



ISOLATION AND IDENTIFICATION OF HALOPHILIC BACTERIA OBTAINED FROM RAW SALT

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Key words: salt, PCR-DGGE, halophilic bacteria

Introduction. Halophilic bacteria have developed various strategies to maintain their biochemical structure and cell function under high osmotic pressure environments (1). Studies of the bacterial microbiota of Cotija cheese -an artisanal dairy product that is manufactured in Mexico- and of other traditional fermented foods in the world, had showed that salt is a potential source of microorganisms involved in their fermentation (2).

The aim of this work was the isolation and identification of halophilic bacteria from the artisanal raw salt used in Cotija cheese manufacture (SCC) and to compare it with the one isolated from a commercial raw salt (SMC) from Sonora, by means of culture and molecular methods.

Methods. Two salt samples were used: an artisanal raw one from Coyutlán, Colima (SCC) and a commercial iodized one from Obregón, Sonora (SMC). Each salt sample was enriched in four different selective media for halophilic bacteria (3, 4). Seven CFU from each culture media were randomly selected, from which DNA was extracted. PCR and denaturing gradient gel electrophoresis (PCR-DGGE) were used for the amplification of V3 16S rDNA region (5). Some bands of each sample were excised and sequenced. The sequences were compared by BLAST and the identity was confirmed by the Neighbor-Joining method with bootstrap of 1,000 replicas.

Results. Microscopic morphology of all isolated UFCs was Gram-positive rods and their colonies had a similar morphology in the culture media used. In figure 1, an example of amplicon migration in DGGE from both salt samples enriched in one culture media is shown. Microheterogeneity of the V3 16S rDNA was observed for all isolated microorganisms. The following bacteria were found in both salt samples: *Bacillus* sp., and *Virgibacillus dokdonensis*. The latter was found in all culture media. *Bacillus flexus* was exclusive in the SCC salt sample and it was found only in one of the tested culture media, while *Bacillus licheniformis*, *Virgibacillus halodentrificans* and *Bacillus megaterium* were exclusive of SMC salt, and they were found in different culture media. Although common species were

observed in both salt samples, their DGGE profile was different between samples but similar among individuals of the same salt sample; thus, they belong to different strains.

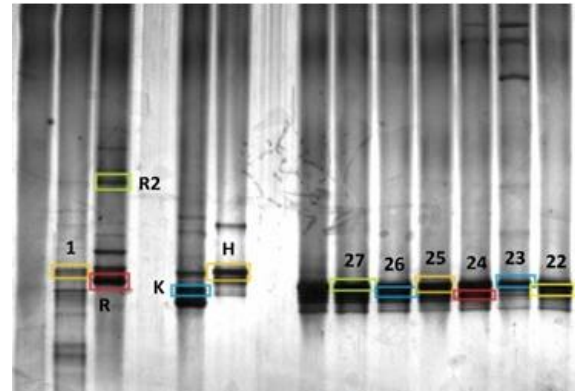


Fig. 1. Amplicon migration profile in DGGE using V3 16S rDNA as target gene. Samples, numbers are from artisanal salt from Coyutlán, Colima (SCC) and capital letters are from commercial iodized salt from Sonora (SMC).

The DGGE profile found in *Bacillus* sp, from the Colima salt sample was similar to the one observed in *Bacillus* strains isolated from Cotija cheese. Thus salt could be its source of inoculation.

Conclusions. Cultured salt microbiota, regardless its origin, was composed by sporulated microorganisms, mainly from the genus *Bacillus*. Although different culture media were used in order to provide different nutrients to support various nutritional needs, the same bacterial genera were capable to grow in the media used.

Acknowledgements. PAPIIT IN230511.

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