Effects of Probiotic Lactobacillus Paracasei Treatment on the Host Gut Tissue Metabolic Profiles Probed via Magic-Angle-Spinning NMR Spectroscopy


Department of Biomolecular Medicine, Division of Surgery, Oncology, Reproductive Biology and Anaesthetics, Faculty of Medicine, Imperial College London, Sir Alexander Fleming Building, South Kensington, London SW7 2AZ, UK, and Department of Nutrition and Health and Bioanalytical Sciences, Nestlé Research Center, P. O. Box 44, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland

Received November 13, 2006

We have used a simplified gnotobiotic mouse model to evaluate the effects of single bacterial species, Lactobacillus paracasei NCC2461, on the metabolic profiles of intact intestinal tissues using high-resolution magic-angle-spinning $^1$H NMR spectroscopy (HRMAS). A total of 24 female gnotobiotic mice were divided into three groups: a control group supplemented with water and two groups supplemented with either live L. paracasei or a $\gamma$-irradiated equivalent. HRMAS was used to characterize the biochemical components of intact epithelial tissues from the duodenum, jejunum, ileum, proximal, and distal colons in all animals and data were analyzed using chemometrics. Variations in relative concentrations of amino acids, anti-oxidant, and creatine were observed relating to different physiological properties in each intestinal tissue. Metabolic characteristics of lipogenesis and fat storage were observed in the jejunum and colon. Colonization with live L. paracasei induced region-dependent changes in the metabolic profiles of all intestinal tissues, except for the colon, consistent with modulation of intestinal digestion, absorption of nutrients, energy metabolism, lipid synthesis and protective functions. Ingestion of $\gamma$-irradiated bacteria produced no effects on the observed metabolic profiles. $^1$H MAS NMR spectroscopy was able to generate characteristic metabolic signatures reflecting the structure and function of intestinal tissues. These signals acted as reference profiles with which to compare changes in response to gut microbiota manipulation at the tissue level as demonstrated by ingestion of a bacterial probiotic.

Keywords: colon • duodenum • HRMAS $^1$H NMR spectroscopy • ileum • intact tissue • intestine • jejunum • Lactobacillus paracasei • Metabolomics • Metabonomics • Chemometrics • O-PLS-DA

Introduction

The gastrointestinal system has a histologically diverse epithelial structure superimposed on a complex longitudinal and cross sectional ordering of multiple connective and muscular cell types. The functional complex of the gastrointestinal tract is responsible for digestion, absorption, and propulsion of dietary components through the digestive tract and the ejection of waste. The intestinal surface is an important host organism-environment boundary and interactions of gut microbes in the gastrointestinal tract are important in immune system development. Symbiotic microbial species in the gastrointestinal tract also help to protect against opportunistic pathological infection, and the gut environment allows the intimate coexistence of dissimilar species that exert mutually beneficial effects on the host organism. This is well illustrated in a recent study on gnotobiotic mice colonized with Bacteroides thetaiotaomicron, where these micro-organisms were shown to devote a significant proportion of their genomic resource to allow an otherwise poorly accessible source of nutrients to be utilized by host metabolism. In addition, the presence of enteric gut microbiota affect intestinal morphology, physiology and modulate gut motility. Hence, understanding the interactions that occur between gut microbiota and their host may provide new insights into the aetiologies of many gastrointestinal diseases. As a consequence, much attention has recently been focused on the use of probiotics as food supplements, as a means of preventing allergies and inflammatory disease and for promoting gut health.

One approach to aiding the understanding of the interactions between micro-organisms and their hosts is to utilize a simplified mammalian ecosystem in the absence of bacteria and to evaluate the effects on host functions after colonizing the gut with a single microbial species. In the current study, the effects of Lactobacillus paracasei, a promising probiotic for human...
health-care, have been investigated in animals that were raised without any resident micro-organisms. It has been shown in vitro and in vivo that L. paracasei is able to shape the intestinal physiology, aid digestion and absorption of nutrients, as well as improve intestinal microbial balance and prevent infection of pathogenic bacteria. Moreover, L. paracasei has been shown to produce antagonist metabolites and antioxidants, to stimulate cells of the immune system in vitro, to induce a homeostatic micro-environment that favors tolerance rather than allergy in gnotobiotic mice, and to contribute to the normalization of gastrointestinal disorders.

