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Research Paper

Gene expression profile in the small intestine of mice fed *Lactobacillus acidophilus* LAFTI L10

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ABSTRACT

The effects of *Lactobacillus acidophilus* LAFTI L10 on gene expression in the small intestine of mice was evaluated using microarrays. Male BALB/c mice were orally fed with 10^8 colony forming units of *L. acidophilus* in skimmed milk powder for 14 days. Control mice received skimmed milk powder without *L. acidophilus*. After 14 days, distal end of the small intestine was excised for microarray analysis of gene expression. *L. acidophilus*-fed mice altered the expression of genes such as CD40 ligand, CD200 receptor-3 and trefoil factor 2, which are involved in host defence mechanisms. Overall, the expression of functional genes influenced by *L. acidophilus* in the small intestine of mice offer as a basis for further investigation into its probiotic effects.

Keywords: Functional foods, Gastrointestinal tract, *Lactobacillus acidophilus*, Probiotic bacteria

INTRODUCTION

The consumer acceptance of functional foods that can deliver potential health benefits is considerably growing in recent years. Specific strains of bifidobacteria and lactobacilli are the most commonly used as probiotic supplements in functional foods due to their health promoting attributes. According to definition by FAO/ WHO (2002), probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". Probiotic effects are strain-specific, therefore it is not appropriate to generalise the beneficial effects of a probiotic bacteria by comparing to bacterial strains belonging to the same species. Scientific evidence suggests that certain probiotic bacteria are capable of modulating the signalling mechanisms in the gastrointestinal (GI) tract (Thomas

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and Versalovic, 2010). To gain further understanding into the beneficial effects of probiotic bacteria, functional genomics approach were pursued to unravel their role in regulating genes involved in various host physiological responses (Chang *et al*., 2009; Shima *et al*., 2008; Yanagihara *et al*., 2012; Paturi *et al*., 2010). In our previous studies, orally administered *Lactobacillus acidophilus* LAFTI L10 enhanced various immune functions in mice (Paturi *et al*., 2010, 2008; Paturi *et al*., 2007). However, the mechanisms by which *L. acidophilus* promotes host health remains largely unknown. In the present study, we investigated the effects of *L. acidophilus* on gene expression patterns in the small intestine of mice using microarray analysis.

MATERIALS AND METHODS

L. acidophilus, mice and feeding procedure

Lactobacillus acidophilus LAFTI L10 used in this study was obtained from DSM culture collection (DSM Food Specialties Ltd., Sydney, Australia). The bacterial strain was grown anaerobically using GasPak System (Oxoid, Adelaide, Australia) for 24 h at 37°C in deMan Rogosa Sharpe broth (Oxoid). Eight-week old male BALB/c mice purchased from Biological Resources Centre (The University of New South Wales, Sydney, Australia) were housed in individual cages at 23 ± 1°C under 12 h light-dark cycle and given *ad libitum* access to food pellets (Standard mouse chow; Gordon's Speciality Stock Feeds, Sydney, Australia) and water throughout the trial. All experiments were performed with the approval from Animal Care and Ethics Committee of University of Western Sydney, Sydney, Australia. After 1 week of acclimatisation, mice were randomly allocated to control and *L. acidophilus* groups (n = 6 per group). Mice were fed daily by oral gavage with 10^8 colony forming units (CFU) of freshly grown *L. acidophilus* in 50 µl of 10% (w/v) skimmed milk powder for 14 days. As a control, mice received 50 µl of skimmed milk powder without *L. acidophilus*. After 14 days, mice were euthanised by carbon dioxide inhalation and a section of the distal end of small intestine was removed and stored at –80°C for gene expression analysis.

RNA extraction and microarrays

Gene expression analysis in the small intestine of mice was carried out using Compugen mouse 22 K oligonucleotide microarray as described by Paturi *et al*. (2010). In briefly, total RNA was extracted from the small intestine tissue using TRIzol reagent (Invitrogen, Melbourne, Australia) and purified with the RNeasy kit (Qiagen, Melbourne, Australia). The pooled RNA from control and *L. acidophilus* group mice was amplified using a Super Script Indirect RNA Amplification System (Invitrogen).The hybridisation of fluorescent cRNA samples to microarray slides and washing were carried out according to the protocols provided by the Adelaide Microarray Facility (The University of Adelaide, Adelaide, Australia). Microarray slides were scanned using a GenePix 4000B Scanner (Axon Instruments, Foster

City, USA). The SPOT software package (http://www.hca-vision.com/product_spot.html) was used to extract the Cy3 and Cy5 fluorescent signal intensity of each gene on the array. Bayesian statistical approach and linear modelling of the normalised data produced a list of genes that were likely to be consistently differentially expressed on all arrays. The genes that meet the cut-off criteria of fold change ≥ 1.5 and $P < 0.05$ were chosen to analyse through Ingenuity Pathway Analysis (IPA, 8.0 version; http://www.ingenuity.com).

