Microbiota Differences in Infancy: Lasting Impacts on Metabolism and Immunity

Shannon Weiman

The impact of gut microbiota on human health begins at birth, and may be influenced by factors such as mode of delivery and early infant diet, according to several researchers who presented recent findings during the 2015 ASM General Meeting held in New Orleans last May. These early influences on microbiome composition can have lasting consequences, particularly on metabolism and immunity, they say. While some researchers document differences in microbial communities, others pinpoint particular species and mechanisms that control host responses, which may prove useful in treating metabolic and immune diseases.

Epidemiologic studies point to an association between Caesarean section delivery and increased fat in later childhood, along with increased rates of autoimmune diseases, including type 1 diabetes, food allergies, and celiac disease, according to Martin Blaser of New York University in New York, N.Y. “Microbiota interactions in infancy may be critical determinants of long-term host metabolic effects,” he says. Further, transient perturbations in the microbiome during infancy, induced by antibiotic treatment of mice, have a long-term impact on fat deposition, he pointed out. “Altering the intestinal microbiota during a critical development window has lasting metabolic consequences...and can result in syndromes of metabolic dysfunction.”

One bacterial species that may protect against such metabolic dysfunction later in life is Akermansia muciniphila, says Clara Belzar of Wageningen University in the Netherlands. “A. muciniphila treatment reversed high-fat diet-induced metabolic disorders, including fat-mass gain, adipose tissue inflammation, and insulin resistance,” she says, describing studies in mice. Metabolites of this bacterium alter expression of various transcription factors and genes involved in host cellular growth, lipid metabolism, lipolysis and satiety, including fasting-induced adipose factor, G-protein coupled receptor 42, histone deacetylases, and peroxisome proliferator-activated receptor gamma, she says. These bacteria also can increase endocannabinoid levels, which control inflammation, gut barrier function, and gut peptide secretion.

Diet can influence gut microbial populations during infancy, which alters immune development, according to Nicole Narayan of the University of California, Davis. Studying rhesus macaques, she finds that breast-fed infants develop different microbiota and more robust immune systems compared to their formula-fed counterparts. "Early infant diet has significant impact on the gut microbiota...which, in turn is associated with different immune systems in infancy," she says. “Breast-fed animals manifested greater T cell activation and proliferation and harbored robust pools of T helper 17 cells.” Moreover, these immunologic differences can be sustained for at least 3 to 5 years.

Lactobacillus reuteri, also a native bacterium of the human gut microbiota, can modulate host immune re-
sponses, suppressing proinflammatory cytokines while alleviating colitis in mice, says Chunxu Gao of Baylor College of Medicine in Houston, Tex. These bacteria convert the amino acid L-histidine into histamine, which signals via H2 histamine receptors on myeloid cells to suppress the mitogen-activated protein kinase pathway and thus block production of pro-inflammatory tumor necrosis factor (TNF). The presence of L-histidine in the diet is essential for alleviating Lactobacillus reuteri-mediated colitis, he says. “These findings point toward a new strategy for controlling intestinal inflammation via probiotics by dietary interventions or microbiome manipulation.”

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NEW FROM ASM

Genomics Outpaces “Grind and Find” in Search for Useful Natural Products

Jeffrey L. Fox

Although natural products remain “the best source of antibiotics,” finding ways to accelerate their discovery remains a critical challenge for would-be developers of new drugs, says Ben Shen from the Scripps Research Institute in Jupiter, Fla. The recipient this year of the Promega Biotechnology Research Award, he spoke during the plenary session, “The Third Age of Antimicrobials,” convened at the 2015 ASM General Meeting held in New Orleans last May.

The traditional “grind-and-find” approach of cultivating microorganisms and then extracting natural products from them to search for those with promising antimicrobial or other useful biological activities, is simply too slow, Shen says. For instance, it took about 15 years to comb through more than 80,000 strains and to examine some 250,000 extracts before researchers identified platensimycin, a metabolite of Streptomyces platensis. This natural product has promising antibiotic activity and is part of a new structural class. Another drawback is that this extraction stage typically is a mere prelude to the medicinal chemical manipulations required to convert promising leads into genuine candidate drugs with appropriate pharmacologic properties. Although a solid traditional approach to drug discovery, it can be glacially paced and dreary, he suggests.

