GLOBAL INVESTIGATION OF METHANOGEN PREVALENCE ACROSS GUT MICROBIOME

By

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CERTIFICATE

This is to certify that the thesis titled **“Global Investigation of Methanogen Prevalence across Gut Microbiomes”** being submitted by **Akanksha Singh** to the Indraprastha Institute of Information Technology Delhi, for the award of the Master of Technology, is an original research work carried out by her under my supervision. In my opinion, the thesis has reached the standards fulfilling the requirements of the regulations relating to the degree.

The results contained in this thesis have not been submitted in part or full to any other university or institute for the award of any degree/diploma.

August, 2025

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I deeply appreciate his steadfast dedication and accessibility. Irrespective of the time or condition, he has consistently been available to respond to my queries, regardless of the lateness of the hour or the difficulty of the situation. The level of commitment he has shown towards my development has been truly extraordinary, frequently devoting more of his time to mentor me than he may have allocated for his own family. The remarkable level of support and encouragement I have received has not only motivated me but also compelled me to exert my utmost effort towards this project.

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Lastly, I would like to express my gratitude to my family for their consistent and unwavering support, both in terms of financial assistance and emotional encouragement, throughout my entire life. The unwavering faith they have in me has consistently provided me with the fortitude to confidently and resolutely pursue my academic and research aspirations.

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ABSTRACT

The human gut serves as home to trillions of microorganisms, forming a complex and dynamic ecosystem that influences nearly every aspect of human health. While much of the research so far has focused on bacteria, the gut also harbours another fascinating but less explored group of microbes—archaea. Among them, methanogens stand out for their ability to produce methane gas in the gut, a function that may play important roles in digestion, microbial balance, and overall gut physiology.

Unlike the bacterial microbiome, which has been studied extensively and is known to vary widely across individuals depending on geography, diet, lifestyle, medication, environment, and genetics, the gut archaeome remains comparatively underexplored. Its diversity, distribution, and functional contributions to human health are still poorly understood. In particular, how archaeal populations change across different conditions and how they interact with bacterial communities are key questions that remain unanswered.

To address this gap, we conducted a large-scale analysis of 55,788 human gut metagenomes, focusing specifically on the distribution and ecological associations of methanogens. Using relative abundance data from 7,471 gut microbiomes, we found that methanogen prevalence is far from uniform; instead, it displays distinct patterns strongly shaped by geography, lifestyle, and host-associated factors. Interestingly, certain populations showed markedly higher carriage rates of methanogens, suggesting that environmental exposures, dietary practices, and cultural traditions may play a crucial role in shaping archaeal communities.

Our analysis further revealed important host-related associations. When examining body mass index (BMI), we observed a negative correlation between methanogen prevalence and BMI. Methanogen levels were highest in underweight individuals and progressively decreased toward obese individuals. Among species, *Methanobrevibacter smithii* emerged as the most dominant archaeon, though prevalence patterns varied notably between males and females.

We also explored the potential links between methanogens and human disease. Our findings suggest that methanogen presence is not random but may be closely tied to health outcomes. For example, *M. smithii* has been implicated in conditions such as Parkinson’s disease and intestinal polyps, while also showing positive associations with gut-related disorders, type 2 diabetes, and other metabolic conditions. These associations hint at a possible role of methanogens in either contributing to or modulating disease pathways, though the exact mechanisms remain to be clarified.

Finally, through co-occurrence network analysis, we observed that methanogens tend to cluster with specific bacterial communities. This points to potential ecological interactions and niche specialization within the gut ecosystem, where methanogens may work in tandem with or compete against other microbes, thereby influencing broader microbiome structure and function.

Together, these findings underscore the importance of moving beyond bacteria-focused studies and paying closer attention to the archaeome. Methanogens, long overlooked in human microbiome research, may hold critical clues about how microbial ecosystems function, how they interact with host factors, and how they influence health and disease. A deeper exploration

of their ecological and functional roles could open new avenues for microbiome-based diagnostics and therapeutics.

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CHAPTER 1

INTRODUCTION:

Gut health is an essential aspect of overall well-being, as it influences nutrition, immunity, and disease resistance. The World Health Organization has emphasized that gastrointestinal diseases, including colorectal cancer and inflammatory bowel disorders, remain among the leading causes of global illness and death[1].Colorectal cancer ranks as one of the most common cancers worldwide, while conditions such as inflammatory bowel disease and functional gut disorders affect millions of people across different regions[2].These challenges highlight the urgent need to better understand the factors that maintain a healthy gastrointestinal environment.

In recent years, the gut microbiome has emerged as a central focus of research. This term refers to the wide community of microorganisms including bacteria, archaea, viruses, and fungi that inhabit the gastrointestinal tract. The diversity of the gut microbiome is remarkable. Each individual carries a unique microbial signature. This diversity is not only in the number of species but also in their functional capacities. These microbes play crucial roles in digestion, nutrient absorption, energy balance, and immune. Such functional diversity explains why the microbiome is considered a vital organ in itself, performing tasks that the human body cannot achieve alone[2,3].

While bacteria have traditionally been the main focus of gut microbiome research, other groups of microorganisms are now gaining recognition. Within this diverse microbial ecosystem, archaea represent a unique and less-studied group. Although once considered minor players, recent studies have shown that archaea influence the efficiency of digestion, energy harvest, and the composition of the microbial community as a whole. Unlike bacteria and eukaryotes, archaea form a separate domain of life with distinct biological features. In the human gut, archaea are dominated by methanogens, a group of microbes that generate methane by utilizing hydrogen, carbon dioxide, and other fermentation products produced by bacteria[4].By removing excess hydrogen, methanogens improve the efficiency of bacterial fermentation and influence the overall stability of the gut environment[5].

As research progresses, the gut microbiome is emerging as both a marker and a target for health interventions. Microbiome analysis can provide diagnostic clues about disease states, while therapeutic strategies such as probiotics, prebiotics, dietary modifications, and faecal microbiota transplantation aim to restore a healthy microbial balance. These approaches hold promise not only for digestive disorders but also for conditions far beyond the gut, including autoimmune and neurological diseases[6].

Altogether, the gut microbiome represents one of the most complex and dynamic ecosystems in the human body, and archaea particularly methanogens, are a vital but underexplored part of it. Understanding how these organism function, how they interact with bacteria, and how they influence host health will be essential for developing innovative approaches to prevent and treat gastrointestinal as well as systemic[4,6].

CHAPTER 2

BACKGROUND AND OBJECTIVES:

2.1 Literature:

The human gut hosts trillions of microorganisms that collectively form the gut microbiome, a complex ecosystem essential for maintaining health. A well-balanced microbiome supports good health while disturbances in this community known as dysbiosis[1,2] have been linked to a wide range of disorders, including obesity[7], diabetes[8], cardiovascular disease[9], liver disorders[10,11], inflammatory bowel disease[12], and colorectal cancer[1,13].

