Living in a microbial world

Our personal ecosystem of microbes is shed on everything we touch and everyplace we go. Charles Schmidt reports on efforts to harness this information to improve human health and activity.

At this January's JP Morgan Healthcare Conference in San Francisco, investors and executives had their cell phones swabbed by StuckOnU, a metagenomics research project being run by Weill Cornell Medical College. A mere 36 hours later, "personalized molecular footprints" of 96 attendees were reported, which revealed, among other things, that sell-side people have more pets than buy-side. Most of what was coating the phones was skin bacteria, but at least one person's phone contained huge amounts of fungi. "We confidentially let that person know about this intriguing result," says Christopher Mason, who leads the project.

This is one of many studies sampling the microbiome from the human environment, and what researchers are finding is that indoor microbial communities are profoundly affected by their interactions with people. The microbial residues left on surfaces by human contact "provide a molecular echo of the places you've been, the things you've eaten and the people you've encountered," says Mason. Scientists are scouring environmental samples for microbial genes, not just to catalog and describe these hidden communities, but also to exploit them for advancing health and improving productivity[1].

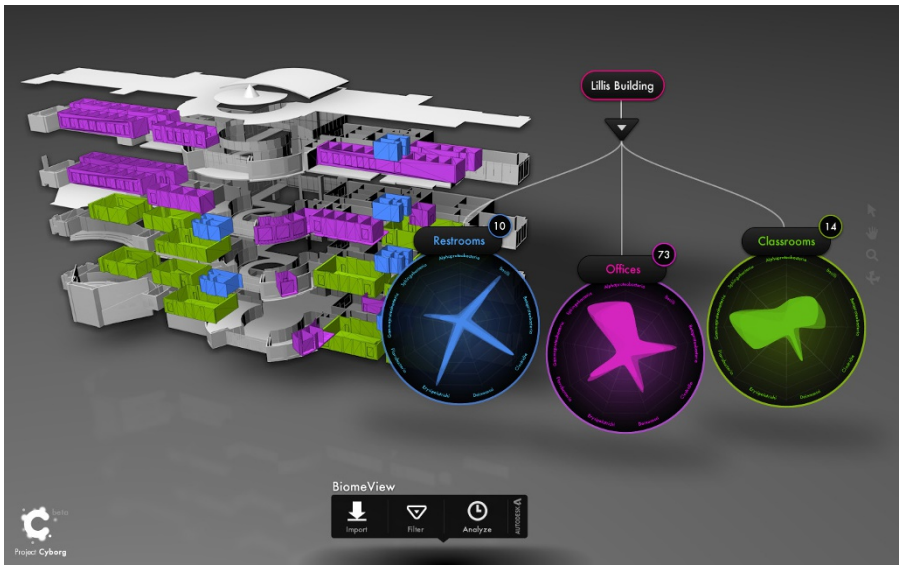
It's early days still, and fundamental challenges exist, among them, what constitutes a healthy microbial assemblage. "We're dealing with microbial systems that are enormously heterogeneous," says Joan Bennett, professor at Rutgers University, in New Brunswick, New Jersey, and chair of the Microbiomes of the Built Environment study being conducted by the National Academies of Sciences, Engineering, and Medicine. "The complexity can get dizzying," says Bennett.

If you build it, they will come

Studies of the gut microbiome have been generating headlines for years, but the focus on external microbiomes—those residing outside the human body—have received less attention. Microbiomes in urban and man-made environments (the 'built environment') are of particular interest, given their close association with humans. Recent years have seen a spike in surveys of the microbiomes of homes, hospitals, ATM machines, subways, sewers and even the International Space Station[2,3]. Increasingly researchers believe that by isolating themselves from nature and using antimicrobial products to kill off beneficial germs in their living spaces, humans could inadvertently be making themselves sick[4]. "We're testing whether antimicrobials in hundreds of commercial products promote the spread of antibiotic resistance," says Jessica Green, co-director of the University of Oregon's Biology and the Built Environment Center (BioBE) in Eugene, and co-founder and chief technology officer of the biotech Phylagen. In 2014 Green and Harrison Dillon founded Phylagen, a data harvesting and analytics company commercializing microbiome technology to improve business performance.

But whereas companies are already marketing probiotics targeting the gut microbiome, commercial applications for the built environment are still years away, cautions Jack Gilbert, a professor at the University of Chicago and a group leader in microbial ecology at the Argonne National Laboratory, in Lemont, Illinois. Scientists have only scratched the surface in terms of understanding how indoor microbial communities assemble and evolve, and how they differ from outdoor communities. "We have a good handle on how to interrogate the microbiome, and we're getting better at cataloguing bacterial, fungal and viral structure and functionality," Gilbert says. "What's harder is transitioning towards useful applications for architectural sciences or the clinic. It comes down to what the microbes do and how we can manipulate them."

