TINY MICROBES, BIG YIELDS: THE FUTURE OF FOOD AND AGRICULTURE

EDITED BY: Phillip Myer and Liesel Schneider
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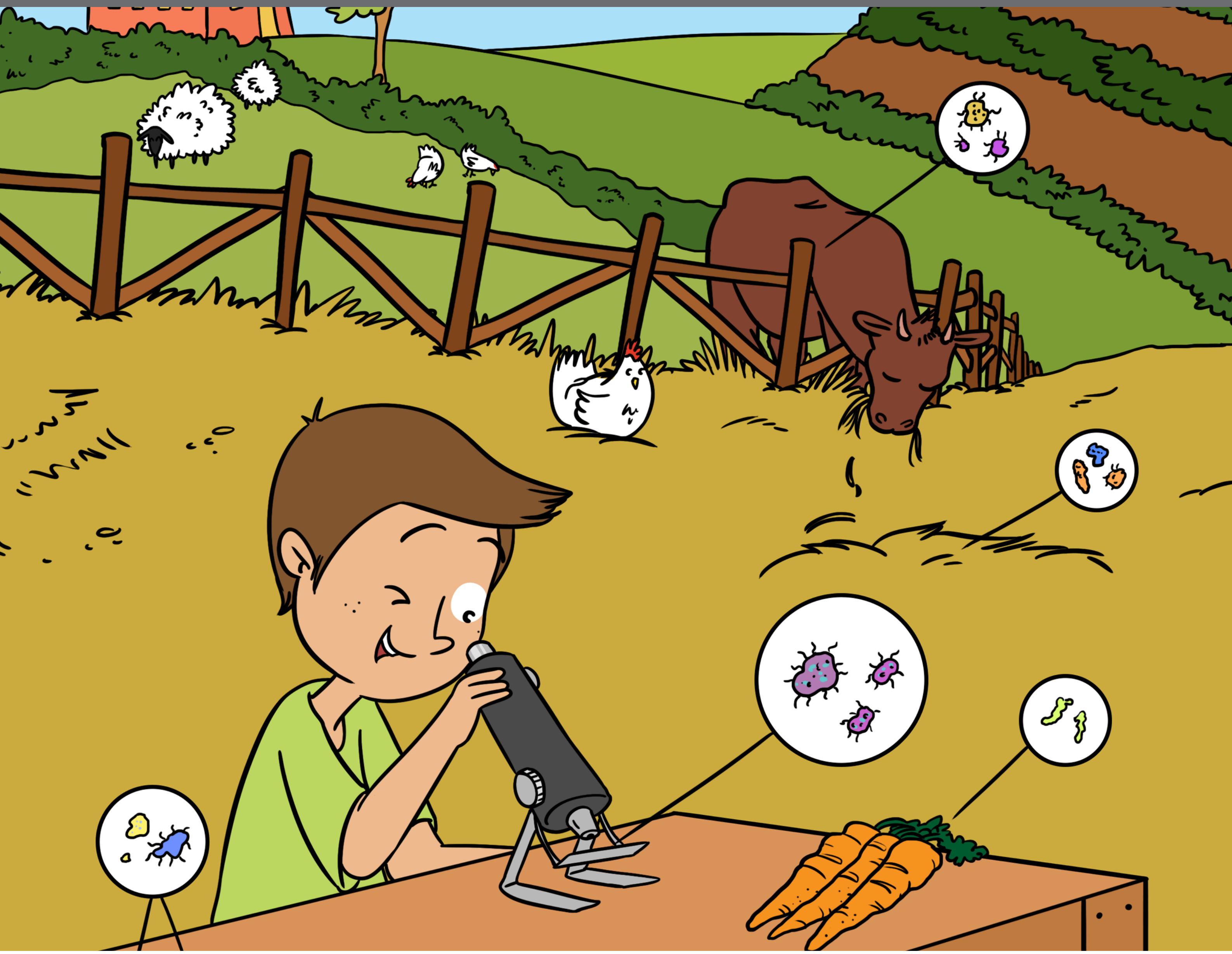

frontiers FOR YOUNG MINDS

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TINY MICROBES, BIG YIELDS: THE FUTURE OF FOOD AND AGRICULTURE

Topic Editors:
Phillip Myer, The University of Tennessee, United States
Liesel Schneider, The University of Tennessee, United States

Our world is made up of countless tiny living beings. There are so many of them, that they make up the largest number of living beings on the planet. These microscopic organisms, called microorganisms or microbes, cannot be seen with the naked eye. We encounter them daily and we interact with them through the air we breathe, the food we eat, and the natural processes within our own organ systems. Microbes have evolved with life on Earth to be important for its survival. They act as food for plants and animals, help humans and animals digest food, break down dead material, and even serve as guardians against bad microbes. Whether we realize it or not, humans rely on microbes to help make the food we eat every day, and understanding how they work helps us to improve our foods and agriculture.

