A metagenomic assessment of bacterial community in spices sold open-air markets in Saint-Louis, Senegal

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Abstract

Natural spices are reputed to play an essential role in human nutrition and well-being due to their organoleptic and therapeutic properties. Moreover, they are increasingly being processed on various scales, exposing them to potential sources of contamination. This study aimed to describe the bacterial community in spices sold in Senegal. Thyme, Curcuma, a mixture of 7 spices and "Local Spices or Herbs" (LSH) samples were collected in selected open-air markets in August 2022 in Saint-Louis, Senegal. DNA extraction was performed using the Omega E.N.Z.A Food DNA kit. PCR assays were based on a genomic fragment encoding bacterial 16S rRNA, which was subsequently sequenced using Oxford Nanopore technology (ONT). Sequencing was carried out on two batches of samples, one containing part of the "Local Spices or Herbs" (n = 10) designated as "a mixture of food seasonings", and the other, samples of a mixture of 7 spices, Curcuma, Thyme and the other part of the "Local Spices or Herbs" (n = 39). Analysis of the sequencing data showed high bacterial diversity and the predominance of *Escherichia coli*, *Salmonella enterica* and *Escherichia marmotae* in the samples, with a total number of reads of 65744 and 165325 for the two batches, respectively. The sample category "Homemade mixture of food condiments (ready to use)", which includes all "Local Spices or Herbs" samples, showed remarkable bacterial diversity. These were followed by Curcuma, a blend of 7 spices and thyme, respectively. These results highlight a highly diverse genomic profile of the microbial community, including pathogenic bacteria, in spice samples. This is the first metagenomic study to assess microbial diversity and analyze microbial community structures in spices in Senegal.

1. Introduction

Spices have been used for centuries as an essential component in traditional Asian medicine [1–2]. Some of them draw special attention due to their use in the cosmetics and pharmaceutical industries [3]. Spices are reputed to be a source of bioactive compounds [4] with sometimes rich extracts in polyphenolic compounds, including high levels of antimicrobial, antioxidant and anti-inflammatory activity [5–6]. Preventive and curative actions of spices against cancer, gastrointestinal disorders, psychological disorders, diabetes, obesity, metabolic syndromes and neurological disorders were also reported [7–8]. Aromatic plants and spices have thus become well established over time, justifying their use by around 80% of the world’s population, mainly in developing countries [7]. Spices can be divided into several groups: strong spices (pepper, chilli, ginger), mild spices (paprika, coriander), aromatic spices (cinnamon, curcuma, cloves, cumin, anise, celery), dried herbs (basil, bay leaves, dill, marjoram, tarragon, thyme) and aromatic vegetables, for instance, onions or garlic [9].

From a nutritional point of view, China and some West African countries have begun to use condiments and seasonings to remedy significant dietary deficiencies, particularly in iodine, iron and vitamin A [10]. According to several studies, various micronutrients are added to condiments depending on the deficiencies demonstrated in the target population. The bioavailability of the micronutrient remains highly important, as it must be absorbed by the body in sufficient quantities to contribute effectively to improving these deficiencies [10, 11]. Despite the awareness messages to limit the overconsumption of

processed food products while promoting natural products in the diet [12–14], the use of industrial ingredients remains a concern [15]. However, spices and broths made from natural products can also be at risk for consumers. Indeed, due to transformation processes such as extraction and packaging processes, these can be subject to contamination by bacteria, viruses, parasites and pathogenic fungi due to handling [16–17]. Furthermore, the diversity and abundance of microbial communities in these food matrices can influence their compositions, nutritional benefits and even the functioning of the gut ecosystem [18–20].

Traditional studies of bacterial diversity in foods have relied on culture methods, whereas only around 1% of microorganisms present in the natural environment can be grown by these methods [21–22]. Culture-independent approaches involving molecular biology methods such as gradient gel electrophoresis (DGGE) polymerase chain reaction (PCR) and extended later to metagenomic methods to assess microbial diversity and analyze microbial community structures in diverse environments, including food matrices [22–25]. This study aimed to describe the genomic footprint of the microbial community present in spices sold in open-air markets in Senegal using the sequencing method.