To streamline this process, Shen recommends putting microbial genomics to use in setting microbial strain priorities. This newer approach includes a study of the specific genes involved in producing a natural product such as platensimycin, and then using them to probe other strains that can or cannot make particular strategic intermediates of that natural product. An alternate screening strategy is to look more narrowly for genes encoding those enzymes that are called into action very late in the synthesis of a particularly useful natural product, he says. Such information helped him and his collaborators to identify a half-dozen similar but not identical platensimycin-producer strains that had been collected from widely distributed sites around the world, affording an opportunity to tease out strains with higher efficiencies of production or with modified end products.

Analysis of the genes and intermediates involved in this metabolic pathway helped to uncover a repressor molecule that effectively chokes overall productivity, according to Shen. Blocking that repressor boosts output, enabling the producer strain to over-produce metabolites, in turn, making it easier to

MINITOPIC

Microbiology Policy Bulletin Board

Recent national and international developments involving microbiology and related science policy matters include:

- Following the meeting of the G7 countries in Germany last June, the leaders declared their full support for the WHO Global Action Plan on Antimicrobial Resistance, urging the prudent use of antibiotics and pledging to stimulate basic research as well as development of new antibiotics, alternative therapies, vaccines, and rapid-point-of-care diagnostics.
- The U.S. Government Accountability Office (GAO) in July issued a preliminary report on efforts by the Department of Defense (DoD) and the Centers for Disease Control and Prevention (CDC) to address weaknesses in their management of high-containment laboratories. Earlier, GAO recommended mandating a single federal entity to conduct strategic planning for such laboratories and to develop national standards for them to follow. For details, see http://www.gao.gov/products/GAO-15-792T?utm_source=gao.gov/products/GAO-15-792T?utm_src=email
- The National Academy of Sciences hosted the first meeting of the Commission on a Global Health Risk Framework for the Future, whose central task is to recommend an effective global “architecture” for recognizing and mitigating the threat of epidemic infectious diseases.
MINITOPIC
Microbiomes Help To Set or Modify Traits of Foods, Beverages, Consumables

Recent developments involving out-of-the-ordinary relationships among microorganisms, foods, beverages, and other consumables include:

- The coffee borer beetle bears bacteria in its gut that enables it to digest and detoxify caffeine while it does damage to coffee plants. For *Pseudomonas fulva* among those gut bacteria, caffeine can serve as a sole source of carbon and nitrogen, according to Javier Ceja-Navarro of Lawrence Berkeley National Laboratory in Berkeley, Calif., and collaborators from several institutions. Details appeared 14 July 2015 in *Nature Communications* (doi:10.1038/ncomms8618).


- In a similar vein, Richard Splivallo of Goethe University Frankfurt in Germany and his collaborators there and in France review the role that the microbiomes of four truffle species play in the development of aromas associated with this food delicacy. Details appeared 17 July 2015 in *Applied and Environmental Microbiology* (doi:10.1128/AEM.01098-15).

- “Robust hybrids” of the yeast *Saccharomyces cerevisiae*, outperform both wild and conventional strains in fermenting cocoa pulp, leading to “superior chocolate,” according to Kevin J. Verstrepen of the University of Leuven and the Flanders Institute for Biotechnology in Belgium and his collaborators. Details appeared 10 July 2015 in *Molecular Microbiology* (doi:10.1128/AEM.01098-15).

- An enzyme from *Pseudomonas putida* bacteria degrades nicotine and thus might prove useful for smokers trying to quit that habit by destroying this ingredient after it is absorbed into the blood but before it reaches the brain, according to Kim Janda of the Scripps Research Institute in La Jolla, Calif., and his collaborators. Details appeared 6 August in the *Journal of the American Chemical Society* (doi:10.1021/jacs.5b06605).

produce greater amounts of potentially useful analogs of platensimycin—or, by generalizing this strategy, other altogether different drug candidates from very different strains.

