae</i>	<i>Agrobacterium</i>	0.001035	<i>Veillonellaceae</i>	<i>Megamonas</i>
0.009318	<i>Acetobacteraceae</i>	<i>Commensilibacter</i>	0.001035	<i>Acidaminobacteraceae</i>	<i>Fusibacter</i>
0.009318	<i>Campylobacteraceae</i>	<i>Arcobacter</i>	0.001035	<i>Tissierellaceae</i>	<i>Helcococcus</i>
0.009318	<i>Legionellaceae</i>	<i>Legionella</i>	0.001035	<i>Erysipelotrichaceae</i>	<i>Clostridium</i>
0.009318	<i>Halomonadaceae</i>	<i>Kushneria</i>	0.001035	<i>Erysipelotrichaceae</i>	<i>Sharpea</i>
0.009318	<i>Vibrionaceae</i>	<i>Vibrio</i>	0.001035	<i>Caulobacteraceae</i>	<i>Asticcacaulis</i>
0.009318	<i>Opitutaceae</i>	<i>Opitutus</i>	0.001035	<i>Caulobacteraceae</i>	<i>Nitrobacteria</i>
0.008283	<i>Aerococcaceae</i>	<i>Abiotrophia</i>	0.001035	<i>Caulobacteraceae</i>	<i>Phenylobacterium</i>
0.008283	<i>Lachnospiraceae</i>	<i>Moryella</i>	0.001035	<i>Kiloniellaceae</i>	<i>Thalassospira</i>
0.008283	<i>Ruminococcaceae</i>	<i>Butyricicoccus</i>	0.001035	<i>Bradyrhizobiaceae</i>	<i>Bradyrhizobium</i>
0.008283	<i>Rhodobacteraceae</i>	<i>Amaricoccus</i>	0.001035	<i>Phyllobacteriaceae</i>	<i>Aminobacter</i>
0.008283	<i>Oxalobacteraceae</i>	<i>Cupriavidus</i>	0.001035	<i>Phyllobacteriaceae</i>	<i>Aquamicrobium</i>
0.008283	<i>Enterobacteriaceae</i>	<i>Enterobacter</i>	0.001035	<i>Rhizobiaceae</i>	<i>Kaistia</i>
0.008283	<i>Chthoniobacteraceae</i>	<i>Candidatus Xiphinema bacter</i>	0.001035	<i>Rhodospirillaceae</i>	<i>Magnetospirillum</i>
0.007247	<i>Nocardiodaceae</i>	<i>Pimelobacter</i>	0.001035	<i>Rickettsiaceae</i>	<i>Rickettsia</i>
0.007247	<i>Rikenellaceae</i>	<i>AF12</i>	0.001035	<i>mitochondria</i>	<i>Arabidopsis</i>
0.007247	<i>Planococcaceae</i>	<i>Sporosarcina</i>	0.001035	<i>mitochondria</i>	<i>Citrullus</i>
0.007247	<i>Lactobacillaceae</i>	<i>Pediococcus</i>	0.001035	<i>Sphingomonadaceae</i>	<i>Sandaracinobacter</i>
0.007247	<i>Coxiellaceae</i>	<i>Coxiella</i>	0.001035	<i>Alcaligenaceae</i>	<i>Alcaligenes</i>
0.006212	<i>Cellulomonadaceae</i>	<i>Actinotalea</i>	0.001035	<i>Alcaligenaceae</i>	<i>Rhodospirillum</i>
0.006212	<i>Nocardiodaceae</i>	<i>Propionicimonas</i>	0.001035	<i>Comamonadaceae</i>	<i>Rubrivivax</i>
0.006212	<i>Bifidobacteriaceae</i>	<i>Gardnerella</i>	0.001035	<i>Methylophilaceae</i>	<i>Methylobacillus</i>
0.006212	<i>Cryomorphaceae</i>	<i>Fluviiicola</i>	0.001035	<i>Desulfarculaceae</i>	<i>Desulfarculus</i>
0.006212	<i>Lachnospiraceae</i>	<i>Roseburia</i>	0.001035	<i>HTCC2188</i>	<i>HTCC</i>
0.006212	<i>Acidaminobacteraceae</i>	<i>Guggenheimella</i>	0.001035	<i>Idiomarinaceae</i>	<i>Pseudidiomarina</i>
0.006212	<i>Planctomycetaceae</i>	<i>Planctomyces</i>	0.001035	<i>Chromatiaceae</i>	<i>Alkalimonas</i>
0.006212	<i>Erythrobacteraceae</i>	<i>Citromicrobium</i>	0.001035	<i>Halomonadaceae</i>	<i>Chromohalobacter</i>
0.006212	<i>Sphingomonadaceae</i>	<i>Sphingopyxis</i>	0.001035	<i>Verrucomicrobiaceae</i>	<i>Luteolibacter</i>
0.006212	<i>Comamonadaceae</i>	<i>Comamonas</i>	0.001035	<i>Verrucomicrobiaceae</i>	<i>Verrucomicrobium</i>
0.006212	<i>Oxalobacteraceae</i>	<i>Janthinobacterium</i>	0.001035	<i>Chthoniobacteraceae</i>	<i>DA101</i>
0.006212	<i>Aeromonadaceae</i>	<i>Aeromonas</i>			

