A Longitudinal study to gain insights into the Gut Bacterial Abundances and Associated Diet Practices of important ethnic tribes of West Bengal

SUBMITTED BY
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REGISTRATION NO.
Ph.D./21/MCB/03

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THESIS SUBMITTED FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY (SCIENCE)
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AFFILIATED TO THE UNIVERSITY OF CALCUTTA

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“Quite literally, your gut is the epicenter of your mental and physical health.

If you want better immunity, efficient digestion, improved clarity and balance,

Focus on rebuilding your gut health”

- Kris Carr

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ABBREVIATIONS

BMI	Body Mass Index
CF	Conversion Factor
DD	Dietary Diversification
EER	Estimated Energy Requirement
EI	Energy Intake
EER	Estimated Energy Requirements
ER	Energy Requirement
FANTA	Food and Nutrition Technical Assistance III Project
FFM	Fat-Free Mass
FFMI	Fat-Free Mass Index
FFQ	Food Frequency Questionnaires
FMI	Fat Mass Index
HWR	Height Weight Ratio
ICMR	Indian Council of Medical Research
MUAC	Mid-Upper Arm Circumference
NFHS	National Family Health Survey
NIN	National Institute of Nutrition
NNMB	National Nutrition Monitoring Bureau
PAL	Physical Activity Level
PBF	Percent Body Fat
PDS	Public Distribution System
RDA	Recommended Dietary Allowances
WHO	World Health Organization
AR	Antibiotic resistance
AMR	Antimicrobial resistance
ARGs	Antibiotic resistance genes
HGT	Horizontal gene transfer
ARB	Antibiotic-resistant bacteria
MDR	Multi-drug resistant
XDR	Extensively drug resistant
pH	Negative of log of hydrogen ion concentration

CFUs	Colony-forming units
NGS	Next-generation Sequencing
EUCAST	European Committee on Antimicrobial Susceptibility Testing
CLSI	Clinical and Laboratory Standards Institute
PCR	Polymerase chain reaction
°C	Degree Celsius
%	Percentage
µg	Microgram
µl	Microliter
µM	Micromole
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
dNTP	Deoxynucleotide triphosphate
g	Gram
mg	Milligram
ml	Milliliter
pM	Picomole
bp	Base pairs
OTUs	Operational Taxonomic Units
rpm	Rotation per minute
TAE	Tris –Acetate-EDTA
NTFPs	Non- Timber Forest Produces
GWAS	Genome Wide Association Study

Abstract:

Tribals of West Bengal are divided into geographically separate regions with distinctive ecological characteristics, and many of them still maintain a traditional lifestyle that excludes the consumption of western foods. Therefore, it can be anticipated that they should possess a pristine gut. Globalization has resulted in considerable growth in the metropolitan sprawl of various cities as the world becomes increasingly urban. Dietary patterns and lifestyle choices have changed dramatically as a result of this spread across individuals. Till date, no study has reported on the gut microbiome of the West Bengal tribal population despite the fact that there are 40 identified tribes in the state comprising 5.8 percent of the total population of the state as per 2011 Census.

Nomadic tribe Sabar currently stationed near Jharkhand, Purulia side; Foothill Mech tribe of Alipurduar; Hilly Bhutia tribe of Lepchakha. Along with these three tribal families we have also recruited an urban family as a control for this longitudinal study over a period of three years. Each family consisted of a male, female and kid member (essentially a male and would not attain puberty during the period of our study). Anthropometric measures such as BMI was evaluated to understand the nutritional status of the subjects. In order to evaluate the gut bacterial diversity, 16S rRNA from first fecal matter was subjected to Illumina Miseq™ sequencing. Quality control (FASTQC), taxonomic (QIIME2) and functional profiling was performed for metagenomic analysis.

Our study involving the Bhutia, Mech and Sabar tribes indicate that association of *Prevotella*, *Bifidobacterium*, *Akkermansia*, *Holdemanella* form a core consortium, which allows consistency in their subsistence to find the alterations in gut bacterial assemblages and finally would been able to map the gut microbial consortia among the tribals and the

urbanized population based on their subsistence pattern. From the urban subjects selected as control, we were able to identify significant increase of genera *Metanobrevibacter*, *Faecalibacterium*, *Alcaligenes*, *Romboutsia* etc. can be correlated with their lifestyle and dietary patterns.

Non-Timber Forest Produces (NTFPs) were also used as a part of their regular diet, and get an insight into their probable utility as drug-like molecules which can be used to address a number of diseases that have become a subject of major concern in recent times. The compounds identified showed satisfactory bioavailability and drug-likeness score we were able to identify functional implications on key disease associated targets using suitable probability indices in the human body involved in various important metabolic pathways and disease regulation.

Our aim was also to be able to explain the disease risk estimation obtained from genome variant analyses performed on the subject candidates and draw a relationship between the lack of phenotypic expression of the diseases even though genetic variants were observed. These observations may be further explored in targeted nutrigenomics studies to evaluate the impact of the dietary components as a preventive arsenal for many diseases. Finally, this study will enable us to create a roadmap for evaluating the interplay between dietary practice and indigenous lifestyle, gut bacteriome structure and function among the Sabar, Bhutia, Mech tribes along with the urban population of West Bengal.

Keywords: *Ethnic Tribes, Pristine Gut, Gut Bacteriome, Anthropometry, Diet, Metagenomics. Microbiological Profiling, GC-MS.*

1. Introduction:

1.1 Overview of Human Gut Microbiota:

The gut microbiota, a vast and dynamic population of trillions of microorganisms in the human gut, is essential to human health and well-being (Afzaal et al., 2022). This diverse population resides in your gut and influences digestion, absorption of nutrients, functions related to the immune system, and even mental health. It is primarily composed of bacteria but also includes fungus, viruses, and archaea (Ortega et al., 2022).

Complex proteins, carbohydrates and dietary fibres that we are unable to digest are broken down by the microbes in our gut. These complicated compounds are converted into advantageous short-chain fatty acids (SCFAs) that maintain gut cell function and enhance general well-being through a procedure known as fermentation (Takihara and Okuda, 2023). Additionally, they are essential for the absorption of critical vitamins and minerals from our diet (Suriano et al., 2023).

Through complex interactions, the gut microbiome has a major effect on how our immune system develops and functions. A balanced gut microbiota protects us against infections and inflammatory diseases and supports a healthy immune response (Earley et al., 2023). For example, it has been demonstrated that segmented filamentous bacteria (SFB) stimulate the growth of T regulatory cells, which are essential for immunological tolerance (Taylor et al., 2020).

Furthermore, the bacteria in our stomach influence how the body uses the energy from meals, which has an impact on blood sugar balance and weight management (Chen and Yan, 2021). According to recent studies, there may be a two-way communication channel between the gut and the brain, and mood regulation and mental health may be influenced

by the gut microbiome (Wu et al., 2011). The gut microbiota has a crucial role in controlling the hypothalamic-pituitary-adrenal (HPA) axis, which is linked to the stress response, as reported in the literature (Sarkar et al., 2020).

The gut microbial constitution is constantly changing for a variety of reasons, with nutrition having the most influence. While a diet high in fat and sugar can upset the balance, a diet high in fibre encourages the growth and activity of good bacteria (Wei et al., 2023). Geographical location, age, genetic composition, prescribed drugs, and environmental conditions are other factors that impact the gut microbiota (Tawfick et al., 2022).

A variety of health problems have been linked to dysbiosis, which is an imbalance in the microbial composition of the gut. This includes autoimmune disorders, neurological illnesses, obesity, diabetes, irritable bowel syndrome, and inflammatory bowel disease (IBD) (Mousa et al., 2022). Personalized Dietary practices are one of the key aspects for promoting the overall well-being of the gut health (Clemente-Suárez et al., 2024).

A multifaceted community of microflora colonizing the human alimentary tract are known for their significant impacts the systemic metabolism in hosts (Finegold et al., 1983). The highly diverse gut microbiota fosters a robust intestinal ecosystem which enables the host to have an adaptable ecosystem, which helps in minimizing the effects of dietary and cultural variations. An adequate layout of the core bacteriome of a specific population can help in the understanding of essential bacterial functioning (Kulkarni et al., 2019). The diversification and composition of resident microbes are demonstrably influenced by a multitude of factors. These factors encompass an individual's age, sex, dietary patterns, genetic makeup, geographic location, and overall health status (Senghor

et al., 2018). Since the gut microbiome has stronger correlations with food preferences than the classic nutritional account usually applied to nutrition studies, changing one's diet can have a profound influence on it. It can be pivotal to understand the importance of diet at the outset of evaluating nutritional status in determining the health status of an individual (Johnson et al., 2019).

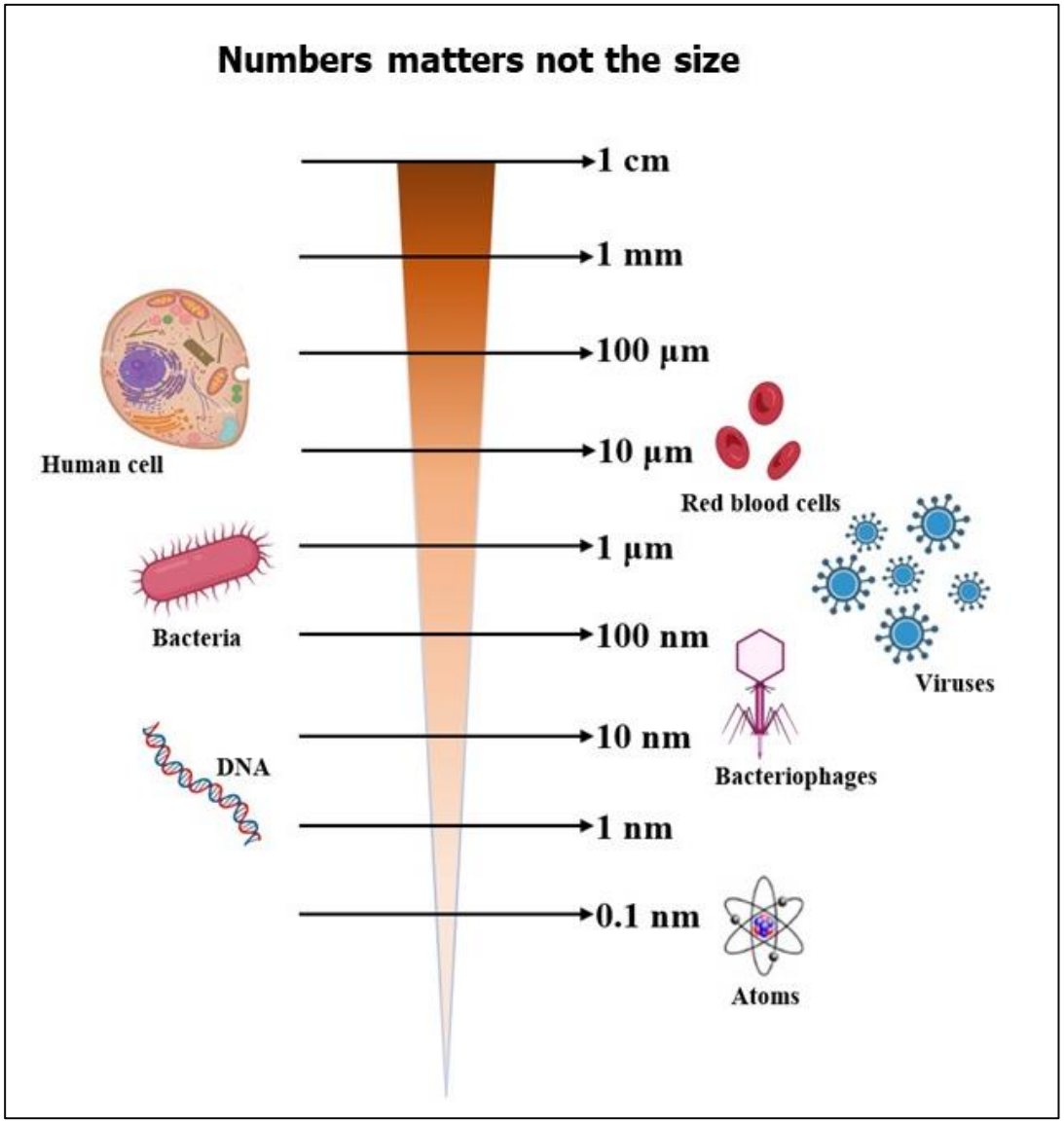


Figure 1.1: Major host cell sizes and their various components in relation to bacteria and viruses (www.biorender.com; Agreement No: WP26T40EKL)

1.2 The Gut Microbiome: A Thriving Ecosystem Shaping Human Health

Residing within our gastrointestinal tract lies a vast and complex ecosystem teeming with trillions of microorganisms – the gut microbiota. This diverse community, primarily composed of bacteria but also encompassing archaea, fungi, and viruses, plays a critical role in human health and well-being (Aggarwal et al., 2023). Far from being passive residents, these microbial partners engage in a symbiotic relationship with their host, influencing numerous physiological processes. Promoting gut health and halting the onset of numerous chronic diseases require an understanding of the complex functions of the gut microbiome (Vijay and Valdes, 2022).

1.2.1 ***Digestive Powerhouse and Nutrient Absorption:*** An essential companion in the digestion process is the gut flora. The enzymes required to break down the complex carbohydrates, proteins, and dietary fibres found in food are absent from human systems. This is when the gut microbiota intervenes, breaking down these intricate compounds through a process known as fermentation (Zhang, 2022). In addition to providing our bodies with essential nutrients, this fermentation generates the good short-chain fatty acids (SCFAs) butyrate, propionate, and acetate (Lange et al., 2023). SCFAs support a healthy gut barrier, feed intestinal cells, and enhance overall health. Furthermore, the absorption of vital vitamins and minerals from our diet, including iron, calcium, and vitamin B12, is greatly aided by gut flora (Rowland et al., 2018). Research has highlighted the importance gut bacteria play in nutrient absorption by showing how they may specifically use complex glycans from plant sources, such as *Bifidobacteria* (Krajmalnik-Brown et al., 2012).

1.2.2. ***Changing Immune Response and Guarding Against Infections:*** The immune system is greatly influenced by the gut microbiota, which shapes the system's growth and operation throughout life (Belkaid and Hand, 2014). Our immune system is strengthened by a well-balanced gut microbiome, which shields us against dangerous infections and inflammatory conditions. An essential barrier separating our internal environment from the outside world is the lining of our stomach (Wu and Wu, 2012).

By encouraging the creation of mucus and fortifying the tight junctions between intestinal cells, gut bacteria support the integrity of this barrier (Gieryńska et al., 2022). Furthermore, certain gut microorganisms induce the immune system to create regulatory T cells, which are specialised cells that are essential for immune tolerance maintenance and the avoidance of immunological overreactions (Pearson et al., 2017). In contrast, a number of immune-associated conditions, including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), have been attributed to dysbiosis, an imbalance in the makeup of the gut microbiota (Quaglio et al., 2022). Atarashi et al. (2015) conducted research that highlights the impact of gut microbiome on immune function by demonstrating how segmented filamentous bacteria (SFB) stimulate the growth of T regulatory cells (Atarashi et al., 2015; Tang and Hazen, 2024).

1.2.3 ***Metabolic Regulation and Mental Health:*** Beyond immune response and digestion, the gut microbiota affects metabolism and may even have an impact on mental health. Blood sugar regulation and weight management are impacted by gut bacteria's role in the food's ability to absorb energy (Chen et al., 2024). The intricate relationship between gut microorganisms and human metabolic processes has been highlighted by studies, which investigated how gut bacteria can affect energy metabolism via altering bile acid metabolism (Canfora et al., 2017). A bidirectional communication link between

the stomach and the brain is suggested by emerging studies, and mood and mental health may be influenced by the gut microbiota (Tawfick et al., 2022). Numerous neurotransmitters, including dopamine and serotonin, are produced by the gut microbiota and are involved in mood regulation (Chen et al., 2021).

The possible connection between the brain and the gut offers intriguing potential for understanding and treating mental health issues, even if the research being conducted in this domain is still in its infancy. The significance of preserving a healthy gut ecology is highlighted by this complex association between the human host and the gut microbes (Ahn and Hayes, 2021). Dietary alterations, lifestyle decisions and perhaps targeted therapies would cultivate a diverse and balanced gut microbiome to unleash the full potential of the amazing community of microorganisms and advancing general wellness and health (Conlon and Bird, 2014).

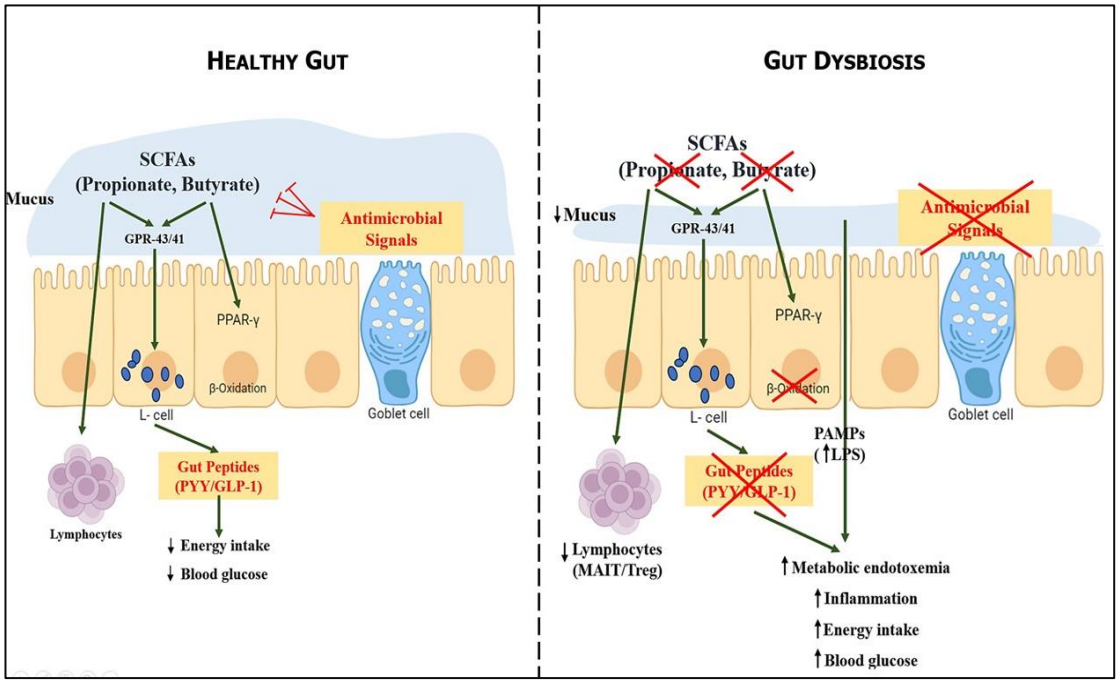


Figure 1.2: Impact of metabolism driving the crosstalk between microorganisms and host (www.biorender.com; Agreement No: IV26UFV9YZ)

1.3 Composition of the Gut Microbiome

1.3.1 Bacterial Diversity:

The compositional bulk of bacteria in the human gut identify with phyla Firmicutes and Bacteroidetes. Other notable phyla include Proteobacteria, Actinobacteria and Verrucomicrobia. These phyla contain thousands of species, and individual species may differ significantly from one another (Thursby and Juge, 2017).

Firmicutes: This group include *Enterococcus*, *Lactobacillus*, and *Clostridium*. They take part in the fermentation process that produces the vital short-chain fatty acids (SCFAs) that gut health depends on (Magne et al., 2020).

Bacteroidetes: This phylum consists of genera such as *Prevotella* and *Bacteroides*. They are necessary for the breakdown of complex carbohydrates and the metabolism of proteins (Fagunwa et al., 2024).

Actinobacteria: This phylum includes *Bifidobacterium*, which is necessary for maintaining gut health and has probiotic properties (Parkin et al., 2024).

Proteobacteria: This phylum contains many dangerous bacteria, including *Escherichia coli*, despite being less widespread (Choi et al., 2024).

Verrucomicrobia: This phylum includes *Akkermansia*, a bacterium that has been connected to the maintenance of the gut barrier's integrity as well as anti-inflammatory qualities (Tilg and Moschen, 2024)

***The Eukaryotic Microbes and Archaea*:** In addition to bacteria, the gut microbiome also includes archaea, which are primarily methanogens that reduce the production of intestinal gas, such as *Methanobrevibacter* (Volmer et al., 2023). Eukaryotic microbes,

such fungi like *Candida*, are less common yet have the ability to impact immune system performance and metabolic processes, both of which can impact health (Jensen et al., 2024).

***Virome*:** The viruses (eukaryotic viruses) and bacteria (bacteriophages) that infect human cells make up the gut virome. Bacteriophages are especially common and play a crucial role in regulating bacterial populations and horizontal gene transfer, which ultimately impacts the gut (Emencheta et al., 2023).

1.3.2 Functions of the Enteric Microbiome

***Digestive Functions*:** Food ingredients that are indigestible to human enzymes must be broken down and fermented by the gut flora. This comprises fibers, some proteins, and complex carbs. SCFAs, such as acetate, propionate, and butyrate, are produced during fermentation and are beneficial to systemic health in addition to providing colonocytes with energy (Baky et al., 2024).

***Immune System Modulation*:** The immune system's development and modulation are immensely influenced by the gut microbiota. It supports immune cell development, the synthesis of peptides with antimicrobial properties and the preservation of the intestinal barrier. Immunological tolerance is enhanced by a healthy microbiome, whereas inflammation and an accelerated immunological response are potential consequences of dysbiosis (Han et al., 2024).

***Metabolic Functions*:** Bile acids, cholesterol, and selected vitamins (including vitamins B and K) are all metabolised by the gut bacteria. They also play a role in drug metabolism and xenobiotic detoxification, which affects the toxicity and effectiveness of pharmaceuticals (Shang et al., 2024).

***Protection Against Pathogens*:** Through the generation of antimicrobial compounds, competitive exclusion, and regulation of the gut environment (e.g., pH and oxygen levels), the gut microbiome functions as a barrier against harmful microbes (Fachi et al., 2024).

1.3.3 Factors Influencing the Gut Microbiome

***Genetics*:** The gut microbiome's makeup and functionality are influenced by host genetics. Genetics can influence the abundance of specific bacterial taxa and the synthesis of metabolites such as SCFAs (Maritan et al., 2024).

***Environment and Lifestyle*:** Environmental factors that have a substantial impact on the gut microbiota include geographic location, sanitation, and antibiotic use. Specifically, antibiotics have the ability to permanently modify microbial communities, resulting in decreased diversity and possibly dysbiosis (Dai et al., 2024).

***Age*:** Throughout the lifespan of an individual, the gut microbiota is subjected to dynamic changes. It begins to develop during delivery of the child and is subjected to modification by feeding practices (formula feeding vs. breastfeeding) and delivery methods (vaginal vs. caesarean section). When it reaches adulthood, it stabilises somewhat, but as it ages, its diversity and composition would fluctuate (Bosco and Noti, 2021).

***Diet*:** Considered amongst the most important factors influencing the gut microbiota, higher proportion of fat and protein diet can boost the abundance of *Bacteroides* and certain *Proteobacteria*, while higher fiber diet encourages growth of host-beneficial bacteria like *Lactobacillus* and *Bifidobacterium*. The microbiome can also be modulated by probiotics, which are live beneficial bacteria, and prebiotics, which are indigestible food elements that promote the growth of helpful bacteria (Fagunwa et al., 2024).

1.3.4 Health Implications of the Gut Microbiome

***Gastrointestinal Diseases*:** Dysbiosis has been found to be linked to a variety of gastrointestinal conditions, including colorectal cancer, irritable bowel syndrome, and inflammatory bowel disease (IBD). For instance, the gut microbiota composition of IBD patients is frequently altered, with a decrease in helpful bacteria and an increase in pro-inflammatory bacteria (Quaglio et al., 2022).

***Metabolic Disorders*:** Metabolic disorders include obesity, Type II diabetes, and non-alcoholic fatty liver disease (NAFLD) are associated with a gut microbiome. Alterations in the gut microbiota can impact systemic inflammation, fat storage, and the ability to obtain energy from the diet. Compared to lean people, obese people frequently have a distinct gut microbiome composition, with a larger ratio of Firmicutes to Bacteroidetes (Leung et al., 2016).

***Immune-Mediated Diseases*:** The makeup of the gut microbiota can affect an individual's susceptibility to autoimmune and allergy disorders. Asthma, multiple sclerosis, and rheumatoid arthritis have all been connected to dysbiosis. The immune system's regulation and the intestinal barrier's integrity are the mechanisms (Kong et al., 2024).

***Neuropsychiatric Disorders*:** The gut microbiome may impact mental health via the gut-brain axis, a communication pathway with bidirectional nature between the central nervous system and the gut microbiota. Autism spectrum diseases, anxiety, and depression have all been linked to dysbiosis. Potential processes include the generation of neuroactive chemicals by gut bacteria and the control of systemic inflammation (Cenit et al., 2017).

1.3.5 Therapeutic Interventions:

***Probiotics and Prebiotics*:** When taken in sufficient quantities, living bacteria known as probiotics can have positive effects on health. They can support better gut health and the restoration of the gut microbiome's equilibrium. Prebiotics are inert food components that specifically promote growth of advantageous bacteria. Probiotics and prebiotics have both demonstrated potential in the treatment of digestive disorders, allergies, and diarrhoea (Sanlier and Kocabas, 2023).

***Fecal Microbiota Transplantation (FMT)*:** In order to rebuild a healthy microbiome, FMT entails transferring faecal matter from a healthy donor to a recipient. It is being investigated for various disorders like IBD and metabolic syndrome. It has proven to be quite successful in treating recurrent *Clostridium difficile* infection (Zhang et al., 2023).

***Diet and Lifestyle Modifications*:** The gut microbiota can be positively impacted by dietary treatments such as increasing fibre intake, consuming fewer processed foods, and including fermented foods in one's diet. Good stress management and regular exercise are examples of lifestyle variables that contribute to a healthy gut flora (Dai et al., 2024).

***Precision Medicine Approaches*:** Metagenomics and metabolomics advances are opening up new avenues for precision medicine strategies aimed at modifying the gut microbiome. Tailored therapies predicated on an individual's metabolic profile and microbiome composition have the potential to improve outcomes in a number of diseases (Mousa et al., 2023).

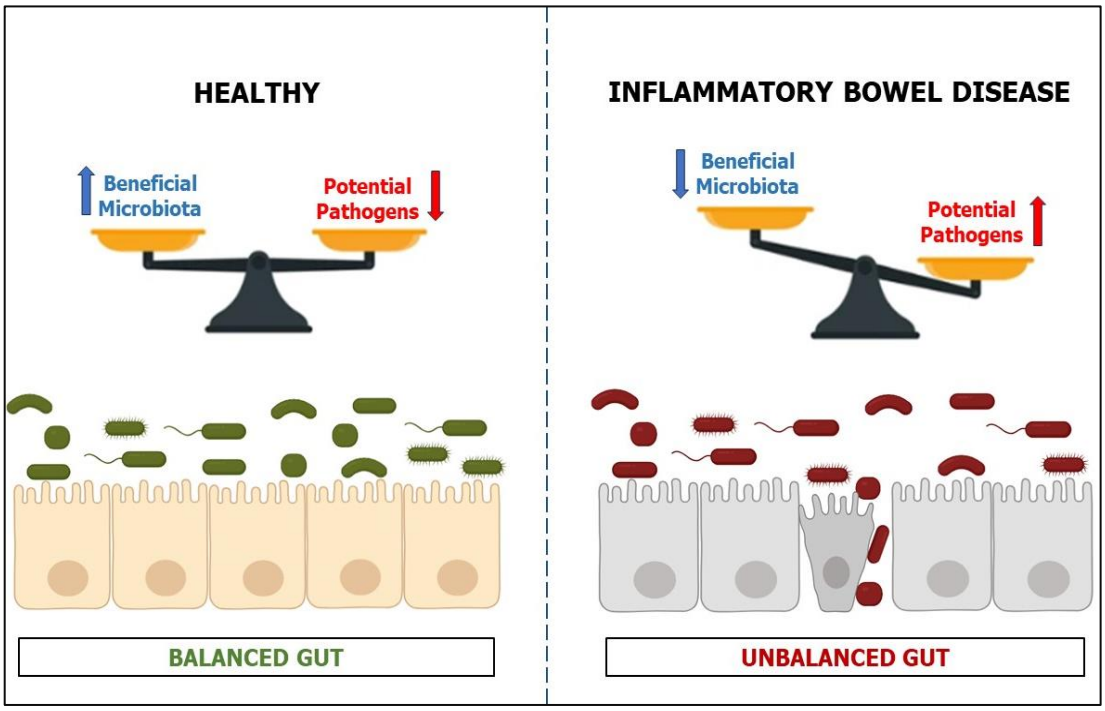


Figure 1.3: Disturbance of gut microbiota between patients with inflammatory bowel disease and healthy individuals. An upward arrow denotes improvement, whereas a downward arrow denotes degradation (www.biorender.com; Agreement No: BQ26T473R2)

1.4 Why Ethnic Tribes are important?

Among 38 different tribal groups in West Bengal, nomadic Sabars are the tenth-largest tribal group with their habitats in the districts like Purulia, Jhargram, Medinipur, Bankura and their adjacent villages (Gorrain et al., 2024). Throughout history, they have typically dwelled within forested regions. However, as a consequence of deforestation, environmental degradation, and the enforcement of the Forest Protection Act, they are undergoing displacement and relocation to areas adjacent to the jungles (Das et al., 2020).

Among the four major ethnic groups of India, the tribal groups with Mongolian descent are receiving significant attention due to their genetic similarity of 20% across the global population (Pederson et al., 2014). Due to the vast array of environmental conditions and ethno-geographical cohorts, Mongolian population has garnered significant research interest (Liu et al., 2016).

The Mongolians evolved their distinctive culture and dietary pattern over centuries. This, in turn, rendered the Mongol Empire one of the most geographically continuous empires in history (Zhang et al., 2014). Nevertheless, there remains limited knowledge regarding the gut microbiota composition among tribes of Mongolian descent.

This particular study specifically targeted the Drukpa Bhutia, a Tibetan-speaking tribe residing in the Lepchakha region of Buxa, Alipurduar district, West Bengal, India. Notably, this Mongolian descendant community inhabits a mountainous environment characterized by geographically isolated settlements (Subba, 2008). They basically rely on available forest resources to sustain their livelihood (Marchang, 2018) and engage in agriculture on small-scale terrains (Nongbri, 1999).

Our data enlightens on the above, in context to the gut bacterial abundance from the foothill Mech tribes of Mongolian descent of North Kamakhyaguri in West Bengal. The tribe has relied on natural produce from nearby forests, which would aid to preserve the gut's pristine state far away from processed foods. Besides farming, they have also shown a keen interest towards small game hunting as part of alternative livelihood and socio-religious practice (Christian, 2018).

In this work we investigate and compare the gut bacterial profiles of separate Sabar, Bhutia and Mech families, each consisting of an adult male and female along with their male kid who is yet to attain puberty, using anthropometric, metagenomic and culture dependent approaches to correlate them with their dietary habits and lifestyle. We have also collected samples from an urban family consisting of an adult male, female and male kid who is yet to reach his puberty to map the gut bacterial assemblages along with the ethnic tribal subjects.

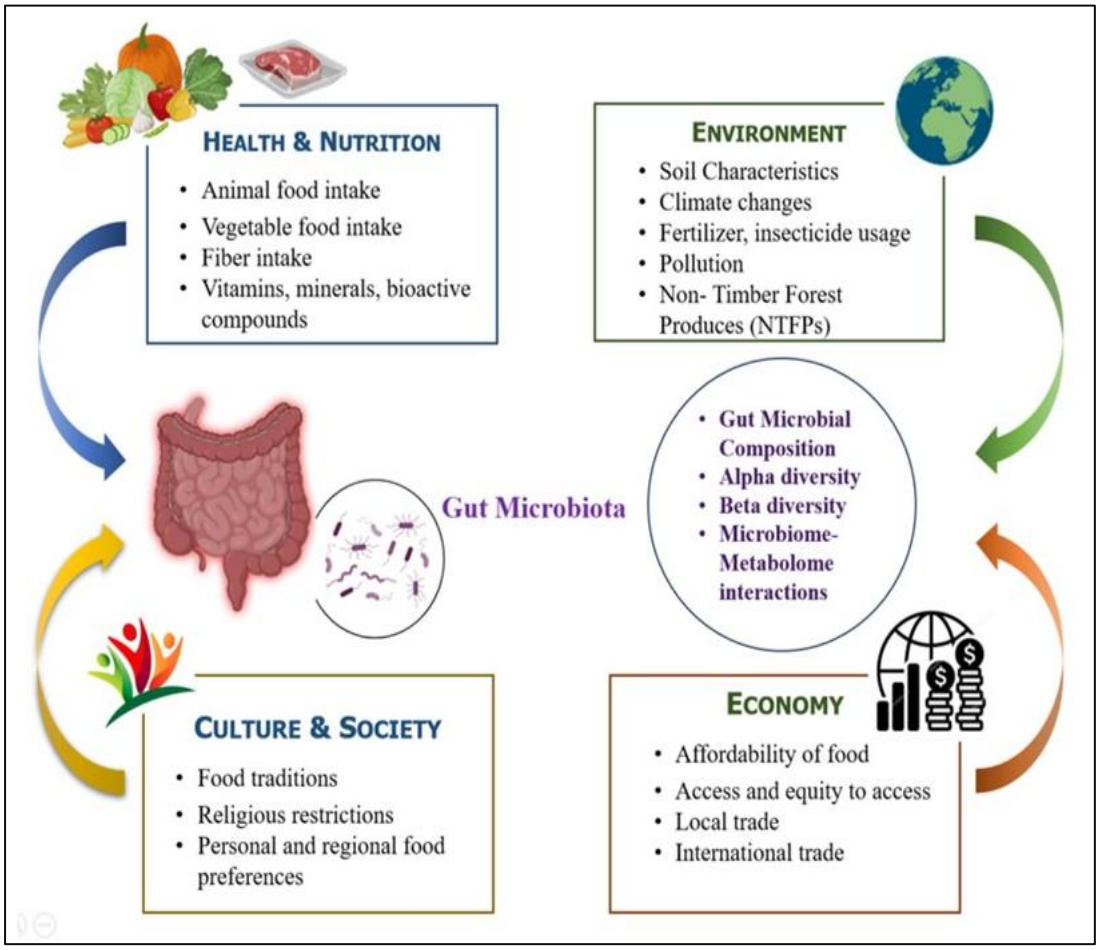


Figure 1.4: Components of a sustainable diet and how they affect gut microbiota

(www.biorender.com; Agreement No: WY26T47Q0V)

2. Review of Literature:

2.1. Global Scenario of Gut Microbiome Research:

Studies regularly demonstrate that, in comparison to urban populations, tribes have a more diversified gut microbiota (Patel et al., 2023). The gut microbiomes of American, Malawian, and Native American people were examined in a seminal study by Yatsunenko et al., 2012, who discovered noticeably greater variety in the tribal groups. This diversity is frequently associated with traditional diets that are low in processed foods and high in fiber (Yatsunenko et al., 2012).

Hunter-gatherer groups, like Tanzania's Hadza, offer important insights into the original state of the gut microbiota. According to a study by Smits et al., 2017, bacteria that can ferment complex plant polysaccharides predominate in the gut microbiome of Hadza people, which is consistent with their diet rich in wild plants and tubers (Smits et al., 2017). Analogous results were observed by Schnorr et al., 2014, who observed that the Hadza microbiome differed significantly from urban populations in that *Prevotella* was more prevalent and *Bifidobacterium* was almost non-existent. The composition of the gut microbiome is mostly influenced by diet. Diverse microbial communities thrive on tribal dietary habits, which are generally low in fats and carbohydrates and high in natural fibers (Schnorr et al., 2014). The significant influence of nutrition is highlighted by recent research, such as those conducted by De Filippo et al., 2017, which compared the gut microbiomes of children in rural Africa with those of their European counterparts. European children displayed larger amounts of bacterial reservoir linked to protein and fat metabolism while African children consuming traditional foods had a greater concentration of microorganisms that break down fiber (De Filippo et al., 2017).

Tribes engaged in agriculture, like those seen in rural South America, also have unique gut microbiota patterns (Skelly et al., 2018). Significant levels of microbial diversity increase and a predominance of bacteria were engaged in the fermentation of plant-based diets of the Matses and Yora tribes of Peru. This is in sharp contrast to communities in the West, where antibiotics and processed foods are frequently consumed (Greene et al., 2018).

The gut microbiota is profoundly impacted by modernization and the food and lifestyle modifications that follow. Traditional lifestyles are becoming more urbanised, which is causing a commensurate decline in the variety of the microbiome and a rise in dysbiosis-related disorders like obesity, diabetes, and inflammatory bowel disease (Wen and Duffy, 2017). Even partial integration into modern lives might result in a considerable decline in gut microbiome diversity for tribes undergoing fast modernization, like the Guarani in Brazil. The correlation between a decline in variety and a rise in the occurrence of illnesses in the west highlights the safeguarding function of a varied microbiome (Clemente et al., 2015).

There exists a close correlation between the gut microbiota composition and health outcomes in indigenous groups. Traditional-living tribes had lower rates of numerous chronic diseases prevalent in industrialised cultures. For example, the rise in autoimmune and allergy illnesses may be related to the decreased microbial diversity in Western societies (Blaser and Falkow, 2009).

The immune system's development and regulation are greatly influenced by the gut microbiota. Tribal cultures typically exhibit stronger immune responses due to their diverse and abundant microbiomes (Stražar et al., 2021). Early childhood exposure to a

wide range of microbes, typical of tribal settings, is crucial for the immune system's correct development and may guard against autoimmune and allergy illnesses (Dzidic et al., 2018).

Recent developments in sequencing technologies have made it possible to analyse the gut microbiota in greater detail. The functional potential and metabolic activities of the microbiome are thoroughly revealed by metagenomic and metabolomic methods. These technologies are used in several studies to compare the gut microbiomes of modern and traditional people, demonstrating notable functional changes that are correlated with lifestyle and diet (Pedroza Matute and Iyavoo, 2023; Satam et al., 2023).

Preservation of tribal populations' microbiological inheritance is becoming more and more important, as traditional ways of life are disappearing so quickly. Documenting and conserving these groups' gut microbiomes is essential for understanding the evolution of the human microbiome as well as for possible medical uses (Mollick and Maji, 2024). Researchers have brought attention to a recent initiative that highlights the significance of biobanking microbiome samples from isolated populations, before modernization causes irreparable alterations to them (Ryan et al., 2021; Annaratone et al., 2021).

Research on the gut microbiome of tribal people sheds light on the connection between microbial diversity, lifestyle, and nutrition. These populations provide insight into the possible health advantages of preserving a diversified gut microbiota due to their robust and diverse microbiomes (Hazarika et al., 2022; Aggarwal et al., 2023). It is critical to comprehend and protect the microbial consortia that contains secrets to human health and disease resilience as modernization continues to have an influence on these communities (Jacob et al., 2023; Kumar and Lakshminarayana, 2024).

The results of these investigations would be crucial in formulating mitigation plans for the detrimental effects of modernization on the variety of the gut microbiome and related health consequences (May, 2023).

2.2. National status of Gut Microbiome Research:

India has the world's second biggest population, dispersed over different geographical places, with immense variation in ethnicity, lifestyle, and food customs (Hullar and Fu, 2014; Lv et al., 2023). There have been limited inquiries into the richness and variation of the gut microbiome among individuals of diverse ethnicities, dietary regimens, and genders, as previously recorded (Chong et al., 2015; Arasu et al., 2023). Firmicutes represent the predominant phylum discovered in the healthy Indian gut, followed by Bacteroidetes, Actinobacteria, Proteobacteria, Spirochetes, Verrucomicrobia, and Fusobacteria (Das et al., 2018). The remote Indian tribal communities endure by preserving traditional lifestyles, dietary practices, and reliance on herbal medicine, largely shielded from the encroachments of modernity. Consequently, they present a distinctive opportunity for examining the human microbiome within an environment untainted by contemporary living, Previous research has unveiled that the gut microbial community of Indian ethnic groups is primarily constituted of genera including *Prevotella, Eubacterium, Faecalibacterium, Collinsella, Clostridium, Roseburia, Dialister, Ruminococcus, Blautia* and *Veillonella* (Bhute et al., 2017; Deb et al., 2020; Dehingia et al., 2015; Mollick and Maji, 2024; Kulkarni et al., 2019).

Tribal populations constitute approximately 8.6 percent of India's total populace. As per the 2011 census, India is home to a total of 104.3 million Scheduled Tribes, with 94.1 million residing in remote rural regions. According to recent trends, India's total tribal

population is anticipated to reach approximately 125 million by 2020, with about 112 million residing in rural areas. More than half of this tribal population inhabits forested regions (Chellasamy and Kannamudaiyar, 2022), where they derive sustenance for their way of life from both forest and land resources. Globalization has led to considerable growth in the metropolitan sprawl of various cities as theworld becomes increasingly urban. Dietary patterns and lifestyle preferences have dramatically changed as a consequence of this spread across individuals (Dehingia et al., 2015).

The stringent anaerobes outnumber the facultative anaerobes organisms that may grow both aerobically and anaerobically, and the aerobes by up to 100-fold in the gut microbial community. The association between the host and microbiome constitutes a crucial factor contributing to the overall health of the host (Yatsunenko et al., 2012). Several gastrointestinal illnesses, including inflammatory bowel disease (IBD) as well as other diseases like obesity, type 2 diabetes, and atopy, have been related to disruptions in the normal gut microbiota (Di Ciaula et al., 2023).

Scientific evidence suggests that gut microbiota and their byproducts influence brain function, blood-brain barrier permeability, and communication pathways between the GI tract and the brain, potentially modulating these systems (Li et al., 2023). Altering one's dietary habits can govern a noteworthy footprint on the constitution of the gut microflora since it is more intimately related to dietary preferences than the traditional nutritional standards commonly used in nutrition research (Smits et al., 2017). Thus, recognizing the role of diet at the onset of assessing nutritional status is pivotal for discerning an individual's health status. Communities upholding traditional livelihoods exhibit greater heterogeneity and divergent microbiome compositions as juxtaposed to the urban gut population (Hazarika and Babu, 2024). The tribal people, in most cases, don’t have access

to the daily dose of over the counter (OTC) medications that are readily available in the urban areas. The comprehensive understanding of how ethnomedicinal practices influence the preservation of the ethnic composition of gut bacteria remains an ongoing endeavor, alongside its potential correlation with their unique lifestyle. Therefore, the examination of tribal dietary habits and lifestyle serves as crucial conjectures in the exploration of the impact of the environment on gut bacterial profiles (GBP) and individual health status ((Li et al., 2023).

Indian tribes have unadulterated gut profile due to traditional diet and established ethnomedicinal practices. Primitive traits, geographical isolation, distinctive culture and economic and social backwardness are other unique characteristics. Observations have indicated a reliance on foraging and hunting for sustenance, characterized by a pre-agricultural technological era, minimal or negligible population growth, and notably low literacy rates. Practitioners of the traditional way of life exhibit a more diverse gut flora compared to urban populations (Li et al., 2023). The widespread adoption of globalized food patterns has facilitated the widespread abandonment of traditional dietary patterns. This dietary shift is in turn may contribute to a dysbiosis of the gut microbiome, potentially leading to a myriad of adverse health outcomes in human (Thursby and Juge, 2017).

Recent studies have begun shedding light on the gut microbiome of Indian tribal populations, revealing intriguing insights into their unique microbial compositions and potential health implications. Studies have underscored the impression of diet, lifestyle, and environmental concerns in shaping the gut microbiota within indigenous populations. For instance, research conducted by Hazarika et al., 2022 revealed discernible differences in microbial profiles between Indian tribal populations and urban residents, suggesting

that traditional dietary practices and ecological variables contribute to the diversity of the gut flora (Hazarika et al., 2022).

Furthermore, investigations by Patel et al., 2023 demonstrated variations in gut microbial composition among different tribal groups within India, emphasizing the significance of cultural and geographical factors (Patel et al., 2023). Moreover, investigations have delved into the possible correlations between gut microbiota and overall health of tribal populations, revealing connections between microbiome diversity and dysmetabolic conditions such as type 2 diabetes mellitus and adiposity (Vijayanna et al., 2024).

Additionally, newly emerging research has commenced unravelling the contribution of the gut microflora in mediating the properties of traditional medicinal practices among Indian tribal communities, offering valuable insights into novel therapeutic approaches (Basu et al., 2022).

Moreover, newly emerging research has commenced unravelling potential role of gut flora in mediating the effects of traditional medicinal traditions within Indian tribal communities, presenting valuable perspectives on innovative therapeutic pathways. However, gaps in understanding persist, necessitating further interdisciplinary research to elucidate the complex interplay between genetics, environment, and microbiota in tribal communities (Shreiner et al., 2015).

In summary, contemporary literature underscores the values of exploring the gut microbiome diversity in Indian tribal populations, revealing distinctive microbial signatures influenced by cultural, dietary, and environmental features. These findings not only deepen our understanding of human microbial ecology but also hold implications for public health interventions tailored to indigenous communities (Mashford-Pringle et

al., 2023).

2.3. Tribal Origin and Genealogy:

The term 'Sabar' is thought to have originated from the word 'sab,' meaning a deceased body, as they had ancient rituals associated with the dead (Singh, 2008). Alternatively, some studies suggest it might have been derived from *'sagar'*, signifying an axe, due to their practice of carrying axes for hunting since they used to live by hunting animals (Ghosh and Ghosh, 2000; Maiti, 2008).

They are frequently mentioned in Hindu mythology, ancient texts, *purans*, epics, and scriptures. In the Ramayana and Mahabharata epics, they are referred to as *Kirats or Vyadhas*, denoting hunters (Maiti, 2008). For instance, the Ramayana introduces the story of a Sabari, a Sabar tribe girl, who awaited Lord Rama for years and met him during his forest journey (Ghosh, 2013; Ghosh and Ghosh, 2000; Maiti, 2008). In the *Treta Yuga* (the age of Ramayana), Jara Sabar, an ancestor, is believed to have been Angada, the son of King Bali, who was the ruler of the *Vanaras* (monkey people) (Maiti, 2008). In the Mahabharata, the *Vyadha* who killed Lord Sri Krishna with a poisoned spear is said to have been a Sabar (Ghosh, 2013; Maiti, 2008). The Aitareya Brahmana Mahagrantha describes Sabar as the eldest son of Viswamitra (Baskey, 2006; Maiti, 2008; Pramanik, 2017). *Puranic* texts identify them as 'Dakshina Parthavasinha and Vidhyamaulika,' implying the indigenous people of the Vindhya Mountains (Maiti, 2008). According to Roy and Roy (1937), the Sabar people in West Bengal are considered a semi-nomadic community skilled in snake charming, often residing in regions with numerous venomous snakes. They are mentioned frequently in Bengali literature, like 'Manasa Mangal' (Maiti, 2008). In Odisha, the Sabar have strong connections with the worship of Lord Jagannath,

who is said to have originated as a tribal deity and was later incorporated into Puri's religious tradition (Baskey, 2006; Ghosh, 2013; Ghosh and Ghosh, 2000; Maiti, 2008; Ota and Mohanty, 2010). Even ancient Greek works by Ptolemy and Pliny mention the existence of this tribe in India, attesting to their historical significance (Maiti, 2008). In the Amar Khosh Mahagrantha, the Sabar people were described as *mlechhas* (untouchables) who sustained themselves through hunting (Maiti, 2008). This indicates that Sabar is among the most ancient in India, making it plausible for them to be featured in the earliest literary compositions. They share a racial connection with the Proto-Australoid group prevalent among the indigenous populations of Central and Southern India (Ota and Mohanty, 2010). Their presence extends across Central India, encompassing Odisha, Jharkhand, Madhya Pradesh, Andhra Pradesh, and West Bengal. Their language belongs to the Mundari group of the Austric language family, lacking a script (Ota and Mohanty, 2010; Upadhyay, 2008). Their language is a blend of Bengali and Hindi, mixed with their native tongue, and it varies across different villages. They are similar to Mundas in physical characteristics, with dark brown or nearly black skin colour, long heads, broad and flat noses with a slight depression at the root, wavy or curly hair, thick lips, slight prognathism and medium stature (Ota and Mohanty, 2010; Upadhyay, 2008). They mostly live in small groups of 15-20 houses on the outskirts of the villages and maintain a distance from neighbouring communities. Their houses are primarily rectangular in shape having stone or mud walls and the roof is generally covered with leaves or thatched with straw. There may be a single door as the main entrance with one or rarely two windows. But presently, many of them are provided with brick build houses under government schemes. Traditionally they are food gatherers and hunters. Their forest ecology is rich in various edible roots, tubers, fruits, leaves flowers, which

fulfil their need for food. But in recent years, they have been forced to settle outside the forest and lose uninterrupted access to the primary source of their livelihood. Nowadays, most of the Sabars earn their livelihoods by working as wage labourers in agricultural fields, construction sites of roads/buildings, mining sites, etc. (Das et al., 2021; Ghosh and Guchhait, 2017). Most of the Sabar families still practice forest wood collection and selling to support their survival. Studies reported that a low level of education among them reduces the chances of getting jobs in the government and the private sectors (Dhargupta et al., 2009; Ghosh and Guchhait, 2017; Gupta, 2011). The government has given land to them, but in the Purulia district, due to the rocky (non-fertile red laterite soil) nature of the land and their lack of agricultural skills, it was difficult for them to grow crops (Menon, 2020). Thus, the separation from their traditional livelihood, low level of education, and the uncertain as well as strenuous activities as wage labourers are causing livelihood disturbance in their socio-economic life (Das et al., 2020a, 2021; Mukhopadhyay, 1998).

Nestled in the heart of the magnificent Himalayas, the Bhutia tribe has a rich and diverse history. Their story is weaved together like a fascinating tapestry, with references to mythology, archaeological finds, language links, and their enduring Buddhist beliefs (Ram, 2008). Examining these facets provides a more profound comprehension of this intriguing native society. Their cultural identity is based on Bhutia mythology, which provides a fascinating but perceptive look at their origins and values. A well-known creation story describes *Tephka Dorji*, the first Bhutia king, as coming down from heaven. *Tephka Dorji*, chosen by the celestial deity *Menlha*, is credited with bringing civilization and founding the Bhutia lineage (Duff, 2015). This tale demonstrates the Bhutia people's respect for the divine and their conviction that their ancestry was predetermined by God.

