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ECOLOGICAL AND FUNCTIONAL CHARACTERIZATION OF A HUMAN GUT MICROBIOME USING SHOTGUN METAGENOMIC PROFILING

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ABSTRACT

The aim of this paper has been to carry out a metagenomic study of the sample of human gut microbiome. In such analyses, high-resolution taxonomic classification and ecological modeling tools (Kraken2 and Bracken) were employed so that we might analyze patterns of distribution at phylum level, at genus level and, lastly, at species level. We have also done a comparison of the ecological structure with Shannon diversity index, rank abundance modeling as well as cumulative dominance curves. Many different functional gene families are used to measure metabolic potential of the microbiome.

Bacillota (~ 50 percent of the community) and Bacteroidota (~ 44 percent of the community) constituted the bulk of the gut microbiome with a Firmicutes Bacteroidota ratio of 1.14. A species taxonomic classification revealed that approximately 25 to 30 species of microbe formed a relatively stable core microbiome of the overall abundance of more than 80. Also, the analysis of functional profiles correlation showed that the gut microbiome was improved with the genes of carbohydrate metabolic pathways (i.e., carbohydrate fermentation), membrane-bound transportation, and anaerobic energy-generation. The key characteristics that were absolute in the community structure of the gut microbiome that has been assessed were ecological stability, metabolic coherence, and hierarchical dominant structure.

INTRODUCTION

Despite the human gastrointestinal system having one of the most complex microbial ecosystems that biology has ever witnessed, microbial structure of human gut microbiome has been thought to be highly dependent on a plethora of factors including nutrition, age,

gender, and disease condition. Metabolic syndrome, inflammatory disease, and immune dysregulation development has been associated with the changes in the microbiome populations.

The traditional 16S rRNA sequencing provides low degree of taxonomic and little bit of functional information. Shotgun metagenomics enables it to solve species-level, as well as can provide direct inferences concerning the contents of metabolic genes. Kraken2 software relies on k-mer matching (or short sequences of DNA) to fastly and efficiently classify metagenomic sequences (reads). The Bracken software conducts the quantitative abundance estimation of even more precise and quicker classification of metagenomic reads.

The gut microbiome can be most understood in the ecological theory. The characteristic features of steady ecosystems are hierarchical predominance, functional duplication and coexistence of a rich core taxa and numerous rare biospheres. In this manner, the synthesis of the taxonomic profiling and ecological modeling is one of the options that can help to present the comprehensive picture of the organization of the microbes.

The goal of the given research was to perform a systematic ecological and functional characterization of a human gut microbiome specimen under metagenomic and quantitative model approaches.

The gastrointestinal (GI) tract contains one of the most varied, large and complicated areas on the Earth, in terms of microbes. The count of cells in the GI tracts ranges at 1011-1012 cells per gram of the intestinal content. In addition to being a complex of microbes, the gut microbiome is an organ-like biological system, which affects the processes of digestion, immunity, biosynthesis of nutrients, and the creation of a metabolic homeostasis.

Although the microorganisms in the intestine microbiome are large and diverse, the microbiome is not an undirected assemblage of microorganisms but rather, an organized ecological network which is characterized by the following aspects:

- Resource competition
- Niche specialization
- Inter microorganism cross-fertilization of products.
- Spatial heterogeneity

Ecological also establish that steady microbial communities are hierarchically controlled, functionally redundant, and centrally dispersed in respect of rare and dominant species. These predictive models can be characterized with the help of the measured taxonomic and ecological modeling.

The present situation of Shotgun Metagenomics: Shotgun sequencing has become a highly potent technology to perform a complete examination over a large mass of genomic data. Recent innovations in Shotgun Metagenomics: Shotgun sequencing has evolved into a powerful tool to learn in a holistic way a huge amount of genomic information.

The traditional relative technique of sequencing 16s rRNA gene enables determination of the species, but not the functional levels. With shotgun metagenomics technique we can make an appraisal of species identity and it is the direct approach that we can make conclusions of the entirety of the amplified genes. Kraken2 eats the corresponding k-mer corresponding and builds the taxonomic groupings speedily compared to Bracken which improves the abundance estimates; depending on the shared areas of genome and thus minimizing biasness.

When these are in combination with other techniques of investigation, we can possess high-resolution profiles of microbial communities. However, despite such data we must consider the ecological and functional context prior to being able to interpret such data in any meaningful way.