2. Material and methods

Collection and preparation of samples

Samples of Curcuma, a mixture of 7 spices (cloves, ginger, garlic, cumin, lemon, nutmeg and salt), Thyme and “Local Spices or Herbs” (LSH, a mixture of food condiments used to season dishes) were collected from 3 markets in Saint-Louis in August 2022 in compliance with the sampling process [26]. For a mixture of 7-spices, thyme and Curcuma samples, 30 g were purchased, while 100 g was for “LSH”. Samples were placed in an individual sterile zipped bag. “Pseudo-industrial spices” are in dehydrated format and sold in their original packaging, particularly Curcuma, a mixture of 7-spices and Thyme, which were transported and stored at ambient temperature until their use. However, the individuals’ zipped bags of “LSH” samples were placed and transported in an insulated bag maintained at + 4°C to the laboratory, ensuring their preservation. The samples were then kept at -20°C until analysis.

Extraction of Bacterial DNA

Samples were subjected to manual extraction using the *Omega E.N.Z.A Food DNA kit (*Omega Bio-Tek, GA, USA*)* in accordance with the manufacturer’s instructions, with a few minor modifications. To optimize cell lysis, four beads with a diameter of 2.0 mm (https://zymoresearch.eu/products/zr-bashing-bead-lysis-tubes) were added to the lysis buffer before beating the sample with Analog Disruptor Genie (https://www.scientificindustries.com/otuli-mixers-and-shakers/bead-beaters/analog-disruptor-genie.html) during 5 minutes. The DNA concentration was determined by a Nanodrop spectrophotometer One (Thermo Scientific, USA), and the sample was stored at − 20°C until analysis.

PCR Amplification and Sequencing

The region coding for the 16S subunit of bacterial RNA was amplified using the universal primers Fd1 (5’ – AGA GTT TGA TCM TGG CTC AG – 3’) and Rd1 (5’ – AAG GAG GTG ATC CAG CC– 3’) [27]. The reaction volume for amplification was 25 µL and included 12.5 µL of *One Taq® Quick-Load 2X Master Mix with Standard Buffer* kit (New England Biolabs, M0486), 2 µL of primers, 5 µL of DNA and 5.5 µL of molecular grade water. PCR was performed under the following conditions: an initial denaturation of 94°C for 30 seconds followed by 30 cycles of 94°C for 30 seconds, 50°C for 30 seconds, 68°C for 1 minute and a final extension of 68°C for 5 minutes. Amplified fragments were visualized by electrophoresis on a 1% agarose gel using ethidium bromide staining and UV light. Amplicons were then purified using the Zymo DNA clean & concentrator ^{TM} kit-5 (Zymo D4034). Library preparation was performed using the Rapid Barcoding Kit (SQK – RBK 110–96). The sequencing was performed on Flow Cell R9.4.1 (FLO-MIN106D) on a MinIon Mk1C sequencer. A real-time workflow Epi2me platform was used for species identification. Alpha diversity, which shows the complexity of species within a sample, was obtained using four indices: Chao1, Shannon, Simpson and observed species. For this, the Vegan and Phyloseq packages of the R Studio software were used. Beta diversity, which shows the complexity of species across all samples, was measured using Jaccard and x indices. To obtain this diversity, the Vegan and Ecodist packages of the R software were used. All figures were also generated using the R software.

Statistical Analysis

All statistical analysis was performed on R software. For each considered spice group, the Wilcoxon test was performed for differential analysis of diversity. The significance level was set at $p < 0.05$. The degree of significance is proportional to the number of stars shown at the top of the figures (* corresponds to $p < 0.05$, ** corresponds to $p < 0.01$ and *** corresponds to $p < 0.001$). In order to assess the similarity between the different microbial communities in the considered spice groups, beta-diversity was calculated based on the Bray-Curtis dissimilarity distance and the Jaccard index. The result was then represented using a principal coordinate analysis (PCoA).