Seven celestial maidens who married earthly men gave birth to the Bhutia people, according to another fascinating tradition. This anecdote emphasizes how marriage and cross-cultural interactions were possible in Bhutia history. Furthermore, the respect given to celestial entities signifies their affinity with the immense and breathtaking Himalayan terrain (Subba, 2008; Chaudhuri et al., 2020).

One of the main roles in Bhutia mythology and cultural identity is played by the epic poem "*Gesar of Ling*". This epic tells the story of the mythical monarch *Gesar*, who possessed superhuman strength (David-Neel, 2001). Among *Gesar's* valiant accomplishments are his battles with demons, founding of the Golden Age, and defence of righteousness. Bhutia virtues like bravery, justice, and the unrelenting struggle against evil are all reflected throughout the epic. Crucially, the story incorporates *Gesar's* admiration for Buddhism and his contribution to its spread (Arya, 2022). Analysing the themes of the epic provides valuable insights into the Bhutia worldview, their aspirations for a just society, and the significance of Buddhist teachings in their lives. Mythology presents a fancy viewpoint, while archaeological discoveries provide us with a more tangible knowledge of the Bhutia tribe's past (Subba, 2008). There is a sizable Bhutia community in Sikkim, where settlements from the eighth and ninth centuries AD have been discovered. These finds offer concrete proof of a well-established Bhutia presence in the area at this time. More archaeological research may shed light on their social systems, trade routes, and habitation patterns. Another hint to their history can be found in the Bhutia language, which is a member of the Sino-Tibetan language family. This language shares similarities with Tibetan, suggesting a possible connection between the two communities. Similar vocabulary and grammatical structures are revealed by linguistic study, suggesting that the Bhutia and the Tibetan people had a similar ancestor.

Studying the Bhutia language's development can also provide insight into their historical connections with other communities and potential cultural influences (Thurgood and LaPolla 2016; Duff, 2015; Chaudhuri et al., 2020).

The introduction of Buddhism to the Himalayas in the seventh and eighth centuries AD left a lasting impression on the history of the Bhutia tribe. The teachings of Buddha resonated with the Bhutia people, leading to a gradual shift from their indigenous belief system towards Buddhism. Adoption of Buddhism also produced a foundation for social behaviour and a sense of community. Magnificent monasteries like as *Rumtek* and *Phodong* in Sikkim provide witness to the Bhutia people's continuous devotion to Buddhism and their part in spreading the faith across the region (David-Neel, 2001).

A colourful tapestry of music, dance, and artistic manifestations make up their rich cultural legacy. Songs like *"Lungee"* and *"Rumtek,"* which tell stories of love, grief, and bravery, preserve their folklore. They showcase their rich cultural heritage at festivals with vibrant dances like the *'Chham'* (Ram, 2008). Furthermore, age-old skills like wood carving and carpet weaving are still practiced, demonstrating their creative inventiveness and ties to their surroundings. It takes a diversified approach to comprehend the Bhutia tribe, recognizing not only their historical origins but also their dynamic contemporary culture, in order to guarantee that their impact will continue to inspire future generations (Subba, 2008; Chaudhuri et al., 2020).

The mech people, who are primarily from Assam and Arunachal Pradesh in northeastern India, have a rich cultural history. Compiling the mech creation tale is more difficult than it is for the bhutia tribe, who have a well-documented historical origin (Sengupta, 2003). Nonetheless, shards of their mythology, entwined with customs and rituals, provide

intriguing new perspectives. Oral traditions that are passed down through the centuries play a major role in mech mythology (De, 2023). One prevalent story speaks of the divine couple, Sing Raja and Sing Rani, who descended from the heavens. They are thought to be the Mech people's ancestors. Another story describes how the mech was made out of bamboo (Bisht and Bankoti, 2004; Subba, 2023). Despite its seemingly surreal nature, this story may have symbolic meaning, emphasizing the Mech people's close relationship to their surroundings and their dependence on bamboo for many parts of daily life. The worship of natural spirits is at the centre of the mech belief system. They honor gods such as the woodland spirit *Binia*, the sky goddess *Thanephowani* and the sky god *Thanephowa*. It is thought that these deities have an impact on their well-being, harvest, and lives. The mechs' reverence for nature is a reflection of their need on it for survival (Sarma, 2014).

Festivals such as *Majerani* and *Baha Bihu* offer insights into their mythology. *Majerani*, celebrated during the sowing season, involves offerings to appease the earth goddess for a bountiful harvest. *Baha Bihu* honors the spirits of nature and celebrates the entrance of spring, falling around the same time as the assamese Bihu festival (Kumar and Pruthi, 2003). These celebrations serve as a forum for them to recount their origin tales and reaffirm their ties to the divine in addition to marking agricultural cycles. It's difficult to reconstruct the creation myth of the mech. Oral storytelling plays a major role in mech traditions, in contrast to certain tribes that have written epics (Kaman, 2013; Bisht and Bankoti, 2004; Subba, 2023). It is challenging to piece together a single story because of a lack of evidence. Further research and collaboration with the mech communities are crucial to unraveling the mysteries of their mythology.

2.4. Role of Anthropometry in Gut Microbiome Research

In gut microbiome investigations, anthropometric measurements are essential for participant stratification. Through the consideration of variables such as height, weight, body fat percentage and BMI, researchers are being able to identify the precise impacts of the gut microbiome on health outcomes. For example, Osborne et al., 2020 examined the gut microbiome in a population in Bangladesh that is well-known for having a reduced rate of obesity. Specific bacterial taxa that may be connected to leanness were found by researchers by comparing the makeup of gut microbes with anthropometric measurements. This method contributes to a more complex understanding of the function of the gut microbiome in various populations (Osborne et al., 2020).

2.4.1. *How We Shape Our Microbiome?*

Despite chances the body composition is influenced by the gut bacteria. In addition to having a substantial influence on anthropometry; diet and lifestyle choices also have a big influence on the gut microbiota (Hills et al., 2019). Numerous studies suggested that consuming higher amounts of dietary fibre is essential for maintaining a more diverse gut microbiota. This demonstrates how important food choices are for maintaining a healthy gut environment (Williams et al., 2023). It is notable that research suggests exercise might also alter gut bacteria which in turn led towards a positive impact on metabolic health (Ramos et al., 2022).

Although anthropometry related research efforts in gut microbiome is still in its infancy, it has great potential for preventative and personalised therapy. Understanding the interactions between these variables may help us create focused interventions that enhance body composition, support gut health, and fend off chronic illnesses (Aggarwal

et al., 2023).

Functional Applications: Beyond association, future studies ought to investigate the functional attributes of that particular bacterial taxa play in controlling body weight and distribution of fat (Valdes et al., 2018).

Personalised Nutrition: Researchers might create food recommendations to optimize gut health and body composition based on an individual's anthropometry and gut flora analysis (Rinninella et al., 2019).

Prebiotics and Probiotics: Using live bacteria supplements and prebiotics, which are food for the gut flora, to target particular populations of gut bacteria may be a viable approach to controlling weight and metabolic health (Sanlier and Kocabas, 2023).

2.4.2 Anthropometric measurements and its significance:

In India, several studies have progressed in recent years to evaluate the nutritional status of subjects by utilising different anthropometric measures and by far the most common measure is body mass index (BMI) (Bose et al., 2006; Chakrabarty and Bharati, 2008; Das et al., 2020a, 2020b, 2021, 2022; Kshtriya and Acharya, 2016; Laxmaiah et al., 2007; Mukherjee et al., 2022; National Nutrition Monitoring Bureau (NNMB), 2009; Puwar and Saxena, 2016; Rao, et al., 2006; Roy et al., 2016; Sahani et al., 2018). However, researchers also claim that the study of human health based on BMI is not always sufficient enough to produce a complete scenario of nutritional status as BMI uses a simple calculation based on height and weight and does not take into account variations in body composition as well as does not distinguish between fat mass and lean mass, which can lead to misclassification (Drywein et al., 2017; Genovese, 2009). Somatotype is a method first introduced by Sheldon et al. in 1940 with scientific vigour to describe

and measure the human body. The Sheldonian classification of somatotype categorises the human body into three scales: endomorphy (measures relative fatness and is characterised by rounded shoulders, a broad face, a short neck, and a round physique); mesomorphy (measures relative musculoskeletal robustness and includes broad shoulders and chest, as well as firm limbs); and ectomorphy (measures relative linearity and consists of a visibly slim physique, a narrow and flat chest, and long limbs), which was later modified by Heath and Carter (Carter and Heath, 1990; Heath and Carter, 1967; Sheldon et al., 1940). Anthropometric somatotype has proven useful in various fields of study, particularly in growth and aging, body image, and sports profiling (Brukner and Bennell, 2008; Carter, 2002; Carter and Heath, 1990; Heath and Carter, 1967; Hume and Ackland, 2017). Somatotypes reflect inherent genetic predispositions but are influenced by factors such as nutrition, age, gender, and physical activity (Drywien et al., 2016; Chiou et al., 2014; Raschka and Graczyk, 2013). Recent studies have explored the relationship between somatotype and the risk and occurrence of lifestyle diseases, including hypertension (Herrera et al., 2004), digestive system diseases (Koleva et al., 2002), metabolic syndrome (Galić et al., 2016), diabetes mellitus (Urrutia-García et al., 2015; Yeung et al., 2010), blood pressure (Kalichman et al., 2004), abdominal adiposity (Ramos-Jiménez et al., 2019), Alzheimer's disease (Buffa et al., 2007), and certain types of cancer (Bertrand, 2013). Over the past three decades, numerous research works have focused on the influence of somatotypes on nutrition and overall health, with an emphasis on improving functional capacity (Hume and Ackland, 2017; Leonardo et al., 2012). In India, most studies on somatotypes have focused on population variations among children, adolescents, and women (Chandel et al., 2018; Gaur et al., 1999; Mallick et al., 2018). However, such studies, especially in adult tribal populations, are very few and far

between (Bhasin and Jain, 2007; Das et al., 2021).

2.5. Perspectives on Tribal diet:

Ethnobotany is a branch of plant science that highlights the relationship between the human society and their surrounding flora (Ralte et al., 2024). Tribal and indigenous communities, whose livelihoods are based in and around the forests, have developed a sustainable and ecologically viable means of utilising the gifts of the forests over the centuries. These tribes and rural indigenous communities harbour a wealth of valuable ethnomedicinal knowledge on the usage of herbs and other forest products available to them (Kiasi et al., 2023). These traditional knowledge and information are time-tested and have mostly trickled down the generations, often orally. Naturally, such ethnomedicinal practices are community-specific and vary from one tribe to another based on their geographical locations, availability of resources in the areas they inhabit, their socio-cultural and spiritual practices (Shukla et al., 2022). Tribal diet comprises both vegetarian and non-vegetarian food items. Plant-based food items consumed regularly include edible leaves, nuts, wild fruits and berries, roots and tubers, certain flowers, mushrooms and honey. People also hunt for wild rabbits, pigeons, other small fowl and consume their flesh. Water snails and red ants are also a frequent part of their dietary routine (Francis, 2022). A dietary survey conducted on the Santals and Pahariyas, residents of the villages of Rajmahal Hills in the state of Bihar indicates that cereals like rice, maize and dried and powdered jowar and ‘ghagra shak’ (*Xanthium strumarium*) constitutes the staple diet of these tribes. Roots and tubers are taken as food more by the Pahariyas than the Santals while, on the other hand, milk and pulses are not a part of the Pahariya people’s dietary routine. Both the tribes seem to partake in the consumption of fish and meat equally (Moitra & Choudhary, 1991).

A similar study on the dietary preferences and food consumption pattern of the Bhil tribe hailing from Dhar district of Madhya Pradesh in India suggests that the principal diet consumed by the people of the community comprises cereals and pulses. The variety in cereals is mostly confined to jowar, maize, savi, kutki and bhadi, whereas the pulses they consume are mainly of three varieties, 'moong', 'urad' and 'tur'. Milk and milk-product consumption is very rare among the members of the tribe as is the consumption of sugar (Reddy, 2018). Vital nutrients such as minerals and vitamins are obtained from whatever seasonal fruits and vegetables are available in their locality, and if available. Bhils are, without exception, non-vegetarians and consume the flesh of fowl and animals that they hunt. But the consumption of meat is considered prestigious and occurs only on ceremonial occasions. Falling sick entails light food in lesser quantities to almost no food consumption. Both solid and liquid diet are avoided as it is believed to be a faster cure to many common diseases like dysentery, diarrhoea, etc (Goswami and Thakur, 2024). Pregnant women are advised to not have hot and spicy as well as food items with a pungent or strong odour as it can make them feel nauseous, and is also considered unhealthy and bad for the development of the foetus (Qamra et al., 2006).

West Bengal is reportedly home to a diverse variety of flora including lichens (329 species), fungi (539 species), algae (873 species), gymnosperms (21 species) and angiosperms (3850 species). Study suggests that of these floral species identified, 850 species of plants are of ethnomedicinal significance and 1600 of them hold relevance in the field of ethnobotany (Chakravarty et al., 1999). Mandal et al., 2020 have documented 73 plants of medicinal value that are used by the Santal tribes inhabiting the villages of the Alipurduar district of West Bengal to treat 38 different kinds of human ailments spanning from everyday physical issues to very complex diseases. The plant species

catalogued belong to 69 genera and 45 different families with Fabaceae being the largest representative family of medicinal plants. The active drug-like chemicals that can be derived from Fabaceae include coumarins, alkaloids, flavonoids and tannins among others which are widely used for therapeutic purposes to treat diseases such as abdominal pain, dysentery, hypertension, diabetes, cuts and wounds, various skin problems, fractures, female disorders, etc. (Mandal et al., 2020; Wink, 2013). Flavonoids are a major class of phytocompounds that have potential health benefits in treating inflammatory conditions. They play a role in the body's inflammatory pathways by the active inhibition of some of the key enzymes such as cyclooxygenase and lipoxygenase, and the suppression of pro-inflammatory cytokines (Kim et al., 2004; Amic et al., 2007).The part of the plant most dominantly used by the Santals in preparing these traditional remedies were found to be leaves since they largely believe that utilization of any other parts will harm the mother plant (Datta et al., 2014; Raj et al., 2018; Mandal et al., 2020).

Free radicals can be neutralized by antioxidants, which have the potential to reduce the imbalance brought on by oxidative stress (Coulibaly et al., 2014). They have a crucial task of ensuring a properly functional defence mechanism for the body, and protecting plants as well as animals from environmental damage and sickness (Ou et al., 2002). Several naturally-occurring floral compounds are known to be effective antioxidants (Liu and Henkel, 2002). Polyphenols are one of the most abundantly available group of metabolites found in nature with a broad range of antioxidant functions. A substantial number of medicinal herbs have yielded evidence of antioxidative activities even as detailed information on the remedial properties of several other therapeutic plant species is still lacking and requires further research (Coulibaly et al., 2014). Application of

ethnopharmacological knowledge recommends the use of *Euphorbia balsamifera* (balsam spurge) in the treatment of chronic wounds, while *Feretia apodanthera* is found to be effective in healing infective wounds (Inngjerdingen et al., 2004). The antioxidative behaviour of phenols and their derivatives is essentially driven by the chemical structure of free radical scavengers that are also hydrogen donors and the content of it has lesser significance (Dauqan et al., 2011; Dorman et al., 2003). Aromatic compounds such as phenolics are capable of donating hydrogen atoms to free radicals found in reactive oxygen species (ROS) in the body. This eventually converts phenolics and similar compounds to radicals which attain a stable conformation by the delocalization of electrons within the aromatic ring due to the effect of resonance, and become structurally similar to quinones. Thus, an increase in the number phenolic hydroxyl groups inevitably enhances the free radical scavenging capacity (Eleazu et al., 2010). In a similar manner, the antioxidative nature of flavonoids can be broadly termed as a result of their higher content of hydroxyl groups and the presence of an ortho-3,4- dihydroxy group in their structure (Gheldof and Engeseth, 2002; Meena and Patni, 2008).

The leaves of the 'babul' tree (*Acacia nilotica*) of the Fabaceae family of flowering plants demonstrates antibacterial activity against bacteria commonly observed in tooth cavities, and thus serve as a medicinal remedy against toothache which is primarily caused by bacterial infection of the teeth and gums (Hebbar et al., 2004). Other prominent species that have previously exhibited antibacterial properties include *Acorus calamus* (sweet flag), *Calotropis gigantea* (milkweed), *Carica papaya* (papaya), *Jatropha curcas* (ratanjot) etc. are found to be in use in these tribal communities as well (Rao, 2000). The traditional healers of the community believe that common oral diseases like plaque and caries can be treated with the help of the locally found 'thumbai' plant (*Leucas aspera*)

which is reported to have antibacterial, antifungal and antimicrobial activities (Prajapati et al., 2010; Hebbar et al., 2004), and the same is backed by claims made another tribal community of Uttara Kannada, the 'Siddis' (Bhandary et al., 1995).

2.6. Next Generation Sequencing and Gut Microbiome Research:

A complex ecosystem brimming with billions of bacteria, the human gut microbiome is essential in both human health and disease. Before next-Generation Sequencing (NGS) technologies, this complex ecosystem could only be studied using time-consuming, culture-dependent methods that only covered a small portion of cultivable species (Malla et al., 2019). NGS transformed the field of gut microbiome research by removing these barriers and allowing scientists to explore the wide range of microbial diversity found in the gut. This study examines how NGS has revolutionized the field of gut microbiome research, focusing on the most widely used methods and their most recent developments (Wensel et al., 2022).

2.6.1. *Revolutionizing Gut Microbiome Research: The Power of NGS*

Because many gut microorganisms are resistant to cultivation in laboratory environments, the traditional method of gut microbiome study involved the culture of individual organisms, which is fundamentally biased (Arnold et al., 2016). With its culture-independent method for analysing all of the microbial DNA in a sample, NGS became a game-changer. A more realistic representation of the gut's microbial landscape is provided by this thorough investigation, which reveals the existence of species that were previously unknown (Nigam et al., 2019). NGS procedures cover a wider range of methods, which consist of a few unique advantages with considerations. Whole Genome

Sequencing (WGS) and amplicon sequencing are the two main methodologies that play a key role in the studies regarding the gut microbiome (Satam et al., 2023).

2.6.2. *Amplicon Sequencing: A Targeted Approach*

The most commonly used method, amplicon sequencing, concentrates on a particular gene region, usually the 16S ribosomal RNA (rRNA) gene, which is a universal marker for bacterial and archaeal identification (Regueira-Iglesias et al., 2023). Through the usage of polymerase chain reaction (PCR), researchers can produce a huge number of copies of this particular region for further sequencing. After that, the sequences are subjected to bioinformatic analysis in order to determine and characterize the microbial taxa that are found in the sample (Iwaszkiewicz-Eggebrecht et al., 2024).

Amplicon sequencing has a number of benefits. This approach is both economical and high-throughput, enabling researchers to examine numerous samples at once. Furthermore, concentrating on a particular gene region makes data analysis easier and enables comparisons between other studies. There are, nevertheless, some restrictions. Amplicon sequencing may not fully capture the taxonomic diversity and only provides a limited amount of functional information about the lesser abundant species (Notario et al., 2023).

2.6.3. *Recent Advancements in Amplicon Sequencing*

Amplicon sequencing breakthroughs recently are resolving these constraints. To provide a more complete landscape of the gut ecology, researchers are investigating the use of marker genes other than 16S rRNA, such as functional genes or 18S rRNA for eukaryotes (Mthethwa-Hlongwa et al., 2024). Furthermore, better bioinformatics databases and processes would improve taxonomic classification along with resolution and accuracy.

2.6.4. *Whole Genome Metagenomics: Unveiling the Functional Landscape*

WG metagenomics sequences of the genomic DNA that is taken out of a gut microbiome sample, providing a more thorough study. This method yields a plethora of data, including the entire genetic composition of every organism found there, regardless of how cultivable they are (Thakur and Verma, 2023). By examining the genes encoded in the metagenome, scientists can not only identify the microbial species but also investigate their functional potential (Simpson et al., 2023). WG metagenomics provides an unparalleled understanding of the functional potential pertaining to gut microbiome. Researchers can gain insight into the metabolic pathways functioning in the gut and their possible effects on human health by examining the genes that are present. On the other hand, WGS is more costly and computationally intensive than amplicon sequencing (Köndgen et al., 2024). WG metagenomics is becoming more accessible due to recent developments in bioinformatics tools and sequencing methods. Longer read lengths provided by third-generation sequencing technology facilitate better assembly of diverse microbial genomes. Better computing tools are also making it easier to analyze the enormous amounts of data that WGS generates (Scarano et al., 2024).

2.6.5 *Beyond Bacteria: Exploring the complexity of the Gut Microbiome*

The gut microbiome is primarily composed of bacteria, but it also contains a significant number of viruses, fungi, and archaea. These different populations are being explored through the development of NGS techniques. For instance, research has been going on viral metagenomics and fungal-specific marker genes for fungal community analysis and to comprehend the function of viruses in the gut ecology (Santus et al., 2021).

2.6.6. *Emerging Sequencing Technologies*

Third-generation sequencing: Compared to Illumina sequencing, the gold standard for NGS, platforms such as PacBio and Nanopore offer significantly longer read lengths (Cook et al., 2024). This advantage is essential for resolving strain-level variety within the gut microbiome and for assembling complicated microbial genomes, especially those from uncultivated species (Agustinho et al., 2024).

Single-cell sequencing: This method enables scientists to sequence individual microbial cells DNA, offering previously uncommon insights into the functional potential and metabolic activities of particular populations within the gut ecosystem. The potential for single-cell sequencing to decipher the complex interactions among various gut microorganisms is enormous (Madhu et al., 2023).

2.6.7. *Future Directions:*

Personalized medicine: Methods for personalized treatment are made possible by an understanding of the distinct makeup and functions of each person's gut microbiome. Healthcare providers may be able to predict an individual's vulnerability to particular illnesses or create tailored therapies for a variety of ailments by examining that person's gut flora (Pedroza Matute and Iyavoo, 2023).

Gut-brain axis: The importance of gut microbiota in regulating behaviour and brain health is becoming more well-acknowledged. NGS methods will be crucial in understanding the processes behind the gut-brain axis and creating viable treatment plans for mental and neurological disorders (Bicknell et al., 2023).

NGS methods have revolutionized the study of the gut microbiome by providing hitherto unseen insights into this intricate ecology. The further advancements in sequencing

technologies, bioinformatic tools, and our understanding of the gut microbiota may lead to many more groundbreaking findings in the upcoming years (Satam et al., 2023). These findings have the potential to completely transform healthcare by opening the door for innovative treatment options for a variety of ailments and personalized healthcare approaches.

2.7. Advancement of Culture Dependent Techniques in Gut Microbiome Research:

A large fraction of gut bacteria is still unknown, despite the fact that culture-independent techniques such as DNA sequencing have transformed our knowledge of its makeup. Herein lies the value of culture-dependent research, which provides an additional means of revealing the gut microbiome's hidden diversity (Chandarana et al., 2023).

Constraints on Culture-Independent Approaches: While dominant methods such as 16S rRNA gene sequencing offer important insights on bacterial communities, their effectiveness depends on the identified sequences. For fastidious or new bacteria—those with particular growth requirements that are challenging to cultivate in the lab—this creates a blind spot. This vacuum is filled by investigations that rely on culture, which enable scientists to identify and analyze these hitherto unidentified microorganisms (Cheema et al., 2023).

Advancements in Culture Techniques Lately: The study of microorganisms through cultivation, or culturomics, is experiencing a rebirth. Researchers are able to extract a greater variety of gut bacteria thanks to new techniques like enhanced anaerobic chambers, complicated growth media that replicate the gut environment, and high-throughput culturing methods (Hirano et al., 2023). There are so many reports that can utilise culture not only to discover isolated bacteria but also to investigate their functional

properties. Using ex vivo culture methods, researchers also looked into how human gut bacteria metabolize medications. This method can help to create customised treatment plans and offers insightful information on how the microbiota affects medication efficacy (Fekete et al., 2023). The ability to isolate particular bacterial groups selectively is another benefit of culture-dependent techniques. As per reports, this targeted approach would enable researchers to investigate the roles that particular bacterial populations contribute in health and disease (Alsayed et al., 2023).

2.8. Role of Bioprospecting of Non-Timber Forest Produces (NTFPs):

Biodiversity prospecting, shortened to bioprospecting, refers to the methodical search for genetic and biochemical data of active ingredients from bioresources found in nature. Such resources include animals, plants, microorganisms and other living organisms. It is targeted at creating economically viable products having applications in pharmaceutical, cosmetic, and agricultural researches. The assets that can be accumulated from such a systematic mining of bioresources comprises but are not limited to genes, chemical compounds and their structures, various metabolic pathways occurring in nature, proteins and their behaviours (Beattie et al., 2011).

Till date, a majority of anticancer drugs are often derived from natural sources (Alves et al., 2020). A classic example of this is the development of two major drugs used to treat cancer namely vinblastine and vincristine which were originally derived from rosy periwinkle (*Catharanthus roseus*), a plant native to Madagascar. The bark of the cinchona tree (*Cinchona officinalis*) is used even today for the production of quinine and its derivatives, the first known medicines to treat malaria (Frisvold, 2023).

A fundamental challenge that arises with the advancement of biodiversity mining and prospecting is the issue of biopiracy. Over the years, bioprospecting for pharmaceutical usage has drawn strong criticism, with major pharmaceutical industries and corporations around the world, accused of exploiting indigenous ethnomedicinal knowledge and resources without giving proper acknowledgements to the native people who have cultivated and developed it over hundreds of years, and thus violating indigenous intellectual property rights (Wynberg, 2023).

Bioprospecting is a crucial source for acquiring ethnomedicinal knowledge and a key step to drug designing. Numerous drug-like molecules have evolved in nature over millions of years that are yet unimaginable to organic chemists, and have been found to combat ailments through novel pathways (Frisvold, 2023).

Network pharmacology (NP) is a modern tool that gives a novel perspective to understanding the mode of action and pathways of traditional medicines by computationally deducing the interaction of the drug molecules with multiple targets (Zhang et al., 2023; Hopkins, 2007). The recent decades have seen a significantly lower number of successful incidents of drug designing, owing to unforeseen toxicity issues and a lack of efficacy of the drugs (Kola and Landis, 2004). The root of this failure was attributed to a more conservative approach to drug designing that implements the one drug-one target-one disease theory. Quite often, complex ailments such as diabetes, tumours and carcinomas, etc. do not develop because of a single dysfunctional molecule, rather are culmination of very many defects in the functional pathway network which mutationally alters or renders dysfunctional several entity molecules in the entire regulatory network.

Network pharmacology has emerged as an amalgamation of several disciplines in the field of science including network biology, polypharmacology, systems biology, and other allied domains. It involves a study of the molecular interactions that take place within our system at the inter- and intracellular levels that form the building blocks of the various metabolic and signalling pathways in our body. These metabolic pathways are crucial in the proper execution of our body functions at cellular, tissue and organ-level (Redhu and Thakur, 2022). Pharmacology is the discipline of biochemistry that examines the actions of drugs on the living systems, their targets of operation and the therapeutic pathways they undertake. These drugs are essentially either naturally-derived and human-modified or synthetically manufactured (Vallance and Smart, 2006).

Network biology and polypharmacology come together to provide a more exquisite understanding of the robustness of multitarget drugs and how existing drugs can be repurposed for previously unknown therapeutic usages in an attempt to exploit their full potential against various diseases (Ramsay et al., 2018). The working principle of network pharmacology is founded on the "one drug-multiple targets" concept that allows for a deeper and detailed understanding of the multiple effects that a single drug can have in supressing complex maladies by working on several different targets in the human body (Shah et al., 2023). It enhances the study of the efficacy of drug action and their underlying pharmacological mechanisms within the human biological network.

Non-timber forest products (NTFPs) encompass a broad collection of forest produce, not comprising timber, that are often used or has potential benefits for humans. Such resources include a wide array of fruits and berries, aromatic plants of medicinal value, either entirely or in part such as the leaves, roots, tubers, extracts, etc. (Negi et al., 2010). Knowledge about the many utilities of various forest products other than timber and the

know-how to properly use them were primarily contained within tribal communities that lived in and around forests and woodlands (Ahmed et al., 2023). This knowledge was passed on from one generation to the next, often orally. Over the decades, a growing awareness regarding the utility of NTFPs is noticeable among the urban population as well. With greater understanding of their potential medicinal, cosmetic as well as spiritual values, the demand for the same has also increased several folds (Shackleton et al., 2024). But we still lack sufficient information on the means to collect them and make proper utilization of them.

With the development of modern medicines and the fast-growing pharmaceutical industry, the demand for medicinal plants is only ever-expanding. Modern medicines depend heavily upon numerous medicinal herbs and other plant products as their key component (Wang et al., 2023). A rising demand of NTFPs across the world prompts a surge of concerns about the methods of procuring these from the wild and the social and economic viability of it as well as its ecological sustainability. The importance of NTFPs and the potential they hold in revolutionizing the human economy, and particularly the health and pharmaceutical industry is hugely underestimated and yet to be explored (Haokip et al., 2024).

The eastern Indian state of West Bengal is home to several indigenous tribes and boasts of a rich tribal culture and heritage. Once categorized as a ‘criminal tribe’ under the British Raj in India, the Sabars are a nomadic tribe inhabiting the forested and hilly parts of West Bengal, Jharkhand, Odisha, Chhattisgarh and Madhya Pradesh (Bhandari et al., 2001). Being one of the scheduled tribes (ST) of India, the community, hailing from a lower caste, faces utmost social discrimination and ostracization, even in this modern era of scientific rationalism and progress. The 2011 census of India estimated the net

population of the Sabars to be 180,000, making them the 10th largest community in West Bengal and constituting 1 % of the state's total tribal population. They are the one of the prominent few tribal communities left who speak the language of the Mundas (Dhargupta et al., 2009; Census of India 2011). The primitive agricultural practice of slash-and-burn cultivation is predominant in the groups that reside in the rugged, hilly terrains. Their use of material goods and instruments is not the most developed, and the use of hoes as the primary instrument for agricultural activities is observed in the community (Dhargupta et al., 2009). Lack of substantial nutritional and healthcare systems results in poor health and malnutrition, which is further aggravated by the subpar socio-economic status of the community.

The tribal population grossly relies on plants and forest products for their dietary requirements and livelihood. While the various resources from forests are harnessed for their economic worth such as selling forest produce that are consumed as food items and making ornamental and handicraft items for selling in local markets, they also essentially form the primary source of nutrition for aboriginal people (Hazarika et al., 2023). A report on the usage of non-timber forest resources by the Himalayan tribal communities inhabiting regions of Himachal Pradesh states that roughly 141 known species of herbs and other plants are consumed as 'wild edibles' as part of their regular dietary pattern, 109 species are utilized as fodder for their domesticated livestock and nearly 739 species are known to have medicinal significance and are used by the people to treat and cure common ailments as well as some complex diseases (Masoodi and Sundriyal, 2020). This reaffirms the massive dependence of the tribal society on forest resources for their daily survival necessities.

3. Research Objectives:

The following are the major objectives of the work:

- ✓ To identify the core gut microbiome of the indigenous tribal population under study for example: Sabar, Mech and Bhutia living in their natural habitats.
- ✓ Evaluation of anthropometric parameters such as Body Mass Index (BMI) and Somatotype which is influenced by several factors like nutrition. Assessment of the mean dietary intake of the cohort selected for the study from the different tribes will also be performed.
- ✓ A series of conventional culture dependent, morphological, and biochemical and molecular tests would be done to provide additional information at successive levels of microbial physiology to analyze the microbial functionality using omics approaches.
- ✓ 16SrRNA sequencing and whole genome sequencing would be performed to identify and report the prevalence of any antibiotic resistant bacteria identified through the culture dependent methods.
- ✓ Data from culture-independent analysis and culture-dependent method will be correlated along with the anthropometric and nutritional parameters to formulate a prediction pipeline which will enable us to predict the GBP of an individual based on the diet and anthropometric measurements, thus aiding primary healthcare.

Conceptual Framework:

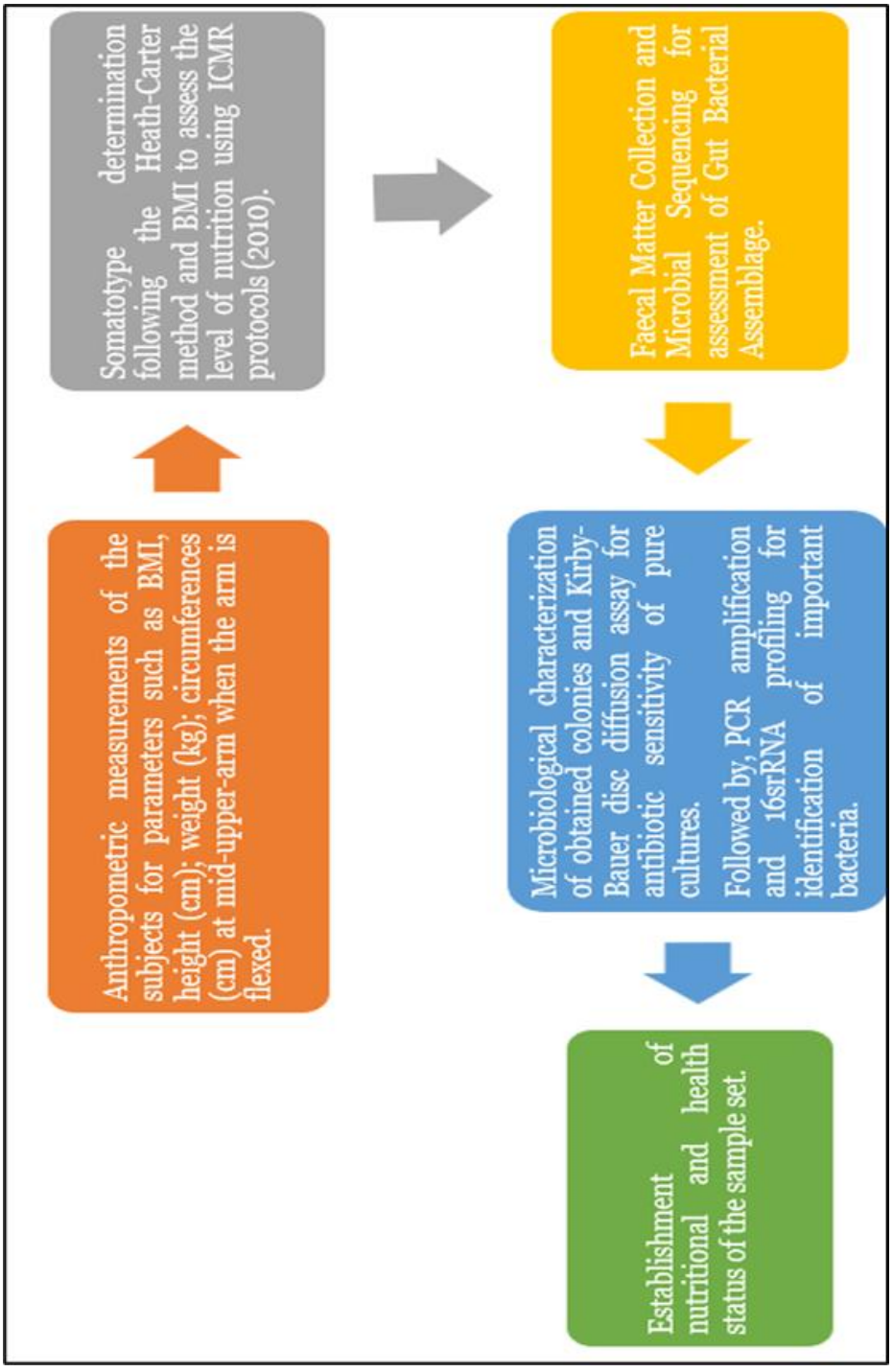


Figure 3.1: Conceptual Framework of the Proposed Study

Figure 3.2: Glimpses of field Exploration and Sampling (A-C: Tribal Subjects; D: Urban Subject; E-F: Morphological differences of Fecal samples)

4. Methodology:

4.1. Data and Sample Collection

Participant selection during the study design was considered a critical aspect which opted me to choose three distinct tribal groups for the longitudinal study to continue. To ensure equal representation within each group, we enrolled families that comprised a male adult, a female adult, and a child participant. The choice for the child subject was based on age range as well as gender; it was preferred that a male pre-pubescent age category. This particular study investigated the Gut Bacterial Profiles (GBP) of three families living in West Bengal, India.

The first family belonged to the nomadic Sabar tribe, whereas the other two families were from the Drukpa Bhutia tribe and the foothill Mech tribe, which trace their roots back to Mongolian lineage. The sample collection from the Sabar family centered on a 30-year-old adult male, a 26-year-old adult female, and their 5-year-old son who had not yet reached puberty. The investigation also included data from a Bhutia family comprising an adult Bhutia male aged 29, an adult Bhutia female aged 27, and their male child of age 5. In parallel with the Sabar family, the gut microbial data from the Mech tribe revealed a similar family structure: a male participant (30 years old), a female participant (26 years old), and their male offspring (5 years old) (Ganguli et al., 2019). The local administration and the respondents were made aware of the necessary approvals and informed consent before the initiation of this study.

One of the main obstacles in conducting such investigations are the seclusion of these settlements. Therefore, the collection team travelled for long hours before reaching the

settlements. Along with the tribal subjects, we also recruited an urban family having the same characteristics feature of a male, female and their non pubertal male kid.

Figure 4.1: Selection of Study Subjects (A. Mech; B. Bhutia; C; Sabar; D. Urban)

Ethics Statement:

This research has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Research and Ethics Committee of Bangabasi College, University of Calcutta (No. 002/2017).

4.2. Anthropometric Evaluation:

Collection was performed through two steps from the aforementioned family:

a) Counselling along with Medical Assessment: The subjects were initially advised regarding the need for the first fecal matter and permitted to consume their regular diet as well as an average of 12 hours of sleep per day. During the sampling tenure, they were found to be healthy and had not suffered from any chronic illnesses or morbid conditions in the previous six months or during the past fifteen days. The study employed calibrated instruments to perform all anthropometric measurements in accordance with a standardized protocol (Weiner and Lourie, 1981).

For the assessment of somatotype, nutritional status, and body composition, anthropometric measurements of height (cm); weight (kg); bi-epicondylar breadth (cm) of humerus and femur; skinfolds (mm) at triceps, biceps, sub-scapula, supraspinale and calf; circumferences (cm) at MUAC (both flexed and relaxed), waist, hip, and mid-calf have been taken following a standardised operating procedure (Lohman et al., 1988). Martin's anthropometer rod, digital weighing machine (OMRON HN 289), Martin's sliding caliper, skinfold caliper (Slim Guide) and calibrated non-elastic measuring tape (Gulick Anthropometric Tape) were used to measure height, weight, breadth of humerus and femur, skinfolds and circumferences, respectively. All the measurements were taken on the left side of the participant with minimal clothing except measuring weight. Technical errors in measurements have been calculated and found within the acceptable limit (Ulijaszek and Kerr, 1999). BMI was calculated following the equation:

BMI (kg/m^2) = Weight (kg) / Height (m^2) (WHO, 1995).

The classification based on BMI (undernutrition: BMI <18.5 kg/m^2, normal: BMI= 18.5-22.9 kg/m^2, overweight: BMI ≥23.0 kg/m^2) proposed by WHO (2000) for the South Asian population was considered here to determine the nutritional status of the studied population (WHO, 2000). Given the limitations associated with using BMI as a measurement, researchers have identified alternative measurements that are useful for nutritional screening, particularly in rural areas with limited resources (Casper et al., 2021; Ahirwar et al., 2023; Sultana et al., 2015). One such widely accepted measurement is MUAC, which researchers use as a substitute for BMI (Casper et al., 2021; Ahirwar et al., 2023; Tang et al., 2017; Van Tonder et al., 2019). Recently in 2017, Tufts University, a collaborator of the Food and Nutrition Technical Assistance III Project (FANTA), funded by the U.S Agency for International Development (USAID), conducted a meta-analysis of individual adult participants from 17 countries, including Africa, South Asia, Southeast Asia, North and South America, and proposed a MUAC cut off (undernutrition: ≤ 24.0 cm) for identifying against low BMI (<18.5 kg/m^2) (Tang et al., 2017, 2020). This MUAC cut-off was used in the present study. Body composition variables like Percent Body Fat (PBF), Fat Mass (FM), Fat-Free Mass (FFM), Fat Mass Index (FMI) and Fat-Free Mass Index (FFMI) were also calculated following standard formulae:

PBF (%) = (1.20 x BMI) + (0.23 x Age) - (10.8 x Sex) - 5.4, where, Sex: Male = 1 (Deurenberg et al., 1991).

FM (kg) = Body Weight (Kg) x (PBF/ 100) (VanItallie et al., 1990).

FFM (kg) = Body Weight (Kg) - FM (Kg) (Lohman, 1992).

FMI (kg/m2) = FM (Kg) / Height (m^2) (VanItallie et al., 1990).

FFMI (kg/m2) = FFM (Kg) / Height (m^2) (Heymsfield et al., 2007).

Somatotype analysis and classification were done by following the Heath-Carter method (Carter, 2002; Carter and Heath, 1990). The following equations were used (Carter, 2002):

Endomorphy = - 0.7182 + 0.1451 (X) – 0.00068 (X^2) + 0.0000014 (X^3)

Where X = (sum of triceps, sub-scapular and supraspinale skinfolds) multiplied by (170.18/ height (cm)).

Mesomorphy = (0.858 x humerus breadth) + (0.601 x femur breadth) + (0.188 x corrected arm girth) + (0.161 x corrected calf girth) – (0.131 x height) + 4.5
Corrected arm girth: arm girth (cm) – (triceps skinfold /10) (mm);

Corrected calf girth: calf girth (cm) – (calf skinfold / 10) (mm)

Ectomorphy = If HWR ≥40.75, Ectomorphy = (0.732 x HWR) – 28.58

If HWR <40.75 and >38.25, Ectomorphy = (0.463 x HWR) – 17.63 If HWR ≤38.25, Ectomorphy = 0.1

Where Height Weight Ratio (HWR): Height (cm) / Weight $(kg)^{1/3}$

After identifying the somatotype components, they were plotted on a 2-D somatochart following X and Y coordinate as:

X-coordinate = Ectomorphy - Endomorphy

Y-coordinate = 2 x Mesomorphy - (Endomorphy + Ectomorphy)

b) ***Analyses of Dietary Patterns:***

The initial phase of the study involved the collection of fecal samples at 5:30 am. Thereafter, dietary intake data pertaining to the prior 24 hours was ascertained from all participants via a 24-hour recall and a Food Frequency Questionnaire (FFQ) (NNMB, 2009). FFQ consisted of few pertaining questions like:

- What are the traditional food types and eating frequency of the studied subjects under study?
- What is the food preference/ avoidance in general?
- Whether the subjects had any allergic reactions to some particular foods or did there any dietary restrictions?
- What is the Meal Intake Pattern during the Breakfast, Lunch, Snacks and Dinner [with time and quantity] of the studied subjects under study?

The fecal matter was collected in RNALater™ (Qiagen Inc.), which is an RNA stabilization reagent, as per manufacturer's protocol. Subsequently, the samples were transferred to sterile containers and secured for transport using Parafilm and duct tape. This ensured sample integrity during delivery to the sequencing laboratory, which occurred within 10 hours of collection.

4.2.1. Dietary Assessment:

The 24-hour dietary recall method was utilised for collection of information regarding the dietary intake of the study participants. All food items consumed for the previous three consecutive days by the individual were documented in detail. The raw amount of all the ingredients used for any preparation was weighed (g) by an electronic digital

kitchen weighing scale (SF-400). Then, the total cooked volume (ml) of each preparation was recorded by filling water in the standardised cups of different sizes (12 cups of 30-1400 ml) and spoons (a tablespoon of 15 ml and a teaspoon of 5 ml) as recommended and used by the National Institute of Nutrition (NIN) of Hyderabad in their national-level diet and nutrition surveys (NNMB, 2009). After assessing the total cooked quantity of any preparation, the portion consumed by the study participant was assessed in terms of cups, following the cup size and volume accordingly. For calculating the raw food equivalent from individual cooked intake, a conversion factor (CF) for any ingredient is used [CF = weight of the raw food used (g) / total cooked quantity of that preparation (ml)] at first, then at the second stage, the individual intake of that ingredient (g) was recorded following the formula = CF (that ingredient) × volume of individual cooked food consumed (ml) (NNMB, 2009). Based on the information collected, the food items were classified into eight groups: cereals, pulses, leafy vegetables, other vegetables, roots and tubers, fats and oils, fruits, and others (foods consumed from outside) (Gopalan et al., 2007). Except for energy, all the nutrients were classified into five groups: macronutrients (protein, carbohydrate, and fat), vitamins (Vit. A, thiamine, niacin, B6, riboflavin, folate, and Vit. C), minerals (calcium, iron and phosphorus), electrolytes (sodium, magnesium and potassium), and trace elements (copper, zinc, manganese, selenium and chromium). The level of Vit. A consumption by the study population was presented here as retinol as this vitamin was present in some animal foods and plant foods as retinol and carotenoids (β-carotene), respectively (ICMR, 2010).

4.2.2. Dietary Habits:

The qualitative methods and techniques, including observation, in-depth interviews, and focus group discussions (FGD), etc., were used to collect information about their daily

food habits, food choices (preference and avoidance), traditional food practices, seasonal food availability, forest collections, game hunting, changes if any in their dietary habits, their perception about nutrition and diet, etc. All the interviews and FGDs were recorded and transcribed the verbal words into written documents verbatim. During data analysis, coding was done from the transcriptions and later, broader themes were generated (Saldana, 2009). A pretested FFQ was prepared and used in the present study to assess the frequency of consumption of different food items following the FFQ used by NNMB in their dietary survey (NNMB, 2012). The frequency of each food was assessed by eleven categories, starting from thrice a day, twice a day, once a day, etc., to never consumed. FFQ include several categories like vegetables (cabbage, pumpkin, eggplant, etc.), cereals (rice, wheat flour, etc.), flesh items (varieties of fish, chicken, field rat, duck, snail, mussel, etc.), drinks (tea, coffee, carbonated drinks, juice, alcoholic beverages, etc.), nuts and legumes (lentils, beans, peas, whole soybeans, etc.), fruits (banana, apple, orange, mango, etc.), outside food items (sweets, ice creams, rolls, noodles, etc). The locally available forest products commonly consumed by the subjects on a regular basis were also recorded.

4.3. Sequencing and Bioinformatic Analyses:

Next-generation sequencing was conducted utilizing the Illumina Miseq™ platform and the bioinformatic analysis was carried out in accordance with the established protocol (Bag et al., 2016) that has been reported previously (Ganguli et al., 2017; Basu et al., 2022; Dhar et al., 2022). Quality control of raw reads and identification of the most prevalent taxa based on the OTU clustering were performed. SILVA v138 (Quast et al., 2013), Greengenes (DeSantis et al., 2006) and RDP (Maidak et al., 1996). Databases were used for taxonomic identification via QIIME2 (Bolyen et al., 2019). Venn analysis of obtained genera was done by using the web-tool Venny v2.1.0 (Oliveros, 2007). Taxon Set Enrichment Analysis (TSEA) was then performed to obtain the disease networks associated with the taxon sets by using the in-built algorithm of Microbiome Analyst webserver (Chong et al., 2020). Using the iVikodak webserver, 16S rRNA gene data was analyzed to identify both dominant and essential functions within the microbial community. These functions were then used to generate network visualizations that depict the relationships between these functionalities (Nagpal et al., 2019).

4.3.1. Identification of the abundances of different microbes in the gut of the subjects chosen and determination of common elements between them:

One of the foremost tasks was to identify the individual microbes present in the gut of the different subjects and obtain their differential abundances. The user-end reads obtained from Illumina sequencing were used as query sequences in the MetaG server and subjected to the LAST algorithm for matching against the RDP_16S_18S database allowing for the analyses of archaeal, bacterial, or eukaryotic matches, at different taxonomic levels, using an alignment score cutoff of 0.8, subsequent to the elimination

of reads having very high e-values (Dhar et al., 2022). The data obtained herein was used for downstream analyses.

Then, starting at the widest taxonomic level, MetaG assigns a taxonomic label to each read. The taxon that receives the most hits is used for this. The analysis continues until a confidence level is breached or numerous taxa are supported by the same quantity of high-quality database hits. The confidence threshold gives the most numerous taxon a statistical measure of support while also reflecting the quality of the underlying alignments. Reads that don't fit the requirements for the assignment are put in the unmatched class. All unmatched or unclassified reads were removed from the data for downstream analyses.

Using Krona tools, discovered taxa and pathogen predictions are displayed in interactive graphs (Dhar et al., 2022). Predicted pathogens are reinforced, where necessary, by predictions about the hosts and drug resistance. Species-level predictions for bacteria and archaea are made as a result of enhanced database naming consistency. Data on bacterial and archaeal pathogens are searched for by MetaG using PATRIC (Manske et al., 2020).

Identification of common elements between the gut microbial consortia of different populations and within different subjects of the same population was done using Venny v2.1.0 (Oliveros, 2007) to generate Venn Diagrams.

4.3.2. Analysis of pathway enrichment networks and functionality prediction of the gut microbial abundance data using iVikodak:

iVikodak is a multi-modular web platform that integrates microbial co-inhabitance patterns and several updated datasets of different curated microbial-function maps, thus, providing a visualization interface and several functional inference and analysis tools that

are logically interconnected. The iVikodak architecture includes three interconnected modules, namely: Global Mapper, Feature Analyzer (ISFA), and Local Mapper, in order to construct a relevant workflow for carrying out amplicon sequencing data-based functional metagenomic analysis.