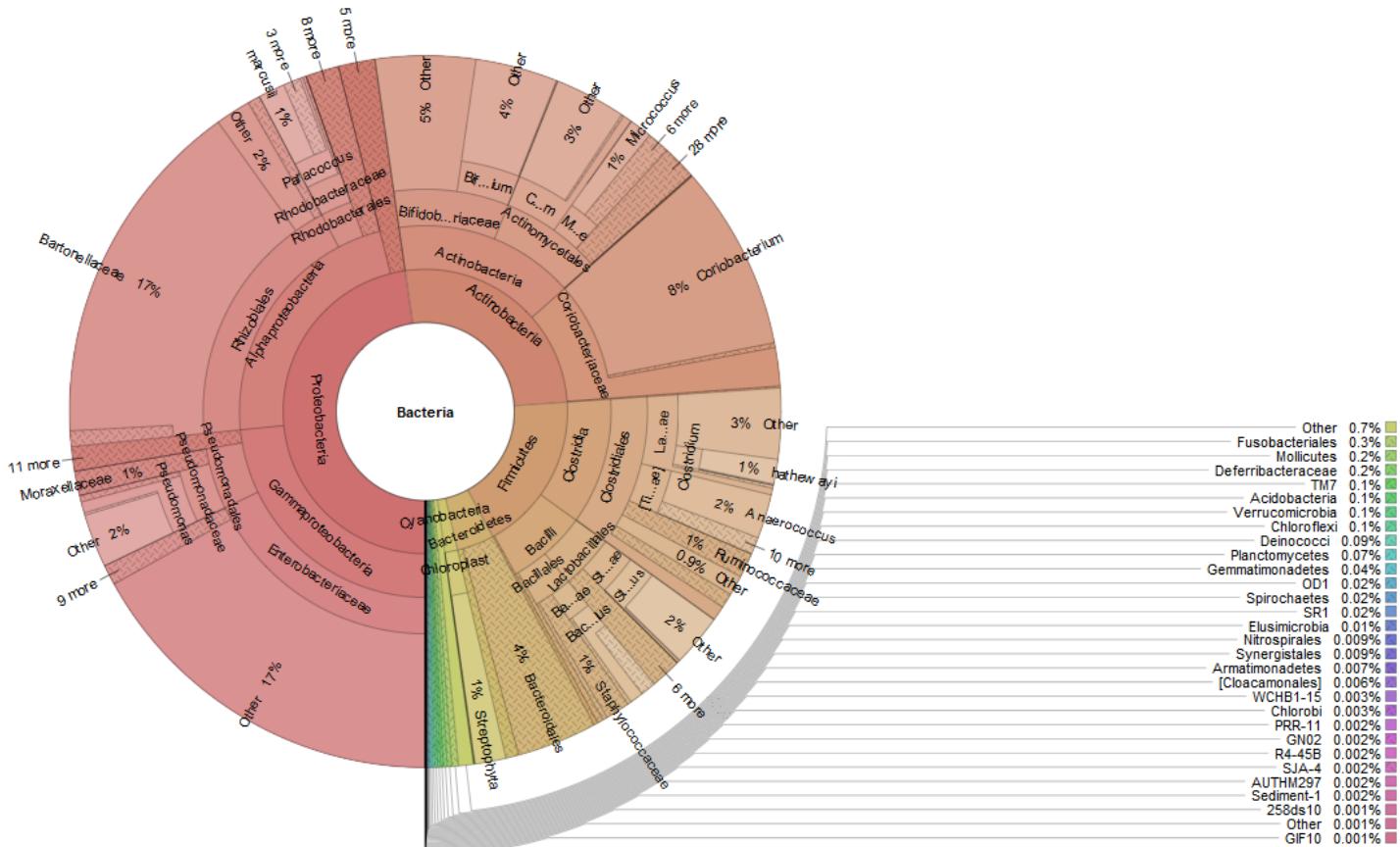


Figure 4 Pie chart showing the distribution of species under Genus level classification

DISCUSSION

Illumina MiSeq technology was applied to unravel the bacterial composition of the gut of *Dysdercus cingulatus* by analyzing the V3 and V4 hyper variable regions of 16S rRNA. In this study, we identified Proteobacteria, Actinobacteria, and Firmicutes as the three major phyla presenting the gut of *D. cingulatus*. The study shows that *Bartonella* was the most abundant genus present in the gut of the pest. *Bartonella* is a collection of gram-negative bacteria belonging to a single genus of the family Bartonellaceae. These are human pathogens transmitted by sand flies, ticks, and fleas. *Bartonella* was identified at species level as *Bartonella bacilliformis* that is known to have a genetic relationship with plant symbionts such as *Rhizobium meliloti*, a nitrogen fixing bacteria (Ihler, 1996). The second largest genus in the gut was an unidentified taxon belonging to the family Enterobacteriaceae followed by genus *Coriobacterium* from family Coriobacteriaceae whose members provide vitamin B to their insect host (H. Salem et al., 2014).

Bifidobacteria was another abundant bacterial genus identified in the gut of *D. cingulatus*. *Bifidobacteria* are fermentative organisms that are present in the gut of various animals and human. They are capable of metabolizing carbohydrate and other types of glycans present in the gut of animals (Pokusaeva, Fitzgerald, & Van Sinderen, 2011). *Bifidobacteria* are also known to be present in insects. Nearly 2-8.4% of the total population was identified as *Bifidobacteria* in the gut of honey bees and homogenous in composition, whereas bifidobacterial population in wasps was heterogeneous (Mrázek, Štrosová, Fliegerová, Kott, & Kopečný, 2008). Here, we report two different species of *Bifidobacteria* namely *Bifidobacterium asteroides* and *Bifidobacterium longum*. In *Apis mellifera*, *Bifidobacterium asteroides* PRL2011 can perform respiratory metabolism (Bottacini et al., 2012).

Corynebacterium is a pantothenic acid producing bacteria in the gut of *Triatoma infestans* (Durvasula et al., 2008). In our study, we identified various species of *Corynebacterium* namely *C. matruchotii*, *C. matruchotii*, *C. tuscaniense*, *C. glaucum*, *C. kroppenstedtii*, *C. suisordis*, *C. bovis*, *C. timonense*, *C. durum*, *C. coyleae*, and *C. riegelii*.

Klebsiella represents 3.5% of gut Microbiome of *D. cingulatus*. *Klebsiella* isolated from guts of larvae of *Aularches miliaris*, *Propylea quatuordecimpunctata* and nymphal stage of *Oxya veloxis* reported to be a cellulose degrading bacterium (Shil, Mojumder, Sadida, Uddin, & Sikdar, 2014). Similarly, *Klebsiella* isolated from *Bombyx mori* also degrades polysaccharides in mulberry leaves. Our study identified *Klebsiella pneumonia* in the gut of *D. cingulatus*. Isolation and characterization of *K. pneumonia* confirmed cellulolytic and xylanolytic properties in the gut of *Bombyx mori* (Anand et al., 2010).