It is amazing to examine how well microorganisms are incorporated into the food we eat, the plants we grow, and the animals we raise. Microbes help ferment foods to make products like cheeses and breads. They work in the soil to provide nitrogen to plants which helps them grow better. Special microbes live in the stomachs of cattle and sheep that allow them to digest grasses that humans cannot eat. Additionally, the energy produced from the microbial digestion of these grasses helps produce meat and milk. However, as with everything, we must take the good with the bad. Although many microbes are helpful, some are harmful and can cause illness. These "bad bugs" must be monitored to ensure they do not enter our food supply. The challenge is to interpret the ways the microbes are positively and negatively impacting food and agriculture and to untangle their complex network to promote improved and more efficient approaches to feed the world.

This collection of articles focuses on understanding more about microbial communities, biodiversity, and their relationships with food and agriculture. This includes, but is not limited to, food and animal production, animal health, food safety, crop safety and production, and agricultural sustainability through microbial-based approaches. What we can learn about these tiny living beings can help provide safe, nutritious, and sustainable food to a growing human global population.

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Table of Contents

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GIVING GOOD BACTERIA TO CHICKENS TO KEEP HUMANS FROM GETTING SICK

Mary Mendoza[1], Rizwana Ali[1], Natalie Roberts[1], Lauren Boop[2], Kristin Bedell[3], Brad Rhew[4], Hosni M. Hassan[1], M. Andrea Azcarate-Peril[5] and Matthew D. Koci[1*]

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YOUNG REVIEWERS:


ANSHUL
AGE: 10


LUVENA
AGE: 12


MARIANA
AGE: 15


PRANATEE
AGE:13


ZARA
AGE: 15

The bacteria *Salmonella* is a major cause of food poisoning. Poultry products are one of the leading foods that cause *Salmonella* outbreaks. While farmers, food processors, and the public health community already do a lot to prevent these illnesses, people are still getting sick. Our group is studying how we can use the "good" bacteria in the intestines of chickens to drive *Salmonella* out of chickens. To test this idea, we used various diets to change the bacterial populations in chicken intestines. We found that changes in the numbers of good bacteria can lead to lower levels of *Salmonella*. We are currently working to identify which bacteria are responsible for the changes in the amount of *Salmonella* in the chicken intestines, with the goal of making a diet that will eliminate *Salmonella* from

chickens. Hopefully, this will reduce the number of people who get sick from eating poultry products.

SALMONELLA BACTERIA CAUSE FOOD-BORNE ILLNESS

Have you ever eaten something and a few days later gotten really sick? You likely had what is commonly called food poisoning, also known as a **food-borne illness**. These illnesses are caused by bacteria or viruses that can infect you if the food you eat is not cooked properly. Different types of food can carry different types of bacteria or viruses, but chicken, eggs, and other poultry products are commonly associated with food-borne illness (Figure 1A) [1].

FOOD-BORNE ILLNESS

Sickness caused by eating or drinking something containing harmful bacteria, parasites, viruses, or chemicals. Symptoms include vomiting, diarrhea, abdominal pain, fever, and chills.

Many kinds of bacteria and viruses can be associated with poultry products but, in the U.S., the bacteria *Salmonella* accounts for the largest number of food-borne illnesses from eating poultry products (Figure 1B) [2]. If you eat a chicken sandwich that is contaminated with *Salmonella*, 48–72 h later you could develop an infection, with symptoms including nausea, abdominal cramps, fever, chills, headache, vomiting, and diarrhea. This infection can last up to 7 days.