OBJECTIVES OF THE STUDY

- Substantiate the taxonomic makeup of a microbiome sample of the human gut.
- Measure the ecological properties of the microbiome according to measures of diversity and dominance modeling.
- Identify microbiome necessary and rare components.
- Using the relative abundance of a specific family of genes finitely infer the metabolic activity.

MATERIALS AND METHODS

1. Dataset

The quality control procedures of DRR895467 metagenomic sequencing dataset include human gut microbiome sample which has gone through with quality control procedure before taxonomical classification has been made.

2. Taxonomic Classification

- Phylum
- Genus
- Species

3. Ecological Analyses

Shannon diversity index was calculated in the following manner:

$$H = -S (pi \ln pi)$$

Pi is the proportion of the ith species.

Rank-Abundance Modeling

The rank-abundance curves were produced on the basis of the rank of the declining abundance of individual species and, therefore, measured the measures of dominance and evenness.

Cumulative Abundance Analysis

Cumulative abundance curves were constructed in order to establish how much species have accumulated to 80 percent of the total community abundance.

Metagenomic reads of the sample DRR895467 (Sequencing Illumina MiSeq) underwent processing through the utilization of the usual quality control measures. Metagenomic reads were compared to a curated microbial reference database and this comparison resulted in Kraken2 taxonomic classification. In order to complete Kraken2 with a taxonomic classification with species-level abundance estimates, Bracken was invited to improve species-level abundance estimates with Kraken2.

Kraken2 and Bracken raw abundance data were processed into relative abundance tables at phylum, genus and species levels. Alpha diversity was determined using the Shannon diversity index. Rank-abundance curves were constructed based on the ranking of the species

in their relative abundance order of highest relative abundance to lowest relative abundance. The measure of cumulative amount of the species with 80 percent of the total community biomass was taken.

The metabolic power was determined with relative abundance of functional gene families. Some of the effector pathways that were discussed were carbohydrate metabolism, fermentation, membrane transport systems and anaerobic process of respiration.

Ecological Interpretation

The human gut is one of the richest microbial ecosystems in biology. The gut microbiota plays a significant role in the digestion of the complex carbohydrates, the synthesis of vitamins, the balance of the immune system, and the protection against infections. It is gaining momentum that demonstrates that various metabolic, inflammatory and immune-related diseases are linked to the alteration in the composition of the gut microbiota.

Classic 16S rRNA sequencing just gives a low taxonomic (or species) resolution and functional information on the microbiota. Shotgun metagenomics may both offer species-level taxonomic detail, and also permit an easy estimate of the abundance of metabolic genes. Kraken2 offers an effective metagenomic read classification system, which applies k-mer matches to a reference database of curated microbial genomes. Bracken enhances the estimation of the abundance of species in the microbiome of the sample.

Ecologically speaking, the gut microbiota is best characterized as an ecological community (i.e. an ecosystem). A stable ecosystem is characterized by three features and characteristics, including hierarchical structure or dominance; functional redundancy; and co-occurrence of the abundant core taxa and a diverse array of rare taxa (the biosphere). A combination of taxonomic evaluation, measurements of the ecological microorganism structure characterizes the full picture of the microorganism arrangement in the gut.

The general aim of the study was to perform a formal ecological and functional characterization of a sample of the human gut microbiome through the metagenomic profiling techniques and quantitative ecological modeling techniques and tools.

Quality-controlled reads of the metagenome (DRR895467) were subjected to the Kraken2 software to taxonomically assign these. Bracken provided derivations of abundance of species in the gut microbiome.

Table of relative abundance were developed at phylum, genus, and species levels. The alpha diversity measured was the Shannon diversity index. Rank-abundance curves were prepared by ranking the species in decreasing abundance and the cumulative abundance of the species was then counted to find out how many species would take 80 percent of the total community biomass.

The richness of functional family of genes was compared to draw general conclusions about the metabolism potential. The metabolic pathways associated with the fermentation, carbohydrate metabolism, membrane transport and anaerobic respiration were evaluated in this analysis.

The taxonomic classification of the microorganisms resulted in 99.9 percent of the reads of the sequences being classified under the taxonomic domain of bacteria which signifies a good quality sequencing and low level of contamination. The most widespread phyla of microbes (i.e., Bacillota; approximately 49-50 percent), Bacteroidota (approximately 43-44 percent) and Pseudomonadota, among others of low abundance also were met.

At the genus level of genera analysis, Bacteroides, Phocaeicola, Ruminococcus, Alistipes and Dysosmobacter were the most notable genera. At the genera level, Phocaeicola vulgatus (approximately 11 percent), Bacteroides caccae (approximately 8 percent), and Bacteroides ovatus (approximately 8 percent), were the most common ones.

