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SHORT COMMUNICATION

Convergence in probiotic *Lactobacillus* gut-adaptive responses in humans and mice

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Probiotic bacteria provide unique opportunities to study the global responses and molecular mechanisms underlying the effects of gut-associated microorganisms in the human digestive tract. In this study, we show by comparative transcriptome analysis using DNA microarrays that the established probiotic *Lactobacillus plantarum* 299v specifically adapts its metabolic capacity in the human intestine for carbohydrate acquisition and expression of exopolysaccharide and proteinaceous cell surface compounds. This report constitutes the first application of global gene expression profiling of a commensal microorganism in the human gut. A core *L. plantarum* transcriptome expressed in the mammalian intestine was also determined through comparisons of *L. plantarum* 299v activities in humans to those found for *L. plantarum* WCFS1 in germ-free mice. These results identify the niche-specific adaptations of a dietary microorganism to the intestinal ecosystem and provide novel targets for molecular analysis of microbial–host interactions which affect human health.

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Probiotic strains of *Lactobacillus* confer health benefits in the human gut and have a long history of safe use in high amounts (Floch *et al.*, 2008). These organisms are therefore attractive models for quantifying the adaptations of gut-adapted commensal microorganisms to the conditions in the human digestive tract. *Lactobacillus plantarum* 299v is a commercially applied probiotic strain known to alleviate irritable bowel syndrome and reduce enteropathogen colonization *in vivo* (de Vries *et al.*, 2006; Klarin *et al.*, 2008). *L. plantarum* 299v and the highly related strain *L. plantarum* WCFS1 (Kleerebezem *et al.*, 2003; Molenaar *et al.*, 2005) are able to survive in the human gut for extended periods of time (Vesa *et al.*, 2000; Molin, 2001). *L. plantarum* WCFS1 was also shown to differentially induce nuclear factor-κB-dependent pathways in the human intestine (van Baarlen *et al.*, 2009) and specific cell products expressed by this strain were found to modulate anti-inflammatory responses *in vivo* (Grangette *et al.*, 2005).

To determine the *L. plantarum* molecular properties expressed in the human intestine, which might be responsible for adaptation to the gut ecosystem and modulation of intestinal cell function, transcript profiling was performed on biopsy samples collected from healthy intestinal tissue during surgery of three colon cancer patients (see Supplementary Materials). For 8 consecutive days before surgery, the subjects consumed 100 ml of a fermented oatmeal drink containing 10^9 colony-forming units of *L. plantarum* 299v per ml. Biopsy samples were collected from normal tissue of the ileum (subject A) and colon (subjects A, B and C). A biopsy sample was also collected from a fourth subject (D) who had consumed a placebo oatmeal product without the probiotic culture. RNA was isolated from mucosal scrapings of the intestinal segments and hybridized to *L. plantarum* WCFS1 microarrays (Supplementary Materials). Microarray hybridization intensities for total mucosal RNA isolated from the biopsies of subjects A to C showed enrichment of *L. plantarum* in the mucosa compared with the placebo control (subject D) (data not

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shown). Real-time reverse transcriptase PCR on *L. plantarum* 16S rRNA from the biopsies of subjects who had consumed the probiotic drink confirmed the presence of elevated amounts of *L. plantarum* over a 40-fold range and ⩾160-fold more *L. plantarum* compared with the control subject (subject D) (Supplementary Materials and Supplementary Table S1). These data confirmed the enrichment of *L. plantarum* 299v RNA in the human mucosa and minimal interference of the indigenous gut microbiota, including *Lactobacillus* species, on the transcriptomes obtained for *L. plantarum* 299v.

Global gene expression profiles of *L. plantarum* 299v were conserved in the ileum and colon within one individual and between the genetically unrelated human subjects (Figure 1). According to principal component analysis, these profiles were distinct from transcriptomes of *L. plantarum* WCFS1 in mono-associated mice fed a diet rich in plant polysaccharides (chow diet) or a 'Western' diet high in saturated and unsaturated fats (41% of the total calories) and sucrose (18% of the chow weight) (Marco *et al.*, 2009) (Figure 1). These differences might have been at least partially due to the use of germ-free mice lacking an indigenous microbiota, which are likely to affect microbe–microbe interactions and nutrient availability in the gut. Moreover, global gene expression profiles of *L. plantarum* in the mice were strongly correlated to host diet (Figure 1) (Marco *et al.*, 2009). According to the first component constituting the majority of the variance between the *in vivo* samples, the expression values in protein biosynthesis and hypothetical genes were least similar and therefore constituted the primary differences in *L. plantarum* responses between the three sample groups (data not shown).

