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Publication date: 2014

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

Celebioglu, H. U., Lahtinen, S. J., Pedersen, S. B., Abou Hachem, M., & Svensson, B. (2014). Interaction of Lactobacillus acidophilus NCFM grown on different carbohydrates with human intestinal epithelial cells: Adhesion Properties and roles of S-layer Proteins. Abstract from 11th international Symposium on Lactic Acid Bacteria, Egmond aan Zee, Netherlands.

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International Symposium on Lactic Acid Bacteria







Abstract Book



August 31 to September 4, 2014

Egmond aan Zee, the Netherlands www.lab11.org

Interaction of Lactobacillus acidophilus NCFM grown on different carbohydrates with human intestinal epithelial cells: Adhesion properties and roles of S-layer proteins

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Adhesion of probiotics to the gastrointestinal tract is considered to be an important criterion for colonization. Lactobacillus acidophilus NCFM (NCFM) is one of the well-defined probiotic strains isolated from humans and used in dietary supplements and yogurts. Although the adhesion process is complex and involves a variety of proteins, Surface layer (S-layer) proteins have been found to have roles in this adhesion. Combination of probiotics with emerging prebiotics has been shown to alter protein expression in a way that might change the functional properties of the probiotic.

The present study aimed at investigating effects of emerging prebiotics on S-layer protein expression and adhesion properties of NCFM. Adhesion differences by growth on the carbohydrates raffinose, cellobiose, and as control glucose were examined by using the human intestinal HT-29 cell line. 2-D gel electrophoresis, image analysis, and protein identification by mass spectrometry were performed to identify differentially expressed S-layer proteins under these growth conditions.

The results showed that NCFM grown on raffinose and cellobiose significantly increased adhesion to HT-29 cells 2.8 and 2.4 fold (p<0.001), respectively, compared to glucose. Protein identification revealed that expression of NCFM SIpX and SIpA proteins was significantly increased in raffinose and cellobiose cultures (p<0.05). In conclusion, increased level of S-layer proteins due to utilization of raffinose and cellobiose leads to increased adhesion of NCFM to human intestinal HT-29 cells.

Acknowledgement: This study was supported by the Danish Strategic Research Council's Programme Committee on Health, Food and Welfare (FøSu); PhD grant by the Republic of Turkey, Ministry of National Education to H.U.C.

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Characterization of immunomodulatory effects of probiotic bacteria in zebrafish model J.G. Guzzo¹, N. Aoudia², N. Yousfi², J. Chluba², G. Jego², C. Garrido³, A. Rieu²

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The zebrafish (*Danio rerio*), already used for the study of the human immune system has an innate response similar to that of humans, representing a relevant model for studying the immunomodulatory properties of probiotic bacteria. The objective of this work is to develop a sensitive vertebrate *in vivo* screening system to assess the ability of probiotic bacteria to colonize the intestine and may also be used to rank the strength of their immunomodulatory effects

A fish feeding protocol with probiotic bacteria and/or with inflammatory agent (TNBS) was established. The epifluorescence microscopy and transmission electron microscopy were used to visualize the location of GFP-expressing bacteria in the gut. The results show that *Lactobacillus casei* ATCC334 attaches to and colonizes the zebrafish intestine Moreover, inflammation response was assessed in the zebrafish by quantitative RT-PCR and the results show a decrease in the expression of genes encoding pro-inflammatory cytokines TNF-α and IL-1β following the probiotic treatment, suggesting that this bacterium is able to limit inflammation caused by TNBS. A decreased number of recruited macrophages is also demonstrated in the same conditions.

This study highlights the importance of this animal model to characterize the immunomodulatory effects of probiotic