

Bacterial Microbiome Report

Sample Name: PEG00002 and PEG00003

Prepared For: Permaculture Gardens

Prepared On: 11/05/2023

PEG00002

Sample Weight	306 mg
DNA concentration post extraction:	3.2 ng/ul
Total high quality 16S bacterial sequences:	74427
Total Unique Sequences:	613
Shannon Diversity:	4.24
Potential plant growth promoting organisms:	84%
Anaerobic indicator organisms:	0.4%
Pathogen Indicator organisms:	2%

PEG00003

Sample Weight	798 mg
DNA concentration post extraction:	4.6 ng/ul
Total high quality 16S bacterial sequences:	139400
Total Unique Sequences:	1006
Shannon Diversity:	4.39
Potential plant growth promoting organisms:	84%
Anaerobic indicator organisms:	0.4%
Pathogen Indicator organisms:	2%

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Alpha Diversity

Alpha Diversity is a measurement of species “richness” or number of species in a sample and “evenness” or how proportionally they are distributed in the population.

We provide two alpha diversity measurements “total unique sequences” which is a species estimate and the Shannon diversity index which takes into account both species richness and evenness.

PEG00002

Total Unique Sequences: 613

Total Observed Genera: 306

Shannon Diversity: 4.24

PEG00003

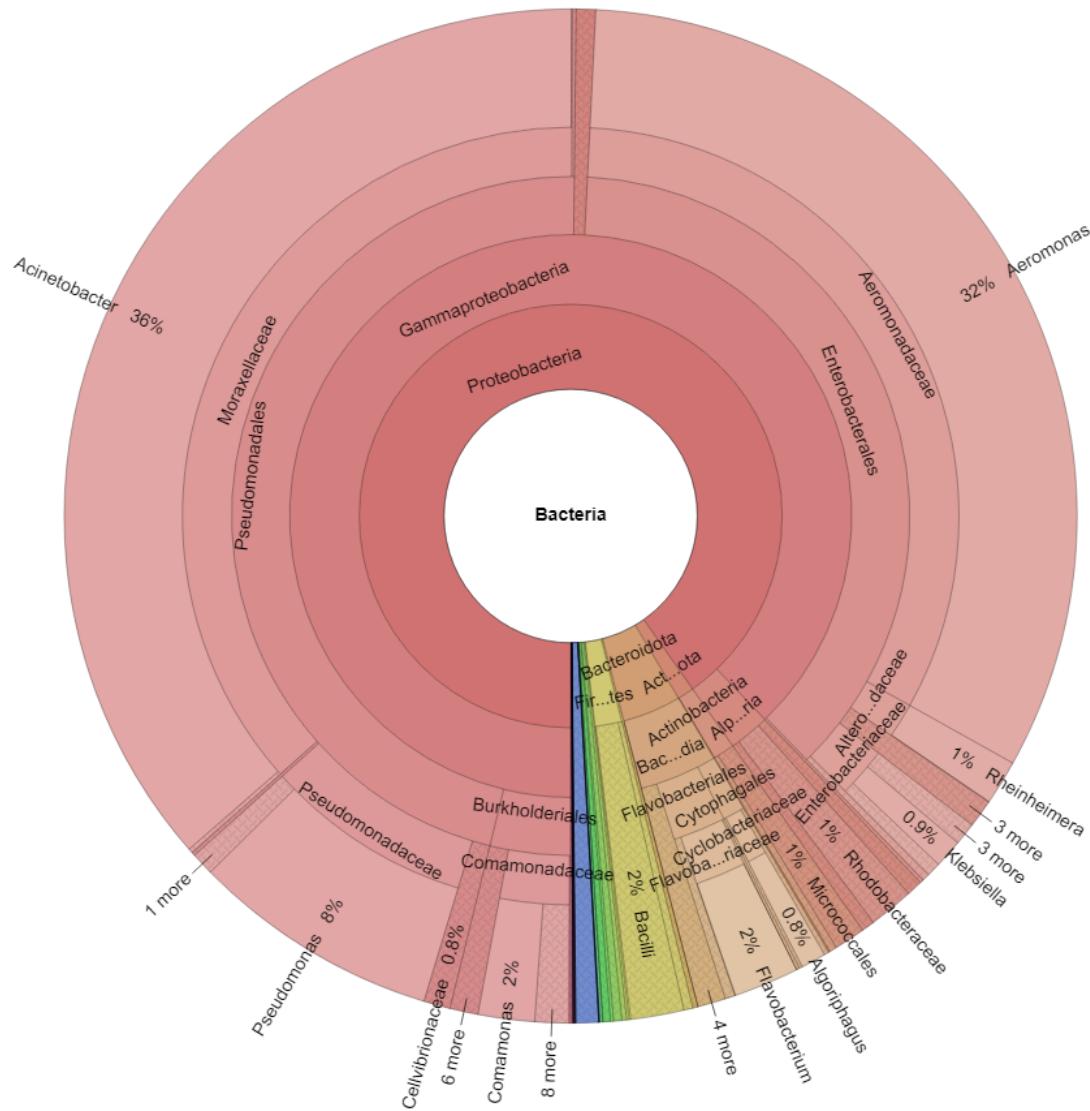
Total Unique Sequences: 2312

Total Observed Genera: 439

Shannon Diversity: 4.40

Krona Plots

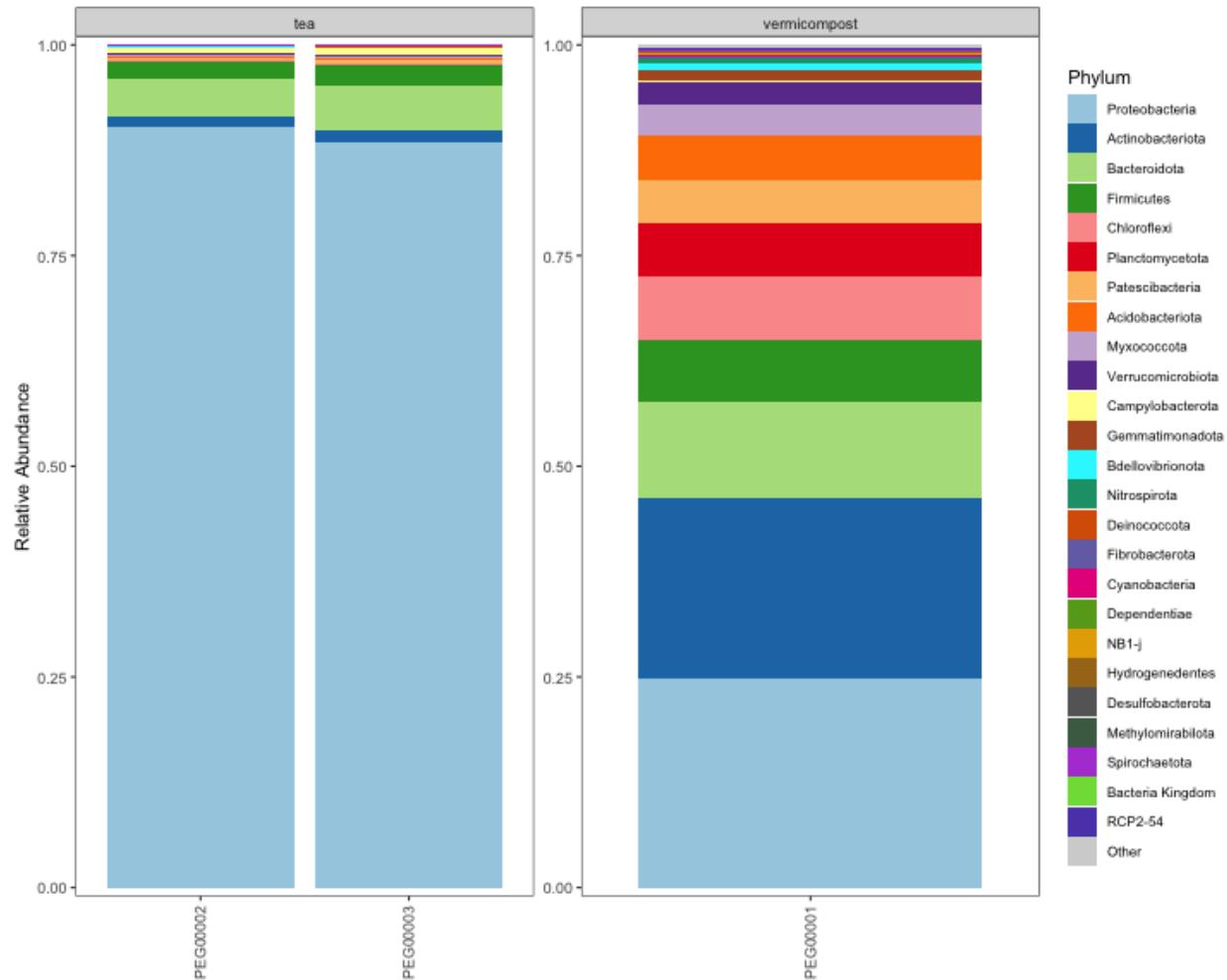
These plots are our preferred way to view sample data as it is an interactive way to explore your sample(s) microbiome from Phylum down to the genus level. Open the files directly from the folder with a web browser. Each sample is listed in the top left and can be individually selected. Double click on any sections of the graph to zoom in. Zoom back out by clicking in the middle of the plot. Statistics about the selected organisms are shown in the top right. Please contact Zack for any help with the plots.



