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Fermented foods, from microbes to functionality

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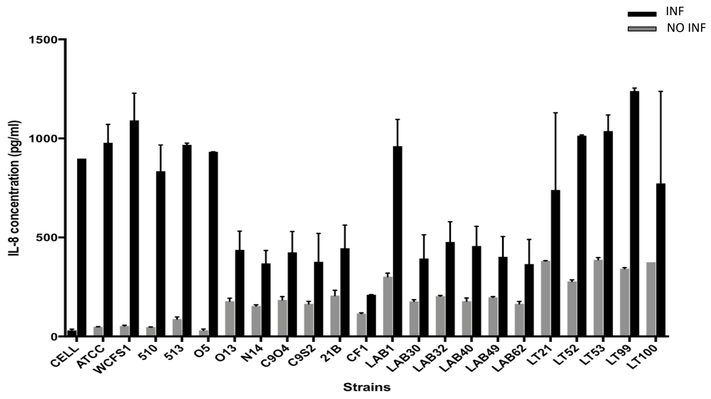
INTRODUCTION

Based on the guidelines of the International Scientific Association for Probiotics and Prebiotics, fermented food-related microbes can be defined as "active" strains since they have some probiotics characteristics exerting beneficial effects on the host (Hill et al., 2014). To provide health benefits, foodborne microbes must reach the gut as viable cells, survive there, persist in the gut and interact with the intestinal epithelium to be effective (David et al., 2014). *Lactobacillus plantarum* has been deeply investigated due to their technological properties as well as probiotic characteristics: they are non-pathogenic, classified as GRAS, suitable for industrial process, acid tolerant and with antimicrobial features. Moreover, it has recently been clarified that food-associated microbes share genetic and physiological traits with probiotic strains (Marco et al., 2017).

In this perspective, a set of *in vitro* tests have been adopted to select potential probiotic microbes among 22 food-associated *Lb. plantarum* strains (Table 1) belonging to UNITE collection. This first approach allowed us a rapid and simple characterization of different strains simultaneously based on the current definition of probiotics: 1) ability to adhere to intestinal epithelium, 2) ability to hydrolyse bile salts by expressing *bsh* genes, 3) modulation of the host immune system and 4) ability to exert anti-genotoxic properties.

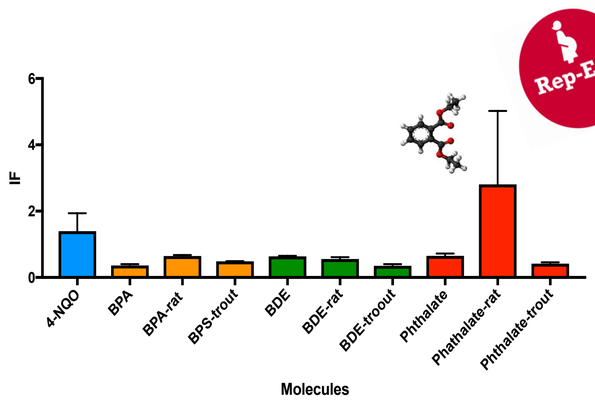
Immunomodulatory Activity

The potential probiotic of food-borne *Lb. plantarum* strains were also examined for their capability to exert an immunomodulatory effect on NCM460 cell line.
Release of cytokines were evaluated by ELISA assay after incubation of inflamed NCM460 cells with *Lb. plantarum* strains.



Quantification of **IL-8** release by NCM460 cells

Anti-genotoxic Activity



It has been demonstrated that food-borne *Lb. plantarum* strains could improve the food safety and quality by displaying antigenotoxic properties, degrading or modifying toxic components (Prete et al., 2017).

Genotoxicity of 3 different molecules (Bisphenol A, BDE99 and Diethyl phthalate) was evaluated using **SOS-CHROMOTEST** (Quillardet et al., 1985).

Genotoxicity, expressed through induction factor (IF), showed **Diethyl Phthalate** as the most genotoxic molecule.

