Research for the 21st Century: Can We Draw a Blueprint of the Bowel Ecosystem?

Gerald W. TANNOCK*

Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand

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The bacterial community of the bowel of humans and other animals derives carbon and energy sources from the diet and secretions of the host. The metabolic products generated by the community, and the antigens and other molecules associated with the bacterial cells, interact with the bowel mucosa. Some bacterial products are absorbed from the bowel and affect the systemic organs of the body. The interactions that occur within the bacterial community and between the community and food components and host tissues comprise a highly interactive web. It is concluded that this interactive matrix characteristic of the bowel ecosystem may be studied by detailed autecological and synecological experiments. The results of these studies could be used to construct a general blueprint of the healthy bowel and might reveal biomarkers predictive of health or disease.

Key words: autecology; synecology; lactobacilli; microbiota; metabolome; immunology; digestion-resistant carbohydrates

The large bowel of humans and other animals contains a bacterial community whose members are biodiverse and numerous. Undigested plant residues (digestion-resistant carbohydrates) and bowel secretions (mucins) provide the major sources of carbon and energy for the members of the community (2, 5). Bacteriological investigations of the community in the late 20th century and early 21st century have concentrated on answering the question “who is there?” (4). An even better question to address in the 21st century with respect to community members is “what are they doing and how are they doing it?” There is a need to define the interactions that occur within bowel communities and to learn about trophic groups within the community and their interrelationships. The preparation of a blueprint (a plan or reference diagram that can be used as a guide in constructing a building or for doing something in the future) of the bowel ecosystem encompassing food-bacterial community-host interactions would be useful. Then, predictive responses of the ecosystem to manipulations of the diet might be possible.

Although research in the 21st century may be dominated initially by high-throughput technologies such as metagenomic and metatranscriptomic sequencing and microarray screens, detailed knowledge of the operation of the bowel ecosystem is likely to be generated by precise ecological and physiological experiments utilizing experimental animal models. These experiments may have autecological or synecological bases. In autecology, the ecology of a single species and the influence of environmental factors on that species are studied. There may be a focus on the effect of an individual species on its surroundings, the impact of the environment on the organism, or the basis for its adaptation to the features and stresses of the site of habitation. In contrast, synecology studies the relationships between the environment and the different organisms that make up a biological complex (community) in a single locale (1).

AUTECOLOGY: THE Lactobacillus reuteri 100-23/ Lactobacillus-FREE MOUSE PARADIGM

*Lactobacillus reuteri 100-23 is autochthonous to the rodent gut as evidenced by the facts that it adheres to the non-secretory epithelium of the forestomach thus forming a biofilm, persists at constant population levels throughout life in the guts of formerly Lactobacillus-free mice inoculated by mouth with a pure culture of strain 100-23 on a single occasion and influences small bowel biochemistry (6). The genome of strain 100-23 has been sequenced through support from the Community Sequencing Program of the Joint Genome Institute (JGI; USA). An annotated sequence of the genome is publicly available (http://genome.ornl.gov/microbial/leu_23).

Lactobacillus-free mice constitute an ideal

*Corresponding author. Mailing address: Department of Microbiology and Immunology, University of Otago, PO Box 56, Dunedin, New Zealand. Phone: +64-3-479-7713. Fax: +64-3-479-8540. E-mail: gerald.tannock@stonebow.otago.ac.nz

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experimental animal model for *in vivo* work. These mice contain a gut microbiota devoid of lactobacilli but which is otherwise equivalent to that of conventional mice (Fig. 1) (8). An important advantage of these mice over monoassociated ex-germfree animals is that they permit the study of *Lactobacillus* microbiota and *Lactobacillus* host interactions against a standard bacteriological background (6). The animals are maintained in gnotobiotic isolators and fed a sterile diet.

Together, *L. reuteri* 100-23 and *Lactobacillus*-free mice provide an excellent paradigm for the study of the molecular mechanisms of autochthony. Insertional mutagenesis of selected genes in strain 100-23 has demonstrated several bacterial attributes that enhance gut colonisation (Table 1). These facilitators have been best demonstrated using *in vivo* competition experiments between wild type and mutant strains.

