

# Meeting Report: Microbiomes in Food Safety, Food Quality, and Human Health

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## SUMMARY

The Institute for Food Safety and Health (IFSH); International Life Sciences Institute, North American Branch; and the Food Research Institute (FRI) of the University of Wisconsin-Madison brought together more than one hundred academics, regulators, and industry experts for a symposium “Microbiomes in Food Safety, Food Quality, and Human Health” on September 28, 2017, in Burr Ridge, Illinois. The human gut microbiome and its relationships with diet and disease were highlighted, while the microbiomes of food, food animals, and food manufacturing environments were also discussed. Metagenomic methods have broad diagnostic, therapeutic, and other impacts that several speakers explored. Starting from these topics, the meeting addressed several important questions: How can microbiome knowledge be used to benefit human health? How is the food industry using this information to improve food safety? And how are regulatory agencies using the flood of microbiome information to inform their decisions?

## OVERVIEW

The microbiome is “the infection intersection: where host, food, and pathogen intersect,” according to speaker Colin Hill (University College Cork, Ireland). He reminded the audience and speakers that a microbiome is more than just bacteria residing in the gut; it consists of the bacteria, viruses, fungi, and their genomes in the specific environment, including the host and its genome.

Microbiome research encompasses more than just studies involving the human gut. Speakers discussed the microbiome of different animal species, plants, foods, and food manufacturing environments. Within the gut, the microbiome is not homogeneous; the microbiota found in an easily obtained fecal sample differs significantly from the microbiota of other parts of the gastrointestinal tract (such as the ileum, meters away along the tube). Even different locations within an organ (the mucosal layer vs. the lumen of the intestine, for example) may host different microbial residents.

Setting the stage for the first half of the meeting, Cindy Davis (National Institutes of Health) provided a general overview of the human microbiome. Humans are compos-



ites of species, with up to ten times as many microbial cells as human cells. Our microbiomes benefit us in many ways: they allow us to harvest nutrients and extract otherwise inaccessible energy; produce vitamins; metabolize carcinogens, and compete with pathogens. Every individual’s microbiomes (which differ depending on body location) are unique to them.

While it is straightforward to obtain a 16S rRNA profile of an individual’s fecal bacteria, the interpretation of this information remains on the cusp of being possible. Although much has been learned about what microbes within our bodies can do, the definition of a healthy microbiome remains elusive. Having a greater diversity of species seems to be important (and can help reduce susceptibility to pathogens), but it remains unknown exactly which organisms and what abundance of them is desirable for health. Although certain shifts in the human microbiome (gut and other locations) have been correlated with various states of disease, no definition for a healthy microbiome currently exists, and we cannot yet predict how broad changes to the microbiome translate into health effects.

### How can you change the microbiome?

Microbiomes are remarkably resilient, but they can be altered by prebiotic and probiotics, antibiotics, fecal transplants, and diet.

Prebiotics, as defined by Maria Marco (University of California, Davis), are typically non-digestible components of food that beneficially affect the host by stimulating the growth or activity of bacteria in the gut. In other words,

prebiotics feed the microbiota. Fiber is probably the best characterized prebiotic. Other prebiotics include polyphenols and the ellagitannins found in red wine, berries, walnuts, and other foods.

Probiotics include living microorganisms (such as *Lactobacillus* or *Bifidobacterium*) that, when provided in sufficient amounts, confer a health benefit to the host. As discussed by Marco, however, only about half of human probiotic studies demonstrated an effect on the gut microbiota (8).

Other factors that impact the gut microbiome include drugs, especially antibiotics, and fecal transplants. Fecal transplants and antibiotics can have a profound effect on the microbiome, while the impact of pre- and probiotics is less extensive. Large-scale changes in the microbiome might not be necessary for benefits to be obtained, however. Marco presented recent work from her lab demonstrating that oral feeding of a probiotic to mice does not significantly alter the microbiome but is still associated with beneficial changes. Subdominant members of the microbiota may exert substantial effects.