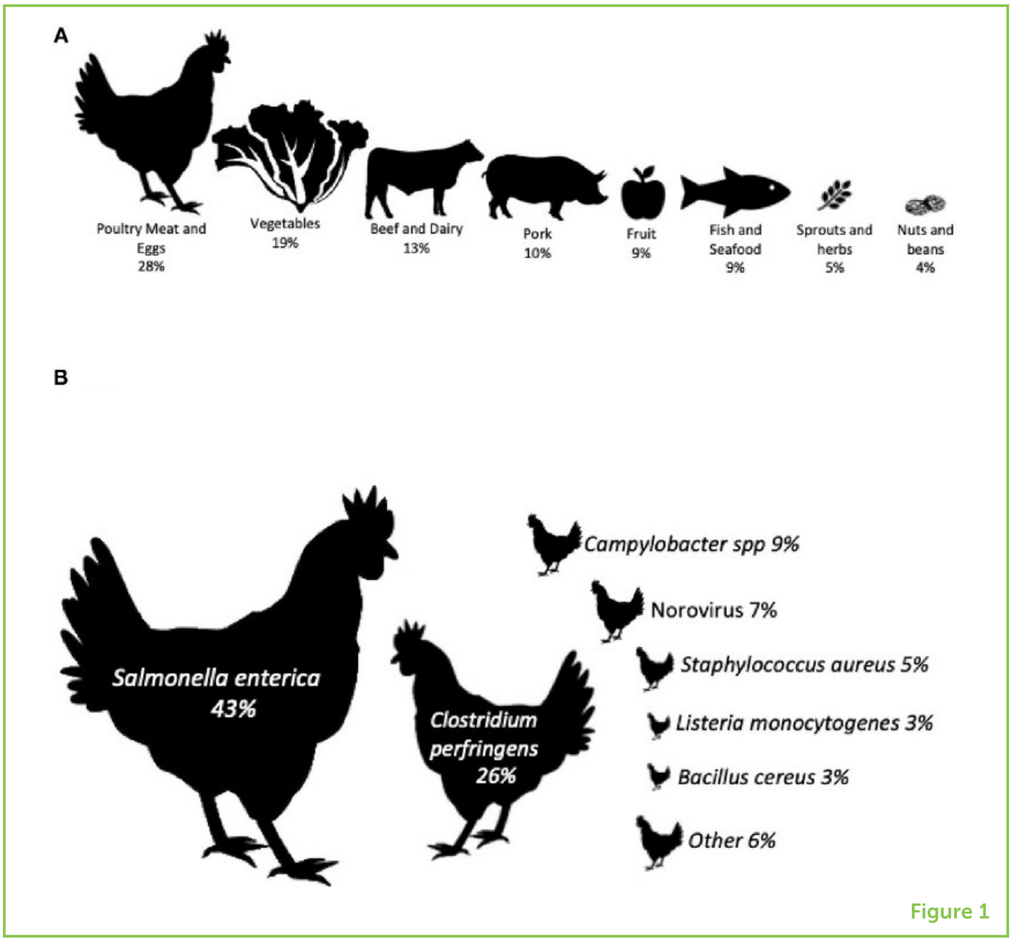

Figure 1

What types of food cause the most food-borne illness? **(A)** In the U.S., there were 5,760 outbreaks of food-borne illness from 2009 to 2015. Of those, 1,281 could be associated with a single food source. Poultry products (meat and eggs) caused the most illnesses, followed by vegetables, beef and dairy products, pork products, fruit, fish and seafood, sprouts and herbs, and finally nuts and beans [1]. **(B)** In 149 food-borne disease outbreaks in which people got sick from eating poultry products, almost half of them (43%) were caused by *Salmonella* [2].

Sometimes the infection can be so severe that you may have to go to the hospital[1].

But how did *Salmonella* get into your chicken sandwich in the first place? *Salmonella* bacteria live in the environment. Chickens can pick up *Salmonella* when they peck at the food on the ground. All chickens, including those sold as organic, free-range, or natural, can pick up the bacteria. While *Salmonella* can make us sick, these bacteria do not make chickens sick. That means *Salmonella* can live inside the intestines (gut) of a chicken without us knowing. From there, *Salmonella* can find its way into the chicken's eggs. Bacteria can also spread from the gut to the meat when a chicken is cut up. If the eggs and meat are stored and cooked properly, any *Salmonella* that may be present will be killed, so it will not make us sick. However, sometimes people do not cook their chicken well enough, or they accidentally spread *Salmonella* from raw chicken to other food[2].

[1] Food poisoning - Causes, symptoms & treatment | Dr. Claudia" *YouTube*, Babylon Health, Jan 16, 2020, https://www.youtube.com/watch?v=JR6yHykfYJE.

[2] "How salmonella spreads from farm to table" YouTube, The Oregonian, Mar 26, 2015, https://www.youtube.com/watch?v=duSDTYFNTi8.

CAN WE FIGHT *SALMONELLA* IN CHICKENS?

Farmers and food producers spend a lot of money trying to keep *Salmonella* out of food [3]. On the farm, chickens are often given vaccines to prevent *Salmonella*. However, there are over 2,600 different types of *Salmonella.* That is too many to make vaccines against all of them. At food processing plants, the eggs and meat go through many steps to wash away *Salmonella* before the food is packaged and sent to grocery stores and restaurants. Even with all of these efforts, poultry products still cause thousands of *Salmonella* illnesses each year [2]. To successfully keep *Salmonella* out of *us*, we need to figure out new ways of keeping *Salmonella* out of chickens.

One approach is to try to get other bacteria to help. Just like humans, chickens have trillions of "good" bacteria that live inside their guts. These good bacteria help chickens digest their food and they also produce nutrients that the chickens cannot make for themselves. These bacterial populations change in response to what the chickens eat. The various types of bacteria in the gut fight over food and resources to survive. We asked whether we could make chicken safer to eat by helping the good bacteria in chickens' guts fight off *Salmonella* [4].

To test this hypothesis, we used three groups of 100 chickens each. We gave normal chicken feed to one group. This was our **control** group, and it was used to show us what the population of bacteria in the chicken gut normally looks like. We fed the second group chicken feed mixed with a type of fiber that only certain good bacteria can use as food. This bacteria food is known as a **prebiotic**. The types of bacteria that can eat the prebiotic make chemicals that should drive out *Salmonella*. We thought that increasing the numbers of these

CONTROL

A group of animals that does not receive any experimental treatment and is used to represent what normally happens and is compared to the other groups of animals to help the researchers determine how the effect the treatment.