Two species of *Streptococcus* namely *S. mitis* and *S. parauberis* were identified in the gut of *D. cingulatus*. *S. mitis* had also been reported in the gut of pine engraver *Ips pini* that colonizes red pine (Jr et al., 2007). Similarly, *Paracoccus aestuarii* and *Paracoccus marcusii* from family Rhodobacteraceae, and *Anaerococcus hydrogenalis*, *Anaerococcus lactolyticus*, and *Anaerococcus prevotii* from family Tissierellaceae were also identified in *D. cingulatus*.

Bacillus is one of the most common bacterial species found in the guts of all the insects. *Bacillus* species isolated from guts of larvae of five different families of wood-feeding Coleoptera collected from tropical forests of Costa Rica possessed β -glucosidase, β -xylanase, and cellobiose hydrolase activities (Rojas-Jiménez & Hernández, 2015). In *D. cingulatus*, *Bacillus* makes 1.48% of the gut bacterial population. *Bacillus* species identified in our study were *B. subtilis*, *B. niacin*, *B. firmus*, *B. badius*, *B. licheniformis*, and *B. megaterium*.

Micrococcaceae is another frequently isolated family of bacteria from the mid guts of various insect species. Three different genera identified in this study were *Rothia*, *Arthrobacter*, *Micrococcus* and *Kocuria* (Rizzi et al., 2013). The most abundant bacterial species identified from this family were *R. mucilaginosa* and *R. dentocariosa*. In humans, *Rothia* has been identified to colonize the upper gastro-intestinal tract and helps in the degradation of gluten.

Similarly, *Micrococcus lutens* is an inhabitant of the human mouth, pharynx, and respiratory tract. Member of genus *Micrococcus* is also found in insects. Gut bacterial composition study had identified *M. lutens* as one of the bacteria present in the pre-pupae and adult stages of sub cortical beetle, *Agrilus planipennis* (Vasantha Kumar, Handelsman, Schloss, Bauer, & Raffa, 2008). Other two bacterial species identified in this study were *Arthrobacter keyseri*, *Kocuria rosea*.

Other important genera present in *D. cingulatus* are *Propionibacterium*, *Prevotella*, *Staphylococcus*, *Acinetobacter*, *Enterobacter*, and *Enterococcus*. We identified three species of *Propionibacterium* namely *P. acnes*, *P. granulosum* and *P. acidifaciens*. Among them, *P. acnes* is abundant compared to other species. *Propionibacterium* spp. are common soil inhabitants with different metabolic characteristics that may be advantageous to their insect hosts. Some species of *Propionibacterium* are lipolytic (Jarvis, Strömpl, Moore, & Thiele, 1998). Similarly, *Acinetobacter* spp. have been correlated to phosphate storage (Rustrian, Delgenes, & Moletta, 1997).

Staphylococcus, an important genus of Firmicutes is present in most of the insects and non-pathogenic. Their abundance and variety differ with different food habits. The comparative study of gut bacteria present in *Dastarcus helophoroides* fed on different artificial diets identified *Staphylococcus* as one of the predominant genera in the gut (Zhang, He, & Li, 2014). Gut bacteria identified at the species level in nine different species of Australian termites

demonstrated the presence of *Staphylococcus* in three different species from family Termitidae (Eutick, O'Brien, & Slaytor, 1978). In *D. cingulatus* we identified four different species of *Staphylococcus* namely *S. sciuri*, *S. epidermidis*, *S. hominis*, *S. pasteurii*, and *S. succinus*.

Enterococci belonging to phylum Firmicutes are known to harbor a large variety of insects which includes beetles, termites, flies and worms. They are mostly present in species feeding on succulent parts of plants and nectar. *E. faecalis* and *E. faecium* are the predominant species but other species appear to lesser extent (Martin & Mundt, 1972). In *Harpalus Pensylvanicus*, *E. faecalis* increases the seed consumption by providing the digestive enzymes to their host (Schmid, Lehman, Brözel, & Lundgren, 2014). *E. durans* is the only bacterial species that was identified from the genus *Enterococcus*.

