A CONNECTED COMMUNITY:
THE RISE OF MICROBIOME RESEARCH
Innovations in Microbiomes
Complete Microbiome-Based Research Solutions from LI-COR

- Identify microbiota function in soil, plants, and animals
- Broaden targeted therapeutics research initiatives
- Advance microbiome-based drug development

Learn More
licor.com/microbiome

---

EZ Species
Microbiome Sequencing Service

Easily identify the bacterial composition of your sample, down the species level

Shot gun level sequencing, 16S methodology
A diet rich with olive oil, fresh fruits and vegetables, legumes, and fish, with minimal red meat, sweets, and alcohol—known as the Mediterranean diet—has been touted for beneficial health effects. In particular, the Mediterranean diet reduces the risk for developing cardiometabolic diseases, including cardiovascular disease and type 2 diabetes. However, few studies have explored how individual gut microbiomes affect the association between a heart healthy diet and disease risk. In a recent paper in the journal *Nature Medicine*, researchers determined how long-term adherence to a Mediterranean diet affected cardiometabolic health as a function of an individual’s gut microbiome.

We often think about a one-way interaction between our diet and our heart health: good food is good for the heart. After all, an apple a day keeps the doctor away. While there is truth to this maxim, our gut microbiome has more influence than we might imagine. Diet affects the microbiome, but the gut microbiome influences the nutrients that reach the body.

To examine the effect gut microbes have on health, Dong Wang and colleagues from the Harvard T.H. Chan School of Public Health turned to a group of more than 300 men who were part of the larger, long-running Health Professionals Follow-up Study (HPFS). In this subpopulation, called the Men’s Lifestyle Validation Study, the researchers acquired longitudinal data from the HPFS on the men’s dietary and lifestyle habits and medical diagnoses to analyze alongside more recent diet questionnaires and stool and blood samples.

The researchers first explored whether dietary patterns affect gut microbiome composition. They found that long-term adherence to a Mediterranean diet associated with an increase in a number of microbial functions, including plant-derived polysaccharide degradation, short-chain fatty acid production, and lactose degradation. Additionally, individuals with greater adherence to the diet had a higher abundance of microbial species that metabolize dietary fiber. These findings are not surprising given that the Mediterranean diet is largely a fiber-rich, plant-based diet.

Previous studies highlighted a number of taxonomic features associated with the Mediterranean diet, such as increases in *Faecalibacterium prausnitzii* and decreases in *Collinsella aerofaciens*. However, these studies have largely been limited to 16S ribosomal RNA gene sequencing, so the scientists could only perform general taxonomic profiling with little to no insight into biochemical functional profiles. In this study, the researchers ran shotgun metagenomic and metatranscriptomic sequencing on the stool samples to acquire taxonomic and functional profiling of individual’s gut microbiomes. Across individuals, 10 microbial species accounted for nearly half of community abundance. An interesting species to crop up was *Prevotella copri* (*P. copri*), which has an unusual distribution pattern in western populations; most individuals carry few of these microbes, but in a minority of individuals, it is highly abundant.

Next, Wang and colleagues wanted to know if the Mediterranean diet had any bearing on cardiometabolic disease risk, and if this association was modulated by certain gut microbes. Indeed, the authors found an overall interaction between the heart healthy diet and reduced risk for developing a cardiometabolic disease. However, this benefit of the diet was not reaped by all. For those who adhered to the diet and carried *P. copri*, there was not a strong protective association between the Mediterranean diet and disease risk.

The study did not show that the Mediterranean diet altered levels of *P. copri*, but instead showed that when *P. copri* is present in the gut microbiome, it interacts with diet, possibly through altered metabolite production. This is an important distinction that has implications for personalized nutrition. The authors suggest that adherence to a heart healthy diet may help individuals lacking *P. copri*, whereas exercise and pharmaceutical intervention may be more beneficial for *P. copri* carriers.