PREBIOTIC

Nutrients used as food by gut bacteria. Prebiotics are used to encourage the growth of "good" bacteria in the gut.

good bacteria might make the gut a place where *Salmonella* do not want to live. Our third group received normal chicken feed, but at the start of the experiment we gave them a *Salmonella* type that had been modified so it cannot make people sick. This was to test the possibility that adding a "safe" *Salmonella* to the chickens' guts might prevent other *Salmonella* types from being able to live there. This is the bacterial equivalent of what you might do when you do not want someone to sit next to you at school: you get other people to sit around you first.

We let each group of chickens eat their assigned foods from the day they hatched until they were 35 days old. This gave the good bacteria plenty of time to grow. By day 35, there could be thousands of different types of bacteria in the chickens' guts.

DIET CHANGES BACTERIA IN CHICKEN INTESTINES

Our hypothesis was that the prebiotic treatment and the safe-*Salmonella* treatment would change the populations of good bacteria in the chicken gut. We needed a way to measure how the gut bacteria in these chickens might be different from the bacteria in the guts of our control group. So how could we measure all the bacteria in the guts of these chickens? It would take a very long time and cost a lot of money to grow all these bacteria in the lab and count them by hand; not to mention that most of the bacteria that live in the gut cannot be grown in the lab. So, we performed a bacterial version of a crime scene investigation—we determined which bacteria were present based on the bacterial **DNA** we found in the gut. To do this, we collected samples of the partially digested food from inside the chickens' guts, and we isolated the bacterial DNA from those gut samples.

To identify which bacteria were present in each gut sample, we tested for a specific bacterial **gene** known as **16S ribosomal RNA**. Testing for this gene could tell us which types of bacteria were in each gut sample *and* how many of each type were there [4]. For each chicken, we then made a list of all the bacteria we found and the percentages of the bacterial population each type made up. We used a computer program that looked at *all* the bacteria we found in *all* chickens, and we grouped the chickens together based on the similarity of the bacterial populations in their guts. This analysis showed us whether there were differences among our groups of chickens.

DNA (DEOXYRIBONUCLEIC ACID)

Material found in cells that contain all the instructions a living thing needs to work and function.

GENE

Specific regions of DNA that contain the instructions for individual components of a living thing.

16S RIBOSOMAL RNA

A gene (segment of DNA) found in bacteria that can be used to identify the types of bacteria in a population.

GOOD BACTERIA CAN REDUCE *SALMONELLA* IN CHICKENS

If our prebiotic treatment or our safe-*Salmonella* treatment affects which bacteria can live in the gut, then we would expect the numbers and types of bacteria to be different between our experimental groups.

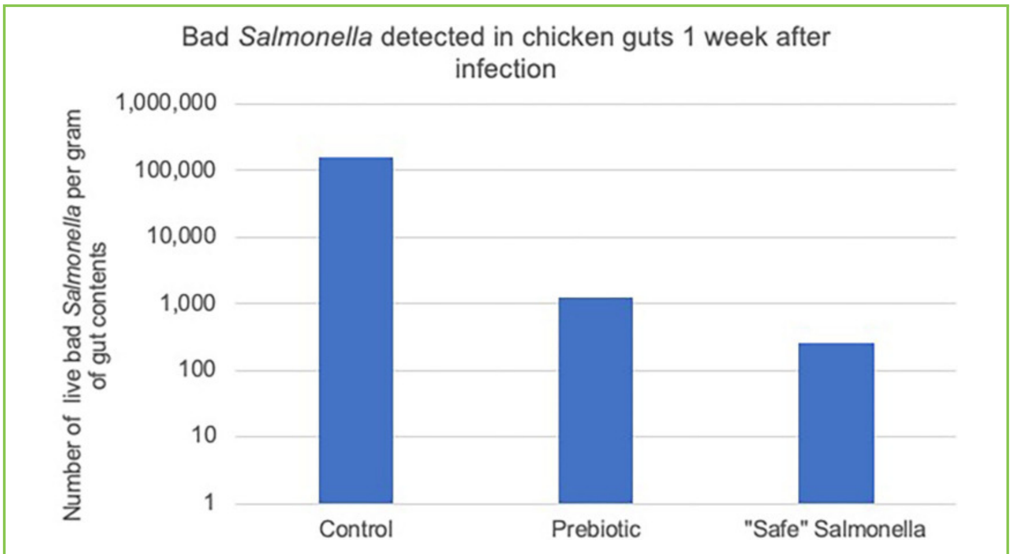

Figure 2

Figure 2

Changing the "good" bacteria in the chicken gut helps get rid of disease-causing *Salmonella*. After 4 weeks on either the control diet, the prebiotic diet, or the safe-*Salmonella* diet, we gave the chickens a dose of disease-causing *Salmonella*. One week later, we collected gut samples and measured how much disease-causing *Salmonella* was in the guts of seven chickens from each of our three groups. The safe-*Salmonella* group and the prebiotic group had less disease-causing *Salmonella* than the control group.