#### Diet affects microbiome, and microbiome affects diet

A key factor that impacts the microbiome is diet. Microbiomes are “resilient,” but long-term food consumption patterns can alter microbial profiles, according to several reports cited by Davis (2, 7). Short-term changes in diet can also impact microbial activity and gene expression (6).

Numerous speakers discussed how diet and the gut microbiota interact to influence health. One of the best-studied examples is that of gut microbes fermenting complex plant polysaccharides into short chain fatty acids (SCFA) such as acetate, propionate, and butyrate. Cindy Davis reviewed work demonstrating that butyrate is a preferred energy source for healthy colonocytes, while cancerous colonocytes are instead inhibited by this microbial metabolite (4).

Expanding on the effect of a fiber-rich diet on health, Maria Marco (UC-Davis) discussed a clinical trial of a

high-fermentable carbohydrate diet (HCD) vs. the typical Western diet. The HCD changed fecal microbiota (9), increased SCFA levels, and improved fasting LDL and total cholesterol levels among individuals on statins, although it did not alter weight or insulin resistance (16). The HCD also reduced intestinal inflammation and improved gut barrier function more than the Western diet did. Notably, individuals demonstrating improvement in intestinal inflammation and barrier function had higher baseline levels of *Akkermansia*, suggesting that certain baseline bacteria are necessary for dietary fiber to have a beneficial impact.

Marco discussed another study in which mice fed a resistant starch (RS) diet (compared to those fed an isocaloric high-fat diet) showed a rearrangement in their gut microbiota toward increased proportions of Bacteroidetes. These microbiome changes were associated with improved markers of intestinal function but were not linked to changes in weight or insulin resistance (3). Interestingly, the resistant starch diet was also associated with reduced levels of branched chain amino acids, a biomarker linked to a loss of insulin sensitivity.

As discussed by André Marette, many of the foods positively associated with gut microbial diversity (e.g., fruits, vegetables, red wine, and tea) is rich in polyphenols. Cranberry extract, for example, protects against diet-induced weight gain and reverses certain manifestations of metabolic syndrome in mice; these effects are associated with an expansion of the *Akkermansia* spp. population in the gut (1).

Marette discussed his laboratory’s recent work with camu-camu, a Brazilian fruit with high levels of polyphenols. In mice, camu-camu extract prevented obesity and decreased insulin resistance and glucose intolerance. The beneficial effects of camu-camu extract were associated with increased overall gut microbial diversity, a decrease in Firmicutes/Bacteroidetes ratio, and an increase in *Akkermansia muciniphila* levels. *Akkermansia* spp. is the main microbes colonizing the gut mucosal layer; increased levels of this organism are associated with the metabolic benefits of both bariatric surgery and the antidiabetic drug metformin. *Akkermansia* spp. are hypothesized to help maintain the integrity of the mucus layer and protect against intestinal permeability. The latter reduces leakage of bacterial lipopolysaccharides (LPS), preventing inflammation and its downstream effects such as metabolic disease.

Camu-camu extract treatment in mice was also associated with a decrease in plasma LPS and an increase in expression of genes associated with energy expenditure. Most interestingly, fecal transplants from camu-camu-treated mice to germ-free mice recapitulated the effects of camu-camu on energy expenditure and weight gain without altering food intake.

Diet influences the microbiome, but the microbiome also impacts the nutritional benefits of food. Federico



Rey (University of Wisconsin-Madison) illustrated how microbial metabolism can affect host nutrition, using gut microbial metabolism of choline as an example. Besides serving as a key methyl group donor involved in 1-carbon metabolism (and therefore epigenetic regulation), choline is also important in cell membrane integrity and serves as a precursor to the neurotransmitter acetylcholine. Choline intake is particularly important during the perinatal period, with deficiencies in utero associated with cognitive and behavioral problems in offspring.

