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Characterization of the food microbiota in ready-to-eat Mexican foods.

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Abstract: The ready-to-eat food microbiota are the microorganisms present in the dishes that are currently consumed during the meals. These microorganisms include those that may have a health benefit, are potentially pathogenic or have not yet been given a function. Foods suitable for consumption are not free of microorganisms, however, within the food industry only yeasts have been given a beneficial function, while other microorganisms such as filamentous fungi and bacteria have been studied for their negative effect on food. We determined the bacterial diversity in samples of high demanded freshly prepared unspoiled ready-to-eat dishes by High-throughput DNA sequencing of 16S rDNA libraries. We found a great bacterial diversity where the most abundant bacterial phyla were Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, TM7, Thermi, among others. These phyla included bacteria with remarkable abundance in some dishes. The alfa diversity analyses showed that the main dishes had the largest diversity. The beta-diversity analyses clustered the bacterial communities of soups, side plates, desserts, and beverages, and some main dishes. Based on our results we conclude that unspoiled ready-to-eat Mexican dishes contain a rich bacterial diversity, which may contribute to the organoleptic properties of the dishes without representing a sanitary risk for the consumers.

Keywords: Food; food microbiota; ready-to-eat food; High-throughput DNA sequencing

1. Introduction

Foods that are suitable for human consumption are not free of microorganisms, the dishes that we consume regularly, in addition to containing nutrients that contribute to our health, are full of microorganisms contributed by each ingredient [1]. The ready-to-eat food microbiota are the microorganisms present in the dishes that are currently consumed during the meals These microorganisms include those that may have a health benefit such as probiotics, or may be potentially pathogenic, in addition to others that have not yet been described or given a function [2]. It is common to describe microbial contamination in food which include pathogenic microorganisms, causing food related diseases, food spoilage or deterioration of the original food properties [3]. There are those that may have a beneficial function for the consumer as the probiotics, or even some that add desirable organoleptic properties to the food by ageing. The cheeses are an example of ingredients in the recipe of some dishes that contribute to the microbiota present in the ready-to-eat food, ingested almost three times every day by the commensals [4]. Although the microbiota which is present in the food ingredients is usually well characterized [5], the reports about the characterization of the microbiota present in ready-to-eat food are scarce [6]. In this work we explored the microbiota present in ready-

to-eat Mexican dishes with the purpose of characterizing the bacterial communities which are ingested in the everyday feeding.

2. Materials and Methods

2.1. Ready-to-eat food sampling

A selection of approximately 35 different dishes with high consumer demand, available in the San-Pedro-Zacatenco area, in northern Mexico City (19.5070° N, 99.1214° W), were aseptically collected and transported immediately to the laboratory to be processed within 30 minutes for analysis (Table 1).

2.2. DNA extraction

For DNA extraction, 100g of each solid dish was homogenized with 100 ml of deionized water using a food hand blender (Braun Cat.# MQ725) in a 500 mL glass beaker. An aliquot of 100 mg of slurry or 200 μl for the liquid samples was used to isolate DNA using the FavorPrep Stool Kit (Cat.# FASTI001-1; Favorgen Biotech Corp; Ping-Tung, Taiwan), recovering the DNA in 80 μL of elution buffer (ddH2O) and stored at -70° C. DNA concentration was measured using the NanoDrop 2000 spectrophotometer (Cat# ND2000 Thermo Scientific; Massachusetts), and the DNA quality was evaluated by electrophoresis in 0.5% agarose gels. Average yield for the DNA extraction was of 37.06 ng/μL.

2.3. Semiconductor DNA sequencing of V3-16S rDNA libraries

The rDNA libraries amplifying the V3 polymorphic region of the 16S rRNA gene were prepared by PCR using barcoded primers, and semiconductor high-throughput DNA sequencing was made in Ion Torrent PGM system as previously described [7].

