The search for *Lactobacillus* proteins that bind to host targets

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Abstract

Interactions between microorganisms and host cells in the gastrointestinal tract are crucial to the host's health. Probiotic bacteria, such as the lactobacilli provide numerous benefits to human health thought to be mediated by bacterial proteins called effectors. *Lactobacillus rhamnosus* HN001 (*L. rhamnosus* HN001) is a cheese-fermenting isolate with probiotic characteristics and *Lactobacillus reuteri* 100-23 (*L. reuteri* 100-23) is a coloniser of the rodent forestomach. Whereas *L. rhamnosus* HN001 was shown to reduce eczema in children, *L. reuteri* 100-23 reduces inflammation in mice. The effector proteins for these strains are largely unknown. In this thesis, phage display technology was used to search for proteins that bind specific ligands. Shot-gun genomic phage display library of *L. rhamnosus* HN001 was affinity screened on fibronectin as bait, leading to enrichment of specific recombinant clones. Analysis of 10 candidate clones, however, determined that these are not genuine binders, but may have been selected due to a potential growth advantage during amplification steps of the library. The *L. reuteri* 100-23 genomic shot-gun phage display library was subjected to two affinity screens on two baits: fibronectin and murine stomach tissue. The aim of the screen on the murine stomach tissue was to identify keratin-binding proteins, as this strain naturally colonises the murine keratinous forestomach. Whereas no enrichment was detected in the screen on fibronectin as a bait, a strong enrichment of a phagemid displaying a short peptide, IGINS, derived from a cell-surface protease of *L. reuteri* 100-23 was identified. Identifying and characterising probiotic bacterial proteins that positively influence health will lead to a greater understanding of gastrointestinal tract interactions. Ultimately, this aids development of probiotic use as therapeutic agents.

Foreword and Acknowledgements

Through perseverance we move forward in life. Perseverance is achieved with ‘One More Try’. This mantra comes from two hardworking men and also an angel who is dearly loved by me. They never give up in the face of hardship. This thesis is not only the product of two years study and experimentation into gastrointestinal interactions, but a reflection on a lifelong journey and pursuit of learning.

My interest in science began at primary school. This involved learning about space, dinosaurs, geology and uncovering the remains of ancient civilisations. My interest in the genetic and chemical sciences began in high school. At University, by majoring in genetics and microbiology, I became highly interested in microorganisms and their significant influence. Therefore, this thesis is the product of all those years, hours and energy studying science and its role in our community.

There are some significant people who have had a positive impact in my life. In particular, I would like to thank the following people: Peter Flynn, for being the greatest maths teacher I have known; Vicki Bothwell, for being the greatest English teacher I have known; Jasna Rakonjac, my supervisor, for her expertise and guidance; Marie McGlynn, my mother, for raising me to do my best and follow my dreams; Catherine Dyhrberg, for her long lasting friendship and advice; Misha Collins, for being a role model for universal love and kindness; Luke Phoenix, for his support and inspiring me to be stronger in all walks of life.

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In memory of my father who would be proud of what I have achieved.

Table of Contents

Abbreviations

Amp	Ampicillin
BLAST	Basic Local Alignment Search Tools
BSA	Bovine Serum Albumin
Cm	Chloramphenicol
DCs	Dendritic Cells
E. coli	*Escherichia coli*
ECM	Extracellular Matrix
GIT	Gastrointestinal Tract
IECs	Intestinal Epithelial Cells
IL_(number)	Interleukin (number) (i.e Interleukin 17)
Kn	Kanamycin
L. rhamnosus GG	*Lactobacillus rhamnosus* GG
L. rhamnosus HN001	*Lactobacillus rhamnosus* HN001
L. reuteri 100-23	*Lactobacillus reuteri* 100-23
M/PAMPs	Microbial/Pathogen Associated Molecular Patterns
NFkB	Nuclear Factor kappa B
NEC	Necrotizing enterocolitis
O.D.	Optical Density
PSA	Polysaccharide A
PBS	Phosphate Buffer Saline
PBST	Phosphate Buffered Saline with Tween-20
PEG	Polyethylene Glycol
pmol	Pico mole
PP	Phagemid Particles
RF	Replicative Form
SDS	Sodium Dodecyl Sulfate
SOC	Super Optimal broth with Catabolite repression
ssDNA	single stranded Deoxyribose Nucleic Acid
TDPs	Transducing Phagemid Particles
2xYT	Yeast Extract Tryptone Broth

LIST OF FIGURES

LIST OF TABLES