For the Inferred Functions of each microbiome under investigation, the iVikodak platform was employed (Nagpal et al., 2019). The common genera from the studied datasets of venny were used to generate the following results. Using the Global Mapper module, the most crucial functions were determined according to KEGG (Kyoto Encyclopedia of Genes and Genomes) (Yousef et al., 2021). Results were represented as:

a. Top functions that correspond to various pathways exhibited by the bacteria at various levels of functional hierarchy.

b. Core functions that show minimum defined abundance in a given environment

c. Functional Correlation networks with nodes representing microbes and the edges in between indicating that they are (potentially) co-contributing to one or more specific functions.

The functions were examined and extensive literature survey was performed to corroborate the obtained data as well as to correlate it with observed findings. This includes the most significant genera (along with their inherent hierarchy) in the dataset which were visualized as heatmaps, exhibiting variations between males, females, and children within individual tribes as well as between the tribes. The functions in the metabolic participation that showed predominance were also visualized and separated using enrichment networks.

4.3.3. Prediction of a disease network based on the functional predictions of the microbiome:

MicrobiomeAnalyst is a web-server comprising four modules of which, the Taxon Set Enrichment Analysis (TSEA), was used to obtain the disease networks associated with our taxon sets (Chong et al., 2020).

The objective of this study was to assess if a given taxon set's members are represented in the user-uploaded taxa list more frequently than predicted by chance. A list like this could include significant traits discovered through differential abundance analysis or those showing comparable behaviors discovered through clustering analysis. Hypergeometric tests are used to calculate the enrichment analysis.

The common gut microbial genera were entered as ‘Mixed Level Taxon Names’ for phenotype mapping and enrichment analysis. 454 taxon sets were found to be associated with host intrinsic factors, (here, diseases). The enrichment network provides a high-level overview of significant taxon sets and their relationships. Each node represents a taxon set, and the size and color of each node are determined by the number of hits and the P value, respectively. If the number of shared hits between two taxon sets is greater than 20% of the total number of their combined taxa, then the two taxon sets are connected by an edge.

4.4. Culture dependent studies for identification of antibiotic resistant isolates:

4.4.1. Microbiological Characterization:

Serial dilution and plating were performed onto the different agar plates (nutrient as well as specialized agar) during the microbiological culture (both aerobes and anaerobes) of the collected raw fecal samples. Identification and characterization of bacteria involves the study of cellular morphology and different physiological and biochemical properties of bacteria. Classification of bacteria based on the morphological, physiological and biochemical features is the most practical way to identify these organisms. All the microbes have their own physiological and biochemical characteristics that are governed by the presence and expression of specific genes that control the enzymatic activity responsible for biosynthesis and biodegradation of specific compounds (Cappuccino and Sherman, 2005). Thus, every microbe has its own biochemical and physiological fingerprint that makes it unique from the others. Anaerobes were cultured using Anaerobic Gas Jar system. This system created an oxygen-free zone for cultivating microorganisms that cannot survive in oxygen (anaerobes). Samples were placed inside a container and a special pouch containing chemical tablet (sodium borohydride and sodium bicarbonate) was added before sealing. Water triggered a reaction within the pouch, producing hydrogen gas and carbon dioxide. The resultant hydrogen gas was used to eliminate any remaining oxygen inside the container, while the palladium catalyst in the lid aided this process. Finally, the carbon dioxide filled the space left by the oxygen, resulting in a completely oxygen-free environment for growing the microbes.

After obtaining pure and isolated colonies, morphological examination of the isolated colonies under 100x magnification of a Compound Light Microscope were being

followed by Gram staining. Presumable identification of bacterial isolates, using conventional morphological as well as biochemical inspections, adhering to the established protocols delineated in Bergey's Manual of Determinative Bacteriology was performed (Bergey, 1994).

4.4.2. Morphological study of Bacteria:

Preliminary characterization of bacteria includes the classification of bacteria according to their cellular and colony morphology. Cellular morphology of a bacterium can clearly be obtained by observing the cell under microscope after proper staining. Bacterial cells can exist either in isolated or in aggregated fashion. Shape of most of the bacteria in the environment are either spherical (Coccus) or rod shaped (Bacillus). Beside these there are many special types of cellular morphology for few bacteria. Those include comma shaped (Vibrio), spiral (Spirillum) etc. In aggregate the spherical coccus formed Diplococcus (aggregate of two coccus), Sarcina (aggregate of four coccus), Streptococcus (Chain of coccus), and Staphylococcus (Bunch of coccus).

Whatever be the shape of the cell, multiplication of bacteria give rise to a visible colony. A rough estimation of the viable cell in a population can be determined by counting the number of colonies developed in the solid culture media after incubation.

The estimated value is then expressed in terms of colony forming units or CFU. Depending upon the association of the colony members and their physical and biochemical properties, each type of bacteria has their distinctive colony morphology (Bergey, 1994). While studying a colony, few standard parameters should be considered.

Shape: Varies from round to irregular to filamentous and rhizoid (root-like).

Size (measure with a millimeter rule): Can vary from large colonies to tiny colonies, less than 1mm = punctiform (pin-point).

Edge/ Margin: Magnified edge shape (use a dissecting microscope to see the margin edge well)

Pigmentation (color of colonies): white, buff, red, purple, etc.

Opacity: Transparent (clear), Opaque (not transparent or clear), Translucent (almost clear, but distorted vision–like looking through frosted glass), Iridescent (changing colors in reflected light)

Elevation: How much does the colony rise above the agar (turn the plate on end to determine height)

Surface: Smooth, Glistening, Rough, Dull (opposite of glistening), Rugose (wrinkled)

Texture: Butyrous (buttery), Viscid (sticks to loop, hard to get off), Brittle/Friable

(dry, breaks apart), Mucoid (sticky, mucus-like).

4.4.3. Biochemical Characterization:

Biochemical testing methods for Starch hydrolysis, Oxidase, Catalase, H_2S production, Nitrate reduction, Urease, Lactose fermentation, Indole production, Methyl red, Voges Proskauer, Citrate Utilisation were performed for profiling and categorization of the isolates (Cappuccino and Sherman, 2005).

4.4.3.1. Starch hydrolysis test:

Monosaccharide and more preferentially glucose is the simplest form of carbohydrate or polysaccharide that is able to be transported into the cell and take part in cellular

metabolism. Many bacteria produce extra-cellular enzymes like amylase, maltase characteristic to their biochemical property. These bacteria can hydrolyse starch, a branched polysaccharide into its building block glucose and thereby utilise this polysaccharide as their source of carbon and energy.

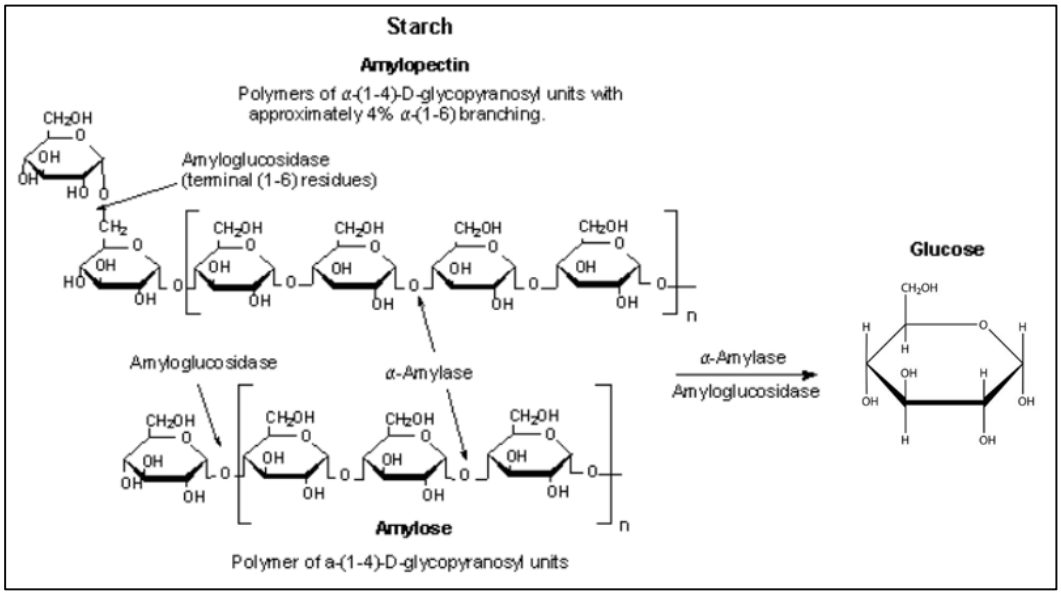


Figure 4.2: Starch hydrolysis test

4.4.3.2. Oxidase test:

The terminal key enzyme of electron transport chain of aerobic organism is cytochrome oxidase which oxidizes the terminal cytochrome $cytaa_3$ using oxygen as terminal electron acceptor. p-aminodimethylaniline oxalate is an artificial substrate that act on behalf of cytochrome as terminal electron donor and oxidized into a blackish compound.

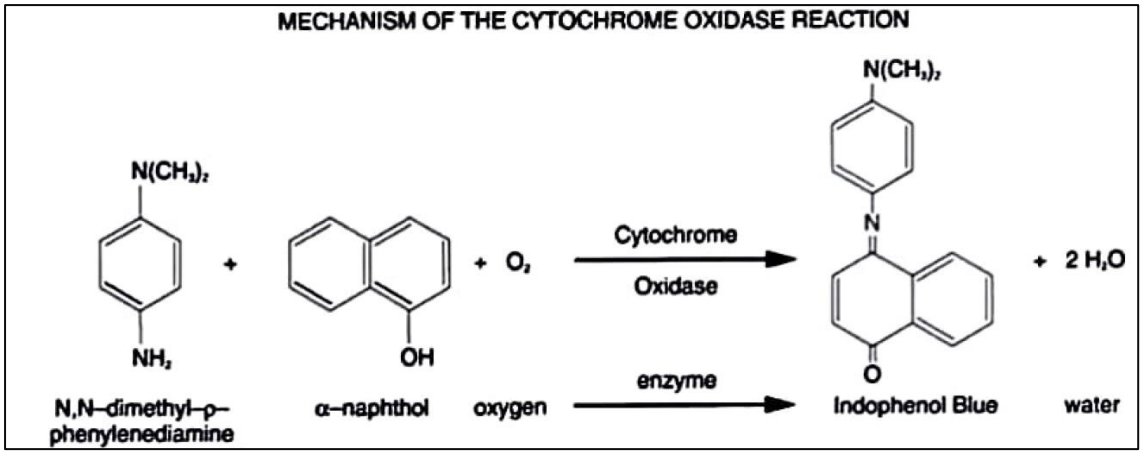


Figure 4.3: Oxidase test

$$\text{TMPD (reduced form)} + O_2 \xrightarrow{\text{Cytochrome C}} \text{TMPD (reduced form)} + H_2O$$

4.4.3.3. Catalase test:

The gut environment facilitates the growth of the microaerophilic organisms. These organisms during their aerobic respiration use oxygen as terminal electron acceptor which results in the generation of hydrogen peroxide and other toxic superoxide. To survive within this toxicity, microrganisms have to produce the enzyme catalase rapidly to degrade the hydrogen peroxide produced into water and oxygen.

Hence production of the enzyme catalase is an important biochemical parameter for the microaerophiles, faculatative anaerobes and strict aerobes.

$$\underset{\text{Hydrogen peroxide}}{2H_2O_2} \xrightarrow{\text{Catalase}} \underset{\text{Water}}{2H_2O} + \underset{\text{Oxygen}}{O_2}$$

4.4.3.4. Hydrogen Sulphide Production test:

Many bacteria are able to produce hydrogen sulphide from sulphur containing amino acid like cysteine or inorganic sulphur compounds. Cysteine loses its sulphur atom by the action of the enzyme cysteine desulphurase and produce ammonia and hydrogen sulphide.

On the other way gaseous hydrogen sulphide may also be produced by the reduction of inorganic sulphur compounds like thiosulphates, sulphates or sulphites by the corresponding reductases. The hydrogen sulphide gas produced reacts with the ferrous ammonium sulphate of the media to give a black precipitate of ferrous sulphide.

$$Pb(C_2H_3O_2)_2 + H_2S \longrightarrow PbS + 2CH_3COOH$$

Lead acetate Hydrogen sul Lead sulfide Acetic acid

4.4.3.5. Nitrate reduction test:

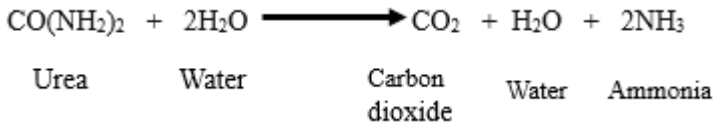
Some aerobic and facultative anaerobic bacteria can utilize nitrate (NO_3^-) or sulphate (SO_4^-) as a source of oxygen under oxygen deplete condition. These bacteria possess an enzyme nitrate reductase that converts the nitrate into nitrite (NO_2^{-}) which in presence of sulfanilic acid and α-naphthylamine gives a characteristic red colour to the medium. A few among them can also reduce nitrite to ammonia or nitrogen. Addition of an oxidative agent in the form of zinc dust will transform these reduced nitrogenous substrates into nitrite that gives positive signal upon reaction with sulfanilic acid and α-naphthylamine.

$$NO_2^- + C_6H_5SO_2NH_2 + C_{10}H_7NH_2 \longrightarrow C_{16}H_{13}N_3O_3S \text{ (Red Azo dye)}$$

Nitrite Sulfanilic acid α-Naphthylamine p-Sulfobenzene-azo-naphthylamine

4.4.3.6. Urease test:

Many microorganisms are able to produce a hydrolytic enzyme urease that breaks the nitrogen and carbon bond in an amide compound like urea and results in the production of ammonia. Among the bacterial kingdom the genus *Proteus* is the most efficient in producing this enzyme along with the other genus. Production of ammonia through urease activity would increase the pH of the media containing urea which changes the colour of the redox dye phenol red from red to pink.



4.4.3.7. Lactose fermentation test:

The lactose fermentation test is a fundamental tool in microbiology used to assess a bacterium's ability to utilize lactose, a sugar commonly found in milk products. This method is essential for differentiating and identifying bacteria, which helps with diagnosis and other scientific uses. The idea of fermentation is fundamental to the test. Lactose is broken down by bacteria that can ferment it for energy and release acidic byproducts as a byproduct. The culture media contains a pH indicator, usually phenol red. The indicator's colour varies according on the acidity level in the vicinity.

The lactose fermentation test does not include a single, direct chemical reaction with lactose. It's a multi-step process that starts with the fermentation of lactose. A summary of the main chemical changes is provided below:

Fermentation promotes the breakdown of lactose in a multi-step process. The main chemical changes are broken down as follows:

Lactose Breakdown: Lactose, a disaccharide, is first broken down by the enzyme beta-galactosidase into its two monosaccharide components, glucose and galactose.

$$C_{12}H_{22}O_{11}\ (\text{Lactose}) + H_2O \rightarrow C_6H_{12}O_6\ (\text{Glucose}) + C_6H_{12}O_6\ (\text{Galactose})$$

Glucose Fermentation: Some bacteria can further ferment these simple sugars. In the context of the lactose fermentation test, we typically focus on homofermentative lactic acid fermentation. Here, glucose is broken down into lactic acid, with the net gain of ATP (cellular energy) through substrate-level phosphorylation.

$$C_6H_{12}O_6\ (\text{Glucose}) + 2\ ADP + 2\ Pi \rightarrow 2\ CH_3CHOHCOOH\ (\text{Lactic Acid}) + 2\ ATP$$

pH Change: As lactic acid accumulates; it lowers the pH of the surrounding medium. This acidity is what causes the phenol red indicator to change color in the lactose fermentation test.

4.4.3.8. Indole Production test:

This test demonstrates how some bacteria may break down tryptophan into indole, an amino acid that builds up in the medium. This is carried out by a series of distinct intracellular enzymes, collectively known as "tryptophanase." It is a component of the IMViC protocols, which are tests intended to identify amongst Enterobacteriaceae family members.

Bacteria expressing the tryptophanase enzyme have the ability to hydrolyze and deaminate the amino acid tryptophan. Tryptophan is converted to indole through the process of reductive deamination using the intermediate molecule indolepyruvic acid. The deamination reaction, which removes the tryptophan molecule's amine ($-NH_2$) group, is catalysed by tryptophanase. The reaction's end products are energy, pyruvic acid,

ammonium (NH_4+), and indole. As a coenzyme, pyridoxal phosphate is necessary. When indole is combined with Kovac's Reagent (which contains hydrochloric acid and p-dimethylaminobenzaldehyde in amyl alcohol) the solution turns from yellow to cherry red. Because amyl alcohol is not water soluble, the red coloration will form in an oily layer at the top of the broth.

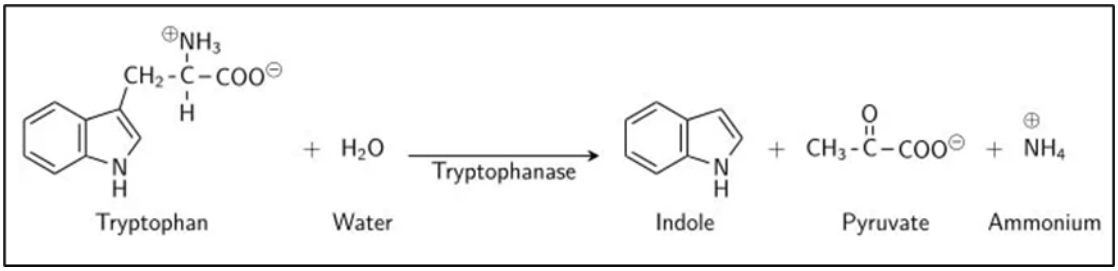


Figure 4.4: Indole Production test

4.4.3.9. Methyl Red test:

An organism's ability to carry out mixed acid fermentation and generate stable acid end products is assessed using the Methyl Red (MR) test. After an enteric gram-negative rod has fully fermented glucose, methyl red is the indicator that measures pH.

Bacteria that produce succinic, lactic, and acetic acids during mixed acid fermentation lower the pH of the medium to less than 4.4.

Methyl red (2-[[4-(dimethylamino) phenyl] diazenyl] benzoic acid), a pH indicator that is red at pH < 4.4 and yellow at pH 5.8, is used to visualise it.

MR Positive: Due to the fermentation of glucose, the culture medium's pH falls to 4.4 or below, causing the medium to colour red.

MR Negative: Less acid is formed by the fermentation of glucose, and the culture medium stays yellow.

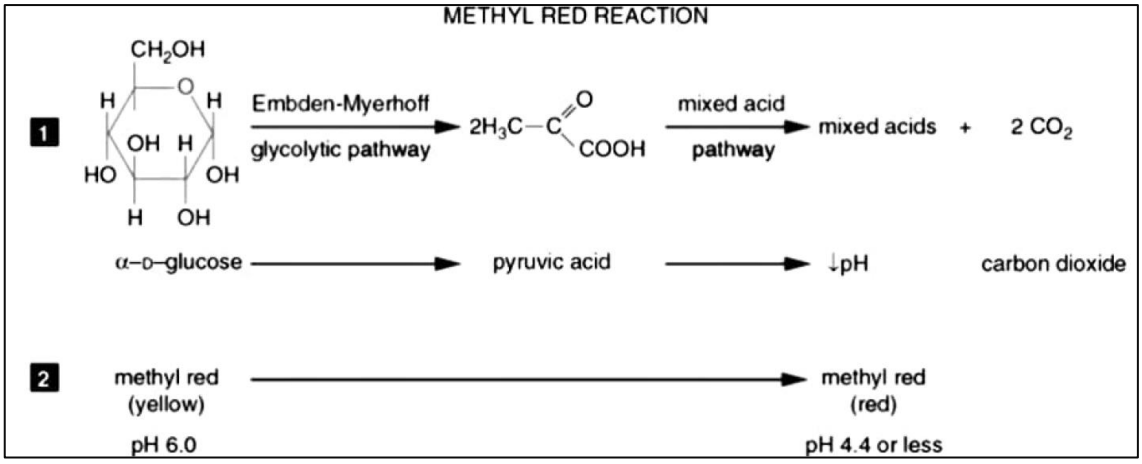


Figure 4.5: Methyl Red test

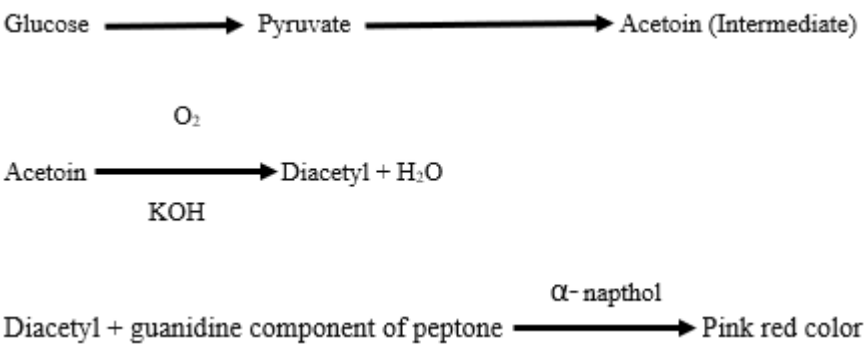
4.4.3.10. Voges Proskauer test:

While most bacteria have the ability to ferment or metabolize glucose, various bacteria may produce different end products from this process. After glycolysis, some proceed via the butylene glycol pathway to make acetylmethylcarbinol (acetoin) and butanediol, while others proceed via the mixed acid fermentation pathway to transform pyruvate into a stable organic acid combination.

The VP test is a biochemical procedure to determine if bacteria can convert pyruvate into acetylmethylcarbinol, also known as acetoin, a neutral intermediate product.

Following glycolysis, pyruvate is generated. This can then be metabolised via the butylene glycol pathway, resulting in the production of two neutral end products: butanediol and acetylmethylcarbinol, which is also referred to as acetoin.

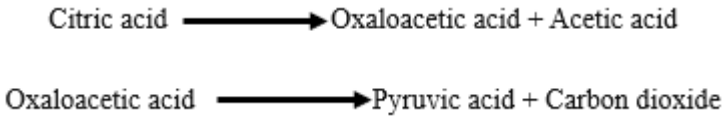
In the presence of KOH (potassium hydroxide) and oxygen (O_2), the acetoin so generated will oxidize to diacetyl. When diacetyl is created in this way and combined with naphthol, it reacts with the guanidine component of peptone to form a polymer that is coloured pink to crimson.



4.4.3.11. Citrate Utilization test:

Citrate can be utilized by some bacteria as a carbon source in absence of glucose or lactose. It is the first major intermediate in the Kreb's cycle. The enzyme citrase breaks citrate into oxaloacetic acid and acetate. These products are then enzymatically converted to pyruvic acid and carbon dioxide.

In spite of the production of acid the medium becomes alkaline due to the formation of sodium carbonate upon reaction with carbon-di-oxide and the sodium ion present in medium. This ultimately changes the colour of the redox dye bromothymol blue to prussian blue.



4.4.4. Antibiotic Resistance Profiling:

In order to ascertain the isolates' susceptibility to various antibiotics, the Kirby-Bauer disc diffusion assay was used.

In clinical microbiology, the Kirby-Bauer disc diffusion assay, sometimes referred to as the disc diffusion test, is a frequently used technique for ascertaining the sensitivity of bacteria to diverse antimicrobial drugs. It offers a qualitative evaluation of the potency of several antibiotics against particular types of bacteria. The actions listed below were taken:

i. To create a consistent lawn of bacterial growth, a bacterial culture is cultured on a solid agar medium, such as Mueller-Hinton agar.
ii. On the agar surface, small discs with a predetermined dosage of antibiotics are positioned.
iii. After that, the plate is incubated in the ideal conditions to support the growth of the bacteria.
iv. The antibiotic diffuses from the disc into the surrounding agar during incubation, resulting in a concentration gradient.
v. Growth inhibition zones, often referred to as zones of inhibition, will develop around the discs if the bacteria are susceptible to the antibiotic. These zones are measured in millimetres and their size reveals how susceptible the bacteria are to the antibiotic. Greater susceptibility is typically indicated by larger zones.
vi. Organisations such as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) establish standardised interpretation criteria that are used to compare the diameter

of the inhibition zones. According to these standards, breakpoints are defined by which the bacteria are classified as susceptible, intermediate, or resistant to particular antibiotics (Vanstokstraeten et al., 2021).

Healthcare providers can choose the best medications to treat bacterial infections by comparing the zone sizes to the interpretation criteria. The Kirby-Bauer test results, in conjunction with additional clinical and patient data, aid in the decision-making process for antibiotic medication.

4.5. Molecular Identification:

Faecal DNA extraction kit was used to perform the further downstream analyses (Sinclair et al., 2023). The steps were written in the following points:

1. 180–220 mg of stool sample was added in a centrifuge tube (preferably 1.5ml), then set the tube on ice.

2. Each stool sample was added with 1 millilitre of Buffer 1. After the stool sample has been completely homogenized, vortex continuously for two minutes.

3. Heat the suspension to 95°C for 5 minutes, then vortex it for 30 seconds.

4. Centrifuge the sample for five minutes at room temperature (RT) at 10,000 rpm to pellet the faeces particles.

5. Fill a fresh 1.5 ml microcentrifuge tube with 15 µl proteinase K.

6. Transfer 200 µl of the step 4 supernatant into the 1.5 ml proteinase K microcentrifuge tube.

7. 200 µl of Buffer 2 was added and mixed for 45 seconds to achieve homogeneity.

8. Then, it was incubated at 70°C.

9. The lysate was mixed with 200 µl of absolute ethanol and vortexed for 45 seconds.

10. Next, add 600 µl of the lysate from step 9 to the spin column. Close the cap and centrifuge for 5 minutes at 10,000 rpm.

11. Then, carefully open the spin column and add 500 µl of Wash buffer 1. Centrifuge for 5 minutes at 10,000 rpm. Finally, discard the tube containing the filtrate and replace the spin column in the collection tube.

12. Gently open the spin column, then fill it with 500 µl of Wash Buffer 2. Centrifuge at 10,000 rpm for ten minutes, RT Reinstall the spin column in the collecting tube after discarding the filtrate-containing tube.

13. Centrifuge at 10,000 rpm for six minutes, RT, to eliminate any remaining buffer.

14. Place the spin column in a fresh, clearly marked 1.5 ml microcentrifuge tube, and then pipette 200 µl of Elution Buffer straight onto the membrane. To elute DNA, incubate for five minutes at room temperature and then centrifuge for five minutes at 10,000 rpm, RT.

15. To prevent DNA breakage, add 500 µl of ice-cold 100% ethanol and 20 µl of 3 M sodium acetate pH 5 2 to the sample. Mix in a figure-of-eight motion.

16. To precipitate the DNA, store at 200°C for an entire night.

17. Centrifuge for 30 minutes at 4 rpm in order to keep the sample from overheating.

18. Remove the supernatant and wash the DNA by adding 600 µl of 70 percent ethanol and centrifuging for 15 minutes at 14000 rpm in reverse orientation.

19. Remove the supernatant and let the particle air dry in a Laminar air flow until the tubes stop emitting an alcoholic odour.

20. Gently tap 30 µl of TE buffer (pH 8.0) to dissolve the DNA.

21. Run the eluted DNA on a single agarose gel to assess its quality.

For amplification of 16 S sequence, set up the PCR reaction as follows:

16S rRNA gene fragment was amplified using the two 16S specific universal primer 27F and 1492R. The total reaction volume was 25 µl. The components of the reaction was tabuled below (Table: 4.5.1). The PCR reaction cycle was started with initial denaturation at 95°C for 5 minutes followed by 30 cycles of denaturation 95°C for 1 minute, annealing at 60°C for 45 seconds, extension at 72°C for 1minute 30 seconds and then a final extension at 72° for 7minutes (Table: 4.5.2) (Sinclair et al., 2023).

For total reaction volume of 25 µl,

Component	Volume
Template	2 µl
$MgCl_2$	1 µl
dNTP mix	2 µl
Forward Primer	1 µl
Reverse Primer	1 µl
Taq Buffer	5 µl
Taq Polymerase	0.5 µl
Molecular grade water	12.5 µl

Table 4.5.1: Components of PCR

Set the PCR reaction in the Thermocycler as below:

No. of cycles- 30, for steps 2-4.

Steps	Temperature	Time
1	95°C	5 min
2	95°C	1 min
3	60°C	45 s
4	72°C	1 min 30 sec
5	72°C	7 min
6	4°C	∞

Table 4.5.2: PCR reaction cycle

4.5.1. PCR Product Purification Using Agarose Gel Extraction

The steps involved in purifying PCR products using a commercially available QIAGEN gel extraction kit (Zeden and Gründling, 2023).

4.5.1.1. Preparation:

Gel Electrophoresis: Prepare a 1% agarose gel, load your PCR products, and run the gel using electrophoresis to separate the DNA fragments.

DNA Band Visualization and Excision: Visualize the separated DNA fragments under UV light and identify the bands containing your desired PCR product. Carefully excise these bands using a sterile scalpel and transfer them to sterile Eppendorf tubes.

4.5.1.1.2. DNA Extraction:

Weight Estimation of Gel Slice: While not explicitly stated, it's crucial to estimate the weight of the gel slices according to the kit instructions. This information is necessary for adding the appropriate volumes of buffers in subsequent steps.

Buffer Addition: Add 3 volumes of Buffer QG (as specified by the QIAGEN Gel Extraction Kit protocol) to the gel slice in the Eppendorf tube. This buffer helps dissolve the agarose and allows the DNA to bind to the silica membrane in the spin column later.

Incubation: Incubate the mixture at 50°C for 10 minutes. This incubation facilitates efficient agarose dissolution and promotes DNA binding to the silica membrane.

Isopropanol Precipitation: Add one volume of Isopropanol and mix thoroughly. Isopropanol helps precipitate the DNA out of the solution, allowing it to bind to the silica membrane during centrifugation.

Spin Column Purification: Load the sample onto a spin column placed in a collection tube and centrifuge. During centrifugation, the flow-through containing buffer, primers, and other contaminants passes through the column, while the DNA binds to the silica membrane. Discard the flow-through.

Washing with Buffer QG: Add another 500 µl of Buffer QG to the spin column and centrifuge. This step washes away any remaining impurities from the isolated DNA.

Washing with Buffer PE: For a final wash, add 750 µl of Buffer PE to the spin column and centrifuge. Buffer PE removes salts and other contaminants that might interfere with downstream applications.

Air Drying: Perform a short air-drying step to remove any residual ethanol from the wash buffer, as ethanol can inhibit enzymatic reactions often used after DNA purification.

Elution: Finally, for elution (retrieving the purified DNA), add 20 µl of deionized water to the spin column and centrifuge. The DNA gets released from the silica membrane and eluted in the water, ready for downstream applications like sequencing or cloning.

4.6. Whole Genome analyses of Resistant isolates:

For bacterial culture, quality assessment of the raw fastq reads of the sample was performed using FastQC v.0.12.1 (Simon, 2023). The raw fastq reads were preprocessed using Fastp v.0.23.4 (Chen et al., 2023), followed by quality re-assessment using FastQC and summarization using MultiQC (Ewels et al., 2016). The processed paired-end reads were assembled into contigs using Unicycler v.0.5.0 (Wick et al., 2017) with assembly polishing using Racon v.1.5.0 (Vaser et al., 2017) and Pilon v.1.20 (Walker et al., 2014) to obtain the final assembled genome. The genome was visualized using Proksee (Grant et al., 2023).

The final assembled genome was annotated using RAST tool kit (RASTtk) (Brettin et al., 2015), Bakta v. 1.8.2 (Database: v.5.0 – Light) (Schwengers et al., 2021) and Prokka v.1.14.6 (Seemann, 2014). Mapping of mobile genetic elements were performed using Alien Hunter v.1.7 (Vernikos and Parkhill, 2006) and mobileOG-db v.1.6 (Beatrix) (Brown et al., 2022) followed by detection of phage genomic regions using Phigaro v.2.3.0 (Starikova et al., 2020) and VirSorter2 v.2.2.4 (Guo et al., 2021). Any CRISPR arrays and their associated Cas proteins were detected using CRISPRCasFinder v.4.2.20 (Couvin et al., 2018), succeeded by detection of resistance genes using the Comprehensive Antibiotic Resistance Database (CARD) Resistance Gene Identifier (RGI) v.6.0.3 (Alcock et al., 2023).

For fungus, a comprehensive quality control (QC) pipeline was implemented to ensure the integrity of the raw sequencing data. Initially, FastQC v.0.11.9 assessed the quality profile of the raw FASTQ reads (Andrews, 2010). Subsequently, Fastp v.0.20.1 performed a pre-processing step to eliminate low-quality sequences (Chen et al., 2018). Following pre-processing, FastQC re-evaluated the quality of the cleaned reads, and MultiQC generated a consolidated quality report (Ewels et al., 2016). Subsequent to rigorous quality control measures and pre-processing steps, the paired-end reads were subjected to sequence alignment against a pre-constructed, KMA-compatible NCBI 2019 reference genome database utilizing the K-mer Mapper (KMA) software. The KMA alignment results were subjected to metagenomic profiling via CCmetagen v.1.4.0, and the generated data was visualized using Krona software for enhanced interpretation (Clausen et al., 2018; Marcelino et al., 2020). MEGAHIT assembler version 1.2.9 was employed to assemble the decontaminated reads into a high-fidelity genome assembly (Lind et al., 2021; Li et al., 2015). Reference based scaffolding of genome assembly was

done using RaGOO (Alonge et al., 2019). The assemblies which are generated in each step were subjected to the BUSCO (Simao et al., 2015) v 5. 4. 3 for quantitative assessment of completeness of the genome and to QUAST v5.0.2 (Gurevich et al., 2013) for quality assessment of the assembly contig FASTA file.

The Genome annotation was done using the nf-core/genome annotator (Ewels et al., 2020) pipeline. The protein sequences generated were subjected to PANNZER2 (Törönen et al., 2018) for gene ontology (GO) annotation and transcript sequences generated were subjected to DIAMOND blastx v_2.0.6 (Buchfink et al., 2015) against KEGG database for KEGG annotation and against NCBI's non-redundant protein database (NRDB). The amino acid sequences were taken as inputs for the InterProScan v 5.56-89 (Jones et al., 2014) for the functional analysis of proteins.

4.7. GC-MS analyses of Non- Timber Forest Produces (NTFPs):

GC-MS is an effective analytical tool that exploits the hybrid technique of coupling gas chromatography (GC) with mass spectrometry (MS). GC allows for the separation of volatile compounds in a test sample of a complex mixture which is then combined with the mass spectrometric technique of fragmentation and identification of the separated compounds based on their molecular weight (Nwachukwu et al., 2024).

The tubers (NTFPs) were collected from the dense forests adjacent to the tribal settlements, which are used by them in their regular diet. After the collection, it was sealed in Ziplock bags to minimize contamination. Upon return to the lab, the sample was carefully removed from the bags and rinsed with water to eliminate any adhering dust or debris. Subsequently, the tubers were thoroughly washed, chopped into fine pieces using a sharp knife and placed over an aluminium foil into a hot air oven for 6-7 hours. After

the sample was dried, a methanolic extract was prepared for subsequent downstream analyses by gas chromatography-mass spectrometry (GC-MS) (Nwachukwu et al., 2024).

A virtual screening of the active phytochemicals obtained from the GC-MS data was performed using the canonical SMILES generated from PubChem (Kim et al., 2023). PubChem is an open database of chemical compounds and substances maintained by the National Institutes of Health (NIH) (https://www.nih.gov/). The PubChem database primarily gives information about small molecules as well as a wide range of macromolecules like carbohydrates, nucleotides, peptides, lipids, chemically-modified large molecules, etc. Some of the information available includes physical and chemical properties of the molecules and their chemical structures, their known biological properties and activities, toxicity data and patents (Kim et al.,2004).

The ADMET profiles of the compounds were obtained from SwissADME and pkCSM databases (He et al., 2022). SwissADME is a free website of the SIB Swiss Institute of Bioinformatics that allows for the computation of the physicochemical properties of one or multiple small molecules simultaneously and provides their ADME parameters, drug-likeness and pharmacokinetic properties required for the evaluation of small molecules for drug discovery (Daina et al., 2017).

Following the screening of the phytocompounds based on their ADMET parameters Lipinski violations and lead likeness, a search for potential human targets from the screened compounds was performed using two databases—Swiss Target Prediction, a webtool that estimates the possible macromolecular targets for small molecules (Daina et al., 2019) and Traditional Chinese Medicine Simplified Integrated Database (TCMSID) (Zhang et al., 2022).

4.8. Genome-Wide association study (GWAS) across the subjects:

GWAS typically focus on associations between single-nucleotide polymorphisms (SNPs) and traits, but can be applied to any other genetic variants and any other organisms. SNPs are the most common type of genetic variation in humans, and they occur when a single DNA building block (nucleotide) differs between people (Yirgu et al., 2023).

The data collected about the potential targets and their interaction with our query compounds were compared and correlated with genome variant detection of the members of the tribes under study with the collaboration of mapmygenome similar to a genome-wide association study (GWAS) based prediction of diseases among members of them. GWAS technique is essentially employed to obtain a whole genome analysis of a large population at once in an effort to identify millions of genetic variants present in individuals of that population and correlate their impact on specific disease phenotypes and non-disease traits (Uffelmann et al., 2021). It is used to draw associations between genotypic variations observed in individuals having the same genomic composition inherited from their ancestors and the phenotypic manifestations of such variations. GWAS primarily identifies single-nucleotide polymorphisms, or SNPs by studying the differences in allelic frequencies of genetic variants among subjects exhibiting distinct phenotypes despite being hereditarily similar. The SNPs, along with copy-number and sequence variants that GWAS can also recognize, statistically implies significant associations with the expression of the particular trait or diseased condition (Uffelmann et al., 2021). With a steady increase in the GWAS sample size, the number of genetic variants discovered and insight into the associated phenotypic implications have enhanced significantly, and these results are catalogued in the NHGRI-EBI GWAS

directory. The GWAS data repository has provided a substantial break-through in applied epidemiologic studies (Munro, 2015).

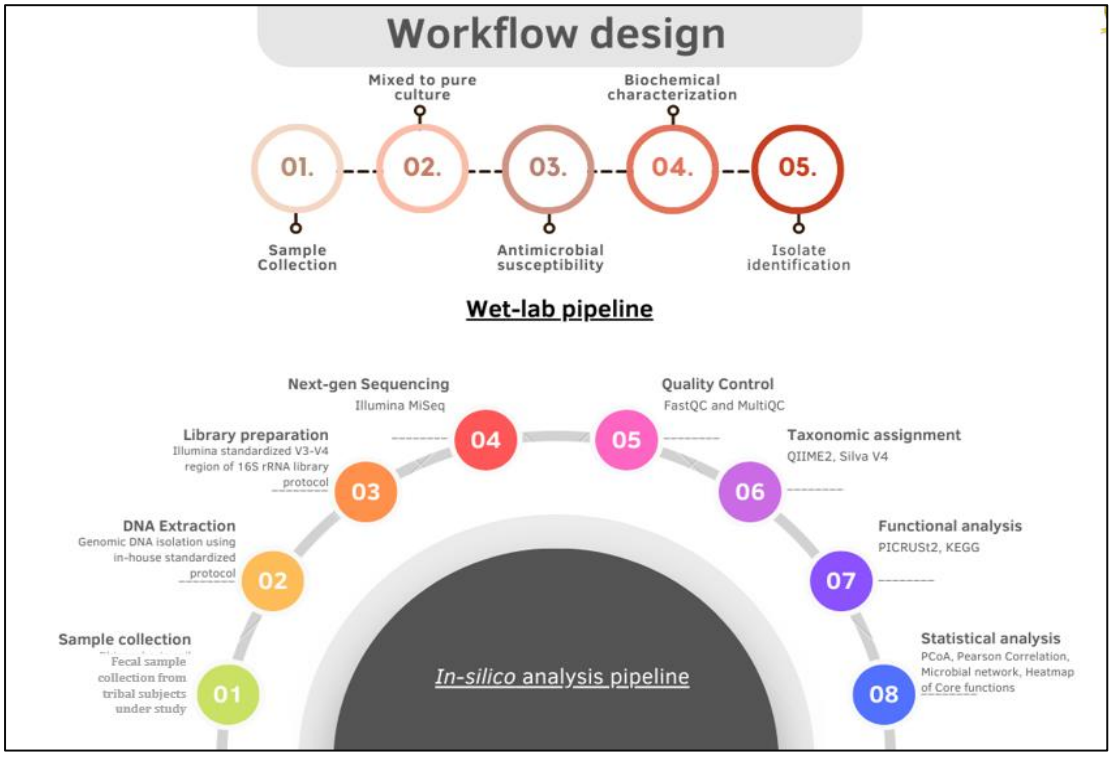


Figure 4.6: Workflow of culture independent and culture dependent analyses

5. Results:

5.1. *Anthropometric Parameters Studied:*

Body Mass Index (BMI), Circumferences, Skinfolds and Body Fat were measured across the subjects under study. In context to BMI, Sabar male member is predicted to be underweight over the period of three years. Whereas, female member can be classified as overweight category. In Bhutia family male and female members gained weight over three years. Therefore, they are classified into overweight category based on their respective BMI. Mech female shows normal BMI whereas mech male exhibits underweight in terms of BMI. Sabar and Mech kids exhibit overweight in context to their BMI whereas Bhutia kid possess normal BMI (WHO, 2000); [Cutoff: < 18.5= Underweight; 18.5-22.9= Normal; 23 & above= Overweight]. In context to Mid- Upper Arm Circumference (MUAC), Sabar male member as well as kid indicates undernourishment whereas female member appears to be normal. Bhutia male and female members remain in the normal category whereas kid indicates undernourishment. Same pattern like Bhutia family has been observed across the Mech members (FANTA, 2017); [Cutoff: <24.0 cm=Undernourished; 24.0 or above= Normal] ((Figure 4). Whereas, in context to urban family we have found normal BMI in male and female member. But the kid showed underweight and undernourished in terms of BMI and MUAC assessment respectively (Figure 5.1.1).

From our somatotype analysis, Sabar male categorised into mesomorphic-ectomorph, female exhibited mesomorphic-endomorph and endomorphic-mesomorph has been observed in context to kid. Bhutia male, female and kid has been categorised into

endomorphic-mesomorph, mesomorphic-endomorph and endomorphic-ectomorph respectively.

In context to Mech male, female and kid exhibited mesomorphic-ectomorph, mesomorphic-endomorph and endomorphic-mesomorph respectively. Urban male, female and kid exhibited endomorphic-mesomorph, mesomorphic-endomorph and balanced mesomorph respectively (Figure: 5.1.2;5.1.3).

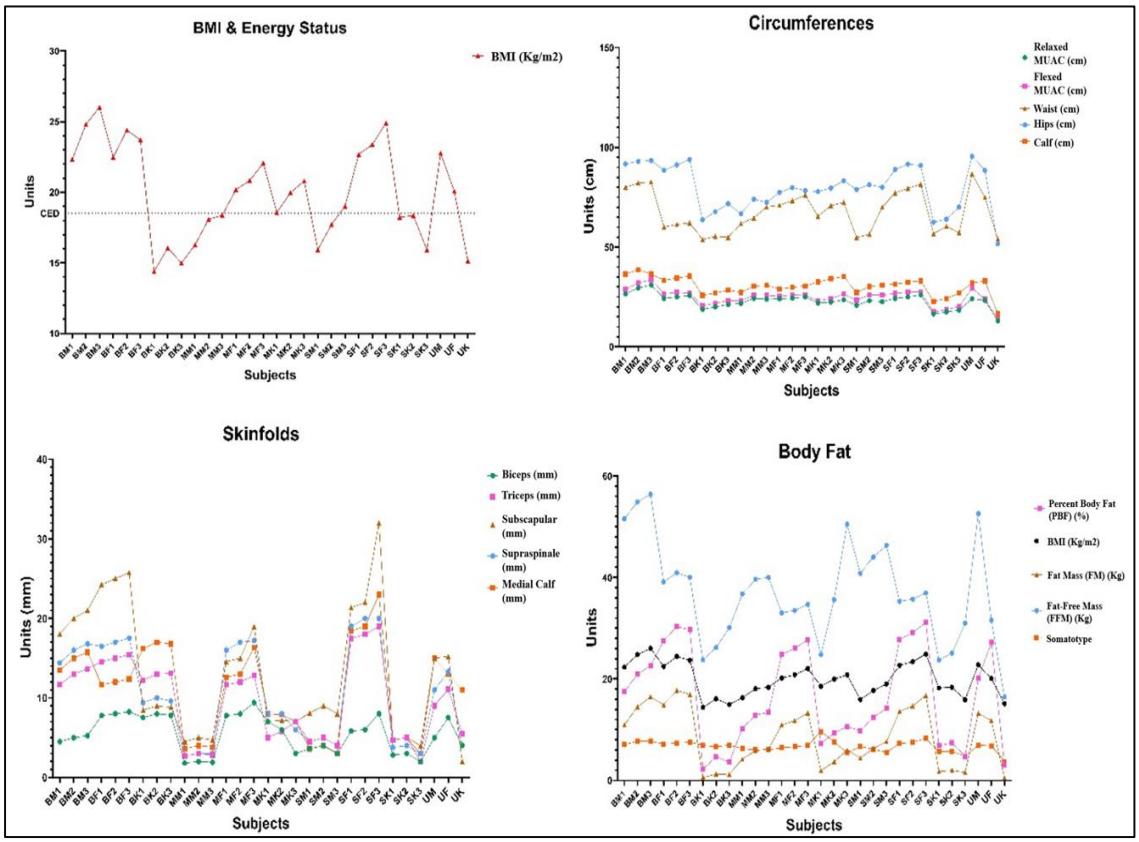


Figure 5.1.1: Anthropometric parameters across the subjects under study

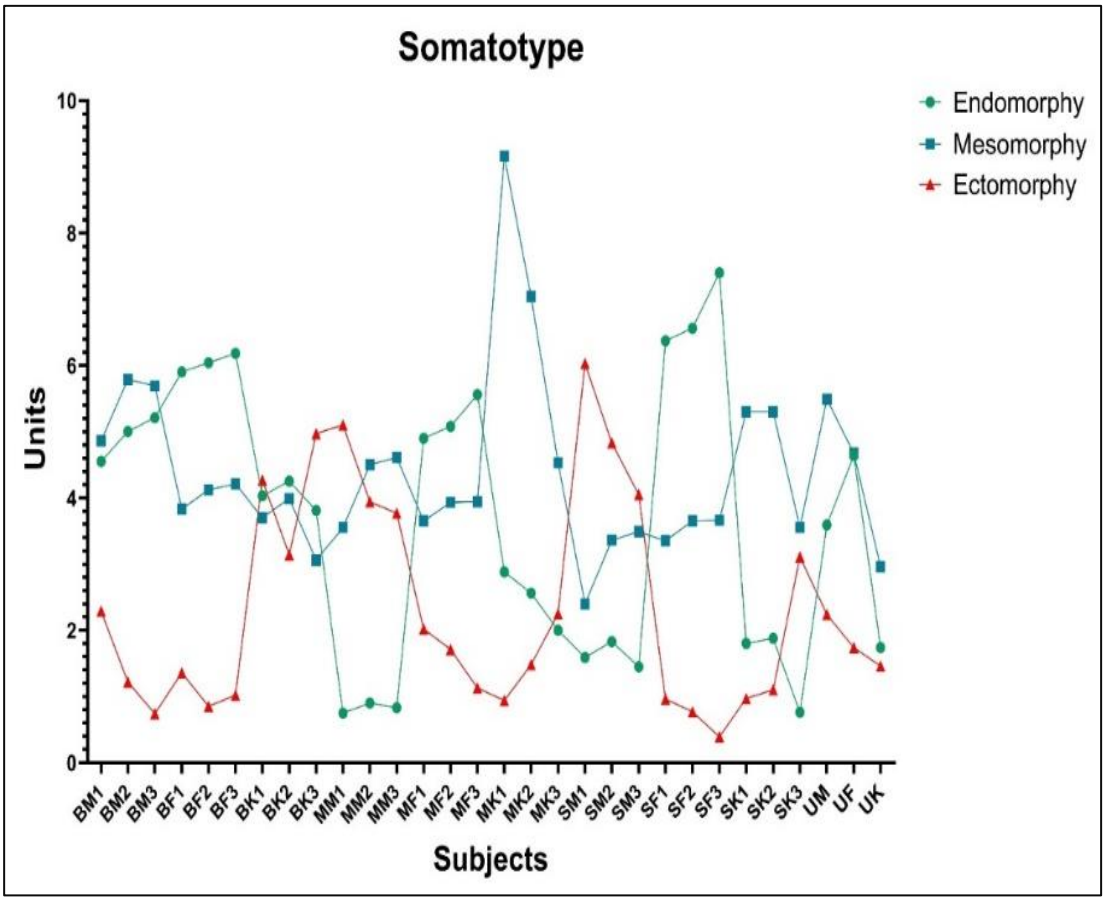


Figure 5.1.2: Somatotype trends across the subjects under study

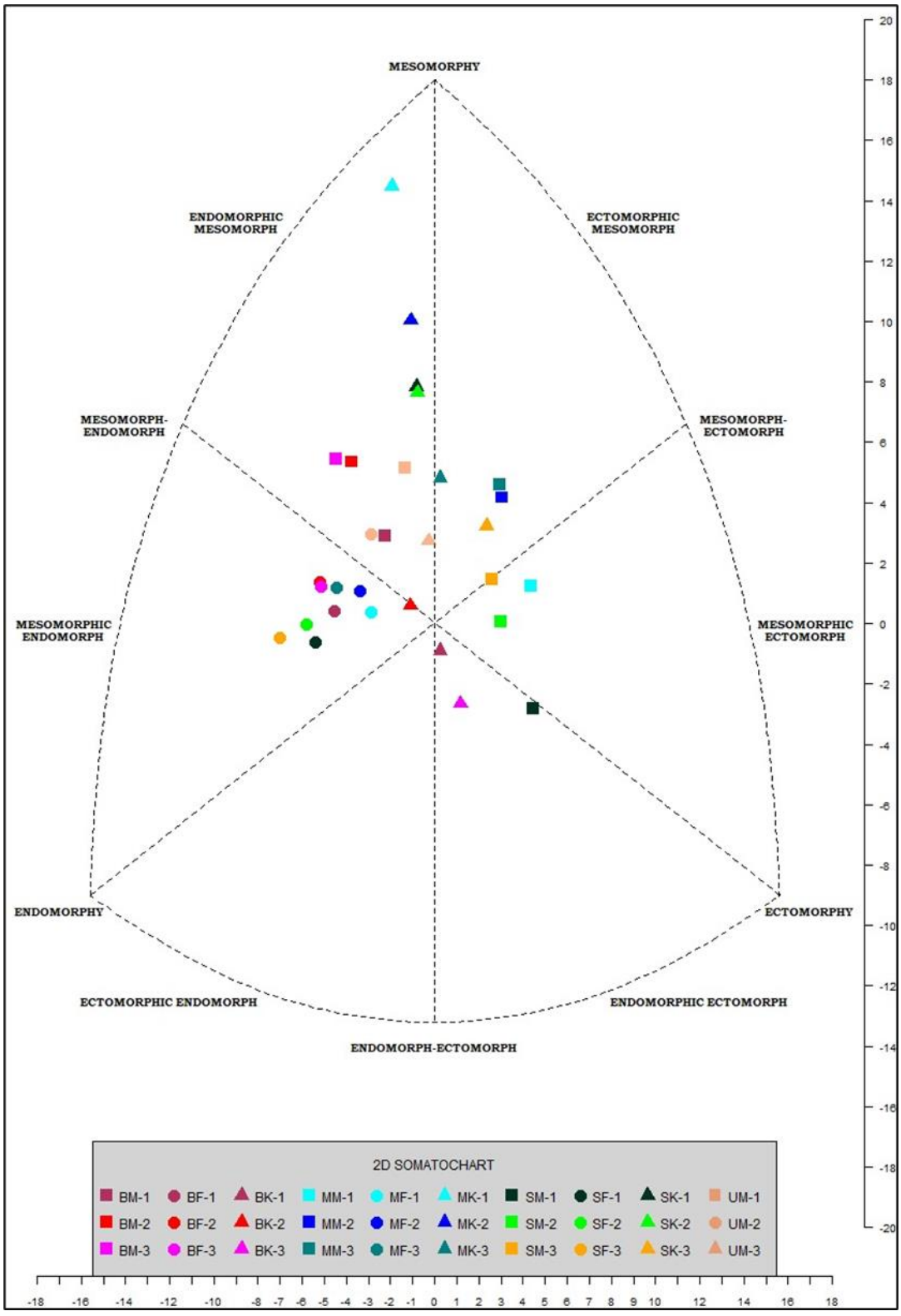


Figure 5.1.3: Somatochart Analysis of our subjects under study

5.2. *Insights into Diet Practices:*

The Sabar community follows a non-vegetarian diet. Rice is their main staple, which they eat twice a day, along with seasonal vegetables such as cabbage, cauliflower, eggplants, tomatoes, pumpkins, radishes, raw papayas, and other wild forest produce. They also include flesh items such as fish, rats, chickens, snails, snakes, which are collected from nearby water bodies or agricultural field. Some individuals in the community have a habit of drinking tea liquor (without milk and sugar) with salt. Additionally, the consumption of alcoholic beverages, both local and traditional, is common among them on a regular basis (Das et al., 2022). Our field studies revealed that these people are still fond of consuming non-timber forest products like roots, tubers, wild fruits, vegetables and wild animals like rats, snakes, civets, rabbits, birds, etc. They have become habituated with everyday staple foods like rice, pulses, vegetables etc., available from the Public Distribution System (PDS) and in the local markets. They used to domesticate cows and buffaloes for obtaining and consuming milk. However, at present, due to the reduction in pastoral area and the high price of those animals, domestication has been vastly reduced.