2.4. Data analysis

The data obtained from the sequencing was analyzed using the QIIME program (Quantitative Insights Into Microbial Ecology, v1.9.0) pipeline [8] to determine the relative abundance of bacteria. The alpha diversity was characterized with the Shannon, Simpson, Chao1 indices and observed species using phyloseq (v1.22.3) and ggplot2 (v3.1.0) packages in R program (v3.4.4) [9]. Beta diversity dissimilarity index was calculated by UniFrac distance metric as % of total variability in different axes of the plot and visualized by principal coordinate analysis as described in [10].

3. Results

3.1. Abundance of phyla in the ready-to-eat food.

We found a great bacterial diversity among all studied ready-to-eat dishes (Table 1). The most abundant bacterial phyla were Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, TM7, Thermi, among others. In the dairy products (DA) for example, the phyla Firmicutes and Proteobacteria were the most abundant except for DA1 that corresponds to a sample of Kefir which exhibited a large abundance of the phylum Proteobacteria (Fig. 1). A similar situation was observed for the side dishes (SD) where the SD3 corresponding to a sample of “chef's salad” was the one with a large relative abundance of Proteobacteria. The soups such as SO1 and SO5 (Table 1) had a large abundance of Proteobacteria and Bacteroidetes respectively, while the soups SO2, SO3, and SO4 exhibited similar large abundance of Firmicutes (Fig. 1). The main dishes MD1 to MD7 (Table 1), including dishes consisting mostly of cooked meat of chicken, pork or beef, had Firmicutes as the most abundant phylum, with the exception of MD2 (soft taco with pork rind), which had Proteobacteria the most abundant phylum. When the microbiota of the sample of desserts DE1 to DE6 were analyzed, again the Firmicutes were the most abundant phyla with exception of “sugary churros” (DE1) which contained high abundance of Actinobacteria and “chocolate bread-flan” (DE6)

which contained Proteobacteria (Fig. 1). The three beverages we studied (Table 1) made mostly with uncooked vegetable ingredients, showed high abundance of Firmicutes (BE1 and BE3) and Proteobacteria (BE2) (Fig. 1).

3.2. Abundance of orders, families, and genera in the ready-to-eat food.

Among the most abundant taxa, the families Streptomycetaceae (Actinobacteria), Rikenellaceae (Bacteroidetes), and the genera *Oscillospira* (Firmicutes), *Prevotella* (Bacteroidetes), *Faecalibacterium* (Firmicutes), *Bacteroides* (Bacteroidetes), and *Pseudomonas* (Proteobacteria), are present in almost all the food dishes categories (Fig. 2). In the DA group, the "Kefir sample" (DA1) shows high abundance of *Enterococcus* (Firmicutes), Erisipelotrichaceae (Firmicutes), f Leuconostocaceae (Firmicutes), *Pseudomonas* (Proteobacteria), *Lactococcus* (Firmicutes), *Acinetobacter* (Proteobacteria), Lactobacillales (Firmicutes), *Acetobacter* (Proteobacteria), and f S24_7 (Bacteroidetes); while a "drinkable yogurt" (DA4) had high abundance of *Oscillospira* (Firmicutes), Streptomycetaceae (Actinobacteria), *Prevotella* (Bacteroidetes), *Faecalibacterium* (Firmicutes), *Bacteroides* (Bacteroidetes), *Weisella* (Firmicutes), *Staphylococcus* (Firmicutes), Rikenellaceae (Bacteroidetes), and Aeromonadaceae (Proteobacteria). Two side dishes had remarkable abundance of bacteria; for instance the "chicken salad" (SD1), had *Thermus* (Deinococcus–Thermus), Leuconostocaceae (Firmicutes), Acetobacteraceae (Proteobacteria), and *Geobacillus* (Firmicutes); while the "chef's salad" (SD3) exhibited high abundance of *Staphylococcus* (Firmicutes), Rikenellaceae (Bacteroidetes), Lactobacillales (Firmicutes), and Acetobacteraceae (Proteobacteria). For the case of soups, only the "chinese style rice" (SO5) had a comparable high abundance of *Bacteroides* (Bacteroidetes). Main dishes like "soft taco with pork rind" (MD2) had high abundance of Aeromonadaceae (Proteobacteria), Acetobacteraceae (Proteobacteria), and *Geobacillus* (Firmicutes), this last Firmicutes was also observed in high abundance in "Egg with chorizo" (MD3). The f S24_7 (Bacteroidetes), Acetobacteraceae (Proteobacteria), and g *Geobacillus* (Firmicutes), were abundant in the "Stew taco dish" (MD4); while in the same category, the "Mexican ham torta", "Mexican enfrijoladas" and "Mexican pork leg torta" carried high abundance of *Weisella* (Firmicutes), *Thermus* (Deinococcus–Thermus), *Acetobacter* (Proteobacteria), and Acetobacteraceae (Proteobacteria). Among the sweets, only the "Chocolate bread-Flan" carried *Pseudomonas* (Proteobacteria) in a high abundance comparable to the other dishes, same was observed in the beverages where only the Orange juice carried comparable high abundance of *Lactococcus* (Firmicutes) (Fig. 2).

