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

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degree of Master of Science in Microbiology

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## 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<sub>1</sub>-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- $\beta$ -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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