



MARINE OBLIGATE ACTINOBACTERIA FROM A SEDIMENT SAMPLE IN MEXICO AND USE OF GENETICS TOOLS FOR BIODIVERSITY

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Introduction. Actinobacteria are widely distributed in soil, water and other natural environments. However, their diversity and ecology in marine habitats is still poorly understood. The genus *Salinispora* (1,2) is a marine obligate organism which within the *Actinobacteria* that was the first to be properly described with such unique feature (2). Members of this genus (3 species) are distributed around the world and have been isolated particularly from tropical sediments (1, 3). Interestingly, *Salinispora* species have shown to produce bioactive unique compounds.

Methods. This work focuses on the isolation of marine obligate actinobacteria, from a sub-tropical sediment collected at 10 meters depth in Bahía de los Santos, Baja California Sur, Mexico. The isolation procedure consisted in serial dilutions from wet marine sediment (10^{-1} to 10^{-4}), two different media (GYM and GYEA; Glucose-Yeast-Malt-Extract and Glucose-Yeast-Extract, respectively) and two different dilutions (1:10 and 1:100) from each media. All media were supplemented with Rifampicin and Nystatin. One hundred milliliters from each sediment-dilution were distributed onto the surface of Petri dishes and incubated at 30° for 4 weeks and checked by eye until colonies resembling actinobacteria were observed.

Results. Seventy two isolates were selected from the Petri dishes. Sixty-five isolates (90.3%) resemble the typical morphological features of members of the family Micromonosporaceae, namely, the genera *Micromonospora*, *Verrucosipora* and *Salinispora*. Sixty actinobacterial isolates (92.3%) required marine water for growth (Figure 1). Five (7.7%) isolates are non halophilic bacteria. The isolates showed a positive PCR reaction when using a set of specific actinobacterial primers and both family and genus specific primers. Partial sequencing of their corresponding 16S rRNA gene confirmed that all of

them belong to the class *Actinobacteria* and some of them to the genus *Salinispora*. Genetic fingerprinting tools (ie. REP-PCR and AFLP) confirmed the intrinsic diversity of the isolates as they generated unique fingerprinting patterns (data not shown).

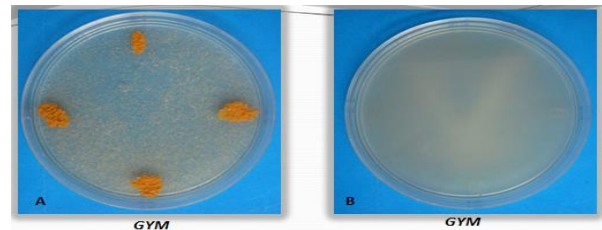


Fig. 1. Marine actinobacteria. A) four isolates growing in GYM medium prepared with marine water and B) without. Plates incubated at 30°C for 2 weeks.

Conclusions. A procedure was developed for the selective isolation of marine obligate actinobacteria from marine sediments and the use of genetic tools suggested they could well represent novel species. Genetic fingerprinting tools are of use when high numbers of morphologically similar microorganisms are isolated.

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