Gut bacteria can convert choline into trimethylamine (TMA), depleting plasma choline levels. When germ-free mice are colonized with recombinant bacteria lacking the enzyme needed to convert choline to TMA, the mice have higher plasma levels of choline. Rey's group demonstrated that reduced plasma choline levels resulting from gut microbial choline utilization can trigger problems similar to those associated with dietary choline deficiency. Current dietary recommendations for choline do not take into account microbial metabolism of the nutrient.

TMA produced from choline by gut bacteria can be converted by the host to trimethylamine N-oxide (TMAO), a proatherogenic metabolite linked to cardiovascular atherosclerosis, diabetes, and other diseases. Rey commented that in some stages of life (for example, during perinatal development) choline depletion may be more problematic than TMAO accumulation.

#### **Perturbations in the microbiome can perturb health**

When alterations in the microbiome are linked to negative health consequences, the shift is referred to as dysbiosis. André Marette (Université Laval, Québec City, Canada) described how dysbiosis can cause obesity-linked cardiometabolic diseases. Gut microbiota from obese donors (mice or humans) can also transfer metabolic syndrome to germ-free mice (15, 19). Maintaining integrity of the gut mucosal layer appears critical: emulsifiers (found in some processed foods) may cause dysbiosis leading to colitis and metabolic syndrome by perturbing the microbiological composition of the gut mucosal layer (5).

Anne Marie Singh (University of Wisconsin-Madison) described how the gut microbiome influences immune system development, thereby impacting the development of food allergies. Increased levels of *Staphylococcus aureus* were found in fecal samples from children with food allergies. An association between atopic dermatitis and colonization with *S. aureus* has also been noted, suggesting that certain *S. aureus* proteins may act as "super antigens" that trigger broad immune responses, subverting the development of oral tolerance to foods. Studies in mice strengthen the hypothesis: oral tolerance can be elicited by oral administration of peanut or egg protein, but such tolerance is prevented when *Staphylococcal* enterotoxin B is given concurrently.

#### **Studying the microbiome**

Techniques used to study the microbiome were addressed throughout the day. Several speakers, including Rey, described studies in which germ-free mice were colonized with defined species of microbes. An important strength of these types of studies is that they allow both host and microbial genetics to be manipulated.

Paul Morley (Colorado State University) discussed the microbial ecology of antibiotic resistance in cattle. The lack of consistent correlation between administration of antibiotics and antimicrobial resistance is a puzzle. In a recent study by Morley's group, beef cattle raised without antibiotics did not show substantially different abundances of antibiotic resistance genes in their microbiome compared with cattle raised conventionally with antibiotics (20).

Morley's research integrates a genomic approach to find out "who is there" with a transcriptome approach to see which microbes are active and a metabolomic approach to identify what microbial products are generated. Significant nasopharyngeal microbiota changes occur in cattle from the time of weaning to arrival at (and during the time at) a feedlot. Microbiome changes precede illness, suggesting that times when the microbiota are in rapid flux are opportunities for pathogenic infection (18). Morley provided evidence that the "resistome," or collection of genomic sequences encoding antibiotic resistance genes, varies greatly among the feces, soil, and water in a feedlot. The abundance of antibiotic resistance genes decreases through the time of slaughter (10), with no resistance genes found in samples collected after slaughter.

#### **Microbiome research being conducted now is predicted to drive significant health-related benefits**

Personalized nutrition may someday be based on an individual's unique microbiome. A recent study cited by Cindy Davis described how an individual's glycemic response to a food can now be predicted based on his or her microbiome (21). Dietary requirements may change to reflect colonic microbial requirements to maximize host health.

Microbiome-based anti-infectives are being investigated, as discussed in detail by Colin Hill. The microbiome plays an important role in preventing infection with foodborne pathogens, as germ-free mice are exquisitely sensitive to infection. Hill described a recent clinical study in which oral administration of a probiotic plus a prebiotic prevented sepsis among infants in rural India (13). Hill's group has developed probiotics that are efficacious against a variety of microbial diseases in various animal species, including porcine salmonellosis, bovine mastitis, and murine listeriosis.

