

PRELIMINARY GUT MICROBIOTA FROM TRADITIONAL LIFESTYLE INDIGENOUS IN THE BRAZILIAN AMAZON EXPERIENCING A PROCESS OF TRANSITION TO URBANIZATION

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ABSTRACT:

The Yanomami are one of the more ancient indigenous tribes in the Amazon and direct descendants of the first to colonize South America 12,000 years ago. They are localized in the Venezuela and Brazil border, being uncontacted on the Venezuelan side. Although they maintain a hunter-gatherer society behaviour, in Brazil, they are currently experiencing contact with urbanized populations. Human gut microbiota from traditional communities is the subject of recent studies because the Westernization of the diet and the introduction of antibiotics and other chemicals have affected the microbial diversity in indigenous populations, threatening their existence. Here we preliminarily characterized the diversity of the gut microbiota from the Brazilian Yanomami in terms of Bacteroidetes and Firmicutes. Likewise, its diversity was compared with the Manaus-AM population. A metagenomic approach of the 16S rRNA gene was carried out in the faecal samples. Differences were found between the two populations, mainly concerning the genera abundance (*Prevotella* and *Bacteroides*) and the highest values of the phyla Bacteroidetes over Firmicutes, being significant only in the Yanomami. However, diversity was statistically equal between them. In conclusion, the composition of the Yanomami gut microbiota still maintains the profile characteristic of a community with a traditional lifestyle; however, our results suggest an underlying homogenization process between Yanomami and urbanized populations such as Manaus.

Keywords: Bacteroidetes, Firmicutes, Gut, 16s rRNA Sequencing, Yanomami.

INTRODUCTION

The human gut microbiota of isolated Yanomami indigenous communities in Venezuela, was the subject of a study by (1) and it became a matter of fundamental importance, as it showed the most diverse microbiota ever seen in humans, a direct relationship with the health, and clarified the idea that a low bacterial diversity may be associated with several diseases in westernized populations (2). The study of the gut microbiota of isolated tribes becomes a window toward the patterns of the human past (3,4), but is also the subject of recent studies because the Westernization of the diet and the introduction of antibiotics and other chemicals have affected the microbial diversity in indigenous populations, threatening their existence. The Yanomami are one of the more ancient indigenous tribes in the Amazon, and direct descendants of the first to colonize South America 12,000 years ago. The Yanomami are a hunter/farmer society who live in isolated villages in the north of the Amazon rainforest in Venezuela and Brazil but have recently experienced contact with Westernized societies localized in Brazil. The Yanomami feed on seeds, roots, and fruits of the forest, fish, and occasionally meat (5). The Yanomami villages in Brazil are distributed in the territories of the states of Roraima and Amazonas, some of which have access to nearby cities, such as Barcelos (Amazonas) and Boa Vista (Roraima), and other quite isolated villages (6). Some studies have demonstrated the importance of maintaining high bacterial diversity in our microbiota, including high levels of Bacteroidetes over Firmicutes phyla ratio, something that has already been verified in inhabitants of traditional communities, such as societies of hunters and food collectors, for example, from Africa, Peru, and Papua Nova-Guinea (4). On the other hand, urbanized/westernized populations present in their intestinal bacterial composition an absence of this diversity (including low levels of Bacteroidetes over Firmicutes phyla ratio: dysbiosis), which is a medical treatment concern result, since this low diversity of bacteria and dysbiosis has been linked to obesity, diabetes, and several autoimmune diseases, such as allergies, Crohn's disease, and ulcerative colitis (7,8). Here, we tested the hypothesis that, although it is known from previous studies that isolated populations of Yanomami in Venezuela have the greatest gut microbiota diversity known to date (1), the westernization of the Yanomami of northern Brazil may be decreasing its diversity to the point of becoming similar to the largest urban center in Amazonia, the city of Manaus.

OBJECTIVE

To characterize the diversity of the gut microbiota from the Brazilian Yanomami in terms of Bacteroidetes and Firmicutes, and compare it with westernized populations such as Manaus-AM.

METHODOLOGY

The collection of fecal samples from the indigenous people was carried out at CASAI in Boa Vista-RR, where 18 healthy Yanomami individuals were selected: two babies aged 1 to 11 months, six children up to 10 years old, five young adults up to 35 years old and five adults from 50 to 65 years old, classified here as Seniors. Stool samples were collected by the indigenous people themselves with the help of the CASAI medical team. From the group in the Manaus population, the same number of individuals were collected at the same ages as the

Yanomami, for comparative purposes. The bacterial DNA was isolated using the PowerSoil DNA Isolation Kit (MoBio) (1) following the manufacturer's instructions. For amplification, we implemented a Polymerase Chain Reaction (PCR) of the variable region V1-V2 of the rRNA 16S gene, following Watts et al. (2017)(9). Finally, the library sequencing was performed in IonTorrent PGM (Life Technologies, USA). Data analysis was performed using Mothur (10). We used the “pooled samples” approach by age, developed by Ray et al. (2019)(11) to determine community-level microbiome diversity, increasing the statistic robustness and depth-coverage per locus. The sequences were aligned using the SILVA database and split into Operational Taxonomic Units (OTUs) up to the genus level. Differences in the relative abundance of Firmicutes and Bacteroidetes within and between Yanomami and Manaus populations were explored through a non-parametric multiple comparison test of Kruskal-Wallis ($P < 0.05$), using the software IBM SPSS Statistics v.22. Likewise, it was used to configure a U-Mann-Whitney test of independent samples to detect differences in Ace diversity indices.

RESULTS AND DISCUSSION

The identification of microbiota from Yanomami and Manaus reached 147 OTUs. Among these genera, 40 are shared between the two populations; three are exclusive for the Yanomami and 11 for Manaus. Regarding the composition of the Yanomami gut microbiota, the largest OTU was the *Prevotella* genus. On the other hand, the Manaus population presented a great abundance of the *Bacteroides* genus, while the Yanomami population presented very low levels of this genus for all age groups. Differences were found between the two populations, mainly concerning the highest values of the phyla Bacteroidetes over Firmicutes, being significantly dominant only in the Yanomami. However, no statistical difference was found between them in terms of alpha diversity ($p = 0.3972$). Here, we found that lifestyle, and especially westernization, have a great impact on the composition of the human gut microbiome, as demonstrated by Obregon-Tito et al. (2015)(4) and Clemente et al. (2015)(1). For instance, our results confirmed that the gut microbiome of nonindustrialized societies is enriched with the phyla Bacteroidetes, and industrialized populations have a gut microbiome enriched with the phylum Firmicutes (12). Likewise, it was confirmed that within the phylum Bacteroidetes, *Prevotella* abundance is more significant in nonindustrialized societies, whereas the abundance of *Bacteroides* genus is more significant among industrialized societies (7,12). Analogous findings have been shown in other non-westernized populations from Africa, Papua New Guinea, and Perú (4). One possible explanation for this difference is that in nonindustrialized societies, people tend to eat more plant-derived carbohydrates and dietary fiber, known to have a higher bacterial diversity in their microbiome (13) to favor the colonization of *Prevotella* (14) as compared with the western diet (including high fat and cholesterol, high protein, high sugar, and excess salt intake). However, the lack of difference in total diversity between groups, suggests a warning about a Westernization process underway for the Yanomami. **Conclusion:** The composition of the Yanomami gut microbiota still maintains the profile characteristic of a community with a traditional lifestyle; however, our results suggest an underlying westernization process for Yanomami by loss of diversity.

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