In vitro process for adapting the probiotic *Lactiplantibacillus plantarum* with enhanced mucus adhesion properties using the SHIME gastrointestinal model

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Oral probiotic administration is suggested to help prevent microbial dysbiosis, or act as a therapeutic medication to help restore gut homeostasis (1,2). The major challenge of oral probiotics efficiency is ensuring gut colonization. For effective colonization and proliferation, probiotics need to reach their target alive and functional, adhere to the mucus layer and be in sufficient numbers. Increased mucus adhesion is expected to improve gut colonization, thereby increasing the potential of the probiotic to have a positive effect on both gut microbiota and host health. Using a simplified version of the M-SHIME® (Simulator of Human Intestinal Microbial Ecosystem) including a mucosal compartment, ***Lactiplantibacillus plantarum*** **(ATCC14917)** was exposed to different challenging gastrointestinal conditions, including prolonged exposure to low pH and pancreatic juice, to induce spontaneous mutants with an enhanced ability to adhere to mucus under adverse conditions. After 72 hours of anaerobic fermentation in the simplified M-SHIME model without microbiota (hereafter referred to as BATCH fermentation), 8 potential mutant strains with improved mucus adhesion potential were isolated. Next, the potential of these 8 mutant strains to adhere to mucus in the presence of human gut microbiota was assessed. Using human fecal gut microbiota from 3 healthy donors, 10 short-term BATCH fermentation assays were carried out in triplicate, using the original strain as the control. After 72 hours of incubation, 50% of mucin adhered bacteria samples were isolated and plated on MRS agar, while the rest of the samples were processed for metataxonomic and metabolite analysis. Furthermore, samples from the luminal content were collected daily to assess the changes in microbial communities and metabolite concentrations following the addition of an improved mucus adhering probiotic. We demonstrate an improved adaptation of ***Lactiplantibacillus plantarum*** to low acidic conditions, with enhanced capability to adhere to mucus.

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