

## 16S rrna gene oligotyping reveals the correlation retween food process and food $q_{UAL_{ITY}}^{\S k_3}$

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## Introduction

Introduction
The new frontiers of microbial ecology are concerned with how microbes
a complex ecosystem, such as food, and how microbes The new frontiers of interest are involved in a complex ecosystem, such as food, and how the are involved in a complex ecosystem, such as food, and how the are involved in a compensation of a condition (e.g., changes in the process parameters, storage environmental condition of a starter culture or the use of antienvironmental conditions (of a starter culture or the use of antimicrobial temperature, the addition of a starter and functioning of misorial temperature, the addition of a starter culture or the use of antimicrobial temperature, the additional temperature, the additional temperature, the additional temperature, the additional temperature and functioning of microbial agents) can affect the development and functioning of microbial agents) can affect the development and functioning of microbial agents. agents) can affect the development agents agents amount amplican target sequencing is most frequently applied to throughput (HT) amplican target sequencing is most frequently applied to throughput (171) amplitude to 16S gene) of the foods in order to find a study the microbiota (targeting the 16S gene) and the norder to find a study the interest the external perturbations and the evolution of the microbial composition (Ferrocino and Cocolin, 2017). The current methodology to analyze HT amplicon data is limited by the common use of a de novo clustering approach, relying on the clustering of sequences into operational taxonomic units (OTUs) at 97% or 99% of similarity threshold Large areas of the 16S reference tree offer a poor taxonomic resolution due to lack of such isolated representatives and the clustering often resulting in phylogenetically mixed units. This approach makes it difficult to identify organisms in communities that differ from each other by a very small number of nucleotides. The 16S rRNA gene has limited specificity yet it is very sensitive, since a single nucleotide difference at the 16S rRNA gene level can predict remarkable genomic variation (Eren et al., 2013). Unlike clustering methods, which compare all the positions in sequence reads to assess similarity, oligotyping utilizes the nucleotide positions that have been identified as the most information-rich by Shannon entropy calculations and allows resolution at a species level or even below (Eren et al., 2013). oligotyping was used in environmental studies as a tool to identify environmental studies as a tool to identify oligotyping was used in environmental studies as a tool to identify executly oligotyping was used in environmental studies as a tool to identify the studies are considered as a tool to identify the studies are considered as a supplies of the studies are considered as a oligotyping was used in confidential studies as a tool to identify evel differences across samples (De Filippis et al., 2016) or to level different adaptation efficiency of strains of the same Recently level differences across samples (De Filippis et al., 2016) or to sub-of-U level different adaptation efficiency of strains of the same species sub-of-U level different adaptation efficiency of strains of the same species sub-of-U level different adaptation et al., 2017). In this study we expand the scope of oligotyning discover the difference successfully resolve key miles of al., 2017 and 2017 anbO1U the different adaptation entreiency of strains of the same species discover the different adaptation entreiency of strains of the same species discover the different adaptation entreiency of strains of the same species of oligotyping and discover the different adaptation entreiency of strains of the same species of the same species of strains of the same species of the same species of strains of the same species of the same species of strains of the same species of the same species of strains of the same species of the same species of strains of discover and 2017). In this study we expand the scope of oligotyping and stellato et al. 2017). In this study we expand the scope of oligotyping and stellato et al. 2017) in this study we expand the scope of oligotyping and color of the study resolve key microbial diversity among the study in the study in the study of the distribution of the distribution of the distribution of the study in th (Stellar) that it can successfully resolve key microbial diversity among demonstrate that it can successfully important microbial taxa in the food matrix in demonstrate and ecologically important of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of a specific taxon in a factor of the distribution of demonstration and ecologically important microbial taxa in the food matrix in numerically and ecologically important microbial taxa in the food matrix in a food-order to have a better idea of the distribution of a specific taxon in a food-order to have a system.

Materials and Methous

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Were analyzed through ripening or storage to three different process and the microbial community due to different process and the microbial community due to different process based system. March different tood systems were analyzed through ripening or storage to three different process parameters three shifts in the microbial agents (ii) and the addition of a starter out the use of antimicrobial agents (ii) The shifts in the microbial agents (ii) and the addition of a starter culture.

(i) the use of antimicrobial agents (ii) and were selected T.... (i), the use of antimicropial agents (ii) and the addition of a starter culture.