Recently, gene expression profiling approaches have been applied to elucidate the molecular mechanisms underlying symbiotic host-bacterial relationships. Here, a complementary metabonomic approach has been employed for the biochemical characterization of metabolic changes triggered by gut microbiota. Metabonomics involves the study of the dynamic multi-parametric metabolic response of complex cellular organisms to biological stimuli or genetic modification, using in this case high resolution 1H magic-angle-spinning nuclear magnetic resonance (HRMAS NMR) spectroscopy coupled with multivariate statistical methods. MAS averages major line broadening factors including dipole–dipole interaction, chemical shift anisotropy and magnetic field inhomogeneities and results in narrow line width. HRMAS NMR spectroscopy is nondestructive and can provide molecular structural information as well as relative quantitative information on small intact tissue samples by generating a profile that contains physiological and pathological information. Recently, a HRMAS NMR-based approach has been successfully applied to characterize the biochemistry of rat intestine development and healthy human gastric mucosa, and to study multi-organ effects of drug-induced toxicity in animal models, metabolic compartmentation in the rat heart and cardiac mitochondria and to investigate the “microbe-mammalian metabolic axis” interactions in a Trichinella spiralis-induced Irritable Bowel Syndrome mouse model.

In the present study, we have extended our metabolic studies to characterize the biochemical composition of intact intestinal mouse tissues in the absence of microbiota and then to evaluate the impact of colonization of gnotobiotic animals with both live and y-irradiated L. paracasei.

Materials and Methods

Animal Handling Procedure and Sample Collection. All animal studies were carried out under appropriate national guidelines at the Nestlé Research Center. A total of 24 gnotobiotic C3H female mice, age 6 weeks, were randomly assigned to 3 groups of 8 mice and all received the same standard diets. L. paracasei, strain NCC2461, was obtained from the Nestlé Culture Collection. At day 0, one group of gnotobiotic mice was kept as control and received water only by oral gavage, one group received a single dose of live bacteria (log_10 cells = 8.78) and one group received y-irradiated bacteria (log_10 cells = 9.18) both in their spent culture medium. The maintenance of pathogen-free conditions and the intestinal establishment of L. paracasei were evaluated by bacterial counting using single freshly collected fecal pellet at timed intervals over the duration of the experiment. Briefly, for each mouse 1 fecal pellet was homogenized in 0.5 mL Ringer solution (Oxoid, UK) supplemented with 0.05% (w/v) l-Cystein (HCl), weighed, homogenized, and plated onto MRS (Man Rogosa Sharpe) agar plates (Oxoid). Plates were incubated anaerobically for 48 h at 37 °C. As a secondary objective of the study that is not evaluated here, all mice also received a single intra-gastric inoculation of whey proteins at day 15 after the start of the experiment for induction of tolerance to β-lactoglobulin. Five days after oral tolerance induction, at day 19, the animals were immunized with specific β-lactoglobulin antigen via subcutaneous injections. Animals were euthanized at day 30 by isoflurane anaesthesia followed by exhaustive bleeding by sampling the cardiac aorta. After sacrifice, the intestine was removed from each animal. The first 1 cm of gut after the stomach was designated as duodenum and the rest of the intestine to the caecum was divided into three sections, the first 2/3 were designated as jejunum and remaining 1/3 as ileum, and 3 cm samples were excised from the middle of each section. The colon was separated into two parts, defined as proximal and distal colon. Microbiological tests were carried out on jejunum, ileum and colon tissues to verify absence of contamination and the L. paracasei population, as described by Guigoz et al. Each sample was flushed using an iso-osmotic phosphate buffer solution (pH 7) and then snap-frozen in liquid nitrogen and then preserved at −80 °C prior to NMR spectroscopic analysis.

Sample Preparation and 1H NMR Spectroscopic Analysis. Intact intestinal tissue samples were bathed in 0.9% saline D2O solution. A portion of each tissue (approximately 15 mg) was inserted into a zirconium oxide 4 mm outer diameter rotor, using an insert to make a spherical sample volume of 25 μL. A drop of deuterated isotonic saline solution was added to provide a field-frequency lock for the NMR spectrometer. All 1H NMR spectra were recorded on a Bruker AV-600 NMR spectrometer (Rheinstetten, Germany) operating at 600.11 MHz for 1H, equipped with a high-resolution magic-angle-spinning probe at a spin rate of 5000 Hz. Sample temperature was regulated using cooled N2 gas to 283 K during the acquisition of spectra to minimize biochemical degradation.