Among the diverse microbial inhabitants of the gut, methanogenic archaea represent a particularly intriguing group. The best characterized species is *Methanobrevibacter smithii*, although other taxa such as *Methanosphaera stadtmanae* and members of the order *Methanomassiliicoccales* are also present. These organisms are notable for their ability to produce methane as a metabolic by-product, and their abundance varies considerably between individuals and populations[2].

The prevalence of methanogens varies among individuals and populations. Apart from Host genetics, Environmental factors such as geographic location, ethnicity, lifestyle, diet, age, gender, Body Mass Index (BMI) and medications influence their abundance[15-19]. People in rural communities consuming high-fiber, plant-based diets often show higher methanogen levels, while urban populations with Western diets rich in fat and sugar tend to have fewer. Associations with body weight have also been observed: methanogens appear more common in lean individuals, while their levels may decrease in obesity[7].

Investigating methanogen prevalence on a global scale is therefore critical. Human health is shaped not only by genetics but also by environmental and lifestyle factors that differ markedly across regions. Understanding how methanogen distribution varies worldwide may help explain population-level differences in disease risk for conditions such as obesity, diabetes, and cancer. It could also open the door to new interventions, including diet-based therapies, probiotics designed to support archaea, and targeted microbiome modulation. By focusing specifically on this understudied domain of archaea, the study aims to address an important gap in microbiome research and to shed light on the potential roles of methanogens in human health and disease.

2.2 Objectives:

1. Data compilation and homogenization.
2. Analysis of Methanogen Prevalence across Age groups, Lifestyle cohorts, Geography, and BMI categories.
3. Investigating Methanogens Alteration in Different Diseases.
4. To identify the association of Methanogens with Non-Methanogens.

CHAPTER 3

MATERIALS AND METHODS:

3.1. Compiling a Data Repository

Any Metagenomic analysis needs two basic things: Species Profile data and Metadata. Species Profile data is the relative abundance data that is prepared by sequencing the sequence taken from the patients. Metadata is the information of the patients with respect to age, gender, disease, country, BMI, sequencing type, sample site, etc.

Compiled data consisted of 162 study cohorts, 120 out of them were accessed from HACK Index research article[20] and the rest 42 were collected from others.

All the data compiled was stored in the repository, irrespective of which type of data it is. Study names wise separation was done between the samples to know all the studies have their own disease and control matched samples.

The Species profile data and Metadata were then homogenized for further analysis.

3.2. Data Homogenization

Data Homogenization was done to uniform the data compiled. Initial step was to perform normalization using Total Sum Scaling (TSS). The row abundances were normalized by dividing each feature count by the total count of that sample and the resulting relative abundances were then used for detecting the microbiomes having significant presence using a threshold of >0.0001 relative abundance. The resulting data was then processed to detect methanogens out of all the microbiome data using National Center for Biotechnology Information, National Institutes of Health; listing of Methanogens.

3.3. Statistical analysis using R

The species abundance and Metadata for every study cohort was imported in R for statistical analysis.

3.3.1. Methanogen Prevalence Analysis

In a meta study on Methanogen prevalence, one needs to analyse its prevalence on the basis of factors affecting the methanogen or on mass level, the gut microbiome. For the same purpose, we analysed the prevalence of Methanogens on the basis of its Geography, Age, Cohort Lifestyle and BMI. Prevalence was calculated as the proportion of samples in which a given species had a non-zero abundance, relative to the total number of samples in the dataset.

3.3.1.1 Methanogen Prevalence according to continents

Country data of patients from Metadata was used for this analysis. The countries were mapped to their respective continents and clubbed according to study names to plot them on the box plot.

3.3.2.1 Methanogen Prevalence according to Age

Age Category data of patients from Metadata was used for this analysis. There were three age categories Newborn, Children and Adult. Species Abundance data was clubbed according to study names and age category to plot them on the box plot.

3.3.3.1 Methanogen Prevalence according to Cohort Lifestyle

Cohort Lifestyle data of patients from Metadata was used for this analysis. Cohort Lifestyles were categorized into Industrialized and Non- Industrialized. Species Abundance data was clubbed according to study names and cohort lifestyles to plot them on the box plot.

3.3.4.1 Methanogen Prevalence according to BMI

BMI data of patients from Metadata was used for this analysis. Standard BMIs according to their categories were opted - Underweight (below 18.5), Healthy weight (18.5–24.9), Overweight (25.0–29.9), and Obesity (30.0 and above) and the data was categorized likewise. Species Abundance data was clubbed according to study names and BMI Categories to plot them on the box plot.

3.3.2. Alteration of Methanogens in Different Diseases using Wilcox Test

To find how the methanogens are altering in various diseases we used Wilcox test which is non-parametric and single variate statistical test. Wilcox test takes two data at a time to see the significant abundance difference in two groups – disease and control [21]. This Wilcox test was run for every disease separately as shown in *Figure 3.3.2.*

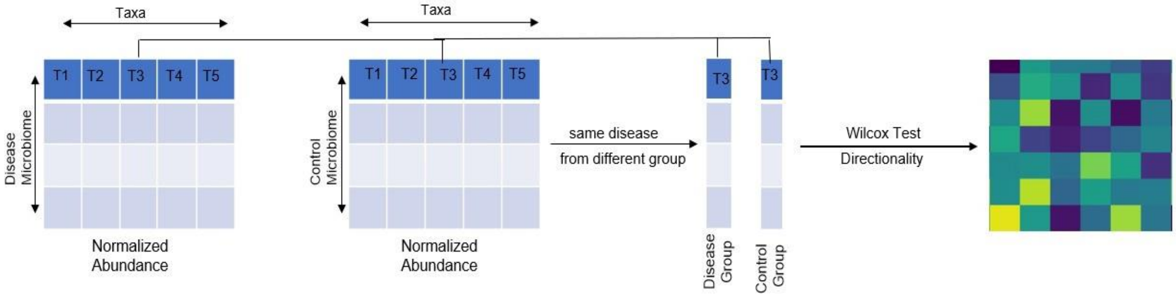


Figure 3.3.2: Workflow of the Wilcox Test and Directionality

Wilcox test yields P-value, estimate, etc. Along with this we calculate the difference of mean abundance of disease and control samples for every disease. Based on P-value and mean we calculate the directionality of the species as positive or negative towards disease and graphically present it with a heatmap.