Three farms producing Lard d'Arnad were selected. Two plants, i) Three industrial process characterized either by low matterials. Three farms producing Laid d'Arnad were selected. Two plants, in the industrial process characterized either by low maturation reflecting the industrial process characterized either by low maturation (plant A [10% NaCl, 2°C]) or by using a language of the contraction of the cont the industrial process characterized either by low maturation reflecting the industrial process characterized either by low maturation (plant A [10% NaCl, 2°C]) or by using a low NaCl reflecting the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected while the industrial process characterized either by low maturation (plant B [2.5% NaCl, 4°C]) were selected whil temperature (plant A 11070 NaCl, 2°Cl) or by using a low NaCl temperature (plant B [2.5% NaCl, 4°C]) were selected, while the third was concentration (plant B [2.5% NaCl occurrence of the concentration of the concentrat concentration (piant a ratisanal process (plant C [30% NaCl, 8°C]). Lard characterized by an artisanal at time 0 and after 7. 15 30 60 573 000. characterized by an artistical process (plant C 150% NaCl, 8°C]). Lard samples were obtained at time 0 and after 7, 15, 30, 60, and 90 days of samples (Ferrocino et al., 2017).

maturation (removing et al., 2017).

ii) Beefsteaks pre-treated with aqueous ozone (AO) or electrolyzed water iii) Beefsteaks pre-treated with aqueous ozone at refrigered. ii) Beefsteaks pre-treated with addeous ozone (AO) or electrolyzed water (EW) and vacuum packed. After storage at refrigerated condition the (EW) and vacuum problems at refrigerated condition the microbiota was analyzed over 15 days. Analysis of volatile compounds by GC-MS was also assessed (Botta et al., 2017).

GC-MS was also used as a starter culture during Satawiii) Lactobacillus plantarum was used as a starter culture during Sataw-Dong fermentation (fermented stink bean in brine, made with Parkia Dong letinemation (contained and plus speciosa). Two fermentations were performed: spontaneous (S) and plus speciosa). The brine microbiota was analyzed throughout fermentation for starter (ST). The brine microbiota was analyzed throughout fermentation for 15 days by 16S HTS and by GC-MS (Jampaphaeng et al., 2017).

Samples were collected across time/condition and were subjected to nucleic Samples were controlled by PCR amplification of the V3-V4 region of the 16S rRNA and then sequenced on a MiSeq Illumina platform. The sequences were quality filtered and an OTUs clustering approach was then applied to select the main OTUs. Reads from selected OTUs were ther extracted and the Shannon entropy analysis was used to find only the significant discrimination in sub-species (oligotypes) by focusing on the variable sites. To minimize the noise, each oligotype was required to appe in at least 1 sample, occur in more than 1.0% of the reads for at least o

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sample, represent a minimum of 500 reads (M option) in all sample, and have a most abundant unique sequence with a minimum Wileoxon tests were used in order. sample, represent a minimum of 300 testas (at option) in all sample, combined, and have a most abundant unique sequence with a sample, combined, and have a most abundant unique sequence with a minimum combined, and have a most abundant unique sequence with a sample, combined of 50. Pairwise Wilcoxon tests were used in order to determine the sample of 50. Pairwise Wilcoxon tests were used in order to determine the sample of 50. Pairwise Wilcoxon tests were used in order to determine the sample of 50. sample, represent a most abundant unique sequence with a sample, combined, and have a most abundant unique sequence with a sample, combined, and have a most abundance used in order to determine abundance of 50. Pairwise Wilcoxon tests were used in order to determine abundance according to the combined of the combined abundance of 50. Pairwise Wilcoxon to abundance abundance of 50 pairwise wilcoxon to digotype abundance according significant differences in specific oligotype abundance according to determine significant differences. Spearman correlation was also assessed benefit abundance according assessed between samples/time/treatment. Spearman correlation was also assessed between samples/time/treatment. oligotype and VOCs.

Results
i) The main taxa identified in lard samples by sequencing were inhusonii, Psychrobacter, Staphylococcus equorum, S were i) The main taxa identified action of the main taxa Acinetobacter johnsonu, I sydnamonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas and Vibrio and differences in Pseudomonas fragi, Brochothrix, Halomonas fragi, Brochothrix, Brochothrix, Brochothrix, Brochothrix, Brochothrix, Brochothrix, Brochothrix, Brochothr Pseudomonas fragi, prochounts their relative abundance distinguished samples from the individual plants a plants a plants a plants a plants. their relative abundance distinguished was more similar among plants A and B The composition of the find was characterized by the higher presence of (industrialized process) and was characterized by the higher presence of (industrialized process) and had a magnet presence of taxa recognized as undesired bacteria in food processing environments, reads of Acinetobacter and Halams After Entropy analysis reads of Acinetobacter and Halomonas were After Entropy analysis leading to the extracted and analyzed. A higher number of oligotypes was found within extracted and analyzed and within Acinetobacter. 9 oligotypes were more abundant in A samples [10% NaCl. 10 prayabled in R samples [2, 5% NaCl. 10% NaCl. 100 prayabled in R samples [2, 5% NaCl. 100 prayabled in R sampled Acinetobacter. 7 ongosper (10% NaCl, 4°C). Only (PDR <0.05), while 10 prevailed in B samples [2.5% NaCl, 4°C]. Only 4 oligotypes were characteristic of C samples. Seven Halomonas oligotypes were found more abundant in A samples while 4 characterized B samples and only 3 were associated with C samples [30% NaCl, 8°C].

