

Host-microbe dialogue: evaluation of food-associated *Lactobacillus plantarum* adhesion property to human intestinal epithelium

N. Garcia-Gonzalez^{†1}, R. Prete^{†1}, N. Battista^{1*}, A. Corsetti^{1*}

¹ Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy.

[†]These authors contributed equally to this work

*nbattista@unite.it; *acorsetti@unite.it

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, food-associated 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). Here, we report the ability of *Lactobacillus plantarum* strains to adhere to both Normal-derived Colon Mucosal (NCM-460) cell line and mucus, by using qualitative and quantitative approaches.

Bacterial Culture

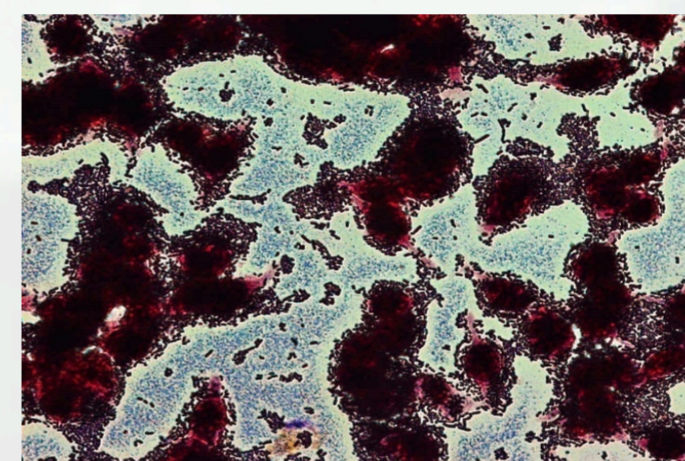
L. plantarum strains

Origin

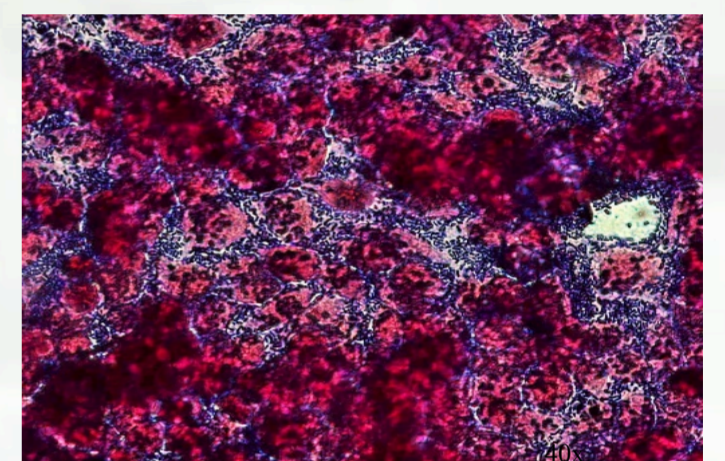
WCSF1	Human saliva
ATCC® 14917™	Pickled cabbage
IMC 510®	Synbiotec s.r.l.
IMC 513®	Synbiotec s.r.l.
C9O4/C9S2/N14/O13	Table olives
O5	
21B	Sourdough
CF1	
LT21/LT52/LT53	Raw-milk cheeses
LAB40/LAB49/LAB62	
LT100/LAB30	
LAB1/LAB32	
LT99	

Fermented food-related *L. plantarum* belong to UNITE collection and they were previously isolated and characterized till strain level.