3.3.3. Association of Methanogens with Non-Methanogens using Spearman Correlation Test

The Spearman correlation test was used to examine the relationship between methanogens and non-methanogenic taxa. The Spearman correlation test is a non-parametric statistical method used to measure the strength and direction of the association between two ranked variables. This test is rank-based and does not assume normal distribution, making it suitable for microbiome data[21]. Spearman test takes two data at a time, here we provided, Species profile for Methanogen and Non- Methanogen. The test resulted in P – value and spearman correlation value, also known as spearman rho, using which we calculated directionality matrix as correlation being positive, negative or neutral to finally present it on heatmap.

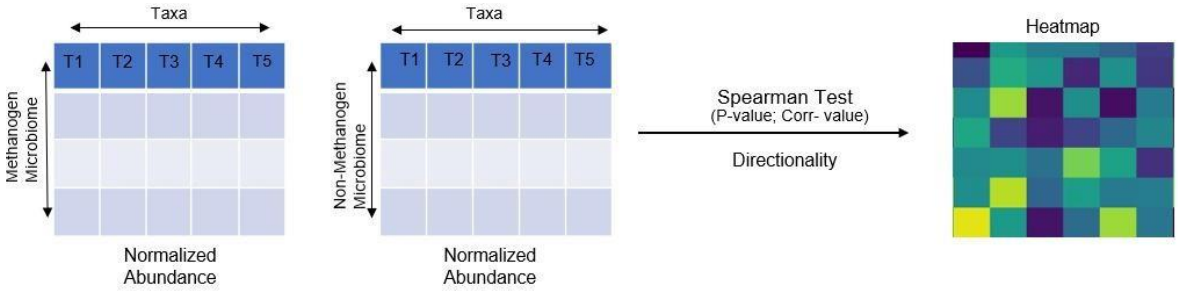


Figure 3.3.3: Workflow of Spearman Correlation Test and Directionality

CHAPTER 4

RESULTS:

We compiled and homogenized a total 55,788 samples from 162 study cohorts belonging to 45 different countries comprising 28 diseases. The outcomes of each of them are as follows:

4.1. Graphical Representation of Data compiled:

The data compiled has been represented in *Figure 4.1*. Data compilation is from a total 45 countries where the largest collection is from the United States, covering about 28 different diseases. Out of 55,788 samples, 39,055 were Control samples, while remaining 16,733 were samples from people having disease. Of total 55,788 samples, only 10,795 samples belong to Non-Industrialized samples, while 44,993 samples belong to Industrialized samples.

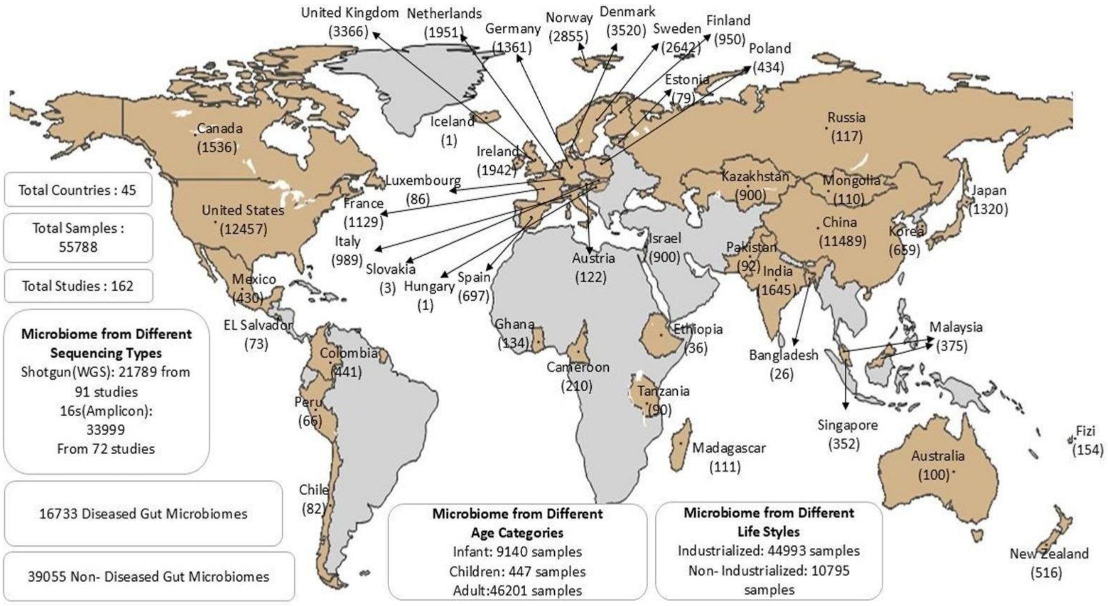


Figure 4.1: Data Representation of Compiled Data

4.2. Overall Methanogen Prevalence across Globe:

Our investigation on Methanogen prevalence across the globe results in variable signatures across the geography. The figure 4.2 represents the prevalence of Methanogens all across the globe where legend depicts the range of prevalence, greater than or equal to zero to more than thirty. As we can see in the figure, the country having most prevalence is Hungary which is present in Europe while the country having the least prevalence is Bangladesh which is in the continent Asia.