This is essentially what we found! At the start of the experiment, there were no differences between the groups. The gut bacteria found in the chickens from all three groups were the same. However, 4 weeks after the start of the experiment, there were three distinct groups of bacterial populations: one for the control group, one for the prebiotic group, and one for the safe-*Salmonella* group.

Once we knew that our diet treatments could change the bacterial populations in the gut, we wanted to see if the altered bacterial populations could prevent disease-causing *Salmonella* from living in the gut. Four-week-old chickens from each group were given disease-causing *Salmonella. One* week later, we collected gut samples from the chickens, like before. This time, we grew the bacteria in the lab, so we could count how many disease-causing *Salmonella* were in each chicken and compare those numbers among the three groups. In the control group, we found that chickens had an average of 100,000 disease-causing *Salmonella* bacteria per gram of gut material. However, chickens that had either the prebiotic treatment or the safe-*Salmonella* treatment had much lower levels of disease-causing *Salmonella* in their guts (Figure 2).

KEEPING HUMANS HEALTHY

From these experiments, we learned that feeding chickens a prebiotic or giving them a safe *Salmonella* changes the kinds and amounts of bacteria living in chickens' guts. We also learned that these changes can lead to lower levels of disease-causing *Salmonella*. Now we are trying to find out exactly which bacteria in the chicken guts are responsible for decreasing the growth of disease-causing *Salmonella*. We hope that by identifying the specific bacteria that prevent disease-causing *Salmonella* from living in chickens, and understanding exactly how these good bacteria manage to stop the *Salmonella*, we will be able to develop diets for chickens that will completely eliminate *Salmonella* from their guts. Since *Salmonella*-contaminated eggs and poultry products are a major source of *Salmonella* outbreaks in humans, eliminating *Salmonella* in the guts of chickens could greatly reduce the number of people who get sick from food-borne illnesses in the future.

ORIGINAL SOURCE ARTICLE

Azcarate-Peril, M. A., Butz, N., Cadenas, M., Koci, M., Ballou, A., Mendoza, M., et al. 2018. A *Salmonella*-attenuated strain and galacto-oligosaccharides accelerate clearance of *Salmonella* infections in poultry through modifications to the gut microbiome. *Appl. Environ. Microbiol*. 84:e02526-17. doi: 10.1128/AEM.02526-17

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4. Azcarate-Peril, M. A., Butz, N., Cadenas, M., Koci, M., Ballou, A., Mendoza, M., et al. 2018. A *Salmonella*-attenuated strain and galacto-oligosaccharides accelerate clearance of *Salmonella* infections in poultry through modifications to the gut microbiome. *Appl. Environ. Microbiol*. 84:e02526-17. doi: 10.1128/AEM.02526-17

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CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

ANSHUL, AGE: 10

Hello! My name is Anshul and I am a fifth grader in North Wales, Pennsylvania, which is close to Philadelphia. I am very interested in Biology and Entomology. I am an active member for the Johns Hopkins CTY program, and my favorite hobby is to read.

LUVENA, AGE: 12

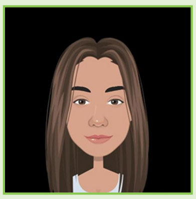
Hi, my name is Luvena! I love music, sports, and food. My favorite subjects in school are math and language arts. In my spare time, I enjoy playing piano and reading books with my sister. When I grow up, I would like to be a neurosurgeon.

MARIANA, AGE: 15

Hi! My name is Mariana and I am 15 years old. I like reading books, spending time with animals, and swimming. I consider every day brings an opportunity for me to learn something new and for being happy.

PRANATEE, AGE: 13

Hello! I love to bake, especially tarts and pies. In school, my favorite subjects are science, lunch, and recess. I like spending time outdoors and going hiking. I also love going to the beach and have an interest in photography. Watching my favorite TV shows, painting, listening to music, singing, and hanging out with friends are my favorite things to do in my free time. In the future, I would like to either like to be a scientist, or a singer/songwriter and actress.



ZARA, AGE: 15

My name is Zara and I am 15 years old. In my spare time, I enjoy reading, helping my community, and playing volleyball. I think it is important to work hard for achieving our dreams and for being a better person every day.

AUTHORS

MARY MENDOZA

Mary Mendoza is a research specialist at North Carolina State University. After graduating from the University of Maryland with a B.S. in Biology, Mary worked at the National Institutes of Health studying the signaling pathway of mast cells.

Upon relocating to North Carolina in 2008, Mary joined Hosni Hassan's lab at North Carolina State University as a research specialist where current research has focused on *Salmonella* and its interaction with the microbiome of the chicken.

