Talk

Isolation and identification of acetic acid bacteria in artisan sourdough



Garrido Romero, Manuel(1*), Floriano Pardal, Belén (1), Garzón Villar, Andrés (1) (1) Molecular Biology and Chemical Engineering Department, Experimental Sciences Faculty, Pablo de Olavide University of Seville, Ctra. Utrera km 1. 41013, Sevilla, Spain. Tutor académico: Belén Floriano Pardal y Andrés Garzón Villar

Keywords: acetic acid bacteria; sourdough; bakery

ABSTRACT

Motivation: In recent years the production of bakery products with sourdough is increasingly successful due to consumer demand for more natural, tasty and healthy products (1). Sourdoughs are a mixture of flour and water in which a very complex biological ecosystem is developed, consisting mainly of lactic acid bacteria and yeasts. However, in the last decade, it has been discovered that acetic acid bacteria are also part of this microbial consortium, although there is little information on their importance in the development of the sourdoughs and their role in the final bakery product (2,3). For this reason, this project aims to isolate and identify acetic acid bacteria from bakery sourdoughs from Andalusian artisan bakeries with the aim of studying the genera and species that comprise it.

Methods: To isolate acetic acid bacteria from sourdough a culture-dependent microbiological analysis was performed from a sample of artisan sourdough. Appropriate dilutions were plated in two different culture media and grown at 30°C for 72 hours under aerobic conditions. Cell concentration was calculated as CFU/g of sourdough. The morphology of the colonies was studied by a Gram stain and a catalase assay was performed. For those Gram-negative and catalase positive, a molecular identification was carried out, extracting the DNA according to the protocol described by Cold Spring Harbor Laboratory (4). A REP-PCR was performed with oligo (GTG)5 (5) to group the candidate bacteria according to their pattern of bands and, subsequently, a 1,5kb fragment of the gene encoding the 16S rRNA was amplified with the oligonucleotides f27 and pH (6) for a pair of candidates of each pattern and sent to an external company for sequencing.

Results and conclusions: It has been proven that the most appropriate culture medium for the isolation of acetic acid bacteria is the one that contains ethanol, acetic acid, an antibiotic and an antifungal in its composition. The concentration of acetic acid bacteria obtained in a solid bakery sourdough was $1,16 \cdot 10^{4}$ CFU/g. The colonies obtained have been found to be catalase positive and Gram-negative and a REP-PCR was performed, managing to group the candidates into at least 5 different groups according to their pattern of bands. The poster will present the results of the identification by sequencing of a couple of candidates from each group, with the aim of studying which genera of acetic acid bacteria are present in the studied sourdough.

REFERENCES

- 1. Comasio A, Verce M, Van Kerrebroeck S, De Vuyst L. Diverse Microbial Composition of Sourdoughs from Different Origins. Front Microbiol. 2020;11.
- Chavan RS, Chavan SR. Sourdough Technology-A Traditional Way for Wholesome Foods: A Review. Compr Rev Food Sci Food Saf. 2011;10(3):169– 82.
- 3. Minervini F, Lattanzi A, De Angelis M, Di Cagno R, Gobbetti M. Influence of artisan bakery- or laboratory-propagated sourdoughs on the diversity of lactic acid bacterium and yeast microbiolas. Appl Environ Microbiol. 2012;78(15):5328–40.
- 4. https://dnabarcoding101.org/lab/protocol-2.html
- 5. Papalexandratou Z, Cleenwerck I, De Vos P, De Vuyst L. (GTG)5-PCR reference framework for acetic acid bacteria. FEMS Microbiol Lett. 2009;301(1):44-9.
- 6. Chakravorty S, Sarkar S, Gachhui R. Identification of new conserved and variable regions in the 16S rRNA gene of acetic acid bacteria and acetobacteraceae family. Mol Biol. 2015;49(5):668–77.

1