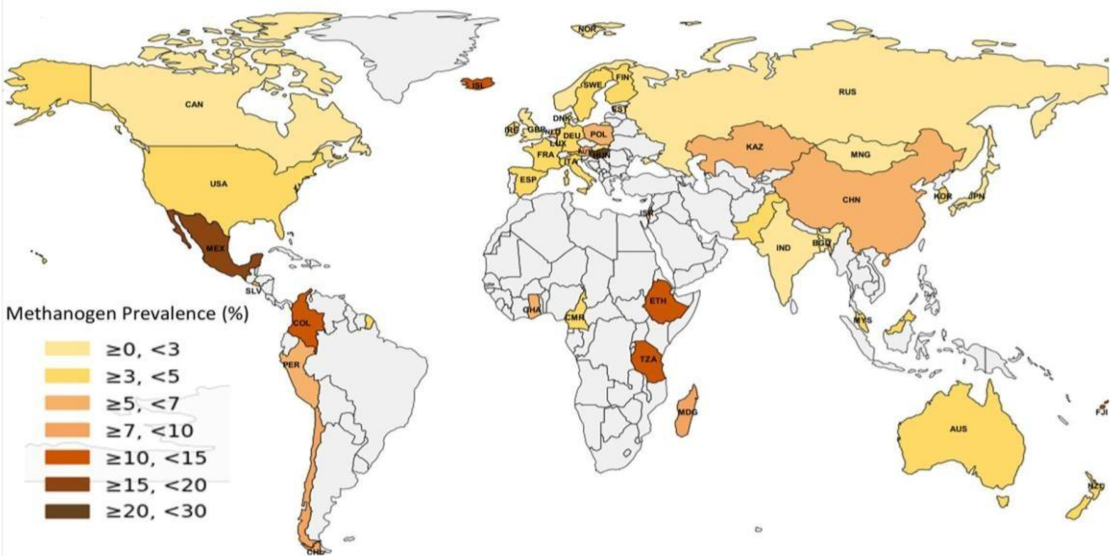


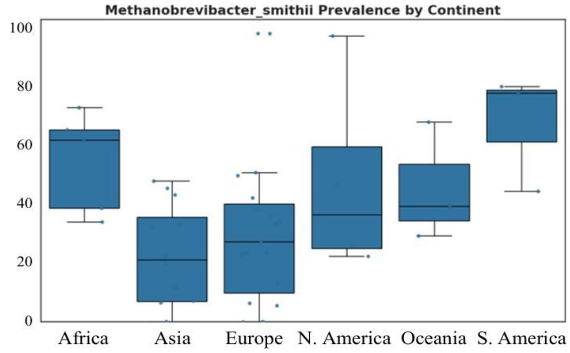
Figure 4.2: Representation of overall methanogen prevalence across the globe

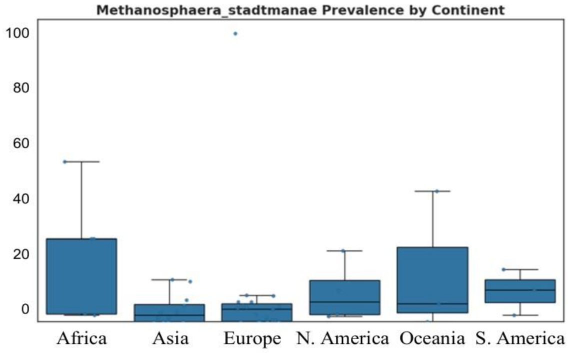
4.3. Analysis of methanogen prevalence:

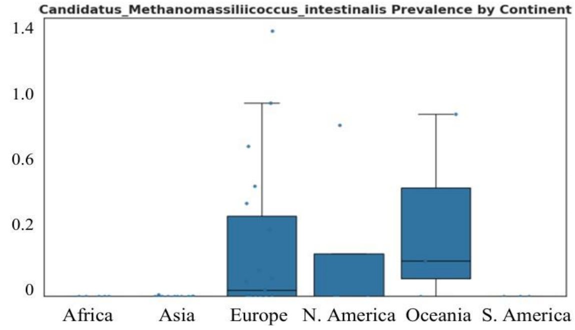
4.3.1 Methanogen prevalence across the continents:

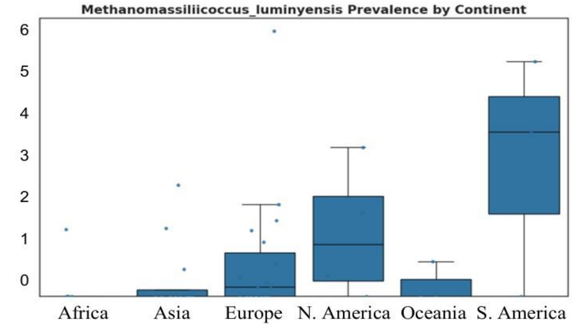
The results demonstrate that methanogens distribution is not uniform but is strongly shaped by geography as seen in *figure 4.3.1*. We see in the figure the overarching trend in the prevalence and several important exceptions that highlight the unique role of different species.

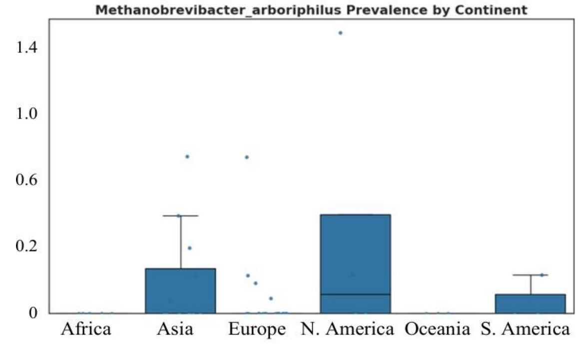
The results demonstrate that higher prevalence is found in the continent Africa, Oceania and America. Both common and rare methanogens prevalence are consistently higher in these continents. While Europe and Asia are showing the lowest prevalence. One thing to notice here is the scale of the *Methanobrevibacter smithii*. While we discuss other methanogens in the single digit percentage *Methanobrevibacter smithii* prevalence is often over 40- 60% hence it suggests that not only it is the dominant but also the most common methanogen in the human gut globally**.**

