Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 50 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

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Host-microbiome interactions impacting pathogen and mutualist colonization within defensive symbioses

By

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Chapter 5: Gut Microbes: Good Versus Illness

The Wisconsin Initiative for Science Literacy invites doctoral candidates in science and engineering to include a chapter in their Ph.D. thesis that describes their scholarly research to non-science audiences. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress. WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors.

Symbiosis is defined by relationships. Symbiosis refers to any unlike organisms living together, and the relationships between these organisms can vary widely. If two partners benefit each other, like bees getting nutrients while pollinating flowers, they are called mutualists. If one partner exploits the other, like ticks biting hosts to feed on blood and harming the host, the relationship is parasitic. The bulk of our relationships with microbes is beneficial or at least not particularly damaging. Although most microbes are not harmful to us, those that are (often known as pathogens) may have a terrible impact on our health: *Salmonella*, norovirus, influenza, etc. In the current coronavirus pandemic of 2020, these impacts are not limited to our individual health, but even our collective societal functions.

Though often more attention is paid to our microbial nemeses, microbes can also be our best defenders against pathogens. If you count up all the cells of our bodies, approximately half of those cells are microbial, not human (Sender, Fuchs, and Milo 2016). Most of those microbes reside in the gut and are collectively known as the gut microbiome or microbiota. With large numbers, and large diversity (hundreds of gut microbial species may be found in one person) (Qin et al. 2010), come many interactions: microbes interacting with our bodies, and microbes interacting with other microbes, including pathogens.

In my work, I have explored how these microbes respond to infection with *Salmonella*. *Salmonella* is a group of pathogens that cause illnesses including food poisoning and typhoid fever. Even as early as the 1950s researchers found that beneficial microbes had an effect on *Salmonella*. Early studies in mice showed that mice had much less resistance to *Salmonella* when treated in advance with antibiotics, which disrupt the existing microbes in the gut (Bohnhoff, Drake, and Miller 1954).

Today, we know a great deal more about *Salmonella'*s interactions in the gut environment. *Salmonella*, a rabble-rouser in the gut, first triggers the immune system, causing inflammation. The body releases reactive chemicals containing oxygen which disturbs the normally low-oxygen environment of the gut. Oxygen is highly reactive and can kill cells by damaging cell walls, which people rely on when using hydrogen peroxide (H_2O_2) to treat a cut for bacteria. The wily *Salmonella* bacteria conveniently take advantage of the newly released chemicals and the disrupted gut environment, growing to large numbers in the gut so *Salmonella* can then be shed and transmitted via the fecal-oral route (for example, preparing food after not washing hands in the bathroom) to the next unlucky host.

From the defensive microbes' perspective, this situation is less than ideal. A better outcome for us and our beneficial microbes is if the microbes prevent *Salmonella* from gaining a foothold in the gut. The microbes have many options--they can try to change the immune system's response, take up space and food, and make compounds that stop the growth or control the pathogen. To picture this on a macroscopic scale, you can imagine the efforts to maintain a garden against weeds. Some of your plants may naturally outcompete weeds, perhaps by shading them or using up the nutrients in the plot fastest. Garden plots also benefit from hand-weeding, which we can imagine as the equivalent of the immune system role. Plants also have their own chemical warfare from chemicals they produce to prevent growth of other species (also called allelopathy) akin to herbicides. On the microscopic scale, chemical battles are especially potent

in bacterial competition since microbes are excellent chemical engineers, with incredibly unique and diverse enzymes for making different compounds. For this reason, microbes are also a major source of antibiotics and other drugs (Chevrette and Currie 2019).

Figure 1. Housing of germ-free mice

With the help of Dr. Federico Rey's lab, I used germ-free mice--laboratory mice kept in sterile bubbles or cages, without any outside contact to any microbes (Figure 1). Using these mice allowed me to colonize them with whatever microbes I wanted. In my first set of experiments, I gave them specific strains of bacteria that had been previously isolated from humans in order to "humanize" the mice. A few mice I left germ-free.

After 2 weeks waiting for these communities to stabilize in the mice, I infected some of the mice with microbiota and the still germ-free mice with *Salmonella enterica* Typhimurium (a strain that infects both mice and humans, although it causes somewhat different symptoms). By comparing these two groups, I could find compounds made during infection only when the microbiota was present. In addition, I had a third group of mice with a microbiota that were not infected so I could eliminate compounds made normally by the microbes and focus on those made during infection (Figure 2).

