ANALYSIS OF UNCULTURED MICROBIAL DIVERSITY IN A SUGAR CANE SOIL DURING EARLY STAGES OF THE PRODUCTION CYCLE

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Introduction. The cultivation of sugar cane is preponderant and important in the economic and social activity in Mexico. Despite its importance, there are no reports that consider the effect of agricultural practices employed on bacterial diversity associated with the soils used for cultivation**.** Molecular techniques such as denaturing gradient gel electrophoresis (DGGE) has been used to explore non-cultivable bacterial diversity present in environmental samples such as soil by separation of conserved/variable regions of 16S rDNA from bacterial origin, and has resulted in detection of a remarkable bacterial diversity in analyzed samples (1).

The purpose of this study is to determine the fluctuation of non-cultivable bacterial diversity and its overall metabolic profile in soil used for cultivation of sugar cane, during the first four stages of the production cycle and the implications of agricultural practices carried out on bacterial diversity.

Methods. Soil samples were collected from a sugar cane cultivation zone located in the Tlalquitenango Municipality, Morelos State, Mexico, during: soil plow [T0]; after planting sugar cane and watering [T1]; after herbicide (GESAPAX H-375 EC, Syngenta) application [T2]; after fertilizing (sugarcane formulation 18:4.5:3) [T3]. Total bacterial DNA was extracted from each soil sample using the PowerMix Soil DNA Isolation kit (MoBio) according to the instructions of manufacturer. Variable (V6-V8) region of bacterial 16S rRNA genes present in extracted soil bacteria DNA were PCR amplified and DGGE analyzed as described previously. (2)

Selected bands were excised from acrylamide gel and purified using the Ultrafree-DA columns kit (Millipore Corp.) and sequenced using the primer UNI1401 Rv (2). The obtained sequences were compared against to the non-redundant GenBank database by using the BLAST on-line application, in order to identify submitted sequences.

Results. Figure 1 shows that DGGE profiling of 16S rRNA variable region V6-V8 amplified from total bacterial DNA extracted from soil samples T0 – T3, is relatively constant among samples and replicates, indicating that non-cultivated bacterial diversity present originally in plowed soil was no affected after planting, watering, herbicide treatment and fertilizing.

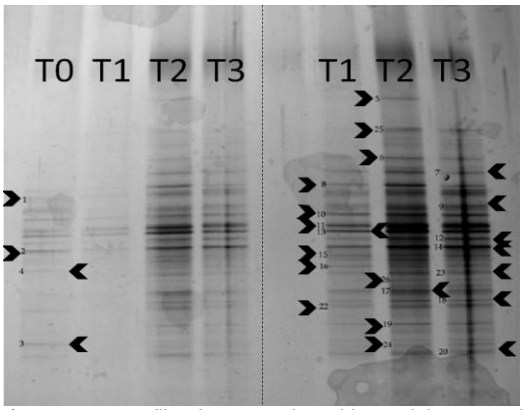

Fig.1 DGGE profiles from uncultured bacterial community of soil sample for sugar cane crop during four stages of cultivation. Selected bands are marked in arrows.

Identification of selected bands observed in DGGE profiling by sequence comparison allowed to identify mainly uncultured soil bacteria. Interestingly, this result contrasts with changes in cultivable bacterial diversity previously observed (3).

Conclusions.

The non-cultivable bacterial diversity present in soil sample used for sugar cane production was not affected during early agricultural practices including planting, watering, herbicide, and fertilizing treatment. Identification of selected DGGE bands by sequence comparison allowed identifying non-cultivable bacteria.

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