E. durans isolated from the gut of *O. velox*, *A. miliaris* and *P. quatuordecimpunctata* was identified as cellulolytic bacteria along with bacteria from other genera. Genus *Anaerococcus* is a butyrate-producing species. The seed-parasitic wasp of genus *Megastigmus*, *Anaerococcus* was the major Firmicute (Paulson, Aderkas, & Perlman, 2014). Our study also identified *Anaerococcus* spp. *A. Prevotti*, *A. lactolyticus*, and *A. Hyarogenati* in the gut of *D. cingulatus* with a total abundance of 2%. Butyrate and energy molecule producing other bacteria identified in the gut of *D. cingulatus* were *Butyrivibrio fibrisolvens*, *Butyrivibrio hungatei*, *Dorea formicigenerans*, *Roseburia inulinivorans*, *Roseburia faecis*, and *Roseburia cecicola* from the family Lachnospiraceae (Titus & Ahearn, 1988).

CONCLUSION

Dysdercus cingulatus (Hemiptera: Pyrrhocoridae) is a cotton pest that has noxious effect on the cotton seeds and cotton lint. Bacterial community colonizing the gut of this pest was identified using 16S rRNA Metagenomics sequencing technique. The bacterial species were identified using QIIME. The analysis shows the presence of bacterial species from genera *Coriobacterium*, *Bifidobacterium*, *Corynebacterium*, *Pseudomonas* and many others. These bacteria are highly important in their own self because of their vital bio-processing capabilities. This study will facilitate further in identifying specific roles played by some of these major bacteria identified in the gut of *D. cingulatus*. Identification of such bacterial species that are important for the survival of the insect could be used to initiate new strategies for pest management.