The Bhutia people have a long history of traditional farming methods. Even today, they rely on raising animals, growing their own vegetables and fruits, and making alcoholic drinks and fermented dairy products. This unique diet likely contributes to a gut microbiome rich in diverse, unprocessed microbes. Base foods are staples like rice but occasionally they prefer beef and pork. But their utilisation of crops like millet, barley, and wheat also demonstrates their agricultural prowess. Potatoes are now a staple food in some regions. Even after the main meal, momo and thukpa are being used for sustenance. Vital nutrition and flavour are added to their meals with lentils and pickles (Sanyal, 1973).

The Mech community's diet consists of a diverse range of locally available vegetables, roots and tubers, pulses, dairy products, and various types of meat. Rice is their staple food, consumed three times a day. Mustard oil is commonly used for cooking purposes. They consume meat from animals such as goats, sheep, buffaloes, fowls, frogs, ducks, as well as eat fish. They have also domesticated animals like buffaloes, goats, and ducks, which provide a regular source of milk, dairy products, and meat. Mech tribe have relied on regional farming methods and continues to depend on fresh fruits along with vegetables from nearby forests, which would aid to preserve the gut's pristine state far away from processed foods. Besides farming, they have also shown a significant interest towards small game hunting as part of alternative livelihood and socio-religious practice Like many other indigenous communities, consumption of alcoholic beverages also seen among them. They also take tea (without sugar and milk) with salt frequently (Sanyal, 1973) (Figure: 5.2.1).

Whereas, urban subjects have been more inclined towards processed meals resulting in a higher intake of refined carbohydrates, saturated fats, sugar and sodium. Urban diets tend to be lower in fiber along with micronutrient deficiencies resulting from lack of fresh fruits, vegetables, whole grains etc. (which are rich sources of vitamins, minerals and antioxidants) in their diet. They also had a tendency to take more sugar components into their diet that was quite natural in context to the urbanized dietary practices.

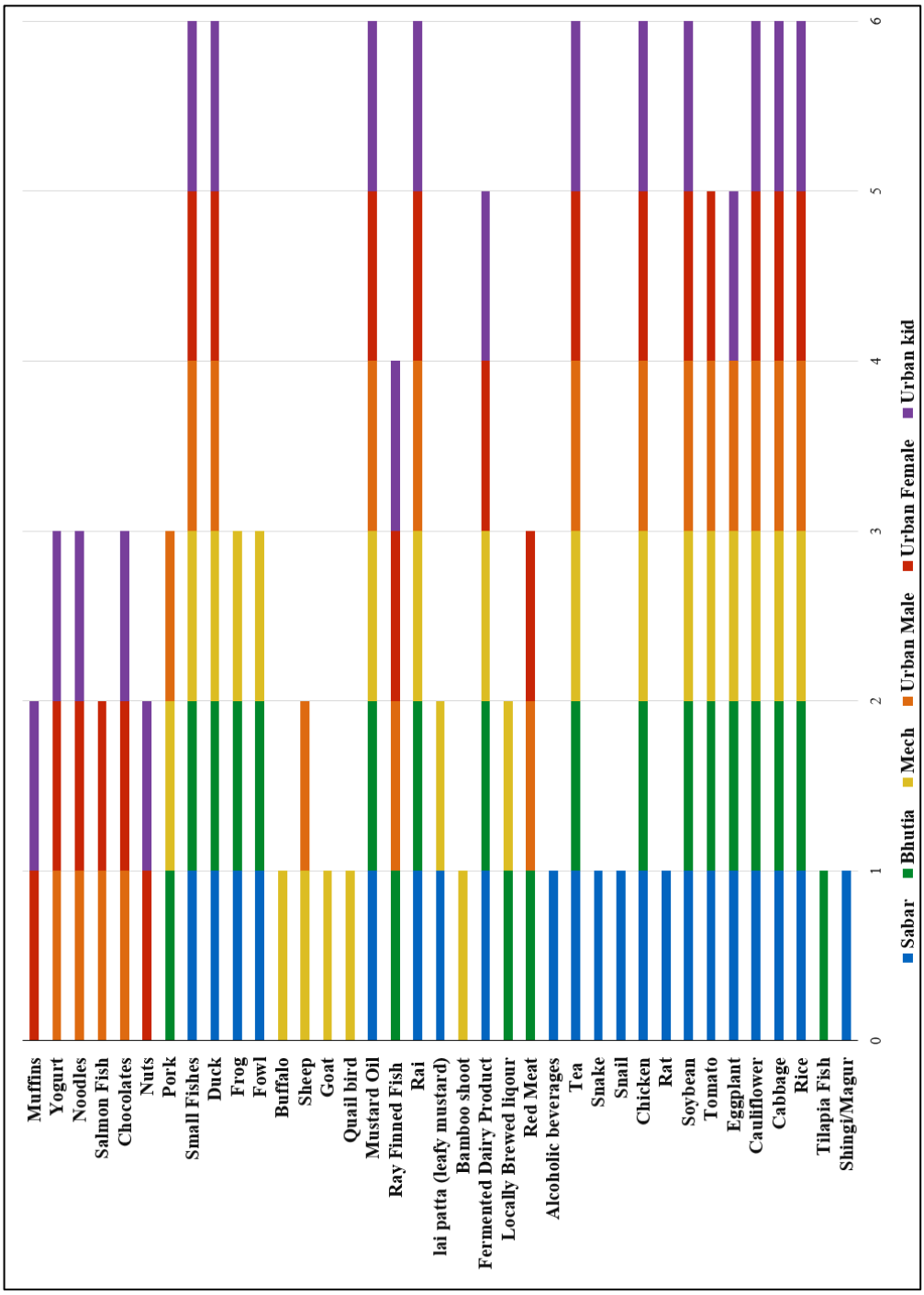


Figure 5.2.1: Dietary Practices of our studied subjects over the years

Sabar (Male, Female and Kid)		Mech (Male, Female and Kid)		Bhutia (Male, Female and Kid)	
Macronutrient	Source	Macronutrient	Source	Macronutrient	Source
Carbohydrates	Starched Rice Roots and Tubers such as Potato, Radish, Tapioca, etc. Wild varieties of vegetables including *Baula audh*, *Chigo*, *Kham audh*, *Churku audh*, *Baya*, *Kulhu audh*, *Muhar audh* and *Bir kundri* Country liquors like *Hadia*, *Mahua* and *Keya*	Carbohydrates	Staple rice Country Liquors including fermented rice liquor and fermented millet liquor Added sugar Maize Wheat Wheat flour Jaggery Puffed rice Flattened rice Rotis Biscuits Potato	Carbohydrates	Staple rice Country Liquors including fermented rice liquor and fermented millet liquor Added sugar Maize Wheat Wheat flour Jaggery Puffed rice Flattened rice Rotis Biscuits Potato Instant Noodles Occasional fast food (Chowmein) Sweetmeat (*Laddoo*)

Proteins	Pulses such as lentils, pigeon pea, Bengal gram and green gram Green leafy vegetables such as leaves of Colocasia, drumstick, cabbage and pumpkin, spinach, water spinach and swampweeds Meats of fowls, fish, pork, rat, snakes, frogs, snails and crabs Eggs	Proteins	Meats of hogs (wild pig), fowls, ducks, pigeons, goats, deers, turtles, hares and snails Lentils Eggs Fish Gram (*Chhola*) Green leafy vegetables	Proteins	Meats of hogs (wild pig), fowls, ducks, pigeons, goats, deers, turtles, hares and snails Lentils Eggs Fish Gram (*Chhola*) Green leafy vegetables

Fats	Mustard Oil Meats of pork and rat Roots and tubers such as potato and Tapioca Wild varieties of vegetables including *Baula audh*, *Chigo*, *Kham audh*, *Churku audh*, *Baya*, *Kulhu audh*, *Muhar audh* and *Bir kundri*	Fats	Ghee Buffalo milk Mustard Oil Occasional fast food	Fats	Ghee Buffalo milk Mustard Oil Occasional fast food (Chowmein) Sweetmeats (*Laddoo*)

Table 5.2.1: Tribal diet profile showing macronutrients and their sources

Urban Male		Urban Female		Urban Kid	
Macronutrient	Source	Macronutrient	Source	Macronutrient	Source
Carbohydrates	Rice, Bread, Pasta, Cereals, Bakery items (cakes, muffins), Noodles	Carbohydrates	Rice, Bread, Pasta, Cereals, Bakery items (cakes, muffins), Fruits	Carbohydrates	Rice, Bread, Pasta, Cereals, Fruits, Sweetened yogurt
Proteins	Meat (chicken, fish, beef), Eggs, Dairy (milk, cheese), Tofu, Lentils	Proteins	Meat (chicken, fish), Eggs, Dairy (milk, cheese), Tofu Lentils	Proteins	Meat (chicken, fish), Eggs, Dairy (milk, cheese), Tofu, Lentils, Beans
Fats	Vegetable oils (olive, canola), Nuts and seeds, fish (salmon, *Tenualosa ilisha* etc.), Butter (in moderation)	Fats	Vegetable oils (olive, canola), Nuts and seeds, Dairy (milk, cheese) in moderation	Fats	Vegetable oils (olive, canola), Nuts & seeds (in moderation), Chocolates

Table 5.2.2: Urban diet profile showing macronutrients and their sources

5.3. Metagenomic Profiling:

Shannon diversity indicating the species richness and evenness of the Gut Bacterial Profiles (GBP) of male, female and kid members of tribal subjects under study. Bray-Curtis Dissimilarity Index was calculated in order to compare the obtained gut bacterial profiles of the sampled tribal and urban individuals (Figure 5.3.1). Quality control of raw reads and identification of the most prevalent taxa based on the OTU clustering were performed. SILVA v138 (Quast et al., 2013), Greengenes (DeSantis et al., 2006) and RDP (Maidak et al., 1996) Databases were used for taxonomic identification via QIIME2 (Bolyen et al., 2019). Multilayered pie chart Krona analyses revealed the prevalence of genera like *Holdemanella*, *Catenibacterium*, *Bifidobacterium*, *Bacteroides*, *Ralstonia*, *Prevotella*, *Faecalibacterium*, *Romboutsia*, *Alcaligenes* across the studied subjects over a period of three years (Figure 5.3.2-5.3.11). We have also identified prevalence of *Holdemanella*, *Catenibacterium*, *Ralstonia*, *Enterococcus*, *Bifidobacterium* etc. across the sabar members under study (Figure 5.3.12). In context to Bhutia we have found the prevalence of *Bacteroides*, *Blautia*, *Faecalibacterium*, *Akkermansia*, *Escherichia* etc. (Figure 5.3.13). Whereas, in Mech family prevalence of *Prevotella*, *Bifidobacterium*, *Streptococcus*, *Catenibacterium* etc. showed higher prevalence over the years (Figure 5.3.14). Urban subjects showed higher prevalence of *Methanobrevibacter*, *Alcaligenes*, *Faecalibacterium, Romboutsia, Hodemanella* etc. (Figure 5.3.15). Intra-Tribal Venn anaysis revealed 14.4%, 17% and 25.8% similarity between the core genera of sabar, bhutia and mech respectively in the yearwise manner (Figure 5.3.16). Whereas 26.6% similarity across the core genera were found in the studied tribes over the years (Figure 5.3.17). Venn analyses exhibited 11.3%, 15.6%, 22.7% and 13.5% similarity between the core genera of sabar vs urban; bhutia vs urban; mech vs urban; all tribes vs urban

respectively (Figure 5.3.18). *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Faecalibacterium*, *Ruminococcus*, *Parabacteroides*, *Blautia*, *Streptococcus*, *Collinsella*, *Dialister*, *Romboutsia*, *Escherichia* were the few representative genera present across the Core GBP of studied subjects under study (Figure 5.3.19). A large number of biological pathways were also predicted, which encompassed both homeotic and response pathways. Surprisingly, we found enrichment of several antibiotic resistance pathways in these datasets, namely vancomycin and cationic antimicrobial peptide (CAMP) resistances. Vancomycin resistance was prevalent in Sabar and Bhutia female as well as in kid gut while CAMP resistance was found in Sabar kid and Mech male gut (Figure 5.3.20). Core Gut Microbial Network Analysis across the subjects under study depicted the bidirectional phenomenon in blue lines whereas unidirectional phenomenon in red lines (Figure 5.3.21). Host intrinsic pathogenic load of the core genera includes lifestyle disorders such as Liver cirrhosis, Inflammatory bowel diseases etc. across the studied subjects (Figure 5.3.22).

Name of Tribe	**Shannon Diversity Index**								
	Male			**Female**			**Kid**		
	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
Sabar	3.64	3.91	2.91	3.64	2.47	2.45	2.99	3.86	2.84
Bhutia	3.24	3.02	3.16	2.89	4.04	2.92	2.91	2.83	3.31
Mech	2.56	3.57	3.08	3.41	3.95	2.24	2.84	3.21	3.70

Table 5.3.1: Shannon Diversity Index of Bacterial Abundances Across Tribes Under Study

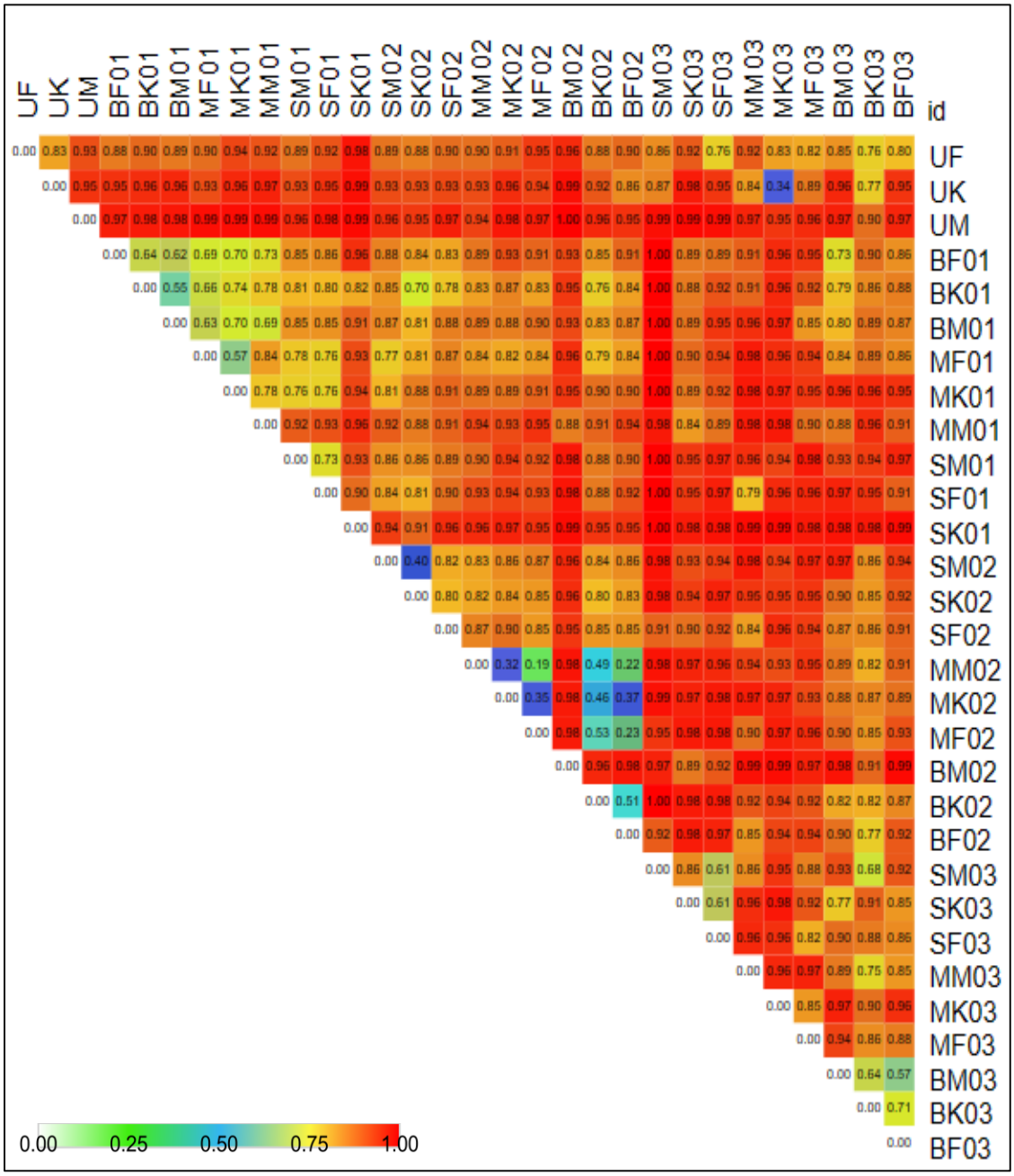


Figure 5.3.1: Beta Diversity across the Tribes and Urban subjects under study

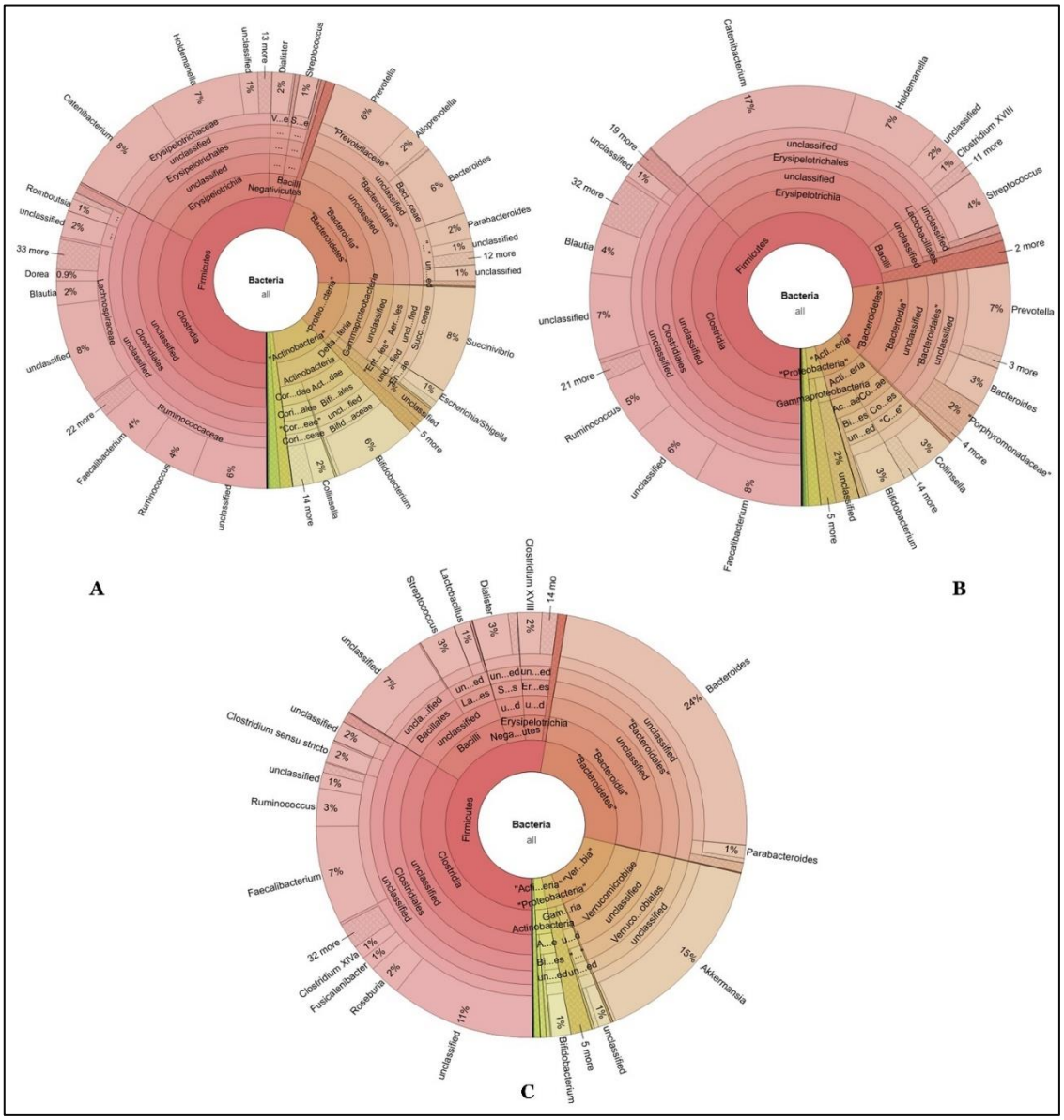


Figure 5.3.2: Krona representation of Sabar tribes under study Year 1 (A: Sabar male; B. Sabar female; C. Sabar kid)

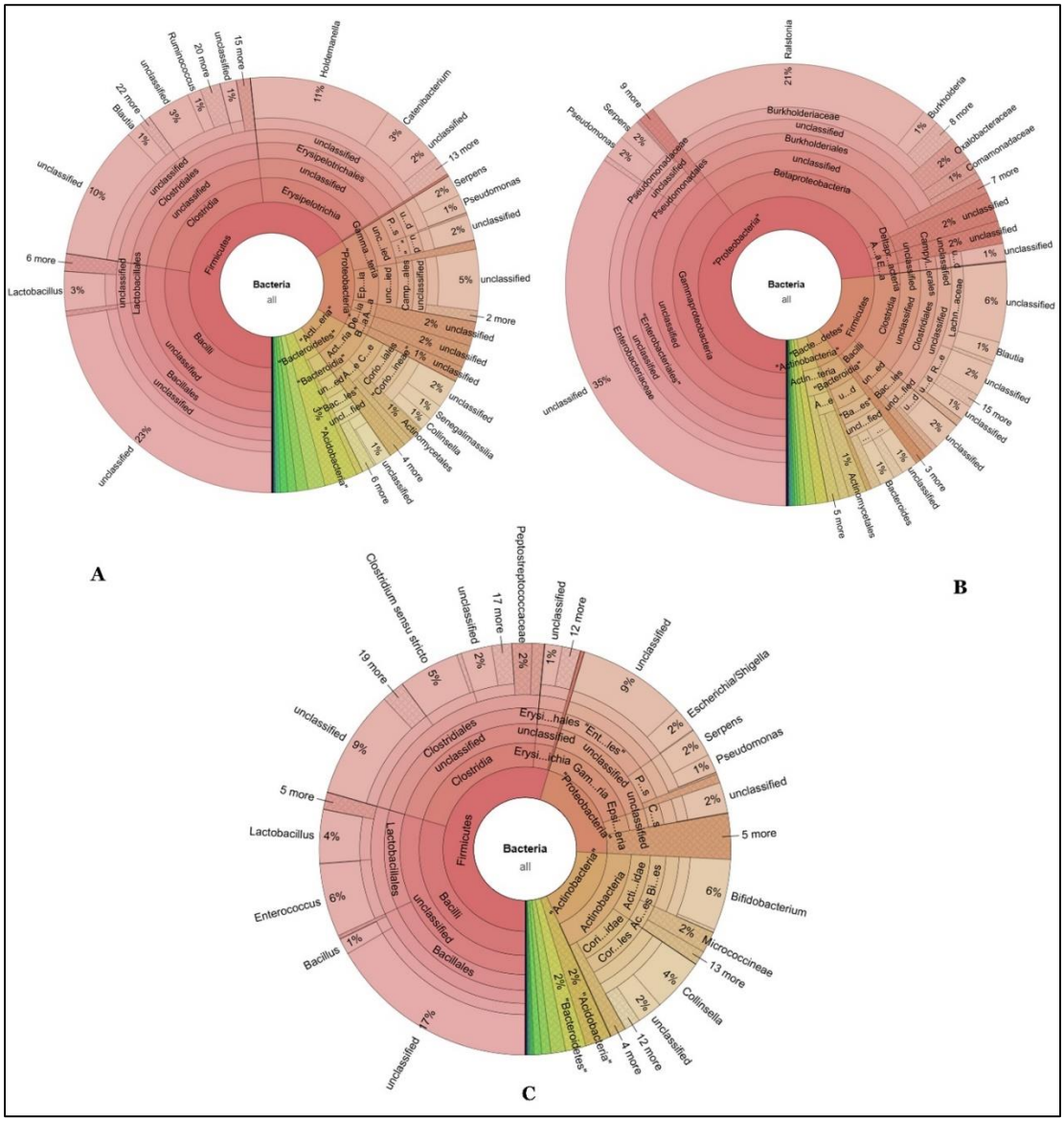


Figure 5.3.3: Krona representation of Sabar tribes under study Year 2 (A: Sabar male; B. Sabar female; C. Sabar kid)

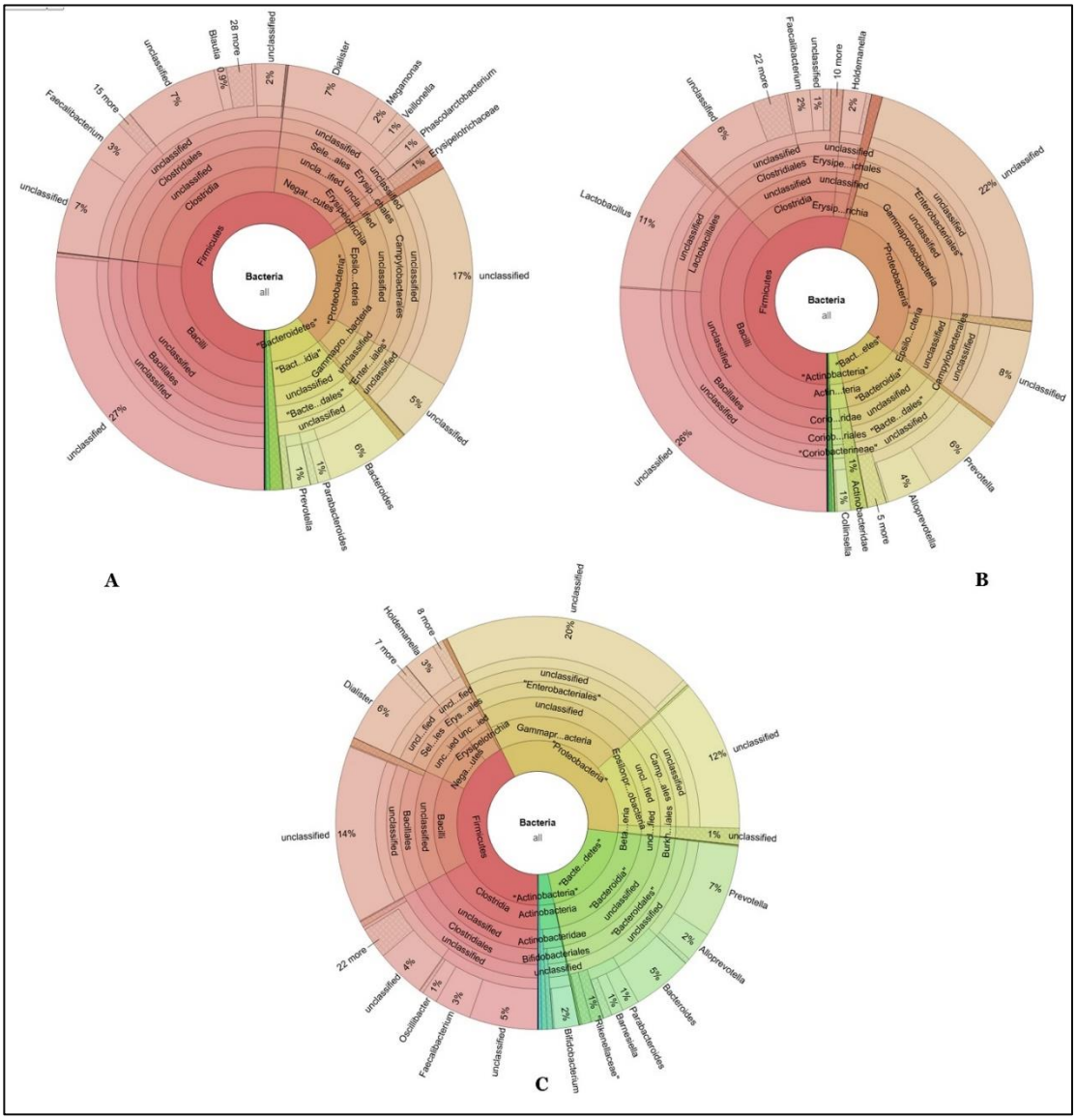


Figure 5.3.4: Krona representation of Sabar tribes under study Year 3 (A: Sabar male; B. Sabar female; C. Sabar kid)

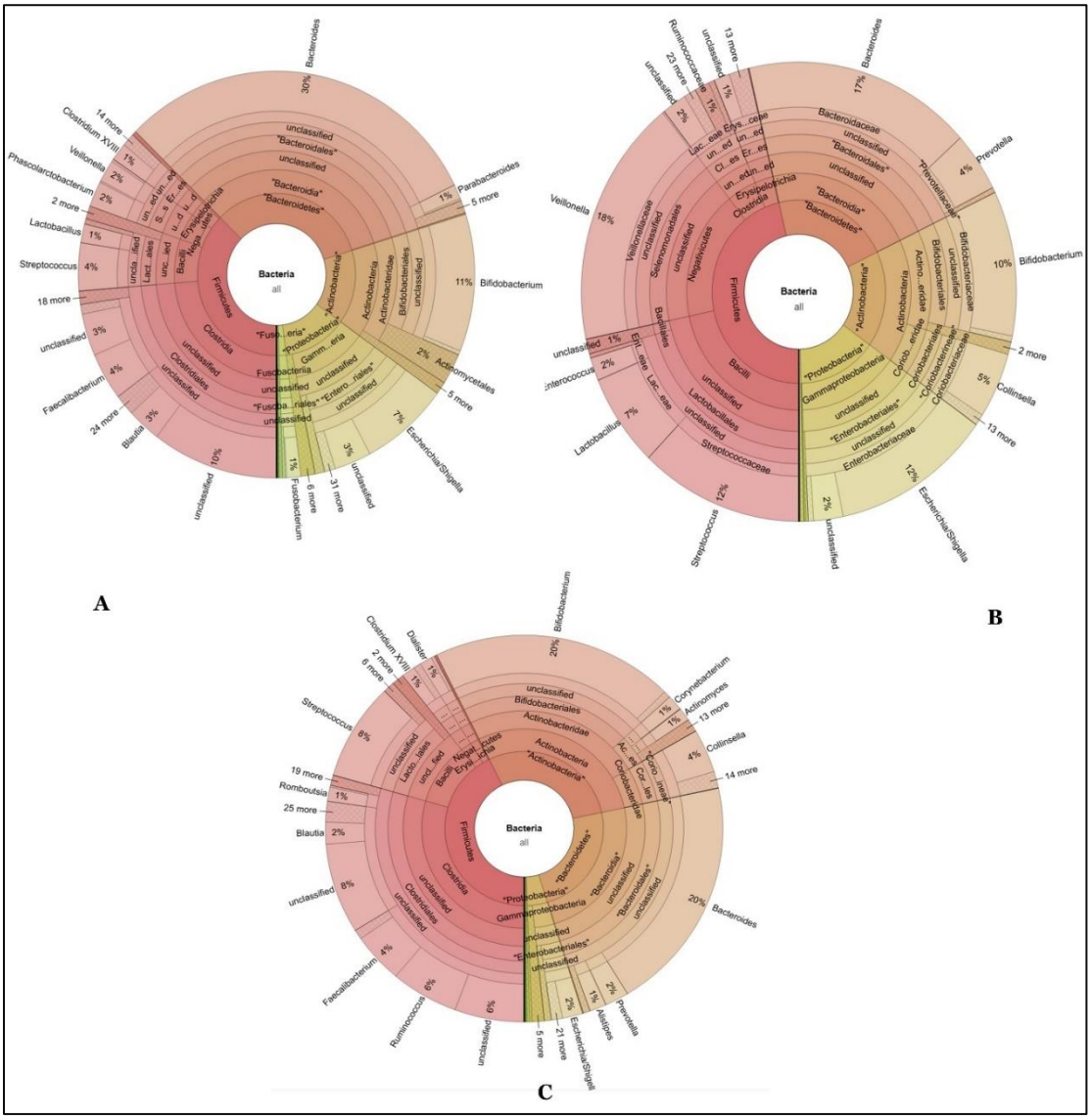


Figure 5.3.5: Krona representation of Bhutia tribes under study Year 1 (A: Bhutia male; B. Bhutia female; C. Bhutia kid)

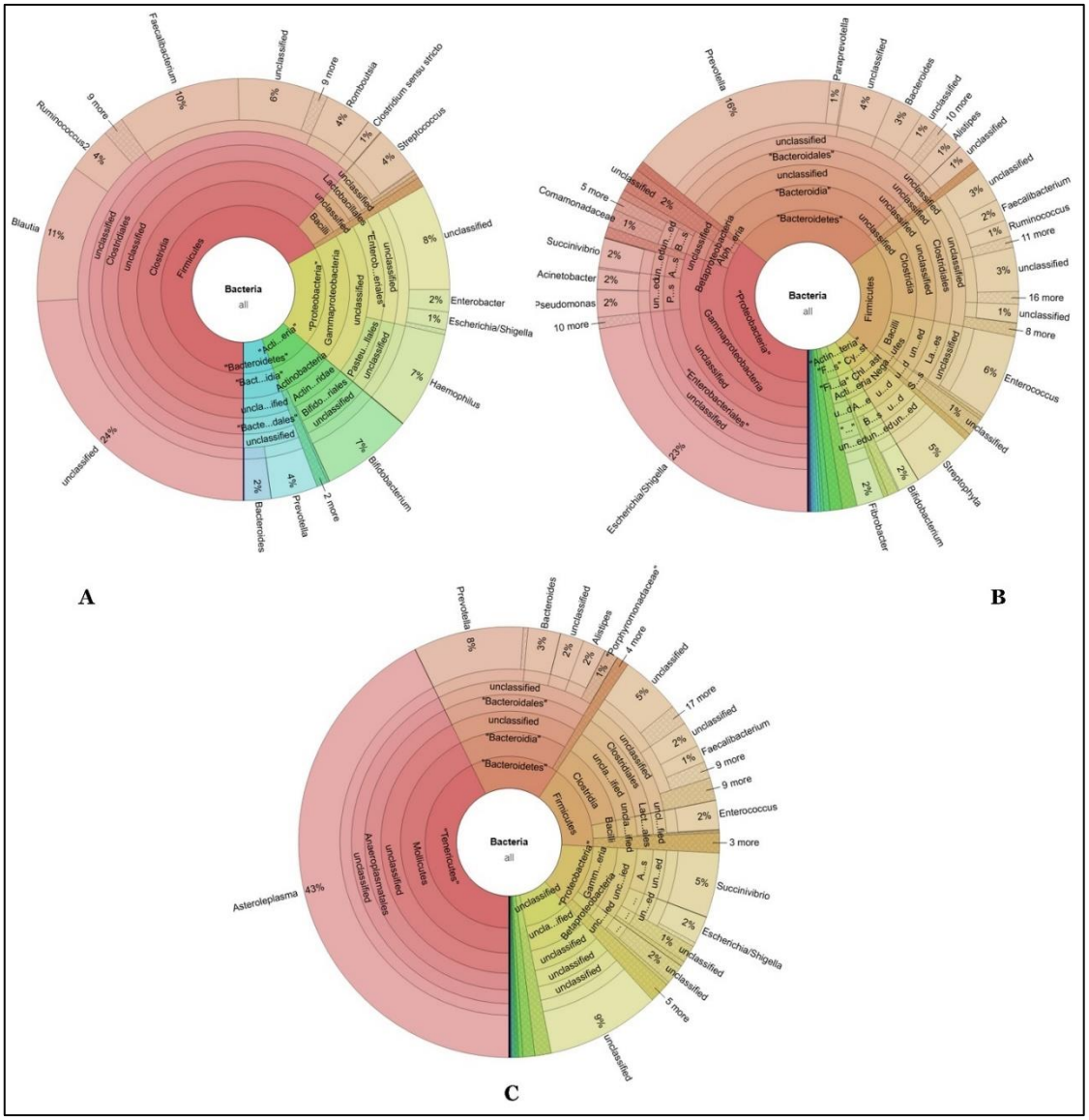


Figure 5.3.6: Krona representation of Bhutia tribes under study Year 2 (A: Bhutia male; B. Bhutia female; C. Bhutia kid)

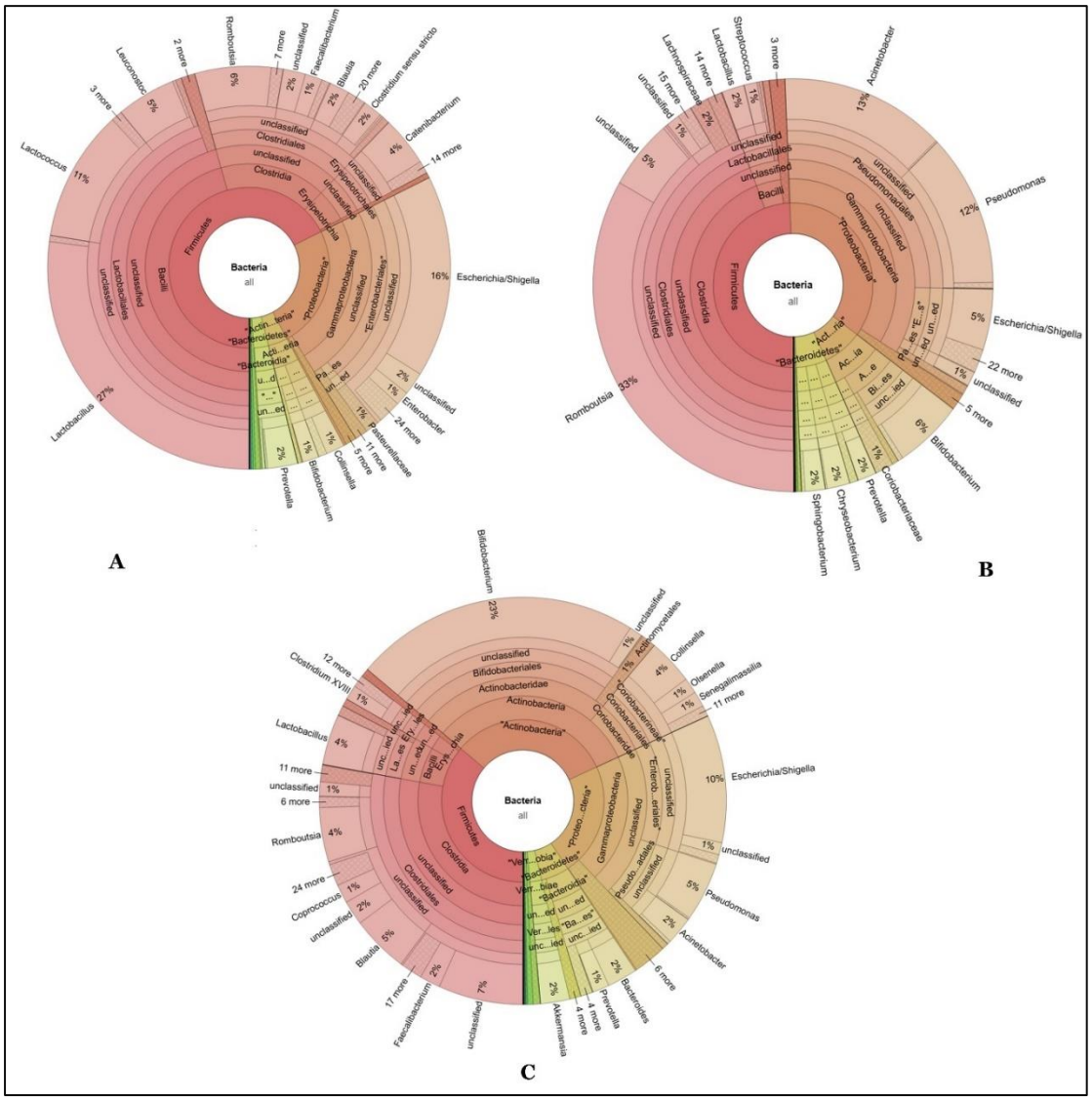


Figure 5.3.7: Krona representation of Bhutia tribes under study Year 3 (A: Bhutia male; B. Bhutia female; C. Bhutia kid)

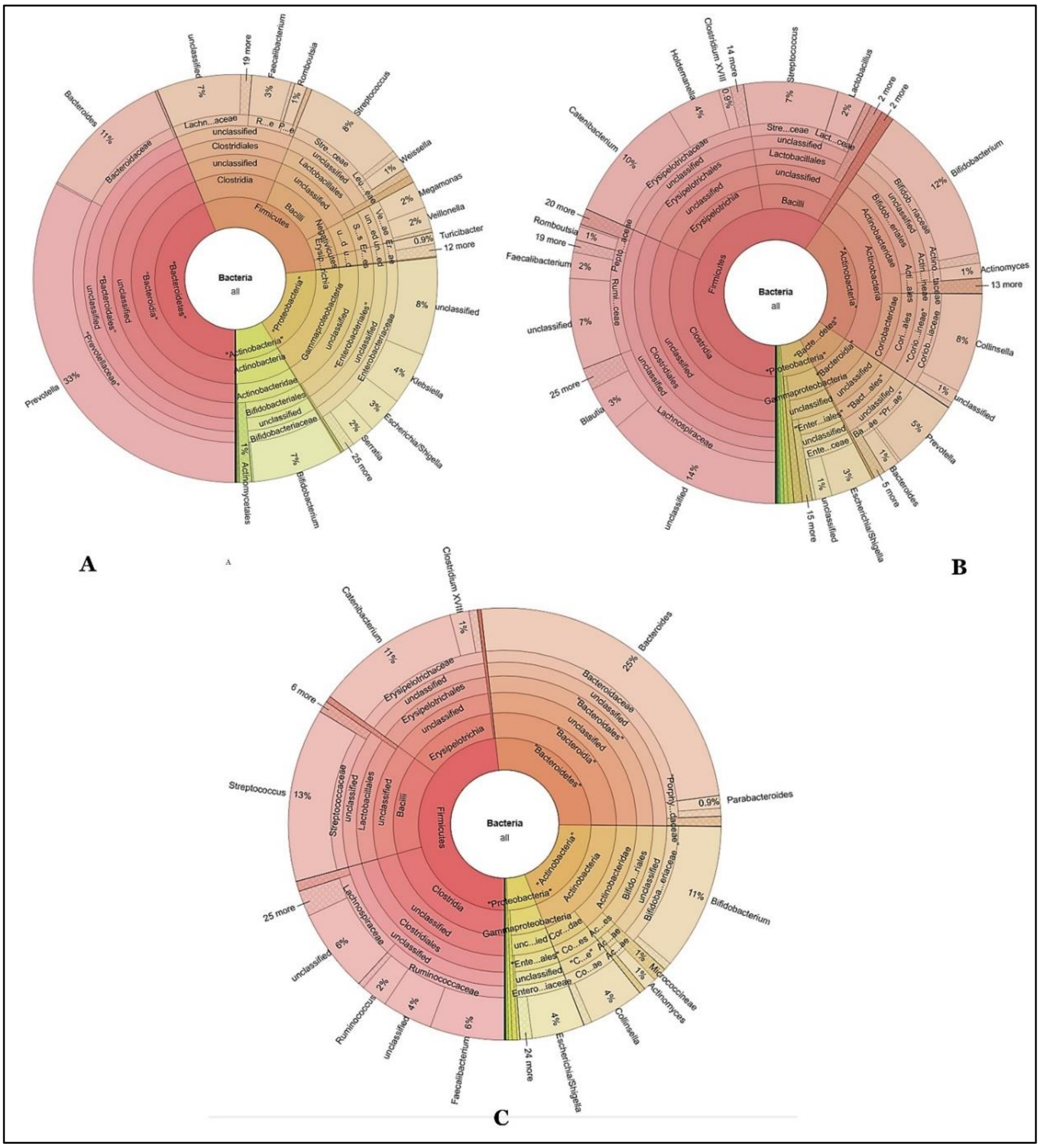


Figure 5.3.8: Krona representation of Mech tribes under study Year 1 (A: Mech male; B. Mech female; C. Mech kid)

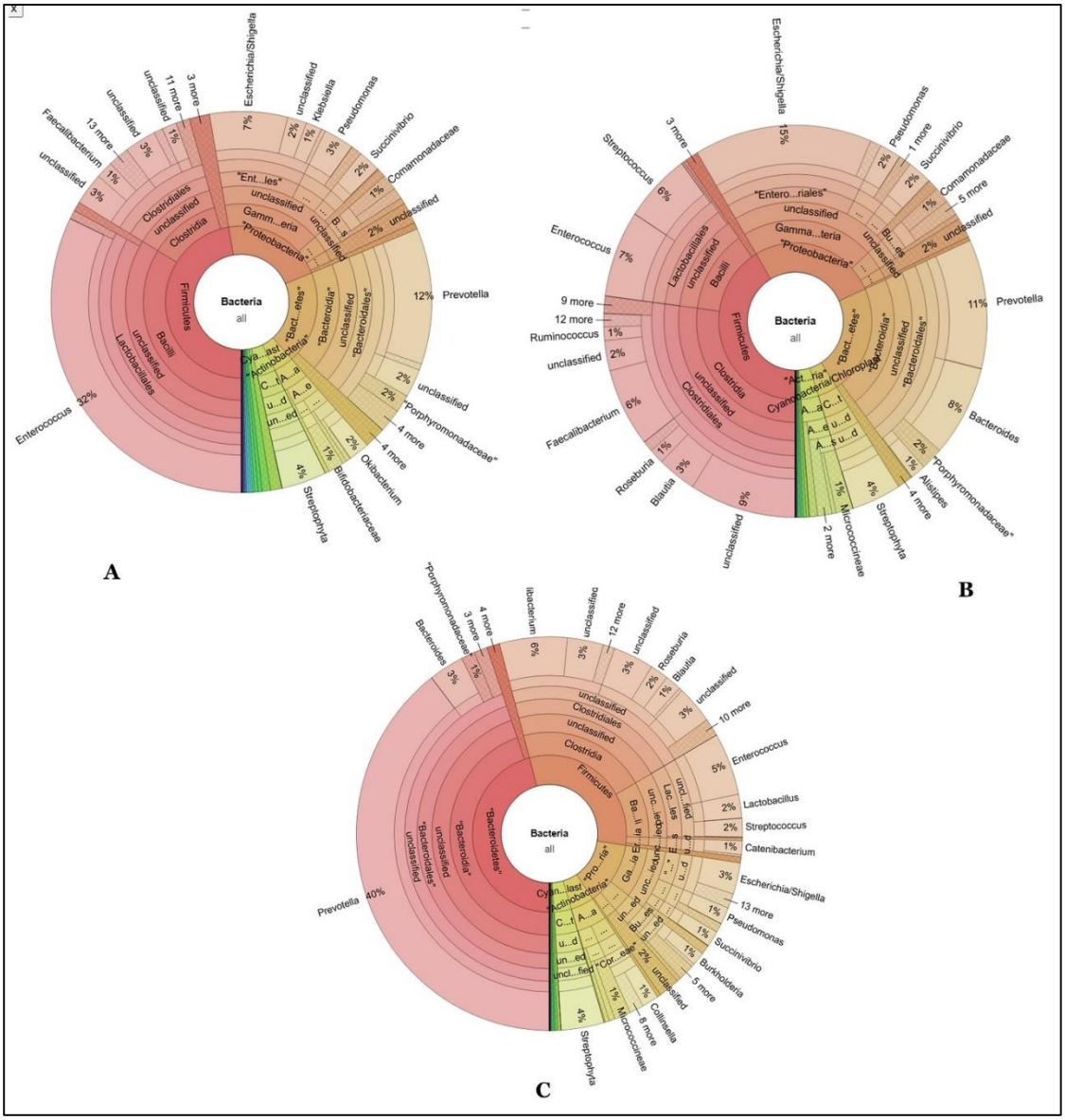


Figure 5.3.9: Krona representation of Mech tribes under study Year 2 (A: Mech male; B. Mech female; C. Mech kid)