Three different types of 1H NMR spectra were collected for each sample, a standard one-dimensional (1D) spectrum with water suppression, a 1D Carr-Purcell-Mel boom-Gill (CPMG) spin−echo spectrum, and a diffusion-edited spectrum. Because the CPMG spin−echo experiment gave the clearest signature of metabolic changes following intervention, with little extra information contained in the higher molecular weight components observed in the other types of spectra, only the results derived from the CPMG experiments are presented here. CPMG spin−echo spectra were acquired using the pulse sequence [RD-90°-(t-180°-t)_n - acquire FID], with a spin−spin relaxation delay, 200 μs, of 200 ms. The 90° pulse length was 9.0−12 μs. A total of 128 transients were collected into 32 K data points. The recycle delay (RD) was 2 s. For assignment purposes, 2D correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY) NMR spectra were acquired on selected samples. In both cases, 48 transients per increment and 256 increments were collected into 2 K data points. The spectral width in both dimensions was 10 ppm. The TOCSY NMR spectra were acquired by using the MLEV-17 25 spin-lock scheme with a spin-lock power of 6 kHz. COSY spectra were recorded with gradient selection. In both 2D NMR experiments,
L. Paracasei Treatment on Gut Tissue

Results

Microbiological Status of the Animals. An absence of bacterial development was observed in the gnotobiotic mice and in the group of mice supplemented with irradiated bacteria. For the mice supplemented with probiotics, an L. paracasei population was established in both the small intestine and the colon. The populations of L. paracasei were significantly greater in the colon than in the jejenum or ileum with values of log10 (Colony Forming Unit ± standard deviation) of 9 ± 0.4 (colon), 6.6 ± 0.9 (jejenum), and 6.3 ± 1.4 (ileum).

NMR Spectra of Intestinal Tissues from Gnotobiotic Mice. Representative metabolic profiles of each intestinal tissue, i.e., duodenum, jejunum, ileum, proximal, and distal colons, were obtained using 1H MAS NMR spectroscopy. Typical examples of 1H MAS CPMG NMR spectra of gut sections obtained from a gnotobiotic mouse are illustrated in Figure 1. From these spectra, 49 metabolites were unambiguously assigned, and their chemical shifts and peak multiplicity are given in Table 1 along with the corresponding 1H NMR chemical shifts and signal multiplicities. Assignment of metabolites was made by comparison with published literature and confirmed by 2D COSY, TOCSY, and STOCSY methods.

The spectra from all tissues contained resonances from a number of amino acids, organic acids, triglycerides, fatty acids, and glycerol as well as choline, inosine, myo-inositol, scyllo-inositol, ethanolamine, and membrane components. Pyrimidine metabolites such as uracil and cytosine were also detected. Other assigned metabolites including creatine, glucose, ethanol, glutathione, trimethylamine-N-oxide (TMAO), betaine, and N-acetylglutamate were identified in some of the tissues.

Visual inspection of the 1H NMR spectra shown in Figure 1 reveals that each tissue produces its own specific metabolite
fingerprint that allowed differentiation between tissues. Among
the most striking observations of intestinal composition, the
duodenum tissues showed high levels of amino acids compared
to other tissues, whereas the jejunum contained higher levels
of lipids. In addition, high levels of taurine, glutathione,
creatine, and oxaloacetate were observed in both the jejunum
and the ileum. An abundance of choline and other membrane components was also observed in the jejunum and ileum. The colon tissues showed higher levels of lipids, phosphorylcholine and uracil than the small intestinal tissues. In particular, the distal colon manifested high levels of myo-inositol and scyllo-inositol and low levels of cystosine.

Multivariate Statistical Analysis of NMR Spectra from Gnotobiotic Murine Tissues. To better characterize the metabolic profile of each intestinal section with respect to the others, O-PLS-DA was performed on 1H MAS CPMG NMR spectra obtained from the 5 classes of gut tissues from gnotobiotic mice. The cross-validated scores plot showed statistically significant separations between the different gut compartments as presented in the type of plot that we term a “differential metabogram” (Figure 2 A), with the strongest differences between the small and large intestine. Discrimination of tissues within the small bowel and colon was also achieved, with some overlap between adjacent compartments (for example, duodenum and jejunum). The corresponding O-PLS-DA coefficients identified the specific metabolites associated with each gut section (positive values) with respect to the rest of the intestinal tissues (negative values) (Figure 2 B–F). The weight of a variable in the discrimination is given by the square of its correlation coefficient ($r^2$), which is color coded from zero in blue to high values (0.6–0.7) in red.

In addition, further characterization of the intestinal metabolic profiles was achieved using O-PLS-DA with pairwise comparisons between intestinal compartments to better identify subtle differences. Statistically significant discrimination between each tissue was achieved, and Table 2 summarizes the characteristics of each model. To better visualize the longitudinal metabolic differences along the intestine, the sequential statistical comparison of each segment to its adjacent segment from proximal to distal intestine is depicted in Figure 3. The coefficients plots (Figure 3) show the dominant metabolites that influence the differentiation between adjacent tissues, and these are listed in Table 3. In addition, relative changes displayed using box-and-whiskers plots showed dispersion of the relative concentrations among the animals which are in agreement with the multivariate data analysis (see Supplementary Figure 1).

