UNIVERSITAT POLITÈCNICA DE VALÈNCIA

V Encuentro de Estudiantes de Doctorado

IIAD

In vitro mucin adhesion assay of *L. salivarius* spp. *salivarius* CECT 4063, inoculated into clementine juice

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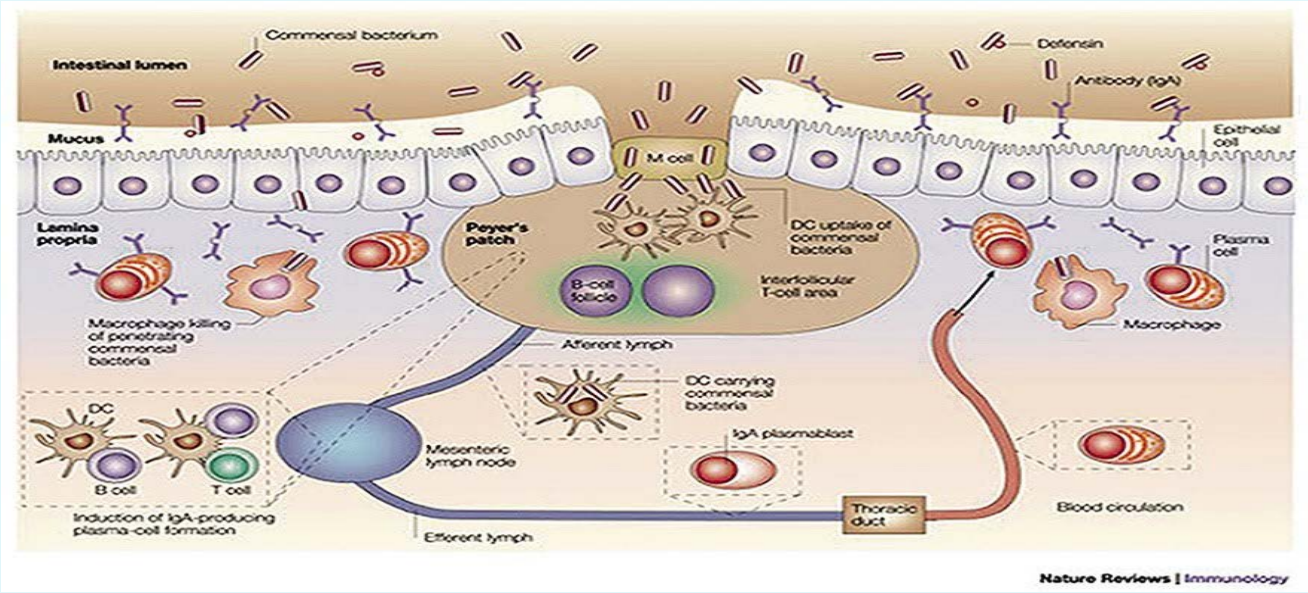
INTRODUCTION

Probiotics are living microorganisms that provide a benefit to the host health. One of the main criteria for selecting probiotic strains is their ability to adhere to intestinal surfaces.



OBJECTIVE

To evaluate *L. salivarius* spp. *salivarius* (CECT 4063) ability to adhere to mucin as affected by its growing in clementine juice, the addition of 10% by weight of trehalose to the juice formulation and/or the juice homogenization at 100 MPa.

MAIN STAGES OF RESEARCH DEVELOPMENT

ST1

- Preparation of juice samples containing *Lactobacillus salivarius* spp. *salivarius* (CECT 4063): with and without 10% by weight of trehalose and non homogenized and homogenized at 100 MPa.

ST2

- 2 steps centrifugation of fermented juices to eliminate the pulp without negatively affect the microbial counts: at 1000 rpm for 10 min. at 4 ºC and then at 3000 rpm for 10 min. at 4 ºC.

ST3

- Preparation of black multi-well polystyrene plate with protein solutions: mucin (0.5 mg/mL), collagen (0.05 mg/mL) and BSA (0.5 mg/mL) and left in incubation overnight. Wash the rest of the protein not adhered with PBS 1X.