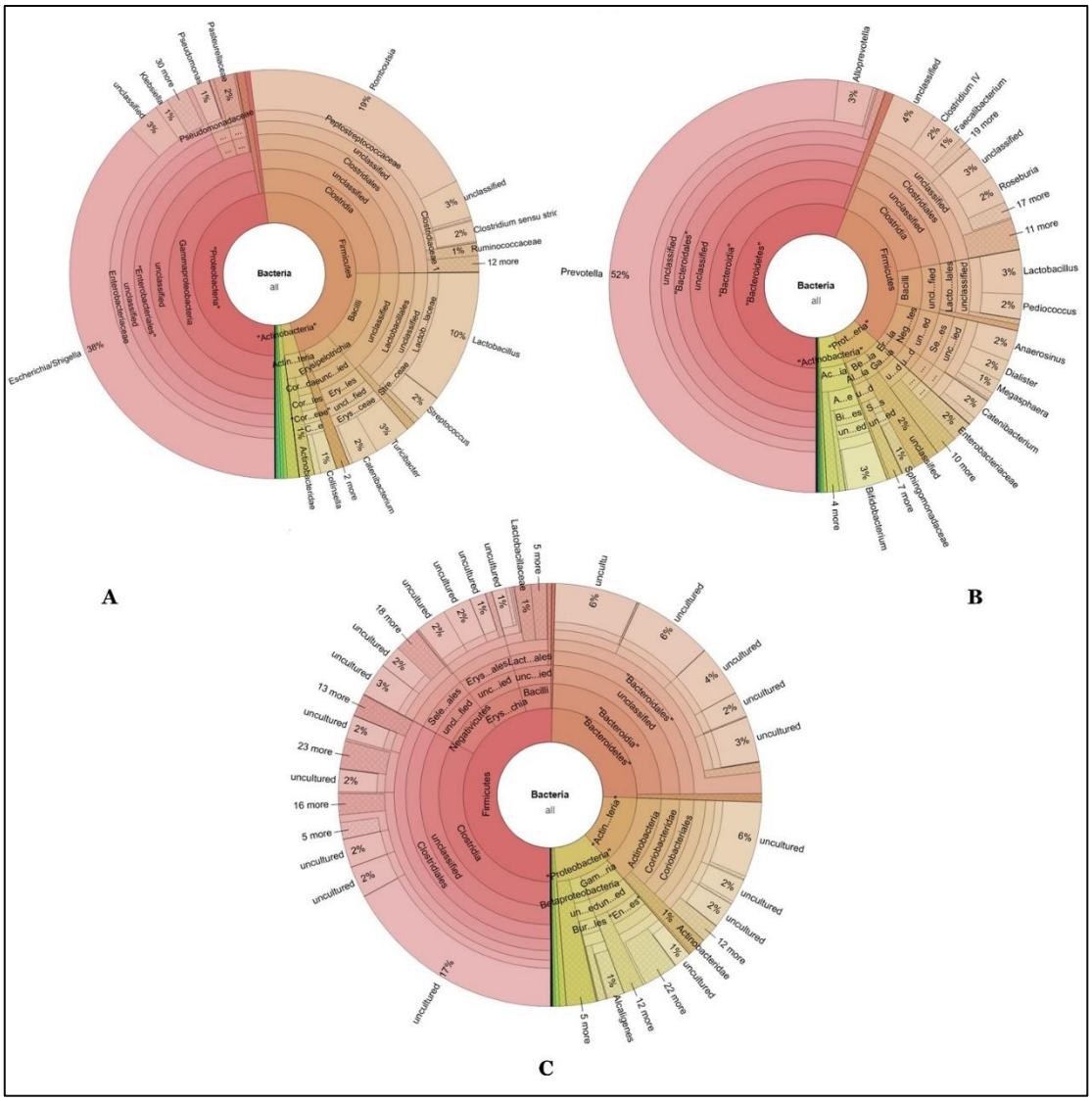


Figure 5.3.10: Krona representation of Mech tribes under study Year 3 (A: Mech male; B. Mech female; C. Mech kid)

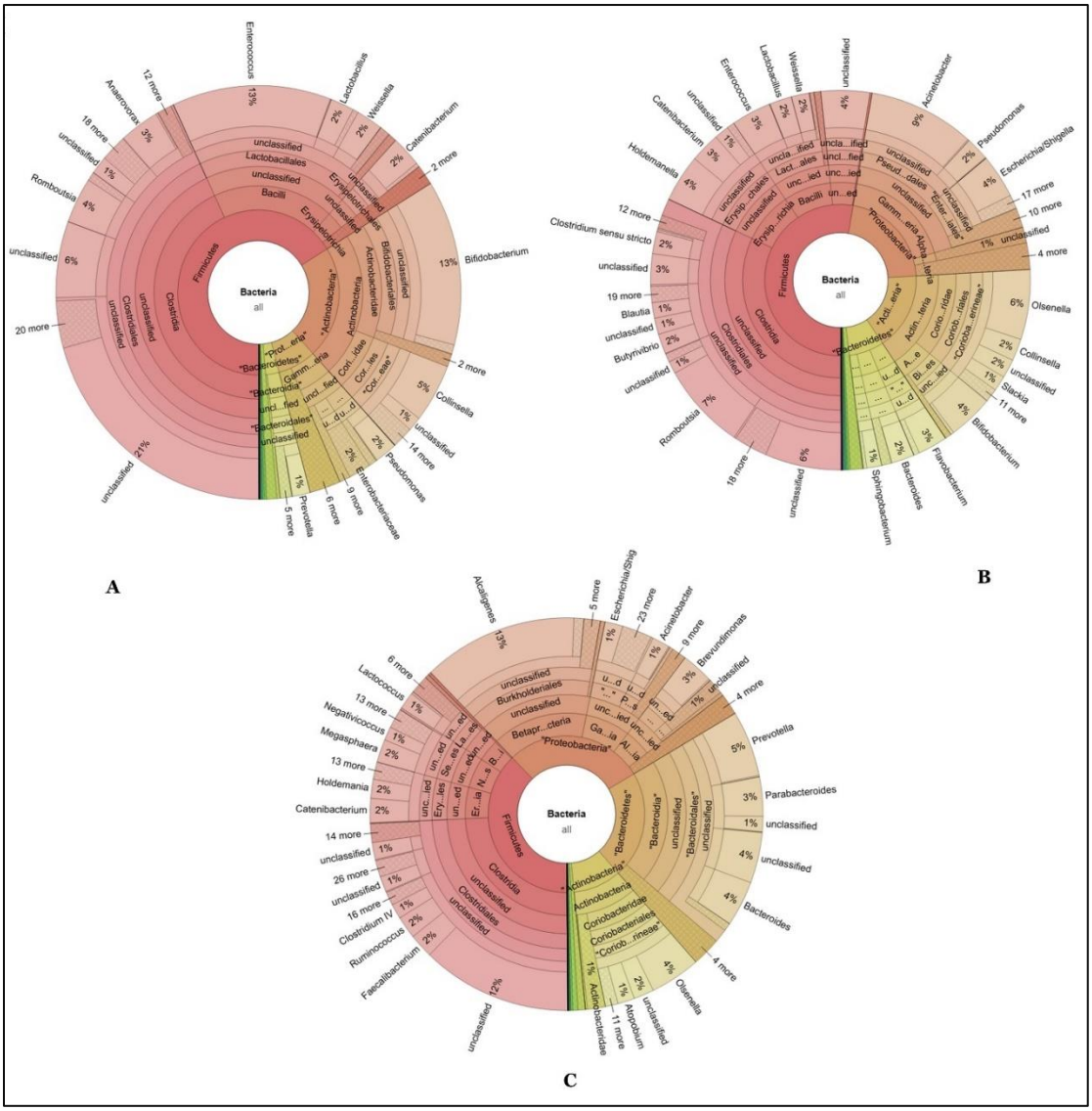


Figure 5.3.11: Krona representation of Urban subjects under study (A: Urban male; B. Urban female; C. Urban kid)

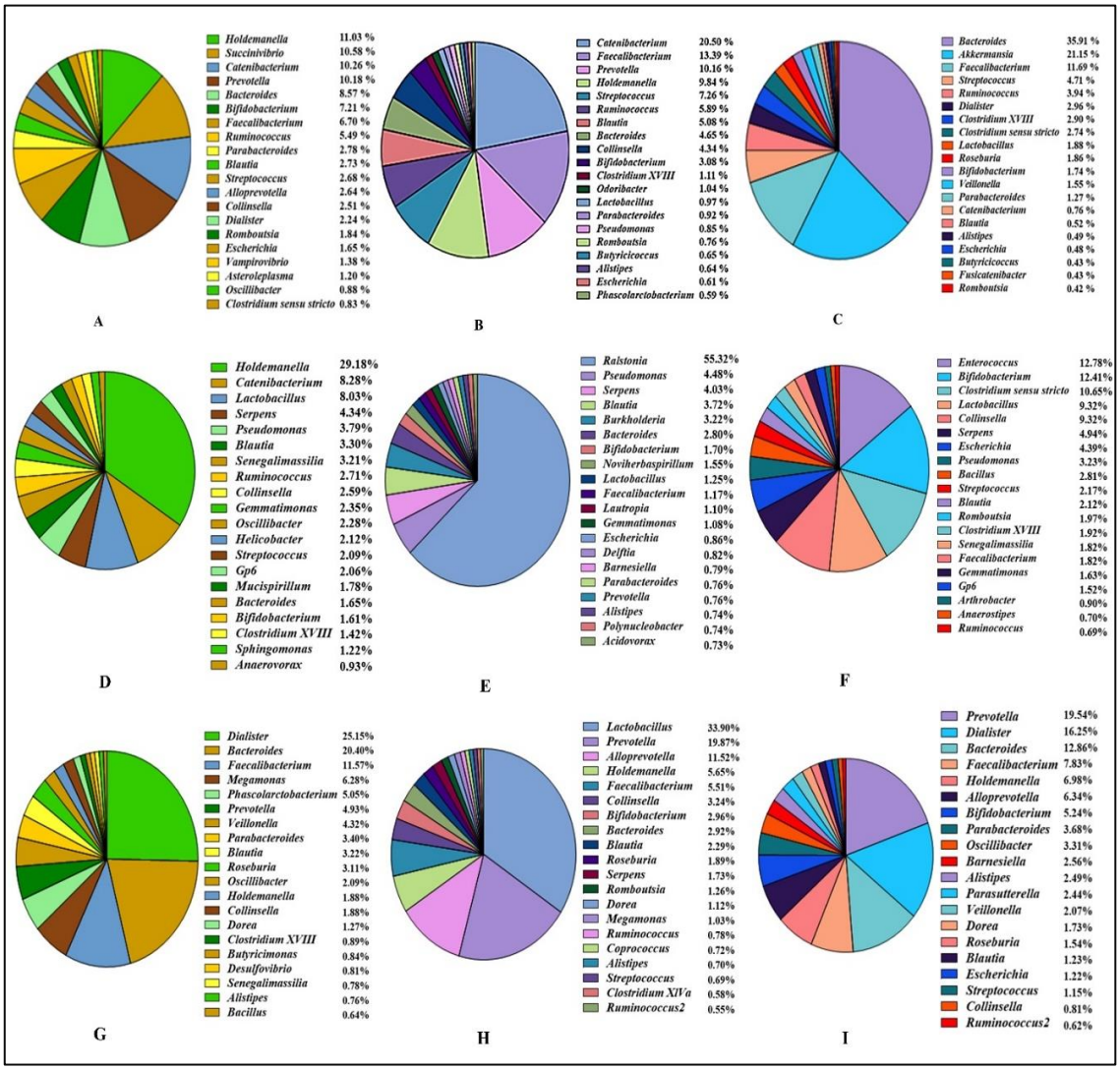


Figure 5.3.12: Bacterial Abundance of Sabar members under study (A, B, C Male, Female and Kid respectively Year 1; D, E, F Male, Female and Kid Year 2 respectively; G, H, I Male, Female and Kid respectively Year 3)

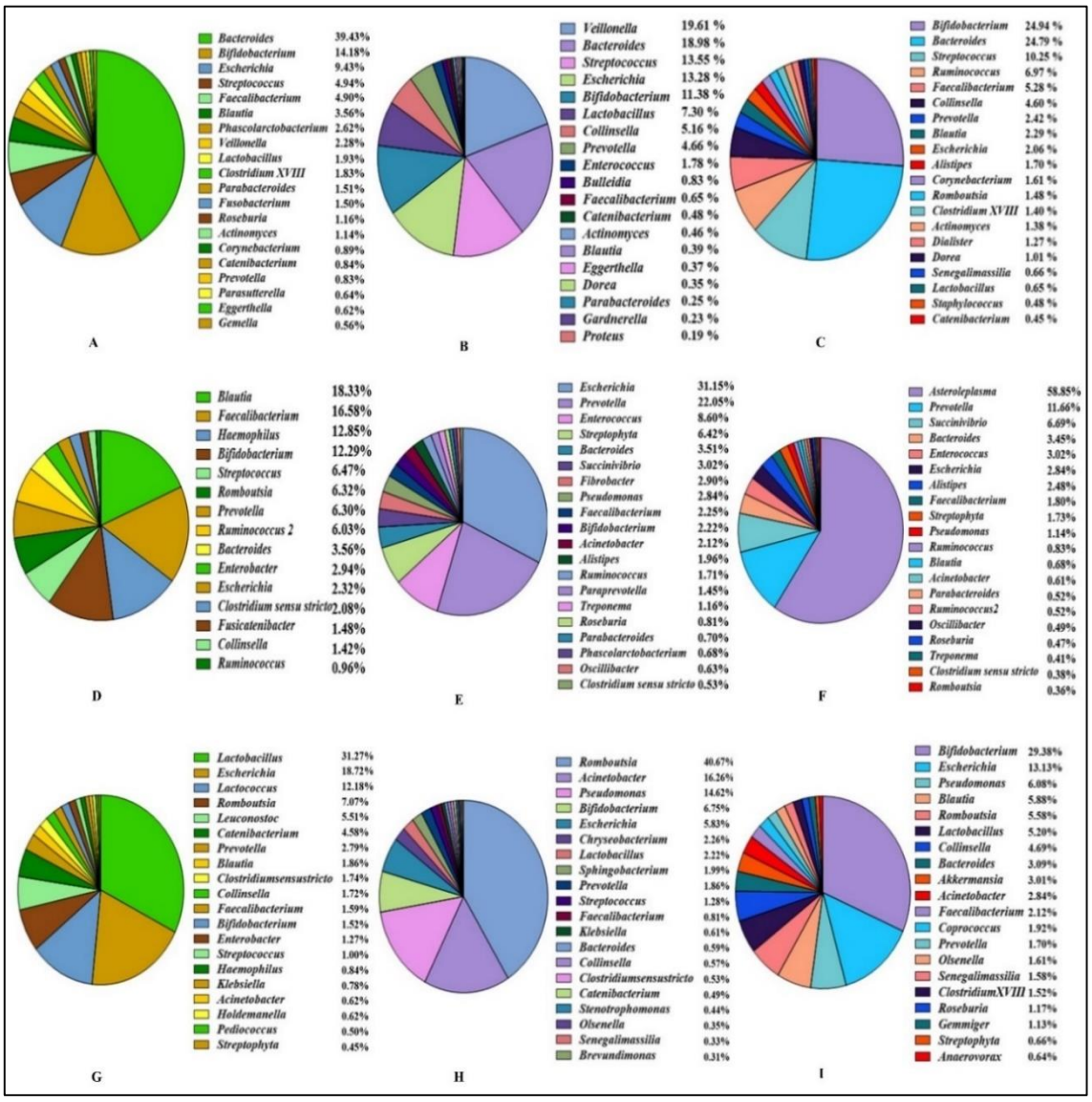


Figure 5.3.13: Bacterial Abundance of Bhutia members under study (A, B, C Male, Female and Kid respectively Year 1; D, E, F Male, Female and Kid Year 2 respectively; G, H, I Male, Female and Kid respectively Year 3)

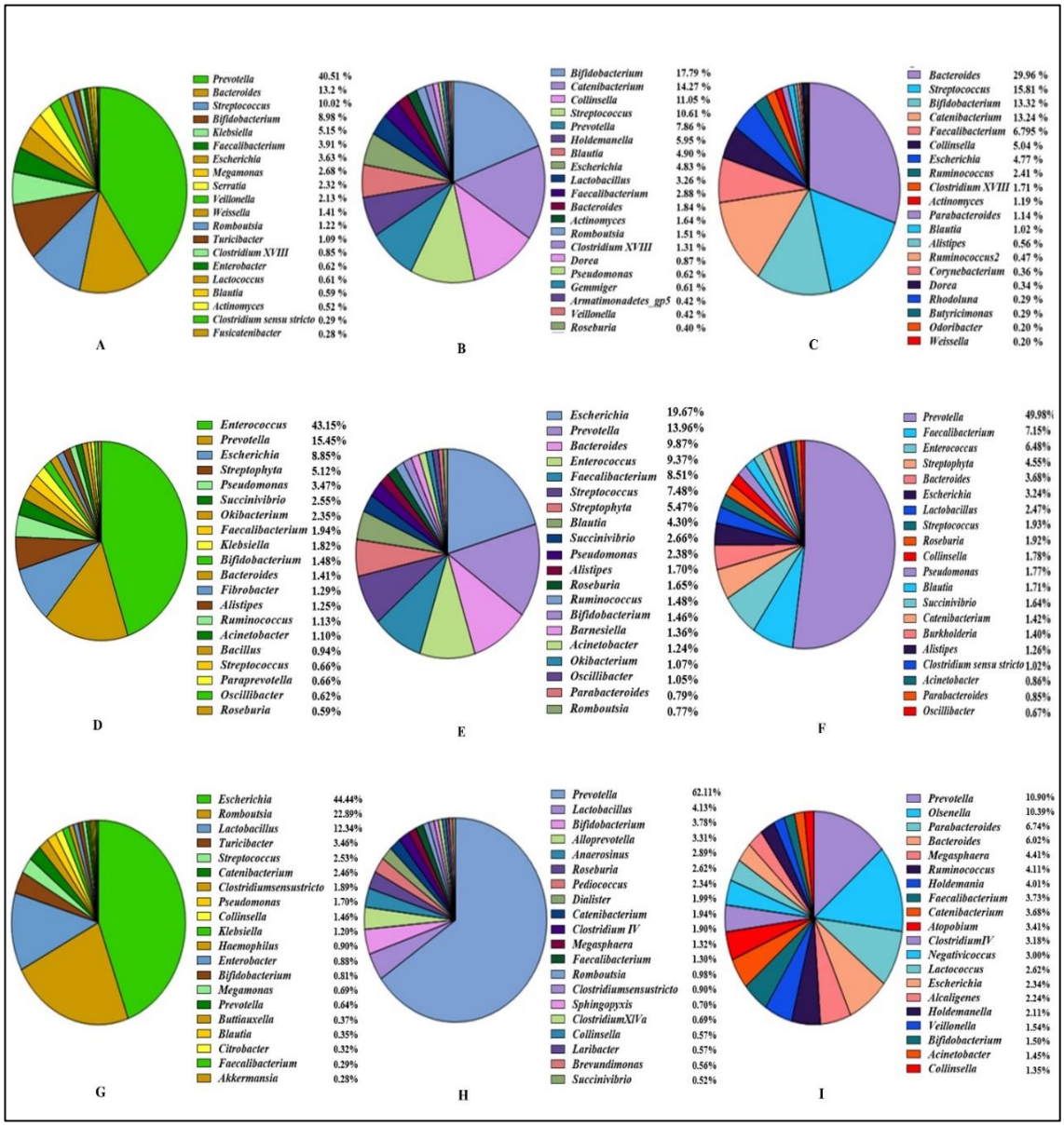


Figure 5.3.14: Bacterial Abundance of Mech members under study (A, B, C Male, Female and Kid respectively Year 1; D, E, F Male, Female and Kid Year 2 respectively; G, H, I Male, Female and Kid respectively Year 3)

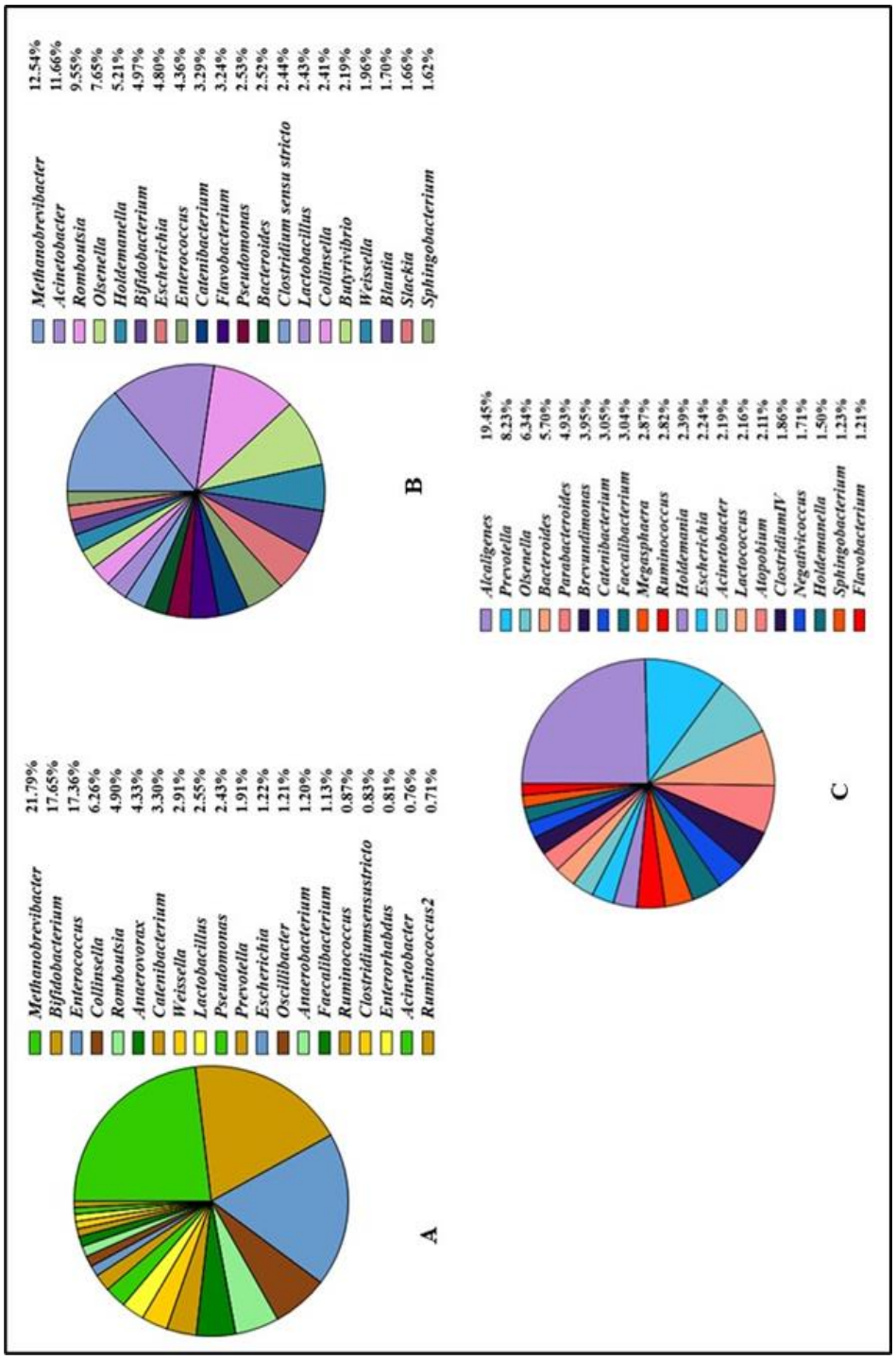


Figure 5.3.15: Bacterial Abundance of Urban members under study (A, B, C Male, Female and Kid respectively)

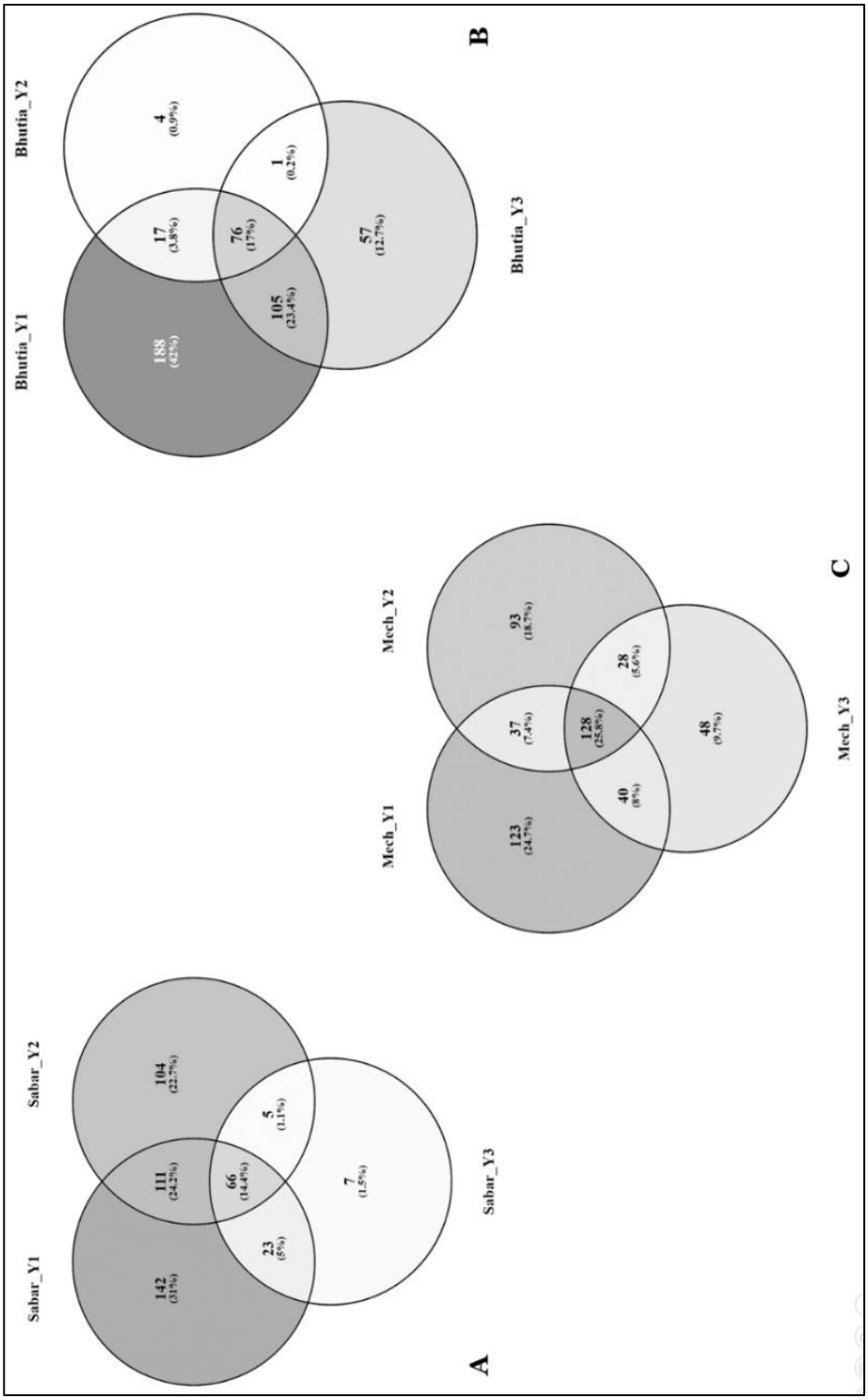


Figure 5.3.16: Intra Tribal Venn analysis of tribal subjects under study (A. Sabar, B. Bhutia & C. Mech in a yearwise manner)

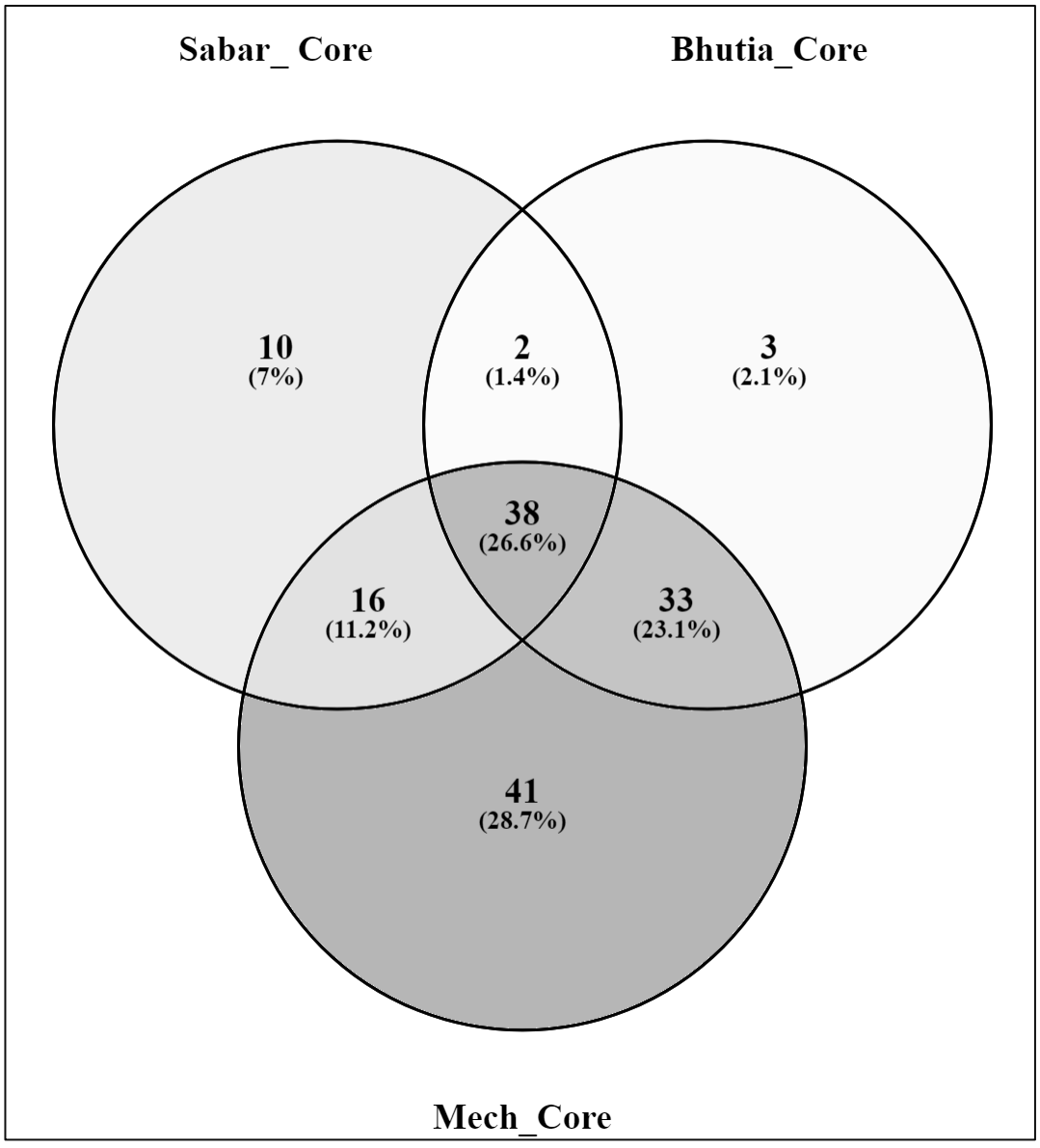


Figure 5.3.17: Inter Tribal Venn analysis of tribal subjects under study

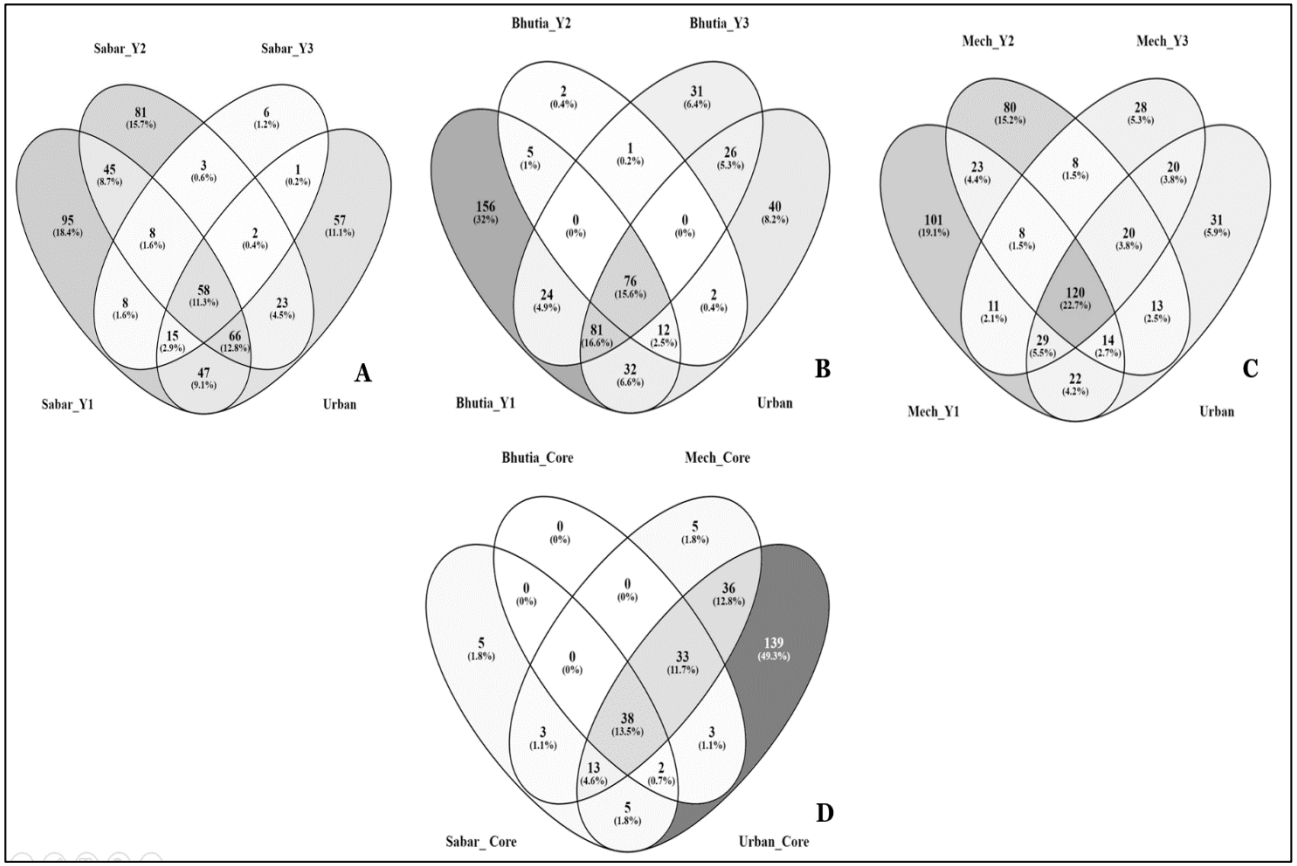


Figure 5.3.18: Venn analyses of the studied subjects under study [A: Sabar Core Vs Urban Core; B: Bhutia Core Vs Urban Core; C: Mech Core Vs Urban Core; D: Tribal Core Vs Urban Core]

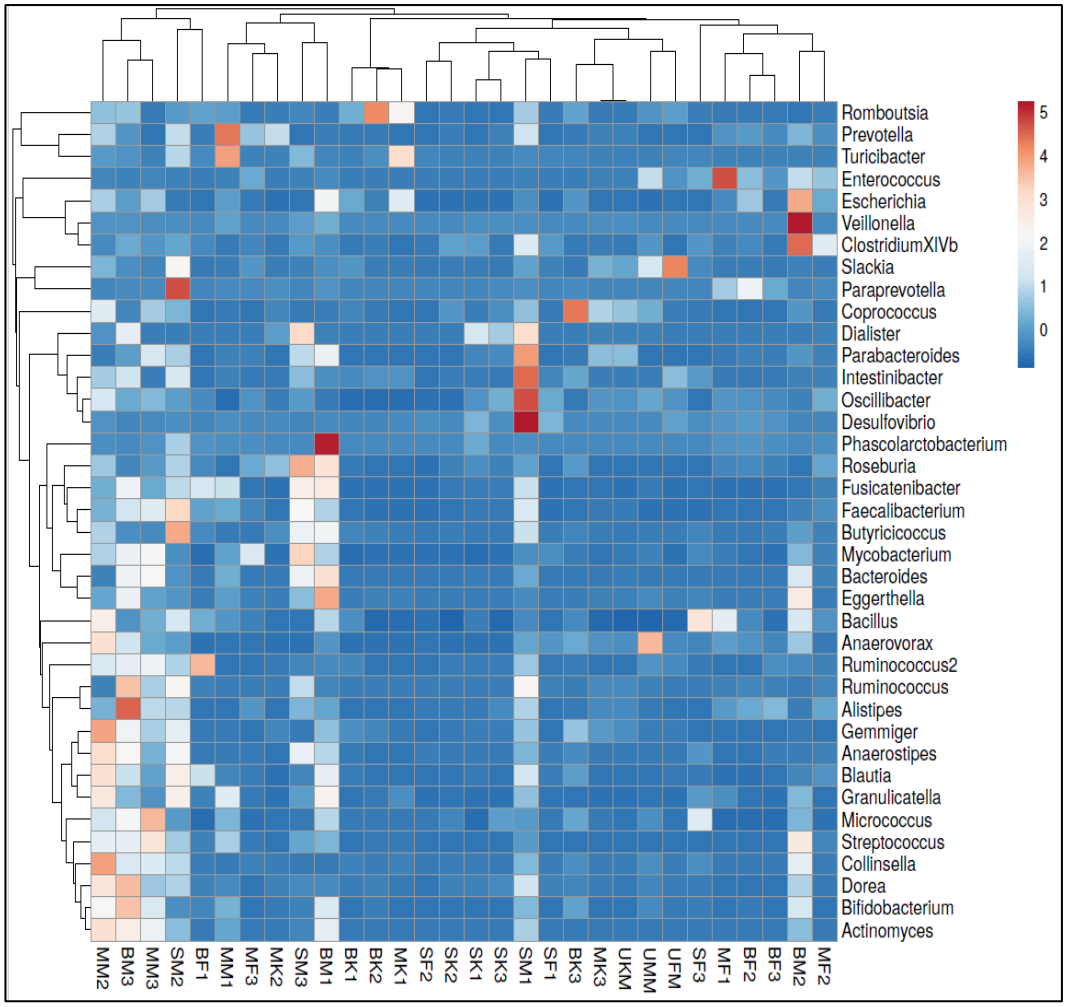


Figure 5.3.19: Heatmap representation of core taxa across the members under study, where SM1= Sabar Male, SF1= Sabar Female and SK1= Sabar Kid; BM1= Bhutia Male, BF1= Bhutia Female and BK1= Bhutia Kid; MM1= Mech Male, MF1= Mech Female and MK1= Mech Kid (Year 1);

SM2= Sabar Male, SF2= Sabar Female and SK2= Sabar Kid; BM2= Bhutia Male, BF2= Bhutia Female and BK2= Bhutia Kid; MM2= Mech Male, MF2= Mech Female and MK2= Mech Kid (Year 2);

SM3= Sabar Male, SF3= Sabar Female and SK3= Sabar Kid; BM3= Bhutia Male, BF3= Bhutia Female and BK3= Bhutia Kid; MM3= Mech Male, MF3= Mech Female and MK3= Mech Kid (Year 3); UMM= Urban Male member; UFM= Urban Female member; UKM= Urban Kid

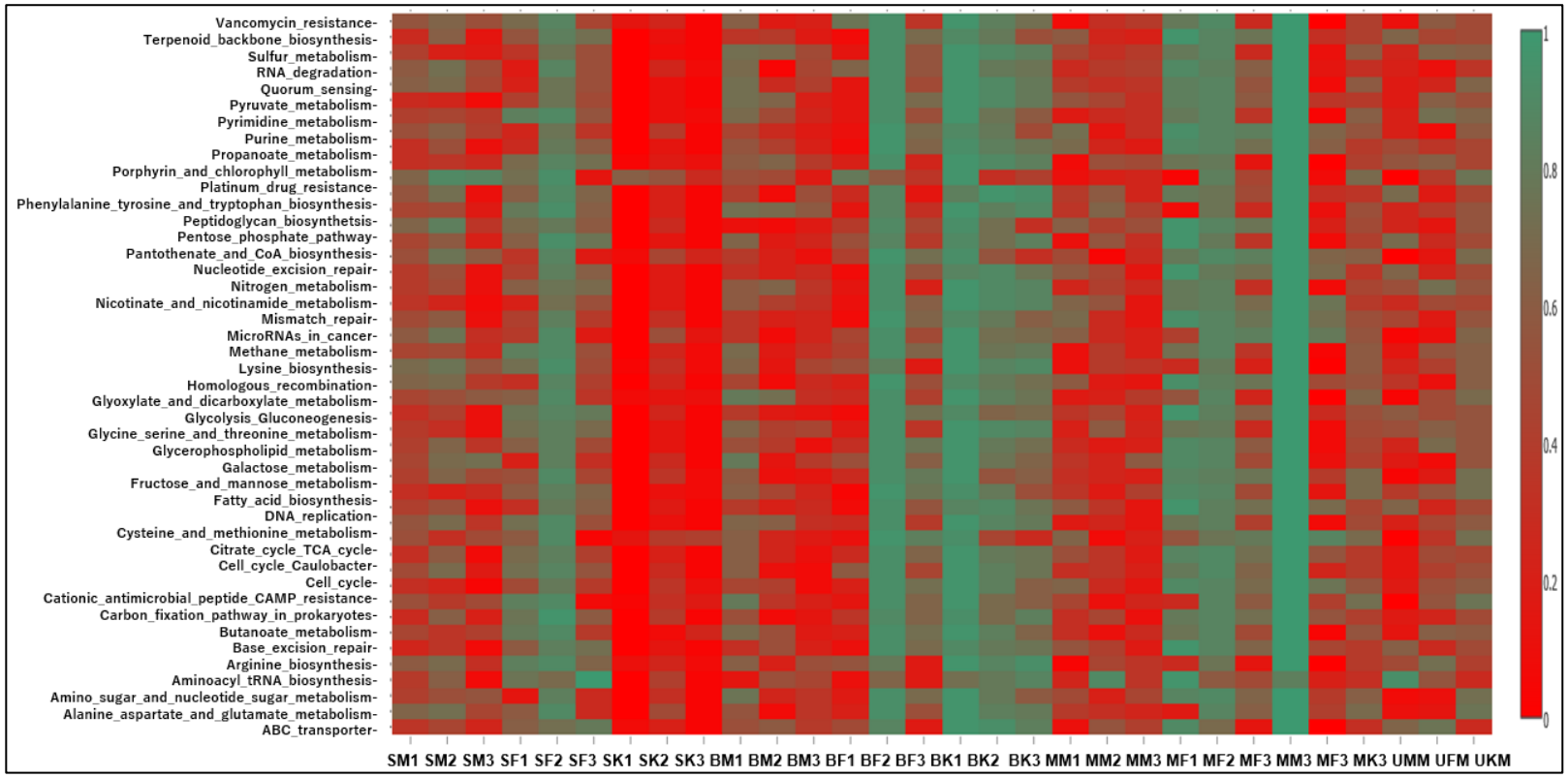


Figure 5.3.20: Enriched Pathways across the subjects under study, where SM1= Sabar Male, SF1= Sabar Female and SK1= Sabar Kid; BM1= Bhutia Male, BF1= Bhutia Female and BK1= Bhutia Kid; MM1= Mech Male, MF1= Mech Female and MK1= Mech Kid (Year 1); SM2= Sabar Male, SF2= Sabar Female and SK2= Sabar Kid; BM2= Bhutia Male, BF2= Bhutia Female and BK2=Bhutia Kid; MM2= Mech Male, MF2= Mech Female and MK2= Mech Kid (Year 2); SM3= Sabar Male, SF3= Sabar Female and SK3= Sabar Kid; BM3= Bhutia Male, BF3= Bhutia Female and BK3= Bhutia Kid; MM3= Mech Male, MF3= Mech Female and MK3= Mech Kid (Year 3); UMM= Urban Male member; UFM= Urban Female member; UKM= Urban Kid Member

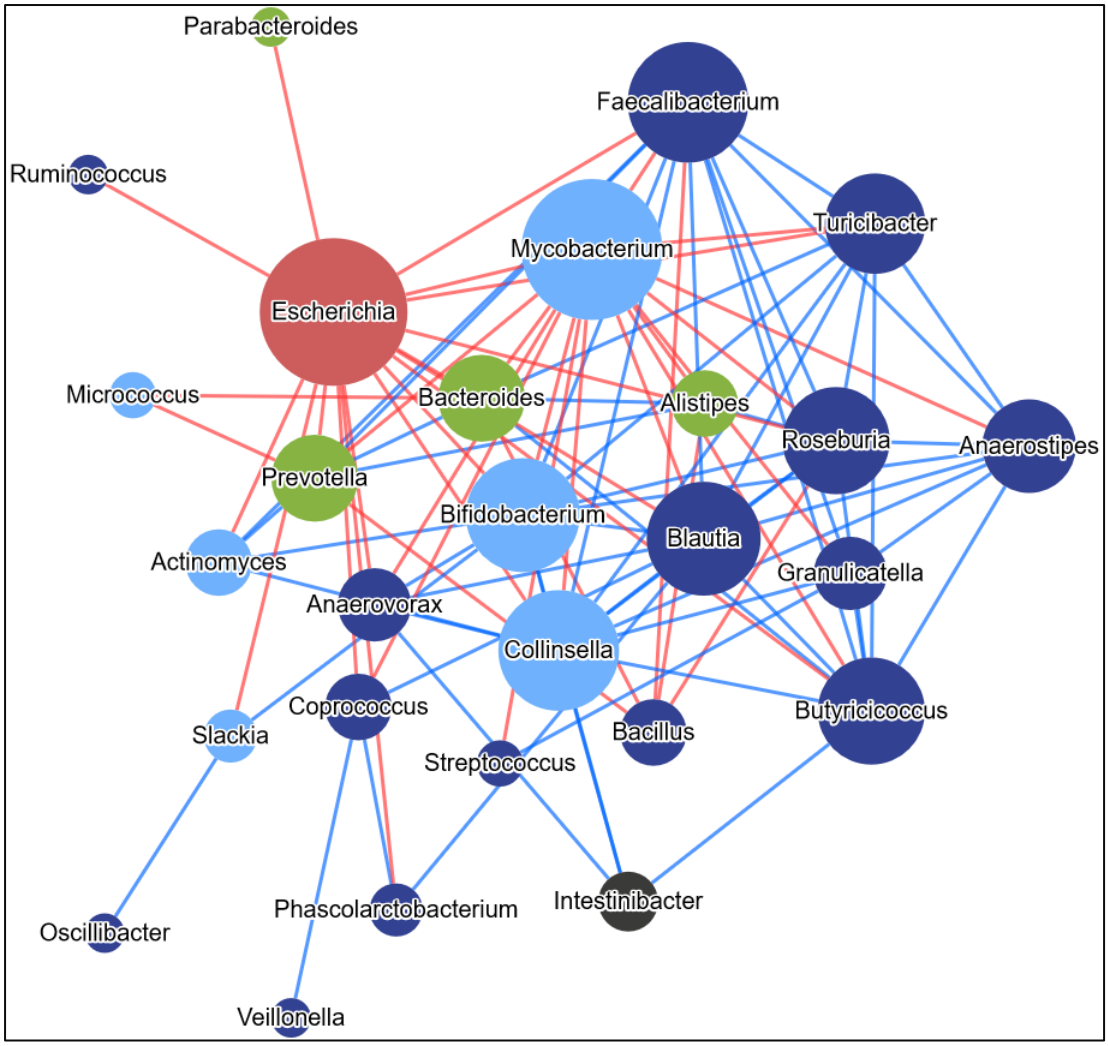


Figure 5.3.21: Core Gut Microbial Network Analysis across the subjects under study

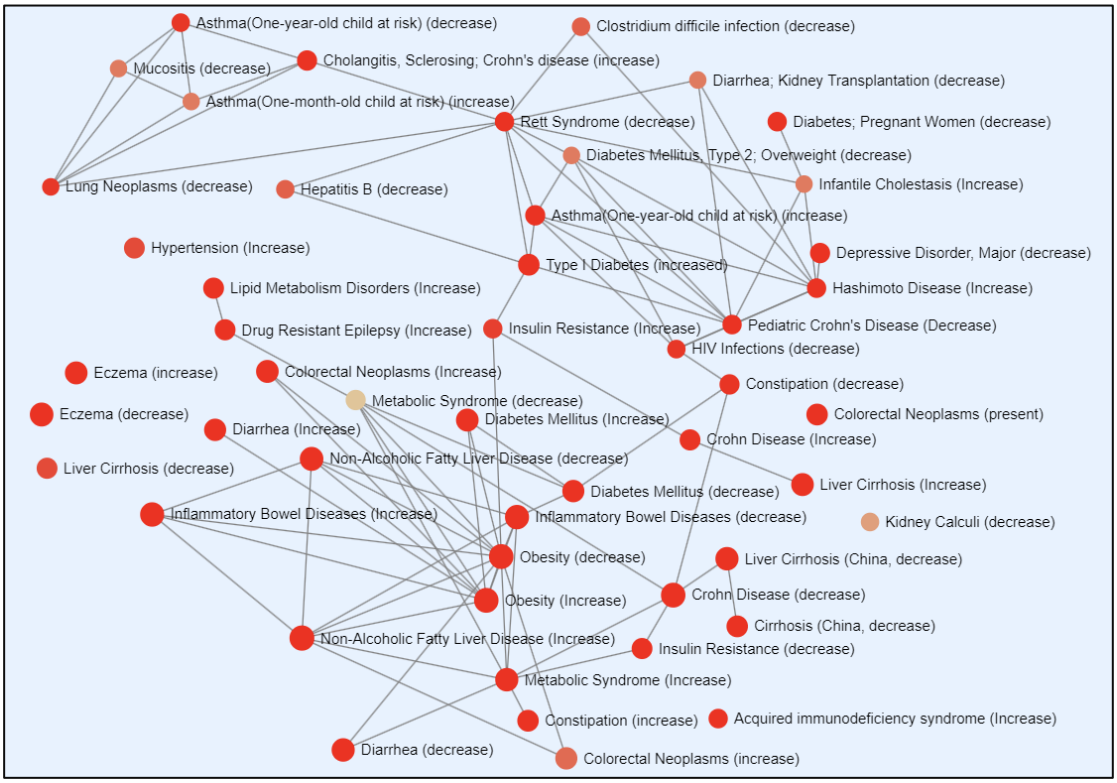


Figure 5.3.22: Core Disease Network Predicting Pathogenic Load across the subjects under study

5.4. Microbiological Profiling and Molecular Characterization:

Once we are done with the metagenomic data, we have proceeded to the culture-based studies in context to both aerobic and anaerobic microbes. We have been able to isolate 143 pure cultures from the Sabar tribe over a period of three years in which 13 isolates are resistant. In context to Bhutia, 12 isolates are showing resistance out of 161 pure cultures. 8 isolates are showing resistance out of 164 pure cultures isolated from Mech tribe. 11 isolates are showing resistance out of 100 pure cultures isolated from urban individuals (Figure 5.4.1). Amidst them, three isolates of interest are ubiquitously present across all three years in the members and infer same pattern of antibiotic resistance across three years. Gram characterization of pure culture is followed by Kirby-Bauer Disc Diffusion Assay across the tribal subjects under study. Antibiotics have been selected based on their classes as well as mode of actions viz ampicillin, tetracycline, nalidixic acid, erythromycin, gentamycin, imipenem, cotrimoxazole (Table 5.4.1.1). Along with the aerobic isolates, we have also performed the initial screening of anaerobic organisms using anaerobic culture jar (Figure 5.4.2). We have identified *Bacillus aerius* as a core gut bacterial member across the all individuals of Sabar family over a period of three years which shows resistance among ampicillin, tetracycline, nalidixic acid, erythromycin, gentamycin, imipenem, cotrimoxazole (Figure 5.4.3). *Bacillus safensis* has been identified as a core gut bacterial member across the all individuals of Bhutia family over a period of three years which shows resistance among ampicillin, nalidixic acid, erythromycin and cotrimoxazole (Figure 5.4.4). In context to Mech family, *Mammaliicoccus sciuri* has been identified as a core gut bacterial member across three years exhibiting resistance to ampicillin, tetracycline, nalidixic acid, erythromycin, gentamycin, imipenem, cotrimoxazole (Figure 5.4.5). We have also performed standard biochemical tests to validate our isolates of interests depicted

in (Figure 5.4.6). Once the biochemical tests are done, we have performed genomic DNA isolation of our isolates of interests followed by 16SrRNA gene amplification (Figure 5.4.7). The very first step of bacterial identification involved genomic DNA isolation from bacterial isolates. Using universal 16S primers 27f (5′AGAGTTTGATCCTGGCTCAG3′) and 1492r (5′TACGGTTACCTTGTTACGACTT3′), the 16S rDNA fragment is amplified and sequenced in the second step (Gerhardt et al., 1994). The third phase is the assembly of raw sequences using the in silico Cap3 Contig Assembly algorithm. Using BLASTntool, all of the acquired sequences can be used finally to identify the bacteria (Johnson et al., 2008). Besides these analyses, we have also performed initial anaerobiosis profiling of the studied subjects (Figure: 5.4.8-5.4.11). From the data, we have found higher antibiotic resistance in the urban gut than the tribal subjects.