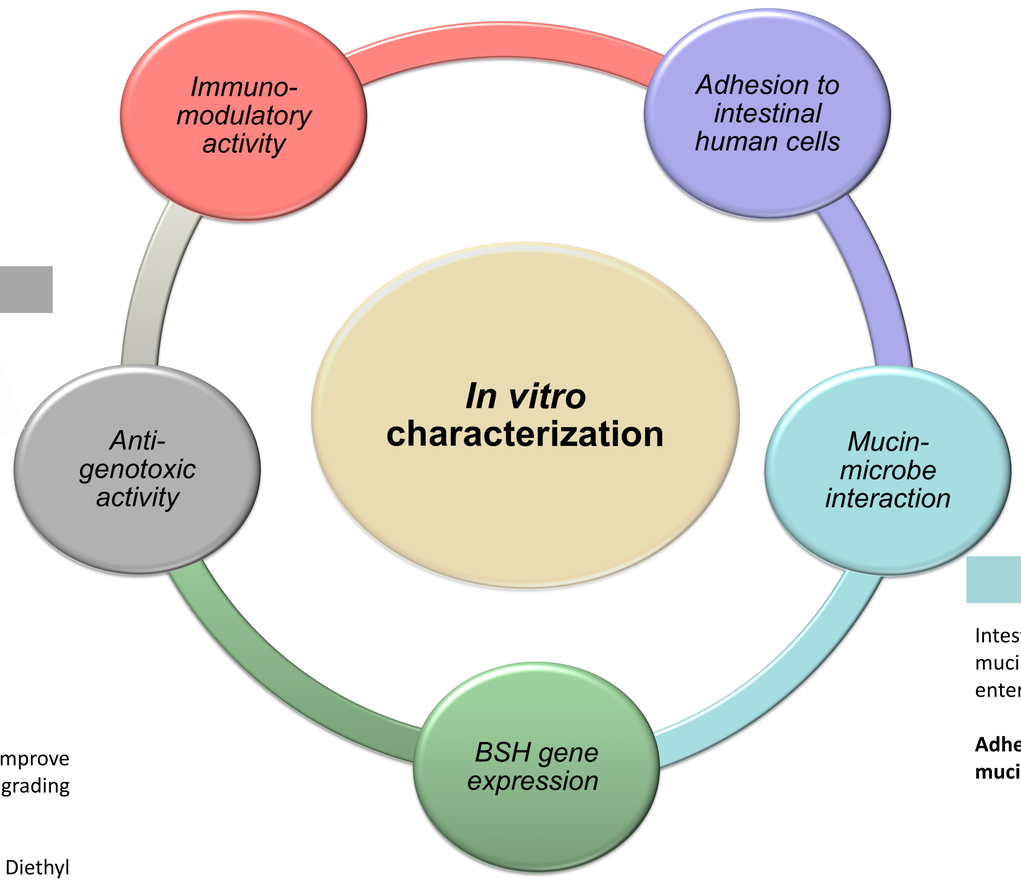
Overnight bacteria cultures were co-incubated with Luria-Bertani (LB) medium and LB supplemented with **0.5% bile extract porcine (type III)** for 90 min.

Bile Salt Hydrolase (BSH) function was evaluated through the expression of *bsh* genes: ***bsh*1, *bsh*2, *bsh*3 and *bsh*4**.

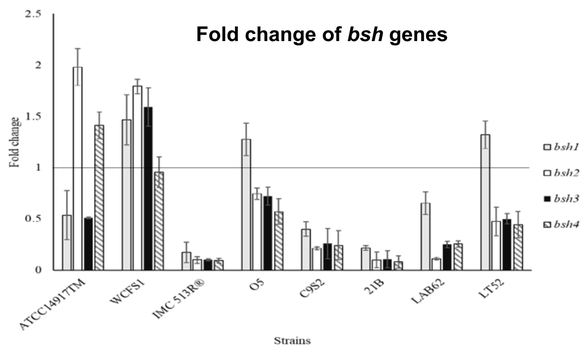
Results are expressed as mean of relative fold change ± SD , with bacteria grown in LB as reference. Data were normalized to the relative expression value of the housekeeping genes *ldhD* and *gyrA* in each respective sample.