RESULTS AND DISCUSSION

The mice body weight and food intake between the diet groups were similar ($P > 0.05$) (data not shown). Microarray analysis was carried out in this study to view the global gene expression profile influenced by *L. acidophilus*. A total of 76 genes meet the cut-off criteria (fold change ≥ 1.5 and $P < 0.05$) in mice fed *L. acidophilus* compared to control group. From those genes, IPA identified the association of 36 genes to a biological function or disease (Table 1). The biological functions significantly affected by *L. acidophilus* were grouped into three IPA categories: (1) diseases and disorders, (2) molecular and cellular functions and (3) physiological system development and functions. In these categories, top five functions and number of genes involved in those functions are shown in Table 2.

In this study, mice fed *L. acidophilus* modulated the immune response genes in small intestine through down-regulation of CD40 ligand and up-regulation of CD200 receptor-3 (Table 1). The co-activation of CD40 ligand and CD40 are known for their functional consequence in inflammatory bowel disease (IBD), with high levels of CD40 ligand observed in lamina propria T-cells in IBD patients compared to normal individuals (Liu *et al.*, 1999). The influence of bifidobacteria strains in reducing CD40 expression levels on dendritic cells was reported earlier (Hart *et al.*, 2004). An up-regulation of CD200 receptor-3 gene was observed in mice fed *L. acidophilus*, which belongs to immunoglobulin superfamily. The CD200 receptor-3 was capable of functioning as an activating receptor on mast cells and basophils to regulate immune responses (Kojima *et al.*, 2007). The mast cells are present in variety of tissues including organs that are exposed to external environment such as GI tract, where they actively involves in innate immune response to bacteria and viruses. Sialyltransferases (ST) relates to family of glycosyltransferase enzymes, which are also known to regulate immune functions. The down-regulation of ST6GAL1 gene was observed in mice fed *L. acidophilus*. In a previous study, ST6GAL1 knockout mice showed damaged humoral immune response evidenced by low levels of immunoglobulin-M and impaired B lymphocyte proliferation (Hennet *et al.*, 1998). *L. acidophilus* also modified the expression of matrix metallopeptidase (MMP)-2, which is an important enzyme implicated in tumor angiogenesis and metastasis (Nakajima and Chop, 1991). A recent study proposed MMP2 and tissue inhibitor of metalloproteinase-2 as predictive markers in cancer (Vasala and Turpeenniemi-Hujanen, 2007). Furthermore, *L. acidophilus* altered immune response genes also include complement component 4B, interleukin-23A and gap junction protein alpha-4.

Table 1: Genes (up- and down-regulated) in the small intestine of mice fed *L. acidophilus* are associated to a biological function or disease[†].