Taxonomy Bar Plots

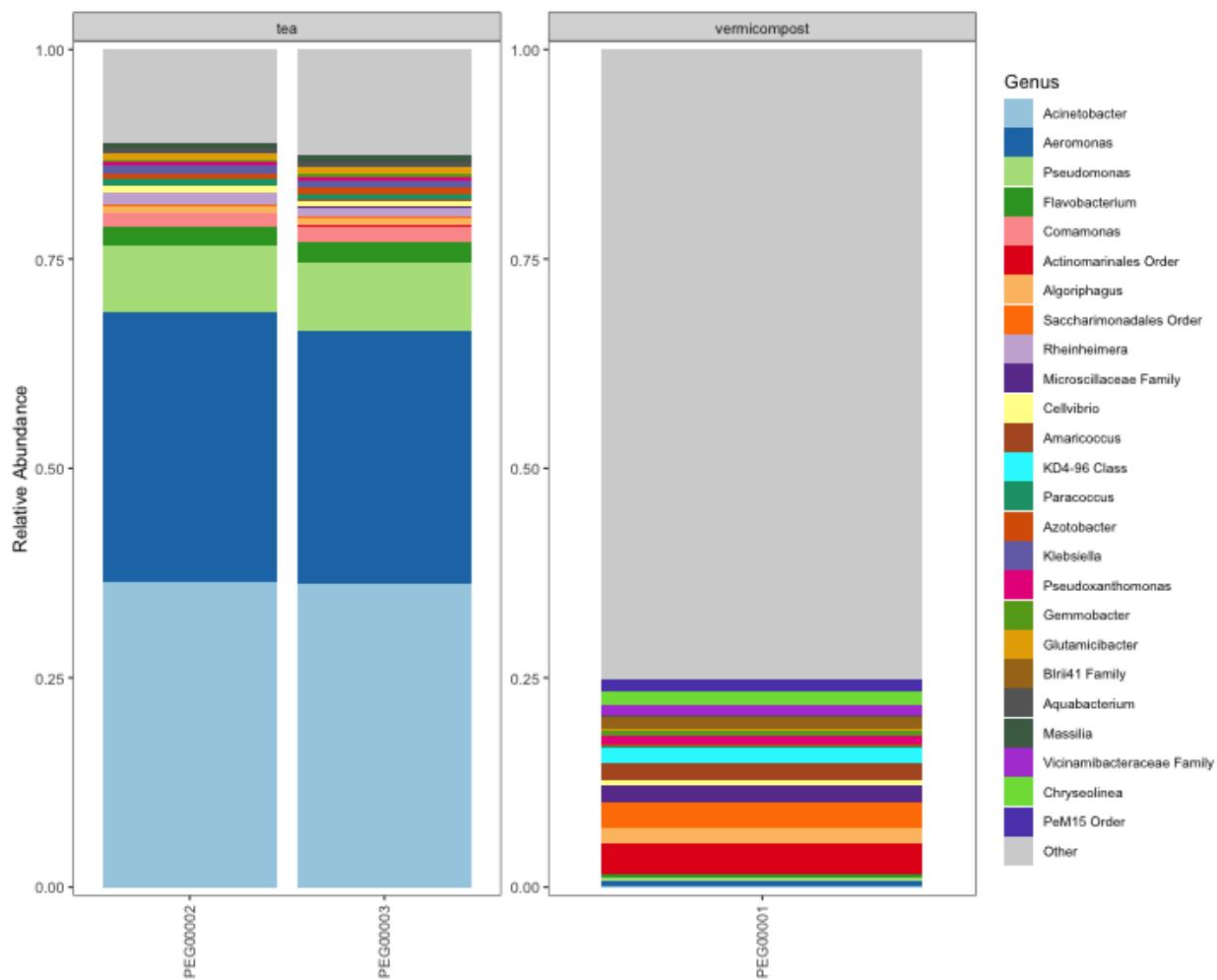
Phylum Level

The top 25 Phyla are shown. This is the highest level of classification.



Genus level

The top 25 genus (one level above species). Large gray “other” bar is due to the high diversity of the sample and represents all organisms outside of the top 25 most abundant.



Beneficial Organism Analysis

Aggrego Data performs a screening for potential beneficial organisms using a proprietary database based on published literature. For vermicompost, the four main groups of beneficial bacteria are Rhizobiales (Order), Actinobacteria (Class), Bacillales (Class) and Gammaproteobacteria (Class). Other potentially beneficial organisms are also included.

Gammaproteobacteria (86%)

Order: Pseudomonadales (46%)

Family: Moraxellaceae(36%)

Genus: Acinetobacter (36%)

- Some isolates were able to produce auxin, while nearly all were able to solubilize phosphorus, and all produced siderophores. Also evidence for fungal pathogen inhibition. Significantly enhanced the shoot height, root length, and root dry weights of pearl millet seedlings in pot experiments when compared with controls, underscoring the plant-growth-promoting potential of these isolates (Rokhbakhsh-Zamin et al. 2011).
- Acinetobacter is a Gram-negative bacterium present everywhere in nature and is usually found in the rhizosphere of many plants. It plays a significant role as plant growth-promoting bacteria (PGPR) and has gained importance in agriculture worldwide. Acinetobacter is known to produce IAA, siderophore, gibberellin, antibiotic, biosurfactants/bioemulsifiers and solubilize phosphate, potassium, and zinc, which make it an important PGPR (Mujumdar et al. 2023).