The duodenum was differentiated from all the other intestinal tissues by relatively high levels of observable glutathione, ethanolamine, glycerophosphorylethanolamine (GPE), and amino acids including alanine, valine, isoleucine, leucine, threonine, methionine, lysine, arginine, tyrosine, phenylalanine, and cysteine. In addition, relative higher levels of choline and glycerophosphorylcholine (GPC) were observed when comparing the duodenum to the adjacent jejunum. Jejunal tissue showed relative lower contents of glutamine and glutamate, higher levels of phosphocholine and phosphorylethanolamine when compared to all other intestinal tissues. Relative elevated triglycerides, uracil, creatine and in particular glutathione were additionally observed in the jejunal tissues when compared to duodenum. Moreover, distinction between the gnotobiotic jejunum and ileum was also observed due to relative higher levels of triglycerides and amino acids including alanine, glycine, glutamate, aspartate, and asparagine in the jejunum (Figure 3).

The ileal profile was associated with relative high levels of glutathione, taurine, oxalacetate, phosphorylcholine, and betaine when compared to other tissues. Pairwise O-PLS-DA also revealed that the ileal tissue contained relative higher levels of acetoacetate and GPC when compared to the jejunum. In addition, levels of lactate, glycine, choline, GPC, ethanolamine, and GPE were relatively higher in ileal tissue than in the colon.

The proximal colon showed relative higher levels of triglycerides, uracil, acetate, and propionate than other tissues whereas the distal colon showed relative high concentrations of creatine, myo-inositol, scyllo-inositol, N-acetyl-glutamate, and lower levels of ethanolamine. When comparing the two regions of the colon, OPLS-DA showed that the distal colon contained relative higher levels of phosphorylcholine, myo-inositol and scyllo-inositol, creatine, N-acetyl-glutamate, and uracil (Figure 3), whereas the proximal colon contained relative higher levels of propionate, acetate, oxalacetate, alanine, formate, and cystosine. The variations in longitudinal metabolic profiles of the gut are likely to have strong functional significance and will be considered later.

**Metabolic Changes Induced by L. paracasei in Intestinal Tissues.** Visual inspection of the 1H NMR spectra shown in Supplementary Figure 2 does not reveal specific metabolite fingerprint that allowed differentiation between tissues from controls and probiotic supplemented mice. To characterize the effects of live and irradiated *L. paracasei* in the intestinal metabolic profiles, O-PLS-DA was applied to 1H CPMG MAS NMR spectra of gut tissues obtained from germ-free mice and those supplemented with live or irradiated probiotics. The comparison of the metabolic profiles from control gnotobiotic mice and those supplemented with live *L. paracasei* indicated that the probiotics induced changes in the small intestine (duodenum, jejunum, and ileum) as described by the statistical model descriptors shown as coefficients plots (Figure 4). These show the metabolites exerting the strongest influence on the separation of the two animal classes, i.e., germ-free and live probiotic supplemented. These significant metabolites are listed in Table 4. Moreover, no probiotics-induced metabolic variation was observed in the colon as indicated by the negative values of the model $Q^2$ parameter meaning that the discriminant function has no statistical power. In contrast with live probiotics supplementation, metabolic profiling established that supplementing mice with irradiated bacteria did not induce significant changes in the biochemical composition of the tissues, as also indicated by the negative values of the $Q^2$ parameter obtained for the statistical model generated.

In the duodenum, establishment of *L. paracasei* increased the relative level of tissue choline, but decreased the amounts of serine and glutamine (Figure 4). In the jejunum and ileum, relative levels of glutamate, glutathione, methionine, acetate, alanine, glycine, creatine, choline, and GPC were decreased with the probiotic supplementation (Figure 4). In addition, the probiotic gavage was correlated with relative decreased levels of cysteine and an unknown compound in the ileal tissues and relative increased concentrations of lactate in the jejunum (Figure 4).

**Discussion**

The aims of the present study were to define a metabolic profile for each intestinal tissue from female germ-free mice and to evaluate the impacts of *L. paracasei* on intestinal tissue physiology and host metabolism. The metabolic variations observed between tissues are discussed in relation to functional biochemical properties of the gut wall pertaining to digestion, contractility and protection from oxidative stress.
Digestive Functions. A major source of the intestinal metabolites comes from further processing of dietary nutrients by intestinal cells after digestion and absorption, and this occurs mainly in the first quarter of the small intestine (duodenum and jejunum). The importance of these nutrients in the tissue metabolic profiles were consistent with previous reports (Figures 2 and 3). For instance, the final digestion of proteins and lipids that produces absorbable products takes...
place among the microvilli, and lipid-soluble components are able to penetrate in the epithelial membranes and diffuse into the mucosal cells.29