Table 1. **Food-borne *L. plantarum* strains belonging to the UNITE collection**

strains	Origin
WCSF1	Human saliva
ATCC® 14917™	Pickled cabbage
IMC 510®	Synbiotec s.r.l.
IMC 513®	Synbiotec s.r.l.
C9O4/C9S2/N14/O13/O5	Table olives
21B/CF1	Sourdough
LT21/LT52/LT53	Raw-milk cheeses
LAB40/LAB49/LAB62	
LT99/LT100/LAB30	
LAB1/LAB32	



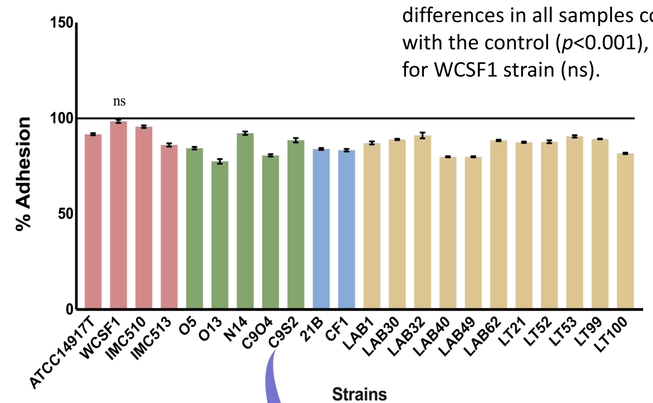
bsh Gene Expression

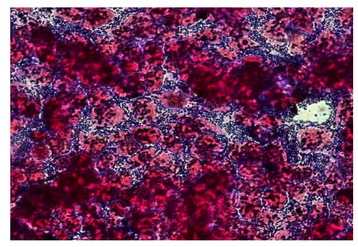


Adhesion to Intestinal Human Cells

Quantitively microbial adhesion ability was assessed by **plating serial dilutions on MRS agar plates**.

Results showed significant differences in all samples compared with the control (p<0.001), except for WCSF1 strain (ns).



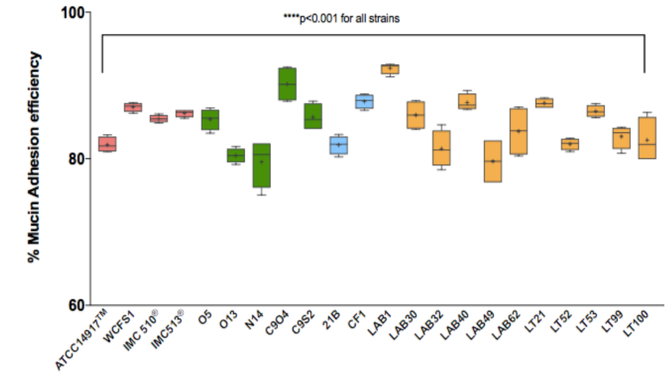


Qualitatively, adhesive strains to human cells were microscopically observed by using **GRAM stain** (Kotzamanidis et al., 2010).

Mucin-Microbe Interaction

Intestinal mucus is a dynamic matrix, normally transparent, composed of mucin glycoproteins sheets which covered the apical surfaces of intestinal enterocytes and is part of the human defense system (Li et al., 2015).

Adhesion to mucus has been performed quantifying adhered bacteria to **pig mucin type III** by plating serial dilutions on MRS plates (Tallon *et al.*, 2007).



Adhesion values are expressed as mean (bold horizontal bars) with min and max values (boxes) ± SD and are reported as percentage of adhesion compared with the control (100%). ANOVA Bonferroni's test showed significant differences (p<0.001) for all samples compared with the control.

RESEARCH QUESTIONS

- ✓ Could food-borne strains display the same properties than probiotics?
- ✓Are all probiotic features displayed at the same level among food-borne *Lb. plantarum* strains?
- ✓Could microbes in fermented foods may contribute to human health in a similar way to probiotics?

CONCLUSIONS

- ✓*Lb. plantarum* strains were able to adhere NCM460 cells and mucin, exert immunomodulatory properties and express *bsh* genes.
- ✓Intrinsic variability among the strains highlights the strain-dependent feature of all the activities evaluated.
- ✓Further *In vivo* studies will be carried out in order to confirm the probiotic features of the selected food-related *Lb. plantarum* strains.

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