Family: Pseudomonadaceae (9%)

Genus: Pseudomonas (9%)

- One of the most highly studied PGPB as they produce any antifungal molecules for disease suppression. They also can produce phytohormones auxin and gibberellin as well as siderophores. (Dorje, Dolkar, and Sharma 2017)
- Same species of pseudomonas can be both plant pathogens and beneficial organisms to plants (Preston 2004).
- Found to be key PGPB in garlic with six different beneficial strains isolated (Zhuang et al. 2021).

Genus: Azotobacter (0.7%)

"Two strains A. chroococcum 67B and 76A were selected for their multiple plant growth promotion activities (N₂-fixing activity, synthesis of siderophores and indole-3-acetic acid) as well as antimicrobial activity against Sclerotinia minor). The two Azotobacter chroococcum strains exhibited high tolerance to salt and drought stresses and could alleviate the negative effects exerted by abiotic stress on tomato plants. Overall results showed that the strains Azotobacter chroococcum 67B and Azotobacter chroococcum 76A could be interesting candidates to be employed as bio-effector in sustainable agricultural systems also under stressful conditions leading to improved economic and environmental benefits." (Viscardi et al. 2016)

Seed bacterization with Azb19 [Azotobacter chroococcum] resulted in increased plant height, shoot height, root volume, leaf area and total plant dry mass. Further, bacterial inoculation also significantly increased macro-and micronutrient uptake by sorghum plants. (Sultana, Desai, and Reddy, n.d.)