**Reference**

HIGHLIGHTS

High consumption of processed foods, liberal prescription of antibiotics, and the increasing use of sanitizers associated with industrialized societies alter the health of our gut microbiomes. Much of the research to date has focused on how the composition and diversity of bacterial species in our gut adapt to these factors. In a paper recently published in the journal *Cell*, a global consortium of researchers explored whether bacterial genomes respond to levels of industrialization in the host’s society by examining rates of horizontal gene transfer (HGT).

Bacteria often employ HGT to move their genes to other bacteria without reproduction (vertical gene transfer). Bacteria gain new functions through HGT to rapidly adapt to changing environments. This process can occur surprisingly fast within an individual’s bacterial genome and stick around for a while, especially when a particular function offers advantages to the bacteria, such as antibiotic resistance. However, it is unclear whether HGT in response to environmental changes can substantially impact an individual’s gut microbiome function, or whether it is simply the introduction of new, functionally-different bacteria over time that drives change.

Although the research team previously provided evidence of HGT in the human microbiome, the lack of human diversity in their samples limited their ability to draw conclusions on when exactly these transfers occurred. To address this, they developed the Global Microbiome Conservancy (GMbC) isolate collection, which includes more than 4,000 cultured, isolated, and whole-genome sequenced gut bacteria isolated from stool samples from 37 individuals living across the world in 14 different locations. After combining this data with 11 more individuals in the Broad Institute-OpenBiome Microbiome Library, the total number of isolate genomes available for analysis reached over 7,700. Next, the researchers categorized these 48 individuals as living in either an industrialized or nonindustrialized society that was either urban or rural.

The researchers wanted a more accurate estimate of how recently bacterial gene transfer occurred. To find out, they identified pairs of genomes of different species with long sequences of identical DNA as candidate HGTs. Next, they compared the frequency of HGTs occurring between bacteria strains isolated from a single host to that observed between the same bacterial strain from different individuals. They found that HGT occurred more frequently within an individual’s lifetime than between the same bacterial strain between people, supporting the hypothesis that HGT can occur within an individual’s lifetime. Some bacteria acquired anywhere from 10 to 100 new genes in a single year.

With evidence that HGT occurs frequently within an individual, the researchers next explored whether rates of HGT differed depending on the level of industrialization. To answer this, they looked at HGT in bacterial species pairs that are shared by pairs of lifestyle groups, such as rural, industrialized populations and urban, nonindustrialized populations. They observed that bacterial species pairs taken from urban industrialized populations exchanged significantly more genes than species pairs in rural nonindustrialized groups. Finally, the researchers investigated whether the genes participating in HGT reflected the selective pressures of living in these different societies. They found that individuals living in nonindustrialized societies had bacteria that frequently exchange carbohydrate-active enzyme genes, which makes sense given the high consumption of nondigestable fiber. Populations living in industrialized regions unsurprisingly housed bacteria that exchanged genes involved in gene transfer but also genes related to virulence. The elevated transfer of antibiotic resistance genes in nonindustrialized populations, where antibiotics are frequently administered to livestock, illustrates just how profound of an impact agricultural practices can have on our microbiomes.

Reference

S
ince 2009, the drug-resistant “killer fungus” *Candida auris* has been spreading through health care facilities worldwide. *Aspergillus fumigatus*, a type of mold, creeps into homes, threatening those with weakened immune systems. New antifungal drugs are sorely needed to keep up with emerging drug-resistant strains. In a recent paper published in the journal *Science*, researchers from the University of Wisconsin-Madison dove into the deep blue sea in search of novel antifungal treatments and resurfaced with a promising target.

Surprisingly, only three classes of antifungal drugs are available today. Drug discovery efforts have been hampered by our shared evolutionary history with fungi, leading to many promising leads failing due to little efficacy and toxic side-effects. Conventional high-throughput screening strategies of pure synthetic compounds often find the same hits over and over again, further hindering treatment development. In search of novel antifungal candidates, Fan Zhang and his colleagues headed off the coast of the Florida Keys.

