



# Lactobacillus Modulates Intestinal Host Defense Peptide Expression

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## ABSTRACT

Modulation of the synthesis of endogenous host defense peptides (HDPs) by probiotics represents a totally unique antimicrobial approach for disease control and prevention, particularly against antibiotic-resistant infections in human and animals. However, the extent of HDP modulation by probiotics is species dependent and strain specific. Within the present study, the porcine small intestinal vegetative cell line (IPEC-J2) cells and neonatal piglets were used as in-vitro and in-vivo models to see whether *Lactobacillus reuteri* I5007 could modulate intestinal HDP expression. Gene expressions of HDPs, toll-like receptors, and carboxylic acid receptors were determined, also as colonic short chain carboxylic acid concentrations and microbiota. Exposure to 10<sup>8</sup> colony forming units (CFU)/mL of *L. reuteri* I5007 for 6 h significantly increased the expression of porcine  $\beta$ -Defensin2 (pBD2), pBD3, pBD114, pBD129, and protegrins (PG) 1-5 in IPEC-J2 cells. Similarly, *L. reuteri* I5007 administration significantly increased the expression of jejunal pBD2 also as colonic pBD2, pBD3, pBD114, and pBD129 in neonatal piglets ( $p < 0.05$ ). This was probably related to the rise in colonic butanoic acid concentration and up-regulating expression of Peroxisome Proliferator-Activated Receptor- $\gamma$  (PPAR- $\gamma$ ) and G Protein-Coupled Receptor 41 (GPR41) ( $p < 0.05$ ), but not with stimulation of Pattern-Recognition Receptors. Additionally, supplementation with *L. reuteri* I5007 within the piglets didn't affect the colonic microbiota structure, PPAR- $\gamma$  and GPR41, but not through modifying gut microbiota structure.

**Keywords:** Lactobacillus, probiotics

## INTRODUCTION

Probiotics are “live microorganisms that when administered in adequate amounts confer a health benefit on the host”. The benefits of consuming bacteria have been known since ancient times, when fermented milk was commonly prescribed to treat an upset stomach. Today, the term “probiotic” has been defined and qualified by the World Health Organization, which put also forward guidelines to support their use. Accordingly, different probiotics are shown to stop or treat a good range of health issues, including tract infections, infectious diarrhea, atopic dermatitis related to cow's milk allergy, infant colic, NEC pouchitis, bacterial vaginosis, Clostridioides (formerly Clostridium) difficile-associated diarrhea, and tract infections.

Currently, commercial probiotic food products contain a spread of various probiotic species and strains. Certain health benefits are common to most or all probiotic species

These effects are considered “core benefits” and include the regulation of intestinal transit, normalization of perturbed microbiota, turnover of enterocytes, competitive exclusion of pathogens, colonization resistance, and short-chain carboxylic acid production. Meanwhile, some probiotic effects are found only among specific species of probiotics. Examples include vitamin synthesis, gut-barrier reinforcement, bile salt metabolism, enzymatic activity, and neutralization of carcinogens. Lastly, certain benefits may only be found among specific strains of bacteria

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This includes neurological effects, immunological effects, endocrinological effects, and therefore the production of bioactives.

Statistical analyses were performed using SPSS 17.0 Software (SPSS Inc., Chicago, IL, USA). All pairwise comparisons for the in vivo and in vitro data were examined using an unpaired Student's two-tailed t-test. Chi square was used to test differences in diarrhea incidence between the 2 groups. The level of significance was set at  $p < 0.05$ . The results were expressed as mean  $\pm$  standard error of the mean (SEM). Principle component analysis (PCA) plots were used to visualize differences in bacterial community composition among samples. The PCA plots were produced supported a euclidean metric. Linear discriminant analysis effect size (LEfSe) analysis was used to identify the OTUs or taxa, which were liable for the differences between the groups. An effect size threshold of two was used for the biomarkers discussed during this study. The metastats program from R-script was used to identify statistically different phylotypes among groups. Only taxa with average abundances greater than  $10^{-3}$ ,  $p < 0.05$  and low Q values (low risk of false discovery) were considered significant.

Data was derived from the Food Label Information Program (FLIP), a database of Canadian food package label information derived from major outlets of the three largest grocery chains in Canada (Loblaws, Metro, and Sobeys) and one major western retailer (Safeway). This database represents 75.4% of the grocery retail market share in Canada and provides a detailed assessment of the nutrition information found on Canadian packaged food labels.

## CONCLUSION

When multiple sizes of a product were available, just one size was collected. However, all flavors and sorts of a product were collected. Information collected for every product included the

Grocery store shelves were systematically scanned, and data for each foodstuff with a Nutrition Facts table (NFt), including all available national and personal label brands, were collected. Data for food products sold at multiple retailers were collected just one occasion.

Universal Product Code, company, brand, price, Nutrition Facts table information (serving size, calories etc.), ingredients, container size, nutrient content claims, disease risk reduction claims, function claims, front of pack symbols, children's marketing, and other claims (e.g., organic, natural, and gluten-free), additionally to the date and site of sampling. The FLIP database is updated every three years. Presently, two collections are completed (in 2010 and 2013) and are described in greater detail elsewhere. The packages were visually inspected, and ingredient lists of the 15,341 unique products collected in 2013 were searched to spot probiotic-containing products. Fermented foods were not considered to be probiotic products unless they were labeled as being probiotic.

The species, strain(s), and dosage found within the 92 probiotic containing products were recorded and tabulated. In July 2016, Loblaws, Metro, and Sobeys were revisited to spot if any probiotic strain and dosage information had changed and to research if new probiotic products had entered the marketplace. Four new probiotic products were identified and included in this study.