ViennaLab® 16S Microbiome + ITS NGS Assay Report [Human Gut]

Sample ID: 70M2_update

Date of analysis: 2024-12-20
Pipeline version: 4.0.2

Bacterial Analysis Statistics

Reads passing QC:	18 215 reads
Unclassified:	116 reads
Reads passing QC identified at species level:	99.4%

Bacterial Results

Firmicutes/Bacteroidetes

F/B ratio: 0.5

< 1.5 ... balanced microbiome composition
1.5–3 ... imbalanced microbiome composition
> 3 ... unfavorable microbiome composition

Enterotype

1

Microbiome Diversity

Shannon index: 3.41

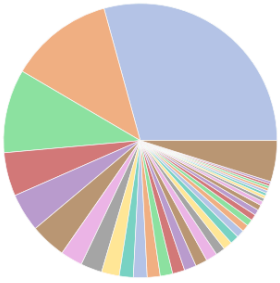
< 3 ... limited diversity
3–4 ... medium diversity
> 4 ... good diversity

Bacterial Phyla

Phylum	Sample Value	Reference Range	Role in Gut
Bacteroidetes	60.22%	30% – 60%	Production of short chain fatty acids for gut health
Firmicutes	31.24%	30% – 60%	Production of short chain fatty acids for gut health
Proteobacteria	3.52%	1.5% – 5%	Produce LPS inducing proinflammatory responses
Verrucomicrobia	1.54%	1.5% – 5%	Contribution to gut health and glucose homeostasis
Actinobacteria	0.15%	1% – 5%	Maintenance of gut health and permeability
Tenericutes	1.33%	0.02% – 0.4%	Pathogens to humans
Euryarchaeota	0.00%	0.01% – 0.06%	Putatively linked to chronic diseases
Fusobacteria	0.00%	0% – 1%	Can promote inflammatory responses
Other	2.01%		

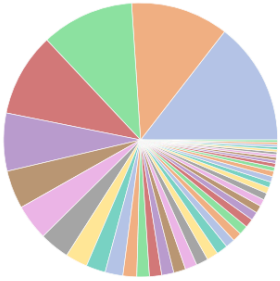
Genus and Species Level Composition

Proportion of QC passing reads
Genus



- Bacteroides 29.28%
- Alistipes 12.29%
- Phocaeicola 9.85%
- Parabacteroides 5.14%
- Faecalibacterium 4.51%
- Ruminococcus 4.22%
- Flavonifractor 2.61%
- Ruminiclostridium 2.52%
- Clostridioides 2.10%
- Eubacterium 1.65%
- Azospirillum 1.63%
- Blautia 1.53%
- Phascolarctobacterium 1.48%
- Butyrivibrio 1.44%
- Akkermansia 1.42%
- Lachnoclostridium 1.41%
- Mordavella 1.34%
- Mesomycoplasma 1.10%
- Anaerostipes 1.07%
- Arsenophonus 1.04%
- Bacillus 0.91%
- Leptothoe 0.83%
- Butyricimonas 0.79%
- Saccharicrinis 0.72%
- Muribaculum 0.66%
- UNKNOWN 0.64%
- Anaerocolumna 0.49%
- Acetivibrio 0.38%
- Mediterraneibacter 0.35%
- Acetanaerobacterium 0.32%
- Prevotella 0.31%
- Eisenbergiella 0.29%
- Thermodesulfomicrobium 0.29%
- Blattabacterium 0.29%
- Massilioclostridium 0.26%
- Other 4.84%

