Ecology and Comparative Analysis of the Gut Microbiome of Cancer Patients Against Healthy Controls

The gut microbiome plays a crucial role in maintaining homeostasis by degrading complex fibers, synthesizing vitamins, modulating the immune system, and preserving gut integrity through mutualistic interactions with the host, as well as the production of metabolites.[1] Patients with acute inflammatory diseases such as IBD, UC, and Crohn's disease exhibit significant differences in the composition and diversity of their gut microbiome compared to healthy individuals.[2] These variations in microbial population and diversity between healthy and diseased individuals are collectively referred to as gut dysbiosis.[3,4] A strong link exists between gut dysbiosis and overall health.[2,5–7] However, the mechanisms by which gut microbiome interacts with the host to contribute to disease phenotypes remain poorly understood. This critical knowledge gap hinders the development of effective treatments for inflammatory bowel disease, highlighting the urgent need to investigate the functional roles of specific bacterial taxa and their interactions with the host.

Gut dysbiosis is a common phenotype observed in IBD, neurodegenerative diseases, and colorectal cancer.[4,8] For instance, the gut microbiome of IBD patients undergoes significant shifts, with an increased presence of aerotolerant bacterial pathogens.[9] The Human Microbiome Project (HMP) established a genetic reference database for gut bacterial taxa, enabling comparative analyses of the gut microbiome in healthy and diseased individuals. Given the rising incidence of colorectal cancer and pancreatic cancer and its association with changes in the gut microbiome, there have been increased efforts to study the relationship between cancer and gut dysbiosis and possibly identify and characterize the effects of bacterial pathobionts in cancer progression.[6]

The overall goal of this project is to investigate the differences in the gut microbiome of pancreatic cancer patients versus healthy individuals. *Our central hypothesis* is that the gut microbiome of pancreatic cancer patients is different in composition and abundance than a healthy individual. We will carry out a meta-analysis of already existing clinical data to do a comparative taxonomic analysis of the gut microbiome of healthy and cancer patients. The rationale for this project is that while lots of studies have shown significant differences in the gut microbiome of patients suffering from colorectal cancer versus healthy individuals, little is known about the pancreatic cancer gut microbiome. Thus, this study aims to identify possible bacterial pathobionts that are associated with pancreatic cancer and possibly use them as biomarkers for early diagnosis of pancreatic cancer.

Aim 1: To investigate the comparative differences in the gut microbiome of pancreatic cancer patients versus healthy individuals. This aims to give us an idea t the phylum level, the differences in the bacterial composition of cancer and healthy individuals. We will use data from the European Nucleotide Archive (project ID: 994901, Accession no..: PRJNA994901, Project title: Healthy human gut microbiome subjected to B9 and B12 treatment, and NCBI with study no: PRJNA542319).

Aim 2: Identify bacterial pathobionts highly abundant in pancreatic cancer patients. We will profile the top species of bacteria that are found in pancreatic cancer patients.

We will utilize R Data analytic tool (version 4.2) and my conversation with Chat GPT 4 to generate codes in R and data.