RIZWANA ALI

Rizwana Ali is a, recently retired, research specialist at North Carolina State University. She has a B.S. in Pharmacy from the University of Karachi and a M.S. degree in Physiology from N. C. State University.

NATALIE ROBERTS

Natalie Roberts is a Research Specialist in Dr. Matt Koci's lab at North Carolina State University. She is excited about exploring how microbes like bacteria affect health and immunity. In her free time, she enjoys reading, gardening and spending time with her three daughters.

LAUREN BOOP

Lauren Boop taught for 9 years in Wake County in North Carolina where she became really interested in intersecting STEM education and the humanities. Through an inquiry-based classroom, she teaches students to explore all aspects of the world around them from tiny microbes to large landscapes. Her own love of exploring the world has brought her to her current role as a middle school teacher at an international school in Portugal.

KRISTIN BEDELL

Kristin Bedell has taught kindergarten through college. She currently writes STEM curriculum for North Carolina 4-H and is working on her Ph.D. in Education at the University of North Carolina at Chapel Hill. Her favorite microbes turn milk into cheese and flour into sourdough bread.

BRAD RHEW

Brad Rhew is the STEM Coach in Winston-Salem/Forsyth County Schools, North Carolina and serves on the North Carolina Science Teachers Association as the District 5 Leader and the Grants Committee Chair. He works closely with his school district and state science leaders to increase hands-on inquiry based learning in the science classroom. He is currently working on his doctorate in Curriculum and Instruction through Gardner-Webb University.

HOSNI M. HASSAN

Dr. Hassan is Professor of Microbiology, Biochemistry, Food Science and Toxicology at North Carolina State University. Dr. Hassan's research focuses on applying microbial physiology, genetics, and molecular biology to understand host/microbe interactions to promote the growth of good bacteria and inhibit the growth of the bad ones (i.e., Pathogenic Bacteria). His research interests include—Oxygen free radical biology, oxidative stress, microbiology and food safety, poultry microbiome and metabolome, developing patented *Salmonella* vaccines, isolation/application of species-specific probiotics, and developing best practices to eliminate human salmonellosis.

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Dr. M. Andrea Azcarate-Peril, is an Associate Professor of Medicine at UNC Chapel Hill. She uses molecular biology, genomics, and next-generation sequencing tools to address questions relevant to the role of the intestinal microbiota in human health and disease.

MATTHEW D. KOCI

Dr. Matthew D. Koci is a professor of immunology and infectious diseases at North Carolina State University. Our bodies encounter millions of bacteria and viruses every day. The vast majority of them do not pose any threat to us and our immune system generally leaves them alone. His research is focused on trying to find how our immune system can tell the difference between the helpful microbes and the bad ones, and why some of the microbes and viruses that make humans sick do not make animals sick. *mdkoci@ncsu.edu

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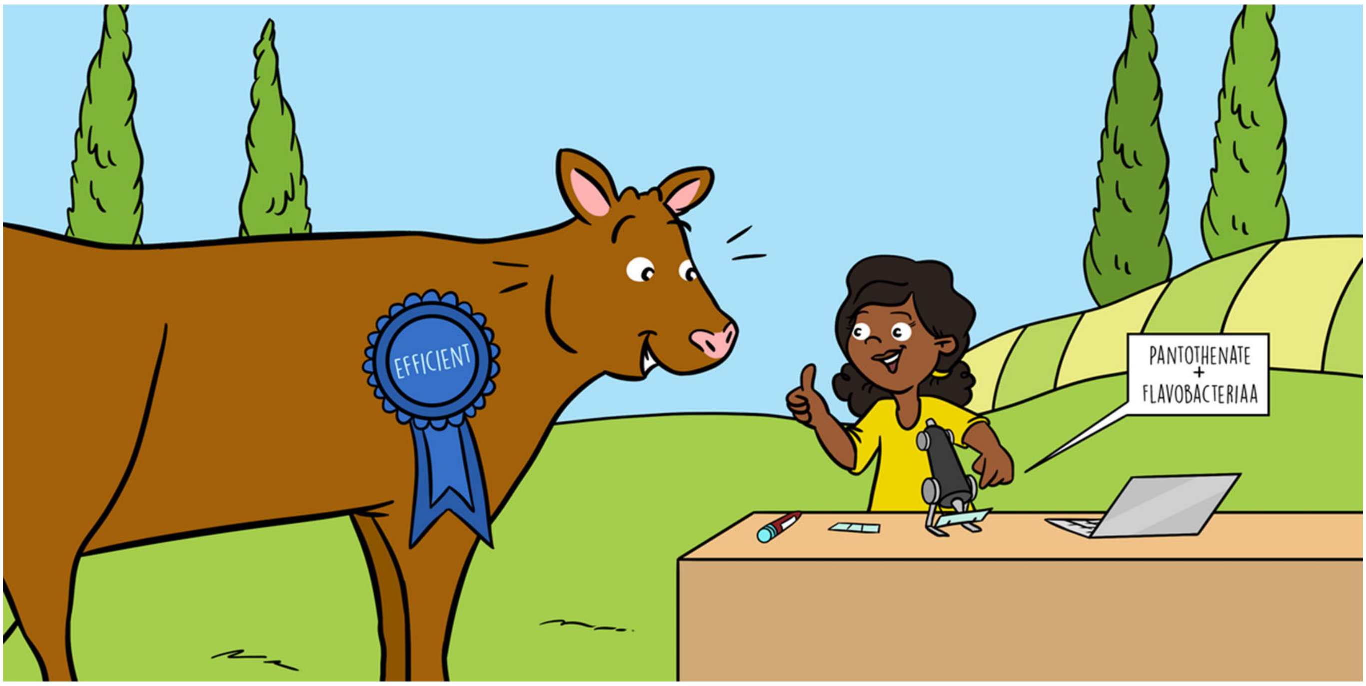