Probiotics may function as anti-infectives through a variety of mechanisms, including the production of bacteriocins that kill or inhibit other bacteria. Hill's group identified thuricin as efficacious against *Clostridium difficile* (14), selectively attacking the pathogen while preventing collateral

damage to the microbiome that occurs with conventional antibiotic therapy. In other work, inactive bacteriocins buried within the genomes of *Lactobacillus* spp. were resurrected in a quest for new bacteriocins effective against a broader range of bacteria. The bacteriocin nisin was also intelligently engineered to resist gastric proteases and even to expand its specificity so that it can destroy *Listeria monocytogenes*. Hill predicts that, in addition to bacteriocins, other bacterial agents borrowed from nature, the bacteriophages, will find increasing use as therapeutics and food additives.

Andrea Ottesen (FDA) described how genomic and microbiome research on plants and elsewhere is being used to make food safer. FDA, together with the National Center for Biotechnology Information, is developing a public library of chloroplast DNA from plant species (23), dubbed “GenomeTrakrCP.” Chloroplast DNA, like genomic DNA, can be used to identify plant species and may allow closely related species to be better distinguished than is possible by standard genomic DNA barcoding (22). GenomeTrakrCP may soon help identify falsely labeled or adulterated foods as well as pinpoint the source of plants associated with foodborne disease outbreaks. Another new program, Metagenome TrakR, generates a fingerprint of all of the organisms present in an environment: host, bacterial, fungal, viral, etc. This combined information can help identify the source of pathogens in food beyond simply sequencing the pathogen.

Drawing upon the recent ice cream listeriosis outbreak (11) and the *Salmonella* outbreak associated with papayas, Ottesen gave examples of how microbiome knowledge can improve enrichment strategies, especially for low-abundance pathogens. Other work done in collaboration with Bob Sanderson (Jonathan’s Sprouts) demonstrated that protists can accumulate in the water used to grow sprouts. These protists could serve as “Trojan Horses,” quietly harboring and releasing bacteria, potentially including pathogens, while sprouts are grown. The potential of air to introduce microbes (including pathogens) that can colonize a plant was also discussed. Plastic plants (as a control) and tomato plants exposed to the same air were associated with similar bacterial taxa over time, suggesting that the environment, including air, plays an important role in determining the microbial composition of the plant phyllosphere (12).

#### **How are the food industry and regulators using microbiome/metagenomic analyses now?**

Food industry leaders and government regulators discussed how they are currently using microbiome and metagenomics analyses (and knowledge obtained from these efforts).

John O’Brien (Nestle) discussed the impact of recent high-profile reports on the negative effects of artificial sweeteners (17) and emulsifiers (5) on the mouse microbi-



ota and their associated impacts on glucose tolerance and colitis/metabolic syndrome, leading food companies to examine their use of these additives. On the positive side, Miguel Freitas (Danone) commented that there is strong continued interest in using probiotics to improve the healthfulness of foods.

Microbiome and metagenomic testing are also impacting food and environmental testing. Greg Siragusa pointed out the dilemma that a company using whole genome sequencing on environmental samples faces: what should they do if they find a pathogen? Mark Allard (FDA) said that FDA is not currently collecting regulatory microbiome samples. They are screening for antibiotic resistance genes in ground meats, however, and will be performing metagenomic analyses during water sampling.

Regulatory agencies are conducting microbiome-related research to directly address significant food safety concerns. Joelle Salazar (FDA) discussed research projects demonstrating that storage of bagged spinach at abusive temperatures for several weeks greatly increases the relative abundance of *E. coli*. In response to the 2010 outbreak of *E. coli* O157:H7 associated with raw milk Gouda cheese, Salazar also described efforts to better understand how native microbes influence pathogen survival in cheese by studying how bacterial composition of cheese is affected by milk pasteurization, by location within cheese (near the rind or not), and by aging.

