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Metagenomic study of diverse ingredients and seasoning material used in table olive elaboration

Table olive has been reported as splendid carrier of benificial microorganisms to the human body. However, scarce information is available about different ingredients used in the processing and packaging, such as salt and seasoning material. In this sense, few molecular studies of the associated microbial diversity to table olives has been development through New Generation Sequencing (NGS) methods (Arroyo-López et al., 2016; Medina, E et al., 2016; De Angelis et al., 2015; Cocilano et al., 2013). NGS such as Metagenomic techniques provide a wide information about the whole biodiversity of an environmental sample without the necessity of a culture media. Is this lack of information which has led to focus this research to the metagenomic study of the microbiota (bacteria and yeast) present in the main ingredients and seasoning material (Figure 1) used in table olive elaboration.

The use of this methodology open a new door to the knowledge of the microbiota associated to table olive processing, limited until the moment by the lack of specific culture media in mostly cases.

MATERIALS AND METHODS

To carry out this study, were obtained different ingredients commonly used in the packaging of green table olives. 5 types of samples were analyzed; fennel, thymus, garlic, red pepper and salt. The first three samples are ingredients used in the elaboration of “Aloreña de Málaga” table olives, having been provided directly from producing industries. The salt sample were obtained from saltworks of the Atlantic coast of Cádiz (Spain), and is used by the industry to prepare fermentation and packaged brines.

B) Sequencing and Analysis of the data

For the microbial ecology study, DNA was extracted according to the instructions in the MoBio PowerFood DNA Isolation Kit. Hypervariable regions of the 16S rDNA were amplified by Polymerase chain reaction (PCR) from total bacterial using the 8F (5’-AGTTTGATCCTGGTCAG-3’) and 357R (5’-CTCCTCGATCTTCCTCGACT-3’) primers. The case of fungal DNA was developed the analysis of the ITS region. The primers used were ITS1F (5’-CTGGTCATTAGAGGAAGTAA-3’) and ITS4 (5’-CTTGGATCCTTATTGATATGC-3’) that specifically amplifies fungal sequences linked to universal 18S rDNA forward (5’-GTTG GAAAACACGGGCGGCACTG-3’) sequence and the ITS4 (5’-TCCTCGATCTTCCTCGACT-3’) primer linked to universal 18S rDNA reverse (5’-CAGCAGGAAAGATCATGACC-3’) sequence (M13-F3T74 and M13-R7T31). Leader sequences and barcodes were designed according to the instructions for 454 sequencing. Data files were processed with QIIME (http://qiime.org/index.html). Operational taxonomic units (OTUs) were identified by sequence similarity among the reads. The identity for each OTU was determined using the SILVA database (RELEASE 108) at the default 97% identity level in the case of the bacterial ecology and UNITE database for fungal ecology.

CONCLUSIONS

The study has revealed the presence of a very high diversity in both bacterial and fungal population, many of them unknown until the moment in this kind of samples. In this way, NGS studies provide information which is not possible to obtain with other techniques. Further studies are necessary to understand how these genres could affect to the safety and processing of table olives.

REFERENCES


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