Direct selection and phage display of the *Lactobacillus rhamnosus* HN001 secretome

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ABBREVIATIONS

BLAST	Basic Local Alignment Search Tools
BRB	Bromphenol blue
BSA	Bovine Serum Albumin
cfu	Colony forming units
Da	Dalton
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleoside triphosphate
ECM	Extracellular matrix
EDTA	Ethylenediaminetetra Acetic Acid Disodium salt
ELISA	Enzyme-Linked Immunosorbent Assay
EtBr	Ethidium bromide
Fn	Fibronectin
HRP	Horseradish Peroxidase
IAA	Iso Amyl Alcohol
IPTG	Isopropylthio-β-D-galactoside
Kbp	Kilobase pair
LAB	Lactic acid bacteria
LTA	Lipoteichoic acid
min	minutes
Mb	megabases
OD	Optical density
ORF	Open reading frame
ori	Origin of replication
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
pfu	Plaque forming units
RBS	Ribosome binding site
s	seconds
SDS	Sodium dodecyl sulfate

SDS-PAGE	SDS-polyacrylamide gel electrophoresis
Tm	Melting temperature
TMB	3,3’,5,5’ tetramethylbenzidine
UV	Ultraviolet
v/v	volume/volume
wt	wild type
w/v	weight/volume

ABSTRACT

Bacteria communicate with their hosts in part via surface, secreted and transmembrane proteins (collectively the secretome) resulting in probiotic (beneficial) or pathogenic (harmful) outcomes to the host. Therapeutic benefits of probiotic bacteria have been shown previously, but the molecular mechanisms and the health-promoting effector components involved are still being elucidated. Some evidence suggests that probiotic bacteria can competitively adhere to intestinal mucus and displace pathogens. The adherence of probiotic bacteria to human intestinal mucus and cells appears to be mediated, at least in part, by secretome proteins.

Secretome proteins-encoding open reading frames can be identified in bacterial genome sequences using bioinformatics. However, functional analysis of the translated secretome is possible only if many secretome proteins are expressed and purified individually. Phage display technology offers a very efficient way to purify and functionally characterise proteins by displaying them on the surface of the bacteriophage. While a phage display system for cloning secretome proteins has been previously reported it is not efficient for enrichment and display of Gram-positive secretome proteins.

In this study a new phage display system has been developed and applied in direct selection, identification, expression and purification of Gram-positive *Lactobacillus rhamnosus* strain HN001 secretome proteins. The new phage display system is based on the requirement of a signal sequence for assembly of sarcosyl-resistant filamentous phage virions. Using this system 89 secretome open reading frames were identified from a library of only 10^6 clones, performing at least 20-fold more efficiently than the previously reported enrichment method. Seven of the identified secretome proteins are unique for *L. rhamnosus* HN001.

A *L. rhamnosus* HN001 shot-gun phage display library was also constructed to capture proteins that mediate adhesion or aggregation, initial steps in establishing host-microbe contact or forming multicellular aggregates, both of which may lead to beneficial effects – colonisation of the gastro-intestinal tract and exclusion of pathogens. In search for proteins involved in adhesion, a *L. rhamnosus* HN001 shot-gun phage display library was screened against the human extracellular matrix component fibronectin commonly used as binding

target by bacteria that colonise diverse tissues. This screen selected, instead of a fibronectin-binding protein, a protein that binds to avidin, used to immobilise biotinylated fibronectin.

Affinity screening of the shot-gun library for binding to *L. rhamnosus* HN001 cells identified a secretome protein, Lrh33, as an HN001-cell surface binding protein. This protein contains two bacterial immunoglobulin-like domains type 3. Analysis of phage-displayed nested deletions of Lrh33 determined that the proximal (N-terminal) immunoglobulin-like domain is not sufficient for binding; only the constructs displaying both domains demonstrated binding to HN001. Lrh33 does not have any similarity to previously identified *Lactobacillus*-binding proteins and no match in the NCBI database (at a cutoff value of $> e^{-13}$), hence it represents potentially a new type of bacterial auto-aggregation protein.