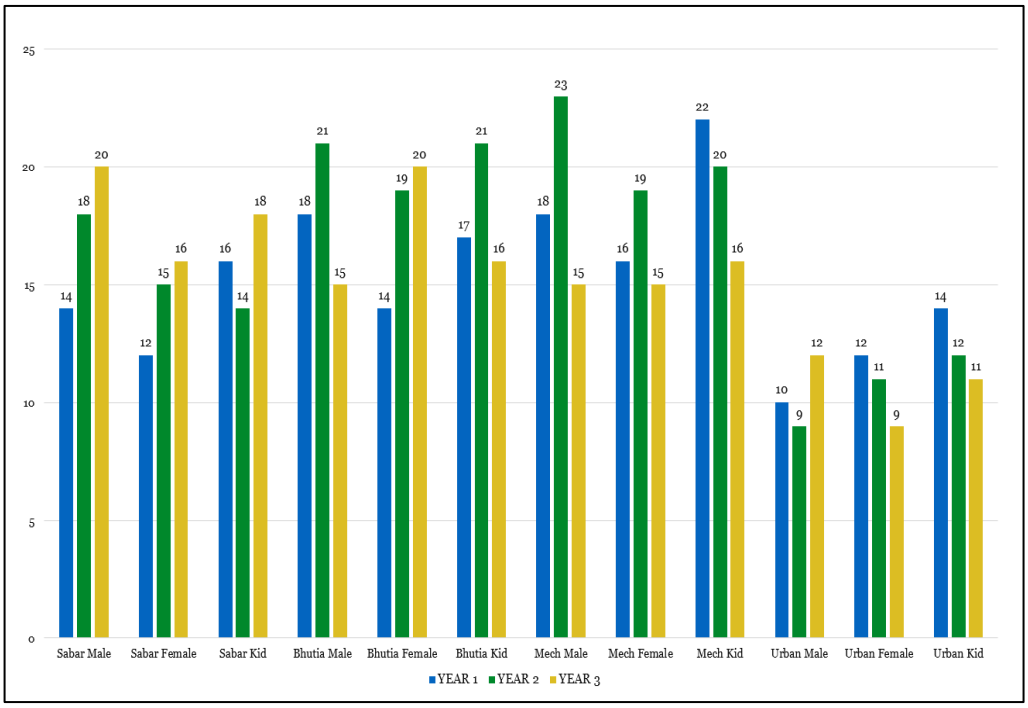


Figure 5.4.1: Isolation of Pure Colonies from each member of the tribes in a year wise manner

Class of Antibiotics	Name	Dosage used (mcg)
Beta Lactam	Ampicillin (AMP)	30
Tetracycline	Tetracycline (TET)	30
Synthetic Quinolone	Nalidixic Acid (NA)	30
Macrolide	Erythromycin (E)	15
Aminoglycoside	Gentamycin (GEN)	10
Synthetic Beta Lactam	Imipenem (IPM)	10
Sulfonamide	Co-trimoxazole (COT)	25

Table 5.4.1.1: Classes of antibiotics used for the study

A **B**

Figure 5.4.2: Anaerobic Culture Jar A. Culture plates along with anaerobic gas production systems and anaerobic indicator tablet B. Indicating Anaerobiosis

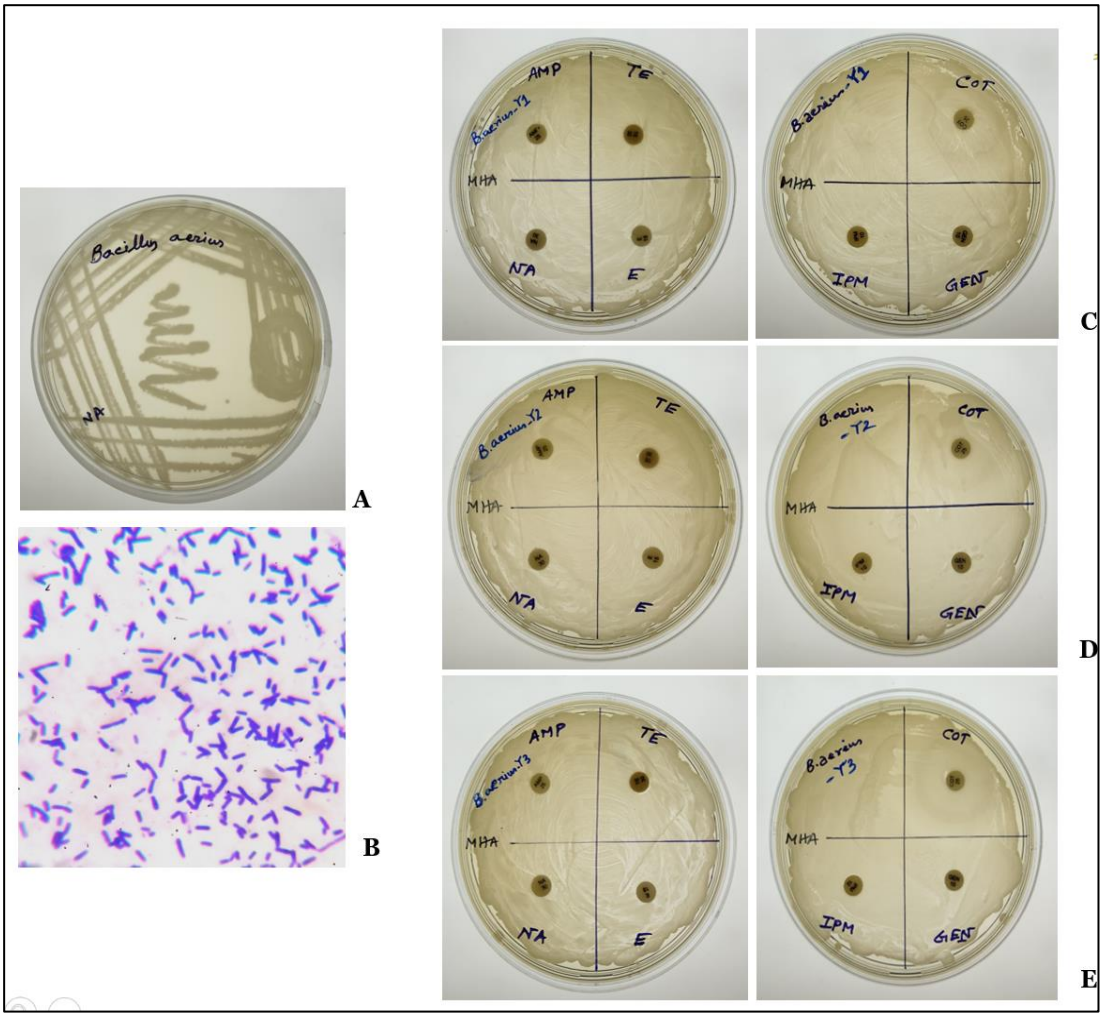


Figure 5.4.3: Microbiological insights of *Bacillus aerius* across the Sabar family under study A. Pure Culture; B. Gram Characterization; C-E. Antibiotic resistance over three years [AMP: Ampicillin; TET: Tetracycline; NA: Nalidixic Acid; E: Erythromycin; GEN: Gentamycin; IPM: Imipenem; COT: Co-trimoxazole]

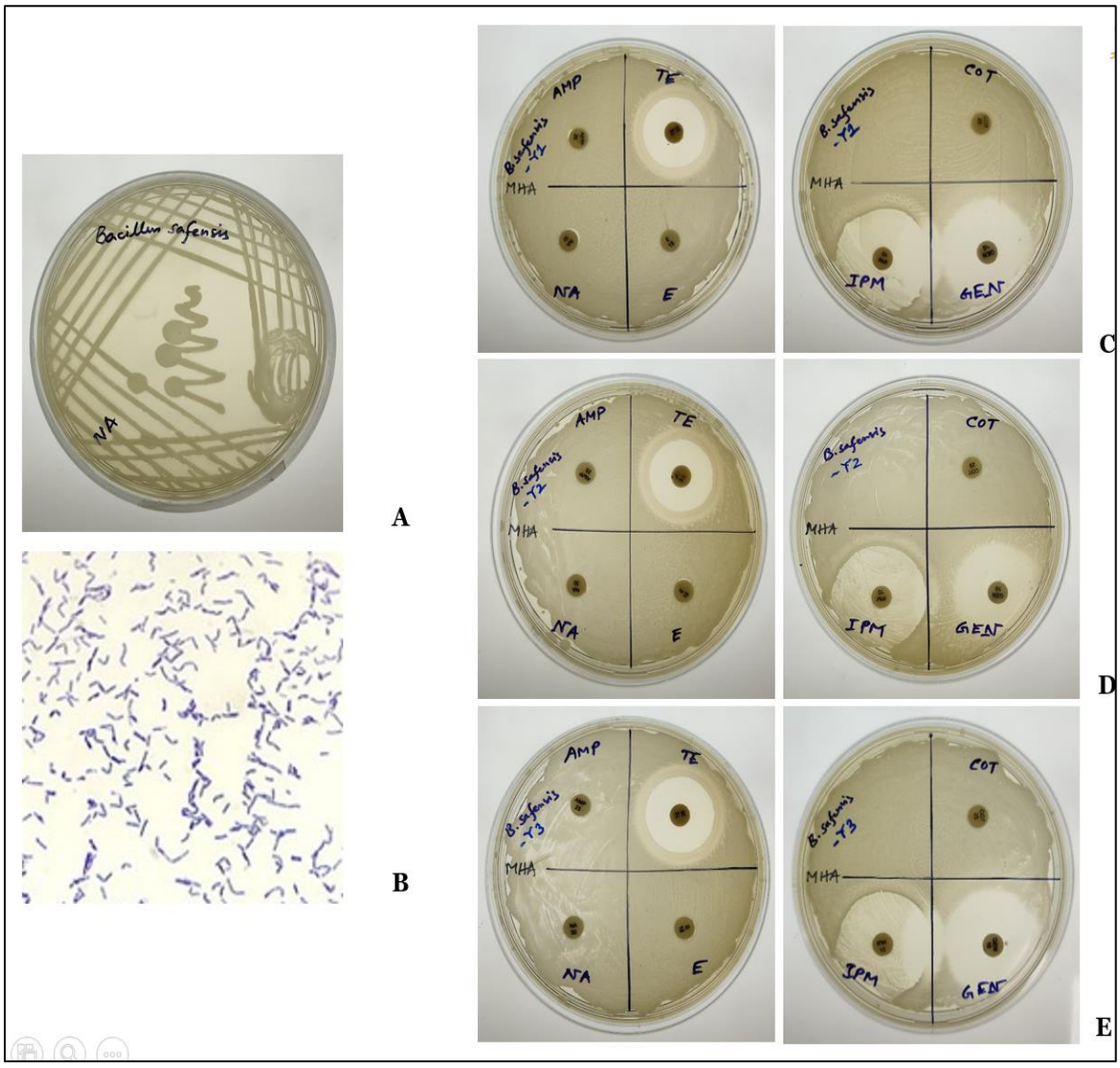


Figure 5.4.4: Microbiological insights of *Bacillus safensis* across the Bhutia family under study A. Pure Culture; B. Gram Characterization; C-E. Antibiotic resistance over three years [AMP: Ampicillin; TET: Tetracycline; NA: Nalidixic Acid; E: Erythromycin; GEN: Gentamycin; IPM: Imipenem; COT: Co-trimoxazole]

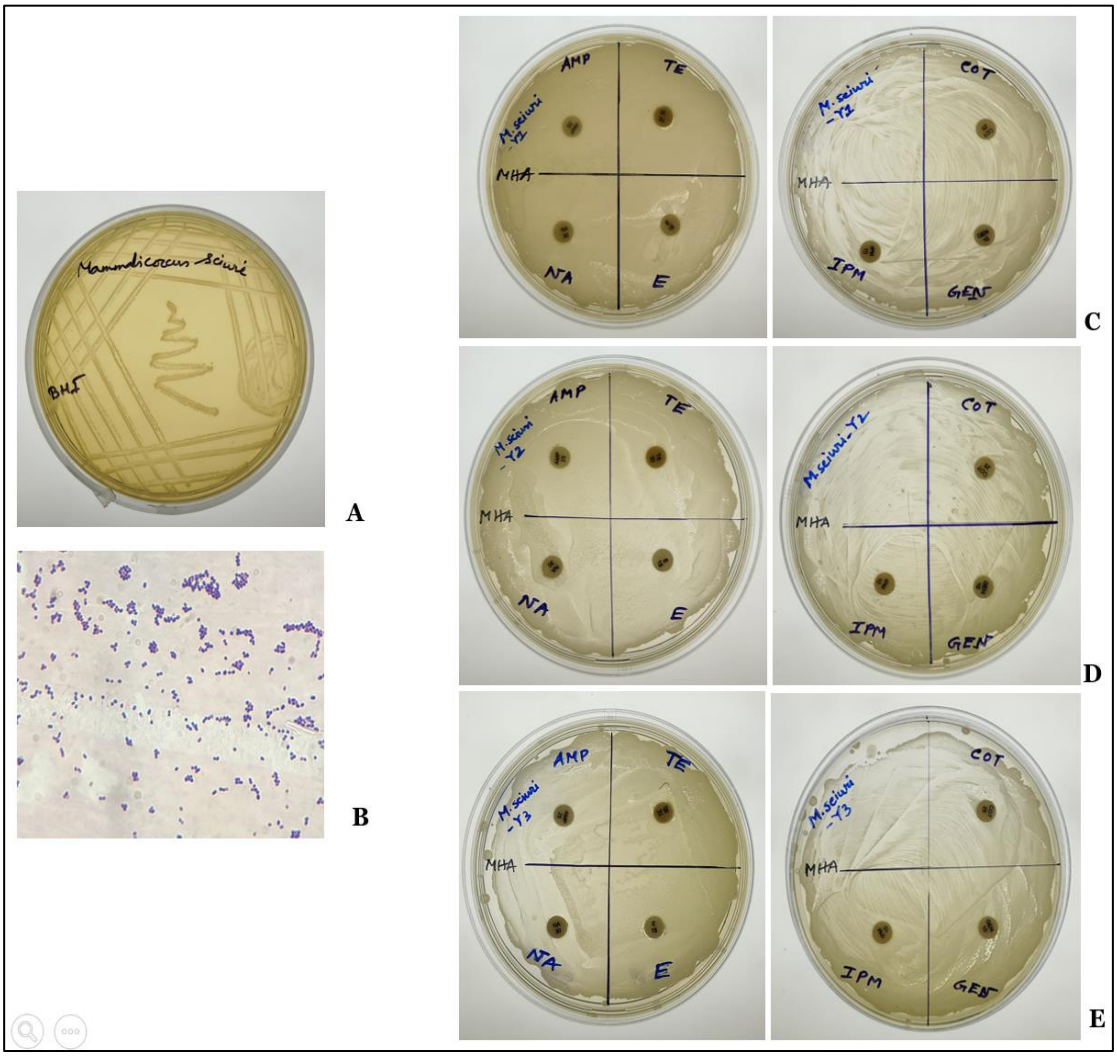


Figure 5.4.5: Microbiological insights of *Mammaliicoccus sciuri* across the Mech family under study A. Pure Culture; B. Gram Characterization; C-E. Antibiotic resistance over three years [AMP: Ampicillin; TET: Tetracycline; NA: Nalidixic Acid; E: Erythromycin; GEN: Gentamycin; IPM: Imipenem; COT: Co-trimoxazole]

Tribes under Study	Isolates of Interest	Antibiotic Resistance						
		AMP	TET	NA	E	COT	GEN	IPM
SABAR	*Bacillus aerius*	√	√	√	√	√	√	√
BHUTIA	*Bacillus safensis*	√		√	√	√		
MECH	*Mammaliicoccus sciuri*	√	√	√	√	√	√	√

Table 5.4.1.2: Antibiotic Resistance among the isolates of interest under study over the three years

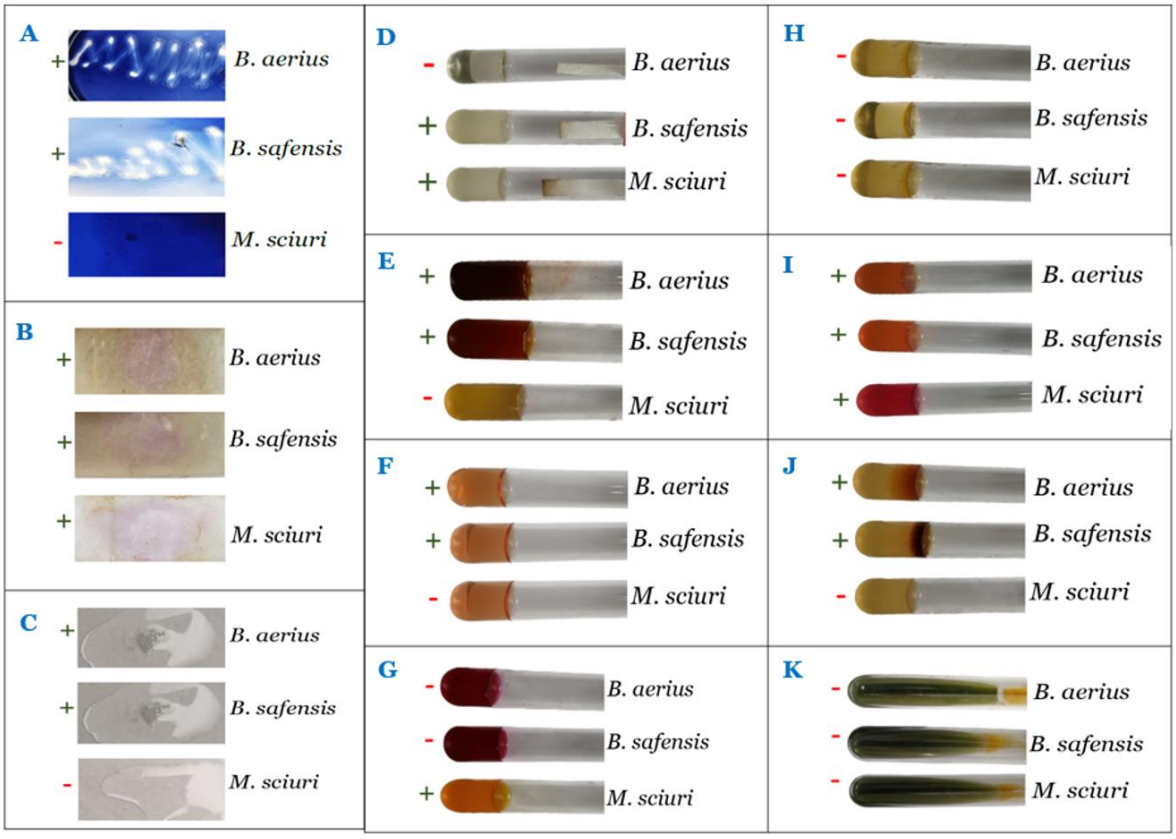


Figure 5.4.6: Biochemical Tests across the isolates of interest A-K [A. Starch hydrolysis; B. Oxidase test; C. Catalase test; D. H2S production test; E. Nitrate reduction test; F. Urease test; G. Lactose fermentation test; H. Indole production test; I. Methyl red test; J. Voges Proskauer test; K. Citrate Utilisation test]

Isolates of Interest	Starch hydrolysis	Oxidase test	Catalase Test	H2S production Test	Nitrate Reduction Test	Urease Test	Lactose Fermentation Test	Indole production test	Methyl Red Test	Voges Proskauer Test	Citrate Utilisation Test
Bacillus aerius	+	+	+	-	+	-	-	-	+	+	-
Bacillus safensis	+	+	+	+	+	-	-	-	+	+	-
Mammalicoccus sciuri	-	+	-	+	-	-	+	-	+	-	-

Table 5.4.1.3: Biochemical tests among the isolates of interest under study over the three years

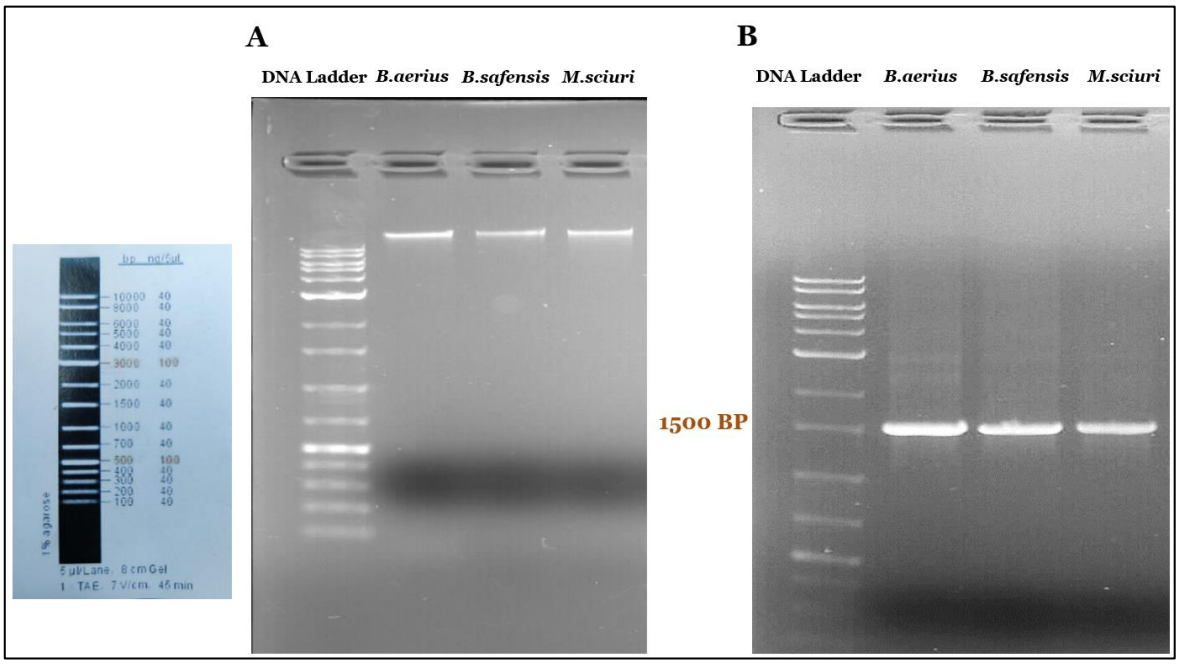


Figure 5.4.7: Genomic DNA Isolation (A) followed by 16SrRNA gene amplification (B) of the isolates under study

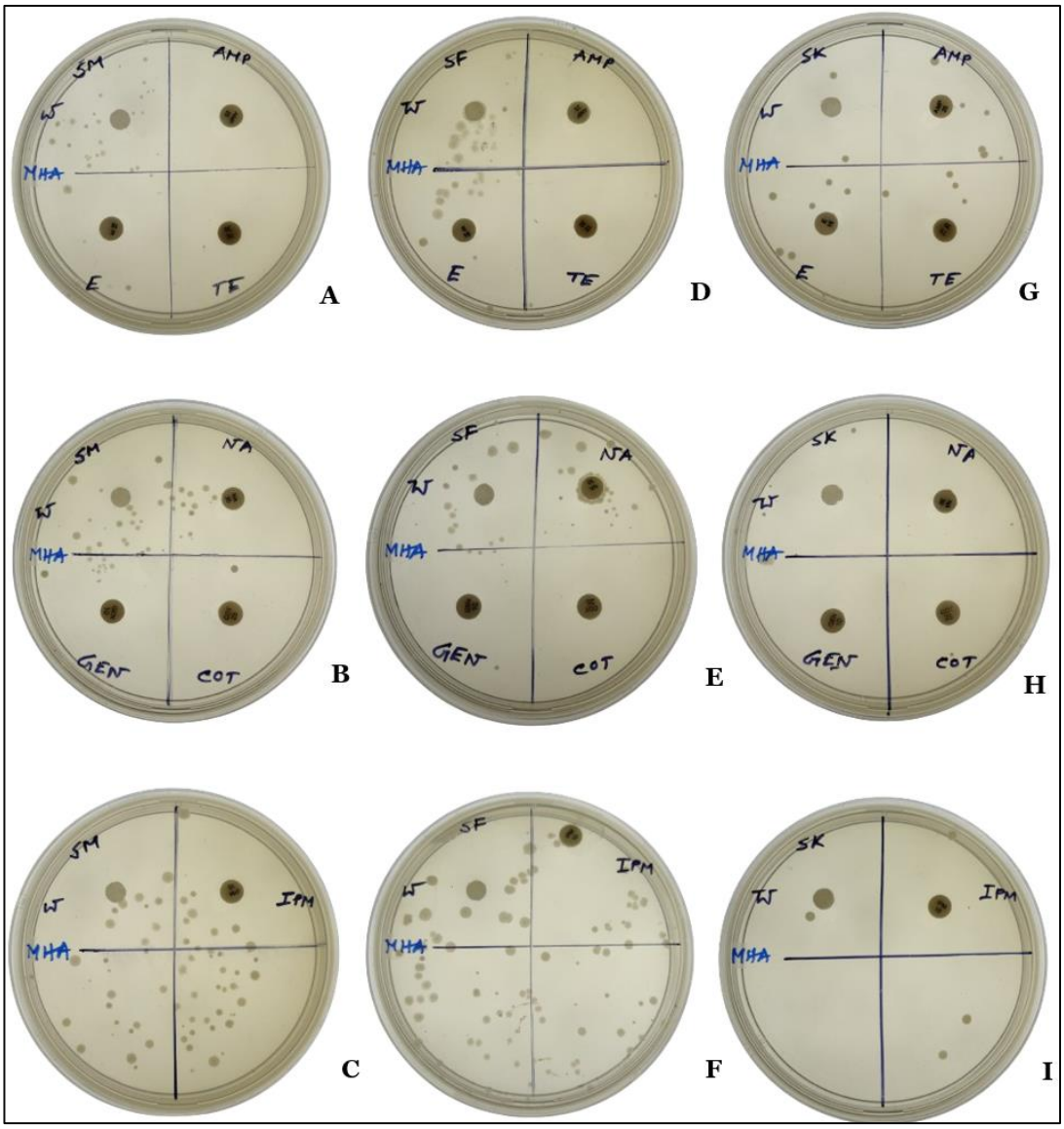


Figure 5.4.8: Anaerobic Profiling of Sabar subjects under study (A-C: Sabar male; D-F: Sabar female; G-I: Sabar kid)

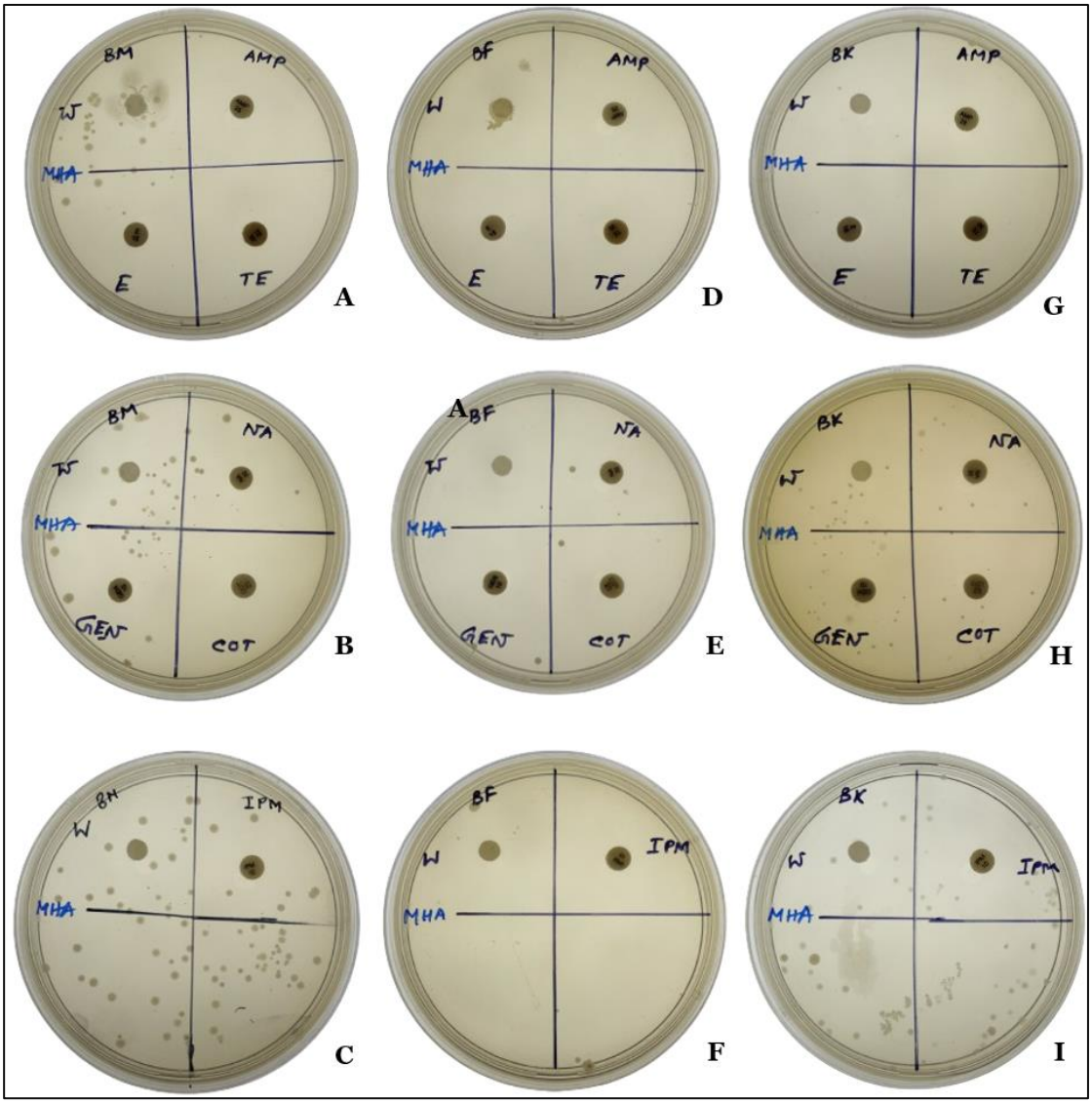


Figure 5.4.9: Anaerobic Profiling of Bhutia subjects under study (A-C: Bhutia male; D-F: Bhutia female; G-I: Bhutia kid)

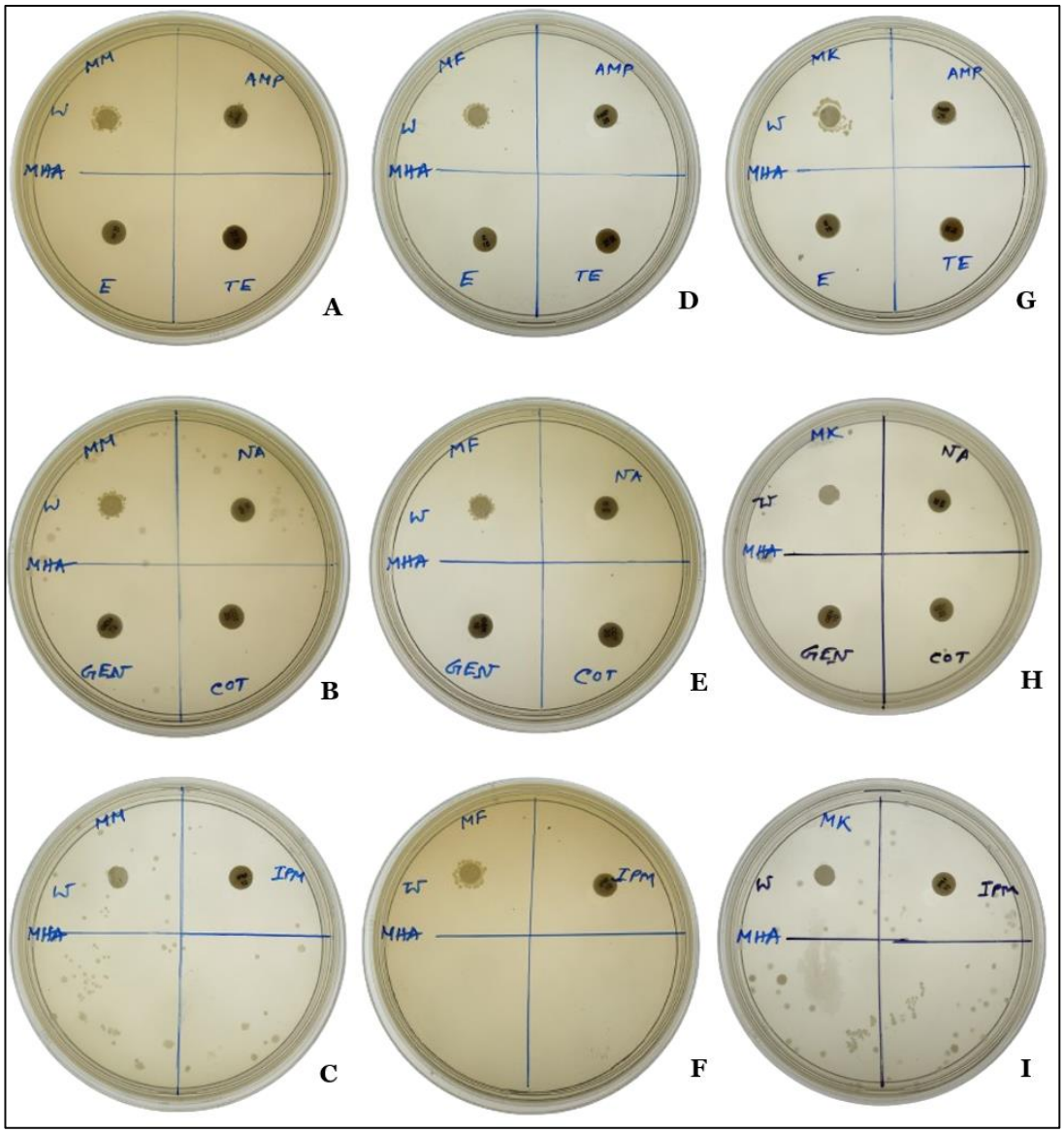


Figure 5.4.10: Anaerobic Profiling of Mech subjects under study (A-C: Mech male; D-F: Mech female; G-I: Mech kid)

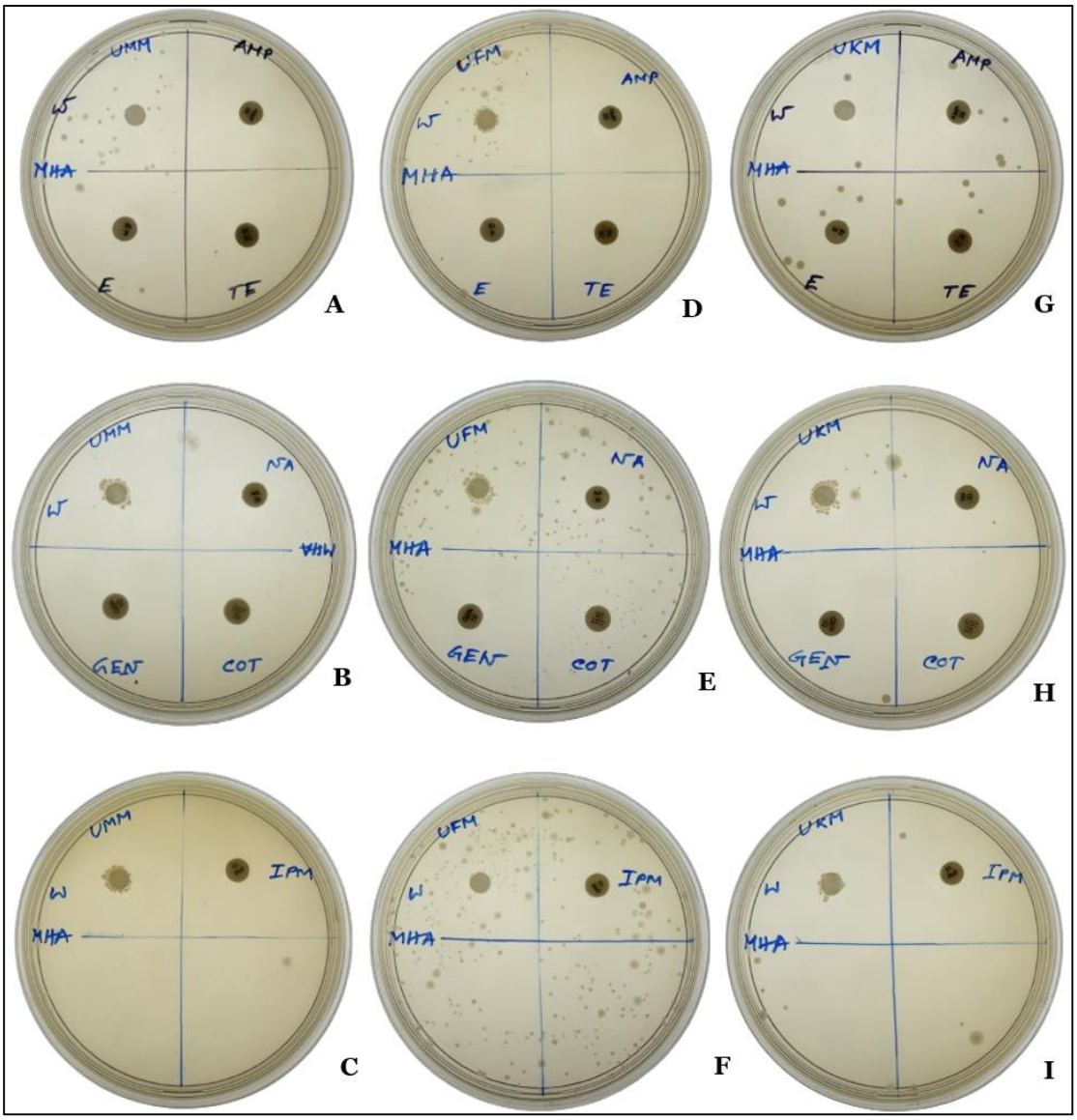


Figure 5.4.11: Anaerobic Profiling of Urban subjects under study (A-C: Urban male; D-F: Urban female; G-I: Urban kid)

5.5. *Whole Genome Analyses of Bacterial and Fungal isolates:*

5.5.1. Bacterial Whole Genome Analysis from Sabar Tribe

There were 23 contigs, an estimated genome length of 3,830,238 bp, and an average G+C content of 41.04%. The N50 length, which is defined as the shortest sequence length at 50% of the genome, is 788,994 bp. The L50 count, which is defined as the smallest number of contigs whose length sum produces N50, is 3.

The taxonomy of this genome is:

cellular organisms > Bacteria > Terrabacteria group > Bacillota > Bacilli > Bacillales > Bacillaceae > *Bacillus* > *Bacillus aerius*

This genome has 4,073 protein coding sequences (CDS), 69 transfer RNA (tRNA) genes, and 3 ribosomal RNA (rRNA) genes.

A circular graphical display of the distribution of the genome annotations is provided (Figure 5.5.1.1). This includes, from outer to inner rings, the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to know virulence factors, GC content and GC skew. Where, Blue colour bar indicates Metabolism; Orange colour bar indicates Protein processing; Purple colour bar indicates Stress response, defence, virulence; Red Colour bar indicates Cellular processes, Green colour bar indicates Energy; Pink colour bar indicates DNA processing; Brown colour bar indicates Membrane transport; Grey colour bar indicates RNA Processing; Light Blue colour indicates Miscellaneous; Olive Green colour indicates Cell envelope; Sky colour indicates Regulation and Cell Signaling.

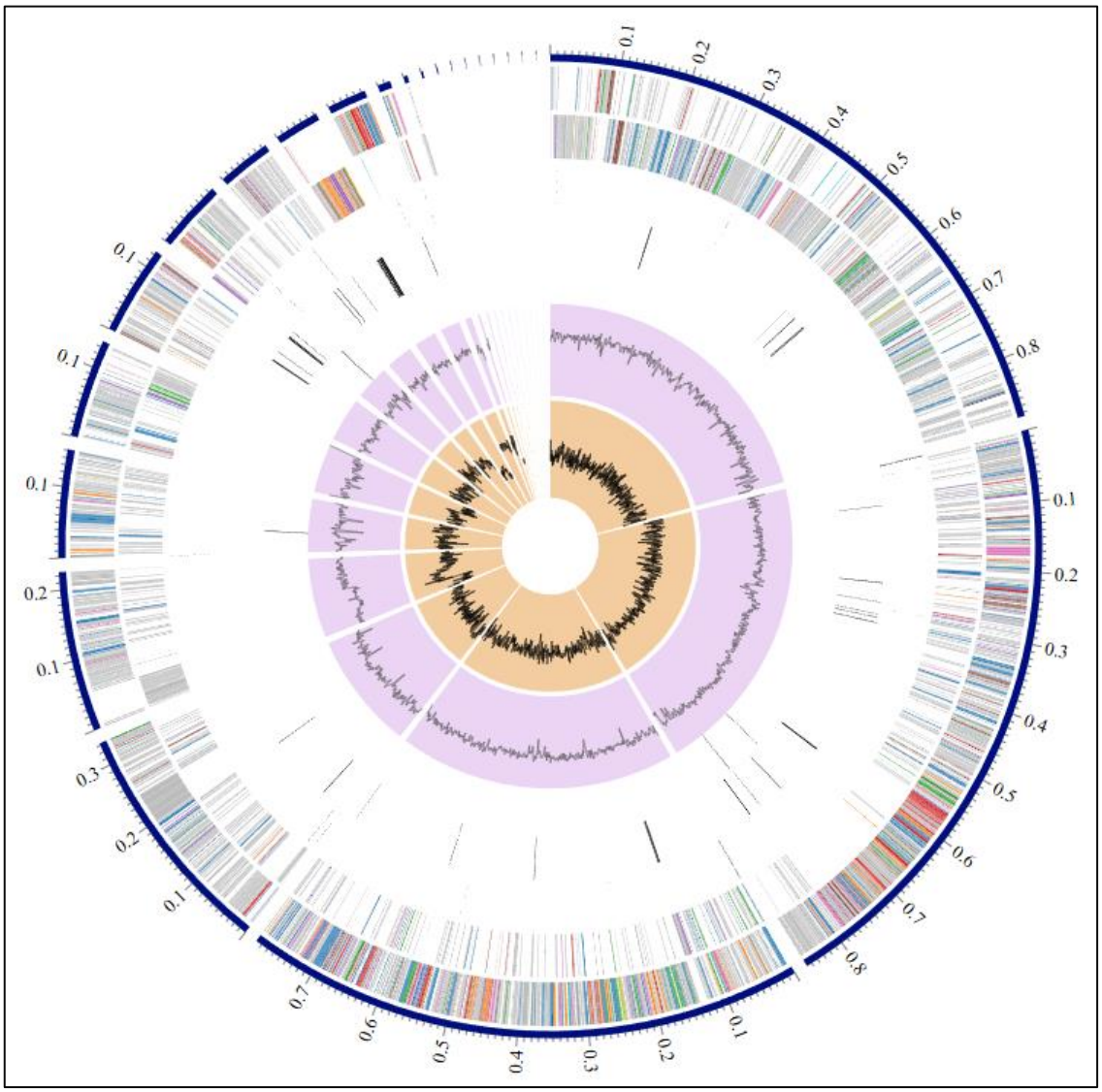


Figure 5.5.1.1: A circular graphical display (Circos) of the distribution of the genome annotations of *Bacillus aerius*

PATRIC provides the reference and representative genomes, and includes them in the phylogenetic analysis that is part of the comprehensive genome analysis. The closest reference and representative genomes to were identified by Mash/MinHash (Ondov et al., 2016). PATRIC global protein families (PGFams) (Davis et al., 2016) were selected from these genomes to determine the phylogenetic placement of this genome. The protein sequences from these families were aligned with MUSCLE (Edgar, 2004), and the nucleotides for each of those sequences were mapped to the protein alignment. The joint set of amino acid and nucleotide alignments were concatenated into a data matrix, and RaxML (Stamatakis, 2014) was used to analyze this matrix, with fast bootstrapping (Stamatakis et al., 2008) was used to generate the support values in the tree (Figure 5.5.1.2).

