A comparative study of two *Lactobacillus fermentum* strains that show opposing effects on intestinal barrier integrity

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ABSTRACT

The *Lactobacillus* species can exert health promoting effects in the gastrointestinal tract (GIT) of humans through several mechanisms, which include pathogen inhibition, maintenance of microbial balance, immunomodulation and enhancement of the GIT barrier function. However, different strains of lactobacilli can evoke different responses in the host and not all strains of the same species can be considered health promoting. Two strains identified as *Lactobacillus fermentum*, namely AGR1485 and AGR1487, isolated from human oral cavities, exhibit opposing effects on intestinal barrier integrity. Studies have shown that AGR1485 maintains trans-epithelial electric resistance (TEER), a measure of GIT barrier integrity, across Caco-2 cell monolayers, while AGR1487 decreases TEER by 12 hours.

This work aimed to test the hypotheses that the varying effects shown by these two *L. fermentum* strains are related to phenotypic differences between the two strains and are mediated by the interaction of secreted and/or cell-associated bacterial components with the GIT epithelial layer. Differences in metabolic events that occur during the various phases of growth in bacteria can impact not only cellular structure and secreted molecules, but may also affect their interactions with the intestinal epithelial cells. TEER assays were conducted to investigate if variation in bacterial secreted molecules and cell wall components associated with various phases of microbial growth can affect Caco-2 cell TEER. The effect on Caco-2 cell TEER caused by both strains was independent of bacterial growth phase. To test the hypothesis that it is the bacterial structural and/or secreted components that influence

Caco-2 cell TEER, assays were conducted with live versus UV-killed bacteria on Caco-2 cells. Results showed that for both strains of *L. fermentum*, dead bacteria have similar effects on Caco-2 cell TEER as live bacteria, implying that direct bacterial contact with Caco-2 cells is necessary for the effects. Analogous to TEER assays, live AGR1487 increased mannitol permeability while UV-killed AGR1487 did not, implying that AGR1487 uses both cell surface structures and/or metabolites through distinct mechanisms to modulate host barrier properties. Subsequent experiments conducted using secreted metabolites from bacteria, Caco-2 cells and bacteria-Caco-2 cell interactions indicated that they have no effect on Caco-2 cell TEER, strengthening the assumption that bacterial cell surface-associated components are involved in mediating these effects.

The bacterial cells were subjected to ultrasonication followed by ultracentrifugation to isolate the bacterial cell wall extract. TEER assays conducted with the cell wall extracts from both strains resulted in decreasing Caco-2 cell TEER, although at high concentrations, further strengthening the role of bacterial cell surface components in influencing barrier integrity of the Caco-2 cells. To narrow down proteinaceous components of the cell wall extracts from both the strains that influence Caco-2 cell TEER, they were fractionated through size exclusion chromatography and the effects of these cell wall fractions on Caco-2 cell TEER were studied. One fraction of AGR1487 CW appeared to decrease Caco-2 cell TEER, although at a high concentration. However, the results could not be repeated when the same fraction was applied at concentrations that the proteins comprising this fraction would be found in

live AGR1487. Even the high concentration tested previously did not decrease Caco-2 cell TEER and the discrepancy in results remains unexplained.

The results reported in this dissertation have added to the knowledge that the two strains of *L. fermentum* AGR1485 and AGR1487 show differences in their genome size and in their phenotypic characteristics. In addition, these bacteria utilise both cell surface and/or secreted metabolites through multiple mechanisms to modulate host response. In the future, identification of specific bacterial effector molecules that influence host response will be a major step towards understanding strain-specific characteristics shown by *Lactobacilli*.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BHI	Brain-heart infusion
BLASTN	NCBI nucleotide Basic Local Alignment Search
BSA	Bovine serum albumin
cDNA	Complementary DNA
CFU	Colony-forming units
CM	Cell membrane
CnBP	Collagen binding protein
CLR	C-type lectin receptor
CPS	Capsular polysaccharides
CW	Cell wall
DC	Dendritic cells
DC-SIGN	Dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin

ddNTPs	Dideoxynucleotides
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide
EDTA	Ethylenediamine tetra-acetic acid
EF-Tu	Elongation factor Tu
EHEC	Enterohemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
EPS	Exopolysaccharides
ERK	Extracellular signal regulated kinases
Fbp	Fibronectin binding protein
FBS	Foetal bovine serum
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
G+C	Guanine plus cytosine
GlcNAc	N-acetyl-glucosamine

GIT	Gastrointestinal tract
H_2O_2	Hydrogen peroxide
IBD	Inflammatory bowel disease
IEC	Intestinal epithelial cells
IFN	Interferon
IKK	IκB kinase
IL	Interleukin
IRAK	IL-1-receptor-asociated kinase
IRF	IFN-regulatory factor
IκB	Inhibitor of NF-κB
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LAB	Lactic acid bacteria
LACOG	*Lactobacillales*-specific clusters of orthologous genes
LTA	Lipoteichoic acids
LPS	Lipopolysaccharides
LSD	Least significant difference

M199	Medium 199
MAMPs	Microbe-associated molecular patterns
MAPK	Mitogen activated protein kinases
MBF	Mucus binding factor
MDCK	Madin Darby canine kidney
MLCK	Myosin light chain kinase
mRNA	Messenger RNA
MRS	Man, Rogosa and Sharpe
Msa	Mannose-specific adhesin
Mub	Mucus binding protein
MurNAc	β-1-4linked N-acetyl-muramic acid
MyD88	Myeloid differentiation primary response gene
NADH	Nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information
NEAA	Non-essential amino acids
NF-κB	Nuclear factor kappa B

NLR	NOD-like receptor
NOD	Nucleotide-binding and oligomerisation-domain
OD	Optical density
O_2^-	Superoxide anion radicals
$^{\cdot}OH$	Hydroxyl radicals
PAMPs	Pathogen-associated molecular patterns
PC	Polycarbonate (Transwell® cell culture inserts)
PCR	Polymerase chain reaction
PG	Peptidoglycan
PKC	Protein kinase C
PLA2	Group IIA phospholipase A2
PRRs	Pathogen recognition receptors
PFGE	Pulsed-field gel electrophoresis
REML	Restricted maximum likelihood
RNA	Ribonucleic acid
RNase	Ribonuclease A

ROS	Reactive oxygen species
rRNA	Ribosomal RNA
SDS	Sodium dodecyl sulphate
SDP	Sortase-dependent proteins
SEM	Standard error of the mean
Slp	S-layer proteins
SN	Supernatant
SOD	Superoxide dismutase
SrtA	Sortase enzyme
TA	Teichoic acids
TDCA	Taurodeoxycholic acid
TEER	Transepithelial electrical resistance
TGF	Transforming growth factor
TJ	Tight junction
TLR	Toll-like receptor
TNF	Tumour necrosis factor

UV	Ultraviolet
WPS	Wall polysaccharides
WTA	Wall teichoic acids
ZO	Zonula occludens