3. Results

A total of 49 samples divided into two categories, including "Homemade mixture of food condiments (ready to use)" (n = 19) and "pseudo-industrial spices" sold dehydrated with primary packaging (n = 30), were collected and analyzed in two batches. The first batch consists of 10 "Local or Spices Herbs" samples. This type of spice is a blend of green onions, peppers, garlic and Sofia bell pepper (Capsicum annum). The second batch, designated as "pseudo-industrial spices, " was made up of thyme, 7-spice mix, curcuma, and the "Local or Spices Herbs" samples. For each sample type, additional details, such as the numbers and collection locations, are reported in Table 1.

Table 1
Distribution of samples according to type and collection sites

Categories	Types of samples	Dry or dehydrated form	Number of collected specimens	Collection sites
“Homemade mixture of food condiments (ready to use)”	Local spices or herbs	no	19	Sor/ Saint-Louis
Pseudo-industrial spices	Mixture of 7 spices	yes	10	Sor, Guet Ndar and Pikine / Saint-Louis
	Curcuma	yes	10	Sor, Guet Ndar and Pikine / Saint-Louis
	Thyme	yes	10	Sor, Guet Ndar and Pikine / Saint-Louis

Sequencing data were processed using Nanopore's Epi2me software. Quality scores were 9.87 and 9.82, with classified read counts of 65744 and 165325, respectively for the two batches analyzed. Figure 1 shows the predominant bacterial species for all the samples processed in this study. Generally speaking, *Escherichia coli* and *Salmonella enterica* are the most represented species in all samples (Fig. 1).

Figure 1

Relative distribution of bacterial species in samples

Analysis of bacterial diversity according to spice type showed the predominance of *Escherichia coli*, *Salmonella enterica* and *Acinetobacter seifertii*, with proportions differing slightly from one category to another (Supplements 1–4). All species with an abundance of less than 1% were merged into an "others" category. This group represented 21.2% of species in the "Local Spices or Herbs" and was mainly made up *of Saccharothrix sp.6-C* (4.7%), *Shigella flexneri* (4.5%) and *Buchnera aphidicola* (4.1%). For Curcuma, the "others" group represented 9.1% and was mainly made up of *Acinetobacter baumannii* (6.1%), *Bacillus circulans* 3.9% and *Kosakonia cowanii* (3%). For the 7 spices and Thyme samples, this group represented 4% and 2%, respectively. The predominant bacterial species were *Acinetobacter baumannii* (16.3%), *Clostridium botulinium* (11.3%) and *Clostridium tatani* (3.6%) for 7 spices, *and Shigella flexneri* (12.4%) and *Pseudomonas fulva* (6.7%) for Thyme.

By considering all the types of spices, alpha-diversity analysis showed that "Local Spices or Herbs" had the most incredible diversity, with an average number of taxa per sample of 115.78. This group is followed by Curcuma, a mixture of 7 spices and Thyme, respectively. The average taxa numbers per sample were 41.1, 14.5 and 10.3, respectively (Supplement 1). About the number of species per spice

group, after using the Wilcoxon test, there was a significant difference between "Local Spices or Herbs" and Thyme ($p < 0.001$), Curcuma and Thyme ($p < 0.001$), and Curcuma and the mixture of 7-spice ($p < 0.01$). However, there was no statistically significant difference between the mixture of 7-spice and Thyme, as shown in Figs. 2A and 2B. Figures 2C and 2D show that species' relative abundance and stability are greater for "Local Spices or Herbs". This group shows a significant difference with Thyme ($p < 0.05$). There is also a significant difference between Curcuma and Thyme ($p < 0.001$) and between Curcuma and the mix of 7-spice ($p < 0.001$).

Figure 2

Alpha-diversity analysis

Beta-diversity was illustrated using PcoA based on Bray-Curtis dissimilarity and Jaccard distance, which showed similarities in bacterial composition between samples from the different spice groups and showed two clusters (Fig. 3). One consisted of the species found in Thyme, Curcuma, a mixture of 7 spices samples and part of the “Local Spices or Herbs” samples. The second cluster consists solely of “Local Spices or Herbs” samples. These results show that the bacterial communities in the samples from the first cluster are closer than those in the second cluster.