REFERENCES

- Ahmad, I., & Schaefer, C. W. (1987). Food plant and feeding biology of the Pyrrhocoridae (Hemiptera). *Phytophaga*, 1, 75–92.
- Anand, A. A. P., Vennison, S. J., Sankar, S. G., Prabhu, D. I. G., Vasan, P. T., Raghuraman, T., ... Vendan, S. E. (2010). Isolation and characterization of bacteria from the gut of *Bombyx mori* that degrade cellulose, xylan, pectin and starch and their impact on digestion. *Journal of Insect Science (Online)*, 10. <https://doi.org/10.1673/031.010.10701>
- Bartram, A. K., Lynch, M. D. J., Stearns, J. C., Moreno-Hagelsieb, G., & Neufeld, J. D. (2011). Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Applied and Environmental Microbiology*, 77(11), 3846–3852. <https://doi.org/10.1128/AEM.02772-10>
- Bennett, A. E. (2013). Can plant-microbe-insect interactions enhance or inhibit the spread of invasive species? *Functional Ecology*, 27(3), 661–671. <https://doi.org/10.1111/1365-2435.12099>
- Bottacini, F., Milani, C., Turroni, F., Sánchez, B., Foroni, E., Duranti, S., ... Ventura, M. (2012). *Bifidobacterium asteroides* PRL2011 Genome Analysis Reveals Clues for Colonization of the Insect Gut. *PLoS ONE*, 7(9), 1–14. <https://doi.org/10.1371/journal.pone.0044229>
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... Walters, W. a. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5), 335–336. <https://doi.org/10.1038/nmeth.f.303.QIIME>
- Durvasula, R. V., Sundaram, R. k., Kirsch, P., Hurwitz, I., Crawford, C. V., Dotson, E., & Beard, C. B. (2008). Genetic transformation of a Corynebacterial symbiont from the Chagas disease vector *Triatoma infestans*. *Experimental Parasitology*, 119(1), 94–98. <https://doi.org/10.1016/j.exppara.2007.12.020.Genetic>
- Eutick, M. L., O'Brien, R. W., & Slaytor, M. (1978). Bacteria from the gut of Australian termites. *Applied and Environmental Microbiology*, 35(5), 823–828.
- Huse, S. M., Dethlefsen, L., Huber, J. A., Welch, D. M., Relman, D. A., & Sogin, M. L. (2008). Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. *PLoS Genetics*, 4(11). <https://doi.org/10.1371/journal.pgen.1000255>
- Ihler, G. M. (1996). *Bartonella bacilliformis*: dangerous pathogen slowly emerging from deep background. *FEMS Microbiology Letters*, 144(1), 1–11. [https://doi.org/10.1016/0378-1097\(96\)00307-2](https://doi.org/10.1016/0378-1097(96)00307-2)
- Janda, J. M., & Abbott, S. L. (2007). 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls. *Journal of Clinical Microbiology*, 45(9), 2761–2764. <https://doi.org/10.1128/JCM.01228-07>
- Jarvis, G. N., Strömpl, C., Moore, E. R. B., & Thiele, J. H. (1998). Isolation and Characterisation of Obligately Anaerobic, Lipolytic Bacteria from the Rumen of Red Deer. *Systematic and Applied Microbiology*, 21(1), 135–143. [https://doi.org/10.1016/S0723-2020\(98\)80017-9](https://doi.org/10.1016/S0723-2020(98)80017-9)
- Jr, I. D., Vasanthakumar, A., Burwitz, B. J., Schloss, P. D., Klepzig, K. D., Handelsman, J., & Raffa, K. F. (2007). Composition of the bacterial community in the gut of the pine engraver, *Ips pini* (Say) (Coleoptera) colonizing red pine. *Symbiosis*, 43, 97–104.
- Kress, W. J., & Erickson, D. L. (2008). DNA barcodes: Genes, genomics, and bioinformatics. *Proceedings of the National Academy of Sciences of the United States of America*, 105(8), 2761–2762. <https://doi.org/10.1073/pnas.0800476105>
- Magoč, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21), 2957–2963. <https://doi.org/10.1093/bioinformatics/btr507>
- Martin, J. D., & Mundt, J. O. (1972). Enterococci in insects. *Applied Microbiology*, 24(4), 575–580.
- Maxwell-Lefroy, H. (1906). Indian insect pests. Calcutta: Office of the Superintendent of Government Printing.
