

TITLE: BACTERIAL DIVERSITY IN WET PROCESSED COFFEE (*Coffea arabica*) FERMENTATION IDENTIFIED BY PHENOTYPIC, GENETIC METHODS AND MALDI-TOF (MS) TECHNIQUES

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Epiphytic microorganisms naturally present in coffee cherries play an important role during fermentation and drying due to consumption of the pulp and mucilage around the fruit and production of compounds that will directly affect the coffee quality. Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) is a technique that can be used to identify microorganisms. The objective of this work was to evaluate the bacterial diversity of the three varieties of coffee, Mundo Novo, Ouro Amarelo, and Catuaí Vermelho during spontaneous wet fermentation. Mesophilic bacteria were enumerated and isolated by spread plating on Nutrient Agar (30 °C) and lactic acid bacteria on MRS agar (35 °C) added nystatin to inhibit growth of yeasts and filamentous fungi. The morphological and phenotypic characteristics of the colonies were recorded. Protein extraction from 321 isolates were made with organic solution (water/acetonitrile/trifluoro-acetic acid) and 1 µl matrix solution [α -cyano-4-hydroxycinnamic acid (CHCA) in organic solution] was added and gently mixed in 96-well MALDI flex target plate. Representative strains from each cluster performed by MALDI-TOF were submitted to molecular analyses. The amplification of the 16S rRNA gene used the primers 27F and 1512R. A total of 42 bacterial species were identified, being 36 mesophilic and 6 lactic acid bacteria. Mesophilic bacteria started with (4.84 - 4.10 CFU/g), however decreased throughout the process until reach values of 2.48 - 2.78 CFU/g. Similar behavior was observed with lactic acid bacteria, however, the population was highest than mesophilic (6.15 - 3.84 CFU/g). The population of mesophilic and lactic acid bacteria was different in each coffee variety. Most of the population values declined until dried coffee. During fermentation, the dominant species of mesophilic bacteria were *Bacillus cereus*, *B. subtilis*, *B. megaterium*, *Cellulosimicrobium cellulans*, *Enterobacter cloacae* and *Pantoea dispersa*. Lactic acid bacteria as *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were often found in all varieties. The bacteriological differences in three coffee varieties (Ouro Amarelo, Mundo Novo and Catuaí Vermelho) fermentations provided useful information about the possible starter cultures to be used in coffee wet processing.

Keywords: coffee varieties, fermentation, bacteria, MALDI-TOF, sequencing.

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