The digestion of alimentary phospholipids produces a variety of intestinal lipids, free fatty acids, phosphoethanolamine, and GPE.30 Furthermore, dietary phosphatidylcholine is hydrolyzed in the intestinal lumen to 1-acylglyceryl-phosphorylcholine, which on entering the mucosal cell is mainly converted to phosphatidylcholine and GPC.31 The observation of relative elevated concentrations of lipids, phosphoethanolamine, GPE, choline, phosphorylcholine, and GPC in the duodenum and jejunum from germ-free mice is consistent with these reports (Figures 2 and 3). In particular, the jejunum contained relative high levels of phosphorylcholine and lipids when compared to the duodenum and ileum (Figure 3). This is consistent with previous studies showing that jejunum is primary location for digested fat storage.32,33 Also, the essential role of choline, phosphorylcholine, and GPC in cell metabolism, signaling processes and membrane constitution, as well as the osmolyte function of taurine and betaine in the intestine have been described previously.34-36 In addition, choline, GPC, and phosphorylcholine also play a crucial role in lipid cholesterol transport and metabolism.35 Hence, their relative high concentrations in the overall composition of the intestinal tissues are consistent with their known functions. Furthermore, relative higher level of taurocholate was observed in the ileum (Figure 3) when compared to other compartments. The ileum is known to actively reabsorb 90–95% of all the luminal tauroconjugated bile acids.37

Similarly to jejunum, colon is characterized by its lipid biosynthetic ability,32,38-40 which is consistent with its relative higher lipid content when compared to the small intestine (Figure 3). Moreover, the distal colon of germ-free mice also showed relative lower levels of propionate, acetate, formate, oxaloacetate, and cytosine when compared to the proximal colon (Figure 3). Acetate and propionate are a significant carbon source for the synthesis of lipids, and in particular for phospholipids, whereas formate and oxaloacetate are intermediates in glyceroneogenesis to synthesize triacylglycerols.38 In germ-free models, it has been suggested that the relative reduced concentrations of these short chain fatty acids and cytosine are related to requirements for intestinal lipid biosynthesis.38,39 Unlike jejunum, the colon does not transport or absorb lipids efficiently.41 Both the proximal and distal colons of germ-free mice showed relative lower levels of choline, GPC and phosphorylcholine as compared to the small intestine (Figures 2 and 3) consistent with reduced lipid transport as suggested by previous workers.34,42

Recent studies established that animals with a normal microbiota have a 30% greater “calorific bioavailability” than their germ-free counterparts.3 This is due to the conversion of many dietary substances into absorbable nutrients by microbial metabolism.3 The investigation of the metabolic profiles from germ-free mice re-colonized with L. paracasei revealed a relative elevation of choline in the duodenum and lactate in the jejunum (Figure 4). Lactate is a major product of Lactobacillus metabolism,43 and data suggest an extended microbial-induced processing of dietary nutrients, including bacterial fermentation, leading to higher absorption of lactate.43 Recently,

---

Table 2. Summary of O-PLS-DA Models for the Different Discriminations among 1H MAS CPMG NMR Spectra of Intestinal Tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Proximal colon</th>
<th>Distal colon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duodenum</strong></td>
<td>Q²=0.81, R²=0.99</td>
<td>Q²=0.76, R²=0.99</td>
<td>Q²=0.86, R²=0.99</td>
<td>Q²=0.89, R²=0.99</td>
</tr>
<tr>
<td><strong>Jejunum</strong></td>
<td>Q²=0.60, R²=0.99</td>
<td>Q²=0.655, R²=0.99</td>
<td>Q²=0.74, R²=0.98</td>
<td>Q²=0.74, R²=0.99</td>
</tr>
<tr>
<td><strong>Ileum</strong></td>
<td>Q²=0.75, R²=0.99</td>
<td>Q²=0.74, R²=0.99</td>
<td>Q²=0.74, R²=0.99</td>
<td>Q²=0.74, R²=0.99</td>
</tr>
<tr>
<td>**Proximal colon</td>
<td>Q²=0.51, R²=0.97</td>
<td>Q²=0.51, R²=0.97</td>
<td>Q²=0.51, R²=0.97</td>
<td>Q²=0.51, R²=0.97</td>
</tr>
</tbody>
</table>

O-PLS-DA models were generated with 1 predictive component and 2 orthogonal components to discriminate between 2 groups of mice. The R² value shows how much of the variation in the dataset X is explained by the model. The Q² value represents the predictability of the models and relates to its statistical validity.
the gut microbiota have been implicated as an important environmental factor that regulates fat synthesis and storage in the host cells. In particular, the jejunum was proposed to be a major site for dietary fat accumulation. Interestingly, mice supplemented with *L. paracasei* showed specific metabolic changes in both the jejunum and the ileum, with relative lower concentrations of choline, GPC and acetate (Figure 4). These metabolites have been described as key intermediates of lipid synthesis, suggesting that *L. paracasei* has the capacity to modulate intestinal fat metabolism.