- Mrázek, J., Štrosová, L., Fliegerová, K., Kott, T., & Kopečný, J. (2008). Diversity of insect intestinal microflora. *Folia Microbiologica*, 53(3), 229–233. <https://doi.org/10.1007/s12223-008-0032-z>
- Paulson, A. R., Aderkas, P. Von, & Perlman, S. J. (2014). Bacterial associates of seed-parasitic wasps (Hymenoptera: Megastigmus) Bacterial associates of seed-parasitic wasps (Hymenoptera: Megastigmus), 1–15. <https://doi.org/10.1186/s12866-014-0224-4>
- Pokusaeva, K., Fitzgerald, G. F., & Van Sinderen, D. (2011). Carbohydrate metabolism in *Bifidobacteria*. *Genes and Nutrition*, 6(3), 285–306. <https://doi.org/10.1007/s12263-010-0206-6>
- Rizzi, A., Crotti, E., Borruso, L., Jucker, C., Lupi, D., Colombo, M., & Daffonchio, D. (2013). Characterization of the bacterial community associated with larvae and adults of anoplophora chinensis collected in Italy by culture and culture-independent methods. *BioMed Research International*, 2013. <https://doi.org/10.1155/2013/420287>
- Rojas-Jiménez, K., & Hernández, M. (2015). Isolation of fungi and bacteria associated with the guts of tropical wood-feeding coleoptera and determination of their lignocellulolytic activities. *International Journal of Microbiology*, 2015. <https://doi.org/10.1155/2015/285018>
- Rustrian, E., Delgenes, J. P., & Moletta, R. (1997). Phosphate release and uptake by pure cultures of *Acinetobacter* sp.: Effect of the volatile fatty acids concentration. *Current Microbiology*, 34(1), 43–48. <https://doi.org/10.1007/s002849900142>
- Salem, H., Bauer, E., Strauss, A. S., Vogel, H., Marz, M., & Kaltenpoth, M. (2014). Vitamin supplementation by gut symbionts ensures metabolic homeostasis in an insect host. *Proceedings of the Royal Society B: Biological Sciences*, 281(1796), 20141838–20141838. <https://doi.org/10.1098/rspb.2014.1838>
- Salem, H., Kreutzer, E., Sudakaran, S., & Kaltenpoth, M. (2012). Actinobacteria as essential symbionts in firebugs and cotton stainers (Hemiptera, Pyrrhocoridae). *Environmental Microbiology*, 15(7), 1956–1968. <https://doi.org/10.1111/1462-2920.12001>
- Schmid, R. B., Lehman, R. M., Brözel, V. S., & Lundgren, J. G. (2014). An Indigenous Gut Bacterium, *Enterococcus faecalis* (Lactobacillales: Enterococcaceae), Increases Seed Consumption by *Harpalus pensylvanicus* (Coleoptera: Carabidae). *Florida Entomologist*, 97(2), 575–584. <https://doi.org/10.1653/024.097.0232>
- Shil, R. K., Mojumder, S., Sadida, F. F., Uddin, M., & Sikdar, D. (2014). Isolation and identification of cellulolytic bacteria from the gut of three phytophagous insect species. *Brazilian Archives of Biology and Technology*, 57(6), 927–932. <https://doi.org/10.1590/S1516-8913201402620>
- Shivas, R. G., Smith, M. W., Marney, T. S., Newman, T. K., Hammelwang, D. L., Cooke, A. W., ... Pascoe, I. G. (2005). First record of *Nematospora coryli* in Australia and its association with dry rot of Citrus. *Australasian Plant Pathology*, 34(1), 99–101. <https://doi.org/10.1071/AP04075>
- Singh, H. (1924). On the Anatomy and Bionomics of the Bed Cotton Bug, *Dysdercus cingulatus* (Fabr.). *Jl. & Proc. Asiatic Soc. Bengal. Calcutta*, 19, 15–42. Retrieved from https://archive.org/stream/mobot31753002183942/mobot31753002183942_djvu.txt
- Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., Kato-Maeda, M., Miller, S., ... Lynch, S. V. (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLoS ONE*, 10(2), 1–22. <https://doi.org/10.1371/journal.pone.0117617>
- Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., & Nishijima, M. (2014). Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PLoS ONE*, 9(8). <https://doi.org/10.1371/journal.pone.0105592>