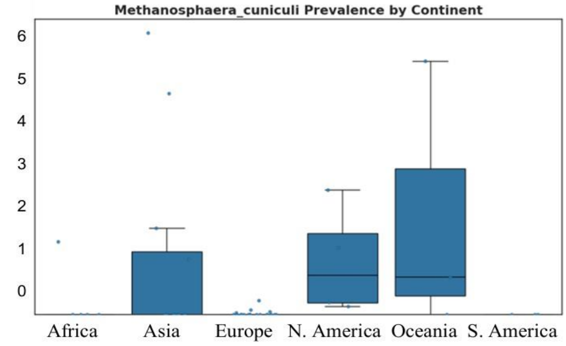

Methanobrevibacter_smithii Prevalence by Continent

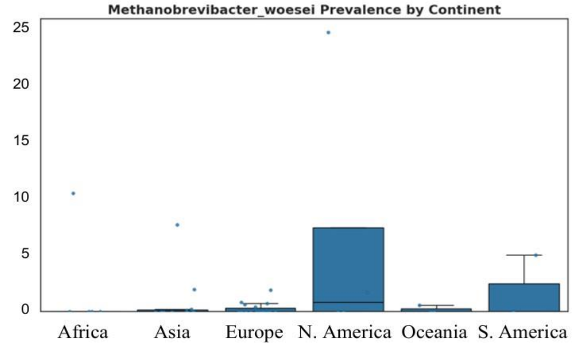

Methanosphaera_stadtmanae Prevalence by Continent

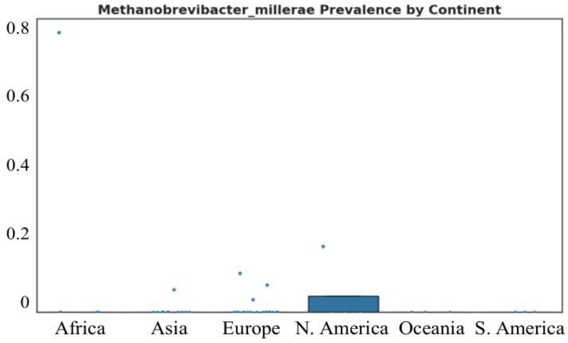

Candidatus_Methanomassiliicoccus_intestinalis Prevalence by Continent

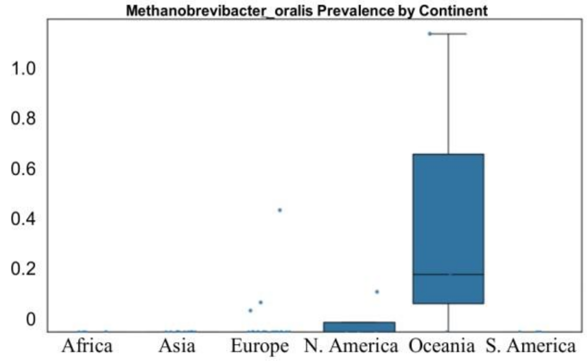

Methanomassiliicoccus_luminyensis Prevalence by Continent


Methanobrevibacter_arboriphilus Prevalence by Continent


Methanosphaera_cuniculi Prevalence by Continent


Methanobrevibacter_woesei Prevalence by Continent


Methanobrevibacter_millerae Prevalence by Continent


Methanobrevibacter_oralis Prevalence by Continent

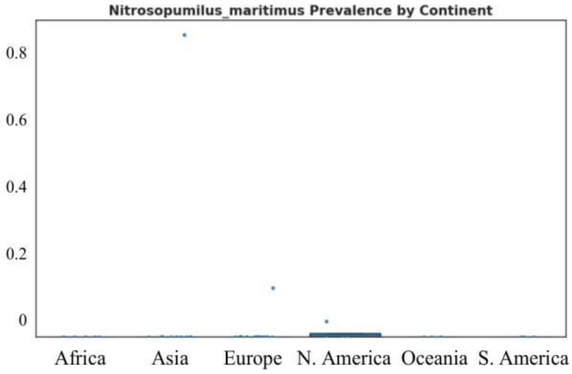

Nitrosopumilus_maritimus Prevalence by Continent

Figure 4.3.1: Methanogens (Methanobrevibacter smithii, Methanosphaera stadtmanae, Candidatus Methanomassiliicoccus intestinalis, Methanomassiliicoccus luminyensis, Methanobrevibacter arboriphilus, Methanosphaera cuniculi, Methanobrevibacter woesei, Methanobrevibacter millerae, Methanobrevibacter oralis, Nitrosopumilus maritimus) prevalence across Continents.

4.3.2 Methanogen prevalence based on lifestyle of the cohort:

Analysis reveals a connection between life style and methanogens presence and abundance. Industrialization was found to have a negative impact on the presence of methanogens, consistent with the results observed in overall prevalence of methanogens. It also suggests that methanogens are sensitive to factors which are associated with industrialized life like diet, environment, etc. While on the other hand, they are more prevalent in non-industrialized continents like Africa and South America which could be the result of high fiber diet, environment, etc. Methanogen prevalences are shown in *figure 4.3.2*.

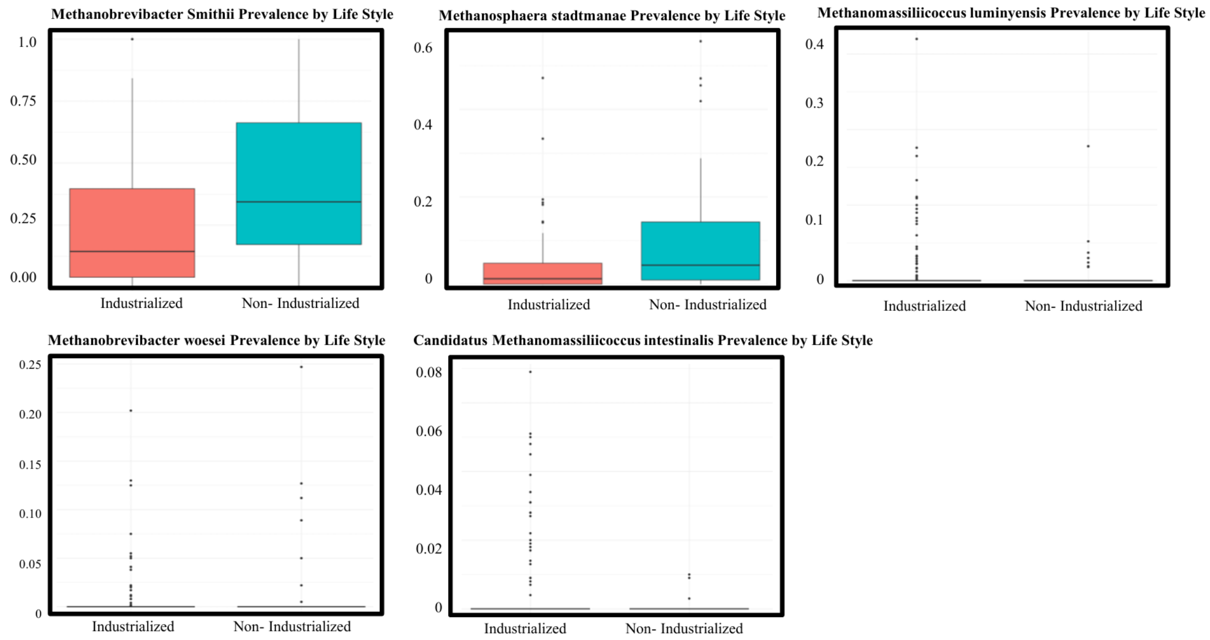


Figure 4.3.2: Methanogens (Methanobrevibacter smithii, Methanosphaera stadtmanae, Candidatus Methanomassiliicoccus intestinalis, Methanomassiliicoccus luminyensis, Methanobrevibacter woesei) prevalence across Cohort Lifestyle.

4.3.3 Methanogen prevalence based on age:

Age is a critical factor in shaping the methanogen community as observed from analysis. Our results show a developmental pattern, where the prevalence of methanogens increases from birth to adulthood.

The most dominant and consistent trend observed across the data was maturation pattern. Methanogens prevalence was lowest in the newborns, increased in the children, and was highest in the adults depicting higher prevalence and diversity with aging. Prevalence plots are shown in figure *4.3.3*.