By avoiding repressor activity, these strains produce about 0.5 g of material per liter, according to Shen. “We can generate all kinds of intermediates and variants, changing linkages to improve activity and stability,” he says. By feeding the producing strains different side chains, it becomes possible to produce a broad spectrum of alternative “final” products, at least a few of which are “as good or better” than the parent antibiotic. This process in culture recapitulates what medicinal chemists do in the lab, while saving time and effort by relying on enzyme-catalyzed metabolic pathways instead of painstaking organic chemistry procedures.

This same genomic analysis-based approach to identifying strains that make natural products with promising biological activities applies as well to the search for anticancer drug candidates as it does to antimicrobial leads, Shen says. “This genomics-based approach holds great promise…and can be applied to discover novel scaffolds and to accelerate drug discovery.”

Carol Potera

A recently developed, high-throughput (HT) method for identifying ligands involved in chemotaxis can also be used for identifying other types of bacterial signal molecules that bind sensor domains, according to Monica Gerth of the University of Otago in Dunedin, New Zealand, and her collaborators. Details appeared in the May 2015 *Molecular Microbiology* (doi:10.1111/mmi.12964).

In developing this method, Gerth and her collaborators combined fluorescence-based thermal shift (FTS) assays with commercial phenotype microarrays from Biolog, Inc., of Hayward, Calif., whose kits contain dozens of compounds that fuel bacterial growth. To begin with, they focused on 95 such compounds that bacteria use as carbon and nitrogen sources and that can trigger chemotaxis. FTS detects protein unfolding, a marker of ligand binding.

“It was a eureka moment when I realized that I could combine the two technologies,” Gerth says. Each assay, done on microtiter plates, requires only about 10 μl of a purified protein and 2 μl of a screening compound as well as access to PCR equipment for subsequent analyses, and takes less than 2 hours to complete.

To put the new test to a test, Gerth and her collaborators identified 43 chemoreceptors of *Pseudomonas syringae* pv. *actinidiae* strain NZ-V13, a plant pathogen that infects kiwifruit, causing wilting, cankers, plant death, and severe economic losses for kiwi growers, according to Gerth. No one else has yet characterized any of its 43 chemoreceptors, she says. One long-
term goal is to prevent kiwifruit infections by disrupting the ability of _P. syringae_ to sense its host. She’s also using the assay to identify chemotaxis receptors in _Phytophthora agathidica_, which is killing kauri, treasured native trees of New Zealand. A close relative of this microorganism caused the Irish potato famine in the 1840s.

After validating the method with a well-characterized amino acid-sensing chemoreceptor from _Pseudomonas aeruginosa_, known as PctA, the New Zealand researchers tested three chemoreceptors from _P. syringae_, called PsaA (the counterpart of PctA), PsaB, and PsaC. None of these chemoreceptors shares binding repertoires with their counterparts from _P. aeruginosa_. For example, PctA strongly binds 18 L-amino acids, whereas PsaA binds only three, namely L-aspartate, L-glutamate, and D-aspartate. Moreover, a single mutation could alter a chemoreceptor’s specificity. “These distinct sensory repertoires may regulate differences in bacterial lifestyles, such as host colonization,” Gerth says.

“Genome sequences reveal vast numbers of genes encoding chemoreceptor proteins,” says John “Sandy” Parkinson, a distinguished professor of biology at the University of Utah in Salt Lake City. “However, discovering the nature of those chemical signals is a tedious process. [Gerth’s] clever assay is now widely accessible to chemoreceptor labs,” and it could “extend to other ligand-binding proteins, such as the vast family of sensor kinases of two-component regulatory systems.”