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Results and Discussion

1. Comparative Gut microbial abundance in healthy and pancreatic cancer patients

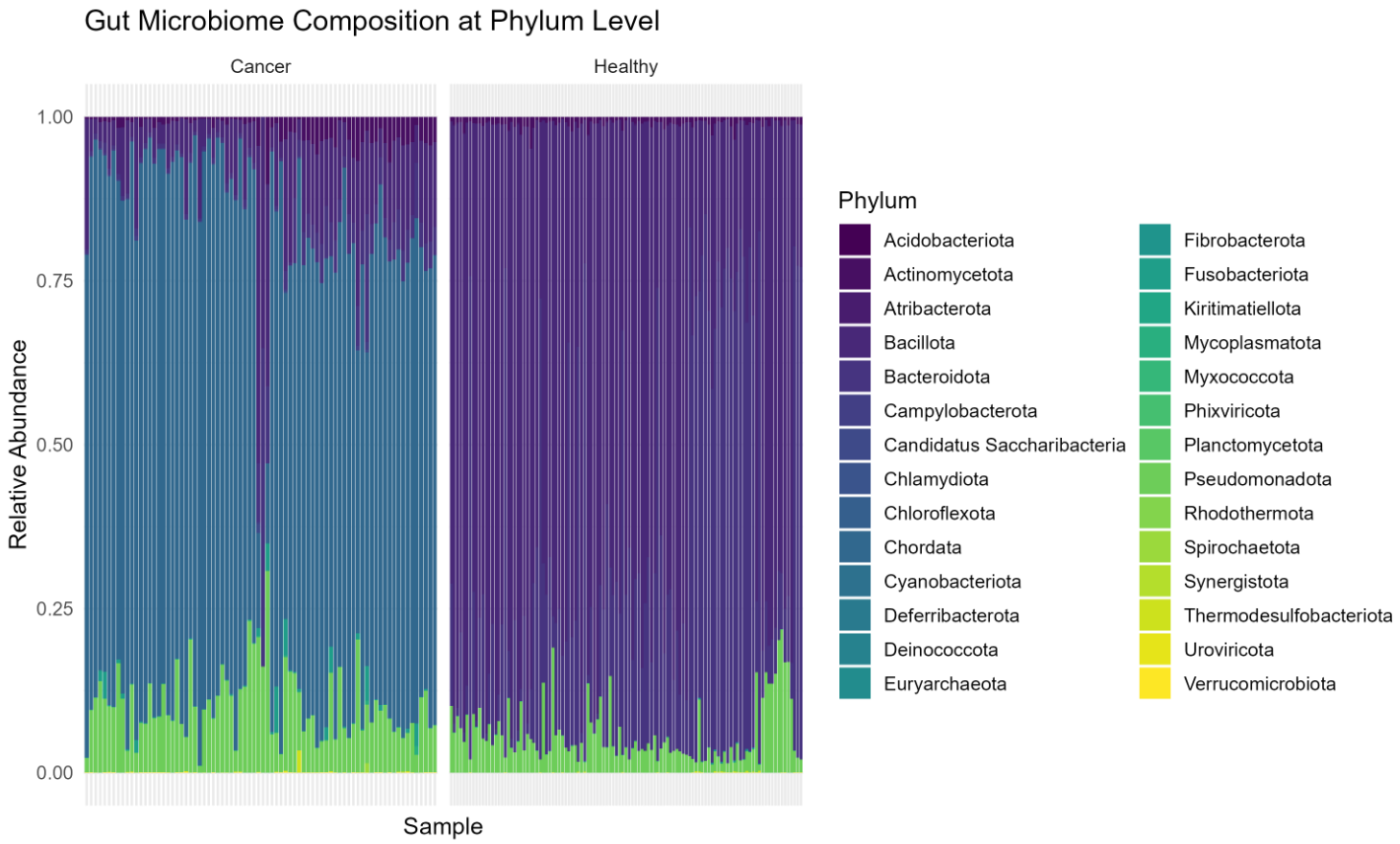

Fig.1: Comparative abundance of the gut microbiome of healthy vs. cancer patients.

Figure 1 shows that there are differences in the compositional abundance of microbial taxa in cancer patients versus healthy individuals. Specifically, cancer patients had more proteobacteria (green colored bars) and also fusobacteria, which are highly associated with gut dysbiosis. This result is consistent with the literature, where proteobacteria and fusobacteria are shown to be highly abundant in a dysbiotic gut environment and colorectal cancer, respectively.

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2. Ecological properties of the Gut microbiome in Healthy and Cancer patients(α-diversity)