Proportion of QC passing reads
Species



- Bacteroides thetaiotaomicron 14.50%
- Alistipes finegoldii 11.55%
- Bacteroides uniformis 10.95%
- Phocaeicola vulgatus 9.85%
- filtered (LOD < 0.25%) 6.83%
- Faecalibacterium prausnitzii 4.51%
- Ruminococcus flavefaciens 4.22%
- Bacteroides cellulosilyticus 3.53%
- Flavonifractor plautii 2.61%
- Parabacteroides distasonis 2.29%
- Clostridioides difficile 2.10%
- Azospirillum brasilense 1.63%
- Phascolarctobacterium faecium 1.48%
- Butyrivibrio fibrisolvens 1.44%
- Parabacteroides merdae 1.43%
- Akkermansia muciniphila 1.42%
- Parabacteroides goldsteinii 1.41%
- [Clostridium] scindens 1.41%
- Mordavella massiliensis 1.34%
- Ruminiclostridium cellobioparum 1.33%
- Mesomycoplasma conjunctivae 1.10%
- Eubacterium limosum 1.08%
- Ruminiclostridium cellulolyticum 1.08%
- Arsenophonus endosymbiont of Dermacentor
- Blautia schinkii 0.98%
- Bacillus cereus 0.89%
- Leptothoe spongobia 0.83%
- Anaerostipes caccae 0.82%
- Saccharicrinis fermentans 0.72%
- Muribaculum intestinale 0.66%
- Butyricimonas virosa 0.66%
- UNKNOWN 0.64%
- Anaerocolumna aminovalerica 0.49%
- Eubacterium ventriosum 0.44%
- Alistipes indistinctus 0.36%
- [Ruminococcus] gnavus 0.35%
- Acetanaerobacterium elongatum 0.32%
- Alistipes onderdonkii 0.31%
- Eisenbergiella tayi 0.29%
- Thermodesulfomicrobium thermophilum 0.29%
- Blattabacterium punctulatus 0.29%
- Blautia wexlerae 0.27%
- Massilioclostridium coli 0.26%

Bacterial Results - Continued

Species Abundance Table

Name	TaxId	Lineage [Kingdom > Phylum > Class > Order > Family > Genus]	Reads	%
Bacteroides thetaiotaomicron	818	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Bacteroidaceae > Bacteroides	2641	14.5
Alistipes finegoldii	214856	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Rikenellaceae > Alistipes	2103	11.5
Bacteroides uniformis	820	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Bacteroidaceae > Bacteroides	1994	10.9
Phocaeicola vulgatus	821	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Bacteroidaceae > Phocaeicola	1795	9.9
Faecalibacterium prausnitzii	853	Bacteria > Firmicutes > Clostridia > Eubacteriales > Oscillospiraceae > Faecalibacterium	822	4.5
Ruminococcus flavefaciens	1265	Bacteria > Firmicutes > Clostridia > Eubacteriales > Oscillospiraceae > Ruminococcus	769	4.2
Bacteroides cellulosilyticus	246787	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Bacteroidaceae > Bacteroides	643	3.5
Flavonifractor plautii	292800	Bacteria > Firmicutes > Clostridia > Eubacteriales > Oscillospiraceae > Flavonifractor	475	2.6
Parabacteroides distasonis	823	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Tannerellaceae > Parabacteroides	417	2.3
Clostridioides difficile	1496	Bacteria > Firmicutes > Clostridia > Eubacteriales > Peptostreptococcaceae > Clostridioides	383	2.1
Azospirillum brasilense	192	Bacteria > Proteobacteria > Alphaproteobacteria > Rhodospirillales > Azospirillaceae > Azospirillum	296	1.6
Phascolarctobacterium faecium	33025	Bacteria > Firmicutes > Negativicutes > Acidaminococcales > Acidaminococcaceae > Phascolarctobacterium	269	1.5
Butyrivibrio fibrisolvens	831	Bacteria > Firmicutes > Clostridia > Eubacteriales > Lachnospiraceae > Butyrivibrio	263	1.4
Parabacteroides merdae	46503	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Tannerellaceae > Parabacteroides	261	1.4
Akkermansia muciniphila	239935	Bacteria > Verrucomicrobia > Verrucomicrobiae > Verrucomicrobiales > Akkermansiaceae > Akkermansia	258	1.4
Parabacteroides goldsteinii	328812	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Tannerellaceae > Parabacteroides	256	1.4
[Clostridium] scindens	29347	Bacteria > Firmicutes > Clostridia > Eubacteriales > Lachnospiraceae > Lachnoclostridium	256	1.4
Mordavella massiliensis	1871024	Bacteria > Firmicutes > Clostridia > Eubacteriales > Clostridiaceae > Mordavella	244	1.3
Ruminiclostridium cellobioparum	29355	Bacteria > Firmicutes > Clostridia > Eubacteriales > Oscillospiraceae > Ruminiclostridium	243	1.3
Mesomycoplasma conjunctivae	45361	Bacteria > Tenericutes > Mollicutes > Mycoplasmatales > Mycoplasmataceae > Mesomycoplasma	201	1.1
Eubacterium limosum	1736	Bacteria > Firmicutes > Clostridia > Eubacteriales > Eubacteriaceae > Eubacterium	196	1.1
Ruminiclostridium cellulolyticum	1521	Bacteria > Firmicutes > Clostridia > Eubacteriales > Oscillospiraceae > Ruminiclostridium	196	1.1
Arsenophonus endosymbiont of Dermacentor variabilis	228913	Bacteria > Proteobacteria > Gammaproteobacteria > Enterobacterales > Morganellaceae > Arsenophonus	189	1.0
Blautia schinkii	180164	Bacteria > Firmicutes > Clostridia > Eubacteriales > Lachnospiraceae > Blautia	178	1.0
Bacillus cereus	1396	Bacteria > Firmicutes > Bacilli > Bacillales > Bacillaceae > Bacillus	162	0.9
Leptothoe spongobia	2651728	Bacteria > Cyanobacteria > > Pseudanabaenales > Leptolyngbyaceae > Leptothoe	151	0.8
Anaerostipes caccae	105841	Bacteria > Firmicutes > Clostridia > Eubacteriales > Lachnospiraceae > Anaerostipes	150	0.8
Saccharicrinis fermentans	982	Bacteria > Bacteroidetes > Bacteroidia > Marinilabiliales > Marinilabiliaceae > Saccharicrinis	132	0.7
Muribaculum intestinale	1796646	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Muribaculaceae > Muribaculum	121	0.7