ii) The targeted HT amplicon sequencing conducted on beefsteak identified Pseudomonas fragi as the most frequent species before and after the treatments with AO and EW, as well as in the untreated control. The tested treatments did not reduce its overall presence, but they did affect the intraspecies distribution of its oligotypes. The oligotype analysis of P. fragi showed a total of 32 oligotypes, 9 of which associated with EW, 2 associated with AO and 3 with the control. When the correlation between P. fragi oligotype abundance and VOC profiles was analyzed, it was possible to observe several positive correlations between specific oligotypes and volatile compounds considered as a spoiler marker.

iii) The microbiota of Sataw-Dong during fermentation with the use of L plantarum as a starter culture revealed the dominance of L. plantarum and L. sanfranciscensis indicating these taxa adapted well in brines. Using a starter markedly affected the microbiota after 1 day of fermentation and clearly influenced VOC profiles. Oligotyping of L. plantarum showed the presence of 53 oligotypes, however when comparing the relative abundance were associated with spontaneous fermentation and 5 with the only 12 were and VOCs showed a street fermentation and 5 with the beautiful fermentat were associated with spontaneous fermentation and 5 with the only 12 were associated with spontaneous fermentation analysis between fermentation and VOCs showed a strong correlation with oligonality and appears abundance and course fermentation and appears to starter fermentation and sta only 1 fermentation (FDR-0.03). The correlation analysis between and VOCs showed a strong correlation with oligotype oligotypes with starter fermentation and esanoic and nonanoic acid acceptable indicating a biobac fermion of the correlation and starter fermentation and esanoic and nonanoic acid acceptable indicating a biobac fermion of the correlation analysis between starter oligotypes abundance and voca showed a strong correlation with oligotype with starter fermentation and esanoic and nonanoic acid as well associated with thus indicating a higher fermentation of carbohydrate with disulfide, thus indicating a higher fermentation of carbohydrate with disulfide. oligory associated with starter refriction and esanoic and nonanoic acid as well associated with disulfide, thus indicating a higher fermentation of carbohydrates and swith disulfide, thus indicating amino acids. On the other hand oligated as with disulfide of sulfur-containing amino acids. as with disulfide, thus indicating a figure rementation of carbohydrates and swith disulfide thus indicating amino acids. On the other hand oligotypes degradation of sulfur-containing amino acids. On the other hand oligotypes degradation with spontaneous fermentation were positively correlated. degradation of surrur-containing annino acids. On the other hand oligotypes were positively correlated with spontaneous fermentation were positively correlated with associated with spontaneal and pentanon. Sensorial analysis elements of the spontaneous fermentation were positively correlated with associated monanal, heptanal and pentanon. degociated with spontaneous icinicitation were positively correlated with associated nonanal, heptanal and pentanon. Sensorial analysis showed that esanal, fermented Sataw-Dong significantly received a positive received and approximately received a positive received and approximately receiv assural, nonanal, neptanan and pentanon. Sensorial analysis showed that esanal, nonanal Sataw-Dong significantly received a positive response in starter-fermented when compared with the natural ones. overall acceptance when compared with the natural ones.

**Discussion**Several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have conditions as a several studies have clearly shown that environmental conditions induce a several studies have clearly shown that environmental conditions induce a several studies have conditions as Several studies have clearly successful that can different biotypes can selection of the microbiota even at strain level and different biotypes can selection that can describe the can describe selection of the intercolors and different metabolic behaviors that can drastically affect the final have different metabolic behaviors (Ercolini et al. 2010) have different included of the products (Ercolini et al., 2010). The use of characteristics a povel computational method can elected. characteristics of the productional method, can elucidate subtle diversity oligotyping, a novel computational method, can elucidate subtle diversity oligotyping, a north and operational units of classification or clustering approaches within the final operation. Here we would be approaches within the line of data information. Here we used this approach to assess by using the 16S data information. Great the approach to assess by using the to process parameters can affect the distribution of the how changes in process parameters can affect the distribution of the how changes in problem of the oligotypes and how this difference can affect the final characteristics of the ongotypes and found that changes in the process parameters (salt products. (said concentration and temperature) during lard d'Arnad manufacturing select the abundance of the oligotypes, clearly showing that the traditional method used to produce cured and ripened lard is effective in reducing the abundance of spoilage bacteria compared with industrial manufacturing. An oversimplified classification of taxa in a homogeneous OTU cannot disclose the possible strain-specific response to treatments, or the strain-specific relationship to VOCs. Analyzing two different food based systems (mean treated with antimicrobial agents and inoculated fermented beans) we clearly observed that different oligotypes from the same species showe distinctive correlation patterns with their volatilome. The selective development was therefore observed to be responsible for the sensori characteristics of the final products.

In conclusion this tool can also be easily applied to food-based studies order to ascertain an association between an oligotype and a process, or have a better idea of the distribution of a specific taxon in a food-ba system. A modulation of the main factors during food preparation

drastically affect the development of the microbiota and require an accurate an accurate order to preserve the food quality from the microbiota modulation drastically affect the development of the developme

The Real Property lies

Keywords
16S amplicon target sequencing, oligotyping, spoilage microbiolage vocs Keywords fermentation, VOCs

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