GenBank	Gene	Symbol	Fold change[‡]
AK007010	Antizyme inhibitor 1	AZIN1	1.53
NM_009756	Bone morphogenetic protein 10	BMP10	2.01
NM_009885	Carboxyl ester lipase (bile salt-stimulated lipase)	CEL	-2.78
AK003088	Carboxypeptidase A1 (pancreatic)	CPA1	-2.23
AK014671	CD200 receptor 3	CD200R3	1.63
NM_011616	CD40 ligand	CD40LG	-2.00
NM_007721	Chemokine (C-C motif) receptor 10	CCR10	-1.67
AK007772	Chymotrypsin C (caldecrin)	CTRC	-1.91
NM_023182	Chymotrypsin-like	CTRL	-2.04
NM_026419	Chymotrypsin-like elastase family, member 3B	CELA3B	-2.80
NM_025583	Chymotrypsinogen B2	CTRB2	-2.42
NM_009780	Complement component 4B (Chido blood group)	C4B	-2.17
NM_008411	CUB and zonapellucida-like domains 1	CUZD1	-1.50
NM_008120	Gap junction protein, alpha 4, 37kDa	GJA4	-1.85
NM_008182	Glutathione S-transferase alpha 5	GSTA5	-1.68
S65735	Glycoprotein M6A	GPM6A	-1.71
M12571	Heat shock 70kDa protein 1A	HSPA1A	1.57
AF277718	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7	HSD3B7	-2.63
AF301619	Interleukin 23, alpha subunit p19	IL23A	-2.28
AF226662	LIM homeobox transcription factor 1, alpha	LMX1A	-1.55
NM_008610	Matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)	MMP2	-1.58
NM_008590	Mesoderm specific transcript homolog (mouse)	MEST	-1.50
Y09010	Mitogen-activated protein kinase kinase kinasekinase 1	MAP4K1	1.58
NM_026925	Pancreatic lipase	PNLIP	-1.86
NM_009430	Protease, serine, 2 (trypsin 2)	PRSS2	-2.62
NM_023333	Protease, serine, 3	PRSS3	-1.89
NM_011160	Protein kinase, cGMP-dependent, type I	PRKG1	1.85
NM_009042	Regenerating islet-derived 1 alpha	REG1A	-1.60
NM_011271	Ribonuclease, RNase A family, 1 (pancreatic)	RNASE1	-1.56
NM_009789	S100 calcium binding protein G	S100G	-1.68
AK015564	Serine/threonine kinase 38 like	STK38L	-2.05
NM_013665	Short stature homeobox 2	SHOX2	-1.53
AK003278	Solute carrier family 46 (folate transporter), member 1	SLC46A1	-1.68
AF177147	ST6 beta-galactosamide alpha-2,6-sialyltranferase 1	ST6GAL1	-1.52
AK014184	Transmembrane protein 59	TMEM59	-1.56
NM_009363	Trefoil factor 2	TFF2	-1.59

[†]The association of genes to a biological function or disease were identified through Ingenuity Pathway Analysis (http://www.ingenuity.com).
[‡]Positive values denote up-regulation, whereas negative values denote down-regulation.

Table 2: The biological functions affected by *L. acidophilus* in the small intestine of mice.

Function†	Number of genes/function
Diseases and disorders	
Inflammatory response	7
Cardiovascular disease	5
Immunological disease	6
Antimicrobial response	1
Connective tissue disorder	5
Molecular and cellular functions	
Lipid metabolism	5
Molecular transport	5
Small molecule biochemistry	11
Vitamin and mineral metabolism	3
Protein degradation	7
Physiological system development and functions	
Haematological system development and function	8
Immune cell trafficking	8
Humoral immune response	3
Cardiovascular system development and function	4
Cell-mediated immune response	2

†Ingenuity Pathway Analysis (IPA; http://www.ingenuity.com) grouped the biological functions into three IPA categories.

The mucosal epithelium of GI tract forms as a protective barrier between host and external environment that can get damaged due to intestinal disorders. However, the restoration of intestinal epithelium rapidly occurs through involvement of several factors like regulatory peptides. Members of trefoil factor (TFF) family peptides are expressed in mucus secreting epithelial cells of the GI tract. The therapeutic potential of TFF2 was demonstrated through accelerated healing of gastric injury in mice (Sun *et al.*, 2009), which was down-regulated in mice fed *L. acidophilus* (Table 1). Furthermore, *L. acidophilus* was able to modulate mitogen-activated protein (MAP) kinase cascade signalling molecules that are involved in cell growth and differentiation through up-regulation of MAP4K1 gene. The genes that are affected by *L. acidophilus* also include carboxyl ester lipase and pancreatic lipase involved in lipid metabolism. The small intestine has a well developed gut-associated lymphoid tissue that consists of lamina propria lymphocytes, mesenteric lymph nodes and Peyer's patches. As a result, several studies investigated the beneficial effects of probiotic bacteria in the small intestine (Paturi *et al.*, 2007; Paturi *et al.*, 2010; Castillo *et al.*, 2011).

CONCLUSION

Microarray analysis demonstrated the ability of *L. acidophilus* modulating the expression of functional genes in the small intestine. Microarray profiling provided a snap-shot view on host gene expression in mice fed *L. acidophilus* and showed potential biomarkers that could be useful for future microbe-host interaction studies.

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