Name	TaxId	Lineage [Kingdom > Phylum > Class > Order > Family > Genus]	Reads	%
Butyricimonas virosa	544645	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Odoribacteraceae > Butyricimonas	120	0.7
UNKNOWN			116	0.6
Anaerocolumna aminovalerica	1527	Bacteria > Firmicutes > Clostridia > Eubacteriales > Lachnospiraceae > Anaerocolumna	90	0.5
Eubacterium ventriosum	39496	Bacteria > Firmicutes > Clostridia > Eubacteriales > Eubacteriaceae > Eubacterium	81	0.4
Alistipes indistinctus	626932	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Rikenellaceae > Alistipes	66	0.4
[Ruminococcus] gnavus	33038	Bacteria > Firmicutes > Clostridia > Eubacteriales > Lachnospiraceae > Mediterraneibacter	64	0.4
Acetanaerobacterium elongatum	258515	Bacteria > Firmicutes > Clostridia > Eubacteriales > Oscillospiraceae > Acetanaerobacterium	58	0.3
Alistipes onderdonkii	328813	Bacteria > Bacteroidetes > Bacteroidia > Bacteroidales > Rikenellaceae > Alistipes	56	0.3
Eisenbergiella tayi	1432052	Bacteria > Firmicutes > Clostridia > Eubacteriales > Lachnospiraceae > Eisenbergiella	53	0.3
Thermodesulfomicrobium thermophilum	380392	Bacteria > Proteobacteria > Deltaproteobacteria > Desulfovibrionales > Desulfomicrobiaceae > Thermodesulfomicrobium	53	0.3
Blattabacterium punctulatus	164514	Bacteria > Bacteroidetes > Flavobacteriia > Flavobacteriales > Blattabacteriaceae > Blattabacterium	52	0.3
Blautia wexlerae	418240	Bacteria > Firmicutes > Clostridia > Eubacteriales > Lachnospiraceae > Blautia	50	0.3
Massilioclostridium coli	1870991	Bacteria > Firmicutes > Clostridia > Eubacteriales > Clostridiaceae > Massilioclostridium	48	0.3