The value of the Shannon diversity index of approximately 3.6 implies that the community was diverse or very diverse. The rank abundance distribution was also examined such that the slope of the rank-abundance curve was large in the beginning of the curve, and the curve had a long flat tail of the rare species which is typical of a log-normal distribution in ecology. Approximately 25 to 30 species make up over 80 percent of the total count of all species in the community, and hence, are the functional core microbiome.

The functional gene profiles analysis showed that the functional genes were highly enriched in the sample and the roles of the metabolism were primarily glycolysis, polysaccharide-degrading enzymes, fermentative metabolism, and membrane transport.
The findings of the Firmicutes to Bacteroides ratios on the sample population of healthy adults confirm the previous research works that revealed that the two groups of bacteria are complementary and create a stable gut ambiance an indicator of a healthy gut microflora. The

percentage of Firmicutes/Bacteroides that was obtained in this paper was far much within the span of the normal and healthy microbiomes of adult humans (approximately 1.14).

The hierarchy of domination being created is operated according to the ecological theory in that, only a limited number of taxa dictate the biomass and majority of the species that serve to create the diversity have extremely small biomass. This is supported by the fact that there are many more fibre degrading taxa in the sample used in this study, which is indicative of the fact that, such taxa are functionally redundant and this provides some resilience to some dietary or environmental disturbance.

Within the context of role of the rare biosphere in genetic reservoir that can be expounded under stressful situations, the functional coherence between taxonomic dominance (i.e., taxonomic composition of community) and gene enrichment is the one that augments the degree of confidence in the biological explanations.

Even though the current data set is on a single sample of carrases, without any environmental and host data, the cross-analysis framework applied in the study supports the permanence of the analytical framework and provides a basis on further comparative research.

Planet earth has a very dense GI tract of microbes. The gut microbiome is significant in the digestion of complicated polysaccharides, synthesis of vitamins, immune system control, and resistance to pathogens. It is being demonstrated that recent studies, changes in the composition of microbial communities are linked with the development of metabolic pathologies, inflammatory and deregulations of the immune system.

Traditional 16S rRNA sequencing is not taxonomically resolute and does not provide much functional information. However, shotgun metagenomic sequencing can provide species-level resolution and has the capability to determine directly the content of microbial metabolic genes. A tool that can be used to quickly classify metagenomic reads, using k-mer matching, is Kraken2, and Bracken can be used to give a more accurate picture of abundance.
The ecological theory is most appropriate in understanding the gut microbiome. More hierarchically dominant (i.e. explicit hierarchy of organisms) stable ecosystems are better placed to host organisms that are functionally redundant together with large numbers of abundant organisms and biosphere rich with rare organisms. It therefore results in the merit

of a taxonomic profiling and ecological modeling combination to gain more insight into how organisms are arranged in a microbial ecosystem.

The main objective of this study was to characterize the human gut microbiome under metagenomics and quantitative modelling as a systematic ecological and functional description of a sample human gut microbiome.

Results

Summing up, in this paper, the various or types of sequencing have been classified in terms of their level of application and the results that are expected.

The rate of produced reads with Kraken2 came to about 25,312, which were identified adequately, whereas the rate to which read was not given a classification was less than 0.1%. The percentage of the total number of reads in the classified sequences were 99.9% bacterial kingdom - which makes the sequencing data highly integrity himself.

Community Structure Phylum Level

The gut microbiome was found to be comprised of the two subsequent phyla (in percentage of abundance):

The Two Subsequent Phyla	**(in percentage of abundance)**
Bacillota (Firmicutes)	~49-50%
Bacteroidota	~43-44%
Other minor phyla	**(in percentage composition)**
Pseudomonadota	~3%
Verrucomicrobiota	<2%
Thermodesulfobacteriota	<1%

The Firmicutes/Bacteroidota ratio was 1.14 and hence a stable and balanced community structure.

- **Genus-level Composition**

Five overarching genera of the sequencing reads were:

a. Bacteroides
b. Phocaeicola
c. Ruminococcus
d. Alistipes
e. Dysosmobacter

All of the above genera have been identified as polysaccharide degraders and have been known to give short-chain fatty acids.

- **Species-level Dominance**

The most common ones were:

1. Phocaeicola vulgatus (~11%)
2. Bacteroides caccae (~8%)
3. Bacteroides ovatus (~8%)
4. Ethanoligenens harbinense (~6%)
5. Ruminococcus bicirculans (~6%)

- **Alpha Diversity**

A Shannon Diversity Index of 3.6 corresponds to a moderate-high level of diversity implying that the ecological complexity of the gut microbiome was large and also none of the species was over dominant.