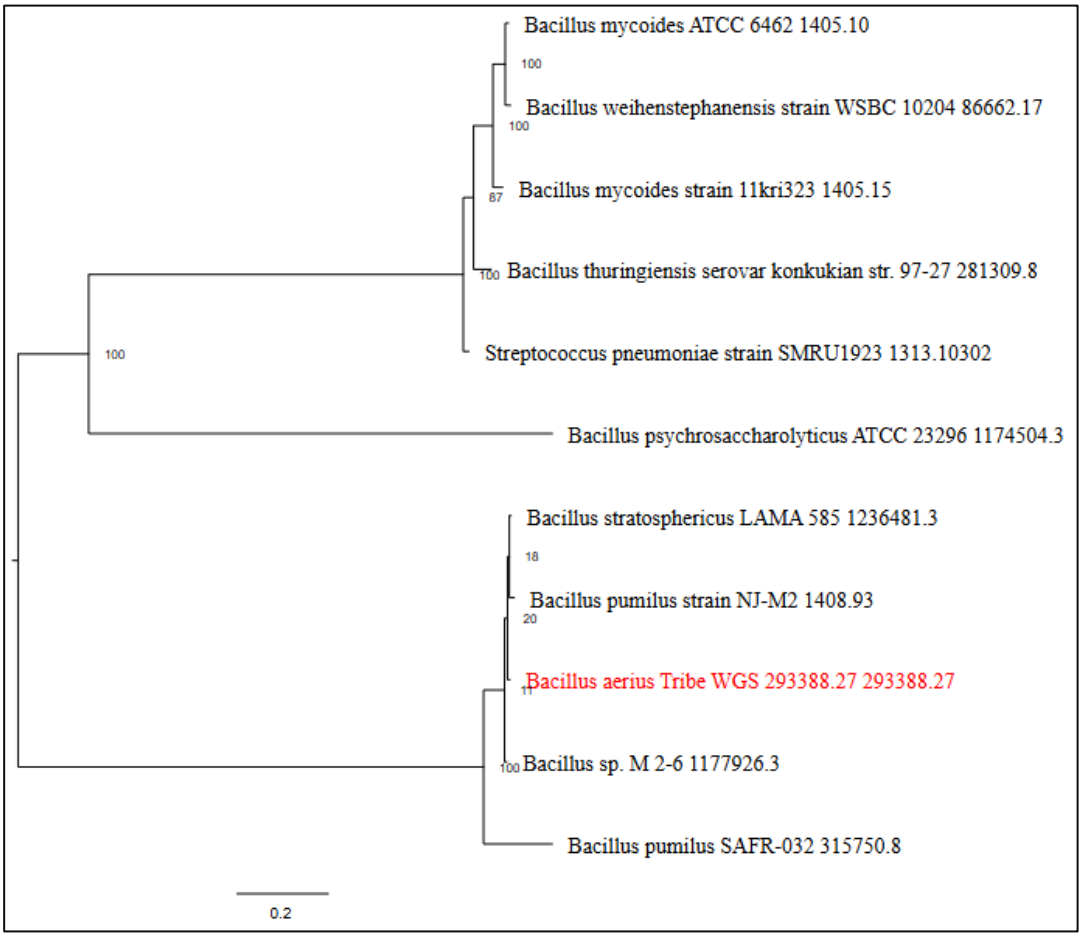


Figure 5.5.1.2: Phylogenetic tree representation using bacterial sequence

5.5.2. Bacterial Whole Genome Analysis from Bhutia Tribe

There were 19 contigs, an estimated genome length of 3,706,068 bp, and an average G+C content of 41.47%. The N50 length, which is defined as the shortest sequence length at 50% of the genome, is 968,630 bp. The L50 count, which is defined as the smallest number of contigs whose length sum produces N50, is 2.

The taxonomy of this genome is:

cellular organisms > Bacteria > Terrabacteria group > Bacillota > Bacilli > Bacillales > Bacillaceae > *Bacillus* > *Bacillus safensis*

This genome has 3,874 protein coding sequences (CDS), 58 transfer RNA (tRNA) genes, and 3 ribosomal RNA (rRNA) genes.

A circular graphical display of the distribution of the genome annotations is provided (Figure 5.5.1.3). This includes, from outer to inner rings, the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to know virulence factors, GC content and GC skew. Where, Blue colour bar indicates Metabolism; Orange colour bar indicates Protein processing; Purple colour bar indicates Stress response, defence, virulence; Red Colour bar indicates Cellular processes, Green colour bar indicates Energy; Pink colour bar indicates DNA processing; Brown colour bar indicates Membrane transport; Grey colour bar indicates RNA Processing; Light Blue colour indicates Miscellaneous; Olive Green colour indicates Cell envelope; Sky colour indicates Regulation and Cell Signaling.

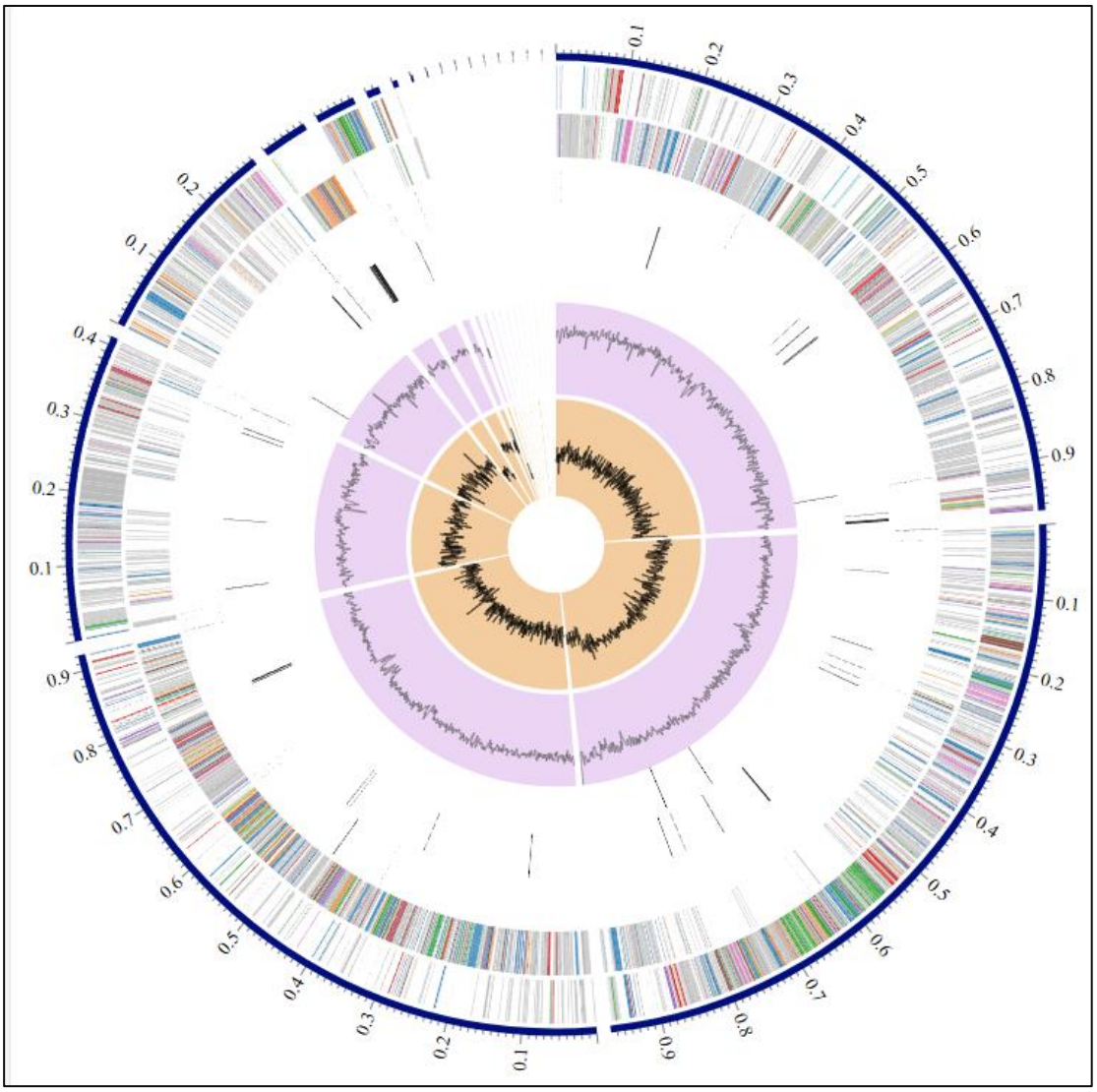


Figure 5.5.1.3: A circular graphical display (Circos) of the distribution of the genome annotations of *Bacillus safensis*

PATRIC provides the reference and representative genomes, and includes them in the phylogenetic analysis that is part of the comprehensive genome analysis. The closest reference and representative genomes to were identified by Mash/MinHash (Ondov et al., 2016). PATRIC global protein families (PGFams) (Davis et al., 2016) were selected from these genomes to determine the phylogenetic placement of this genome. The protein sequences from these families were aligned with MUSCLE (Edgar, 2004), and the nucleotides for each of those sequences were mapped to the protein alignment. The joint set of amino acid and nucleotide alignments were concatenated into a data matrix, and RaxML (Stamatakis, 2014) was used to analyze this matrix, with fast bootstrapping (Stamatakis et al., 2008) was used to generate the support values in the tree (Figure 5.5.1.4).

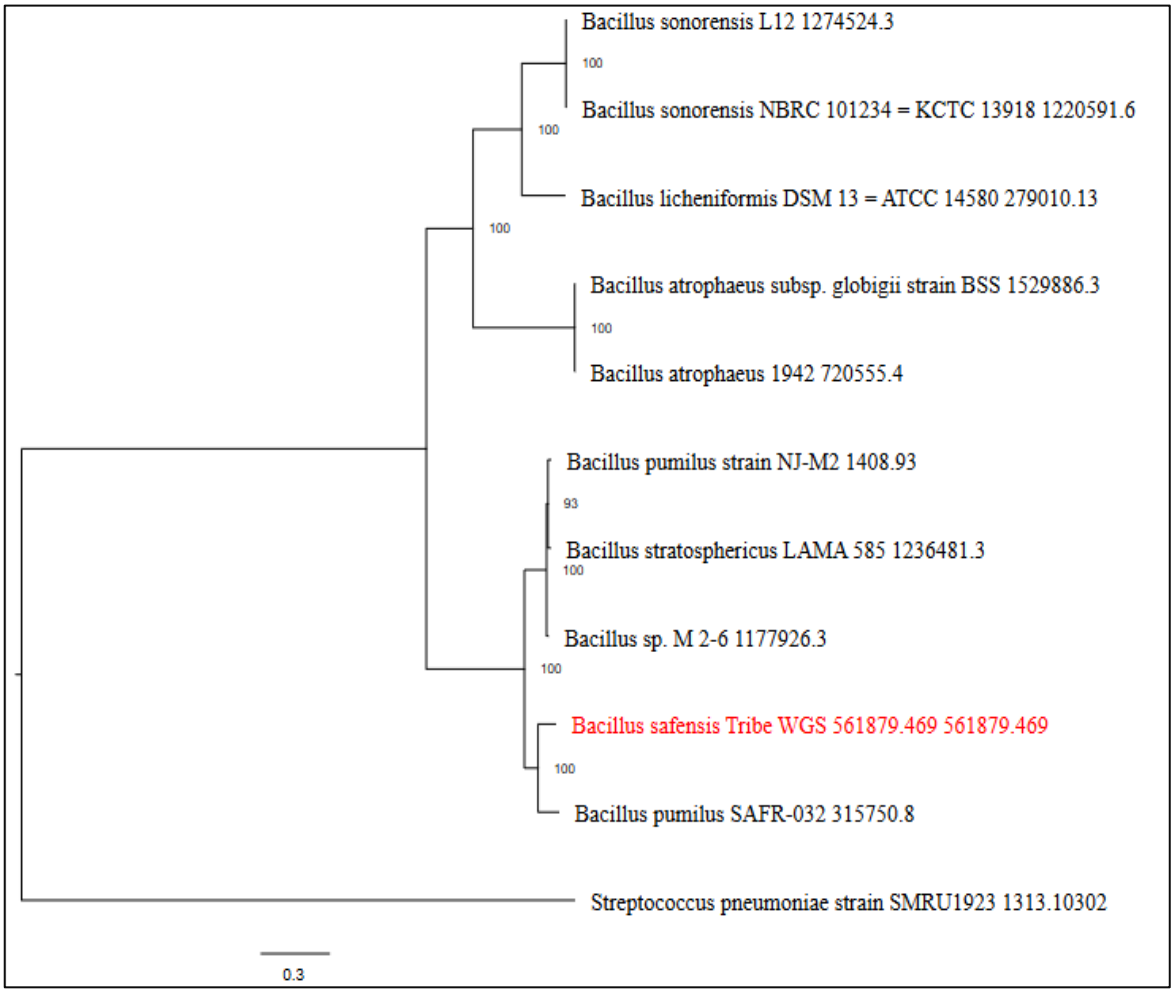


Figure 5.5.1.4: Phylogenetic tree representation using bacterial sequence

5.5.3. Bacterial Whole Genome Analysis from Mech Tribe

There were 40 contigs, an estimated genome length of 3,830,623 bp, and an average G+C content of 42.98%. The N50 length, which is defined as the shortest sequence length at 50% of the genome, is 1,002,477 bp. The L50 count, which is defined as the smallest number of contigs whose length sum produces N50, is 2.

The taxonomy of this genome is:

cellular organisms > Bacteria > Terrabacteria group > Bacillota > Bacilli > Bacillales > Staphylococcaceae > *Mammaliicoccus* > *Mammaliicoccus sciuri*

This genome has 4,050 protein coding sequences (CDS), 55 transfer RNA (tRNA) genes, and 11 ribosomal RNA (rRNA) genes.

A circular graphical display of the distribution of the genome annotations is provided (Figure 5.5.1.5). This includes, from outer to inner rings, the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to know virulence factors, GC content and GC skew. Where, Blue colour bar indicates Metabolism; Orange colour bar indicates Protein processing; Purple colour bar indicates Stress response, defence, virulence; Red Colour bar indicates Cellular processes, Green colour bar indicates Energy; Pink colour bar indicates DNA processing; Brown colour bar indicates Membrane transport; Grey colour bar indicates RNA Processing; Light Blue colour indicates Miscellaneous; Olive Green colour indicates Cell envelope; Sky colour indicates Regulation and Cell Signaling.

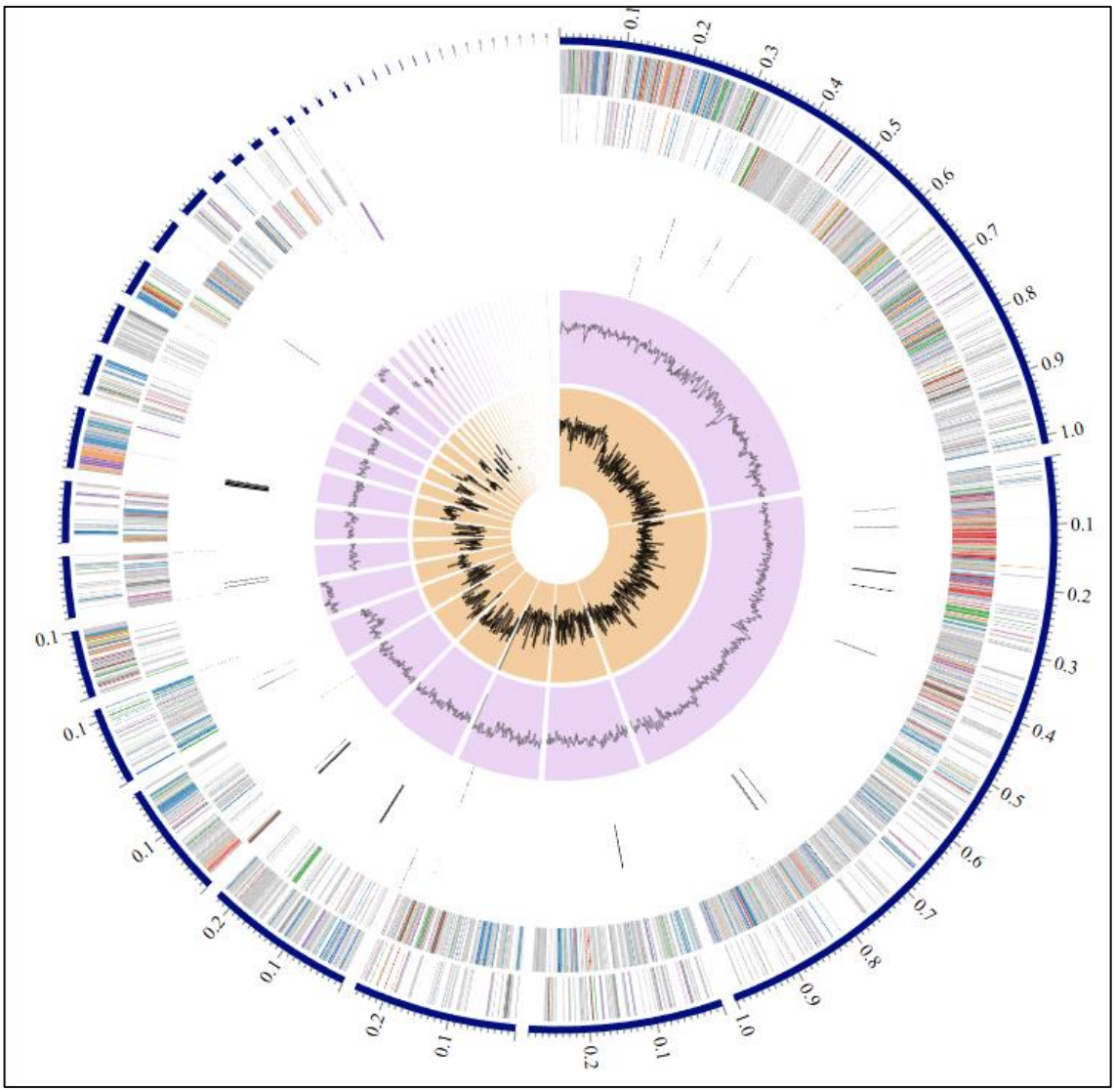


Figure 5.5.1.5: A circular graphical display (Circos) of the distribution of the genome annotations of *Mammaliicoccus sciuri*

PATRIC provides the reference and representative genomes, and includes them in the phylogenetic analysis that is part of the comprehensive genome analysis. The closest reference and representative genomes to were identified by Mash/MinHash (Ondov et al., 2016). PATRIC global protein families (PGFams) (Davis et al., 2016) were selected from these genomes to determine the phylogenetic placement of this genome. The protein sequences from these families were aligned with MUSCLE (Edgar, 2004), and the nucleotides for each of those sequences were mapped to the protein alignment. The joint set of amino acid and nucleotide alignments were concatenated into a data matrix, and RaxML (Stamatakis, 2014) was used to analyze this matrix, with fast bootstrapping (Stamatakis et al., 2008) was used to generate the support values in the tree (Figure 5.5.1.6).

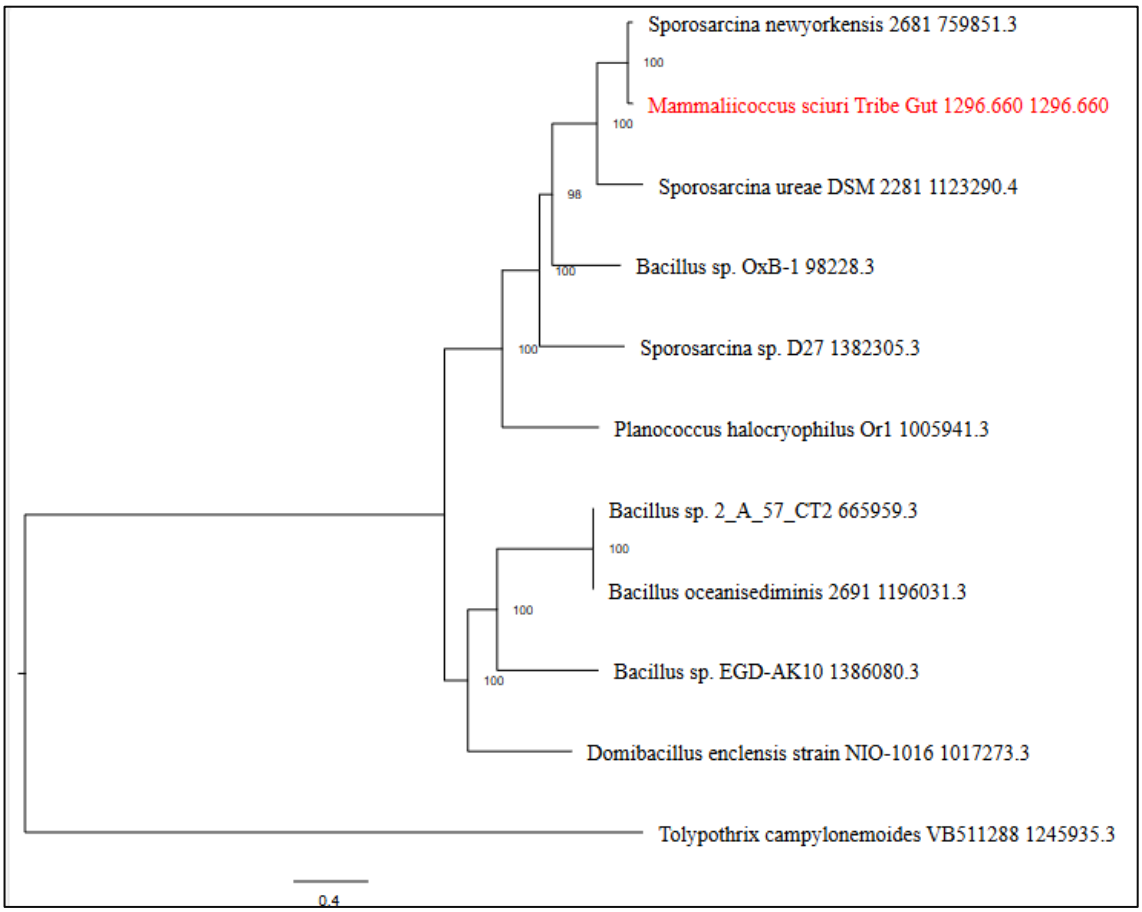


Figure 5.5.1.4: Phylogenetic tree representation using bacterial sequence

5.5.4. Whole Genome Analysis of Fungal Isolate from Bhutia Tribe:

Along with the identification of the above-mentioned antibiotic resistant bacteria, we were also able to identify a fungus related to the yeast community named as *Pichia*. One of the most main features of *Pichia*, that has been previously reported as an important candidate which possess probiotic property. Once the WGS data are available to us, we performed various analyses, ranging from phylogenetic analysis to identification of different metabolic clusters (Figure 5.5.1.7-5.5.1.9). A GC-MS based analysis was also performed, which enabled the identification of various chemicals, both volatile and non-volatile components, of the fungus that could potentially be used for future bioprospecting studies. (Figure 5.5.1.10; Table 5.5.1.1).

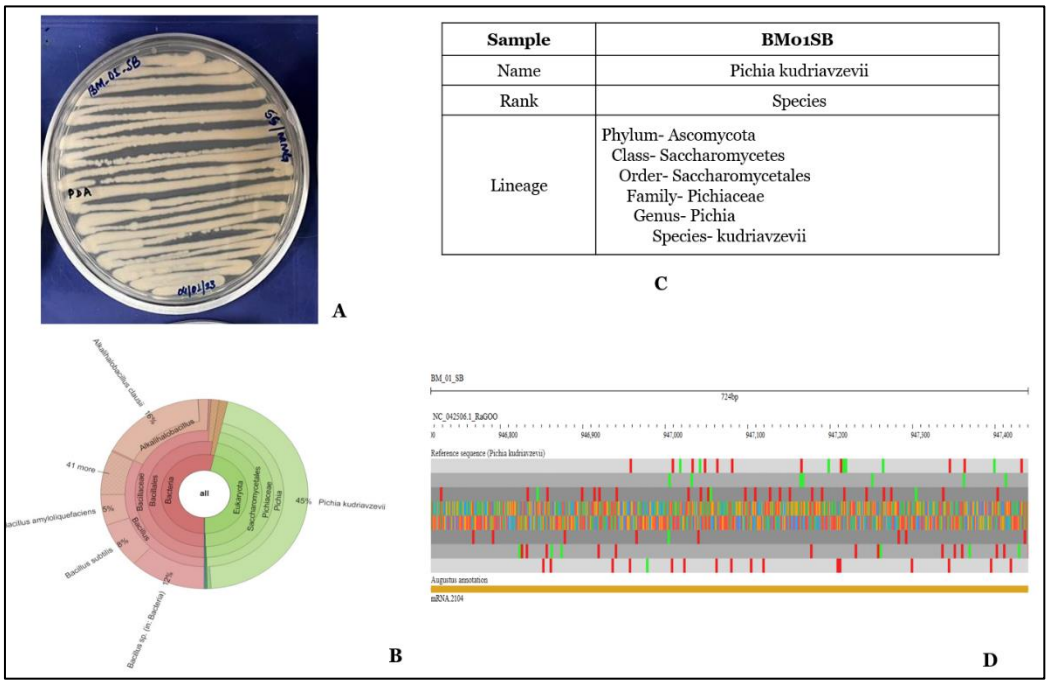


Figure 5.5.1.7: Characteristics of the isolate cultured in PDA media (A) followed by Krona representation (B); taxonomic lineage (C) using CC Metagen and GC content representation against reference sequence (D) Genome Track representation of annotating DNA reference sequence positions

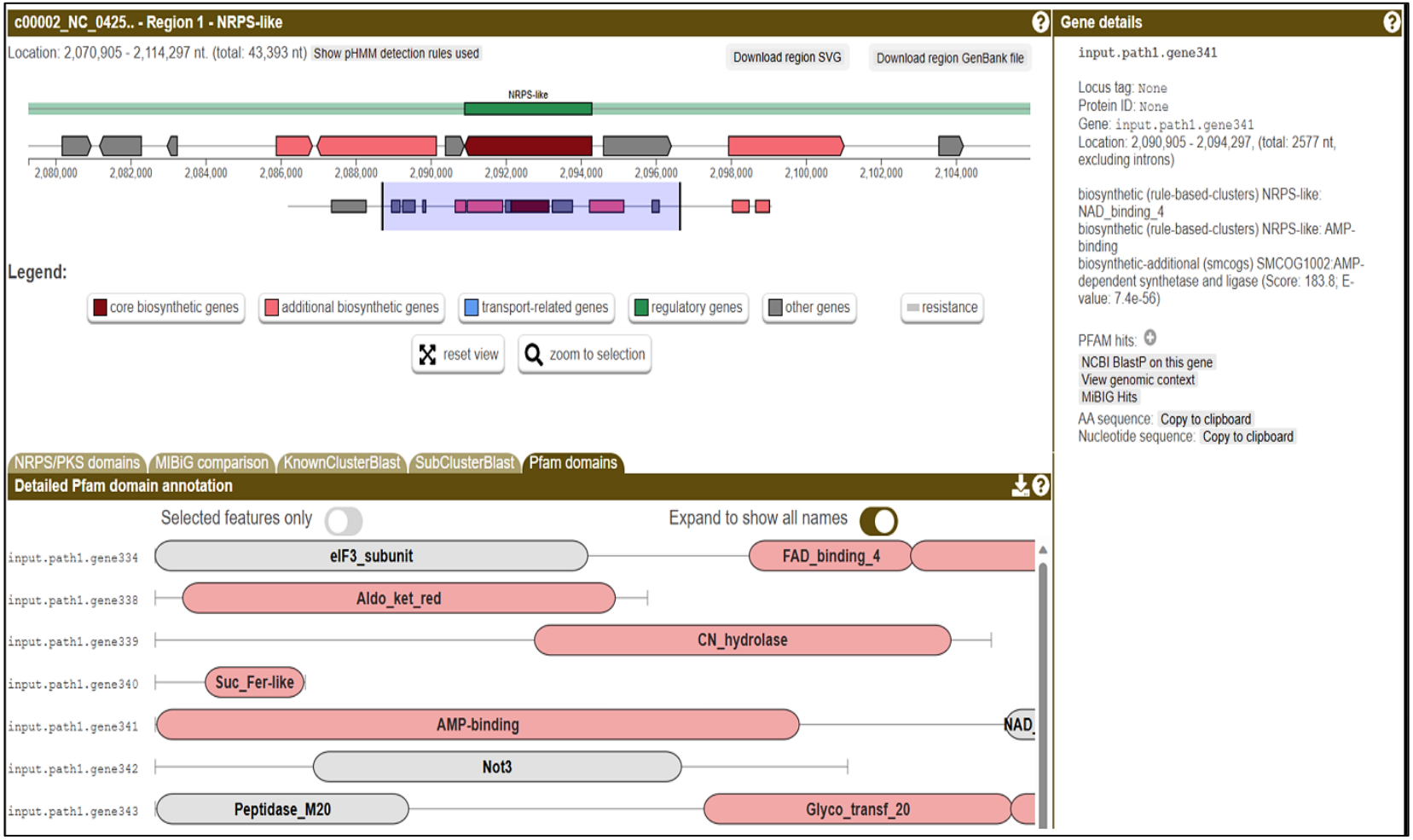


Figure 5.5.1.8: Detection of secondary metabolite gene clusters in Fungal genome using AntiSMASH (Core biosynthetic genes; Additional biosynthetic genes; Transport-related genes; Regulatory genes)

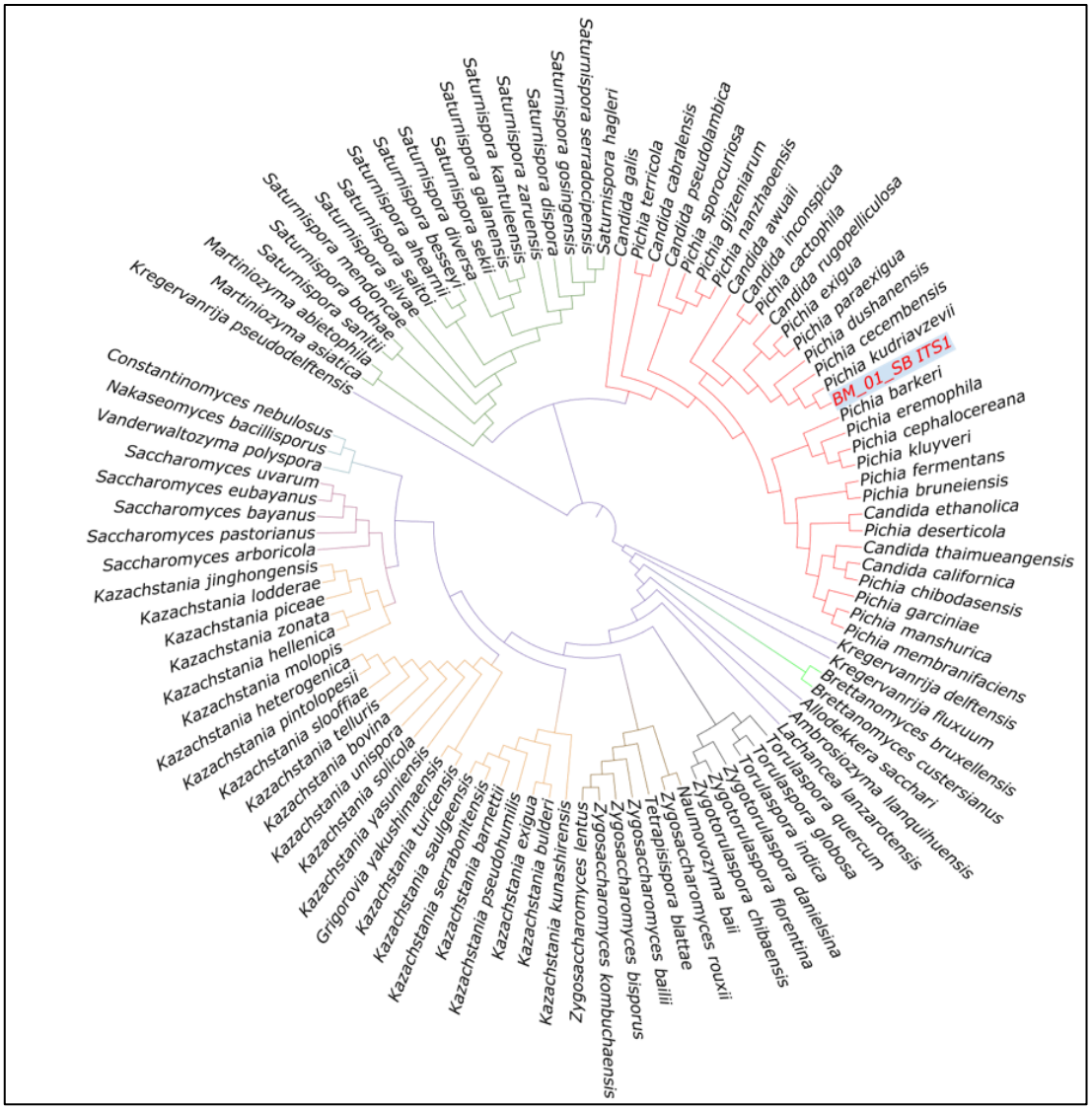


Figure 5.5.1.9: Phylogenetic tree representation using fungal sequence

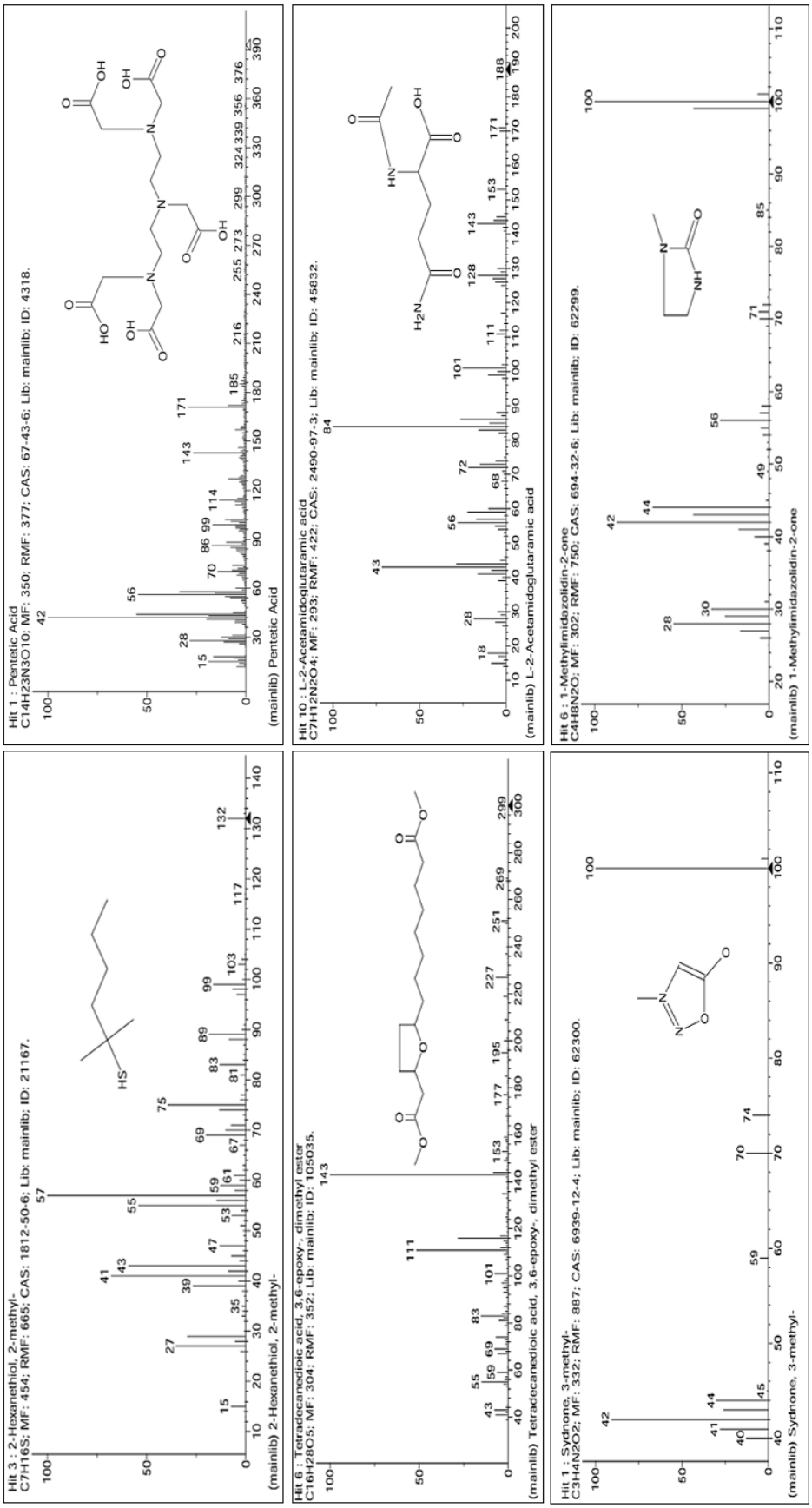


Figure 5.5.1.10: GC-MS Spectra of derived compounds

2-Hexanethiol, 2-methyl; Pentetic Acid; Tetradecanedioic acid, 3,6-epoxy-, dimethyl ester; L-2-Acetamidoglutaramic acid; Sydnone, 3-methyl; 1-Methylimidazolidin-2-one (From Top left to right direction respectively)

Name of Compound	Function
2-Hexanethiol, 2-methyl-	❑ Antioxidant and anti-inflammatory properties. ❑ Anti-microbial properties. ❑ Can be used as preservative.
Diethylenetriaminepentaacetic acid (DTPA)	❑ DTPA, along with its calcium and zinc trisodium salts, are the only FDA approved agents for the treatment of internal contamination by transuranics.
Tetradecanedioic acid, 3,6-epoxy-, dimethyl ester	❑ Anti-inflammatory effect via reduction of the pro-inflammatory cytokine IL-6. ❑ Antioxidant effect by reducing ROS generation. ❑ Found to reduce the levels of cholesterol and triglycerides in both *in vivo* and *in vitro* studies.
L-2-Acetamidoglutaramic acid	❑ Anti-diabetic and anti-hypertensive effects. ❑ Anti-proliferative effects via modulation of expression of various genes involved in the regulation of cell cycle progression.
Sydnone, 3-methyl-	❑ Anti-inflammatory and analgesic properties. ❑ Antioxidant, antibacterial and antifungal properties.
1-Methylimidazolidin-2-one	❑ Inhibits growth of *E. coli* and *S. aureus*. ❑ Antioxidant, anti-inflammatory and anti-cancer activities *in vitro*. ❑ Demonstrated neuroprotective effects in animal models of neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease.

Table 5.5.1.1: Bioactive compounds identified from *Pichia kudriavzevii*

5.6. *Comparative Analysis:*

The heatmap shows inter-genera correlations and correlations between genera and anthropometric factors (Figure 5.6.1). The blue squares indicate a positive correlation, red squares indicate a negative correlation while white squares indicate zero correlation. *Prevotella* and *Clostridium* exhibit a concomitant increase in abundance indicating a symbiotic crosstalk in context to the subjects under study. *Roseburia* and *Clostridium* portray potentially antagonistic interaction while *Bifidobacterium* and *Prevotella* are mutually exclusive, as interpreted from the Pearson Correlation heatmap. Increasing trends of abundance in *Prevotella* and diminishing prevalence of *Phascolarctobacterium* have been explored to be correlated with the ectomorph somatotype of the tribes under study.

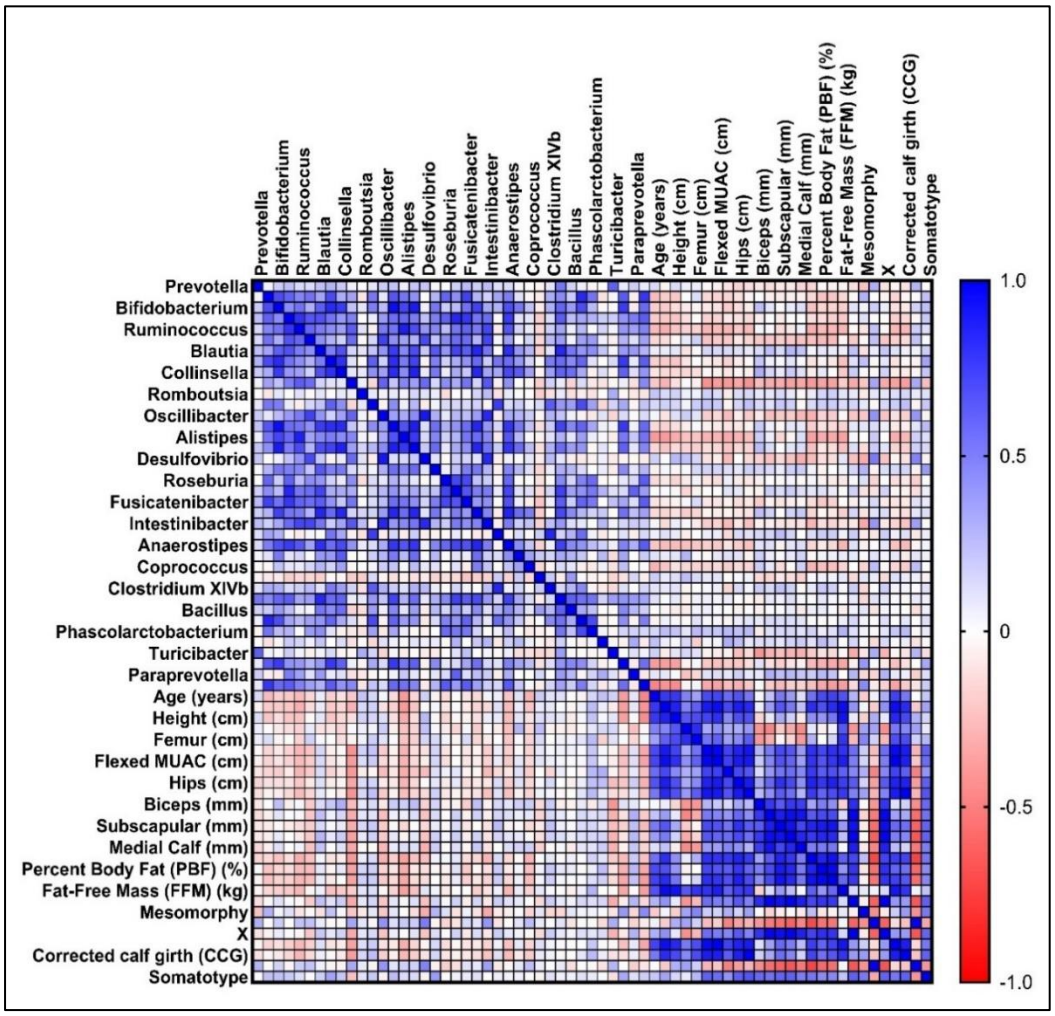


Figure 5.6.1: Heatmap of correlation matrix across core genera and anthropometric parameters of all subjects under study

5.7. *GC-MS analyses of Non- Timber Forest Produces (NTFPs):*

Non-timber forest products (NTFPs) encompass a diverse range of biological materials sourced from forests beyond timber.

Figure 5.7.1: Field exploration of collecting Non-Timber Forest Products (A-I). A. *Kulahu*; B. *Baya*; C. *Chigo*; D. *Kham*; E. *Nyekhla*; F. *Purundi*; G. *Mefry*; H. *Baola*; I. *Meshta*

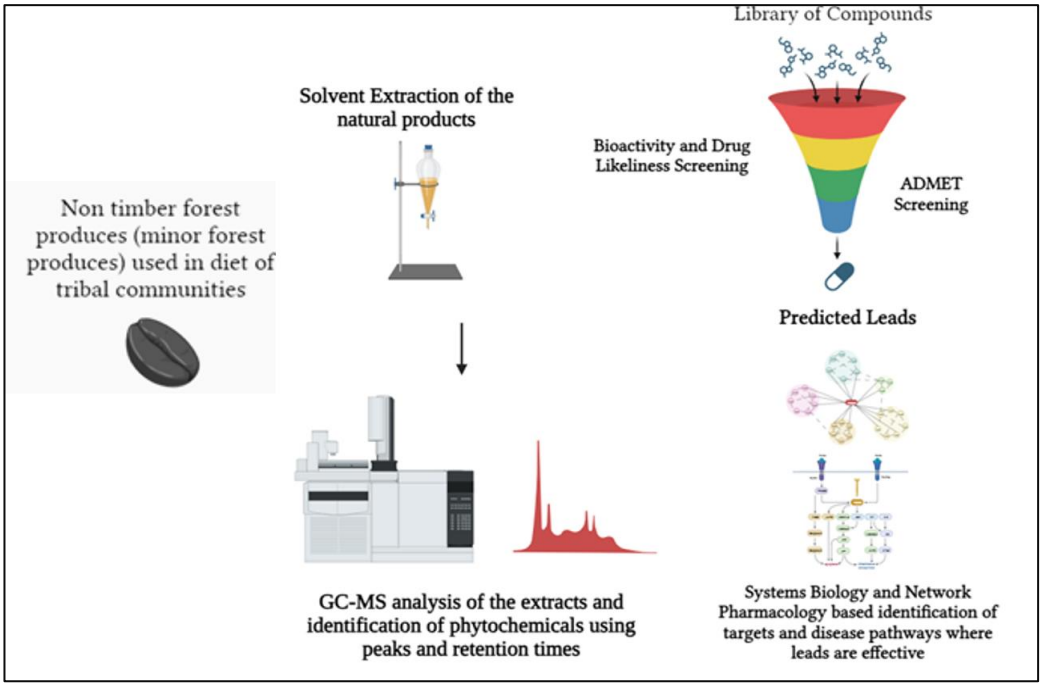


Figure 5.7.2: Methodology of capture and identification of promising therapeutic leads from NTFPs using GC-MS coupled with high-throughput virtual screening

These products, including fruits, nuts, tubers, resins, and fibers, play a vital role in the livelihoods of local communities and hold immense potential for bioprospecting, the search for valuable biological resources. However, responsible utilization and sustainable practices are crucial to ensure the long-term benefits of NTFPs for both people and the environment (Shackleton, 2015). Indigenous communities, intrinsically linked to forest ecosystems, hold ancestral knowledge passed down through generations. This intricate understanding fosters sustainable practices and a deep-seated respect for the delicate balance within these environments (Garnett et al., 2018). Metabolomic profiling identified a total of 90 compounds from the collected NTFP. Subsequent evaluation focused on drug likeness, considering physicochemical and structural properties critical for influencing the pharmacokinetics and pharmacodynamics of these molecules. Four significant compounds viz. Cyclohexylmethane-1,1-diol diacetate, Oxazolidin-2-one, 3-

(2-aminoethyl)-4-hydroxy-4-methyl-5-spiro-cyclohexane, Heptanoic acid, 9-Anthracenepropanoic acid were identified from the above-mentioned pipeline. In accordance with Lipinski's rule of five, the compounds adhered to acceptable ranges for lipophilicity, solubility, and bioavailability. These parameters are associated with absorption, distribution, metabolism, and excretion, collectively influencing the safety and efficacy of the molecule as a potential drug (Figure 5.7.3).

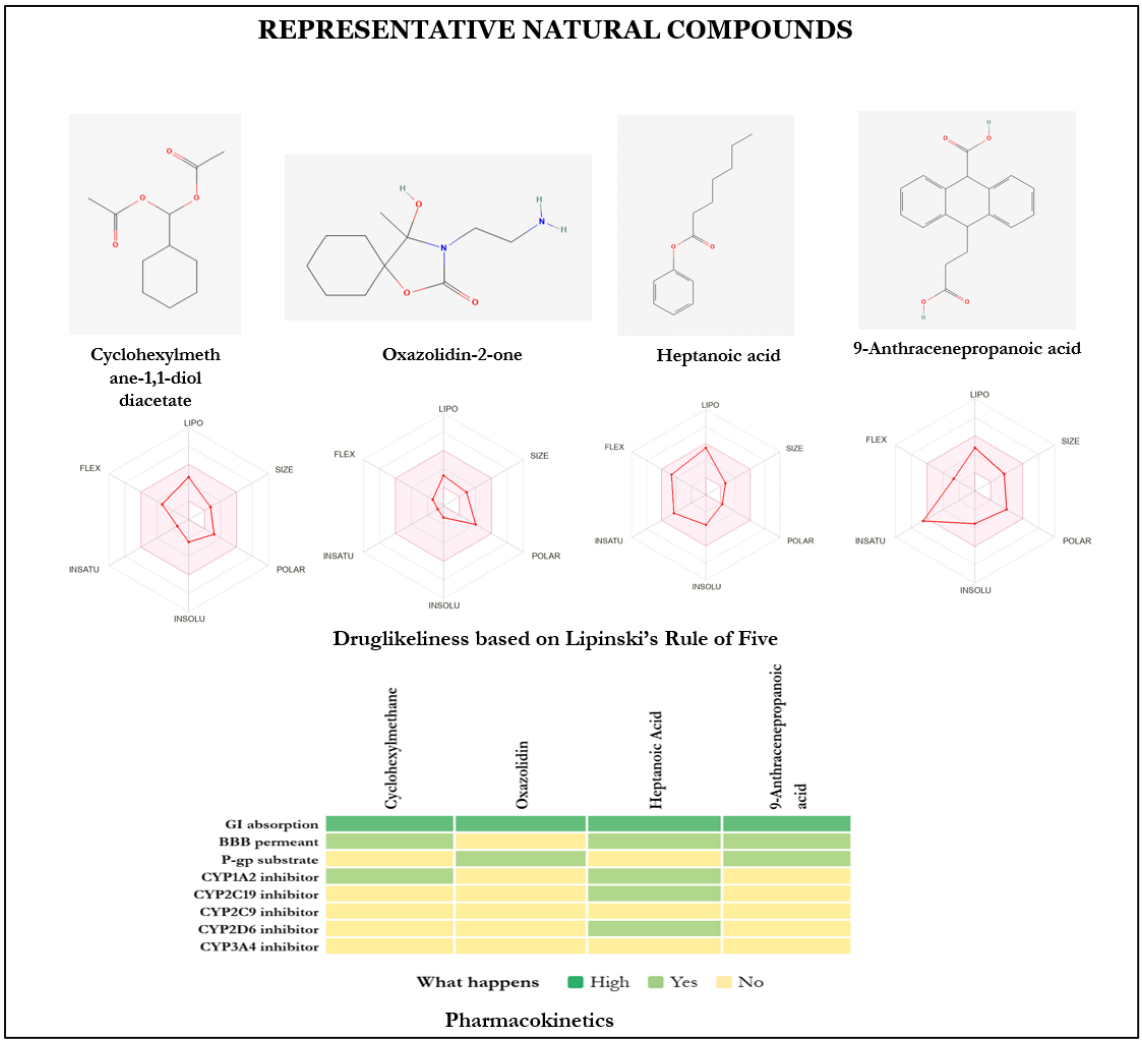


Figure 5.7.3: 2-D Structures of the selected natural ingredients isolated from NTFP along with their drug-likeness and pharmacokinetic properties

Using our approach, we observed a complex network of polypharmacological interactions involving two of our identified small molecules, namely Cyclohexylmethane and Heptanoic acid. These molecules demonstrated communication with multiple targets associated with distinct disorders (Table 5.7.1; Figure 5.7.4).