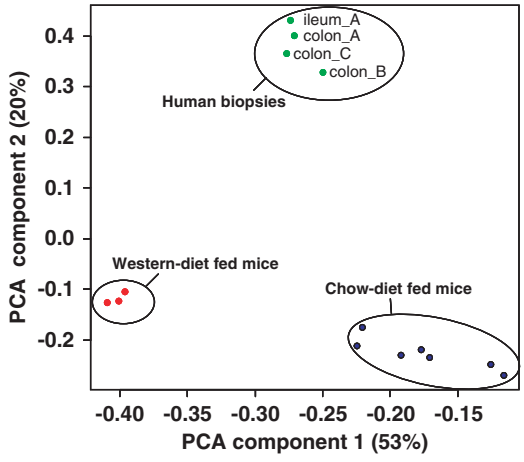

Figure 1 Clustering of *L. plantarum* transcriptomes in the human intestine by principle component analysis. The *in vivo* profiles were normalized against transcriptomes obtained for *L. plantarum* WCFS1 grown in standard laboratory conditions (mid-exponential phase cells in de Man, Rogosa, and Sharpe (MRS) culture medium) to adjust for microarray platform-specific effects between the mice and human data sets. The first two components are shown, representing 53% and 20% of the variance, respectively.

To specify conserved responses by the ingested probiotic among the human subjects and the mono-associated mice, the *in vivo* transcriptomes were quantified against a diverse set of transcriptome patterns identified for *in vitro* laboratory cultures of *L. plantarum* WCFS1 (Supplementary Materials). The *in vitro* transcriptome profiles included *L. plantarum* responses to growth on rich (undefined) and chemically defined media containing known quantities of essential amino acids, vitamins and nucleotides. These media supported different growth rates, included exposure to environmental stresses and contained diverse individual carbohydrates or complex mixtures of plant polysaccharides. Remarkably, *L. plantarum* 299v upregulated genes in the human gut were largely restricted to only a few gene classes and this response was conserved for *L. plantarum* WCFS1 in mice. Induction of transport systems and cell envelope- and cell wall-localized genes constituted the conserved functional response in humans and mice on both dietary regimes relative to laboratory growth (Supplementary Figure S1). Amino acid biosynthesis and central intermediary metabolism were also upregulated in all human subjects (Supplementary Figure S1). In mice fed the Western diet, amino acid biosynthesis was induced, whereas genes associated with energy metabolism were significantly induced in the chow-fed mice (Marco *et al.*, 2009) (Supplementary Figure S1).

Carbohydrate metabolism and amino acid biosynthesis pathways were significantly enriched in the core gut metagenome of the indigenous human intestinal microbiota (Turnbaugh and Gordon, 2009). Genes required for the uptake and degradation of carbohydrates by *L. plantarum* are distributed among several major functional gene classes of this organism and were collectively significantly induced *in vivo* (human subjects, $P = 1 \times 10^{-6}$; mice fed with chow diet, $P = 0.0001$; mice fed with the Western diet, $P = 0.0009$, χ^2-test) (Supplementary Figure S1 and Supplementary Table S2). Induction of both carbohydrate and amino acid biosynthesis gene classes of *L. plantarum* in the human gut confirms the central relevance of these cellular processes in microbial adaptation to the human intestinal ecosystem extending beyond the indigenous microbiota to a transient microbial resident entering the gut through foods and beverages.

Substantial overlaps in the expression patterns of *L. plantarum* carbohydrate metabolism genes in humans and mice were shown on hierarchical clustering of the gene expression profiles (Supplementary Table S2) and projections of these data at pathway levels (Figure 2). Genes encoding the transporters and enzymes for the utilization of maltose, cellobiose, lactose/galactose and melibiose were induced in humans and mice on either diet (Figure 2 and Supplementary Table S2). These disaccharides are constituents of plant-derived polysaccharides and the digestion of such