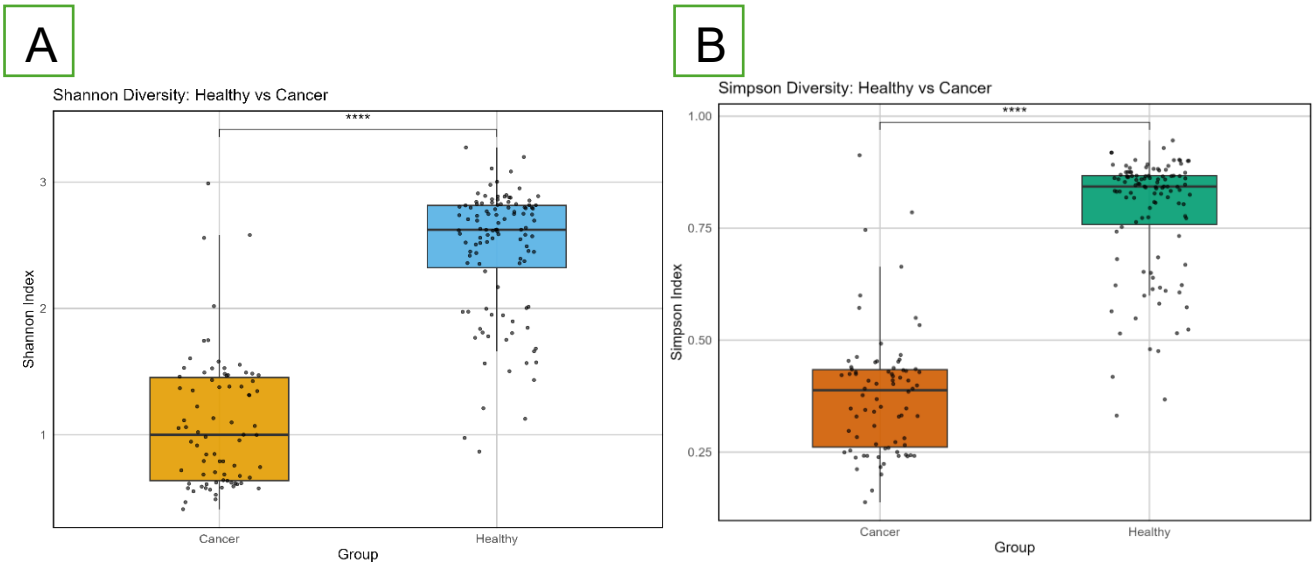

Fig. 2: Alpha diversity of gut microbiome for healthy and cancer subjects. A) Shannon diversity index. B) Simpson diversity index.

The analysis of bacterial diversity across different species shows a significant difference between cancer patients and healthy subjects, with cancer patients exhibiting lower bacterial diversity compared to healthy controls (Fig. 2A). This finding is also consistent with the reduced evenness of bacterial species observed in Fig. 2B, as indicated by the Simpson index. These results suggest that the gut microbiome of cancer patients is dominated by a few unique taxa, unlike the more balanced microbial communities seen in healthy individuals. This observation aligns with several studies which report that reduced diversity and evenness are hallmarks of a dysbiotic gut.

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3. Ecological properties of the Gut microbiome in Healthy and Cancer patients (β-diversity)