ST4

- Centrifugation of the juice supernatants, washing the precipitated cells and adjustment of the absorbance at 600 nm to 10^7-10^8 CFU/mL.

ST5

- Adhesion of the bacteria labeled with cFDA to the multi-well plate with proteins and incubation 1 h at 37 ºC. Washing of non-adherent bacteria with PBS 1X. Reading the fluorescent signal of the bacteria adhered to the mucin with a Clariostar fluorescence equipment at 485 nm and 538 emission.

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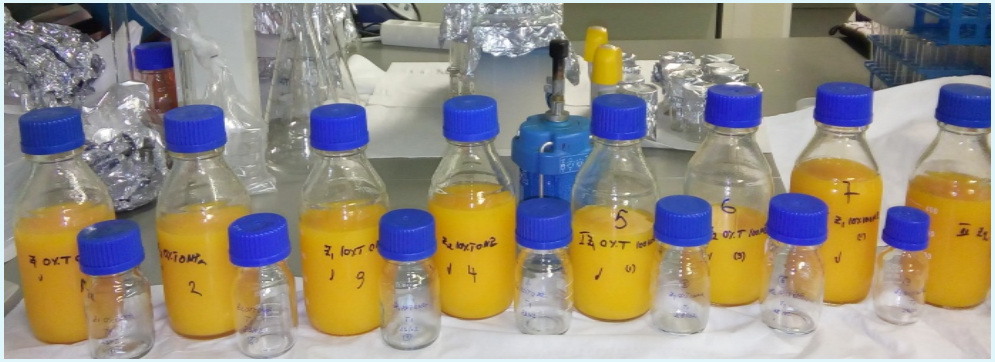

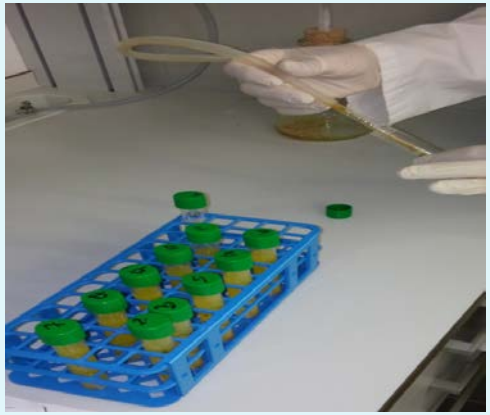

ST2: 2 steps centrifugation of fermented juices to eliminate the pulp without negatively affect the microbial counts: at 1000 rpm for 10 min. at 4 ºC and then at 3000 rpm for 10 min. at 4 ºC.