The rank abundance distribution is the most used distribution in meteorology.

The curve of the rank abundance distribution was found to possess two characteristics:

There is a dramatic decline in the richness of the highest-ranked species, and

❖ A long tail of rare taxa.

The patterns are typical of log-normal distribution in the condition of healthy and steady state microbial ecosystem.

❖ Microorganisms Statistics: Rare Microbiome and Core Microbiome.

The most of it may be due to the fact that there are about 25 to 30 species (which forms the core microbiome), and the other species form the rare biosphere since they are hardly abundant.

❖ Profiling of Family of the functional gene.

It was enriched with the subsequent groups of genes: glycolysis; polysaccharide degradation; short-chain fatty acid (SCFA) production; membrane transport; anaerobic metabolism, which demonstrates that the microorganisms of our fermentative microbiome are metabolically active.

The read was further taxonomically classified and more than 99.9% of the reads were bacteria that was an indication of good and clean sequencing. Bacillota (~49%-50%), Bacteroidota (~43%-44%), and Pseudomonadota and a few additional contributions of other low-abundance phyla were the largest bacteria of the microbial community.

The most common genera at the genus level were Bacteroides, Phocaeicola, Ruminococcus and Alistipes and Dysosmobacter. The commonest species were the following: Phocaeicola vulgaritas (~11%); Bacteroides caccae (~8%); and Bacteroides ovatus (~8%).

The microbial diversity of this sample of people based on Shannon Diversity Index is about 3.6 that is an indicator of a medium to high result of microbial diversity in these individuals. The form of the rank-abundance distribution had a slope of the floor in the origin and then a long tail of comparatively rare species which symbolized log-normal ecology. The cumulative abundance of total species that accounted over 80 percent of the total cumulative abundance was estimated to be approximately 25 percent-30 percent of the total species or a functional core microbiome.

The enrichment of the functional genes analysis revealed that the glycolytic pathways were numerous, the number of polysaccharide-degrading enzymes was large, the number of fermentative metabolism genes and a great number of the systems of anaerobic respiration were numerous.

DISCUSSION

- Ecological Stability

The proportions of the phyla are equal; the Shannon diversity indices are moderate all of them are indicators of an ecologically stable situation.

- Functional Redundancy

It has a number of fiber-degrading taxa, useful redundant taxa which provide perturbation-insensitivity.

- Core Microbiome Metabolism

However, the adequate amount of microorganisms supports the production of SCFAs, who ferment cellulose and produce energy to the host.

- Rare Biosphere as Reservoir

A low abundance of taxa may allow them to be more flexible to dietary or environmental changes.

- Integration of Function and Taxonomy.

The ecological coherence can be seen by the similarity between the phylogenetically dominant taxa and the functionally enriched metabolic pathways.

The results of the current study prove that the Firmicutes/Bacteroidotata ratio was equal in the sample group and this indicates that there was a stable state that has been achieved in the gut microbial community of healthy people. The fact that the Firmicutes/Bacteroidota ratio is high is usually an indication of a more severe metabolic imbalance, and a balance ratio (=1.14) as it is in the current case is typical of healthy individuals.

Another way the hierarchical structure of the dominance supports the ecological theory is that few taxa taxa will signal a significant percentage of the biomass, whereas many species of lower abundance will add up to the diversity of the entire gut microbial community. The fact that there are several fiber-distributing species shows some degree of functional redundancy which makes them less prone to disruptions caused by dietary or environmental alterations.

It is even possible that the rare biosphere could act as a genetic reservoir which could grow when the environment was stressful. The correlation of Largest common taxonomic groups functional coherence and enrichment of functional genes results in high confidence of the biological explanation of data.

Although the current paper is confined to the investigation of only one sample without any metadata of the host, the power of the cumulative analysis framework that was developed during the work should be regarded as the driving force of the comparative research in the future.

The other reason that the hierarchical nature of the dominance aids the ecological theory is that a small group of taxa taxa will contribute to a large percentage of the biomass and a large number of species with low abundance will contribute to the diversity of the overall gut microbial community. The numerous species that are fibre-distributing indicate that there is some level of functional redundancy and thus they are not easily affected by dietary changes or environmental pressures.