Gilbert's research played a pivotal role solidifying one of the field's basic tenets, namely, that humans populate indoor spaces with their own bacteria. In 2014, he published



The indoor microbiome is influenced by building design and operations, human inhabitants and their activities. Source: Biology and Built Environment Center at the University of Oregon and Autodesk, Inc.

results from the Home Microbiome Study, which he directs. Seven families, including three that moved during the study period, provided daily swabs from various parts of their bodies and those of their pets, doorknobs, light switches, floors, countertops and other indoor surfaces. DNA sequencing showed that microbiomes from each home were unique—human skin bacteria dominated countertops, while pet bacteria dominated the floor samples. And when families moved, their microbes went along. Within a day, new microbes completely replaced those left by the prior occupants[5].

This may be unsurprising to scientists at the BioBE who found that humans emit bacteria at rates of over a million biological particles per hour[6]. Subjects placed in sterile climate chambers can be identified by their own microbial clouds in four hours or less. Gilbert found something similar; within five to eight hours of being completely sterilized, public restrooms at a school campus were full of human skin and gut bacteria, most of them completely harmless[7].

Researchers are also mining microbiome data sets for insights into pathogen behaviors and movements. Researchers from the Technical University of Denmark, in Lyngby, sampled toilet waste from 18 commercial flights arriving in Copenhagen from South Asia, North Asia and North America, and reported that waste from the South Asian flights were enriched for *Salmonella enterica* and Norovirus (both causes of food poisoning) and genes encoding antimicrobial resistance. In contrast, *Clostridium difficile* bacteria were more abundant in wastes from North American flights[8]. Frank Aarestrup, a professor at the university's National Food Institute, attributes the geographic differences to heavy antibiotic use in South Asia, where the drugs can often be purchased over the counter. "We are currently analyzing sewage from 64 countries, and again, the anti-microbial resistance genes are especially abundant in the South Asian samples," Aarestrup says.

A cautionary tale

Mason heads an international consortium called The Metagenomics and Metadesign of Subways and Urban Biomes (MetaSUB) that's been sampling urban microbiomes throughout the world (http://metasub.org/). In 2015, he swabbed turnstiles, emergency exits, benches, handrails and trashcans in New York subways and reported that the nearly 1,700 microbial taxa detected were dominated mostly by human skin bacteria, and to a lesser extent by microbes from the human gastrointestinal and urogenital tracts[9]. Almost half the DNA present on the subway surfaces matched no known organism. And though results showed that the bacteria found in the subways were mostly harmless, Mason detected several pathogenic agents, including fragments of the plague and anthrax genomes. Mason and his co-authors emphasized that these fragments didn't appear to be prevalent, and neither did they put city residents at risk.

The findings of *Yersinia pestis* in the subway received wide coverage in the lay press, causing some alarm among New York residents, and prompted sharp rebukes of the study's authors for misinterpreting the data. Rob Knight, a professor in the department of pediatrics at the University of California, San Diego, calls this type of error "a failure of bioinformatics," in that Mason had assumed the gene fragments were unique to the pathogens, when in fact they can also be detected in other common non-pathogens' DNA sequences, and are conserved across a diversity of taxa. "You need to be careful that you don't draw the wrong conclusions," Knight says. Indeed, Knight himself had found sequence matches to the duck-billed platypus in a survey of bacteria in a Virginia tomato field[10].

Mason says his paper merely conveyed results generated by the bioinformatic tools that were available at the time. He has since published a new online tool called OneCodex that discriminates the pathogenic species from other closely related species.

In what's widely seen as the more accurate study, Curtis Huttenhower, a computational biologist at Harvard University's T.H. Chan School of Public Health, sampled train lines and stations in the Boston metropolitan transit system. He found that hanging grips, seats, poles, walls and touchscreens were dominated by harmless human mouth and skin bacteria, including varieties of *Propionibacterium*, *Corynebacterium*, *Staphylococcus* and *Streptococcus*[11]. Huttenhower concludes that built environments don't seem to contain anything overtly dangerous. He feels the more pressing question might be, 'to what extent do indoor spaces shut out potentially beneficial organisms?' "This is a more open question that's much harder to deal with," he says.

The microbiome–asthma connection

Consensus is building that by spending most of their time inside built structures, people have cut themselves off from the microbes they evolved with, a habit that might contribute to some pervasive health problems. In 2016, Knight co-authored a study showing that microbiomes from open huts in the Amazon jungle derived mainly from outdoor soils, whereas those in urban Brazilian apartments were mostly human-derived[12]. Studies of asthma may provide the best evidence yet that indoor spaces dominated by human bacteria aren't necessarily healthy to live in.

A key figure in that research is Erika von Mutius, a pediatrician at the Dr. von Hauner Children's Hospital of the University of Munich, Germany. In 2011, von Mutius reported that children raised in farming households in Europe had lower rates of asthma and atopy—a genetic predisposition to allergic diseases—than children from a non-farming reference group[13]. Later, von Mutius compared Amish children living on traditional farms with livestock to children from a different ethno-

Box 1 Sampling the environmental microbiome

Researchers investigating environmental microbiomes rely on two analytical methods. One of them screens for variations in a 16S ribosomal RNA sequence that is unique to prokaryotes. The other method, called metagenomic or shotgun sequencing, breaks DNA up into fragments that are then reassembled by looking for regions of sequence overlap.