Taylor, C. M., Coffey, P. L., DeLay, B. D., & Dively, G. P. (2014). The importance of gut symbionts in the development of the brown marmorated stink bug, *Halyomorpha halys* (Stål). PLoS ONE, 9(3).

<https://doi.org/10.1371/journal.pone.0090312>

Titus, E., & Ahearn, G. a. (1988). Short-chain fatty acid transport in the intestine of a herbivorous teleost. The Journal of Experimental Biology, 135, 77–94.

Vasantha Kumar, A., Handelsman, J., Schloss, P. D., Bauer, L. S., & Raffa, K. F. (2008). Gut microbiota of an invasive subcortical beetle, *Agrilus planipennis* Fairmaire, across various life stages. Environmental Entomology, 37(5), 1344–1353. [https://doi.org/10.1603/0046-225X\(2008\)37](https://doi.org/10.1603/0046-225X(2008)37)

Xiang, H., Xie, L., Zhang, J., Long, Y. H., Liu, N., Huang, Y. P., & Wang, Q. (2012). Intracolonial differences in gut bacterial community between worker and soldier castes of *Coptotermes formosanus*. Insect Science, 19(1), 86–95.

<https://doi.org/10.1111/j.1744-7917.2011.01435.x>

Zhang, Z. Q., He, C., & Li, M. L. (2014). Analysis of intestinal bacterial community diversity of adult *Dastarcus helophoroides*. Journal of Insect Science, 14(114), 1–13. <https://doi.org/10.1093/jis/14.1.114>