After collecting more than 1,400 bacteria from marine invertebrates, Zhang and colleagues profiled the strains using liquid chromatography-mass spectrometry and metabolomics to examine the chemical diversity in their samples. The researchers identified 174 chemically diverse strains; one strain in particular caught their eye. When they tested these strains, the *Micro
c

c

monospora* strain WMMC-414 found in the sea squirt microbiome showed extremely high potency and chemical diversity and produced a number of metabolites. The researchers focused their efforts on one of these metabolites: turbiminic.

Determining the safety and efficacy of turbiminic was the first order of business. After establishing the minimum drug concentration needed to produce fungicidal activity in a number of fungal pathogen isolates, the researchers ran experiments to characterize the reductions in fungal growth over time across different doses. In the “killer fungus” *Candida auris*, higher doses of turbiminic inhibited fungal growth in a matter of hours. To identify the maximum tolerated dose, Zhang’s team administered a single injection of turbiminic to mice, with doses starting at 1 mg/kg and going all the way up to 256 mg/kg. Amazingly, none of these doses were toxic.

While the in vitro efficacy data and in vivo safety profiles were promising, Zhang and his colleagues wanted to test the in vivo efficacy of turbiminic. They turned to an immunocompromised mouse model where they measured fungal growth in the kidneys following injections of *Candida auris* into the bloodstream. After 4 injections over a 24-hour period, the researchers observed dose-dependent efficacy in reducing fungal burden when compared to vehicle-treated mice. Furthermore, the researchers included the standard-of-care treatment micafungin and found its effects to be comparable to vehicle-treated mice.

To better understand the mechanism of action of turbiminic, the researchers used *Saccharomyces cerevisiae* DNA-bar-coded knockdown and knockout libraries of essential genes and nonessential gene mutants, respectively. From the essential gene library, *SEC14* showed the largest reductions following turbiminic exposure. Given that *SEC14* encodes Sec14p, a transfer protein important for correct trans-Golgi network dynamics, turbiminic may impair vesicle-mediated transports via inhibition of Sec14p. This idea was bolstered by data from the nonessential gene knockout libraries showing turbiminic sensitivities in genes directly involved in vesicle-mediated trafficking, including *ERV14*, *GUP1*, and *BST1*. Finally, to directly measure turbiminic’s impact on membrane trafficking in *S. cerevisiae*, the researchers used the model cargo protein green fluorescent protein (GFP)-Snc1. While untreated samples showed the expected accumulation of GFP-Snc1 on the plasma membrane, turbiminic led to a buildup of GFP-Snc1 trapped in the Golgi and endosomes.

Zhang’s team identified a novel antifungal target with good safety profile and clinical efficacy in mouse models. Although further studies exploring the safety and efficacy in patient populations are warranted, these preclinical findings are sure to make waves in the fight against drug-resistant fungi.

Reference

LEGUME TREES HOLD THE MICROBIAL KEY TO UNLOCKING ESSENTIAL SOIL NUTRIENTS

Nitrogen-fixing trees and their soil microbiomes play an important role in fertilizing the tropical forest community.

By Danielle Gerhard, PhD

Tropical forests soak up a remarkable amount of carbon from the atmosphere, which protects the global biosphere from rising greenhouse gas emissions. However, in many tropical forests, the surrounding soil is highly weathered and nutrient-poor. In a recent study published in *Proceedings of the National Academy of Sciences*, researchers described their search to understand how tropical trees still flourish in the face of these limitations.

Nitrogen is an essential element for plant growth and continued health. For the nitrogen floating around in the atmosphere to become plant food, microorganisms in the soil convert it to nitrogenous compounds in a process called nitrogen fixation. These microorganisms have a symbiotic relationship with certain plant types and live among the roots of these plants, known as N₂-fixers. However, plants need more than nitrogen. Although large amounts of other essential minerals like phosphorus and molybdenum are found in tropical soils, they are largely trapped inside silicate rock until weathering breaks down the rocks and minerals.

