Perspective

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Fulfilling the promise of microbiomes to revolutionize medicine

Perspective

The microbiome is the sum of all organisms living within a specific ecological niche. Until recently, only common, culturable members of a community could be identified. The development of novel technologies for inexpensively sequencing hundreds of thousands of DNA strands has allowed us to see the broad spectrum of bacteria, fungi, and even viruses inhabiting various niches on and within the human body. High throughput sequencing detects organisms that have not been cultured, revealing richer biodiversity than was previously thought to exist. Early microbiome studies involved creating libraries of cloned 16S rDNA or random DNA fragments. This method did not require organisms to be cultured, but was strongly biased towards the most abundant species in a sample. The advent of Roche 454 barcoded pyrosequencing and Illumina fluorescent dye-based sequencing freed researchers to more deeply characterize microbial communities.[1]

Microbiome studies have led to surprising discoveries, such as the role of the microbial flora in promoting or preventing obesity, inflammatory bowel disease, chronic rhinosinusitis, skin diseases, and many other diseases.[2–6] The American Gut Project (americangut. org) has analyzed the fecal flora of over 3,000 volunteers. This project has revealed that a person's diet, age, exercise level, and alcohol consumption influence microbiome composition and diversity. Each individual harbors his or her own unique microbiome; however, some patterns have emerged. The microbiomes of diseased individuals often differ from those of healthy individuals. Pathological alterations are termed "dysbiosis".

Common forms of dysbiosis include oral thrush, vaginosis, and small intestinal bacterial overgrowth (SIBO). Antibiotics or antifungals have historically been used to treat dysbiosis; however, these treatments can create additional problems by killing beneficial members of the microbiome and leaving patients vulnerable to infection with pathogenic organisms such as *Clostridium difficile*. Research on the use of probiotics as sole treatments or in addition to antibiotics has intensified over the past decade.[7–9] Probiotic therapy involves administration of a limited number of microbial species, such as Lactobacilli and Bifidobacteria. Probiotic treatment is limited to culturable organisms. Oral probiotic treatment has been used with limited success to treat ulcerative colitis, allergic rhinitis and sinusitis.[10–12] The effects of oral probiotics on allergy are likely due to immune system shifts.[10] The disadvantage of these treatments is that there is no evidence that the microbiome is permanently altered by probiotics, meaning that long-term treatment is required.[13] Shifts in the microbiome associated with disease typically involve changes in the prevalences of many species, rather than the absence of a few species. Therefore, a large shift in the microbial composition may be required to achieve lasting disease remission.

In addition to the above diseases, bacteria are increasingly being recognized as mediators of carcinogenesis. *Helicobacter pylori* were the first bacterial species classified as a carcinogen.[14] *H. pylori* is known to cause stomach cancer and is suspected of causing some colorectal cancer and a rare peritoneal cancer.[15,16] Other bacteria are being investigated for their roles in cancer as well. Mouse models of

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colorectal cancer clearly demonstrate that certain bacteria promote or inhibit colon cancer development when germ-free mice are infected with one or a few bacterial species.[17–22] Investigators are attempting to dissect the role of bacteria in human colorectal cancer by studying microbiomes of healthy and cancer patients.[23]

Microbiome studies have provided a wealth of promising data, but determining which bacteria are present within a given location is only the start. As we know, correlation is not the same as causation. Are certain bacteria causing a disease, or are they present due to host physiological changes that result from the disease? The bacteria that initially promote carcinogenesis-associated cellular changes may be different from the bacteria that later drive continued tumor growth.[24] Some tumors have necrotic regions that are favored by anaerobic bacteria. The anaerobic bacteria present within the tumor may not have anything to do with tumor development or progression. It is believed that *H. pylori* are often cleared from the stomach as gastric cancer progresses.[25] Therefore, the causative agent may no longer be present by the time the cancer is detected. Zackular et al.,[23] have attempted to address these issues by studying the gut microbiomes of healthy individuals compared with patients who have developed colonic adenomas, a precursor of colon cancer, and with those who have progressed to carcinoma.[23] Significant microbiome differences were seen among these three groups, and this may lead to improved diagnosis.

In spite of the copious data provided by high throughput sequencing methods, existing methods and analysis software have shortcomings. Current microbiome sequencing methods are only able to detect relatively abundant microbial species ($>10^6$ microorganisms per gram of feces).[26] This leaves out certain important pathogens that may be present in small numbers, such as intestinal *H. pylori* and *Salmonella typhi*. The depth of sequence analysis will have to be increased still more in order to find needle-in-a-haystack species that could impact health. Roche-454 and Illumina sequencing platforms generate prodigious amounts of data, but read lengths are less than 500 base pairs. This allows sequencing of one to three 16S rDNA variable regions, which is not always adequate to identify organisms at the species level.[1] Newer technologies promise longer reads; however, analyzing this amount of data requires more computing power than standard desktop computers can provide. One must also devote a considerable amount of time to mastering the existing microbiome

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analysis software. Given the speed of progress over the past decade, the above problems will no doubt be overcome.

