# Characterisation of adhesion of a probiotic bacterium *Lactobacillus rhamnosus* HN001 to extracellular matrix proteins and the intestinal cell line Caco-2

A thesis presented to Massey University in partial fulfilment of the requirement for the degree of Master of Science in Microbiology

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**2003**

# ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr. Mark Lubbers and Dr. Michael Collett from Fonterra Research Centre for all their time, expertise, and support that made this study possible. I would like to thank my supervisors Dr. Paul O'Toole for his time and expertise during the first year of this study and Prof. Pat Sullivan for his assistance in the final stage of this study. I would like to thank John Lumsden from the Institute of Veterinary Science, Massey University, for the preparation of the antisera used in this study. I would like to thank Ellen Hausman for her assistance with the enumeration of Caco-2 cells and the Caco-2 cell line maintenance, and Nini Ripandelli for her assistance with the Caco-2 cell maintenance. Finally, I would like to thank all the members of the Microbial Genetics group at Fonterra Research Centre for their wonderful support during this study. I would like to acknowledge the financial support of Fonterra Research Centre, which made this study possible.

# ABSTRACT

This study focuses on *Lactobacillus rhamnosus* HN001, a potential candidate for use as a probiotic. Probiotics are microorganisms that can exert a beneficial effect on a host. It is believed that the ability of a probiotic to colonise gastrointestinal surfaces is important in its ability to exert a beneficial effect on the host. In order to do so, it is thought the microorganism must be able to adhere to molecules found on intestinal cells. HN001 has been shown to adhere to human intestinal cell lines (Gopal *et al.*, 2001). This study characterises the molecular species involved in the adherence of HN001 to intestinal molecules and cell lines, which may be important in the ability of HN001 to exert health benefits in a host.

Both liquid and solid-phase binding assays were used to characterise HN001 binding to extracellular matrix (ECM) components found in intestinal tissues. Of the ECM components investigated, HN001 bound fibronectin with the highest affinity. This interaction was specific, saturable and dependent on the growth phase of HN001. HN001 bound immobilised fibronectin in preference to soluble fibronectin through a protein-dependent interaction. HN001 was also found to bind to the N-terminal heparin binding domain of fibronectin and the C-terminal part of the first type III repeat in the fibronectin molecule ($III_1$-C). HN001 adhered to the human intestinal cell line, Caco-2, in a dose-dependent manner that was enhanced by a pH-sensitive factor present in the spent culture supernatant.

Since fibronectin-binding was identified as a possible mechanism for adherence of HN001 to intestinal tissues, HN001 genome DNA sequence was examined for genes encoding putative fibronectin-binding proteins. Fbl (Fibronectin-binding like) was identified through its similarity to fibronectin-binding proteins from *Streptococcus pneumoniae* (Holmes *et al.*, 2001) and *S. pyogenes* (Courtney *et al.*, 1994). Fbl was expressed by a GST fusion system and used to compete with HN001 adhesion in liquid-phase binding assays to ascertain its function. Since difficulties were experienced when expressing and purifying soluble Fbl, an insertional disruption of the *fbl* gene was created and its phenotype investigated in liquid-phase, solid-phase and Caco-2 binding assays to determine Fbl function.

# ABBREVIATIONS

| | |
|---|---|
| BLAST | Basic Local Alignment Search Tools |
| BSA | Bovine Serum Albumin |
| cFn | Cellular fibronectin |
| cfu | Colony forming units |
| CIII | C-terminal part of the first type III repeat in fibronectin |
| cpm | Counts per minute |
| Da | Dalton |
| ECM | Extracellular matrix |
| EDTA | Ethylenediaminetetra Acetic Acid Disodium salt |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| Em | Erythromycin |
| Fbl | Fibronectin-binding-like protein |
| Fn | Fibronectin |
| FRC | Fonterra Research Centre |
| FPLC | Fast Performance Liquid Chromatography |
| GST | Glutathione S-transferase |
| HRP | Horseradish Peroxidase |
| IAA | Iso Amyl Alcohol |
| IPTG | Isopropylthio-β-D-galactoside |
| LAB | Lactic acid bacteria |
| LTA | Lipoteichoic acid |
| NEB | New England Biolabs |
| OD | Optical density |
| ORF | Open reading frame |
| ori | Origin of replication |
| PCR | Polymerase chain reaction |
| pFn | Plasma fibronectin |
| SCS | Spent culture supernatant |
| SDS-PAGE | SDS-polyacrylamide gel electrophoresis |
| sFn | Super fibronectin |
| Tm | Melting temperature |
| TMB | 3,3',5,5' tetramethylbenzidine |
| WT | Wild type |

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