“Overall, the assay is very flexible,” says Gerth. Other researchers studying chemotaxis have told her about using HT-FTS assays with other ligand libraries, as well as using other Biolog plates to investigate ligand binding in other types of proteins. “Hopefully, this will prove to be a useful technique for a wide variety of research,” she says.
MINITOPIC
Template-Assisted Ligation Model, Collapsed Ribosomes Raise Primordial Questions

Although unrelated, these two recent developments address fundamental questions about how life might take shape. “Even if all you have is template-assisted ligation, you can still bootstrap the system out of primordial soup,” says Sergei Maslov at the University of Illinois, Urbana-Champaign and Brookhaven National Laboratory. In their new model for template-assisted replication, he and his collaborator Alexei Tkachenko argue that the joining of two polymers by using a third, longer one as a template could have enabled polymers to become self-replicating. Template-assisted ligation in this model thus “allows for heritable transmission of the information,” they note. Details appeared 28 July 2015 in the Journal of Chemical Physics (doi:10.1063/1.4922545). In a separate development, ribosomes can be collapsed from two subunits into one, according to Alexander S. Mankin of the University of Illinois, Chicago, and his collaborators. By engineering a hybrid ribosomal RNA (rRNA) composed of both small and large subunit rRNA sequences, they produced a ribosome whose subunits form a single entity that not only functions in vitro, but also supports the growth of Escherichia coli cells in the absence of wild-type ribosomes. Details appeared 29 July 2015 in Nature (doi:10.1038/nature14862).

with importance far beyond oral infectious disease, in that it reports novel principles likely to apply also to biofilm dynamics in other infectious diseases, as well as in environmental biofilms,” says Ann Progulske-Fox of the University of Florida, Gainesville. Moreover, Frias-Lopez and his collaborators were deft in combining metagenomics, which identifies organisms present in the sample, with metatranscriptome analysis, which identifies genes being expressed by a group of organisms based on the mRNA molecules that are being detected, to study this human disease, she says. Further, by determining the dynamics of sRNAs being expressed over the course of this disease, their findings implicate the biofilm rather than any individual species as driving virulence gene expression, she points out.

“Periodontitis is a polymicrobial disease caused by the coordinated action of a complex microbial community that leads to inflammation of tissues supporting the teeth,” says Frias-Lopez. It is the sixth most disabling health condition, currently affecting 743 million worldwide, or 10% of all humans. Nearly half of American adults have moderate periodontitis, while 10% have the severe form, and this condition is responsible for half of all tooth loss in adults. The disease is considered a contributing risk factor for a series of other chronic health conditions, including diabetes and cardiovascular and respiratory diseases. Even so, efforts to reduce its prevalence have had limited success.

David C. Holzman is the Microbe Journal Highlights Editor.

NEW FROM ASM
Endofungal Bacteria May Determine How These Symbionts Affect Host Plants

Shannon Weiman
Bacteria living within fungi influence host metabolism, reproductive activity, and ecosystem effects, according to several researchers who spoke during the symposium, “Microbes in Microbes (Russian Dolls),” at the 2015 ASM General Meeting, held in New Orleans last May. Thus, for example, some endofungal bacteria and their host fungi form partnerships with plants that range from beneficial to pathogenic. In some cases, these symbioses promote growth among all participants while, in others, the microbial symbionts thrive at the expense of their host plants.

Endophytic fungi and their bacterial symbionts, while previously recognized for their role in plant rhizomes, are also widespread in plant leaves, according to David Baltrus and his collaborators Elizabeth Arnold and Kayla Arendt, all of the University of Arizona, Tucson. “These bacteria occur in living hyphae of phylogenetically diverse endophytes isolated from various plant lineages and in multiple biogeographic provinces,” says Arnold. Earlier, Arnold and her collaborators identified 15 distinct bacterial species, primarily of the Proteobacter lineage, within 414 species of leaf endophytic fungi. These bacterial species differ from those found within endophytic fungi in other plant tissues, suggesting they play special roles within leaves.

By treating these systems with antibiotics, Baltrus and his collaborators “cure” the endophytic fungi of their bacterial symbionts, thus dissecting the bacterial influences from the purely fungal impacts of these species on various metabolic properties and ecological functions within the host plants. “Plant-associated fungi harbor bacteria that can alter fungal interactions with host plants in diverse ways,” Arnold says. For example, Luteibacter bacteria increase cellulase activity of the host fungus Pestalotiopsis, which may help the latter to colonize its plant hosts.

