

Radiation Threats to Humans in Space and an alternative approach with Probiotics

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Abstract

Space type radiation is an important factor to consider for scientists on International Space Stations, especially high linear transfer energy (LET) since it has imminent effects on microorganisms. The abundances of bacteria are a good indicator of how radiation influences the gut microbiome. The current study is an attempt towards this; thus, we have employed a public dataset (Bioproject code PRJNA368790) of 80 mice samples treated with a range of doses from 0Gy to 1Gy and feces samples were collected at different time points of post radiation treatment. Metagenomic analysis was performed on this data to understand the effect of radiation doses on the abundance of microbial species or microbial diversity implementing the DADA2 and Phyloseq pipelines. Our analyses have shown that 0.1Gy high LET radiation had the significant effect on the species of bacteria. There is a significant decrease in four types of bacteria, i.e., Bifidobacterium longum, Bifidobacterium castoris, Lactobacillus gasseri and Lactobacillus johnsonii, with p-value of 7.05×10^{-5} , 0.020, 0.057 and 0.020, respectively. Additionally, pathway analysis indicates the protein coding products of these bacteria are involved in the GABAergic synaptic pathway. Further, our study has shown the significant difference between post radiation time points, i.e., 10 days vs. 30 days and suggested the acclimatization period could be around 10 days for these bacteria.

Introduction

High linear energy transfer (LET), which is a type of ionizing radiation, causes damage on several locations along the DNA that are close together (Takahashi, Ikeda, & Yoshida, 2018). The effects of radiation on somatic cells extends to micro-organisms, too (Maalouf, Durante, & Foray, 2011). Johnson & Foster (2018) also state that bacterial species in the Lactobacillus and Bifidobacterium species affect the nervous system and hence psychology through metabolites like GABA. Gama aminobutyric acid is known to be the chief inhibitory neurotransmitter in the CNS, it is produced by the enzyme GAD (glutamate decarboxylase) (Duman, Sanacora, & Krystal, 2019). A lack of GABA or the decreased stimulation of GABA receptors causes neurobehavioral disorders like depression and anxiety (Kalueff & Nutt, 2007). In this article data from previous studies from NCBI was used to test if the properties of high LET radiation are true and if the abundances of bacteria decrease significantly.

Methodology

For metagenomic analysis of paired end 16S rRNA the DADA2 pipeline is recommended since it can identify between species even with just one nucleotide difference (Callahan et al., 2016). T-BioInfo server ([Tauber Bioinformatics: Making sense of big data in BIOINFORMATICS \(t-bio.info\)](http://Tauber Bioinformatics: Making sense of big data in BIOINFORMATICS (t-bio.info))) was used to analyze the samples obtained. As shown in Figure 3 DADA2 was used to calculate the operational taxonomic units (OTU); whereas PHYLOSEQ was used for other quantitative analyses like Shannon Index. The parameters set for expected number of reads forwards and backwards was 150. The expected errors were estimated as 2 and the min/max value for overlaps was approximated as 12/5. The mean abundance was established as 150 after optimization. Once the OTU table was generated the results were scanned for changes in diversity.

