

LECTINS OF PROBIOTIC *LACTOBACILLUS RHAMNOSUS* GG AND THEIR POTENTIAL FOR PATHOGEN EXCLUSION

Mariya Petrova¹, Sarah Lebeer¹, Tine L.A. Verhoeven¹, Ami De Weerd¹, Ingmar J.J. Claes¹, Sigrid C.J. De Keersmaecker¹, Jan Balzarini², Jos Vanderleyden¹

¹ Centre of Microbial and Plant Genetics, K.U.Leuven, Kasteelpark Arenberg 20, bus 2460, B-3001 Leuven Belgium

² Rega Institute for Medical Research, K. U. Leuven, B-3000 Leuven, Belgium

E-mail: Mariya.Petrova@biw.kuleuven.be

Introduction

An important mechanism of probiotic action is the exclusion or inhibition of pathogens by producing antimicrobial compounds and/or competing for adhesion sites. Molecules on the cell surface of the probiotic strains that can directly interact with the pathogens or host cells are important for these beneficial functions. Various pathogens (bacteria and viruses) infect host cells via binding to specific glycans at the host surface through specific carbohydrate-binding proteins, which are named lectins. Probiotic bacteria also have lectins at their cell surface that could play a role in pathogen exclusion by (1) competitively binding to the same glycans on the host surface, thereby blocking adhesion, or (2) by binding glycans at the pathogenic surfaces, thereby blocking virulence mechanisms such as invasion and infection of host cells. In this study, the role of lectins of the clinically well-documented and model probiotic strain *Lactobacillus rhamnosus* GG (LGG) is studied in relation to pathogen exclusion of gastrointestinal pathogens

Material and Methods

The genome sequence of LGG is screened for genes encoding putative lectins. Corresponding knock-out mutants are constructed using our in-house developed genetic methods and phenotypically characterized in comparison with LGG wild-type. Assays include adhesion experiments with intestinal epithelial cells and biofilm formation on various surfaces, in the presence or absence of competing pathogens.

Results and discussion.

The first putative lectins that were characterized in LGG are the SpaCBA pili that bind to heavily glycosylated human mucus via the tip adhesin SpaC that contains a von Willebrand factor adhesion domain (Kankainen *et al.*, PNAS, 2009). Follow-up assays involving duo-species biofilms of LGG and *Salmonella* showed a role for these SpaC-type pili in pathogen displacement of pre-established *S. Typhimurium* biofilms. Comparative mutant analysis suggests that the SpaCBA pili are important for making initial close contacts with the *Salmonella* biofilms, after which lactic acid produced by LGG can more efficiently kill the pathogenic bacteria. The second adhesin of LGG that was characterized by mutant analysis was a large-sized surface protein containing 26 repetitive DUF1542 domains (called MabA) that can also modulate binding to intestinal epithelial cells and biofilm formation, possibly via its repetitive domains, but its overall role in LGG adhesion is a minor one as compared to that of the SpaCBA pilus (Perea Vélez *et al.*, FEMS Immunol Med Microbiol., 2010). Currently, the LGG genome sequence is screened for the presence of putative mannose-binding lectins, since various pathogens target host cells via mannose-glycoproteins. The corresponding mutants are being constructed and will be investigated in various pathogen exclusion experiments.