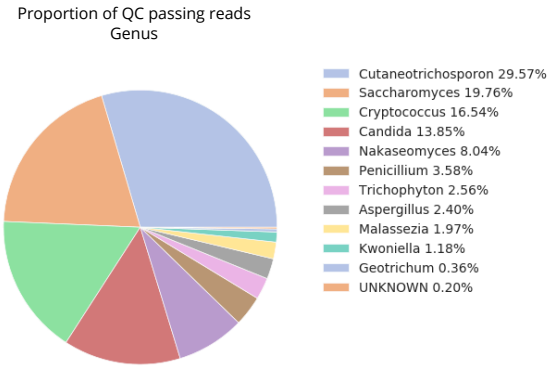
The table displays species identified at proportions greater than 0.25% of reads.

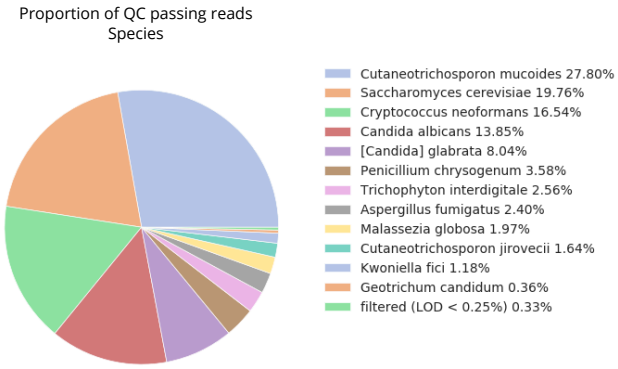
Fungal Analysis Statistics

Reads passing QC:	3 047 reads
Unclassified:	6 reads
Reads passing QC identified at species level:	99.8%

Fungal Results

Genus and Species Level Composition





Species Abundance Table

Name	TaxId	Lineage [Kingdom > Phylum > Class > Order > Family > Genus]	Reads	%
Cutaneotrichosporon mucoides	82522	Eukaryota > Basidiomycota > Tremellomycetes > Trichosporonales > Trichosporonaceae > Cutaneotrichosporon	847	27.8
Saccharomyces cerevisiae	4932	Eukaryota > Ascomycota > Saccharomycetes > Saccharomycetales > Saccharomycetaceae > Saccharomyces	602	19.8
Cryptococcus neoformans	5207	Eukaryota > Basidiomycota > Tremellomycetes > Tremellales > Cryptococcaceae > Cryptococcus	504	16.5
Candida albicans	5476	Eukaryota > Ascomycota > Saccharomycetes > Saccharomycetales > Debaryomycetaceae > Candida	422	13.8
[Candida] glabrata	5478	Eukaryota > Ascomycota > Saccharomycetes > Saccharomycetales > Saccharomycetaceae > Nakaseomyces	245	8.0
Penicillium chrysogenum	5076	Eukaryota > Ascomycota > Eurotiomycetes > Eurotiales > Aspergillaceae > Penicillium	109	3.6
Trichophyton interdigitale	101480	Eukaryota > Ascomycota > Eurotiomycetes > Onygenales > Arthrodermataceae > Trichophyton	78	2.6
Aspergillus fumigatus	746128	Eukaryota > Ascomycota > Eurotiomycetes > Eurotiales > Aspergillaceae > Aspergillus	73	2.4
Malassezia globosa	76773	Eukaryota > Basidiomycota > Malasseziomycetes > Malasseziales > Malasseziaceae > Malassezia	60	2.0
Cutaneotrichosporon jirovecii	82518	Eukaryota > Basidiomycota > Tremellomycetes > Trichosporonales > Trichosporonaceae > Cutaneotrichosporon	50	1.6
Kwoniella fici	2878307	Eukaryota > Basidiomycota > Tremellomycetes > Tremellales > Cryptococcaceae > Kwoniella	36	1.2
Geotrichum candidum	1173061	Eukaryota > Ascomycota > Saccharomycetes > Saccharomycetales > Dipodascaceae > Geotrichum	11	0.4

The table displays species identified at proportions greater than 0.25% of reads.