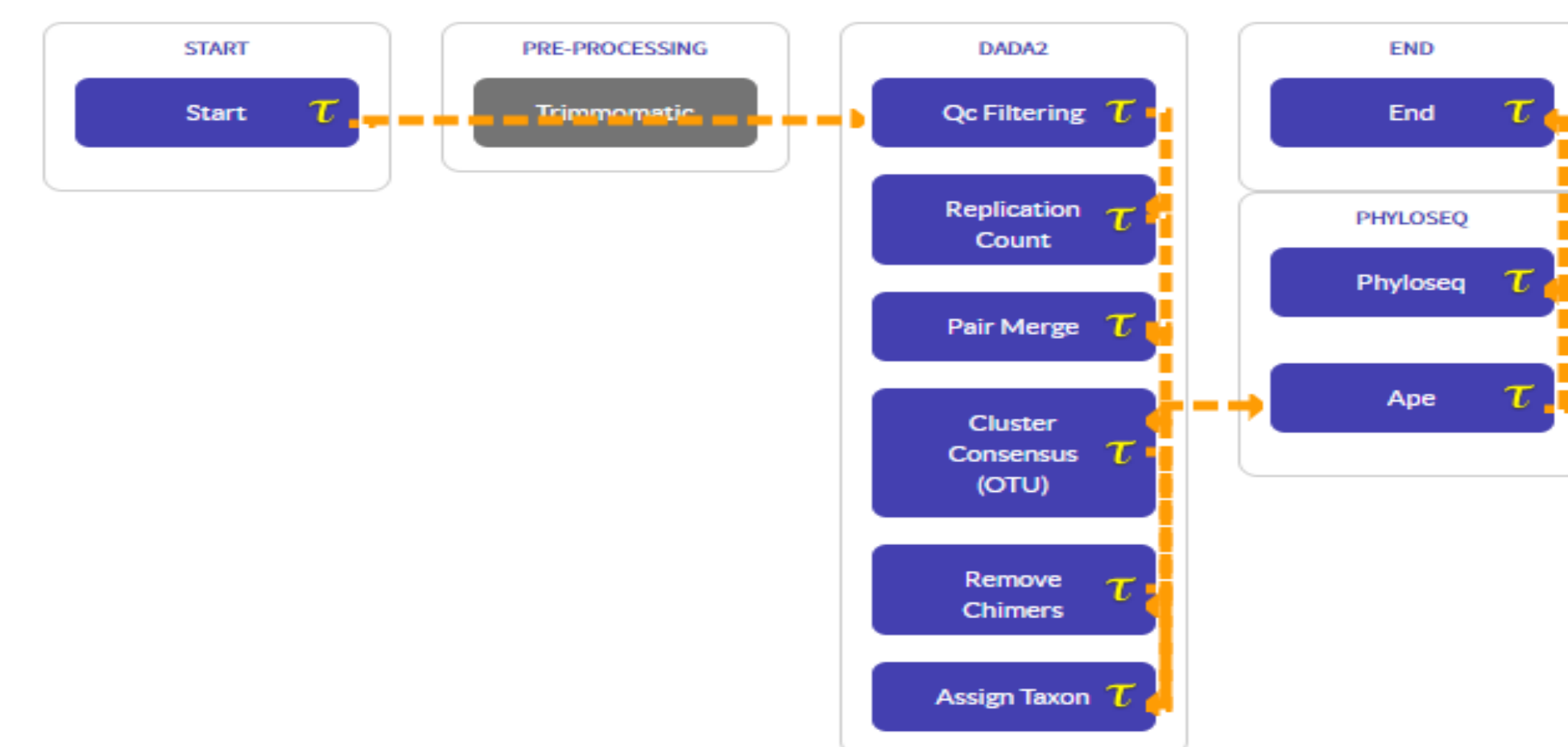


Figure 1. The pipeline used for metagenomics analysis of the gut microbiome of mice that were exposed to radiation. This study utilized both DADA2 and PHYLOSEQ from the T-BioInfo server

Another step used for confirmation was clustering. The type of clustering used was hierarchical clustering (H-clustering), which groups the data depending on how closely related they are. To confirm that the observations from the PHYLOSEQ pipeline output ANOVA and T.test was calculated in excel.