4. Discussion

In this work we characterized the bacterial diversity present in ready-to-eat Mexican dishes, habitually consumed by adult Mexican workers. We found a remarkable bacterial diversity in the unspoiled food from which DNA was extracted after homogenization, with bacterial members of the phyla Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, TM7, and Thermi. The Mexican healthy adult population which consume this type of food, have a fecal microbiota characterized by members of the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, that includes commonly members of the order Rhizobiales, Cytophagales, Nitrospirales, families f_Sphingomonadaceae, f_Cytophagaceae, f_Chitinophagaceae, f_Sphingomonadaceae, and genera such as *Bulleidia*, *Agrobacterium*, *Lentzea*, *Nitrospira*, and *Sphingomonas* [10]. We believe that in addition to environmental and genetic factors, every day food supplies a defined set of bacteria where some members either get established in the gut or influence the establishment of other taxa.

5. Conclusions

Based on our results we conclude that unspoiled ready-to-eat Mexican dishes contain a rich diversity in the bacterial community, which may contribute to the organoleptic properties of the dishes, and also might contribute to the gut microbiota in a daily basis, without representing a sanitary risk for the consumers.

Table 1. ready-to-eat samples.

ID	Local Mexican Spanish name	English name	Description
DA1	Leche-Búlgara	Kefir	Fermented dairy product.
DA2	Leche-Liconsa	Milk	Synthetic milk.
DA3	Leche-Saborizada	Flavored milk	Strawberry flavored milk.
DA4	Yogurt-bebible	Drinkable yogurt	Yogurt with a liquid consistency.
DA5	Yogurt-Griego	Greek style yogurt	Yogurt with a higher amount of fat than normal.
SD1	Ensalada-de-pollo	Chicken salad	Vegetable salad that includes chicken for protein.
SD2	Ensalada-de-jamón	Ham salad	Vegetable salad that includes ham as a source of protein.
SD3	Ensalada-del-chef	Chef's salad	Vegetable salad with chicken, cubed cheese, and boiled egg.
SD4	Ensalada-de-frutas	Salad with fruits	Vegetable salad that includes fruits within its ingredients.
SD5	Ensalada-de-broccoli	Broccoli salad	Broccoli al vapor acompañado de verduras frescas
SO1	Sopa-de-papa	Potato soup	Tomato broth with diced potato chunks
SO2	Arroz-a-la-Mexicana	Mexican rice	Rice with tomato, carrot, and pea as main ingredients
SO3	Crema-de-poblano	Poblano cream	Crepe prepared with poblano pepper
SO4	Sopa-de-verduras	Vegetable soup	Tomato broth with diced vegetables
SO5	Arroz-chino	Chinese style rice	Rice prepared as a typical oriental recipe
MD1	Alambre	Beef kabob cooked on a grill.	Cubes of broiled beef, bell pepper, onion, bacon, and melted cheese.
MD2	Taco-de-canasta con chicharrón	soft taco with pork rind.	Steamed soft corn tortillas stuffed with fried pork rind.
MD3	Huevo-con-chorizo	Egg with chorizo	Fried egg accompanied by Spanish sausage.
MD4	Taco-de-guisado	Stew taco	Taco with Mexican tyle rice with sausages with tomato.
MD5	Torta-de-jamón	Mexican ham torta	Mexican bolillo (crusty roll) filled with mayonnaise, avocado, ham, and basket cheese.
MD6	Enfrijoladas	Mexican enfrijoladas	Fried tortillas, dipped in a slurry of refried beans, and stuffed.
MD7	Torta-de-pierna	Mexican pork leg torta	Mexican bolillo (crusty roll) filed with mayonnaise, beans, avocado, tomato, smoked pork leg.
DE1	Churros-azucarados	Sugary churros	Fried-dough pastry in oil, with granulated sugar.
DE2	Pay-de-Piña	Pineapple Pie	Pie filled with pineapple jam.
DE3	Frituras de maíz con limón y sal	Corn chips with lemon and salt	Deep fried corn flour chips with lemon and salt.
DE4	Dona glaseada	Glassed donut	Dough fried in oil and glazed with sucrose after cooking.
DE5	Gorditas-de-la-Villa	Villas's soft cookies	Cornmeal (cacahuazintle), sucrose, baking soda, eggs, vanilla essence, water, and vegetable shortening.
DE6	Chocoflan	Chocolate bread-Flan	Chocolate flavor bread with a layer of sweetened egg custard with caramel topping.

BE1	Jugo de naranja	Orange juice	Juice obtained from orange fruit
BE2	Agua de melón	Melon water	Water accompanied by blended melon and sugar to sweeten.
BE3	Jugo verde	Green juice	Juice obtained by blending spinach, pineapple, orange juice and celery.

DA, dairy product; SD, side dishes; SO, soups; MD, main dishes; DE, deserts; BE, beverages.