Dietary supplementation with live *L. paracasei* reduced the levels of glutamine and serine in both the duodenum and jejunum (Figure 4), which were specifically characterized as crucial metabolic fuels used by intestinal enterocytes for rapid division, repairing of epithelial cells, nutrient transport, and protein turnover (Figures 2 and 3). Nutrient utilization is known to be less efficient in gnotobiotic as compared to conventional mice. Therefore, germ-free intestines generally show changed gut morphology with increased surface area to compensate for the less efficient nutrient usage. These malformations include atypical epithelial structure, longer villi, and shorter crypts containing fewer cells. As a consequence, energy requirements for cell division and growth are augmented in gnotobiotic intestines as suggested by the relative reduction of glutamine and serine seen in the intestinal tissues from germ-free mice supplemented with live *L. paracasei*. The results presented here are consistent with the reported contribution of symbiotic bacteria to the amino acid homeostasis and nutritional status of the host.

### Key Observed Metabolic Differences between Sequential Intestinal Compartments

<table>
<thead>
<tr>
<th>metabolite (key)</th>
<th>δ (ppm)</th>
<th>duodenum vs jejunum</th>
<th>jejunum vs ileum</th>
<th>ileum vs proximal colon</th>
<th>proximal vs distal colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetate (9)</td>
<td>1.91(s)</td>
<td>0.73 (5)</td>
<td>−0.71 (6)</td>
<td>0.58 (12)</td>
<td></td>
</tr>
<tr>
<td>acetoacetate (46)</td>
<td>2.29(s)</td>
<td>−0.84 (1)</td>
<td>−0.71 (6)</td>
<td>0.66 (8)</td>
<td></td>
</tr>
<tr>
<td>acetyl-glutamate (41)</td>
<td>1.95(s)</td>
<td>−0.62 (6)</td>
<td>0.64 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alanine (6)</td>
<td>1.47(d)</td>
<td>0.77 (3)</td>
<td>0.74 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>arginine (7)</td>
<td>1.63(m)</td>
<td>−0.84 (2)</td>
<td>0.77 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>asparagine (16)</td>
<td>2.94(m)</td>
<td>−0.76 (4)</td>
<td>0.77 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aspartic acid (15)</td>
<td>2.68(m)</td>
<td>−0.77 (3)</td>
<td>0.77 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>betaine (47)</td>
<td>3.90(s)</td>
<td>0.85 (1)</td>
<td>0.76 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>choline (19)</td>
<td>3.21(s)</td>
<td>0.76 (4)</td>
<td>0.71 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>creatine (17)</td>
<td>3.03(s)</td>
<td>0.79 (10)</td>
<td>0.71 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cysteine (43)</td>
<td>3.10(m)</td>
<td>0.78 (11)</td>
<td>0.76 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cytosine (37)</td>
<td>5.92(d)</td>
<td></td>
<td>0.76 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unknown (40)</td>
<td>4.41(s)</td>
<td>0.65 (9)</td>
<td>0.67 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ethanol (4)</td>
<td>1.18(t)</td>
<td>0.67 (17)</td>
<td>0.63 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ethanolamine (18)</td>
<td>3.15(t)</td>
<td>0.89 (9)</td>
<td>0.79 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>formate (34)</td>
<td>8.45(s)</td>
<td>0.82 (7)</td>
<td>0.84 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>glutamate (12)</td>
<td>2.34(m)</td>
<td>0.82 (7)</td>
<td>0.84 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>glutamine (14)</td>
<td>2.44(m)</td>
<td>0.88 (2)</td>
<td>0.84 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>glutathione (24)</td>
<td>2.55(m)</td>
<td>−0.74 (13)</td>
<td>0.74 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>glycine (23)</td>
<td>3.55(s)</td>
<td>0.68 (16)</td>
<td>0.74 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPC (21)</td>
<td>3.22(s)</td>
<td>0.82 (7)</td>
<td>0.74 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPE (45)</td>
<td>4.12(m)</td>
<td>0.81 (8)</td>
<td>0.74 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>isoleucine (1)</td>
<td>1.02(d)</td>
<td>0.87 (3)</td>
<td>0.74 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lactate (5)</td>
<td>4.11(q)</td>
<td>0.67 (11)</td>
<td>0.74 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>leucine (2)</td>
<td>0.91(d)</td>
<td>0.88 (2)</td>
<td>0.74 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lipids (25)</td>
<td>1.27(m)</td>
<td>−0.76 (12)</td>
<td>0.67 (8)</td>
<td>−0.62 (10)</td>
<td></td>
</tr>
<tr>
<td>lysine (8)</td>
<td>1.72(m)</td>
<td>0.86 (4)</td>
<td>0.74 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>methionine (13)</td>
<td>2.13(s)</td>
<td>0.87 (3)</td>
<td>0.74 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myo-inositol (30)</td>
<td>4.06(i)</td>
<td>0.77 (2)</td>
<td>0.58 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxaloacetate (29)</td>
<td>2.38(s)</td>
<td>−0.77 (2)</td>
<td>0.58 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>phenylalanine (28)</td>
<td>7.40(m)</td>
<td>0.81 (8)</td>
<td>0.58 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>phosphorylcholine (20)</td>
<td>3.22(s)</td>
<td>−0.84 (6)</td>
<td>0.82 (2)</td>
<td>−0.64 (10)</td>
<td></td>
</tr>
<tr>
<td>phosphorylthanolamine (44)</td>
<td>4.00(m)</td>
<td>0.7 (15)</td>
<td>0.82 (2)</td>
<td>−0.64 (10)</td>
<td></td>
</tr>
<tr>
<td>proline (11)</td>
<td>2.00(m)</td>
<td>0.84 (6)</td>
<td>0.82 (2)</td>
<td>−0.64 (10)</td>
<td></td>
</tr>
<tr>
<td>propionate (39)</td>
<td>1.06(t)</td>
<td>0.66 (8)</td>
<td>0.74 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>scyllo-inositol (31)</td>
<td>3.35(s)</td>
<td>−0.8 (1)</td>
<td>0.74 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>serine (42)</td>
<td>3.96(m)</td>
<td>0.81 (8)</td>
<td>0.74 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>taurine (22)</td>
<td>3.40(t)</td>
<td>−0.72 (14)</td>
<td>0.57 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>threonine (10)</td>
<td>4.25(m)</td>
<td>0.9 (1)</td>
<td>0.57 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMAO (26)</td>
<td>3.26(s)</td>
<td>0.72 (14)</td>
<td>0.57 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tyrosine (27)</td>
<td>6.87(dd)</td>
<td>0.8 (9)</td>
<td>−0.66 (8)</td>
<td>−0.65 (9)</td>
<td></td>
</tr>
<tr>
<td>uracil (35)</td>
<td>5.78(d)</td>
<td>−0.66 (8)</td>
<td>−0.65 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>valine (3)</td>
<td>1.04(d)</td>
<td>0.9 (1)</td>
<td>−0.65 (9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Key numbers correspond to those shown on the 600 MHz spectra in the Figures. *a*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *m*, multiplet; *dd*, doublet of doublets. *b* Ranking is computed from the selected variables for each model according to their O-PLS weights.
These changes were associated with relative increased amount of alanine and glutamine suggested fewer interconversions of amino acids to produce energy via Cori cycle. These results are in agreement with other reports of the crucial role of *L. paracasei* in regulating hypercontractility of intestinal muscle and microbial importance in modulating gastrointestinal motility.