Results

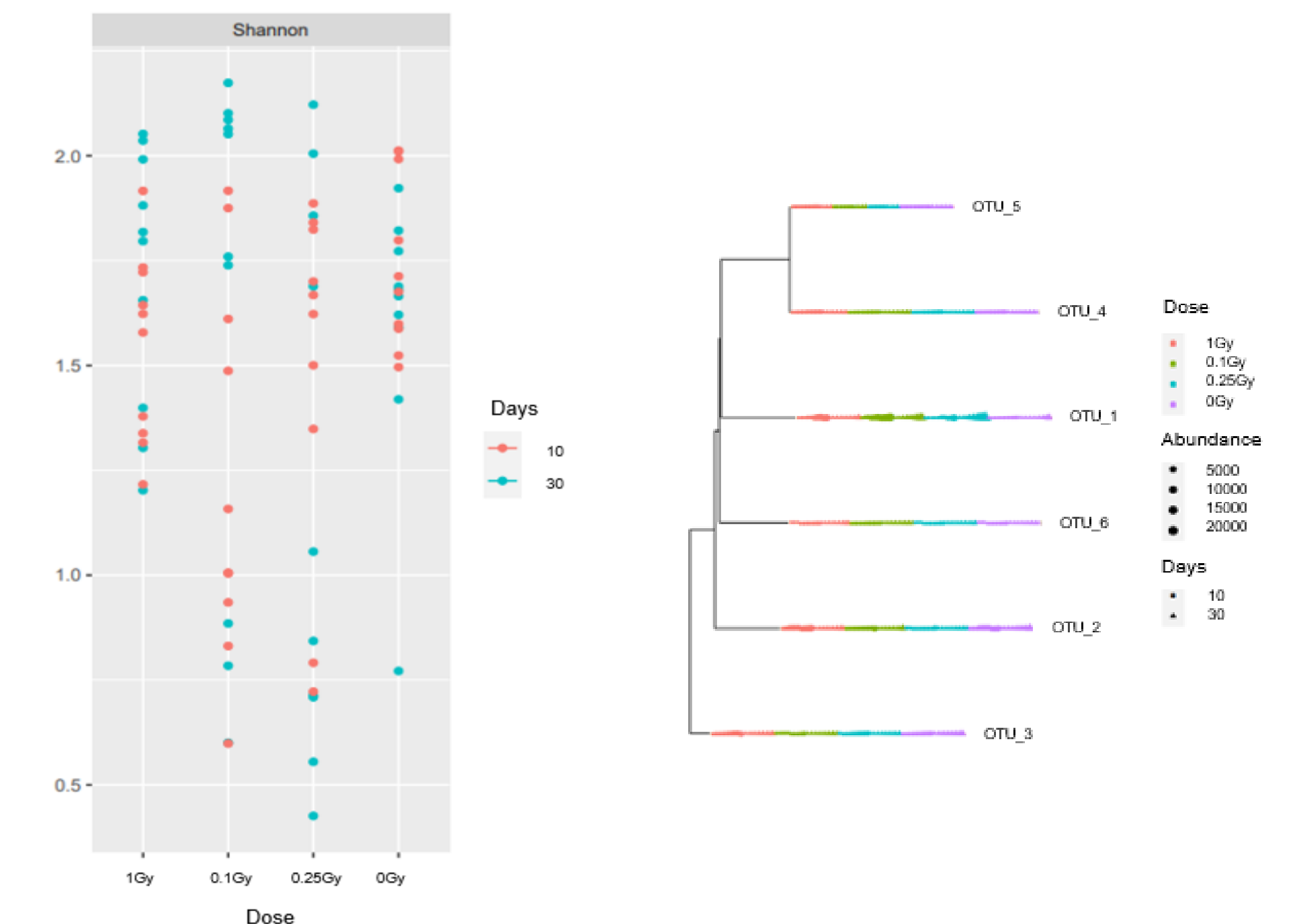


Figure 2. a. The Shannon Index for the four different doses of radiation. **b.** The phylogenetic tree displaying the significant OTUs with their respective abundances correlating with days and dosages.

Aligned with previous findings from the PHYLOSEQ pipeline, the significant OTUs are 3, 4, 5, and 8. With further evidence backing up the significant change in the abundances of these four OTUs, their genus and species were identified using the JGI/IMG database as follows: OTU 3 is *L.gasseri*, OTU 8 is *L.johnsonii*, OTU 4 is *B.longum* and OTU 5 is *B.castoris*. The species in the Lactobacillus genus code for proteins involved in both glutaminase (GLS) and glutamine synthase (GS) pathways in the nervous stem. On the other hand, the OTUs in the Bifidobacteria genus were engaged in only one of the pathways in the GABAergic synaptic pathway, which is glutamine synthase (GS). These are both enzymes that process glutamine before it is converted to the neurotransmitter GABA.

Figure 3. Heatmap representing the OTU abundances in mice that are radiated with dose of 0.1Gy and 0Gy radiation at 10 days.

