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Gut microbiota correlates with energy gain from dietary fibre and appears to be associated with acute and chronic intestinal diseases

M. Ukhanova[1], T. Culpepper[1], D. Baer[2], D. Gordon[3], S. Kanahori[3], J. Valentine[4], J. Neu[5], Y. Sun[6], X. Wang[1] *and* V. Mai[1]

1) Microbiology and Cell Science, Emerging Pathogens Institute, University of Florida, Gainesville, FL, 2) US Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Beltsville, MD, USA, 3) Matsutani Chemical Industry Co. Ltd, Itami City, Hyogo, Japan, 4) Division of Gastroenterology, Gainesville, FL, 5) Division of Pediatrics, Gainesville, FL and 6) ICBR University of Florida, Gainesville, FL, USA

Abstract

Improvements in high-throughput sequencing technologies have spurred a large number of studies aimed at obtaining a better understanding of the composition and the dynamics in gut microbiota and its associations with various human diseases, especially those in the intestinal tract. Here we briefly summarize results from three different such studies from our group, all of which used 454 based high-throughput 16S rRNA sequence analysis combined with other microbiota profiling methods to determine faecal microbiota composition. In the first study, a controlled feeding trial, we establish that energy gain from the consumption of up to 50 g/day of a resistant maltodextrin depends on the prevalent microbiota composition. Over time, resistant maltodextrin supplementation increased the proportion of total faecal bacteria as well as potentially beneficial bifidobacteria. Thus, energy gain from resistant maltodextrin in an individual appears to vary over time and depend on the adaptation of gut microbiota. We then illustrate the power of molecular tools for identifying (i) distortions in early microbiota development in pre-term infants and the presence of potentially novel pathogens contributing to necrotizing enterocolitis and (ii) a specific microbiota signature, based on discriminant analysis of the 16S rRNA sequences, that correlates with the prevalence of an early risk marker associated with colorectal carcinogenesis, intestinal adenoma, in elderly adults.

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Corresponding author: Volker Mai, Department of Microbiology and Cell Science and Emerging Pathogens Institute, University of Florida, 2055 Mowry Rd, Gainesville, FL 32611-0700, USA
E-mail: vmai@ufl.edu

Introduction

The role of host-associated microbiota, especially those residing in the intestinal tract, has received renewed interest for potential associations with human health. Molecular 16S rRNA based tools have long helped to overcome earlier limitations of conventional microbiological plating methods in studying gut microbiota composition [1,2]. More recently, the developments of high-throughput parallel sequencing methodologies have increased our ability to analyse microbiota at a previously unimaginable depth. These tools have sparked novel microbiota research including suggestions of associations between the proportion of Bacteroidetes and obesity [3–5]. Although it appears clear from multiple studies that obesity status is associated with prevalent microbiota composition, at least in humans the evidence that gut microbes significantly contribute to the development of obesity needs further investigation. Gut microbes can contribute to obesity by two major means. First, they can generate additional energy in the form of short chain fatty acids from substrates, such as dietary fibre, that reach the large colon. Second, they can affect obesity-associated pathways in the host through signalling, such as the upregulation of Fiaf [6] in response to gut microbes. We present below data suggesting that energy gain from resistant maltodextrin (RM) is dependent on resident gut microbes and conversely that RM shapes microbiota composition.

Due to extensive research funding that recently became available in the USA through the National Institutes of

Health Human Microbiome Project, and similar projects in other regions of the world, there has been an explosion in the number of studies that evaluate associations between microbiota and various disease states. Early results from many of these studies suggest that microbiota is affected by a variety of diseases, but the cross-sectional character of many studies is not well suited for determining associations with disease aetiology. We present below early data from a prospective cohort study in pre-term infants that allowed us to investigate in detail the potential contributions of a distortion in early microbiota development and the presence of novel pathogens to the development of necrotizing enterocolitis (NEC). We then summarize findings from a colonoscopy screening study in adults that shows the utility of advanced 16S rRNA data mining tools [7] to reveal a microbiota signature pattern associated with the prevalence of colorectal polyps. All three studies were approved by the appropriate institutional review boards. Our findings illustrate the need for (i) well controlled feeding studies to link specific dietary components to changes in microbiota composition, and (ii) prospective cohort studies to establish causal links between microbiota and human disease states.