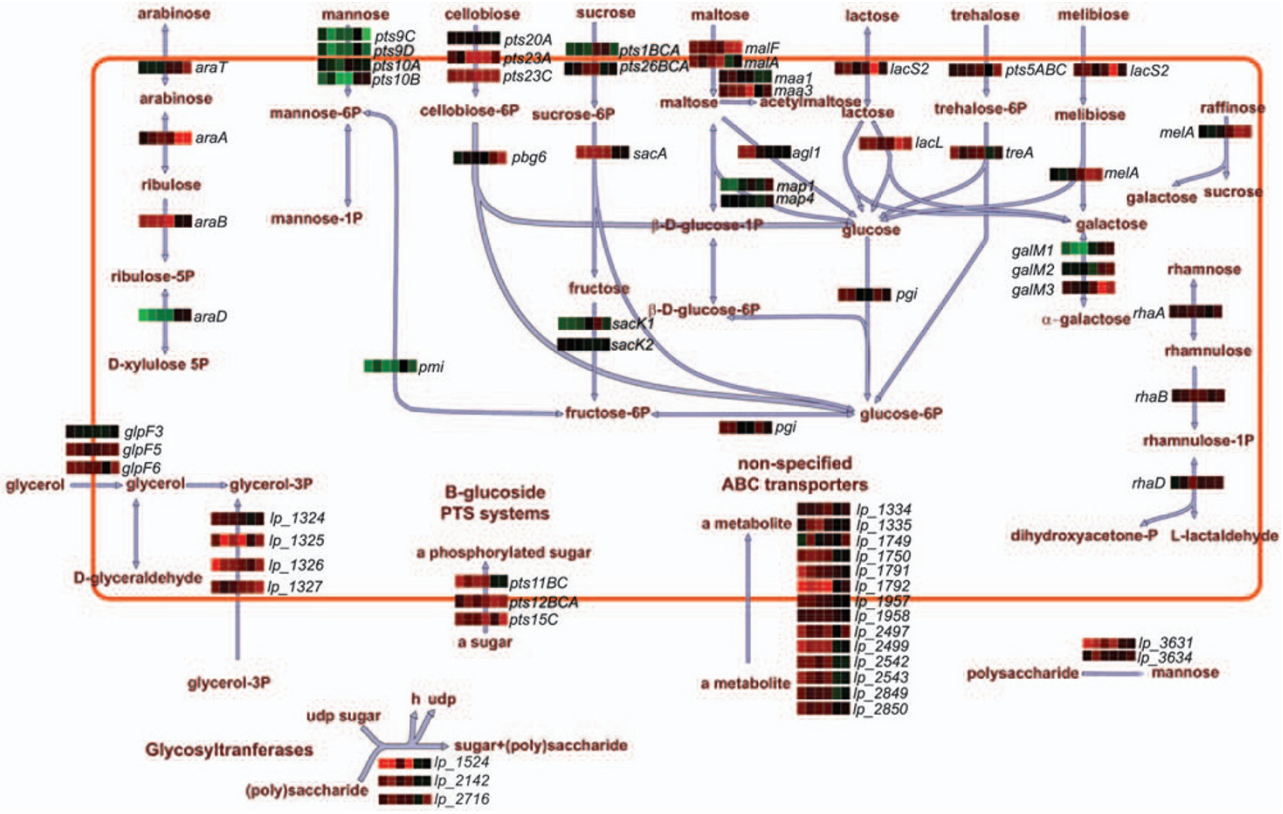

Figure 2 *L. plantarum* carbohydrate and sugar alcohol metabolism in the intestines of humans and mice. The map is based on metabolic model of *L. plantarum* according to Simpheny (Teusink *et al.*, 2006). The heat-map plot from left to right contains the following: ileum_subject A, colon_subject A, colon_subject B, colon_subject C, average of mice fed with chow diet and average of mice fed with the Western diet. The intensity gradient is based on a −1 (green) to 1 (red) ranked difference in expression.

compounds by *L. plantarum* in the mammalian gut might be expected for an organism known for its dominant roles in plant fermentations (de Vries *et al.*, 2006). Furthermore, *L. plantarum* in humans and mice fed with the Western diet preferentially upregulated glycerol and glycerol-3-phosphate transporters, two α-mannosidases, three cell surface-localized glycosyltransferases, and seven ATP-binding cassette sugar transport systems for which the carbohydrate source is presently not known (Supplementary Table S2). Induction of the same sugar metabolism genes in humans and germ-free mice on the Western diet signify a common nutrient resource pool for *L. plantarum* in both mammals, which might be dependent on the nutritional landscape provided in the colonized human gut resembling that found in germ-free mice fed with the Western diet. The overlap in the expression of specific *L. plantarum* genes in humans and germ-free mice confirms the significance of animal models in studying gut-associated microorganisms, particularly when dietary specifications are included in the analyses.

L. plantarum cell surface properties were also significantly modified *in vivo* (Supplementary Figure S1). In humans, an average of 50 out of 133 genes in the cell envelope category encoding extracellular proteins and polysaccharide biosynthesis were induced (Supplementary Figure S2). In contrast, peptidoglycan, lipoteichoic acid and cell membrane biosynthesis pathways were either not affected or downregulated *in vivo* (data not shown). Among the upregulated genes, 23 were also induced in mice fed with the Western diet and a total of 9 genes were upregulated in humans and mice on either diet and constituted significant conservation in *L. plantarum* cell surface modifications *in vivo* ($P = 0.01$, χ^2-test) (Supplementary Figure S2). The *L. plantarum* response in all *in vivo* samples was dominated by cell-surface protein clusters (*csc*) predicted to be involved in carbohydrate acquisition (Siezen *et al.*, 2006) and other genes with putative glycolytic or proteolytic functions (Boekhorst *et al.*, 2006) (Supplementary Table S3). Two genes (*lp_0800* and *lp_2940*) induced in the intestine of humans and germ-free mice on either diet were also identified as gut-inducible in conventionally raised mice (Bron *et al.*, 2004; Marco *et al.*, 2007) (Supplementary Table S3). The survival and persistence of a *L. plantarum* WCFS1

lp_2940 deletion mutant was severely compromised in the mouse digestive tract (Bron *et al.*, 2007). Cell surface proteins such as Lp_2940 for which the functional properties are currently unknown are among a growing repertoire of *Lactobacillus* cell products providing specific contributions in the mammalian gut, which are not related to host diet (Konstantinov *et al.*, 2008). These proteins are prime targets for unraveling the mechanisms by which probiotic bacteria affect human health through targeted interactions with intestinal epithelial and immune cells in ways that encompass the integrated contributions of the gut microbiota and host diet on the human body.

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Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)