In addition, the fungal-bacterial symbiosis benefits the host plant by producing the phytohormone indole-
3-acetic acid (IAA), which stimulates plant growth, according to Arnold. "The endohyphal bacterium significantly enhances IAA production, but does not itself produce measurable IAA when grown outside of the fungus," she says. Thus, the bacterial-fungal relationship sustains the fungal-plant symbiosis. This cooperative IAA production may also diminish plant defenses against the fungal endophyte, a topic for future research.

However, other bacterial-fungal symbioses can prove detrimental to host plants, according to Laila Partida-Martinez of Cinvestav in Irapuato, Mexico. The phytopathogenic fungus *Rhizopus microspores*, which causes rice seedling blight, requires the endo- fungal bacterium *Burkholderia* to produce the phytotoxin rhizoxin, she says. Genomic sequencing reveals that these bacteria provide a critical enzyme, polyketide synthase, in the rhizoxin biosynthetic pathway. Evidence of metabolic symbiosis implies a mutualistic relationship between the bacteria and fungi. "Prediction of primary metabolic pathways and transporters suggests that endosymbionts consume host metabolites like citrate, but might deliver some amino acids and cofactors to the host," she says.

Furthermore, these bacteria are indispensable to the host fungus, playing an essential role in their reproduction. "The persistence of this fungal-bacterial mutualism through symbiotic-dependent sporulation is intriguing from an evolutionary point of view and implies that the symbiont produces factors that are essential for the fungal life cycle," Partida-Martinez continues. "Reproduction of the host has become totally dependent on endo-fungal bacteria, which, in return, provide a highly potent toxin for defending the habitat and accessing nutrients from decaying plants."

NEW FROM ASM

**New Insights on Eisosomes**

Fungal eisosomes are shallow, trough-shaped invaginations of the plasma membrane, of unknown function, that are ubiquitous in fungi. Fungal eisosome assembly requires two conserved proteins carrying "BAR" domains, triple-coiled-coil motifs associated with generation of membrane curvature. Now Ursula Goodenough of Washington University, St. Louis, et al. have identified eisosomes in a subset of red and green microalgae and in cysts of a ciliate. "Microalgal eisosome assembly is correlated with the presence and nature of cell walls," she says. Though sequenced microalgae lack fungal BAR proteins, she has identified two lineage-specific BAR encoding gene families that are candidate eisosome organizers. "The presence of eisosomes in algae, fungi, and ciliates indicates that these membrane differentiations were present in ancient eukaryotic common ancestors," she says. "Experiments probing function in microalgae may yield functional insights. Some fungicides are known to bind to ergosterol, which is enriched in fungal eisosomes." And this, she says, "is the first report of a stable structural patterning of membrane" (J.-H. Lee, J.E. Heuser, R. Roth, and U. Goodenough. 2015. Evisosome ultrastructure and evolution in fungi, microalgae, and ichens. Eukaryot. Cell. Online ahead of print 7 August 2015; doi: 10.1128/EC.00106–15.)

NEW FROM ASM

**Skin Microbiome Influences Common Sexually Transmitted Disease**

In the first such prospective study, Stanley Spinola and Julia van Rensburg of Indiana University School of Medicine, Indianapolis show that individuals with a particular skin microbiome can effectively clear bacteria that cause chancroid, a sexually transmitted disease common in developing countries that has been linked to enhanced HIV transmission. They infected eight volunteers on the arm with *Haemophilus ducreyi*, sampling the microbiome before, during, and after infection. Four volunteers resolved the infection, and four formed abscesses. Resolvers all had similar microbiomes prior to infection, while the microbiomes of those who formed abscesses were different from one another prior to infection, becoming more similar as infection proceeded. "This suggests that the immune response to *H. ducreyi* infection causes the microbiomes to become more similar," says Spinola and van Rensburg. They suggest that the more prevalent bacteria in the resolvers may protect the skin by outcompeting the pathogen, by keeping the immune system primed to fight invaders, or may reflect a more resistant immune system. Ultimately, the skin microbiome could be used to identify people at risk for infection, says Spinola.