Figure 3

Beta-diversity analysis

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4. Discussion

This study aimed to describe the microbial community present in spices sold in open-air markets in Saint-Louis using the sequencing method. The high-throughput sequencing method used in this study allowed us to detect a broad-spectrum genomic footprint of micro-organisms, including those usually not detected by culture methods. Indeed, since the revolutionary improvement in DNA sequencing technologies, direct high-throughput analysis of the genomic DNA of an entire community without prior culture has become the most common approach, overcoming the constraints of conventional microbiological approaches [28]. In addition, from a technological point of view, MinION sequencing is mainly used to address metataxonomy [29].

Our results show the presence of many pathogenic bacteria in the spice samples studied, with their abundance varying according to the type considered. Studies in countries like Brazil have reported similar

results [30]. In contrast to other studies in Vietnam, Iran, Indonesia, India, and the Netherlands, analyzed spices meet established microbiological standards and were of satisfactory quality [29–30]. These were particularly spices, herbs, and dried spices produced on a small scale.

The significant bacterial communities found in the “Local Spices or Herbs” samples analyzed compared to the others could be explained by the difference in water activity. The “Local Spices or Herbs” samples, consisting of a mixture of aromatic herbs and food condiments, represent a more favourable environment for bacterial growth than the other dried spices. Nevertheless, despite their packaging giving them “stable physicochemical characteristics”, the dehydrated products, such as Curcuma, the mixture of 7-spice and thyme, have a relatively large bacterial community (Supplements 1–4). These foods generally undergo drying processes at ambient temperature at the place of production. Moreover, in the developing countries from which they originate, harvesting and production technologies do not necessarily comply with optimal sanitary conditions [29–31]. The non-compliance with hygienic measures at all stages of the process, particularly during agricultural production, harvesting, washing and sun-drying, may explain the results of this study. Packaging hygiene and storage conditions are not always respected either, which is a probable cause of food contamination (FAO, https://www.fao.org/3/w7429f/w7429f0r.htm). All these factors lead to high levels of microbial contamination, which means these products may not be suitable for human consumption [29, 32, 33].

The presence of *Escherichia coli*, *Salmonella spp*. and *Bacillus spp*. in most of the analyzed samples could potentially be harmful in herbs and in various food matrices such as spices, which were the subject of this study [36]. Moreover, the bacterial spores introduced by spices can withstand various preparation processes, including heat treatment [37]. The high presence of *Escherichia coli* in all samples may indicate faecal contamination of various origins. In the case of fat-in-house spices, including horticultural products, faecal contamination could be irrigation water from wastewater systems used without prior biological treatment [37–42]. As for the other spices studied, faecal contamination could be due to a hygiene failure by operators handling the processing (reduction to powder, mincing, etc.), storage and transport stages.

The analysis of distance matrices containing dissimilarity information can effectively capture significant and subtle compositional differences between the samples studied [44]. Other techniques, such as the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), an agglomerative hierarchical clustering method widely used in practice, can also be considered. Step by step, it combines the two closest groups or elements into a higher-level group, and the distance between the new group and any other group is calculated as the arithmetic mean distance between the elements in the different groups or clusters [45–46].

The small sample size (n = 49) in this study constitutes a limitation. Furthermore, the presence of genetic material cannot provide information on the level of bacteriological contamination. Therefore, the description of the microbial community obtained by the metagenomic approach could have been

accompanied by conventional microbiological analyses to highlight the fraction of viable bacteria in the analyzed samples.

5. Conclusion

This study aimed to describe the bacterial community in spices sold in open-air markets in Saint-Louis, Senegal. It revealed that spices, whether fresh (“Local Spices or Herbs”) or dehydrated (Curcuma, Thyme, a mixture of 7 spices), can carry bacteria, which could be pathogens such as *Salmonella spp*, *Shigella spp*, *Bacillus spp*, *Enterobacter spp*, *Pseudomonas spp*, *Klebsiella spp*. In addition, it highlighted potential defective hygiene in the production chain of these spices through the presence of indicators of faecal contamination such as *Escherichia coli* and *Salmonella enterica* species. These results suggest that efforts must be made to raise awareness among the various stakeholder in the production chain. In addition, further research with a more significant number of samples of spices should be carried out to identify other potential contaminants that could harm to human health.