Description

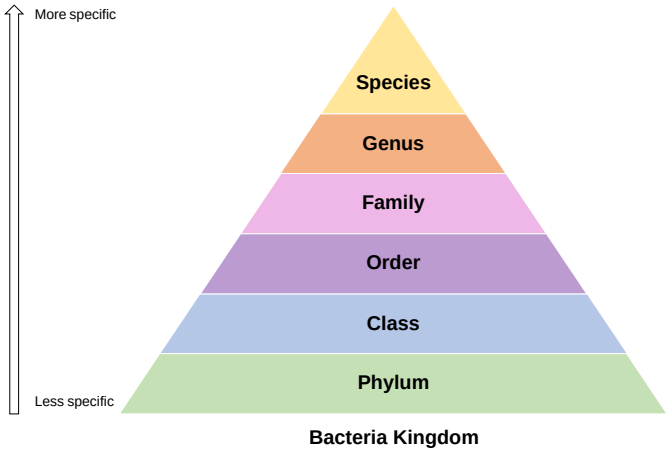
1 Introduction

The human gut microbiome is a diverse and dynamic environment, which consists of bacteria, viruses and fungi. The composition of the human gut microbiome varies between individuals and changes throughout human life, depending on age, lifestyle, diet, antibiotic intake, etc. Imbalances in microbiome composition have been associated with human diseases, such as obesity, type II diabetes, inflammatory bowel disease and asthma. Indeed, due to the growing evidence of the microbiome's impact on human lives, it is critical to understand the microbial gut composition and its role in human pathogenesis [1].

Currently, there are several methodologies for analyzing the human microbiome, ranging from genes to whole genome sequencing. The 16S rRNA is a highly conserved component of the bacterial transcriptional machinery, consisting of conserved and variable regions. The V3 and V4 regions jointly bear the highest variability between different bacterial species, making these regions well-suited for in-depth analysis of bacterial diversity and composition. The same is true for the ITS2 region which has been proposed as a molecular barcode for fungal classification. The ITS2 region is more suitable for unbiased taxonomic classification than the ITS1 region due to its lower length variation and more universal primer sites [2]. Targeted amplification of the V3-V4 variable regions of the 16S rRNA gene and the ITS2 region provides a quick and easy way to assess the microbial composition in the human intestine and is therefore the basis of the ViennaLab 16S Microbiome + ITS NGS Assay.

1.1 Taxonomic Hierarchy

Taxonomy is the science of organism classification into groups based on shared characteristics or evolutionary relatedness. Taxonomic classifications are valid for all living organisms and go from Kingdom (most general hierarchy) to Species/Strain (most specific) [3].



2 Bacteria

2.1 Enterotype

1

The microbiome of each individual is unique. However, microbiomes can be categorized into three general groups called enterotypes, which are dominated by a different bacterial genus: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2), and *Ruminococcus* (enterotype 3). The enterotype is independent of age, gender or geographical origin, and is mainly affected by the individual's genetics and eating habits. Enterotype 1 is enriched in *Bacteroides* and it is more prevalent in individuals eating a protein and animal fat-rich Western diet, while *Prevotella* (enterotype 2) is linked to a carbohydrate rich diet in vegetarian individuals. *Ruminococcus* enrichment is a characteristic of enterotype 3, specific to the resistant starch diet and it consists of bacteria able to degrade mucin [4].