Ingredients	Interacting Gene	Encoded Protein	Associated Diseases
Cyclohexylmethane	ADH1B	All-trans-retinol dehydrogenase [NAD]	Liver Cirrhosis, Esophageal Cancer, Liver Cancer
	PRSS1	Trypsin-1	Gingivitis, Gallstones, Orchitis
	AAVS1	adeno-associated virus integration site 1	Liver Cirrhosis
Heptanoic Acid	ISYNA1	Inositol-3-phosphate synthase 1	Dysmenorrhea, Liver Cirrhosis, Female Infertility, Lymphadenitis, Constipation, Goiter
	PLA2G1B	Phospholipase A2	Hemorrhoids, Crohn Disease, Hypertension, Rheumatoid Arthritis
	AR	Androgen receptor	Acute Glomerulonephritis, Hypertension, Nephrotic Syndrome, Liver Cirrhosis, Heart Failure, Eczema, Orchitis, Diabetes, Amenorrhea, Cervical Cancer, Erectile Dysfunction, Optic Neuritis, Menstrual Irregularities, Acne, Breast Cancer, Baldness, Gingivitis, Metrorrhagia, Vaginitis, Oral Ulcers, Coronary Heart Disease, Leukemia, Female Infertility, Prostatitis, Hemorrhoids, Hepatitis, Thrombocytopenic Purpura, Male Infertility, Testicular Cancer, Liver Cancer, Menopausal Syndrome, Ascites

. Table 5.7.1: summarizing the ingredient-target gene-disease association

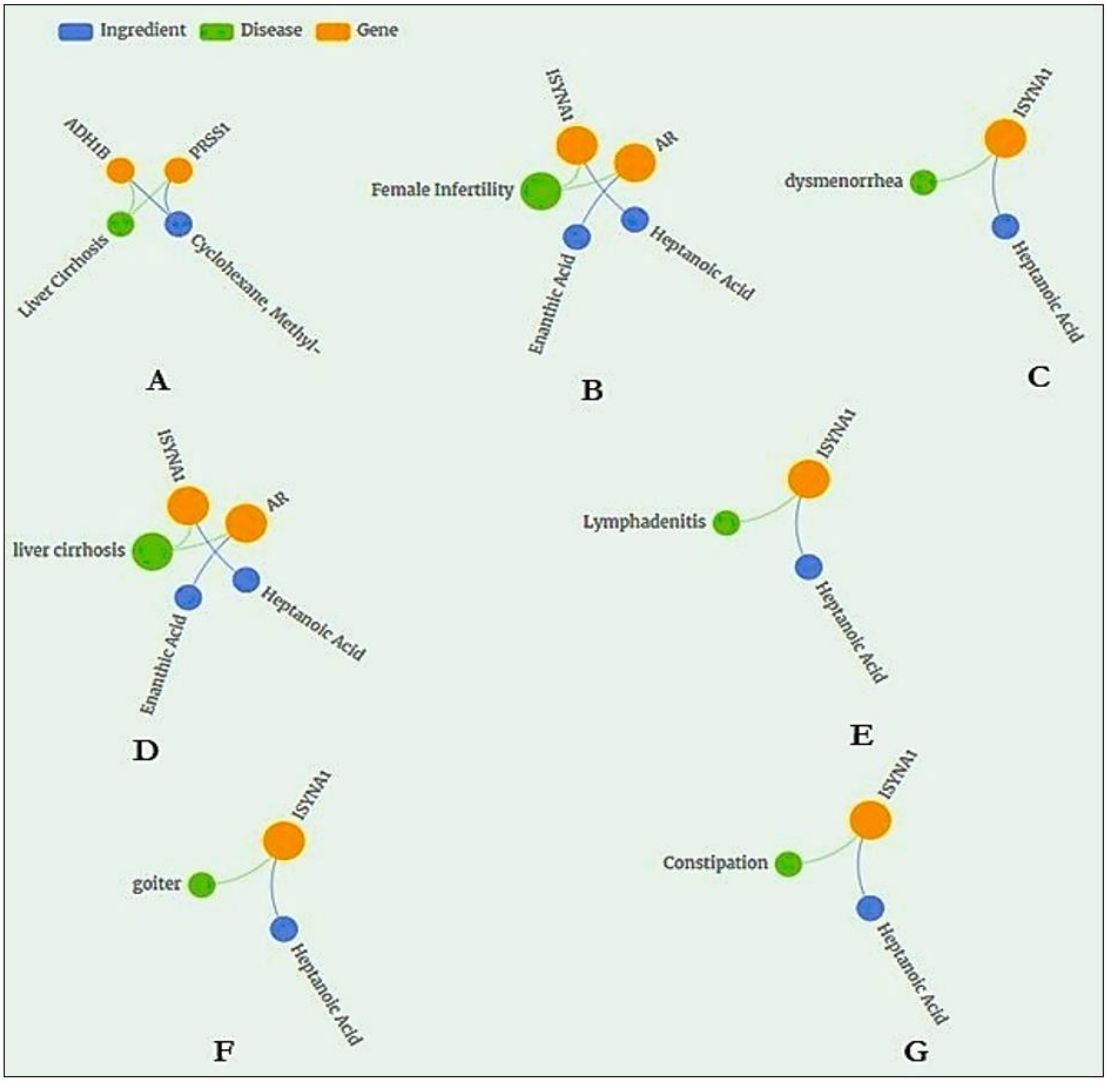


Figure 5.7.4: Network depicting the ligand-target-disease pathway relationship, where each ligand interacts with multiple discrete targets, corelating to a single disease phenotype; A: Cyclohexylmethane with liver cirrhosis; b: Heptanoic Acid with female infertility; C: Heptanoic Acid with dysmenorrhea; D. Heptanoic Acid with liver cirrhosis; E. Heptanoic Acid with lymphadenitis; F. Heptanoic Acid with goiter; G. Heptanoic Acid with constipation

5.8. GWAS studies interpretation across the subjects:

GWAS-based approach was used to detect single nucleotide polymorphisms (SNPs) in their genetic profile that genetically predisposes them to develop certain disease phenotypes. All three participants showed a high genetic tendency to develop biliary cirrhosis and parkinson's disease, with the highest risk being observed in the child. Both the adult and the juvenile male participants also showed high genetic risk of developing prostate cancer, testicular cancer and male pattern baldness. However, female participants exhibited higher risk towards parkinson's disease that can be correlated with the questionnaire data during the sampling of our studied subjects under study (Table:5.8.1.1-5.8.1.3). It was, however, established that the participants, despite having a genetic inclination to develop certain diseases, did not show a phenotypic expression of the same. Thus, we can say probably their gut bacterial diversity and abundance in sort of providing a protective role in preventing the incidences from these diseases mainly the lifestyle ones.

This indicative set of results, we believe can serve as a stepping stone for future GWAS studies for identifying more microbial signature which can act as a potential biomarker for maintenance (Figure: 5.8.1) and protection against lifestyle diseases even when the subjects were predisposed to them based on their SNP profile of their genome and thus pave a new avenue towards personalized gut-based therapies.

	DISEASE	CANDIDATE GENES
SABAR MALE	Atrial Fibrillation	4q25, KCNN3, PITX2
	Prostate Cancer	SRRM1P1-POU5F1B, MSMB, HNF1B, JAZF1, HNF1B, CASC17, CASC8, VDR
	Biliary Cirrhosis	SPIB, MMEL1, IL12A-AS1, IL12RB2, TNFRSF1A, PNPLA3
	Parkinson's Disease	PARK16, MCCC1, CRHR1, SIPA1L2, INPP5F, GAK, MIR4697HG, TMEM175, LOC10537732, LRRK2
	Male Pattern Baldness	TARDBP, AUTS2, SETBP1, AR, AR), AR, PAX1
SABAR FEMALE	Basal Cell Carcinoma	1p36 (PADI6), 1q42 (RHOU)
	Celiac Disease	ITGA4, CD28, LPP, HLA-DQ1, CCR1, LPP
	Biliary Cirrhosis	SPIB, MMEL1, STAT4, PNPLA3
	Parkinson's Disease	PARK16, SNCA, MCCC1, CRHR1, INPP5F, BCKDK, GAK, SNCA, TMEM175, LOC105377329
	Restless Leg Syndrome	MEIS1, BTBD9, PTPRD
SABAR KID	Prostate Cancer	SRRM1P1-POU5F1B, MSMB, HNF1B, JAZF1, HNF1B, CASC17, CASC8, VDR
	Testicular Cancer	BAK1, SPRY4
	Parkinson's Disease	SPIB, MMEL1, TNFRSF1A, STAT4, PNPLA3
	Biliary Cirrhosis	PARK16, SNCA, MCCC1, CRHR1, SIPA1L2, INPP5F, BCKDK, GAK, SNCA, MIR4697HG, TMEM175,
	Restless Leg Syndrome	MEIS1, BTBD9, PTPRD

Table 5.8.1.1: Top 5 diseases along with candidate genes across the Sabar family members

	DISEASE	CANDIDATE GENES
BHUTIA MALE	Renal Cell Carcinoma	ZEB2. 12p12 (BHLHE41), SCARB1
	Prostate Cancer	SRRM1P1-POU5F1B, MSMB, HNF1B, CASC17, CASC8, PRNCR1, PCAT2, VDR
	Parkinson's disease	PARK16, NUCKS1, SNCA, MCCC1, CRHR1, INPP5F, BCKDK, SNCA, TMEM175
	Asthma	HLA-DQ, IL18R1, SMAD3, IL2RB, IL13, RAD50, GSDMB-ORMDL3, GSDMB
	Male Pattern Baldness	HDAC9, TARDBP, SETBP1, PAX1 and FOXA2, AR, PAX1
BHUTIA FEMALE	Atrial Fibrillation	4q25, PITX2
	Ankylosing Spondilytis	Intergenic, 2p15 (near B3GNT2), IL23R, Il1R2, 21q22 (near PSMG1)
	Parkinson's disease	PARK16, NUCKS1, SNCA, MCCC1, CRHR1, INPP5F, BCKDK, SNCA, TMEM175
	Rheumatoid Arthritis	HLA-DRB1, HLA, IL2RA
	Age Related Macular Degeneration	ARMS2, HTRA1
BHUTIA KID	Prostate Cancer	SRRM1P1-POU5F1B, HNF1B, CASC17, CASC8, PRNCR1,PCAT2, VDR, CASC19,PCAT1
	Atrial Fibrillation	4q25 , PITX2
	Parkinson's disease	PARK16,NUCKS1, SNCA, MCCC1, CRHR1, INPP5F, BCKDK, SNCA, TMEM175
	Rheumatoid Arthritis	HLA-DRB1, HLA, CD40, TRAF1, IL2RA, C5-OT1, MMEL1
	Male Pattern Baldness	HDAC9, TARDBP, SETBP1, PAX1 and FOXA2, AR, PAX1

Table 5.8.1.2: Top 5 diseases along with candidate genes across the Bhutia family Members

	DISEASE	CANDIDATE GENES
MECH MALE	Atrial Fibrillation	ZFHX3, ZFHW3, 4q25, PITX2
	Stroke	NINJ2, 4q25, TWIST1- HDAC9
	Prostate Cancer	MSMB, HNF1B, PRNCR1, PCAT2, VDR
	Male Pattern Baldness	HDAC9, TARDBP, SETBP1, AR, PAX1, PAX1
	Parkinson's	PARK16, SNCA, MCCC1, CRHR1, SIPA1L2, INPP5F, BCKDK, TMEM175, LOC105377329
MECH FEMALE	Sudden Cardiac Arrest	BAZ2B
	Parkinson's	PARK16, SNCA, MCCC1, CRHR1, INPP5F, SNCA MIR4, 697HG, TMEM175, LOC105377329
	Vitiligo	6p21, TYR, GZMB, UBASH3A, C1QTNF6, CLNK, SLA, CD44, TICAM1
	Systematic Lupus Erythromatosus (SLE)	STAT4, TNFSF4, BANK1, TYK2, TNIP1, UBE2L3, PRDM1, ATG5, ITGAM, STAT4
	Celiac Disease	ITGA4, CD28, LPP, HLA-DQ1, TAGAP, CCR1, LPP
MECH KID	Atrial Fibrillation	ZFHX3, ZFHW3, 4q25, PITX2
	Stroke	NINJ2, 4q25, TWIST1- HDAC9
	Celiac Disease	ITGA4, LPP, HLA-DQ1, TAGAP, CCR1
	Vitiligo	RERE, IL2RA, TYR, GZMB, UBASH3A, C1QTNF6, SLA, CD44, TICAM1
	Systematic Lupus Erythromatosus (SLE)	STAT4, BANK1, TYK2, TNIP1, STAT4

Table 5.8.1.3: Top 5 diseases along with candidate genes across the Mech family members

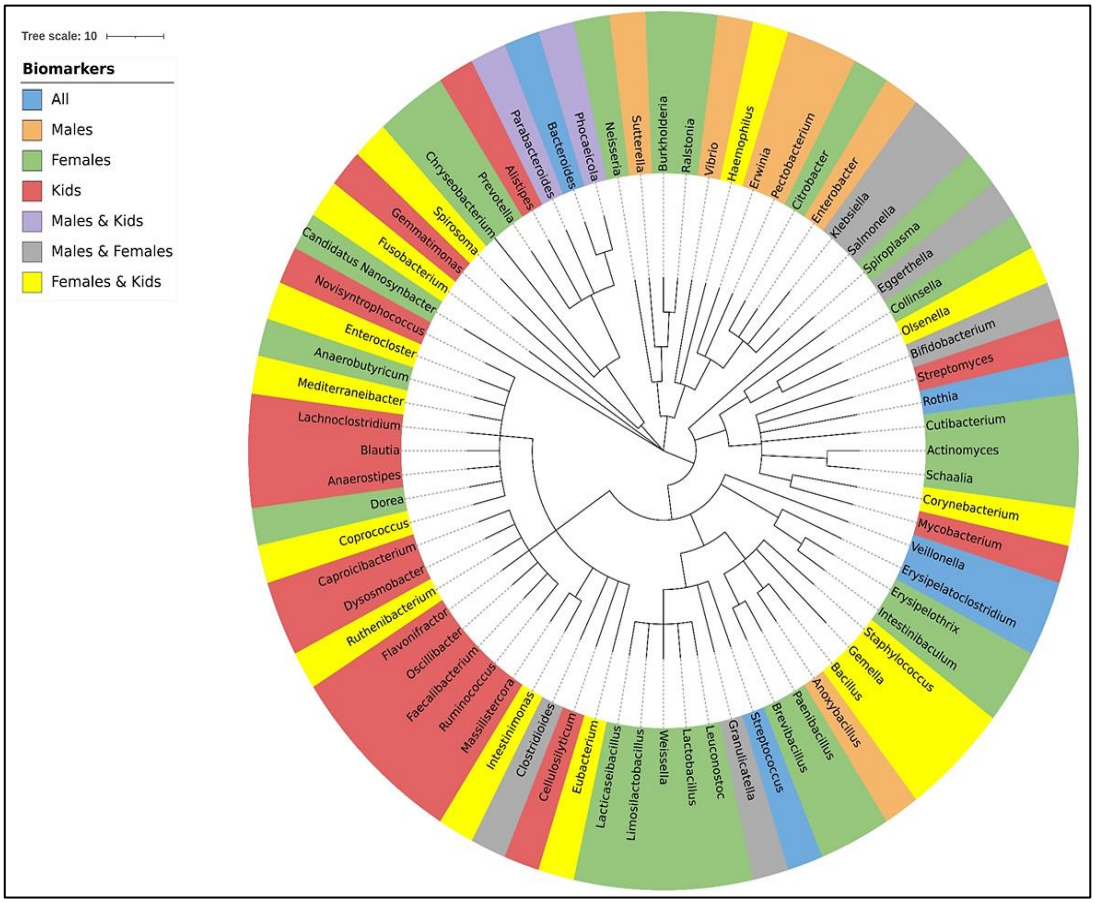


Figure 5.8.1: Biomarkers obtained from LefSE analysis

6. Discussion:

The data contributed to the fact that the male individuals of Sabar and Mech tribe under the category of Chronic Energy Deficiency which is an indicator of malnutrition and aligns with their current socio-economic status and lifestyle (Vernocchi et al., 2020).

When comparing the results of the current study with previous research conducted on other tribal communities of West Bengal, a high prevalence of undernutrition was also found among adult male Bhumij (52.3%), Munda (50%), Koras (51.9%), Santals (55.0%), Oraon (46.2%), and Lodhas (45.2%), and lower among Birhor (19.4%) (Bisai and Bose, 2012; Das and Bose, 2014; Ghosh and Bose, 2015; Ghosh and Malik, 2007; Kshatriya and Acharya, 2016). The present study also revealed that the tribal male members exhibited a higher prevalence of undernutrition when compared to the Sabars (38.0%) in Keonjhar, Orissa (Bose et al., 2006), Sabars (40.3%) in Cuttack and Khurda, Orissa (Chakrabarty and Bharati, 2010), and Sabars of Bankura in West Bengal (46.8%) (Ghosh et al., 2018). This may be because of changes in food habits and physical activities, which were identified as the common reasons for the increase in undernutrition in some of the studied tribal populations (Das and Bose, 2014; Kshatriya and Acharya, 2016). Malnutrition can result from a combination of various factors, spanning both biological and administrative realms. Genetic predisposition plays a role in how individuals absorb and adapt to nutrients from their diets, whereas food and viable means of sustenance hinge mainly on policy decisions. Indigenous populations often find themselves disadvantaged on both fronts due to their challenging environments and their isolation from more developed areas. Consequently, scholarly discussions are the primary source of epidemiological and cognitive information, while older people from indigenous communities are frequently overlooked due to their limited ability to contribute to

livelihoods. Several studies have shown that in recent years, urban market centres were developed close to the habitat of indigenous people in most of the low and middle-income countries, and these people became exposed to the urban lifestyle and have exhibited a broad shift explicitly in their structure of diet and physical activities (Cheema et al., 2014; Kshatriya and Acharya, 2016; Misra and Khurana, 2008). Given the limitations of using BMI as a measurement, MUAC was incorporated as an alternative to BMI in the present study. There is a dearth of studies in the Indian context that have used the newly proposed cut-off by to assess underweight (Tang et al., 2020). Sabar kids exhibited overweigh category in context to BMI; whereas MUAC indicated undernourishment. It can be correlated with several instances like not getting sufficient amount of nutrients like vitamins along with minerals from their dietary practices (Kiani et al., 2022). Significantly, their meal pattern has been altered due to the boarding school along with the mid-day meal program that can be correlatable with the reflection in MUAC value (Shanker and Arora, 2022). BMI was not able to provide more holistic insights therefore somatotype prediction has also been performed to have a clearer vision in context to their nutritional status (Banik et al., 2021).

The Sabar community follows a non-vegetarian diet. Rice is their main staple, which they eat twice a day, along with seasonal vegetables such as cabbage, cauliflower, eggplants, tomatoes, pumpkins, radishes, raw papayas, and other wild forest produce. They also include flesh items such as fish, rats, chickens, snails, snakes, which are collected from nearby water bodies or agricultural field. Some individuals in the community have a habit of drinking tea liquor (without milk and sugar) with salt. Additionally, the consumption of alcoholic beverages, both local and traditional, is common among them on a regular basis (Das et al., 2022).

The Bhutia tribe has relied upon traditional agricultural practices and is still dependent on livestock farming, locally produced vegetables and fruits for their livelihood, along with liquor and fermented dairy products, making their gut prototypical for unadulterated microbes (Subba, 2016). The unique microbial metabolic pathways show abundance in amino acid, carbohydrate and lipid metabolism pathways which are similar to the composition of microbiota in Mongolians that are attributed to be functional in imparting stronger metabolism of the population coupled with high meat and fermented diet uptake (Liu et al., 2016). Along with this, the appearance of *Bacteroides* and *Clostridium* have been correlated with the metabolism of saturated fatty acids, amino acids and animal protein. Inflated intake of milk products and fermented products have also resulted in the abundant existence of *Bifidobacterium* and *Lactobacillus* in their gut which is remarkably evident in other hilly tribes like Lepcha, Bhutia and Nepalis of Sikkimese origin (Tamang et al., 2012). Besides dietary habits, drinking water sources which are composed of different minerals and chemical com-pounds may influence the gut microbial community among the studied population as well (Jha et al., 2018).

The Mech community's diet consists of a diverse range of locally available vegetables, roots and tubers, pulses, dairy products, and various types of meat. Rice is their staple food, consumed three times a day. Mustard oil is commonly used for cooking purposes. They consume meat from animals such as goats, sheep, buffaloes, fowls, frogs, quail, ducks, as well as eat fish. They have also domesticated animals like buffaloes, goats, and ducks, which provide a regular source of milk, dairy products, and meat. Like many other indigenous communities, consumption of alcoholic beverages also seen among them. They also take tea (without sugar and milk) with salt frequently (Sanyal, 1973).

The gut microbiome of urban individuals harbors various bacterial genera, each playing distinct roles. Higher abundance of *Methanobrevibacter* in context to the urban male and female can be correlated with their dietary practices. The methane gas produced by Methanobrevibacter can contribute to bloating, a common symptom of IBS (Ghoshal et al., 2016). Whereas, interestingly urban kid exhibited the presence of *Alcaligenes* that can be correlated with the commensal activity of the particular genera that would prevent the attack from the opportunistic pathogens (Wang et al., 2020). *Faecalibacterium* is a beneficial bacterium producing short-chain fatty acids (SCFAs) that promote gut health and potentially reduce the risk of inflammatory bowel disease (Saxami et al., 2023). *Clostridium* is a diverse genus encompassing both beneficial and harmful species. Beneficial *Clostridium* species like *C. butyricum* and *C. coccoides* produce SCFAs, while others contribute to complex carbohydrate breakdown. However, some *Clostridium* species like *C. difficile* can cause severe complications like antibiotic-associated diarrhea (Czepiel et al., 2019). Whereas, *Lactobacillus*, a genus of lactic acid bacteria with probiotic properties, ferments carbohydrates, creating an acidic environment that inhibits harmful bacteria. This may contribute to improved digestion, reduced diarrhea risk, and potentially manage certain inflammatory conditions. It's essential to keep in mind that the makeup of the gut microbiome differs greatly throughout people and is impacted by variables other than the mere existence of these particular bacteria (Rastogi and Singh, 2022).

The gut microbiota of tribal groups includes much higher levels of *Bifidobacterium* and *Turicibacter* bacteria than those of urban persons. This can be correlated with their traditional diets, which are frequently high in fermented foods and prebiotic fibers that encourage the growth of *Bifidobacterium*, have an impact on this (Leth et al., 2023).

Furthermore, their number may be further enhanced by early exposure to a variety of gut microorganisms through intimate interaction with nature and animals in tribal cultures. In a similar vein, the little-known *Turicibacter* also grows well in these conditions and may be involved in immunological control and the fermentation of carbohydrates (Smits et al., 2017). While more research is needed to determine the precise mechanisms and long-term health effects of this higher prevalence, the different gut microbial composition of tribal populations in comparison to urbanized people emphasizes the substantial influence of dietary patterns and environmental factors on the gut microbiome.

Based on the relative bacterial abundance profile, the core functions were predicted and visualized as a heatmap. A large number of core functional pathways were also predicted, which encompasses both homeotic and response pathways. These tribal guts are still protected from over-the-counter (OTC) medicines and antibiotic resistance should also be unusual amidst them. Surprisingly, we found enrichment of several antibiotic resistance pathways in these datasets, namely vancomycin and cationic antimicrobial peptide (CAMP) resistances. Vancomycin resistance was prevalent only in Sabar female and kid gut while CAMP resistance was found in Sabar kid and Mech male gut. This can be explained by the Sabar female having suffered from colitis for which she had taken vancomycin as a prescribed drug. On the other hand, presence of both vancomycin and CAMP resistance has been correlated with presence of soft tissue infections by *Staphylococcus* which suggests that the Sabar kid may have suffered from a latent infection (Hatlen and Miller, 2021). CAMPs are important innate immune compounds that prevent the colonization of pathogens and contribute to infection clearance. The Mech male's high prevalence of CAMP resistance may be attributed to the presence of a higher load of pathogens in his system (Band and Weiss, 2014).

As per recent literatures, a vegetarian diet can stimulate the development of *Fecalibacterium*, *Bacteroides*, *Prevotella* and *Clostridium* in the gut (Rajoka et al., 2017). The increasing counts of the above-mentioned gut bacterial species in the gut of Sabar population supports the claim of their consumption of green leafy as well as wild varieties of vegetables in their diet. *Prevotella*, which is an important gut commensal, is found in abundance in both male and female guts of sabar and mech tribes. Its presence can be attributed to high intake of plant-based carbohydrate (Salonen and De Vos, 2014). Regular consumption of green leafy vegetables also correlates with increased *Bifidobacterium* abundances across all members under analysis (Seo et al., 2020). This observation is similar to reports of similar abundances in the core bacteriome of a few primitive tribes across the world (Smits et al., 2017; Rinninella et al., 2019).

Polyphenols (e.g., from tea) increases abundance of intestinal barrier protectors (*Bifidobacterium* and *Lactobacillus*), butyrate-producing bacteria (*Fecalibacterium* and *Roseburia*), *Bacteroides* and *Akkermansia* but decreases lipopolysaccharide producers (*Escherichia* and *Enterobacter*) (Moreno-Indias et al., 2016). In our study, the Sabar kid displayed an increased *Bacteroides*, *Akkermansia*, *Lactobacillus*, *Faecalibacterium* and *Roseburia* content which can be attributed to his consumption of tea. Similarly, higher abundances of *Bifidobacterium*, *Faecalibacterium* and *Bacteroides* as well as lower abundances of *Escherichia* and *Enterobacter* in Sabar male and female gut can be attributed to their tea consumption. High meat diet correlates with a higher abundance of *Bacteroides* and *Clostridium* and lower counts of *Bifidobacterium* than a meatless diet, as it is evident from our data which shows higher prevalence of *Bacteroides* in all tribal gut bacterial profiles (Salonen and De Vos, 2014). It is interesting to note that both sabar and mech kids have a much higher *Bacteroides* abundance as compared to their parents

which may be attributed to the cultural practice in rural households of allocating the bigger chunk of delicacies such as meat to the children in the family.

Our study provides first report of genus *Holdemanella* in the gut bacterial profile of Sabar and Mech tribes. Abundance of this genera has been positively correlated with the consumption of meat such as duck and mutton, dairy products and algae vegetables and high abundance of this genera in Sabar male and female can be justified by their greater consumption of meat products and vegetables (Zhang et al., 2022).

More and more people are realizing how important the gut microbiota is to human health. Despite the significant differences in microbiome makeup amongst worldwide lifestyles, studies on microbiomes are strongly skewed towards industrialized people in the West. Low microbiome diversity is a characteristic of industrialised communities (Carter et al., 2023). It has been proposed that a number of lifestyle factors, such as the use of baby formula and caesarean delivery, high rates of antibiotic use, hygienic living conditions, and less direct contact with soil and animals, are responsible for this decline in diversity. Non-industrialized human communities, such as the study subjects, have very high microbiome diversity and do not live with these features (Abdill et al., 2022; Sonnenburg and Sonnenburg, 2019; Smits et al., 2017; Vangay et al; 2018; Martinez et al., 2015). Microbial taxa that are specifically linked to both industrialised and non-industrialized populations are known as VANISH (volatile and/or negatively associated with industrialised societies of humans) and BloSSUM (bloom or selected in societies of urbanization/modernization) taxa, respectively (Yatsunenko et al. 2012; Carter et al., 2023; Clemente et al., 2015).

In context to the vanishing taxa of sabar tribe, we have identified few interesting genera including *Lentibacillus, Zymomonas, Enteractinococcus* etc. *Lentibacillus* may aid in plant polysaccharide degradation and produce antimicrobial compounds (Saravanan et al, 2024). *Enteractinococcus* may be involved in gut epithelial cell adhesion and modulation of the immune system (Tinnirello et al., 2024). The presence of these two genera can be correlated by the livestock management of the subjects under study (Das et al., 2021). *Zymomonas* is involved in ethanol production and may contribute to gut fermentation processes (Li et al., 2022). It can be correlated with the occasional drinking of alcohols by the sabar communities (Das et al., 2021).

The vanishing taxa *Bilophila, Eikenella, Elusimicrobium,* and *Johnsonella* have been found to be present in the gut microbiome of the Bhutia tribe. These microorganisms are believed to play a crucial role in the tribe's adaptation to their unique diet and environment. *Bilophila* potentially involved in the breakdown of sulfur-containing compounds whereas *Eikenella* may be contributed to the degradation of complex polysaccharides (Dostal Webster et al., 2019; Onyango et al., 2021). *Johnsonella* may be involved to play a role in the metabolism of amino acids (Le Bastard et al., 2020). These can be correlated with the fermented food products consumption of the studied tribe (Kahve, 2023). The loss of these taxa, potentially due to modernization and changes in lifestyle of the Bhutia tribe (Basu et al., 2022).

In context to the Mech tribe, we have found *Murimonas, Balneola, Frondihabitans* etc. as vanishing microbes. *Frondihabitans* has been reported to be resided in the plant leaves that can be correlated with the consumption of plant based dietary pattern of the studied subjects (Longa et al., 2022). *Murimonas* and *Balneola* have been linked with the decomposition of organic matter in water. The shift in the dietary pattern along with the

source of the groundwater can be attributed to the fact of identifying these genera as vanishing one (Calvigioni et al., 2023; Núñez Salazar et al., 2020).

Anaerobacter, *Methanocorpusculum*, *Giesbergeria* etc. appeared as a BloSSUM taxa in the urban individuals under study. The presence of these genera in the urban gut can be attributed to the overall shift towards the processed foods which may influence the composition of gut microbiota, potentially reducing some beneficial bacteria and allowing others to thrive (Singh et al., 2023; Chibani et al., 2022; Bouchali et al., 2024).

Culture dependent study provides an important insight on the tribes under study. An extensive drug resistant species *Bacillus aerius* has been found in the gut of the sabar individuals can be correlated with the consumption of catfish like (*Clarias batrachus*; local name Magur and *Heteropneustes fossilis*; local name Shingi) in their regular diet (Meidong et al., 2018). Whereas, consumption of tilapia fish from the local market by the bhutia individuals exhibit the presence of multi-drug resistant *Bacillus safensis* in their gut (Wu et al., 2021). Mech tribes exhibited a dietary practice of eating the meat of quail bird that can be correlated with the presence of an extensive drug resistant species of Mammalicoccus sciuri in their gut (Martinez-Laorden et al., 2023). Interestingly, we have also found *Pichia kudriavzevii* in the gut of the Bhutia male member. It can be correlated with the FFQ data that was obtained during our sampling. Existing literature also exhibited the daily consumption of fermented food products can be attributed to the presence of this fungus (Kahve, 2023).

The tribal practices of using wild as well as cultivated herbs and trees for various medication purposes is ancient and well-documented. The knowledge, however, has been transmitted mostly orally across generations and suffers a gradual loss with time, even

more so in recent decades as the influence of western medicine grips the present generation insurmountably (Bushi et al.; 2021; Uniyal et al. 2006).

The ancient Indian medicinal system had categorically documented the significance and the health and nutritional benefits of thousands of medicinal plants found in the wild that has been traditionally used for centuries to cure an extensive range of diseases from common cuts and wounds, fever, nausea, diarrhoea and dysentery to more complex ailments such as diabetes, pneumonia and jaundice (Basu et al., 2022; Kumar et al., 2021). Among the Santal tribes of West Bengal, some of the recorded known uses of ethnomedicinal utilities include the use of leaf extracts of *Hygrophila auriculata* in the treatment of anemia, *Amaranthus viridis* to counter the venom from deadly snake bites, and the flowers of *Celosia cristata* in the cure of dysentery (Basu et al., 2022). Evidence of medicinal significance in terms of cytotoxic and apoptotic effects have been established in *Calotropis procera* (Apocynaceae), *Millettia pinnata* (Fabaceae) and *Basela alba* (Basellaceae) against lung cancer cell lines (Kumar et al., 2019). Leaf extracts from *Moringa oleifera* (Moringaceae) exhibited potential antioxidative function but poor anti-cancerous activities. Hence there is limited work on the tribal subjects of our interest.

Androgen receptor acts as a transcription factor and nuclear receptor that binds male steroid hormones, and exerts its effect on signaling pathways that regulate the development of tumorigenic growth in the prostate (Davey and Grossmann, 2016). A similar inference can be drawn about the absence of symptoms for colorectal cancer in the female candidate. Glutamate Ionotropic Receptor Kainate Type Subunit 3 (GRIK3) is a vital neurotransmitter receptor in the human body involved in multiple neurodegenerative disorders (Xiao et al., 2019). The upregulation of GRIK3, however,

has also been reported in a number of patients diagnosed with colorectal and breast cancers (Fang et al., 2021; Xiao et al., 2019). The compound, Oxazolidin, identified from our study has been speculated to interact with GRIK3 receptor which might be a probable explanation for the prevention of cancer, in spite of having a genotypic tendency for the same (Mehra, 2008).

Much of the nutrigenomic focus has been on single-nucleotide polymorphisms (SNPs), DNA sequence variations that account for 90% of all human genetic variation. SNPs that alter the function of *"housekeeping genes"* involved in the basic maintenance of the cell are assumed to alter the risk of developing a disease (Babushkina and Kucher, 2023). Dietary factors may differentially alter the effect of one or more SNPs to increase or decrease disease risk (Hong et al., 2023). Recent work exhibited that the unique physiological and genetic characteristics of individuals influence their reactions to different dietary constituents and nutrients. This notion is the foundation of personalized nutrition (Lagoumintzis and Patrinos, 2023). Though significant progress has been made in understanding the impact of genetic variants on macronutrient and micronutrient levels and the individual's responsiveness to dietary intake, the exact routes of how the impact of these variants is increased or decreased still remains unelucidated largely (Nacarelli et al., 2024). Our network pharmacology-based predictions provide possible insights on the targets of the phytocompounds in the human system and their corresponding disease associations. These insights need to be explored further to comprehensively understand the actual mechanisms.

7. Conclusion:

Concealed into the depths of our gastrointestinal tract, a conglomeration of microorganisms comprising of bacteria, fungi and viruses, continues to thrive and contribute significantly to our daily activities including our brain and digestive functioning.

Gut microbiome is a specialized niche present in each one of us, exhibiting commonality as well as uniqueness. When we started our journey of unraveling the secrets of the gut microbiome in our fellow ethnic tribal populations, we were able to understand, how the gut of these ethnic people has been shaped by their dietary intakes, which still contains natural forest produce in significant quantities, apart from the traditional grain crops and flesh.

Our study involving the Bhutia, Mech and Sabar tribes indicate that association of *Prevotella, Bifidobacterium, Akkermansia, Holdemanella* form a core consortium, which allows consistency in their subsistence. Along with the tribal population, we have also selected an urban family to find the alterations in gut bacterial assemblages and finally would been able to map the gut microbial consortia among the tribals and the urbanized population based on their subsistence pattern. Whereas, we have found significant increase of genera *Metanobrevibacter*, *Faecalibacterium, Alcaligenes, Romboutsia* etc. can be correlated with their lifestyle and dietary patterns.

However, specialized metabolites present in the NTFPs and their microbe processed byproducts holds the promise of performing functions such as hepatoprotection, ameliorating IBS and lactose intolerance if cutting edge microbial consortium therapy is employed.

Furthermore, the pristine gut profiles of these ethnic groups serve as eye opener to understand how, switching to a more popular, readily available, processed and fast-food diet, adversely affects our metabolic cascades.

Further investigations are being made so that we can propose a comprehensive therapeutic protocol.

8. Key Findings:

- This study provides the first insights into the Gut Bacterial abundance, of the three tribes from West Bengal, studied over a period of three years.
- This longitudinal study enabled the mapping of the transitional gut bacteriome across the three tribes and was also instrumental in identification of vanishing taxa.
- Anthropometric assessment was instrumental in identification of the essential parameters which stem the fact that the male individuals of Sabar and Mech tribe came under the category of Chronic Energy Deficiency. This is an indicator of malnutrition and aligns with their current socio-economic status and lifestyle (Figure 5.1.1).
- Comparison across the tribes and urban individuals exhibited more enriched pathways due to the shift towards urbanized diet (Figure 5.3.20).
- It seems that traditional diet practices have resulted in the maintenance of a standard core gut bacterial composition for these members of the Sabar, Bhutia and Mech tribe (Figure 5.4.3-5.4.5).
- Therefore, the consumption of traditional Non-Timber Forest Products (NTFPs) like Baya, Chigo, Kudri, Baola, Churku, etc. by the tribals may enhance maintenance of gut bacterial diversity (Figure 5.7.1-5.7.4).
- Detection of gut microbial biomarkers associated with genetic markers of disease in future GWAS studies in a wider cohort may aid the development of personalized therapeutics (Table 5.8.1.1.- 5.8.1.3; Figure 5.8.1).

9. Raw Data and Other Database Submissions:

BioProject	SRA Accession Number	Description
PRJNA525343	SRX5459385	Gut Microbiome analysis of Sabar Male
	SRX5459389	Gut Microbiome analysis of Sabar Female
	SRX5459403	Gut Microbiome analysis of Sabar Kid
PRJNA723462	SRX10651311	Gut Microbiome analysis of Bhutia Male
	SRX10650646	Gut Microbiome analysis of Bhutia Female
	SRX10655654	Gut Microbiome analysis of Bhutia Kid
PRJNA726860	SRX10766711	Gut Microbiome analysis of Mech Male
	SRX10759329	Gut Microbiome analysis of Mech Female
	SRX10752763	Gut Microbiome analysis of Mech Kid
PRJNA1085049	SRX23865137	Gut Microbiome analysis of Sabar Male
	SRX23865170	Gut Microbiome analysis of Sabar Female
	SRX23865518	Gut Microbiome analysis of Sabar Kid
PRJNA1085478	SRX23877341	Gut Microbiome analysis of Bhutia Male
	SRX23877437	Gut Microbiome analysis of Bhutia Female
	SRX23878900	Gut Microbiome analysis of Bhutia Kid
PRJNA1085558	SRX23967157	Gut Microbiome analysis of Mech Male
	SRX23967286	Gut Microbiome analysis of Mech Female
	SRX23975142	Gut Microbiome analysis of Mech Kid
PRJNA1131779	SRX25207935	Gut Microbiome analysis of Sabar Male
	SRX25207952	Gut Microbiome analysis of Sabar Female
	SRX25208019	Gut Microbiome analysis of Sabar Kid
PRJNA1131795	SRX25209278	Gut Microbiome analysis of Bhutia Male
	SRX25209296	Gut Microbiome analysis of Bhutia Female
	SRX25209306	Gut Microbiome analysis of Bhutia Kid
PRJNA1131813	SRX25209544	Gut Microbiome analysis of Mech Male
	SRX25209561	Gut Microbiome analysis of Mech Female
	SRX25209562	Gut Microbiome analysis of Mech Kid
PRJNA1131964	SRX25211870	Gut Microbiome analysis of Urban Male
	SRX25211872	Gut Microbiome analysis of Urban Female
	SRX25211946	Gut Microbiome analysis of Urban Kid

Table 9.1.1: Metagenomic Data submitted in NCBI

GenBank Submission ID	Description
SUB14588049	*Bacillus aerius* strain SG_MMG_ SSB_1 16S ribosomal RNA gene, partial sequence
SUB14588030	*Bacillus safensis* strain SG_MMG_ SSB_2 16S ribosomal RNA gene, partial sequence
SUB14588062	*Mammaliicoccus sciuri* strain SG_MMG_ SSB_3 16S ribosomal RNA gene, partial sequence

Table 9.1.2: 16SrRNA Data submitted in NCBI

BioProject	BioSample	Description
PRJNA1132388: *Bacillus aerius* strain	SAMN42341730	Whole genome sequence of *Bacillus aerius* strain isolated from Sabar tribal subjects
PRJNA1132389: *Bacillus safensis* strain	SAMN42341731	Whole genome sequence of *Bacillus safensis* strain isolated from Bhutia tribal subjects
PRJNA1132400: *Mammaliicoccus sciuri* strain	SAMN42341848	Whole genome sequence of *Mammaliicoccus sciuri* strain isolated from Bhutia tribal subjects
PRJNA1050018: *Pichia kudriavzevii* strain: ATCC 6258	SAMN38724434	Whole genome sequence of fungal isolate *Pichia kudriavzevii* from Bhutia male subject

Table 9.1.3: WGS Data submitted in NCBI

10. Future Prospects:

Exploration of the gut's microbial profile and its comparison with other communities may help us to comprehend the structural and functional diversity of the microbial consortia at play in the tribal along with the urban guts. Assessing how the dietary practices can modulate the gut microbiota of the subjects under study will open the doorway towards development of novel dietary therapeutic interventions which may be more acceptable to their indigenous way of life and will have less perturbatory effects on their gut composition, thereby maintaining the pristine state of these guts in comparision with the urban ones. Few interesting pointers emerge from the study regarding the impact of diet and underlying gut bacteriome of both adult and child in the tribal family. It seems that traditional diet practices have resulted in the maintenance of a standard core gut bacterial composition for these members of the Sabar, Bhutia and Mech tribe with respect to the urban individuals. Metagenomic predictions based on gut bacterial abundances have predicted the prevalence of several diseases whose propensity to manifest due to genetic susceptibility needs to be validated through SNP mapping studies in the future.

Forest in a valued trove of diverse ethnobotanical and ethnomedicinal resources. A comprehensive documentation of the utility of these resources and the knowledge of indigenous practices to treat common as well as complex maladies necessitates more extensive research on the same in the near future. The current rate of deforestation and unchecked depletion of forest resources is also a matter of growing concern, and needs to be addressed with utmost urgency in an effort towards the conservation and sustainable use of ethnobotanical resources and the preservation of indigenous learnings.

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DIETARY ASSESSMENT OF SUBJECTS

Name: Age:

Occupation:

1. What is the type of diet you take? Veg/Non-veg/ other (.....)

2. Are you practicing any dietary restrictions? Yes/No

3. If yes, who advised? Doctor/Self/Peer/neighbours/Mass media influence/ Religious/ others/NA

4. Do you use additional salt during your meal? Yes/No

5. Do you know the consequences of using additional salt on health? Yes/No

6. Commonly used oil(s) for cooking (√): Mustard oil / Groundnut oil / Soyabean oil / Sunflower oil / others (.....)

7. How often you consume major meals from out-side/functions/festivals (√):

Daily/4-6 times in a week/2-3 times in a week/weekly once/once in a fortnight/once in a month/ occasionally/ never

8. Generally what foods do you prefer to have from out-side:

9. Food preferred or avoided during sickness:

Illness	Food preferred	Reasons	Food avoided	Reasons

A. Individual Dietary Food Intake

Routine diet pattern of the individual (√):

Breakfast:

Mid-morning Lunch (MML)/ Lunch:

Evening Snacks:

Dinner:

B. Food Frequency Questionnaire

Sl No.	Food Items	Frequency

NUTRITIONAL ASSESSMENT OF SUBJECTS

A. Anthropometry:

Sl No.	Category	Name of Measurement		Instrument Used
1	Body mass	Weight (kg)		Digital Weighing Machine
2	Lengths	Height (cm)		Anthropometer Rod
3	Breadths	Humerus (cm)		Sliding Caliper
		Femur (cm)		
4	Circumferences	Mid Upper-arm Circumference (MUAC) (cm)	Relaxed	Flexible Steel Tape
			Flexed	
		Waist (cm)		
		Hips (cm)		
		Calf (cm)		
5	Skinfolds	Biceps (mm)		Skinfold Caliper
		Triceps (mm)		
		Subscapular (mm)		
		Supraspinale (mm)		
		Medial Calf (mm)		

B. History of Morbidity during previous fortnight

i. Fever ii. Diarrhoea iii. Dysentery iv. Acute Respiratory Infection

v. Measles vi. Others (.....) vii. Nil

SUBJECT CONSENT FORM

Study Title: A Longitudinal study to gain insights into the Gut Bacterial Abundances and Associated Diet Practices of important ethnic tribes of West Bengal

Statement to be made by the subject willing to participate in the Study

I have read the subject information sheet completely / the subject information sheet read out to me. All my doubts have been cleared. I have been informed that all the information about me in the proposed study will be kept confidential and it will not be revealed to anybody except the principal investigator / co-investigator at any time. I have been informed that there is no risk to me. I was told that I can withdraw my participation at any time if I feel so.

I want to participate in this study myself by my own free will.

I have been offered a copy of my consent form.

By signing this form, I have not lost any of my fundamental rights.

_____

Name of the Subject Signature Date

_____

Name of the Witness Signature Date

(in case of non-literate)

_____

Name of the Researcher Signature Date

PUBLICATIONS:

1. Basu, S., Das, K., Ghosh, M. M., Banerjee, R., Bagchi, S. S., & Ganguli, S. (2022). First report of gut bacterial dataset of a tribal Bhutia family from West Bengal, India. Data in brief, 41, 107859. https://doi.org/10.1016/j.dib.2022.107859

2. Basu, S., Gupta, S., Das, K., Bagchi, S.S. & Ganguli, S. (2022). Tribal Ethnomedicine: a rich resource for future drugs. Indigenous Traditional Knowledge, ISBN: 978-81-955847-0-3. DOI: 10.5281/zenodo.6418656

3. Sarkar, M., Mondal, M; Bhattacharya, D., Basu, S., Mitra, AK., Ganguli, S (2023). Computational modeling for exploring the therapeutic repertoire of lantibiotics. In Advances in Biotechnology and Bioengineering, Lantibiotics as Alternative Therapeutics, Academic Press, ISBN 9780323991414. https://doi.org/10.1016/B978- 0-323-99141-4.00012-6

4. Bhattacharyya, S., De, S., Basu, S., Dhar, GA., Ganguli, S (2022). Comparative Profiling of Rice Endospheric Bacterial Assemblages to Identify Climate Independent Core. Journal of Environment and Sociobiology, Vol 19, No 2, 137-145.

5. Ghosh, S., Dawn, S., Basu, S., Ganguli, S (2022). Microbial Enrichment in Global Wastewater Niches Under Impact of Climate Change- A Computational Study. Journal of Environment and Sociobiology, Vol 19, No 2, 221-243.

6. Gupta, S., Basu, S., Ganguli, S (2022). Repetitive Sequences in Monocot microRNAS- Targets for Future Bioengineering strategies for Climate Resilient Crops. Journal of Environment and Sociobiology, Vol 19, No 2, 295-306.

7. Basu, S., Das, R., Gupta, S., & Ganguli, S. (2021). Does Air Quality Influence the Spread of the Sars -Cov2 In Metropolitan Cities? -A Case Study from Urban India. Current World Environment, 16(2). DOI: 10.12944/CWE.16.2.27

Communicated Manuscripts:

1. Das, K., Basu, S., Mukherjee, K., Ganguli, S., & Bagchi, SS. Assessment of Nutritional Status, Energy Intake and Energy Requirement: A Cross-sectional Study among the Sabar males of West Bengal, India: communicated in ***Papers on Anthropology***
2. Basu, S., Dhar, GA., Das, K., Singha, P., Ghosh, MM., Banerjee, R., Bagchi, SS., & Ganguli, S. Comparative Analysis of gut bacterial abundance and dietary habits of two Ethnic Tribes of West Bengal, India - A pilot study: communicated in ***Applied Biological Research***
3. Basu, S., Dhar, GA., Das, K., Ghosh, MM., Banerjee, R., Bagchi, SS., & Ganguli, S. Whole Genome Analyses of *Pichia kudriavzevii* isolated from Bhutia male gut having a probiotic potential: communicated in ***Scientific Reports***
4. Dhar, GA., Karmakar, R., Mukhopadhyay, S., Basu, S., Ghosh, MM., Ganguli S. Insights into Natural Product based Drug Discovery using a Systems Biology Approach- communicated in a book entitled: ***Potential Bioactive ingredients for healthcare & wellness Industry: Recent Advances and Future Perspectives.*** Publishers: Springer Nature

PRESENTATIONS:

1. Poster Presentation at an International conference of 21st All India Congress of Genetics and Genomics (AICGG,2024) in Jadavpur University (5th -7th February, 2024). The title of the presentation is "Insights into the therapeutic potential of Non-Timber Forest Products across the Ethnic Tribes, West Bengal, India".

2. Poster Presentation at an International conference of Modern Perspectives of Chemistry in Biology (MPCB,2024) in St. Xavier's College (Autonomous), Kolkata (6th January, 2024). The title of the presentation is "A Nutrigenomics Perspective into the Ancient Gut – Role of Non-Timber Forest Products".

3. Poster Presentation at an International conference of Student Conference on Conservation Science (SCCS 2023) in University of Cambridge (28th -30th March, 2023). The title of the presentation is "Insights into the Gut, Dietary Practices and Subsistence patterns from an Indian Foothill Tribe."

4. Oral presentation at an International conference of Translating Human Evolutionary History to Precision Medicine (ADNAT 2023) in Banaras Hindu University (10th-12th March, 2023). The title of the presentation is "Correlating Diet Practices and Gut Bacterial Profiles from a few Ethnic Tribal groups of West Bengal, India."

5. Poster Presentation at a symposium of the Society of Biological Chemists (I), Kolkata Chapter in Sister Nivedita University (9 th- 10 th April, 2022). The title of the presentation is "Dietary intake and uniformity of the gut microbiome - A case study from a Bhutia Tribal Family of West Bengal."

6. Oral Presentation at Indian Anthropological Congress (IAC) in collaboration with Department of Anthropology, University of Hyderabad organized by INCAA on Anthropology and Bio-Cultural Diversity in India: Retrospect & Prospect (21st- 23rd February, 2022). The title of the presentation is “Insight into the applications of Pharmacogenomics on Gut Microbiome and Tribal Ethnomedicine.”

7. Oral presentation at International Symposium in Amrita Vishwa Vidyapeetham, CHARM at University of California San Diego, Bugwork Inc. and C-CAMP organized by School of Biotechnology on Anti-Microbial Resistance (24th-26th February, 2021). The title of the presentation is “What does the tribal child gut microbiome tell us- a comparison of Bhutia, Mech and Sabar guts.”