Lead author Dimitar Epihov from the University of Sheffield and his colleagues wondered whether N₂-fixers were capable of locally increasing silicate weathering. To find out, the researchers traveled to the Agua Salud Secondary Forest Dynamics Network, an experimental watershed that allows for monitoring the health of young (secondary) forests. Legume trees are among the most abundant N₂-fixers, so the researchers buried bags of silicate rock around the roots of N₂-fixing legume trees, nonfixing trees in a legume-rich area, and nonfixing trees far away from N₂-fixers. After 8 months, they collected the silicates and ran an elemental analysis using X-ray fluorescence.

Epihov’s team observed that N₂-fixing legume trees exhibited greater weathering compared to the nonfixing trees, evidenced by lower concentrations of magnesium, that occurred at a rate twice as fast as non-fixers. Interestingly, silicates collected from nonfixing trees that were nearby legume trees showed more weathering than nonfixers far away from legume trees, showing that the benefits of N₂-fixers extend to neighboring trees.

The soil microbial community mediates the nitrogen fixing process. Just like gut microbiomes affect the nutrients we extract from food, soil microbes alter nutrient availability to nearby trees. To examine the impact of the local soil microbiome on silicate rock weathering, the researchers ran shotgun metagenomic sequencing on collected mineral samples. They observed a significant correlation between weathering rate and the abundance of genes involved in high level metabolic pathways including nitrogen metabolism, respiration, and the Krebs cycle. These findings suggest that microbes living below N₂-fixing legume trees alter the soil biochemistry to promote weathering, a benefit they extend to nearby nonfixing trees.

Additional gene analysis of the soil minerals revealed enrichments in iron reduction orthologs and an increased potential for anaerobic metabolism underneath N₂-fixers. Anaerobic iron reduction is a microbially mediated process for releasing iron-bound phosphorous into the soil. As this process preferentially occurs in low pH environments, iron-reducing bacteria are generally acidophilic. When the researchers ran taxonomic profiling on the three different soil samples, they found that *Acidobacteria* were the dominant class of bacteria and the more abundant *Acidobacteria* was in a soil sample, the greater the iron-reducing potential. To dig a little deeper into the soil microbiome, the researchers ran genome assemblies and taxonomic annotations, and made an exciting discovery. The soil underneath N₂-fixers and their neighboring trees was rich with a never before identified bacterial genus that they have called Candidatus *Acidoferum*. Furthermore, Candidatus *Acidoferum* abundance was greater in soil with a lower pH and smaller carbon to nitrogen ratio, which characterizes soil around legume trees.

Nutrient-poor tropical soils can impair forest growth and recovery in the face of continued environmental threats like logging, mining, and agriculture. Epihov’s team’s findings highlight the community-wide impact legume trees have on nutrient cycling and availability and have implications for reforestation initiatives.

Reference
NGS Standards for Microbiome Research

The complexities associated with microbiome research have made assay standardization challenging. ATCC has the solution: microbiome standards. Throughout each stage of your workflow, ATCC® Microbiome Standards enable you to optimize your diverse research applications with confidence and improve the consistency and reproducibility of your data run after run. These standards support a broad array of applications ranging from method optimization to data interpretation, and they serve as superior controls for microbial community testing and assay development on any platform.

Raise the standards of your microbiome research at www.atcc.org/microbiome.

Unbiased microbiome workflows* for true representation of your sample.

DreamPrep™ NAP featuring Zymo Research

Jump start your current and future nucleic acid processing with a customizable solution

www.tecan.com/dreamprepnap

*Please make a copy of these methods in order to customize them to accommodate and validate your workflows according to your desired intended use and laboratory protocols. Tecan makes no claims regarding the performance of the example methods.

For research use only - not for use in diagnostic procedures.