Conclusion

Bacteria in Lactobacillus and Bifidobacterium taxon decreased significantly, which can be connected to the decrease in GABA. Since these bacteria have protein coding genes that affect the nervous system, we concluded that astronauts afflicted by anxiety and depression could also be suffering from dysbiosis. Hence a recommendation of probiotics rich in lactobacillus and bifidobacteria is fundamental. These studies should be extended to testing on fecal samples of astronauts who have been on long duration space travel in order for a better understanding of how problems arising from the extreme conditions in space and the ISS can be overcome. Besides in reality high LET radiation is combined with other types of radiation, microgravity and high sterility, therefore increasing the complexity of both the problem and the solution.

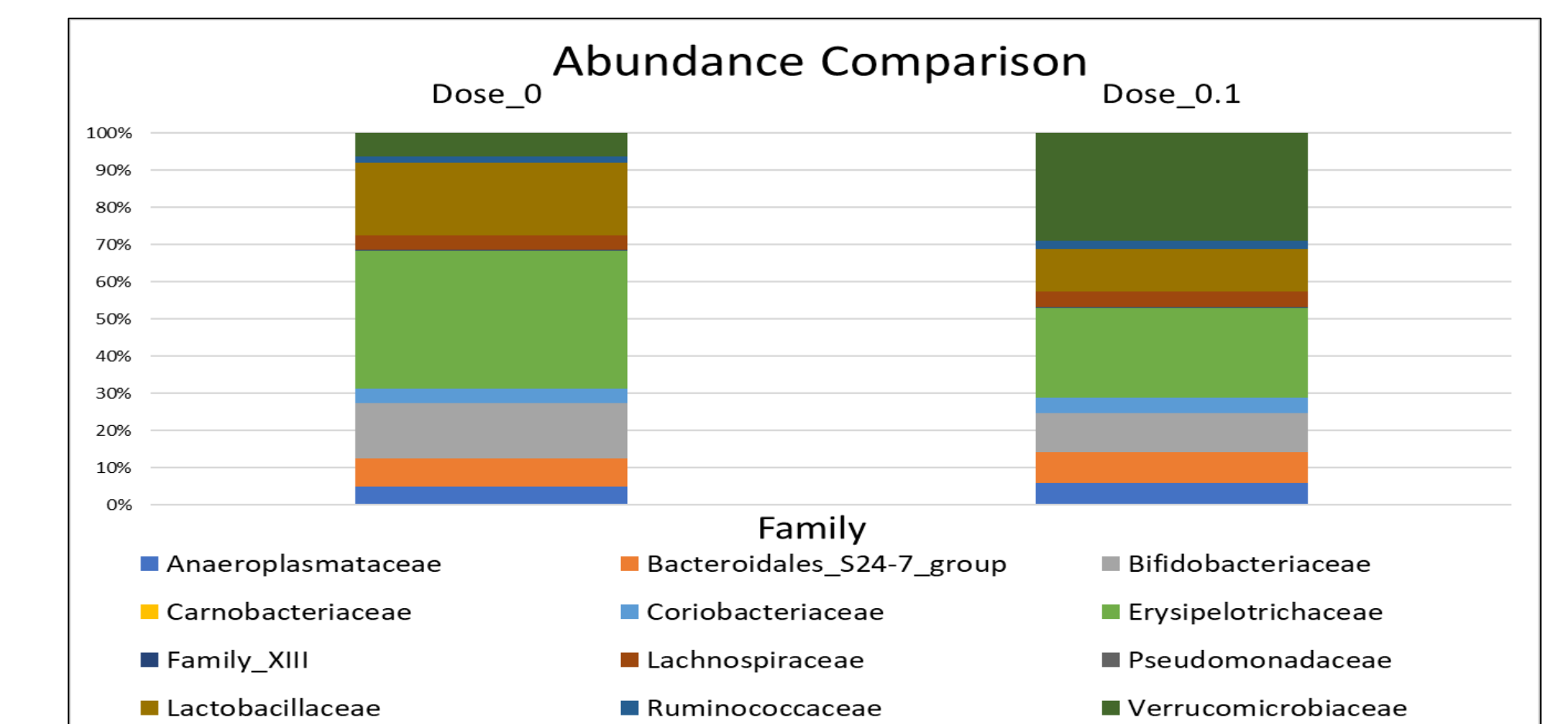


Figure 4. Bar graphs representing the abundances of different families of bacteria in mice that were not radiated and that were radiated at 0.1Gy high LET radiation.

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