2.2 Microbiome Diversity ratio

The human gut microbiome is a complex ecosystem, densely colonized by thousands of microbial species. The gut microbiota has a role in important processes that define the physiology of the host, such as regulation of the immune system, epithelial cell injury and drug response, as well as energy metabolism [5]. Overall, a wide diversity of bacteria is believed to make our gut microbiome more capable and resilient.

2.2.1 Shannon index

Shannon index: 3.41

< 3 ... limited diversity
3–4 ... medium diversity
> 4 ... good diversity

The Shannon index is one of the most accepted methods to describe the diversity of a microbiome sample. It takes into account the richness as well as the evenness of species in a sample to quantify biological diversity [6]. Richness is defined as the number of different kinds of organisms present in the sample, while evenness compares the population size of each of the species. Generally, when either species richness or evenness increases, the Shannon index will increase as well. Some researchers point out that a normal diversity index is 3.0, while others say that values below 4.0 might represent a low diversity [6, 7]. Consequently, the present report considers an index of 3-4 to be an intermediate stage, while values above 4.0 can be linked to a good microbial diversity.

2.3 Firmicutes/Bacteroidetes ratio

F/B ratio: 0.5

< 1.5 ... balanced microbiome composition
1.5–3 ... imbalanced microbiome composition
> 3 ... unfavorable microbiome composition

The majority of the bacteria that live in our gut can survive without oxygen and are therefore called anaerobes. Bacteroidetes and Firmicutes are anaerobes that together can represent more than 90% of the human gut microbiota [8]. These two phyla have the ability to ferment dietary fibers to short-chain fatty acids (SCFA). SCFA are essential for keeping our gut integrity and health, and have also been linked to various positive effects on our bodies, such as reducing appetite, body weight and risk of developing diabetes [9]. Although the relative abundance of the Firmicutes and Bacteroidetes phyla is highly variable between populations [10], many scientific studies support that Firmicutes bacteria have a better capacity to ferment and metabolize carbohydrates and lipids. Therefore, they might increase the risk of obesity. On the other hand, reduced Firmicutes and increased Bacteroidetes abundance has been linked to inflammatory bowel disease [11].

Consequently, a balanced ratio between these two phyla remains an important indicator of a healthy gut microbiota. Your F/B composition remains relatively stable in adulthood, but might improve with a healthy diet and physical activity, and become unfavorable by continuous exposure to food additives, antibiotics and contaminants such as heavy metals and pesticides [10].

2.4 Bacterial Phyla

2.4.1 Bacteroidetes

Bacteroidetes 60.22%  Reference range: 30% – 60%

Bacteroidetes are gram-negative bacteria which successfully colonized all habitats on Earth and are commonly found in human large intestines. Bacteroidetes are important to maintain properly functioning guts by protecting us from pathogens and supplying nutrients to other beneficial microbes, producing vitamins and anti-inflammatory mediators, stimulating our immune system, etc. [12, 13].

2.4.2 Firmicutes

Firmicutes 31.24%  Reference range: 30% – 60%

Firmicutes are the most abundant type of bacteria that live in the human gut; the phylum includes both probiotic bacteria, such as *Lactobacillus*, and pathogens, such as members of *Clostridium* and *Staphylococcus*. Members of this phylum can ferment carbohydrates in the gut and produce vitamins and short-chain fatty acids, such as butyrate, which helps prevent inflammation, or acetate, another healthy short-chain fatty acid [11, 14, 15].

2.4.3 Proteobacteria

Proteobacteria 3.52%  Reference range: 1.5% – 5%

Bacteria from this phyla are gram-negative and produce lipopolysaccharides (LPS), which induce strong proinflammatory immune responses that can lead to septic shock or even death. Proteobacteria include known pathogens such as *Escherichia*, *Legionella*, *Salmonella*, *Vibrio* and *Yersinia* [7]. Thus, the abundance of Proteobacteria in the human gut needs to be kept low and in balance. An expansion of Proteobacteria in the gut reflects imbalance or an unstable gut microbial community structure [16].