Abbreviations

DGGE: Gradient Gel Electrophoresis; **DNA**: Deoxyribonucleic acid; **FAO**: Food and Agriculture Organization of the United Nations; **GRBA-BE:** Applied Biotechnologies and Environmental Bioprocesses Research Group; **IRESSEF** : Institute for Health Research, Epidemiological Surveillance and Training; **ISEP** : Higher Institute for Vocational Training; **ITNA** : Institute of Applied Nuclear Technology; **LABAAM** : Biological, Agronomic, Food Sciences and Complex Systems Modelling Laboratory; **LAE** : Analysis and Testing Laboratory; **LSH**: Local Spices or Herbs; **ONT**: Oxford Nanopore Technology; **PCoA**: Principal Coordinate Analysis; **PCR**: Polymerase Chain Reaction; **RNA**: Ribonucleic Acid; **UPGMA**: Unweighted Pair Group Method with Arithmetic Mean; **USSEIN** : Sine Saloum El Hadj Ibrahima Niass University

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Data Availability: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

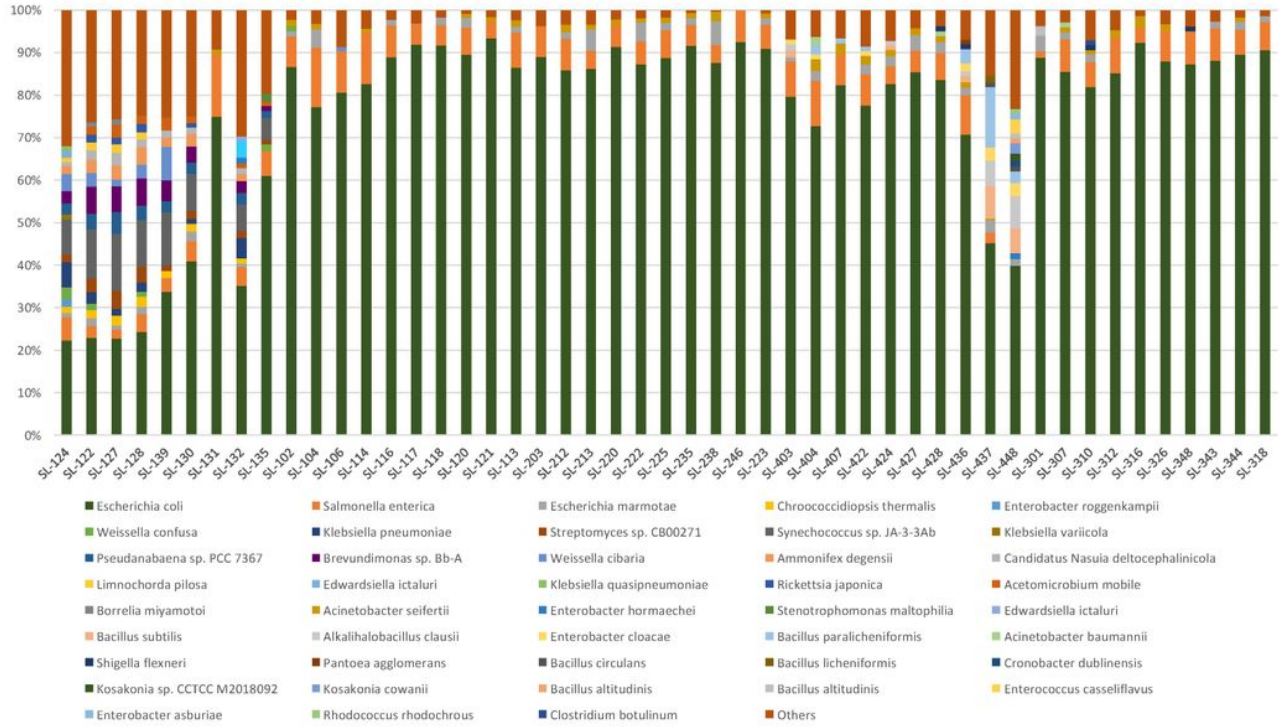

Figure 1: Relative distribution of bacterial species in samples

Figure 1

See image above for figure legend.