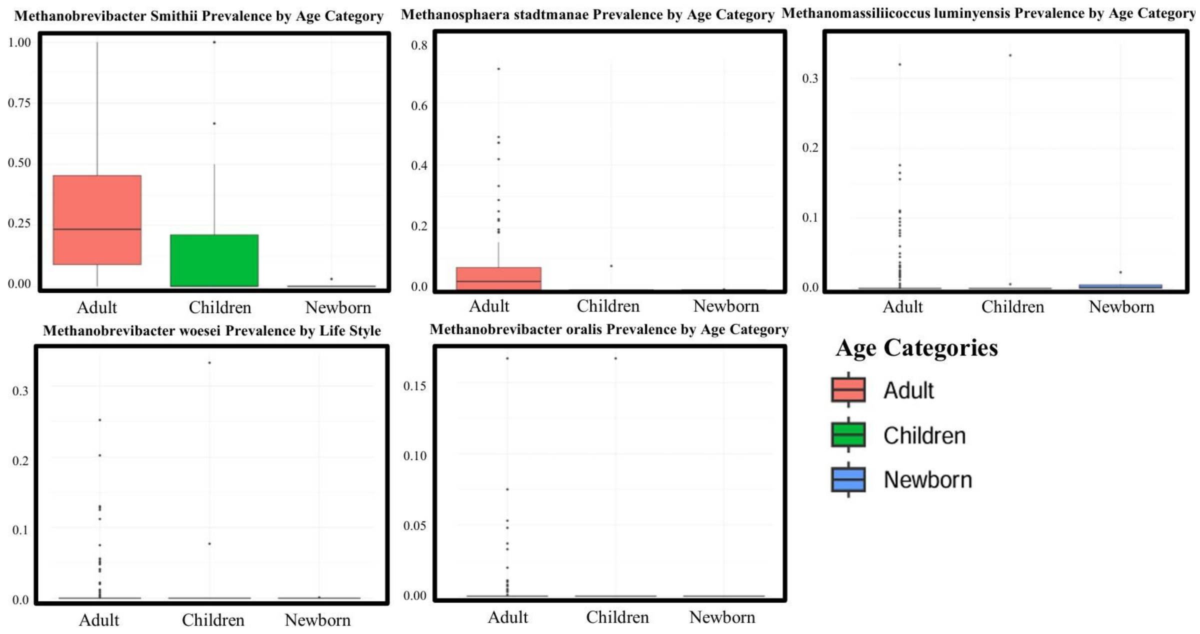


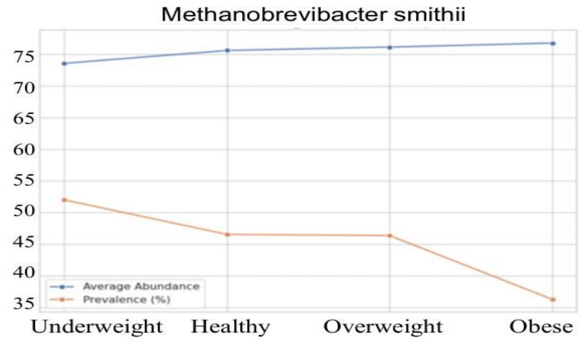
Figure 4.3.3: Methanogens (Methanobrevibacter smithii, Methanosphaera stadtmanae, Candidatus Methanobrevibacter oralis, Methanomassiliicoccus luminyensis, Methanobrevibacter woesei) prevalence across Age Categories.

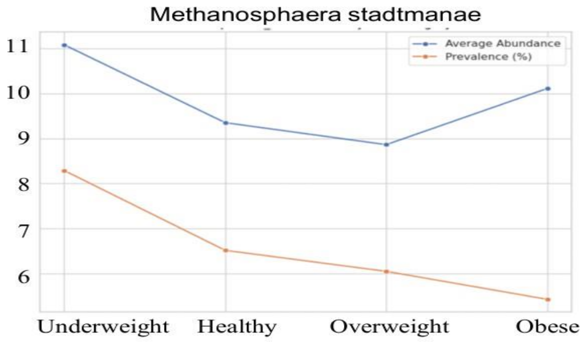
4.3.4 BMI based analysis of Methanogens:

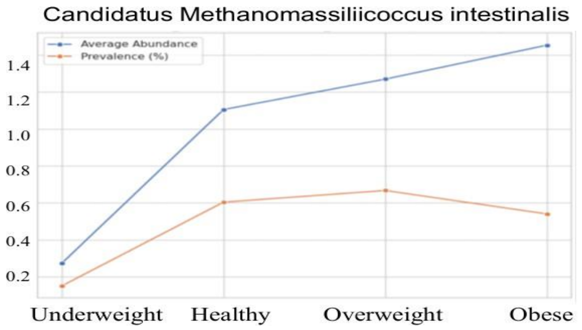
Body Mass Index (BMI) and the gut methanogen community are linked by the types of methanogens present in the gut. The most consistent pattern observed across the majority of methanogen species is inverse association with BMI.

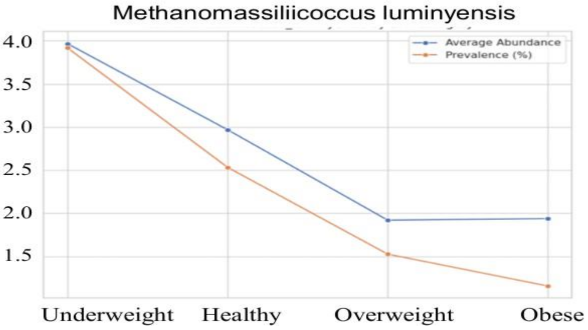
For methanogens like *Methanosphaera stadtmanae* and *Methanomassiliicoccus luminyensis*, both their prevalence and their average abundance decreases as BMI increases from underweight to obese.

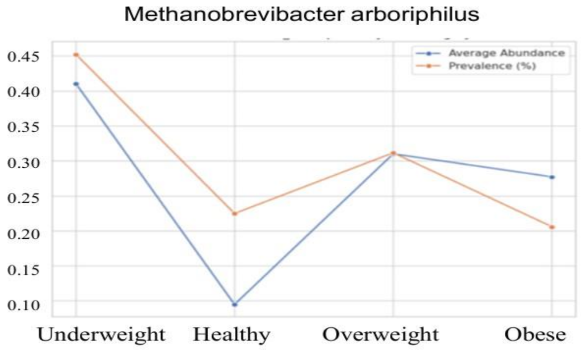
While in contrast to this trend, *Candidatus Methanomassiliicoccus intestinalis* increases with the increase in BMI depicting that it becomes more common in heavier people and may aid in obesity. *Figure 4.3.4* shows the variation of methanogens prevalence and abundance with increasing BMI.

