Cell

Bacteria from Diverse Habitats Colonize and Compete in the Mouse Gut

Henning Seedorf,[1,8] Nicholas W. Griffin,[1,8] Vanessa K. Ridaura,[1] Alejandro Reyes,[1] Jiye Cheng,[1] Federico E. Rey,[1] Michelle I. Smith,[1] Gabriel M. Simon,[1] Rudolf H. Scheffrahn,[2] Dagmar Woebken,[3] Alfred M. Spormann,[3] William Van Treuren,[4] Luke K. Ursell,[4] Megan Pirrung,[4] Adam Robbins-Pianka,[4] Brandi L. Cantarel,[5] Vincent Lombard,[5,6] Bernard Henrissat,[5,6] Rob Knight,[4,7] and Jeffrey I. Gordon[1,*]

[1]Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St. Louis, MO 63108, USA
[2]Fort Lauderdale Research and Education Center, University of Florida, Fort Lauderdale, FL 33314, USA
[3]Departments of Civil and Environmental Engineering and Chemical Engineering, Stanford University, Stanford, CA 94305, USA
[4]Biofrontiers Institute, University of Colorado, Boulder, Boulder, CO 80309, USA
[5]Centre National de la Recherche Scientifique, UMR 7257, 13288 Marseille, France
[6]Aix-Marseille Université, UMR 7257, 13288 Marseille, France
[7]Howard Hughes Medical Institute, University of Colorado, Boulder, Boulder, CO 80309, USA
[8]Co-first author
*Correspondence: jgordon@wustl.edu
http://dx.doi.org/10.1016/j.cell.2014.09.008

SUMMARY

To study how microbes establish themselves in a mammalian gut environment, we colonized germ-free mice with microbial communities from human, zebrafish, and termite guts, human skin and tongue, soil, and estuarine microbial mats. Bacteria from these foreign environments colonized and persisted in the mouse gut; their capacity to metabolize dietary and host carbohydrates and bile acids correlated with colonization success. Cohousing mice harboring these xenomicrobiota or a mouse cecal microbiota, along with germ-free "bystanders," revealed the success of particular bacterial taxa in invading guts with established communities and empty gut habitats. Unanticipated patterns of ecological succession were observed; for example, a soil-derived bacterium dominated even in the presence of bacteria from other gut communities (zebrafish and termite), and human-derived bacteria colonized germ-free bystander mice before mouse-derived organisms. This approach can be generalized to address a variety of mechanistic questions about succession, including succession in the context of microbiota-directed therapeutics.

INTRODUCTION

Understanding the factors that operate to allow microbes to colonize the human gut should help us achieve better understanding of how contact with other humans—including family members—animals, and other microbial reservoirs in our environment impacts diversity in this body habitat at various stages of life. This knowledge could also guide development of new approaches for modulating the risk for ecological invasion by various pathogens, deepen our understanding of how our microbial exposures shape the development of our immune systems, and help direct the design of more effective strategies for introducing members of well-defined species consortia, cultured from the gut microbiota of healthy donors, into already established microbial communities of recipient humans who are at risk for or already have manifest disease.

Macroecologists differentiate the conditions under which an organism can live (its fundamental niche) from the conditions in which the organism actually does live (its realized niche) (Hutchinson, 1957). Studies of macroecosystems have emphasized how a species' realized niche is often more restricted than its fundamental niche because negative interactions with other organisms prevent the species' successful colonization and persistence in areas in which it could live in their absence, or because historical, geographical, or physical processes have prevented that species from reaching certain areas. Colonization resistance, whereby established bacterial communities provide their hosts with some degree of protection against ecological invasion and overgrowth by pathogenic organisms, is a long recognized example of this phenomenon (Bohnhoff et al., 1964).

Gnotobiotic mice provide a powerful system for distinguishing the fundamental versus realized niches of microbes in the gut or other body habitats. Animals reared germ-free (GF) can be colonized at selected stages in their lives with control microbiota from conventionally raised mice or with alien microbiota (xenomicrobiota) harvested from the guts or other body habitats of other mammalian species, other vertebrates or invertebrates, or various highly divergent environmental habitats. A limited 16S rRNA-based analysis of reciprocal gut microbiota transplants involving conventionally raised mouse donors and GF zebrafish recipients, and conventionally raised zebrafish donors

and GF mouse recipients, demonstrated that bacterial taxa from zebrafish that had not been described in the normal mouse intestinal microbiota could persist in the mouse gut (Rawls et al., 2006): i.e., the mouse gut is within the fundamental niches of these microbes, but not in their realized niches. In this previous study, the gene repertoires represented in the gut-selected microbiomes were neither characterized nor were the relative abilities of the transplanted alien communities to invade the normal indigenous gut community of conventionally raised mice assessed.