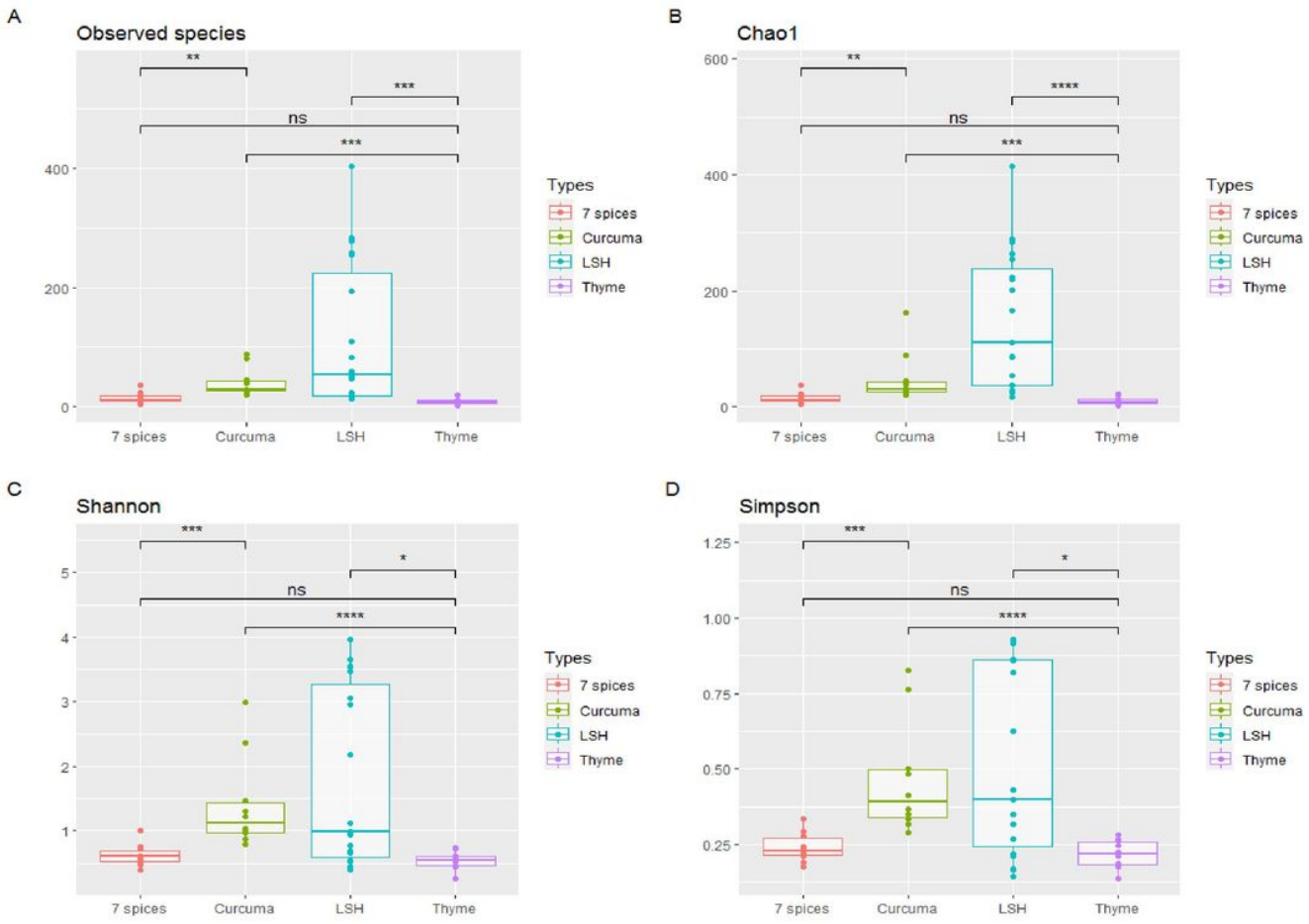

Figure 2: Alpha-diversity analysis

Figure 2

See image above for figure legend.