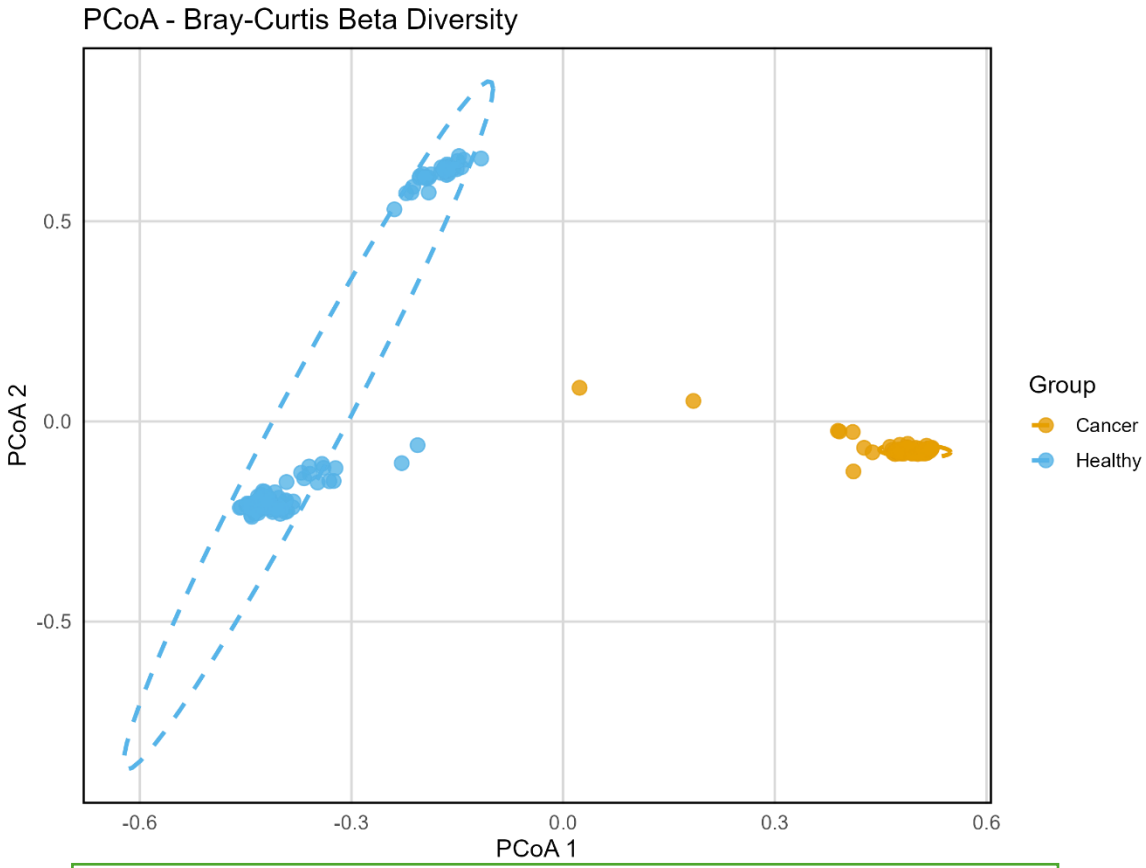

Fig. 3: β-diversity of gut microbiome for healthy and cancer subjects.

Further analysis using Bray-Curtis dissimilarity shows that both cancer patients and healthy subjects possess significantly distinct microbial communities, with greater variation observed among healthy controls compared to cancer patients. This finding is particularly interesting, as no two individuals typically have identical gut microbiomes, and one might expect more inter-individual variation across both groups. However, the data suggest that the unique taxa colonizing the gut microbiome in cancer patients are more similar across individuals, possibly reflecting a shared dysbiotic microbial signature.

4. Comparative Taxonomic Analysis of Gut Microbiome in healthy VS Cancer

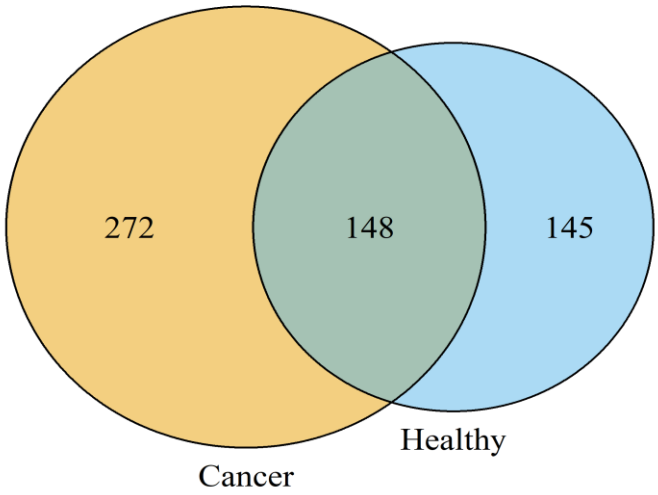

Fig. 4: Differential abundance of microbial species in health and disease states.

subjects

To further characterize bacterial species unique to cancer and healthy individuals, we conducted a comparative taxonomic analysis, as shown in Figure 4. The results reveal differential species abundance between the two groups, with approximately 148 bacterial species shared between cancer patients and healthy subjects. These shared taxa are believed to be opportunistic pathogens—bacteria capable of modulating their phenotype in response to disease, allowing them to adapt to the altered gut environment. However, there is limited understanding of how these organisms maintain their fitness within this niche.