NEW FROM ASM

**Transgenic Mouse Model with Reporter Gene Enables Monitoring of Progression of Inflammatory Disease**

When researchers evaluate the severity of inflammatory disease in experimental mice, they typically must euthanize the mice and then subject tissues to a variety of analytical techniques. For all this, they gain a

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A snapshot of the phenotype, but with no information on the time course of the disease. Now Takashi Moriguchi and colleagues of Tohoku University, Sendai, Japan have developed a transgenic mouse model that enables investigators to monitor progress simply, by means of a luciferase reporter gene. In their experiments they found that their “WIM-6 system” mice (Whole-body in vivo monitoring with human interleukin-6 luciferase transgenic mouse model) showed robust luciferase luminescence in the central nervous system after experimental auto-immune encephalomyelitis induction. They then cross-bred their mice with a model deficient for Nrf2, a master transcriptional regulator of antioxidant genes, and demonstrated that the systemic anti-oxidative stress system is crucial for prevention of inflammatory neurodisease. The new model, Moriguchi says, will help researchers to evaluate the efficacy of candidate drugs, and to find damaged tissue in systemic inflammatory diseases.


NEW FROM ASM

Changes in Chromosome Structure Regulate DNA Replication Initiation

Bacterial chromosomes change organization and structure during the cell cycle, for reasons as yet unclear. Now David Magnan and David Bates of Baylor College of Medicine, Houston, show in Escherichia coli that programmed changes in chromosome structure may regulate initiation of DNA replication. Negative supercoiling, which favors duplex melting, is required to initiate replication. Based on their previous observations, they posited that structural differences before and after initiation of replication changed chromosome supercoiling. They found that artificially tethering the chromosome to the cell membrane decreased negative supercoiling, and blocked replication initiation without affecting other DNA metabolic processes. “This finding may reveal new targets for antibiotics, and may explain why existing antibiotics that affect DNA supercoiling are so effective,” says Bates. “More significantly, this research may lead to better understanding of bacterial cell cycle control, which is a black box, as well as eukaryotic replication initiation, which is also sensitive to changes in chromosome structure.”


NEW FROM ASM

Periodontitis and Heart Disease: Researchers Connect the Molecular Dots

Periodontitis is a risk factor for heart disease. Now doctoral student Boxi Zhang and Torbjörn Bengtsson of the School of Health Sciences, Örebro University, Örebro, Sweden, showed how this happens. Gingipains, virulence factors produced by Porphyromonas gingivalis, boost expression of the pro-inflammatory angiopoietin 2 while dampening expression of the anti-inflammatory angiopoietin 1 in the smooth muscle cells, with the net effect of increasing inflammation. Inflammation is strongly implicated in atherosclerosis. “...stimulation with wild-type P. gingivalis dramatically increases the gene expression of angiopoietin 2 in [aortic smooth muscle cells],” the investigators write.

Angiopoietin 2 boosts migration of aortic smooth muscle cells, which promotes atherosclerosis, says Zhang. The investigators hope to find biomarkers that can aid in diagnosis and treatment of both diseases.


NEW FROM ASM

Long-Distance Travelers Likely Contributing to Antibiotic Resistance’s Spread

Swedish exchange students who studied in India and in central Africa returned from their sojourns with increased diversity of antibiotic resistance genes in their gut microbiomes. Anders Johansson of Umeå University, Sweden, et al. found a 2.6-fold increase in genes encoding resistance to sulfonamide, a 7.7-fold increase in trimethoprim resistance, all without any exposure to the antibiotics before or during the 35 students’ travel. Motivating the research was the observation that as head of a hospital infection control department, “it is very evident that resistance is no longer generated primarily in the hospital,” but that patients have become a source of increasing resistance, says Johansson. “Suppressing further spread after travelers return to their home countries is crucial,” he says.