The biotherapeutic potential of *Lactobacillus reuteri* DPC16 and bovine lactoferrin in controlling some pathogens, genotoxicity and inflammation in the gut

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Abstract

This study investigated the effects of the probiotic bacterium, *L. reuteri* DPC16, alone and in combination with bovine lactoferrin, on intestinal pathogens, intestinal inflammation and carcinogenesis. Human and animal cellular model systems were designed and applied to this evaluation.

The identity of the *L. reuteri* DPC16 strain was confirmed using 16S rRNA analysis and its ability to produce the antibacterial compound, reuterin. It was able to tolerate pH 2 and physiological concentrations of bile salts in a simulated gastrointestinal tract environment in the presence of protective nutrients. It was able to adhere to a Caco-2 human epithelial monolayer (modelling the human GI tract) and it did not degrade mucin.

Both bovine lactoferrin and *L. reuteri* DPC16 inhibited the growth of the intestinal pathogens *Listeria monocytogenes, Staphylococcus aureus, Salmonella typhimurium and Escherichia coli* O157:H7, with much less effect on tested probiotic bacteria. Together, *L. reuteri* DPC16 and bovine lactoferrin showed synergistic inhibitory effects.

L. reuteri DPC16 was also able to remove indole from faecal water. Using human/animal cellular model systems, combined with the use of *E. coli* endotoxin and genotoxic factors present in faecal water, bovine lactoferrin was shown to down-regulate inflammation by affecting the signalling pathway on immune receptors that recognize the endotoxin, while both bovine lactoferrin and strain DPC16 were shown to have the potential to prevent epithelial cell DNA damage.

The study has demonstrated several significant properties of *L. reuteri* DPC16 and bovine lactoferrin, including antibacterial, antigenotoxic, and anti-inflammatory activities, and possible mechanisms for these activities have been proposed. Based on the information obtained from this work, a combination of the probiotic *L. reuteri* DPC16 and bovine lactoferrin could possibly be developed as a novel probiotic formula

for human consumption, to maintain beneficial bacteria while controlling harmful bacteria in the GI tract. However, advanced *in vitro* model systems and *in vivo* studies are suggested to confirm these findings in order to consider the feasibility of commercialisation.

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Abbreviations

AC	Aberrant crypt
ACF	Aberrant crypt foci
AAD	Antibiotic-associated diarrhea
AICR	The American Institute for Cancer Research
ANOVA	Analysis of variance
AOM	Azoxymethane
ATCC	American Type Culture Collection
AFB1	Aflatoxin B1
B(a)P	Benzo(a) pyrene
BCA	Bicinchoninic acid
BLAST	The Basic Local Alignment Search Tool
BLf	Bovine lactoferrin
BHI	Brain heart infusion broth
BM	Basal medium
BSA	Bovine serum albumin
CD	Crohn's disease
CFU/mL	Colony Forming Units per milliliter
CRC	Colorectal cancer
DC	Dendritic cells
DCA	Deoxycholic acid
Dg	Supernatant of DPC16 3h cell suspension in 250 mM glycerol PBS buffer
DMH	1, 2-dimethylhydrazine
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
DS	DPC16 culture supernatant in MRS medium
DSg	DPC16 culture supernatant in 250 mM glycerol-containing MRS medium
CPS	Capsular polysaccharides
EaggEC	Enteroaggregative *E.coli*
EDTA	Ethylenediamine tetra-acetic acid

e.g.	*exempli gratia,* means "for example"
EHEC	Enterohaemorrhagic *E. coli*
ELISA	Enzyme-linked immunosorbent assay
EPEC	Enteropathogenic *E. coli*
EPS	Extracellular polysaccharide
ETEC	Enterotoxigenic *E. coli*
FAO	Food and Agriculture Organization
FDA	The U.S. Food and Drug Administration
GI	Gastrointestinal
GIT	Gastrointestinal tract
HCAs	Heterocyclic amines
HMGB1	High-mobility group protein B1
HIV	Human immunodeficiency virus
HK cells	Heat-killed cells
3-HPA	3-hydroxypropionaldehyde
HFA	Human flora associated
IARC	International Agency for Research into Cancer
IBD	Inflammatory bowel diseases
IBS	Irritable bowel syndrome
i. e.	Abbreviation for id est. Latin meaning "that is,"
IFN-γ	Interferon-γ
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IQ	2- amino-3methylimidazo[4,5-*f*]-quinoline
LAB	Lactic acid bacteria
LCA	Lithocholic acid
LDH	Lactate dehydrogenase
LOS	Lipooligosaccharide
LPS	Lipopolysaccharide
MAM	Methylazoxymethanol

MAP	Modified atmosphere packaging
MIC	Minimum inhibitory concentration
MICA/MICB	MHC class I chain-related genes
MLCK	Myosin light chain kinase
MW	Molecular weight
NAD+	Nicotinamide adenine dinucleotide
NADH	Reduced form of NAD+
NADP	Nicotine adenine dinucleotide phosphate
NK cells	Natural killer cells
NO	Nitric Oxide
NOD2	Nucleotide-binding oligomerization domain containing 2
NPD	4-nitro-o-phenylenediamine
NSP	Non-starch polysaccharides
NZMP	New Zealand milk products
OD	Optical density
PAMPs	pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PAD	Photodiode Array Detector
pH	.-Log10 (aH+)
PGM	Porcine gastric mucin
PMA	Phorbol myristate acetate
PRRs	Pattern-recognition receptors
r DNA	r RNA gene
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
r RNA	Ribosomal RNA
SCGE	Single cell gel electrophoresis
SCFAs	Short chain fatty acids
SD	Standard deviation
SDS	Sodium dodecyl sulphate

SE	Standard error
sIgA	Secretory Immunoglobulin A
TEER	Transepithelial electrical resistance
TNF-α	Tumour necrosis factor-alpha
TLRs	Toll-like receptors
UC	Ulcerative colitis
UV	Ultraviolet light
VLBW	Very low birth weight
WCRF	World Cancer Research Fund
WHO	World Health Organization

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