5. Comparative Taxonomic Analysis of Gut Microbiome in healthy VS Cancer sub

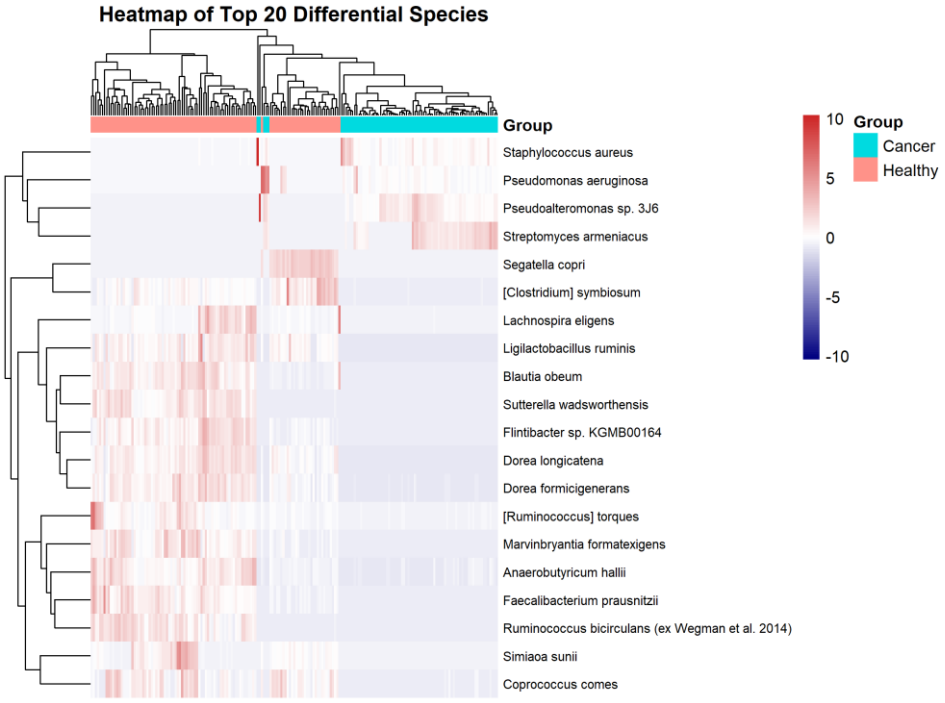

Fig. 5: Top 20 differentially abundant species

We further focused on identifying the top twenty most abundant bacterial species in cancer patients versus healthy controls. The heatmap in Figure 5 complements the results in Figure 1 by highlighting distinct species enriched in each group. Increased abundance of *Streptococcus* and *Pseudomonas* species is strongly associated with inflammatory bowel diseases (IBD). In contrast, species such as *Segatella copri* and *Ruminococcus* spp. are linked to enhanced short-chain fatty acid (SCFA) production in healthy gut environments. These findings suggest that cancer patients may be more susceptible to gut dysbiosis.

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6. Comparative Taxonomic Analysis of Gut Microbiome in healthy VS Cancer subjects

