Identification and functional characterisation of a novel surface protein complex of *Lactobacillus rhamnosus*

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Abbreviations

Amp	Ampicillin
APF	Aggregation-promoting factor
Big-3	Bacterial immunoglobulin-like domain type-3
cfu	colony-forming unit
Cm	Chloramphenicol
COG	Cluster of orthologous genes
CWBD	Cell wall-binding domain
DC	Dendritic cell

DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic Acid
EGF	Epithelial growth factor
EGFR	Epithelial growth factor receptor
Em	Erythromycin
FRDC	Fonterra Research and Development Centre
GALT	Gut-associated lymphoid tissue
GIT	Gastrointestinal tract
GlcNAc	*N*-acetylglucosamine
h	hour(s)
HRP	Horse radish peroxidase
IEC	Intestinal epithelial cell
kbp	kilobase pair
LAB	Lactic acid bacteria
LTA	Lipoteichoic acid
MAMP	Microorganism-associated molecular pattern
MAPK	Mitogen-activated protein kinase
MBP	Maltose-binding protein
min	minute(s)
MRS	Man-Rogosa-Sharpe
MurNAc	*N*-acetylmuramic acid
NF-κB	Nuclear factor κB
NLR	Nucleotide-binding oligomerisation domain-like receptor
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
pfu	plaque-forming unit
PG	Peptidoglycan
PPs	Phagemid particles
PR-1	Pathogenesis response domain 1
PRR	Pattern recognition receptor

PS	Polysaccharides
sec	seconds
Str	Streptomycin
TA	Teichoic acid
TBS	Tris-buffered saline
TBST	TBS-Tween
Tet	Tetracycline
TJ	Tight junction
TLR	Toll-like receptor
TNFα	Tumor necrosis factor alpha
v/v	volume/volume
w/v	weight/volume
WPS	Wall polysaccharide

Abstract

Proteins are the most diverse structures on bacterial surfaces; hence they are candidates for species- and strain-specific interactions of bacteria with the host, environment and other microorganisms. In probiotic bacteria, some surface and secreted proteins mediate interactions with the host and may consequently contribute to the health-promoting effects. However, a limited fraction of surface-associated proteins from probiotic bacteria have been functionally characterised to date. A secreted protein of *Lactobacillus rhamnosus* HN001, SpcA, containing two bacterial immunoglobulin-like domains type 3 (Big-3) and a domain distantly related to plant pathogen response domain 1 (PR-1-like), was previously shown to bind to HN001 cells, however the nature of its ligand on the surface of the cells was unknown. In this study, a series of binding assays first demonstrated that SpcA binds to a cell wall anchored protein of HN001. Next, the SpcA-"docking" protein, named SpcB, was identified using phage display. SpcB is a 3275-residue cell-surface protein that has all the features of large glycosylated serine-rich adhesins/fibrils from Gram-positive bacteria, including the hallmark glycoprotein signal sequence motif KxYKxGKxW and the cell wall anchor motif LPxTG. The *spcA* and *spcB* genes are located in a gene cluster, *spcBCDA,* which is present in 94 out of 100 strains of *L. rhamnosus* species and some strains of *L. casei* and *L. paracasei* whose genome sequences have been determined, but was absent from other *Lactobacillus* clades. To confirm the role of SpcB as the SpcA anchor and investigate the roles of these two proteins in surface properties of probiotic *L. rhamnosus* strains HN001 and GG, stable double-crossover mutations of these two genes were constructed. Binding assays to *L. rhamnosus* mutant cells confirmed dependence on SpcB in both GG and HN001 strains. Comparison of the wild-type and mutant surface properties suggested that SpcB in GG interferes with biofilm formation and aggregation, while it might contribute to the protective effect against TNFα-mediated disruption of the polarised Caco-2 cell monolayer integrity. Deletion of HN001 *spcB* or *spcA* had no effect on functions other than the SpcA binding. Our findings indicate that the roles of a surface protein can vary considerably among the strains of a species, requiring functional data to validate the bioinformatics-based hypotheses.