Identification of the organisms present in various niches is only the beginning. Proving causation and developing new treatment and prevention strategies will require more than analysis of microbiomes. Animal models, *in vitro* models, and clinical trials are needed. Many of the organisms discovered during microbiome analysis have not yet been cultured. Therefore, novel and improved culture methods must be developed. *E. coli* and some other common bacteria can synthesize their own amino acids and use simple carbon sources, such as glucose. Other bacteria have more complex nutritional requirements. Many of the classical bacteriological media and methods were developed before 1940. Perhaps it is time for a renaissance in the study of bacterial culture methods. One pioneering study utilized 212 different culture conditions to isolate fecal bacteria, resulting in greater diversity than had been obtained previously and 31 new species.[27] Human-associated bacteria may be adapted to obtain nutrients from mucins, skin lipids, host iron binding molecules, DNA, and other bacteria. Therefore, mimicking the host environment to the greatest extent possible may increase recovery of novel organisms. Methods for growing bacteria in contact with their natural environments have been very successful in culturing environmental organisms.[28] This involves using semi-porous membranes to physically separate bacteria from the environment while allowing nutrients, bacterial products, and siderophores to flow freely. In some cases, a new species is discovered to grow only in the presence of a helper strain. Thus far, the critical factors provided by the helper bacteria have turned out to be heme, NAD, and catalase;[28] however it is possible that other bacterial or host factors are essential for some organisms.

Once the bacteria are identified and cultured, the big questions remain; which bacteria are doing what and how can the microbiome be safely adjusted to improve health? Although animal models have limitations and do not always predict results in humans, they are currently the best tool available for studying the complex interactions between humans and microbes. Mouse models of inflammatory bowel disease have elucidated interactions between certain components of the immune system and several bacterial genera that contribute to chronic inflammation.[29] Various mouse models of colon cancer have demonstrated that germ free mice are immune to cancer development.[18] Moreover, colonization of mice with some bacterial species (e.g. *Fusobacterium nucleatus, Helicobacter,* enterotoxigenic *Bacteroides fragilis*) promotes cancer while colonization with specific *Lactobacillus* strains does not.[17,19–22]

Current antimicrobial agents are not a suitable means for adjusting the microbiome due to antibiotic resistance and the unwanted side effect of reducing overall diversity. Species- or genus-specific antimicrobial agents or vaccines would be useful, but will likely take many years to develop. Other methods will have to be used to manipulate the microbiome. "Fecal transplants", which involve transferring fecal material from a healthy individual to the intestines of a sick individual, can alter the recipient's microbiome. Fecal transplants have proven far more successful than antibiotics in the treatment of diarrhea and colitis caused by *Clostridium difficile* infection and are gaining acceptance among physicians.[30] Fecal transplants are often successful in treating other inflammatory bowel diseases as well, including Crohn's disease and ulcerative colitis.[30] Thus far, it appears that fecal transplants are more effective for treating inflammatory bowel diseases than treatment with probiotics.[31]

While fecal transplants have saved many lives, the method is too crude and risky for use in less serious diseases, not to mention the "yuck" factor. A core set of bacteria capable of ousting *C. difficile* from the gut must be identified so that a safe, standardized treatment method can be developed. Similarly, the species that promote or prevent obesity, inflammatory bowel disease, cancer, and other disorders need to be identified. Evidence suggests that increased microbiome diversity contributes to disease and allergy resistance,[32–35] but how much diversity is needed and how can diversity be generated? Creating defined mixtures of hundreds of species in a stable form is a daunting proposition, though not impossible. Once a diverse microbiome is generated, it must be maintained. Major dietary alterations can maintain biodiversity, but would it be possible to develop a dietary supplement that would achieve the same goal? Prebiotics are food components that can alter the growth of specific groups of microbes. Currently, nondigestible oligosaccharides are most commonly used as prebiotics. Oligosaccharides are fermented by certain gut bacteria, producing beneficial metabolites.[36,37] Fungal chitin-glucan, plant polyphenols, peptides, and lipids are also being tested.[38,39] There is some clinical evidence that prebiotics can reduce plasma cholesterol, improve glucose tolerance.[40] Enrichment of pasta and bakery products with prebiotics has been proposed as one method for making prebiotics widely available to the general public.[39] The use of live microbes and prebiotics to foster maintenance of healthy microbiomes may one day be as routine as supplementing foods with folate, vitamins, and minerals.

Bacteria are no longer mere "germs" to be feared eradicated. They are also more than food digesters and vitamin providers. Our microbiomes protect us from invaders, shape our immune systems, modulate our metabolisms, and may even alter our moods.[41] Just as determining the structure of DNA and cracking the genetic code were only the first steps in understanding the roles of genes, identifying the members of localized microbiomes is the beginning of a race towards a new form of health management.

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Conflicts of interest

Author declares that there is no conflict of interest.

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