2.4.4 Verrucomicrobia

Verrucomicrobia 1.54%  Reference range: 1.5% – 5%

Verrucomicrobia are bacteria inhabiting the intestinal mucosa that are able to degrade mucin. It has been shown that they are involved in glucose homeostasis contributing to intestinal health. Verrucomicrobia members of the genus *Akkermansia* have been postulated as potential biomarkers of a healthy gut. They are linked to an increase of the metabolic condition in subjects with obesity and type 2 diabetes [17].

2.4.5 Actinobacteria

Actinobacteria 0.15%  Reference range: 1% – 5%

Actinobacteria are one of the major phyla of the human gut microbiota. Although they represent only a small percentage of organisms, Actinobacteria are critical to the maintenance of gut health. They are gram-positive bacteria involved in the modulation of the gut permeability (water and nutrients absorbed from our food into our bloodstream), immune system, metabolism and gut-brain axis (mood, cognition and mental health).

Bifidobacteria are one important example of Actinobacteria that are considered beneficial to health and are often part of probiotic supplements [18].

2.4.6 Tenericutes

Tenericutes 1.33%  Reference range: 0.02% – 0.4%

A phylum of gram-negative bacteria consisting of cells bounded by a plasma membrane that have the distinctive feature of lack of cell walls. Bacteria belonging to this phyla are one of the smallest self-replicating, living structures. *Mycoplasma* are part of this phyla and are known to be human pathogens [19, 20].

2.4.7 Euryarchaeota

Euryarchaeota 0.00%  Reference range: 0.01% – 0.06%

Euryarchaeota belongs to the archaeal domain. Archaea are typically found in habitats with extreme living conditions, and represent a minor group of microorganisms in the human gut. Members of the Euryarchaeota phylum act mainly as fermenters that produce methane. Methane production has been linked to the development of colon cancer and constipation, while other scientific studies linked methane production to anti-inflammatory properties [21].

2.4.8 Fusobacteria

Fusobacteria 0.00%  Reference range: 0% – 1%

Fusobacteria are anaerobic gram-negative bacteria that have been frequently found in inflammatory bowel disease patients with reduced microbial diversity [22]. Research conducted on mice have shown that these bacteria promote intestinal inflammation, specially in the context of a depleted intestinal microbiome [23].

3 Fungi

3.1 General fungal introduction

The human gut microbiome is composed of bacteria, viruses, and fungi. While fungi are less abundant in the gut than bacteria, recent evidence suggests that they may play an important role in maintaining gut health. They may contribute to:

Interactions with Bacteria: Fungi can interact with bacteria in the gut in various ways. Some fungi can compete with harmful bacteria for resources, thus indirectly benefiting the host by preventing the overgrowth of pathogenic bacteria [24, 25].

Dietary Influence: Dietary factors influence the composition of the gut fungi. For example, a diet rich in fiber and plant-based foods may support a diverse fungal community in the gut. Certain fungi have specialized enzymes that aid in the breakdown of dietary components, potentially influencing nutrient absorption and metabolism [26, 27].

Modulation of the Immune System: Intestinal fungi can interact with the host immune system. Some fungal species may stimulate immune responses, helping to maintain immune balance and tolerance. However, dysbiosis or imbalance in the gut microbiota, including fungal overgrowth, can lead to immune dysregulation and inflammation [28].

Role in Health and Disease: Invasive fungal disease is a major health concern worldwide, especially when it affects the bloodstream, lungs, or brain of immunocompromised patients [29]. Although research on gut fungi is still in its infancy, alterations in fungal populations have been associated with several health conditions,

including inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, and metabolic disorders such as obesity and diabetes. Understanding the role of fungi in these diseases may provide insights into potential therapeutic interventions [26, 30]. A possible association between gut mycobiota and colorectal cancer has been suggested [27].

Potential Therapeutic Targets: Manipulation of the gut fungal community, either through dietary interventions, probiotics, or targeted antifungal treatments, could have therapeutic implications for gut-related disorders. However, further research is needed to elucidate specific mechanisms and identify effective interventions [26].