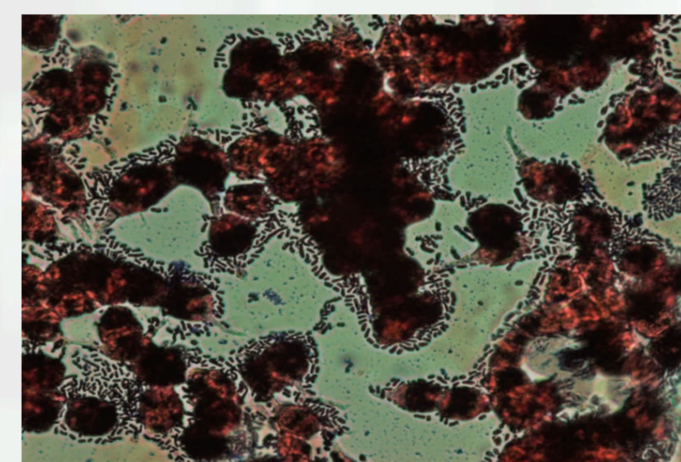
Adhesion to NCM-460



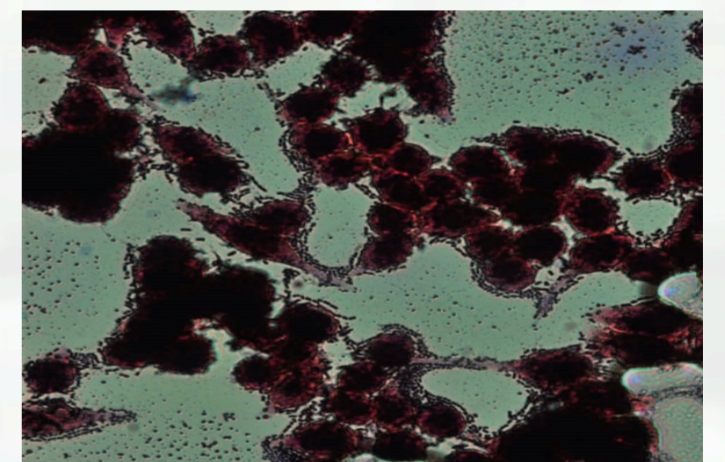
L. plantarum LAB40



L. plantarum C9S2



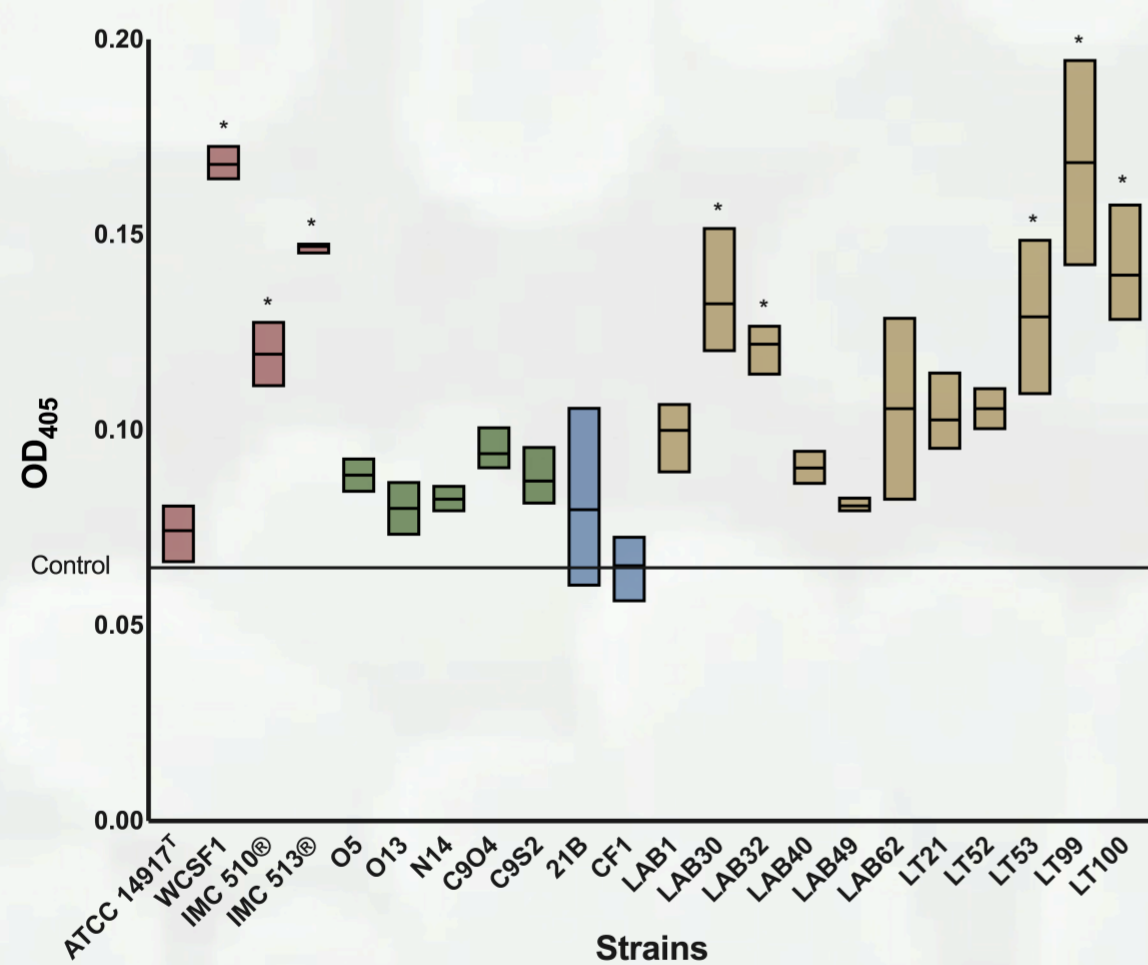
L. plantarum 21B



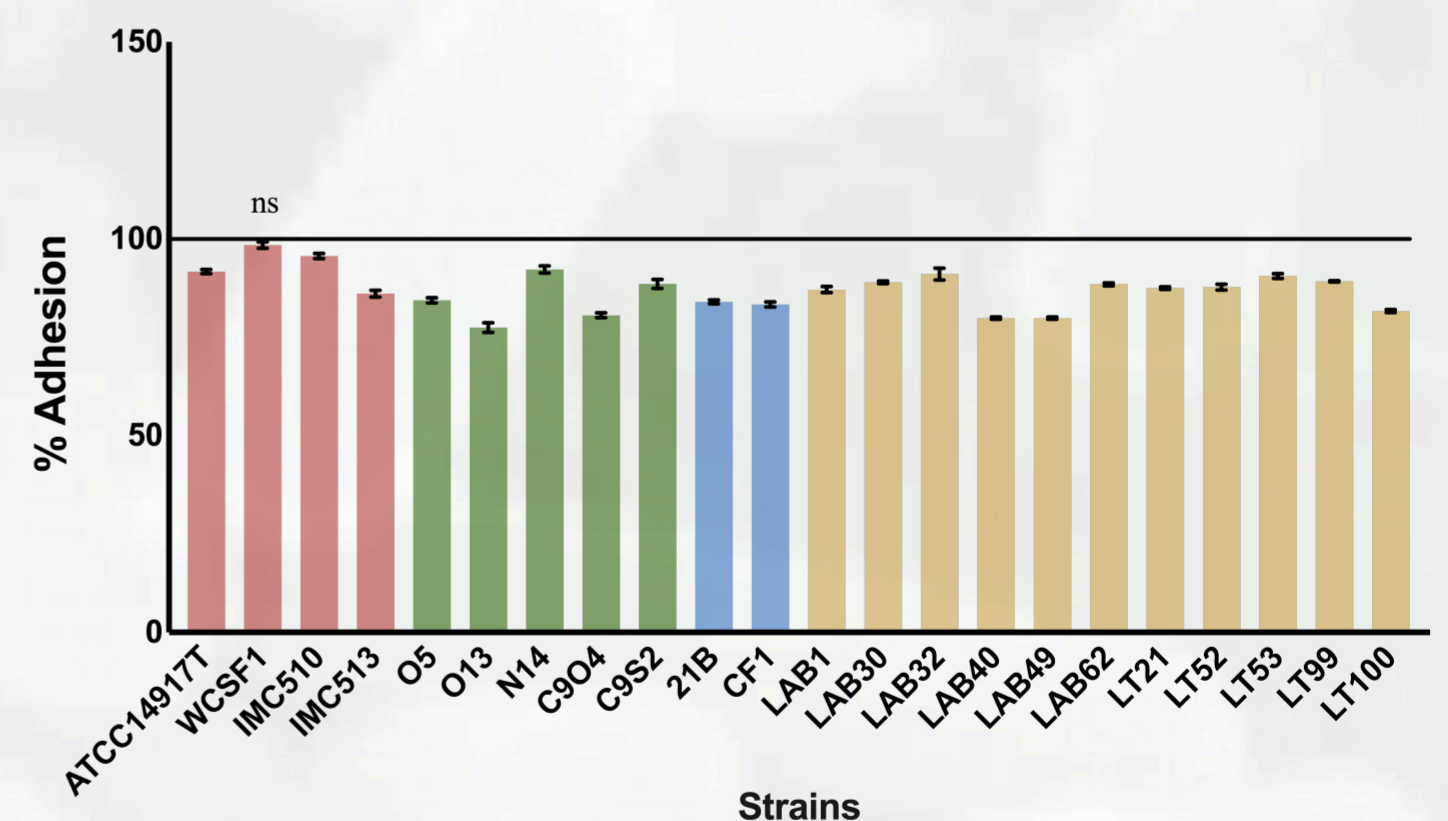
L. plantarum IMC510®

Qualitative adhesion assay was modified from Duary *et al.*, 2010. NCM460 cells and strains were incubated for 1.5 hours at 37 °C in a 5% CO₂ atmosphere. Lactobacilli strains were identified by GRAM staining. Original magnification x40.

Adhesion to mucus



Bacterial adherence to mucus was evaluated as previously reported (Jensen *et al.*, 2014). Strains were incubated with pig mucin type III for 1.5 hours at 37 °C in a 5% CO₂ atmosphere. Adhesion capability to mucus was measured by absorbance reading at 405 nm using an EnSpire multimode plate reader. Values are expressed as mean (bold horizontal bars) with min and max values (boxes). Data were analyzed by means of Prism 7 program (GraphPad Software, San Diego, CA, USA) using the one-way analysis of variance followed by Bonferroni's post hoc analysis. A level of (* $p < 0.05$), was considered statistically significant.



NCM460 cells and strains were incubated for 1.5 hours at 37 °C in a 5% CO₂ atmosphere. To evaluate the percentage of adhesion, bacterial cells were detached by trypsinization, serially diluted and plated on MRS agar.

Values are expressed as mean \pm SEM and are reported as percentage of adhesion compared with the control (100%). ANOVA Bonferroni's test showed significant differences in all samples compared with the control ($p < 0.05$), except for WCSF1 strain (ns).

Conclusions

- All the strains evaluated displayed different ability degrees to adhere to Normal-derived Colon Mucosal (NCM-460) cell line and mucus under *in vitro* conditions.
- Food-borne *L. plantarum* strains showed a similar adhesion properties than probiotics.
- Intrinsic variability among the strains highlights the strain-dependent feature of adhesion capability to mucus and epithelial cells.

References

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- David, L.A., *et al.* 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature letter* doi:10.1038.
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- Jensen, H., *et al.* (2014). Role of *Lactobacillus reuteri* cell and mucus-binding protein A (CmbA) in adhesion to intestinal epithelial cells and mucus *in vitro*. *Microbiol.* **160**, 671–681.