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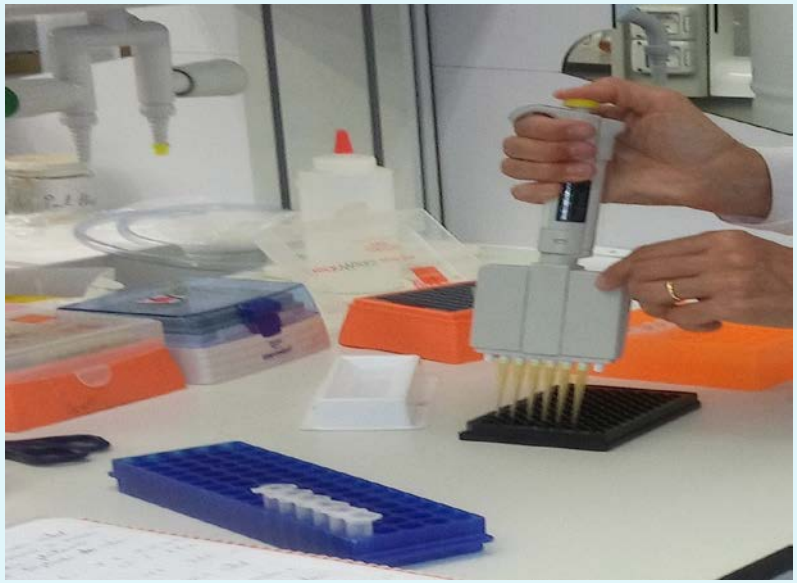
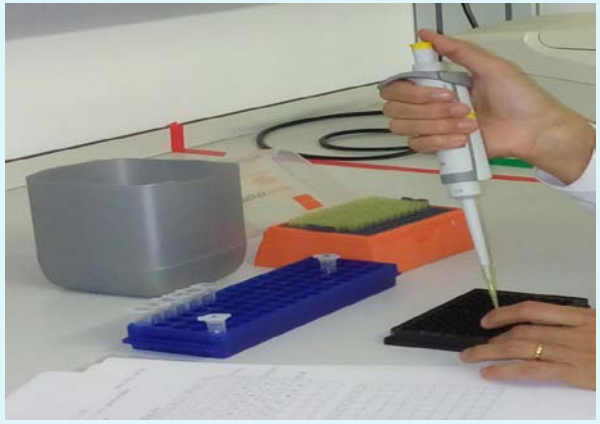
ST3: Preparation of black multi-well polystyrene plate with protein solutions: mucin (0.5 mg/mL), collagen (0.05 mg/mL) and BSA (0.5 mg/mL) and left in incubation overnight. Wash the rest of the protein not adhered with PBS 1X.



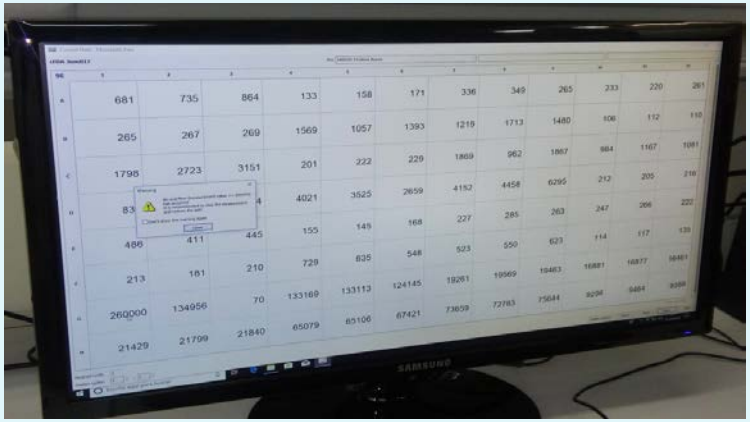
MAIN STAGES OF RESEARCH DEVELOPMENT

ST4: Centrifugation of the juice supernatants, washing the precipitated cells and adjustment of the absorbance at 600 nm to 10^7–10^8 CFU/mL. Mark these bacteria with 20 μL cFDA and leave 1 hour in darkness.



MAIN STAGES OF RESEARCH DEVELOPMENT

ST5: Adhesion of the bacteria labeled with cFDA to the multi-well plate with proteins and incubation 1 h at 37 ºC. Washing of non-adherent bacteria with PBS 1X. Reading the fluorescent signal of the bacteria adhered to the mucin with a Clariostar fluorescence equipment at 485 nm and 538 emission.



EXPECTED RESULTS

***in vitro* adhesion assay showed:**

- on one hand, that clementine juice is a very good food matrix that improves the adhesion of *Lactobacillus salivarius* to mucin and collagen, in comparison to the MRS specific growing media.
- on the other hand, among the variables considered, trehalose addition to the juice formulation was the only improving the *Lactobacillus salivarius* adhesion to mucin and collagen.
- finally, the adhesion of probiotic cells to BSA was lower since this is a protein of bovine origin.

This experimental part was carried out in collaboration with the **Institute of Agrochemistry and Food Tech**nology within the **Laboratory of Lactic and Probiotic Bacteria** and under the supervision of the researcher **PhD Mari Carmen Collado**, to whom I would like to specially thank !

THANK YOU VERY MUCH

FOR YOUR ATTENTION !