3.2 Important fungi for human health

Identifying the most common fungi in the human gut can be challenging due to the complexity and variability of the gut microbiota between individuals. However, some fungal species have been frequently detected in the human gut microbiome in different studies. The most common genera found in the human gut include *Candida*, *Aspergillus*, *Debaryomyces*, *Malassezia*, *Saccharomyces*, *Penicillium*, and *Pichia* [31, 32].

Candida: *Candida* species, particularly *Candida albicans*, are commonly found in the human gut and mucosal surfaces and are therefore considered a normal component of the human intestinal microbiota. While *C. albicans* is usually harmless in healthy individuals, it can cause candidiasis, a fungal infection when overgrowth occurs. *Candida glabrata* and *Candida tropicalis*, can also cause infections, particularly in immunocompromised individuals [28].

Saccharomyces cerevisiae: *Saccharomyces cerevisiae*, also known as baker's yeast, is widely used in the food industry and as a probiotic supplement. It has been detected in the human gut and may have potential health benefits [31].

Aspergillus: Some species of the genus *Aspergillus*, such as *Aspergillus fumigatus*, have been detected in the human gut microbiome. *Aspergillus* species are common in the environment and can cause opportunistic infections, particularly in immunocompromised individuals [31].

Malassezia: *Malassezia* is a genus of fungi that is commonly associated with the skin, particularly the scalp, face, and upper body. *Malassezia* species have been implicated in gastrointestinal disorders such as inflammatory bowel disease (IBD) where increased levels of *Malassezia* have been observed in the gut microbiota of individuals with IBD compared to healthy individuals [31].

Geotrichum candidum: *Geotrichum candidum* is a yeast-like fungus that is widespread in a variety of environments including soil, air, water, plants, and dairy products. It is commonly found on soft cheeses such as Brie and Camembert. It plays an important role in biotechnological processes due to its ability to produce several important enzymes such as beta-glucanases, lipases, and alpha-amylases. The link between food consumption and food-borne diseases has not yet been established [33].

Methods

The ViennaLab 16S Microbiome + ITS NGS Assay uses Next Generation Sequencing to amplify and sequence the V3-V4 region of the bacterial 16S rDNA and the ITS2 region of fungal rDNA.

Read pairs are merged using BBMerge [34] and filtered for minimum length using SeqKit [35]. Cutadapt [36] and ITSx [37] are used for trimming the reads. The read classification pipeline uses the CLARK sequence classification system for species-level classification of reads [38]. The CLARK system is based on discriminative k-mers in a sequence database. The sequence database used by the classification pipeline is constructed based on sequences from the the SILVA [39] and UNITE [40, 41] databases, with additional human sequences from the

NCBI Nucleotide database and the GRCh38 human reference genome assembly. The species taxonomy used by the read classification is downloaded from NCBI (https://www.ncbi.nlm.nih.gov/taxonomy/). Identified human reads and their results are not included in the abundance results. Diversity statistics are calculated from the species level abundance results using MOTHUR [42].

Note: Fungi can be described as a "teleomorph" or an "anamorph" depending on their reproductive stage. The teleomorph stage refers to the sexual reproductive stage, while the anamorph stage refers to the asexual reproductive stage of a fungus. Consequently, many fungal species can have two names corresponding to the respective growth stages, e.g., *Candida famata* (anamorph) and *Debaryomyces hansenii* (teleomorph) or *Candida krusei* (anamorph) and *Pichia kudriavzevii* (teleomorph) [43, 44].

Assay Information / Disclaimer

The 16S Microbiome + ITS NGS Assay targets the V3-V4 region of the bacterial 16S rDNA and the ITS2 region of fungal rDNA. Hence, species level classification accuracy is limited to this region. ViennaLab Diagnostics GmbH is not responsible for diagnoses based on the results at hand. This responsibility relies solely with the medical doctor treating the patient, as well as for prescribed or suggested treatments.

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