Andrew Benson (Metagenome Analytics and University of Nebraska-Lincoln) discussed work that is addressing how the food industry can enter the genomic and metagenomic era. As director of the Nebraska Food for Health Center, Benson described efforts to understand how genetic variations in grains (bred from ancestral strains to improve crop yield, hardiness, etc. but not nutrition) affect utilization by the human gut microbiome, thereby impacting consumer health.

Benson believes the food industry has been slow to use next-generation sequencing (NGS)-based diagnostics because of technical and skill-related challenges as well as negative perceptions driven by regulatory agency efforts

to link outbreaks with environmental samples collected years earlier. Although the ability of NGS to deliver actionable data to the food industry has been largely unproven, Benson described examples of how metagenomic analyses were used to identify the source of a persistent product spoilage problem and how such methods can improve supply chain management.

Metagenomic techniques promise to have more widespread utility in diagnostic applications. Culture-based diagnostic methods remain the gold standard, but the food industry is “poised for a revolution,” according to Benson. As mentioned by Robert Baker (Mars), deep sequencing, which can allow sequencing of rare populations without enrichment, is already being used. As summarized by Benson, NGS technology applications that will become mainstream diagnostic methods in the food industry must have advantages over current technology, be actionable, be cost-effective, and be accepted by regulatory agencies.

### Remaining challenges

Challenges for microbiome research were discussed at the meeting. As described by Paul Morley, it’s easy to collect a fecal sample, but the sample may not tell you what is happening elsewhere in the gastrointestinal tract. Several speakers, including David Klurfeld (USDA), stressed the importance of using diets that reflect actual human diets when conducting microbiome studies. Components of laboratory diets (for example, emulsifiers) can have a significant effect on experimental results, as can the baseline microbiota. It remains unclear what a “normal” or healthy microbiome is or whether microbiome changes induced by diet are good or bad. Terminology needs to be more consistent and better defined.

As mentioned by Maria Marco, it is unclear when food becomes a drug. Tension exists between knowing the mechanism and making claims. While consumers have embraced probiotics, per John O’Brien, making health claims for probiotics is not yet justified. Structure/function claims are allowed (but consumers do not necessarily differentiate). For example, the U.S. Federal Trade Commission challenged Dannon’s label claim for its Activia yogurt (that it relieved irregularity), but this has not affected consumers’ views (or purchase) of the product. Marge Leahy (ILSI North America) invited interested parties to participate in ILSI North America’s working group on the gut microbiome, which aims to further understand the relationship between food and the human gut microbiome and its impact on health.

Challenges exist for metagenomic and NGS diagnostic analyses as well. Industry remains concerned about using NGS methods in environmental and food product testing because it is not known what to do if pathogens are detected. *Salmonella* outbreaks linked to papayas (described by Andrea Ottesen) and cilantro (discussed by Joelle Salazar)

provide examples of how culture/enrichment methods can diminish the sensitivity of pathogen detection and how the overall microbiota of a food may complicate pathogen detection. Turnaround time and cost remain a challenge for NGS methods, according to Greg Siragusa, who predicts that the clinical sector will continue to drive improvements to make these technologies faster and cheaper.



A final concern, expressed by John O’Brien, involves the potential for microbiome and metagenomic research to be overhyped. In the words of one of the meeting organizers, Charles Czuprynski (FRI), “You’d have to be off the grid if you haven’t heard of the microbiome” given the explosion of papers on the topic in academic journals and the popular press. However, as pointed out by Miguel Freitas (Danone), fewer than 16% of consumers understand what the microbiome really is. The promise of these new technologies to improve human health and make food safer is anticipated and appears likely, but more work and time is still needed before its full potential is realized.

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