MICROBES HELP PREDICT MORE EFFICIENT CATTLE

Brooke A. Clemmons and Phillip R. Myer*

Department of Animal Science, University of Tennessee Institute of Agriculture, Knoxville, TN, United States

YOUNG REVIEWERS:


CATHERINE
AGE: 15


HARRISON
AGE: 11


JOSHUA
AGE: 15


MEGAN
AGE: 15

The number of people in the world is rapidly growing and with more people comes the need for more food. Luckily, cattle are a great resource to help provide more food! These animals can produce more protein than they consume, converting human-inedible grasses into high-quality protein for humans to eat. However, we can still improve this conversion of grasses to meat by making cattle more efficient at the process, otherwise known as making cattle more feed efficient. In this study, we identified stomach microbes and blood nutrients of feed-efficient cattle. Using computers, we were able to show that the nutrients in the blood could predict the microbes living in the stomachs of feed-efficient cattle. This work shows that microbes in the stomachs of cattle may help improve feed efficiency and can be used to predict which cattle are feed efficient.

RUMINANTS

Mammals that can get nutrients from plants by digesting them in a specialized stomach with the help of microbes.

MAKING GRASS INTO MEAT

Did you know that **ruminants**, like cattle, sheep, goats, and giraffes, have four-chambered stomachs that are specially made to help them digest plants? The first part of the four-chambered stomach is called a rumen, and this is where most of the plants that ruminants eat get broken down. The rumen of cattle can hold almost 50 gallons of food and water—that is a lot of food! The ruminants are able to break down plants that humans cannot eat because of the tiny **microbes** that live in the rumen. The microbes have special proteins, called enzymes, that can attack and digest the tough materials of the plant. Humans do not have the same enzymes that these microbes do, so ruminants can use plants and other foods that humans cannot use.

MICROBES

Microscopic organisms that live all around us. Microbes include bacteria, fungi, and protozoa.

Since the microbes are the ones mostly breaking down the food that cows and other ruminants eat, they make a huge difference in how well the cows can use different kinds of feed to gain weight. Some cows and microbes are really good at breaking down the food and using the energy and other nutrients that come from it, and some are not so good at it. Cows that are good at breaking down food to get energy are called feed efficient. Feed-inefficient cows are not as good at extracting energy from food.

COMPARING MICROBES AND NUTRIENTS IN DIFFERENT TYPES OF CATTLE

Some of our recent research uses a combination of lab techniques and computer analyses to try to find a quick, easy, and cheap way of figuring out which cows are feed efficient, and how the rumen microbes may be helping them be better at using their food. First, we take blood samples from the cows. Then we collect the microbes in the rumen by placing a tube down the throat to the cow's stomach and pumping some of the stomach contents out. In the lab, we then break open the microbial cells to get the **DNA** from them, which allows us to identify which microbes are present. We also measure the amounts of different nutrients in the blood of cows that are able to gain more weight with less feed and those that do not gain as much weight with the same amount of food. After we find out which microbes are living in the gut of the cattle and the amounts of nutrients in the blood of the different animals, we analyze how these factors are different between feed-efficient cows and feed-inefficient cows. Finally, we use computers to calculate the relationships between the microbes and the nutrients, which allows us to see how the rumen microbes may be helping the cows be better at using the food we give them (Figure 1).

DNA

The material, present in nearly all living things, that is the carrier of genetic information.

WHAT DID WE FIND OUT?