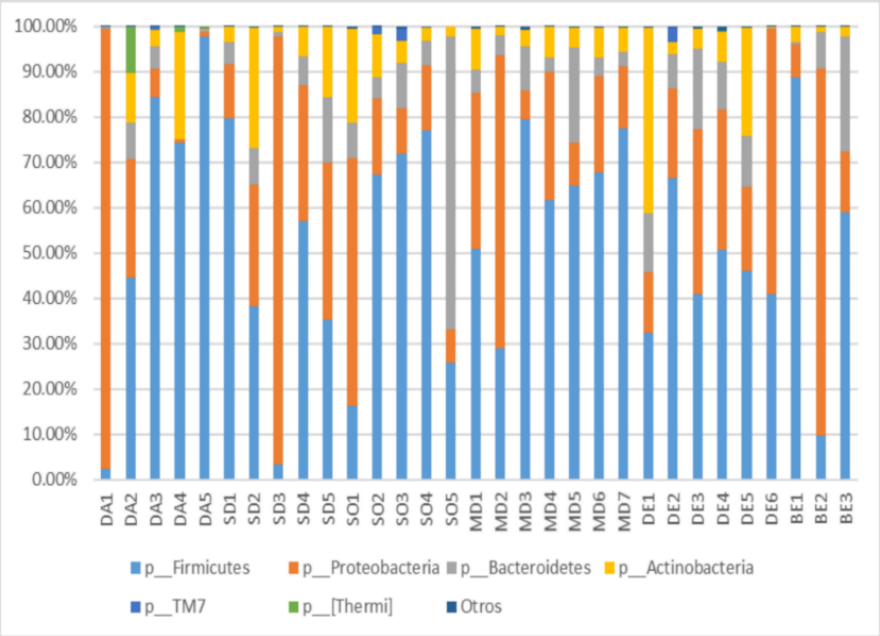

Figure 1. Relative abundance of bacterial phyla in the ready-to-eat dishes. The figure shows a bar graph of relative abundance with the most abundant bacterial phyla in the ready-to-eat dish samples. The phyla are identified with colors as shown below the graph. Others include the phylum Gemmatimonadetes, Synergistetes, and Lentisphaerae. DA, dairy product; SD, side dishes; SO, soups; MD, main dishes; DE, deserts; BE, beverages.

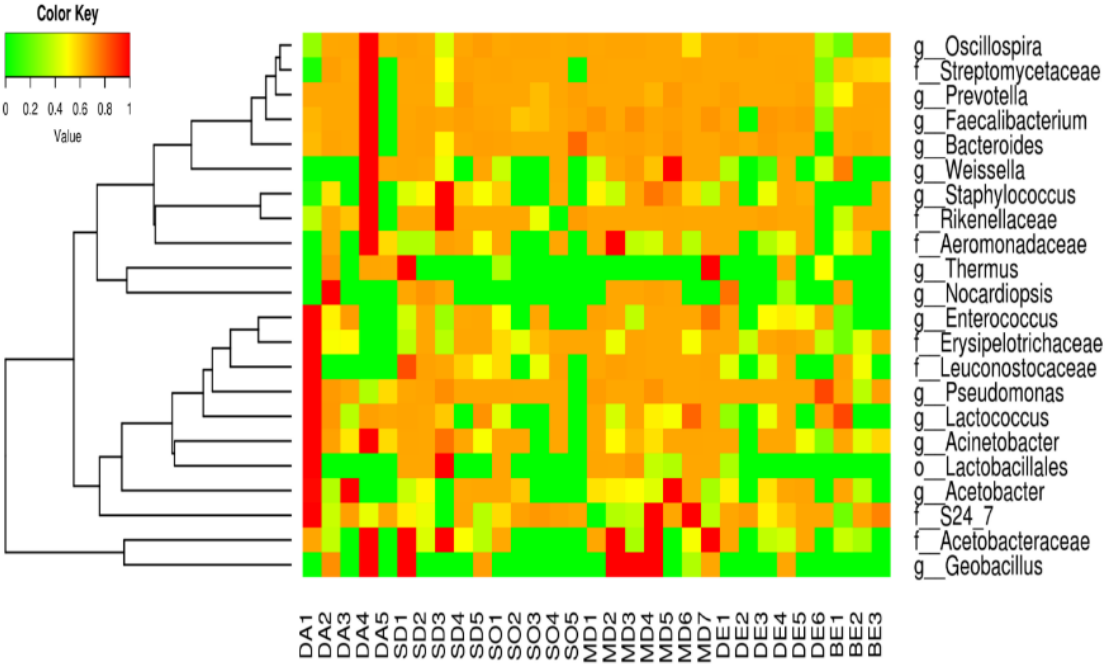

Figure 2. Relative abundance of more abundant bacterial taxa in the ready-to-eat dishes. The figure shows the food groups on the vertical axis and the horizontal axis shows the 20 most abundant bacterial genera in each group which are shared by at least 80% of the dish categories. The scale ranges from green (the least abundant), yellow (the genera with medium abundance) and red (the most abundant bacterial genera). DA, dairy product; SD, side dishes; SO, soups; MD, main dishes; DE, deserts; BE, beverages.

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and JG-M; resources, JG-M; data curation, CF-R, FH-Q, and KN; writing—original draft preparation, CF-R and JG-M; writing—review and editing, CF-R, FH-Q, LEV-F, KN, and JG-M; visualization, JG-M; supervision, JG-M; project administration, JG-M; funding acquisition, JG-M.

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