CONCLUSION

These results of the metagenomic profiles of the gut microbiota in the subjects depict that their gut microbiota are very stable and characterized anaerobic communities that have a certain degree of metabolic coordination. The ecological stability can be substantiated by enough evidence of balance in the composing of the major phyla, moderate to high rates of diversity, a hierarchical structure of dominance of the taxa and functional diversification of the carbohydrate fermentation pathways.

An integrated approach combining a taxonomic profiling approach with ecological modeling and functional inference will provide a strong framework of future research on the microbiome. The future research could be conducted with multi-sample comparisons and longitudinal.

Analysis of this sample of the human gut microbiome through metagenomic analysis has been in a position to depict some of the significant findings that comprise:

- Bacillota Bacteroidota proportional ratios.
- The level of diversity is high/moderate.
- Microbiome hierarchical structure.
- Fermentation metabolism with functional relationship to other members.

There is also the necessity to investigate the taxonomic, ecological, and functional fields of the microbiome to be able to have an overall picture of how the microbiome operates.

In general, this discussion is a global taxonomic, ecological and functional study of a human gut microbial community through shotgun metagenomics profiling, and taxonomic classification with Kraken2 coupled with Bracken refinement, which estimates relative abundance (i.e., quantifying diversity, rank-abundance modelling, cumulative dominance modelling and gene family analysis) to provide a holistic view of the microbiome community.

It has the characteristics of a healthy and functionally integrated mature population of the intestinal flora depending on the microbial population one is studying in this study. The ecological ratio of approximately 1: 1 (Bacillota Bacteroidota): 50: 49: 43) between the two large phyla appears to be an ecological attainment (not a dysbiosis). Ratios commonly found

in normal healthy and stable gut conditions where no report of drastic changes in the microbiota.

Life Presence of a strong hierarchical tendency demonstrates the analysis of species, which suggests that there are a small number of dominant species that make a sum of the total bio matter. A fully operational core microbiome is represented by 80 percent or more of the total amount of microbial abundance comprising the roughly 25-30 species. This central may be the focus of most of the metabolic activity of the system and the hydrolysis of carbohydrates and short-chain fatty acid synthesis may be a primary focus. The dominance of the fiber-fermenting genera Bacteroides, Phocaeicola and Ruminococcusdoes testify to this point of view.

With a Shannon diversity index of about 3.6, there is a great variety of various microbial taxa that are coexisting and active to a moderate or high degree. The overall tendency was that the diversity was not too low (this would imply that the system is unstable) and the highest possible diversity (this would imply transient species or changing the environment). This is a pointer to the fact that such a pattern is an indication that the system is most likely to have well-developed, strong and robust microbial community.

The log-normal distribution should be typical with a rank-abundance distribution, i.e. the slopes of the dominant species are steep in the beginning then there is a long, thin biosphere. They have long tails, which is an indication that the species in the rare biosphere will provide an ecological buffering power. Therefore, rare species can be considered functional reservoirs which will grow and become many as the environment or diet diversity are altered to enhance the resilience and versatility of the ecosystem.

Evidence in the ecological conclusion in the preceding is apparent in functional classification of gene families. An overall study of abundance of genes related to carbohydrates, fermentation pathway, anaerobic energy production systems and membrane transport and the reality that the ranked abundance of individual groups are equal to the pathway abundance of the same are the support of the general hypothesis that the taxa are indicative of the respective ecological niches.

This combination of anaerobic microbiome based on the taxonomic clearance, role prediction and eco-assessment indicates that this specimen may be a symbiotic to the metabolism of complex carbohydrates and energy-gathering. The hierarchical mode of dominance, equal

ratio of phyla, mean level of diversity and redundancy; all are characteristics of a certain measure of ecological stability.

The results in this instance indicate that the abundance correction (Bracken) improves quantitative accuracy of the taxonomic profiling and ecological and taxonomic measures provide more detailed data of the biological systems than the taxonomic metrics.

Even though the data is based on a single sample with no host metadata, the structure described below will become an appropriate basis of the future comparative microbiome research. This would contribute to the discovery of the interaction between the host and his or her microbiomes, and to the dynamics of the process in the ecosystem, in subsequent research where periodic, longitudinal sampling to sample a number of people are used and the research confirmed using metabolomics analysis.

In conclusion, this paper provides evidence that under the conditions of huge metagenomic-informed characterizations and ecological modelling, one is able to make some significant inferences about the framework of the gut microbiome. These results indicate that the human intestinal microbiome is more of a well-organized, resilient and metabolically complementing ecosystem which has a core of functional functionality which is functionally maintained by a large rare biosphere. A combination like this is a solid foundation of future research on microbiome and health and disease and drug-modulated microbiome.

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