In the present study, we extend this line of inquiry by identifying bacteria from a range of communities associated with different gut environments, other human body habitats, and aquatic and terrestrial environments, that successfully colonize the guts of GF mice. Furthermore, we compare the ability of these microbes to colonize empty gut habitats versus those with established microbial communities. The approach used should facilitate identification of successful gut colonizers that have therapeutic utility and the mechanisms that allow them to invade and persist.

RESULTS

Reproducibility of Xenomicrobiota Selection

We introduced microbiota from different habitats into separate groups of adult GF wild-type C57Bl/6J mice (five animals/cage; one gnotobiotic isolator/microbial community type; see stage 1 experiments in Figure 1). These xenomicrobiota included (1) gut-associated communities from a terrestrial vertebrate (human) and an aquatic vertebrate (zebrafish [*Danio rerio*]), plus an invertebrate (termite [*Nasutitermes corniger*]), (2) nongut communities from the same human donor (tongue and skin) so that the colonization success of taxa originating from human body habitats endowed with properties distinct from the gut could be ascertained, and (3) communities from the lower and upper layers of an estuarine microbial mat community and from a terrestrial (soil) community to assess the colonization potential of components of microbiota that reside in nonanimal habitats and contain many bacterial phyla not represented in the mouse gut (Harris et al., 2013; Tringe et al., 2005). Control "conventionalized" (CONV-D) animals received a cecal microbiota harvested from two adult conventionally raised, specific pathogen-free C57BL/6J mice that had been exposed to microbes in their vivarium since birth. Prior to and after transplantation, gnotobiotic mice were maintained on an autoclaved chow low in fat and high in plant polysaccharides ("LF-HPP diet"). Fecal samples were collected from transplant recipients over the course of the 28 days that followed gavage in order to (1) characterize the process of colonization and selection within and between the different groups of recipient animals, (2) determine whether a given community had achieved a stable composition during the period of surveillance, and (3) reference the results obtained from the xenomicrobiota recipients to the control group of CONV-D mice. (See Tables S1A–S1G [available online] for a list of samples characterized by multiplex pyrosequencing of PCR amplicons generated from variable region 2 [V2] of their bacterial 16S rRNA genes and Tables S1H–S1K for samples subjected to shotgun pyrosequencing of community DNA.)

Using the 16S rRNA data sets, we performed pairwise comparisons of communities employing UniFrac, a phylogenetic distance metric (Lozupone and Knight, 2005). Principal coordinates analysis (PCoA) of UniFrac distances revealed that all of the different types of transplanted communities assembled within recipient gnotobiotic mice over the course of 3–7 days and that the temporal pattern of assembly was very consistent within groups of mice that received the same input microbiota. UniFrac, as well as network analysis of shared operational taxonomic units (OTUs; each defined based on grouping of 16S rRNA reads with 97% nucleotide sequence identity [97%ID]), indicated that fecal communities from gnotobiotic mice that received vertebrate gut-derived microbiota generally were more similar to their respective input communities than to those originating from other sources (Figure 2; Figure S1).

To further test the reproducibility of community selection, we transferred the cecal contents of mice from stage 1, sacrificed 28 days after they had received their xenomicrobiota transplants, into a second group of age-matched GF male C57Bl/6J animals (see stage 2 in Figure 1). UniFrac distances between the original input communities and their corresponding stage 1 mouse-selected communities (day 14) were far greater than the distances between the selected stage 1 communities and the selected stage 2 communities (day 14) for all but the human fecal and control mouse cecal communities (Figure 2A). We also transplanted hindgut microbiota from two different colonies of termites and compared the output communities from stages 1 and 2. UniFrac distances were similar between selected termite communities across the two stages and between the two termite communities within a stage (Figure 2A), providing evidence of the reproducibility of the methods used for harvest (Potrikus and Breznak, 1977; Chen et al., 2012) and transplantation, as well as subsequent mouse gut selection of this notoriously fastidious collection of microorganisms.

Differences in the Diversity of Gut-Selected Xenomicrobiota

Bacterial communities selected from vertebrate and invertebrate gut microbiota maintained a significantly greater proportion of the taxonomic richness (97%ID OTUs), biodiversity (Shannon's diversity index), and evenness of relative abundance (Pielou's evenness index) relative to their input communities than did communities from nongut environments (soil; the upper layer, bottom layer, or mixed layers of the microbial mat; human tongue) (Figure S1E). This finding indicates that the mouse gut is within the fundamental niches of a greater proportion of bacterial taxa from other gut environments compared to taxa originating from other nongut habitats.

We identified a total of 1,908 97%ID OTUs in the input communities after rarefaction of the data (Extended Experimental Procedures). These OTUs spanned 76 different bacterial classes from 35 phyla. Most input communities shared very few or no OTUs with other input communities; Jaccard similarity values between input microbiota were zero for most pairs of communities and were higher for bacterial communities from similar sources (e.g., 0.57 for termite A and termite B; 0.31–0.41 for the microbial mat layers) (Figure S1B). This limited sharing of 97%ID OTUs was recapitulated in the recipient gnotobiotic