Resistant maltodextrin feeding study

While the amounts of energy derived from consuming fats, proteins and digestible carbohydrates are well established, the metabolizable energy and net energy value associated with the consumption of dietary fibre has been less studied [8]. RM is a soluble non-viscous dietary fibre that, although recalcitrant to human digestive enzymes, is a substrate for fermentation by the commensal microbiota, primarily that of the large intestine. Due to the chemical composition of RM, determination of its fibre content requires a specific analytical method [9]. We performed a human feeding study to determine if energy gain from increased dietary intake of RM was dependent on resident gut microbiota, and if microbiota adapted to increased RM by increasing microbes that can utilize this specific dietary fibre.

In a randomized, controlled dose–response study 14 healthy males were assigned to a random treatment sequence that consisted of three 28-day treatment periods separated by a wash-out period of at least 14 days, with controlled diets: a placebo diet (0 g/day RM + 50 g/day maltodextrin) and two levels of dietary RM (25 g/day RM + 25 g/day maltodextrin and 50 g/day RM + 0 g/day maltodextrin). When the energy value of RM was calculated by bomb calorimetry for each individual, we detected a wide range among individuals, from 0 to 4 kcal/g with a mean of 2.2 kcal/g. We hypothesized that differences in microbial composition and associated activities caused these differences in energy value for RM. Microbiota composition was determined using a comprehensive 16S rRNA based approach that included denaturing gradient gel electrophoresis (DGGE) for initial profiling and quality control, qPCR and fluorescent *in situ* hybridization for targeted quantification and 454 based 16S rRNA sequencing. DGGE indicated that a particular bacterial signature (band) increased upon RM supplementation. Purification and sequencing of the respective band suggested that bacteria grouping closest to Lachnospiraceae increased with RM. No consistent effects of RM were detected when faecal samples from four individuals were submitted to deep 16S rRNA sequencing. Total numbers of bacteria per gram of faeces increased by 22% in subjects consuming 50 g/day RM (p 0.02). While overall microbiota diversity was little affected, the numbers of bifidobacteria increased during RM periods (qPCR, p 0.03) (Fig. 1). Differences in microbiota composition, specifically higher numbers of bifidobacteria in the group gaining more energy, correlated with the variations in energy gain from RM intake. Our findings are consistent with the hypothesis that energy gain from fibre that reaches the large intestine varies between individuals and is dependent on the resident gut microbiota. The intake of RM microbiota changed the microbiota in individuals, probably towards a composition more adapted to utilizing the additional dietary fibre. This observation suggests that as the microbiota adapts energy gain from fibre, it is changing over time even within an individual.

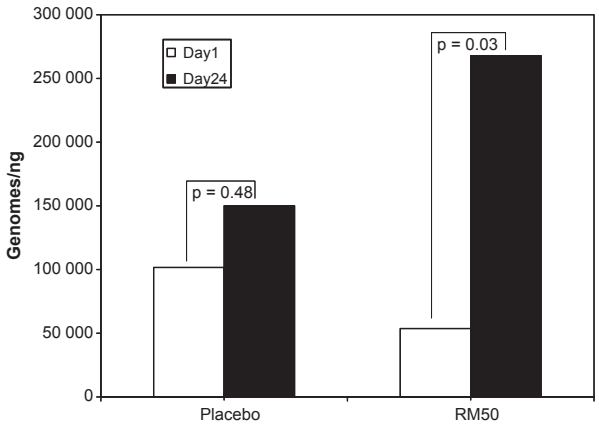

FIG. 1. Change in bifidobacteria by qPCR. The number of genome equivalents for bifidobacteria per nanogram of input DNA are shown for days 1 and 24 during the placebo and the period with 50 g/day of resistant maltodextrin (RM50). p-values are indicated within the intervention periods.