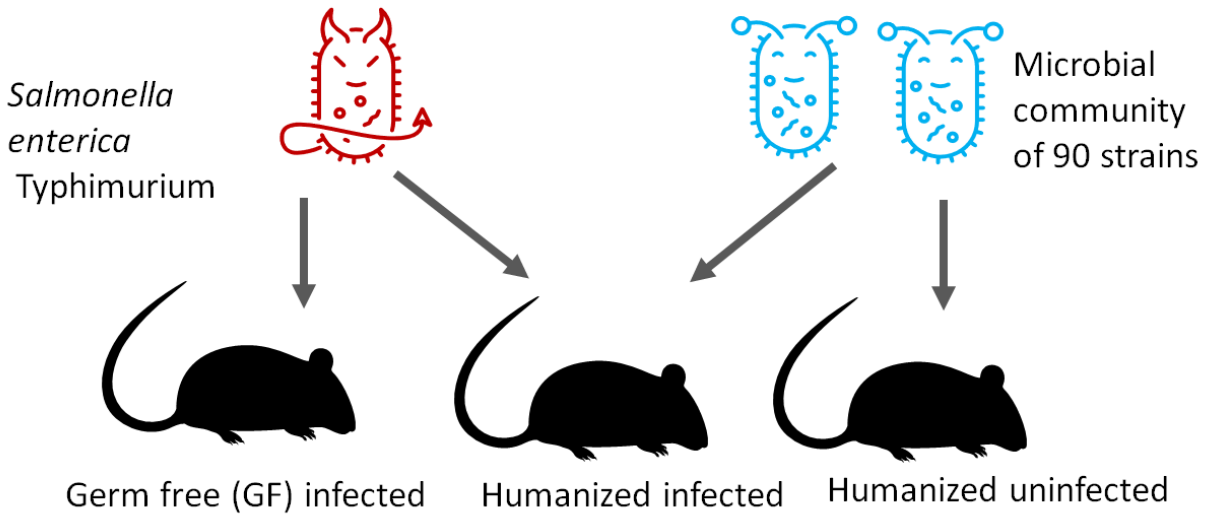

Figure 2. Diagram of experiment setup

By collaborating with Dr. Lingjun Li's lab, I was also able to assess compounds in the guts that are found when both the microbiome and *Salmonella* are present. We used liquid-chromatography mass spectrometry, which can be thought of as splitting up all the compounds in a sample and then measuring their weight (more accurately mass/charge ratios). Many of these compounds are difficult to identify as their "weight" does not match anything in databases of known compounds. Fortunately, we found matches for a few compounds, and could identify them by comparing each to a reference. Of these, two were from the glutathione pathway. Glutathione is an antioxidant, which can help protect from the immune system's reactive

chemicals with oxygen. Potentially, the gut microbes may regulate and produce these metabolites that may impact the infection.

By sequencing DNA from the feces collected over three days of infection, I could get a sense of which microbes were most abundant. I found that without an infection, the microbial communities stayed fairly consistent, but with *Salmonella* they rapidly changed. As had been seen by other researchers, microbes that are more related to *Salmonella* were enriched in the samples after infection. These microbes have similarities in their metabolism to *Salmonella*, perhaps most importantly their ability to tolerate an environment with oxygen (as most of the other gut bacteria live strictly without oxygen).

In the work I have just described, we used one representative microbiome with lab grown strains mixed together. However, each of us has our own individual communities of microbes. This variation might help explain how, with the help of their microbiomes, some people are better able to resist infection than others. How might these different microbiomes with their different strains of bacteria affect which metabolites are produced and our ability to resist disease?

To explore differences among people, I used human microbiome samples (poop) and colonized the mice with these different samples. Then I infected the mice with *Salmonella* and measured how long the mice survived. I found that some people's microbiomes protected the mice better against infection. In addition, I collected samples prior to infection to gain insight into how the microbiome plays a role in preventing *Salmonella* from colonizing, rather than how microbial communities changed after colonization.

From DNA sequencing, I could compare several of the protective microbiomes to see what they shared. I only found a single microbial species that was shared by each of the protective microbiomes, but was not present in the susceptible microbiomes. In the susceptible microbiomes, I also found that several gene pathways were enriched, including those responsible

for degradation of the sugar rhamnose and for creating basic components for cell growth, such as purines, which are compounds used for many things including building DNA.

I compared in detail one of the best communities against one of the worst and found that several different metabolites were enriched in one over the other, although these compounds were different from the kinds I had seen in my previous work. Some of them may have derived from microbial breakdown of soy products. At this point it is unclear if these metabolites play a role in resistance to infection or just happen to be produced in different abundances by the different microbiomes.

Overall, these projects helped us identify microbes and metabolites that may play a role in defending us from *Salmonella* infection. In the future, more experiments could study if compounds identified here play a role in infection and whether those compounds have any potential therapeutic use. The study in which I examined different human microbiomes suggests that there are many compounds and microbial functions that may play a role during infection.

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