Family: Cellvibrionaceae (0.9%)

Genus: Cellvibrio (0.8%)

Nitrogen fixer and positively associated with wheat yield (Anderson and Habiger 2012; Suarez et al. 2014)

Part of core microbiome of Mexican teosinte seeds. (De-la-Vega-Camarillo et al. 2023)

Stimulated by biochar amendments and known to fix N

Order: Enterobacterales (35%)

Family: Aeromonadaceae (31%)

Genus: Aeromonas (0.6%)

- **This genus contains potential human pathogens as well as plant growth promoting organisms. It is unclear which type of organisms are present. Even if we identified the organisms down to the species level pathogenicity might still be unknown. For example the species *Aeromonas caviae* contains organisms that are both plant growth promoting and a human pathogen.**
- Can degrade fungicide MBC and contains plant growth promoting properties include phosphorus and zinc solubilization, auxin production, and HCN production (Silambarasan and Abraham 2020)
- "The species *Aeromonas salmonicida* N264 promoted the largest number of tillers [sugarcane] 30 and 45 DAI [days after inoculation], with 30.26 total

- indoles and 14.83 µg mL⁻¹ phosphate solubilization." (Morgado González et al. 2015)
- "The data reveal that plants inoculated with the bacterial consortium (SAL-21 + SAL-17) [both *Aeromonas* isolates] showed a maximum increase in leaf proline content, nitrate reductase activity, chlorophyll a/b, stomatal conductance, transpiration rate, root length, shoot length, and grain weight over non-inoculated plants grown in saline soil. Also produces auxins, plant stress tolerance hormones, and ACC deaminase. (Nawaz et al. 2020)
 - Under suboptimal root zone temperature nodule number and dry weight of plants receiving *Aeromonas hydrophila* P73 they were increased by 72 and 31% at 17±5 °C RZT when coinoculated with *Brayrhizobium*.(F. Zhang 1997)
 - " *Aeromonas caviae* strain A1-2 showed positive results in in vitro tests for plant growth-promoting traits such as indole3-acetic acid production (7), nitrogen fixation (5), phosphate production (8), 1-aminocyclopropane-1-carboxylate (ACC) deaminase production (9), ammonia production (10), and siderophore production (5)."

Order: Burkholderiales (4%)

Family: Comamonadaceae (3%)

Genus: Comamonas

Shown benefit to rooting kiwi stem cutting and auxin production (Erturk et al. 2010)
 Comamonas was negatively associated with disease severity, relative to the control.(L.-N. Zhang et al. 2019)

Order: Xanthomonadales (0.7%)

Strictly aerobic organisms with diverse metabolisms especially that of hydrocarbons. Contains *Xanthomonas* genus (within the Xanthomonadaceae family below) which is known for many phytopathogens. However, this order also contains species known to beneficial to plants and provide disease resistance.(Stewart et al. 2021)

Family: Xanthomonadaceae (0.7%)

Genus: Pseudoxanthomonas (0.4%)

Isolate seen to solubilize phosphorous, fix N, solublize iron (siderophore production), reduce plant stress (ACC deaminase), and produce auxin (IAA). (Castellano-Hinojosa et al. 2016)

Isolate shown to be arsenic resistant, HCN production, auxin production, and N fixation. Reduced harmful effects of arsenic on plant growth and stimulated plant growth of mung beans. (Huda et al. 2022)

Genus: Arenimonas (0.1%)

- Found in early growth stages of corn and seedlings of tomatoes suggesting plant growth promoting properties but more study is needed (Hu et al. 2020; Li et al. 2014)
- Bioremediation Generalist (Brereton et al. 2020)

Class: Bacilli (2%)

Aerobic spore formers

Order: Bacillales

Family: Bacillaceae (0.2%)

Genus: Bacillus (0.03%)

- *Bacillus Subtilis* is one of the most studied PGPB and one of the most commonly isolated Genus from the soil. It has been shown to fix nitrogen, solubilize phosphorus, produce phytohormones, as well as reduce doubt and salt stress. It has also been shown to inhibit fungal and bacterial plant pathogens. (Blake, Christensen, and Kovács 2021).
- Several other species of this genus have also been identified as PGPB and share properties of *B.Subtilis*. (Sansinenea 2019)

Family: Paenibacillaceae (0.0.7%)

Genus: Paenibacillus (0.02%)

Plant growth promoting organisms known to fix nitrogen, produce antimicrobial compounds, increase nutrient availability through hydrolytic enzymes, and produce auxins, gibberellins and AAC demarinase. (Jeong et al. 2011; Verma et al. 2016).

Family: Planococcaceae (0.4%)

Species within this family are seen to solubilize phosphorus, magnesium and zinc, as well as produce auxins(Verma et al. 2016).