**Possible Protective Mechanisms against Gut Inflammation.** Glutathione was observed in the mucosal cells, and conventional mice have been previously shown to have relative higher concentrations in the duodenal cells when compared to ileal and colon cells. The primary function of glutathione is to protect cells from oxidative stress, and a relation between glutathione concentration and mucosal damage has been established in diverse gastrointestinal diseases. Glutathione plays a important anti-oxidant role and may prevent gut disorders because it neutralizes reactive oxygen species that are possible mediators of inflammatory bowel disease. Here, the metabolic profile of germ-free intestinal tissues showed that the relative highest concentrations of glutathione were in the duodenum and ileum (Figure 2).

When mice were colonized with *L. paracasei*, relative lower levels of glutathione and its precursors glutamate, methionine, cysteine, and glycine were observed in the jejunum and ileum (Figure 4). These observations suggest a probiotic-induced effect on the γ-glutamyl cycle leading to a modulation of the antioxidant ability of the gut and this might be associated with altered sulfur and cysteine metabolism. Additionally, bacterial colonization led to relative decreased levels of taurine and creatine in the small intestine when compared to gnotobiotic controls, which is consistent with previous observations. Other studies showed that microbiota decrease intestinal levels of glutathione, which is in agreement with the work presented here. The probiotic-induced ability to modulate antioxidant enzymes in intestinal tissue may be of important interest in understanding the mechanism of gastrointestinal cancers. Because intestinal amino acid catabolism plays an important role in modulating dietary amino acid availability to extra-intestinal tissues and intestinal synthesis of glutathione, this work suggest that *L. paracasei* may affect enterocyte glutathione metabolism due to its known antioxidant ability and by regulation of the catabolism of dietary nutrients.