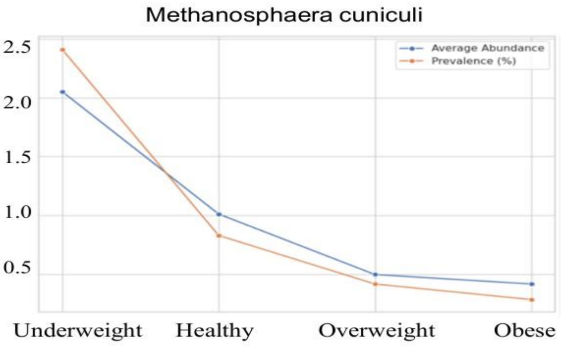

Methanobrevibacter smithii

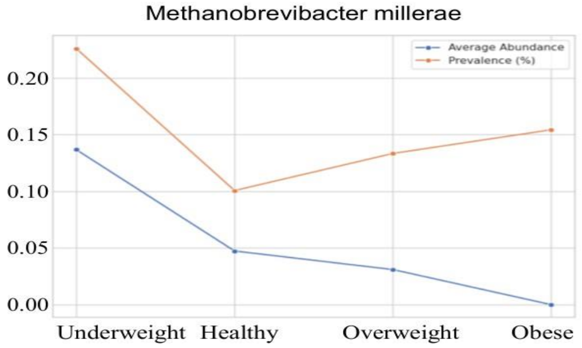

Methanosphaera stadtmanae


Candidatus Methanomassiliicoccus intestinalis


Methanomassiliicoccus luminyensis


Methanobrevibacter arboriphilus


Methanosphaera cuniculi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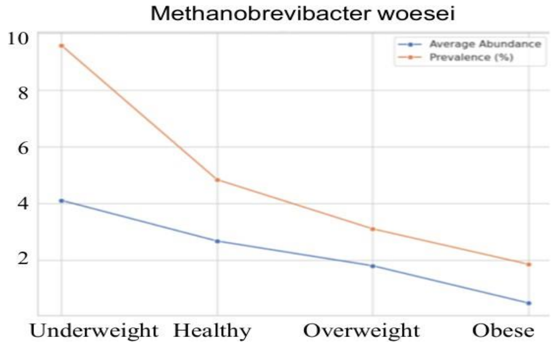
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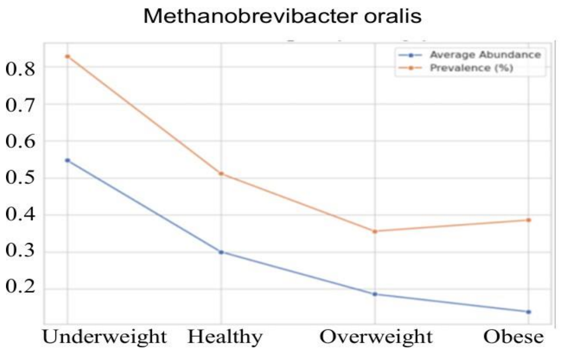

Methanobrevibacter oralis

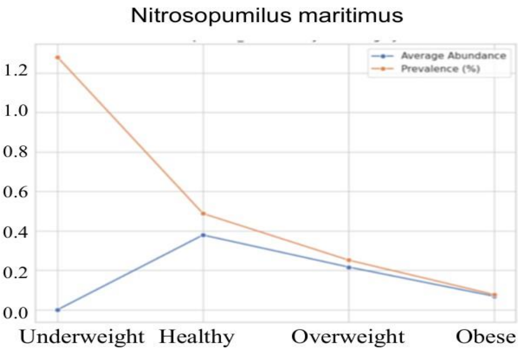
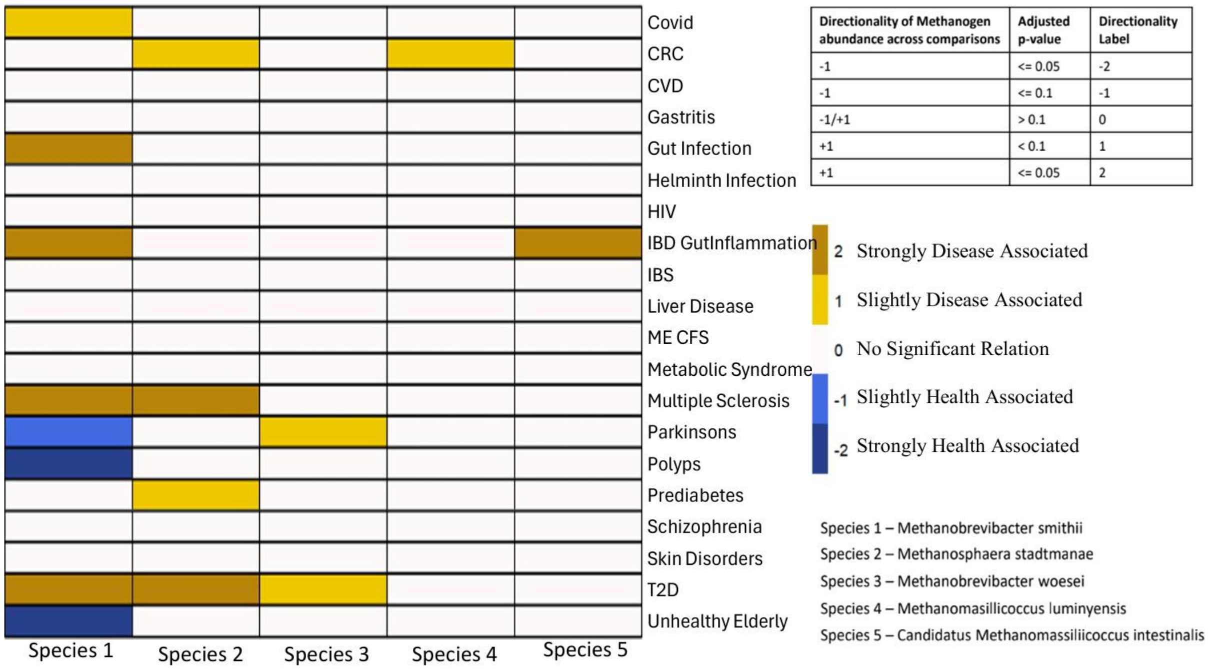

Nitrosopumilus maritimus

Figure 4.3.4: Methanogens (Methanobrevibacter smithii, Methanosphaera stadtmanae, Candidatus Methanomassiliicoccus intestinalis, Methanomassiliicoccus luminyensis, Methanobrevibacter arboriphilus, Methanosphaera cuniculi, Methanobrevibacter woesei, Methanobrevibacter millerae, Methanobrevibacter oralis, Nitrosopumilus maritimus) prevalence across BMI.

4.4. Methanogen Alteration in Different Diseases:

Methanogens have highly specific and complex roles in human health and disease, rather than being beneficial and harmful. Results of analysis are shown in *figure 4.4*. The dominant species like Methanobrevibacter smithii acts as "double edged sword". It is strongly associated with diseases like Type 2 diabetes, IBD and gut Infection, yet it is also linked to better health in the elderly and Polyps disease. Other methanogens appear to be unique biomarkers for their respective specific conditions. Parkinson's disease shows a signature of lower *Methanobrevibacter smithii* but higher in *Methanobrevibacter woesei. Methanomassiliicoccus luminyensis* and *Candidatus Methanomasillicoccus intestinalis* show slight role in CRC and IBD Gut inflammation respectively, suggesting a more neutral role overall.