Order: Flavobacteriales (3%)

Flavobacteriaceae (2%)

Genus: Flavobacterium (2%)

Represent a significant fraction of root- and leaf-associated microbiomes in a broad range of plant species. (Kolton et al. 2016)

34/44 *flavobacterium* isolates were able to solubilize inorganic phosphorus and all isolates were able to produce auxin (Soltani et al. 2010)

Strain showed highest beneficial effects on plant growth parameters for maize inoculum due to nitrogen fixation, auxin production, and siderophore production.(Youseif 2018)

Increased wheat yield by 15% compared to control (Rahmani et al. 2016).

Shown to increased drought and salt stress tolerance (Kim et al. 2020)

Suppressed wilt in tomato plants (Kwak et al. 2018)

Class: Actinobacteria (1%)

One of the most promising classes of organisms for plant growth promotion as some species show nearly all capabilities of plant growth promotion. (Boukhatem, Merabet, and Tsaki 2022)

Order: Micrococcales (1%)

Family: Micrococcaceae (0.8%)

Genus:Glutamicibacter (0.7%)

- Members of the *Glutamicibacter* genus can promote plant growth under saline conditions and antagonize fungi on plates via chitinolytic activity (Fu, Olawole, and Beattie 2021)
- The strain possessed multiple PGP traits such as indole acetic acid production ($166.11 \pm 5.7 \mu\text{g/ml}$), siderophore production ($85.72 \pm 1.06\% \text{ psu}$), and phosphate solubilization ($44.76 \pm 1.5 \mu\text{g/ml}$). Enhanced germination index and germination rate of pea seeds under the LJH19 inoculation further supported the bacterium's PGP potential.(Borker et al. 2021)

Genus: Pseudarthrobacter (0.07%)

- An in vitro test revealed that the strain could fix nitrogen, solubilize phosphate and potassium, and synthesize indole acetic acid. The bacterial strain was identified and characterized as a kind of *Pseudarthrobacter chlorophenolicus*. tomato plants treated with strain BF2P4-5 showed little to no variation with NPK fertilizer treatment, including plant height, stem length, girth, leaf number per plant, and chlorophyll content. (Issifu et al. 2022)

- Gram-positive, aerobic, rod-shaped, auxin-producing bacterium. The strain contains genes related to auxin biosynthesis and heavy metal resistance (Park et al. 2020).

Family: Demequinaceae (0.07%)

Genus: Demequina (0.07%)

Strongly correlated with plant growth in apple orchards (Peruzzi et al. 2017)

Family: Microbacteriaceae (0.09%)

Isolate from wild rice was the highest producer of phytohormone out of >200 isolates (Borah et al. 2018).

Genus: Leucobacter (0.03%)

Has plant growth promoting properties including ACC demaminase (Kadioglu et al. 2018) and auxin production (Yoo, Ji-Yeong et al. 2017).

Genus: Agromyces (0.03)

ACC deaminase and Auxin production(Bal et al. 2013)

Anaerobic Indicator Organisms:

These groups of organisms are commonly found in vermicompost (ideally at low abundances) and include all anaerobic organisms

Class: Clostridia (0.4%)

All organisms in this class lack aerobic respiration and are known to ferment decaying plant material. Unlikely to contain any plant growth promoting properties.

Comment: These are very low abundances for anaerobic organisms. Nearly all vermitea has some amount of anaerobic organisms. They are not “bad” but a large population of them might indicate your composting or storage conditions are not suitable for aerobic beneficial organisms.

Pathogenic Indicator Organisms

These organisms are *potential* human pathogens. We are currently only screening for the family Enterobacteriaceae as it includes human pathogens such as *Salmonella*, *Escherichia coli*, *Klebsiella*, *Shigella*, *Enterobacter* and *Citrobacter*. Members of the Enterobacteriaceae can be referred to as enterobacteria or "enteric bacteria" as several members live in the intestines of animals.

Family: Enterobacteriaceae (2%)

Genus: Citrbacter (0.3%)

Genus: Klebsiella (0.8%)

Genus: Enterobacter (0.3%)

Comment: This is a low percentage but slightly concerning. No vermitea is 100% free of these types of organisms but the addition of molasses has been shown to increase the family Enterobacteriaceae.

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