Each method has pros and cons. 16S sequencing is the workhorse—inexpensive and straightforward, but unable to discriminate organisms at the genus or species level. By contrast, shotgun sequencing analyzes all the DNA in a given sample and can therefore discriminate among different species—even strains within species—while also revealing genes that encode microbial metabolites and proteins. Still, it also has its own drawbacks. "You're working with a mixed bag of DNA pieces from hundreds or thousands of microbial species, many of which we have no reference genomes to base taxonomic or functional inferences on," explains BioBE's Hickey. "So with metagenomic data, we get large chunks that we don't know what to do with because they're not similar to anything we've seen before." *CS*

religious group, the Hutterites, who lived on large, highly industrialized farms without as much exposure to farm animals[14]. Here again, the Amish children, who had comparably more diverse microbial exposures, were protected from allergic asthma, whereas the Hutterite children were not. Thus, von Mutius proposes that more diverse microbial exposures are associated with broader stimulatory effects on innate immunity that protect children from allergic reactions.

Susan Lynch at the University of California, San Francisco has added a mechanistic rationale for that hypothesis. For a 2014 study, she fed laboratory mice house dust that had been collected from homes that had dogs and those that did not, and then she exposed the mice to cockroach allergens. The dog-associated house dust protected against allergen-mediated airway pathology; the mice exposed to it had fewer T cells in airways, less mucin secretion and a downregulation of T helper 2 (Th2)-airway responses associated with innate immunity[15]. The intestinal microflora in the protected mice were enriched for *Lactobacillus johnsonii* bacteria. Lynch found she could protect mice from allergen challenge and respiratory infection simply by feeding them this particular Lactobacillus species. The protection was associated with a significant drop in numbers of activated $CD11c^+/CD11b^+$ and $CD11c^+/CD8^+$ cells and reduced Th2 cytokine expression. However, she couldn't detect the Lactobacillus in the house dust of dog-owning residences. "It's likely that other microbes in the house dust promote an enrichment of gut-resident Lactobacillus in our mouse studies," she says.

Probiotics for home and office

Whether it's possible to capitalize on microbiome research by developing health-promoting probiotics for interior living spaces is still unknown, though studies have shown that bacteria that are closely related to human pathogens tend to be more common indoors than outdoors, particularly in rooms with poor ventilation[16]. Similarly, scientists have found that antibiotic resistance genes accumulate in indoor dust samples from areas with high use of triclosan, a commercial antimicrobial commonly found in soaps, toothpaste, detergents and toys[4]. At least one company, called Better Air, in Hollywood, Florida, is already marketing so-called environmental probiotics that it claims will promote a healthy indoor microbiome, though sources interviewed for this story doubt that its product has any value. According to Better Air's website, "within a few days, the probiotic will take over the microbial indoor environment, consume resources available to other pathogens, allergens and mold and create a protective microflora on every object in the environment." Responding to e-mailed questions, Better Air's CEO, Taly Dery, described the probiotic as "proprietary, but consisting of strains from the bacillus family." However, according to Gilbert, until scientists define specifically what constitutes a healthy indoor microbiome, commercial indoor probiotics will be "totally unproven with regard to treating disease or maintaining health."

Whether health-promoting bacteria can be identified using culture-independent sequencing data is still an open question (**Box 1**), according to Roxana Hickey, formerly a postdoctoral research fellow at the BioBE, now a data scientist at Phylagen. "It really boils down to a philosophical debate," she says. Some scientists say it's crucial to study the organisms by growing them separately in culture, whereas others believe that it is unnecessary, as microbes never exist in isolation.

A similar debate plays out in hospitals when it comes to monitoring for pathogens and antibiotic resistance genes with genetic sequencing tools, says Scott Kelley, of San Diego State University. "There's still a lot of pushback from traditional microbiologists who want to see pathogens growing on a plate," he says. Until hospitals pursue demonstration projects employing the technology, hurdles to investment in or adoption of microbiome sequencing methods will remain. Indeed, in January 2016, sequencing giant Illumina, of San Diego, announced a partnership with the French diagnostics company bioMérieux, headquartered in Marcy-l'Étoile, to launch EpiSeq, a whole genome-sequencing platform to survey hospitals for infections.

Bennett says the authors of the Microbiomes of the Built Environment study plans to release a report this year. Launched in 2016, the study was designed to assess the current state of knowledge on indoor microbiomes, and also to map out research agendas, and advise government agencies on how living spaces can be designed "to support occupant health and wellbeing." She emphasizes that while microbes in the built environment were once considered live pollutants to get rid of, they are now seen as mostly benign organisms in the air we breathe, in what we eat and drink, and covering every surface we touch. "And even with our new analytical tools we're missing an awful lot," she says. "For instance, the microbiome is teeming with viruses in low concentrations that we still don't know about because we can't get enough viral DNA in our samples. And almost no one is amplifying fungal genomes—it's all centered on bacteria."

Mason says concrete applications for health will come in time. "My feeling is that we need to be data-driven and work to improve our computational methods, sampling and data integration," he says. "All that has to get better."

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