

Isolation of lactic acid bacteria and *Bacillus* spp. from tropical fruits and screening for safe candidates for exploring their beneficial properties

Emma Marie Faucher^{1,2}, Giselle Santos Silva¹, Katia Leani¹, Svetoslav Dimitrov Todorov^{1,3}

¹. ProBacLab, Universidade de São Paulo, São Paulo 05508-000, SP, Brazil;

². AMU, Aix-Marseille University, Marseille 13006, France;

³. CISAS, Instituto Politécnico de Viana do Castelo, 4900-347 Viana do Castelo, Portugal;

Lactic acid bacteria (LAB) play a crucial role in the preservation and enhancement of fruits, contributing significantly to food safety, nutritional value, and sensory properties. These beneficial microorganisms are widely recognized for their ability to ferment sugars into lactic acid, which lowers the pH and creates an environment that inhibits the growth of spoilage organisms and pathogens. This natural preservation method extends the shelf life of fruits and fruit products, reducing food waste and ensuring a safer food supply. Moreover, in addition to the preservation properties, LAB also enhance the nutritional profile of fruits by producing bioactive compounds such as vitamins, antioxidants, and antimicrobial peptides. These compounds can improve the health benefits of fruits, making them more appealing to health-conscious consumers. Some LAB can be actively involved in the reduce the content of anti-nutritional factors and allergens in fruits, further enhancing their nutritional value and even can be potential probiotic candidates.

The Brazilian biome is a rich and diverse source of tropical fruits, many of which are little known outside Brazil and Latin America. These fruits are not only appreciated for their gastronomic characteristics but are also used in traditional medicine for their bioactive properties. Exploring the microbiota of these fruits may reveal new strains of bacteriocin-producing bacteria, which have promising therapeutic applications. The project aims to isolate and identify new strains of LAB and *Bacillus* spp. from tropical fruits, evaluate their safety and screen for beneficial properties with aim to be further investigated as putative probiotics.

More than 260 isolates were obtained from banana, Brazilian cheery, lemon, *pitanga*, clementine, pineapple, guava, *atemoya*, *caju* collected in the area of Sao Paulo, SP, Brazil. All fruits were collected with minimal manipulation interference and further macerated in proportion 1:9 with sterile saline. A serial dilutions were prepared and plated on MRS agar and plates incubated for 24-48h in aerobic conditions. Colonies with morphology typical for LAB and *Bacillus* spp. were isolated for further investigation. Morphology was observed after Gram staining and catalase test were performed. Preselection of isolates of interest included tests for hemolytic activity, where 38% were included for further study as β -hemolytic. Isolates were tested for resistance/susceptibility to antibiotics, recommended by EFSA (ampicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, streptomycin, tetracycline and vancomycin) and results served to selected non multidrug resistance isolates. In addition, mucin degradation and lipolytic activity were served as criterium for selection of safe strains. Only 54 from 260 isolated, preselected were subject to differentiated by repPCR and further identified according 16S rRNA partial gene sequencing. In addition to LAB (*Lactiplantibacillus plantarum*, *Levilactobacillus brevis* and *Streptococcus thermophilus*) and *Bacillus* spp. (*Bacillus licheniformis*, *Bacillus aerius* and *Bacillus paralicheniformis*), *Staphylococcus epidermidis*, *Staphylococcus partial*, *Staphylococcus hominis*, *Escherichia coli*, *Escherichia alberti*, *Listeria monocytogenes*, *Kosokinia cowanii* and *Enterobacter cloaca* were identified, a fact that is rising concerns regarding safety of the direct consumption of the not hygienezed fruits.

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