PS4 – Evaluation of *Lactobacillus plantarum* adhesion capacity to intestinal mucosa *in vitro* and *in vivo*

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Abstract: Intestinal microbiota plays a crucial role in human health. More recently it has been discovered that intestinal dysbiosis may contribute not only to the pathogenesis of inflammatory bowel diseases but can have a role also on several diseases, such as diabetes, obesity, cystic fibrosis, Alzheimer and Parkinson¹. The use of probiotics has shown promising results in rebalancing microbiota on such dysbiosis conditions. Their action is carried out at different levels, including the competition with enteropathogens for the intestinal epithelial cells’ adhesion sites.² This work aims at evaluating the adhesion of the strain *Lactobacillus plantarum* ATCC 14917 *in vitro* to cells line Caco-2 and HT-29 (human colon adenocarcinoma) and *in vivo* on *Galleria mellonella’s* gut, innovative model for probiotic screening ³ To evaluate in vitro adhesion, serial dilutions of *Lb. plantarum* was inoculated in the cell lines Caco-2 and HT-29 in DMEM medium. After being incubated for 2h at 37°C, non-adherent bacteria have been removed with PBS. In order to enumerate the adherent bacteria, cells lysates have been serially diluted, plated and counted on MRS agar⁴; to estimate the adhesion *in vivo* 20 *G. mellonella* larvae have been fed through gavage with *Lb. plantarum* suspension and 10 with PBS (control). All the larvae have been sacrificed and their digestive channels diluted, homogenized, and subjected to different analysis.⁵ The study precedes with phenotypic analysis of microbial strains isolated from the *G. mellonella’s* gut, such as bacterial culture, microscopical observation, Gram’s method, and carbohydrates fermentation patterns on API 50 CH. Finally, genetic analysis has been performed on DNA extracted from homogenized intestines, and the amplification by PCR of specific sequences of *Lb. plantarum* has been observed through agarose gel electrophoresis.⁶ This work has shown that the adhesion of *Lactobacillus plantarum* to HT-29 cells is generally higher than to Caco-2. Moreover, the evaluation of the *G. mellonella’s* gut colonization by *Lb. plantarum*, after intestinal transit, appears clearly in the phenotypic analyses. In the control larvae group, there were exclusively cocciform strains (*Enterococcus* and *Staphylococcus*), while in the study group there were also bacilliform strains that were genetically identified as *Lb. plantarum*. In conclusion, the data obtained in this preliminary study have shown a higher adhesion to HT-29 cells in comparison to Caco-2 cell line, and that the adhesive properties of *Lb. plantarum* appeared to be influenced in both cases by the bacterial concentration. This work’s data suggest that *Lb. plantarum* can colonize *G. mellonella’s* gut proving to be a potential probiotic.

References