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Identification of Lactic Acid Bacteria (LAB) in fruits using Matrix-Assisted Laser Desorption Ionization–Time Of Flight Mass Spectrometry (MALDI-TOF MS)

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Statement of the Problem: Matrix-Assisted Laser Desorption Ionization – Time Of Flight Mass Spectrometry (MALDI-TOF MS) can be a replacement for routine 16S rRNA gene sequencing for identification of organisms because it is a fast technique and low cost. This study aimed to isolate and then identify LAB in fruits using MALDI-TOF MS.

Methodology & Theoretical Orientation: Samples of orange (*Citrus sinensis* (L.), grape (*Vitis vinifera* L.), apple (Malus domestica Borkh) and banana (*Musa* spp.) obtained from João Pessoa (Paraíba, Brazil). The samples were diluted, inoculated in MRS agar and incubated at 37° C/48 hr. At least five colonies presenting different morphologies were randomly isolated from plates of each type of fruit and submitted to analyze. Isolates presumptively identified as LAB (catalase negative, gram-positive cocci or rods) were stored at -20°C. Initially, 1 µL of protein extract was deposited in four wells of the sample plate and overlaid with 1 µL of a saturated alpha-cyano-4-hydroxycinnamic acid solution in acetonitrile: water:Tri-Fluoroacetic Acid (TFA). The MALDI-TOF mass spectra measurements of samples were performed using a Bruker Biotyper 3.1. The identification results were expressed by BioTyper log (scores): ≥2.0 is considered accurate species-level identification; ≥1.7-2.0, a probable genus-level identification; and an isolate with a score <1.7 is considered to be unidentified.

Findings: A total of 44 isolates of LAB were identified. Species belonging to *Enterococcus* genus were the most predominant (32/44 isolates; 73%) identified and the other isolates identified belonged to *Lactobacillus* genus (12/44; 27%). *Lactobacillus* are isolated in orange (*L. brevis, L. plantarum, L. paraplantarum*), banana (*L. plantarum*) and grape (*L. brevis and L. plantarum*). *Enterococcus* is isolated from apple (*E. faecium and E. mundtii*) and grape (*E. faecium*). From the identification of LABs isolated from fruits using the MALDI-TOF technique the selection of strains for future studies is possible.

Recent Publications:

- Garcia E F, D E Oliveira Araújo A, Luciano W A, De Albuquerque T M R, De Oliveira Arcanjo N M, Madruga M S, Dos Santos Lima M, Magnani M, Saarela M and de Souza E L (2018) The performance of five fruit-derived and freeze-dried potentially probiotic *Lactobacillus* strains in apple, orange and grape juices. Journal of the Science of Food and Agriculture 98(13):5000-5010.
- 2. Da Costa W K A, De Souza G T, Brandão L R, De Lima R C, Garcia E F, Dos Santos Lima M, De Souza E L, Saarela M and Magnani M (2018). Exploiting antagonistic activity of fruit-derived *Lactobacillus* to control pathogenic bacteria in fresh cheese and chicken meat. Food Research International DOI:10.1016/j.foodres.2018.03.045.
- Luciano W A, Matte T C, Portela I A, Medeiros L L, Dos Santos Lima M, Maciel J F, De Souza E L, Garcia E F and Magnani M (2018) Effects of *Lactobacillus acidophilus* LA-3 on physicochemical and sensory parameters of açaí and mango based smoothies and its survival following simulated gastrointestinal conditions. Food Research International 114:159–168.
- 4. Albuquerque T M R, Garcia E F, Araújo A O, Magnani M, Saarela M and De Souza E L (2017) *In vitro* characterization of *Lactobacillus* strains isolated from fruit processing byproducts as potential probiotics. Probiotics and Antimicrobial Proteins 1:1-13.
- 5. Garcia E F, Luciano W A, Xavier D E, Da Costa W C A, De Sousa Oliveira K, Franco O L, De Morais Júnior M A, Lucena B T L, Picão R C, Magnani M, Saarela M and De Souza E L (2016) Identification of lactic acid bacteria in fruit pulp processing byproducts and potential probiotic properties of selected *Lactobacillus* strains. Frontiers in Microbiology 7:1371.

Biography

Estefania F Garcia has experience in the development of fermented foods and isolation of lactic bacteria to study probiotic potential and application in fermented foods. She has developed studies with isolation of bacteria from fruits and by-products of fruits and insertion in juices, smoothies, breads and cheese, beside evaluation of the quality these products. She recently initiated the study of freeze-dry of lactic bacteria for commercial insertion in foods.

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