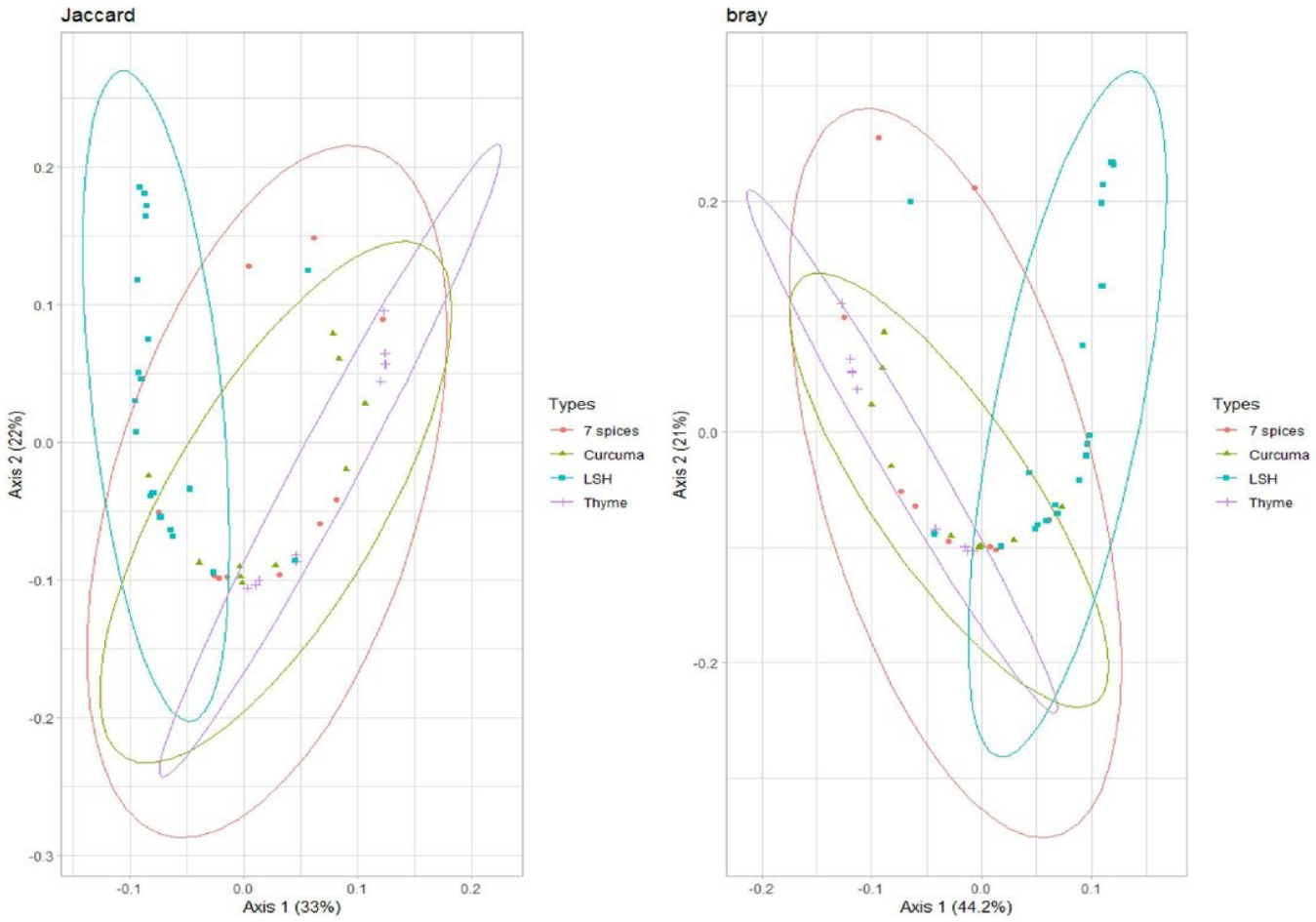

Figure 3: Beta-diversity analysis

Figure 3

See image above for figure legend.

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