Directionality of Methanogen abundance across comparisons	Adjusted p-value	Directionality Label
-1	<= 0.05	-2
-1	<= 0.1	-1
-1/+1	> 0.1	0
+1	< 0.1	1
+1	<= 0.05	2

Species 1 – Methanobrevibacter smithii
Species 2 – Methanosphaera stadtmanae
Species 3 – Methanobrevibacter woesei
Species 4 – Methanomassillicoccus luminyensis
Species 5 – Candidatus Methanomassiliicoccus intestinalis

Figure 4.4: Methanogen alteration in different diseases.

4.5. Association of Methanogens with non-methanogens:

The relation between methanogens and non-methanogens is not random as visible in the *figure 4.5*, two very distinct patterns can be observed in red and blue.

The prominent block of red pattern indicates that methanogen, namely *Candidatus Methanomassillicoccus intestinalis* is highly negatively associated with a large group of bacterias while *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* are negatively associated with some species, namely, Bifidobacterium longum, *Clostridium ramosum*, *Clostridium innocuum*, etc.

In contrast, if we look at the rest of the heatmap the blue color is dominating. This group is led by the methanogens from the Methanobrevibacter genus. These species show widespread positive association with other Non-methanogens. While the blocks in white shows no significant association.

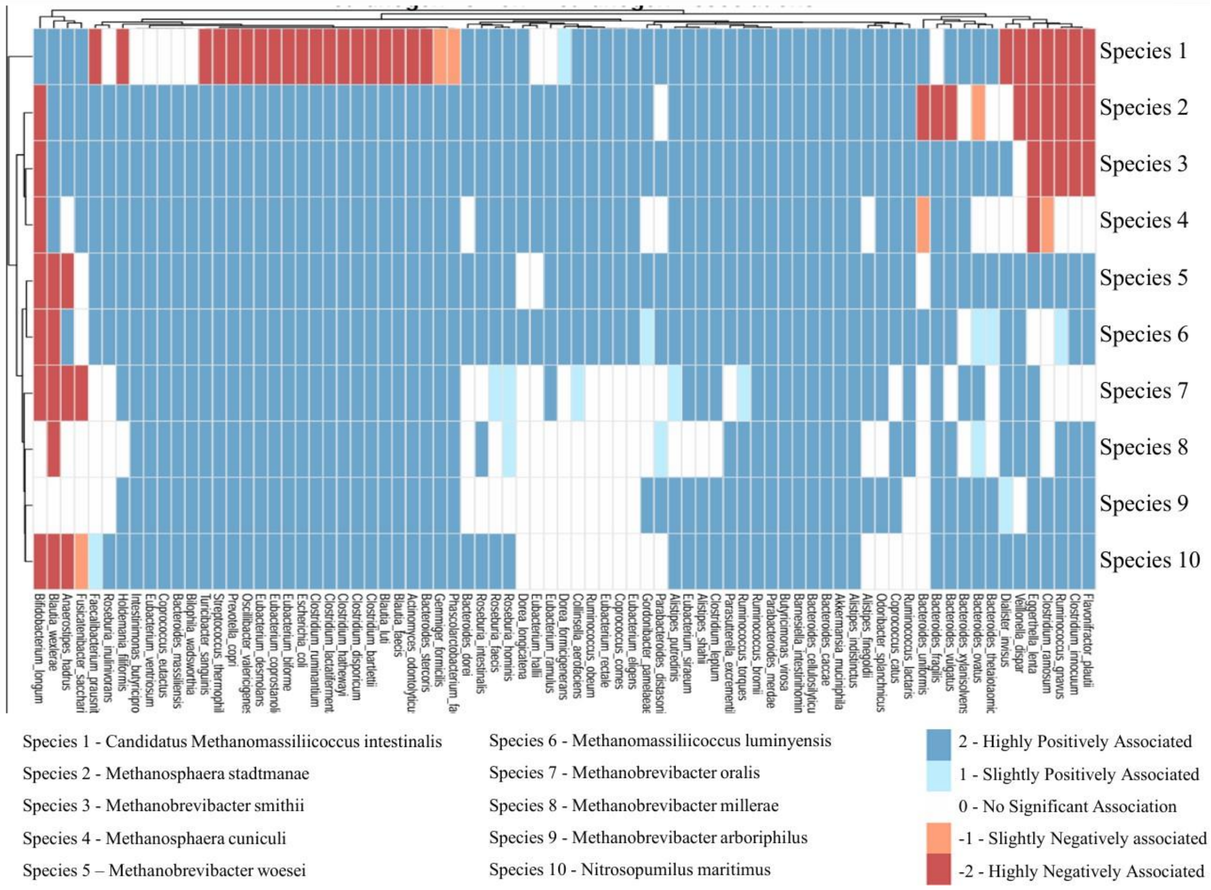


Figure 4.5: Association of Methanogens with Non- Methanogens.

CHAPTER 5

CONCLUSION AND FUTURE SCOPE:

This work brings together data from a large number of countries and provides an overview of how methanogens vary across different populations and conditions. The study shows that methanogen distribution is not uniform worldwide but is strongly shaped by geography and lifestyle. Higher prevalence was observed in non-industrialized regions such as Africa and South America, while industrialized countries showed comparatively lower levels, highlighting the influence of diet and environment. Age was also found to be an important factor, with methanogen levels rising steadily from infancy through adulthood, indicating a clear developmental pattern. Body Mass Index (BMI) further shaped the distribution, as most methanogens declined with higher BMI, except for *Candidatus Methanomassiliicoccus intestinalis*, which showed the opposite trend and may be linked to obesity. The findings also suggest that methanogens play complex roles in human health. For example, *Methanobrevibacter smithii* emerged as the dominant species, acting both as a risk factor in some diseases (like Type 2 diabetes and IBD) and as a beneficial factor in others. Other methanogens were linked to specific conditions and may serve as biomarkers. Associations with non-methanogenic bacteria also revealed patterns of cooperation and competition within the gut ecosystem.

Further work is needed to explain the mechanisms behind these associations. Comparative studies across populations undergoing rapid lifestyle changes could provide new insights into how industrialization impacts methanogen prevalence. Advanced approaches such as metagenomics and metabolomics should be applied to uncover functional pathways and host interactions. Finally, exploring ways to regulate methanogen populations may open new possibilities for microbiome-based therapies and personalized health interventions.

CHAPTER 6

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