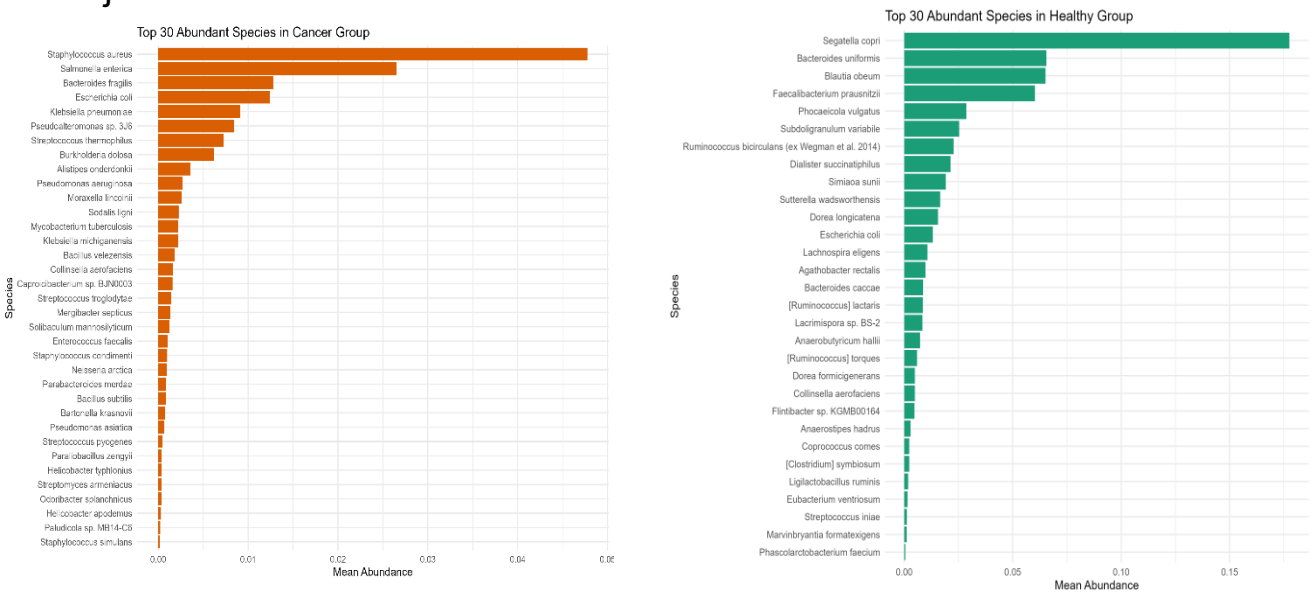

Fig. 6: Top 20 bacterial species in cancer patients and healthy patients

Figure 6 displays the top 20 bacterial species identified in healthy individuals and cancer patients. The graph reveals that these top species are entirely distinct between the two groups. This is a notable finding, as certain species, such as *Staphylococcus* spp., which are abundant in cancer patients, could be further investigated to understand how they persist and adapt in the altered gut environment.

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Conclusion

The data suggests a difference in the richness and diversity of the gut microbiome in cancer patients versus healthy individuals.

There is an increase in proteobacteria in cancer patients compared to healthy individuals, suggesting gut dysbiosis.

Strikingly, unique taxa are common in the cancer gut microbiome, including *Fusobacterium sp., Staphylococcus sp. Fusobacterium* is known to be highly associated with colorectal cancer.

Supplementary Data and R Scripts:

R-Script code:https://raw.githubusercontent.com/okpecallistus/Uchenna5202stuff/refs/heads/main/Codes%20for%20My%20analysis%20tutorial%20Project_Uchenna_Okpe.R

References:

1. Fricker, A. D., Yao, T., Lindemann, S. R. & Flores, G. E. Enrichment and characterization of human-associated mucin-degrading microbial consortia by sequential passage. *FEMS Microbiol Ecol* **100**, (2024).
2. Chamorro, N. *et al.* Landscapes and bacterial signatures of mucosa-associated intestinal microbiota in Chilean and Spanish patients with inflammatory bowel disease. *Microbial Cell* **8**, 223–238 (2021).
3. Zitomersky, N. L. *et al.* Characterization of Adherent Bacteroidales from Intestinal Biopsies of Children and Young Adults with Inflammatory Bowel Disease. *PLoS One* **8**, (2013).
4. Hurych, J. *et al.* Faecal Bacteriome and Metabolome Profiles Associated with Decreased Mucosal Inflammatory Activity Upon Anti-TNF Therapy in Paediatric Crohn's Disease. *J Crohns Colitis* **18**, 106–120 (2024).
5. Lima, I. S. *et al.* Gut Dysbiosis: A Target for Protective Interventions against Parkinson's Disease. *Microorganisms* **11**, (2023).
6. Singh, S. *et al.* Implication of Obesity and Gut Microbiome Dysbiosis in the Etiology of Colorectal Cancer. *Cancers* vol. 15 Preprint at https://doi.org/10.3390/cancers15061913 (2023).
7. Fassarella, M. *et al.* Gut microbiome stability and resilience: Elucidating the response to perturbations in order to modulate gut health. *Gut* vol. 70 595–605 Preprint at https://doi.org/10.1136/gutjnl-2020-321747 (2021).
8. Lima, I. S. *et al.* Gut Dysbiosis: A Target for Protective Interventions against Parkinson's Disease. *Microorganisms* **11**, (2023).
9. Lloyd-Price, J. *et al.* Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* **569**, 655–662 (2019).

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