Metabolomics knowledge can also be generated from the use of the *L. reuteri* 100-23/*Lactobacillus*-free mouse model (Fig. 2). The metabolic footprint of the bowel bacteria is reflected in the metabolome of the animal host because bacterial metabolites are absorbed from the gut lumen into the lymph and blood circulations. Hence the body fluids (blood, lymph, bile, sweat, urine) of the host contain numerous bacterial products that may provide biomarkers of food-microbe-host interrelationships and possible indicators of health or disease (7). For example, as reported by Wang and colleagues, infection of mice...
with the nematode *Schistosoma mansoni* could be diagnosed on the basis of alterations to the urine metabolome in which shifts in bacterial metabolite concentrations were included. These shifts doubtless related to the displacement of baseline murine-bacterial relationships by the introduction of parasites into the
bowel ecosystem (12). The host metabolome represents the end-product of genetic, environmental, and host-bacterial relationships. The study of the host metabolome might therefore contribute to a full systems biology approach to understanding and maintaining bowel health.

Immunological impacts of L. reuteri 100-23 on the murine host have also been measured. Lactobacillus-free mice were inoculated at 8 weeks of age with L. reuteri 100-23. Primary intestinal epithelial cells (small bowel enterocytes) were harvested from colonized mice and from control (uninoculated) mice 2, 6 and 21 days later. At day 6, expression of genes encoding pro-inflammatory cytokines and chemokines was increased in colonised mice relative to controls (Fig. 3). Expression of the gene encoding A20 was decreased. The alterations in gene expression were transient since there was no difference between the data from colonised and non-colonised animals sampled at day 21 (3). Thus L. reuteri 100-23 triggers a pro-inflammatory response in enterocytes when the Lactobacillus population reaches its maximum size in the small bowel. The up-regulation of pro-inflammatory genes results in a mild inflammation of the small bowel that subsides by day 21. The enterocytes no longer react to the presence of the lactobacilli despite the continuing presence of the commensal in the bowel and the renewal of the epithelium. The lack of responsiveness to the commensal presumably reflects the development of a regulatory response by the mucosal immune system by which reactivity of enterocytes to L. reuteri 100-23 is prevented.

**SYNECOLOGY: A RAT PARADIGM OF FOOD-BACTERIA INTERACTIONS**

Digestion-resistant carbohydrates (DRC) in the diet pass to the terminal ileum and large bowel where they may be fermented by resident bacteria. The resident bacterial communities in the bowel of young animals undergo dramatic shifts in composition following weaning. These compositional and associated biochemical changes may have long-lasting impacts on the animal host. A rat model can be used to test whether bacterial communities of predictable composition can be engineered by supplementing the diet of newly weaned animals with DRC. Rats were fed a control diet or diets supplemented with DRC at a concentration of 5% for 28 days. Colonic digesta was collected from each rat to determine short chain fatty acid (SCFA) concentrations and to compare bacterial community profiles (temporal temperature gradient electrophoresis; TTGE). Cluster analysis of TTGE profiles showed that distinctive bacterial communities were enriched by feeding DRC. The community profiles could be clearly differentiated...
from each other and those from control rats (Fig. 4) (Young W, Roy NC, Lee J, Tannock GW. 2008. Feeding digestion-resistant carbohydrates to newly weaned rats modifies the large bowel ecosystem. Poster 2008-A-90-ASM-BM, second ASM Conference on Beneficial Microbes, San Diego, USA). The concentrations of SCFA were also altered with respect to control rats. Therefore bacterial communities in the bowel of newly weaned rats can be engineered by supplementing the diet with DRC. Using this approach, unique bacterial communities with enhanced fermentative capacity and increased uniformity were selected.

CONCLUSION

Autoecology and synecology provide useful experimental approaches to study the bacterial community of the bowel. Modern analytical methods, in conjunction with appropriate model systems, are likely to generate large amounts of data that reflect the food-bacteria-host interrelationships that regulate the bowel ecosystem. Therefore preparation of a blueprint of the healthy bowel is feasible. Preparation of the blueprint of the healthy bowel will, however, require a systems biology investigation encompassing a diversity of scientists from different disciplines. The primary aim of the research will be to understand how all of the heterogeneous parts (dietary components, bacterial consortia, animal physiology and development) are integrated, with a supplementary aim of identifying biomarkers of health or disease. A fusing of biological and computational expertise is clearly required for success.
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