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In vivo Evaluation of LAB Probiotic Potential with the Zebrafish Animal Model

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Abstract : Introduction: It is known that some Lactic Acid Bacteria (LAB) present an interesting probiotic effect. Probiotic bacteria stimulate host resistance to microbial pathogens and thereby aid in immune response, and modulate the host's immune responses to antigens with a potential to down-regulate hypersensitivity reactions. Therefore, probiotic therapy is valuable against intestinal infections and may be beneficial in the treatment of Inflammatory Bowel Disease (IBD). Several in vitro tests are available to evaluate the probiotic potential of a LAB strain. However, an in vivo model is required to understand the interaction between the host immune system and the bacteria. During the last few years, zebrafish (Danio rerio) has gained interest as a promising vertebrate model in this field. This organism has been extensively used to study the interaction between the host and the microbiota, as well as the host immune response under several microbial infections. In this work, we report on the use of the zebrafish model to investigate in vivo the colonizing ability and the immunomodulatory effect of probiotic LAB. Methods: Lactobacillus strains belonging to different LAB species were fluorescently tagged and used to colonize germ-free zebrafish larvae gastrointestinal tract (GIT). Some of the strains had a well-documented probiotic effect (L. acidophilus LA5); while others presented an exopolysaccharide (EPS) producing phenotype, thus allowing evaluating the influence of EPS in the colonization and immunomodulatory effect. Bacteria colonization was monitored for 72 h by direct observation in real time using fluorescent microscopy. CFU count per larva was also evaluated at different times. The immunomodulatory effect was assessed analysing the differential expression of several innate immune system genes (MyD88, NF-κB, Tlr4, Il1β and Il10) by qRT- PCR. The anti-inflammatory effect was evaluated using a chemical enterocolitis zebrafish model. The protective effect against a pathogen was also studied. To that end, a challenge test was developed using a fluorescently tagged pathogen (Vibrio anguillarum-GFP+). The progression of the infection was monitored up to 3 days using a fluorescent stereomicroscope. Mortality rates and CFU counts were also registered. Results and conclusions: Larvae exposed to EPS-producing bacteria showed a higher fluorescence and CFU count than those colonized with no-EPS phenotype LAB. In the same way, qRT-PCR results revealed an immunomodulatory effect on the host after the administration of the strains with probiotic activity. A downregulation of proinflammatory cytoquines as well as other cellular mediators of inflammation was observed. The anti-inflammatory effect was found to be particularly marked following exposure to LA% strain, as well as EPS producing strains. Furthermore, the challenge test revealed a protective effect of probiotic administration. As a matter of fact, larvae fed with probiotics showed a decrease in the mortality rate ranging from 20 to 35%. Discussion: In this work, we developed a promising model, based on the use of gnotobiotic zebrafish coupled with a bacterial fluorescent tagging in order to evaluate the probiotic potential of different LAB strains. We have successfully used this system to monitor in real time the colonization and persistence of exogenous LAB within the gut of zebrafish larvae, to evaluate their immunomodulatory effect and for in vivo competition assays. This approach could bring further insights into the complex microbial-host interactions at intestinal level.

Keywords : gnotobiotic, immune system, lactic acid bacteria, probiotics, zebrafish
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