**Effects of Ingestion of Live and Irradiated Bacteria.** An observation requiring further discussion is the absence of live...
bacterial-induced changes in the metabolic profile of the colon. Different gut microbiota were shown to modulate the host intestinal transcriptome in both the small intestine and colon and also the systemic metabolism and immune responses as compared to gnotobiotic controls.\textsuperscript{2,3,14,65} We have also shown the influences of \textit{L. paracasei} supplementation to conventional mice infected with \textit{Trichinella spiralis} and have demonstrated intricate metabolic changes in the jejunal wall and longitudinal myenteric muscular tissues using NMR-based metabonomics.\textsuperscript{20} The results presented here show intestinal region-dependent metabolic changes triggered by ingestion of live \textit{L. paracasei} in initially germ-free mice. Distinct \textit{Lactobacillus} strains have been suggested to colonize the gut differently and hence to generate divergent host responses.\textsuperscript{8} Colon tissues from animals supplemented with \textit{L. paracasei} were found to be metabolically similar to those from germ-free animals, even though there were ten times more bacteria in the colon than in the small intestine. Interestingly, transcriptomic data have indicated that gut microbiota modulate limited changes in colon when compared to the small intestine. The reported transcriptional changes are related mainly to the biological functions affecting water absorption.\textsuperscript{64} Therefore, it might not be surprising that using NMR-based techniques, no metabolic changes in colon tissues were found upon re-colonization of colon with bacteria. However, an elevation in short chain fatty acids, including lactate and acetate was observed in colon flushes of bacterial associated colon (data not shown). These indicate bacterial fermentation and do not reflect colon metabolic status.

Finally, irradiation-killed bacteria did not change the metabolic profiles of the intestinal tissues when compared to the gnotobiotic controls (Table 3). This indicates that the metabolic changes observed with live bacteria are probably due to genuine host-bacterial interactions rather than to the simple digestion of bacterial cells or to purely sensing of bacterial components by the host. Moreover, although no measurement of animal weight and food intake were recorded, it remains possible that the variations of the tissue phenotypes result specifically from probiotics-induced effect on food intake.LEY et al.\textsuperscript{46} and Turnbaugh et al.\textsuperscript{47} reported recently that the gut microbiota is a contributing factor to the pathophysiology of obesity by stimulating energy recovery from the diet, while reducing nutritional consumption. Our findings also illustrate the influences of gut microbe variations on mammalian biology and the complex metabolic interactions involving more than one genome, which has been termed "the microbial-mammalian metabolic axis".\textsuperscript{12,68}

The biochemical components of the duodenum, jejunum, ileum, proximal, and distal colons in gnotobiotic mice could be characterized by the use of nondestructive HRMAS NMR spectroscopy. Chemometrics methods allowed discrimination of the effects of probiotic treatment in gut epithelial metabolism relating to topographic variation in nutrient absorption, propulsion and detoxification functions. This work also demonstrates the potential of metabolic profiling for studying properties of the "microbe-mammalian metabolic axis" and probiotic modulations of symbiont-host biochemistry. Contrary to the effects induced by live \textit{L. paracasei}, no changes were seen with supplementation of irradiation-killed bacteria. These modifications of the intestinal metabolic profile were shown to be region-dependent and correlated with the intestinal functions as manifested by the effects on digestion, absorption, amino acid homeostasis, fat synthesis, and protection of oxidative stress. The results presented here demonstrate the importance of the transgenomic interactions between \textit{L. paracasei} and the host, at the metabolic level to modulate the gut development.\textsuperscript{67} We have also demonstrated that \textit{1H} MAS NMR spectroscopy produces a stable metabolic signature for each intestinal compartment with respect to their structure/function and has also generated reference profiles with which to compare changes in response to gut microbial manipulation.

**Abbreviations**: CFU, colony forming unit; COSY, \textit{1H}–\textit{1H} correlated spectroscopy; CPMG, Carr-Purcell-Meiboom-Gill; GPC, glycerophosphorylcholine; GPE, glycerophosphorylethanolamine; HRMAS, high resolution magic-angle-spinning; MRS, Masson Marchesini, and Catherine Schwartz. This work received financial support from Nestlé to F.-P.J.M. and Y.W.

**Supporting Information Available**: Supplementary Figures 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

**References**

L. Paracasei *Treatment on Gut Tissue*


(49) Metges, C. C. *J. Nutr.* 2000, 130, 1857S – 1864S.


PR060596A