First, we looked at the microbes in feed-efficient and feed-inefficient cattle [1]. Since there are hundreds of different kinds of microbes that live in the rumen, we wanted to see if the feed-efficient and feed-inefficient cows had different kinds of microbes living in their rumen. We did not see any differences in the number of

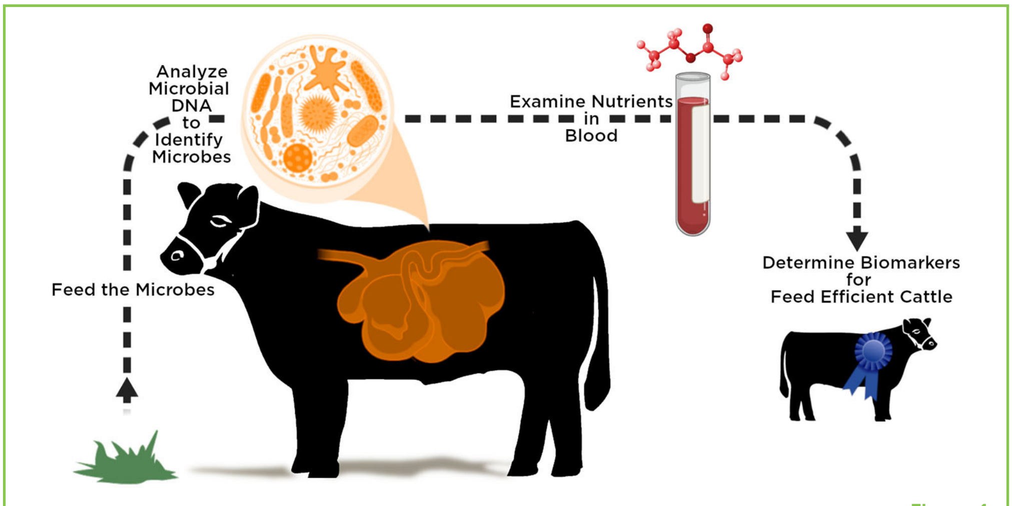

Figure 1

Finding ways to identify feed-efficient cattle through microbes. When cows eat grass, the grass feeds the microbes, which in turn feed the cow. Researchers can examine the microbes in the rumen and the nutrients in the blood of cattle to see whether feed-efficient cattle have different microbes or nutrients than feed-inefficient cattle do.

different kinds of microbes or any changes in how related the microbes were between the feed-efficient and the feed-inefficient cows. However, we did see differences in the numbers of specific microbes between the two groups of cattle. For example, the bacteria group of Flavobacteriia were greater in feed-efficient cows. We also observed that three different kinds of microbes seemed to be the ones responsible for causing the variation in the ability to use nutrients.

MICROBES MAKE A DIFFERENCE!

But even if there are different microbes, are they really making a difference in what nutrients the cows are getting? To answer this question, we looked at the nutrients found in the blood of the cows. First, we found that the feed-efficient and feed-inefficient cows had different amounts of certain nutrients [2]. One of those nutrients, called **pantothenate** , was greater in feed-efficient cows. Pantothenate is used to make a special molecule called **coenzyme A (CoA)**. Coenzyme A is really important for many animals because it is involved in turning food into energy and in building muscle [3]. However, animals do not have the tools to turn pantothenate into CoA, but certain microbes do.

PANTOTHENATE

Commonly known as Vitamin B5, and is important for the breakdown of the foods we eat into substances the body can use.

COENZYME A (CoA)

An abundant and essential molecule that is involved in a lot of energy-related processes.

Knowing this, we wanted to see if there were any microbes from the rumen that could turn pantothenate into coenzyme A. We found that one group of bacteria, called Flavobacteriia, was greater in number in the rumen of feed-efficient cows. We then used computers that could perform very complex math to determine the relationship between the pantothenate and the Flavobacteriia. We found that the amount of pantothenate in the blood of cows could "predict" the amount of Flavobacteriia that was living in the rumen. So, the lower the amount of pantothenate in the blood, the lower the amount of Flavobacteriia living in the rumen of the cows. As the amount of pantothenate in the blood gets higher, the more Flavobacteriia are found living in the rumen.

This is really important for a couple of reasons. First, both pantothenate and Flavobacteriia are more abundant in feed-efficient cows. This might mean that both pantothenate and Flavobacteriia can be used by scientists and farmers to decide which cows are going to be better at using their food. Choosing and growing cows that are better at using their food means that the cows can grow faster and use less food, which is better for the environment [4]. Our findings also mean that the microbes in the rumen do change how the cows use the nutrients in food and the nutrients produced by microbes. Sometimes the small differences that scientists find between two groups of organisms do not actually affect the organisms. But our work provides more evidence that the differences in the rumen microbes can affect how the cows' bodies work to extract energy from food [5].

HOW ARE WE GOING TO USE THIS INFORMATION IN THE FUTURE?

We are hoping to be able to use this information in several ways. First, we hope to eventually develop a test that can use a little bit of blood to measure how efficient cows are at using the nutrients from their feed. Pantothenate may be one of those nutrients that can be used to measure **feed efficiency**, but we would also like to find others. We also hope to find more microbes that are changing or producing the nutrients that make ruminants better at using their food. If we know which microbes are making the nutrients that are helping the ruminants use their food efficiently, we may be able to feed more of those microbes to the ruminants to help them use their food even more efficiently. There is still a lot of work to do before we get to that point, but the work is very important because happy, healthy cows are better for people and better for the environment.

FEED EFFICIENCY

A simple measure to determine the ability of cows to turn their feed into milk, milk components, or body weight.

ORIGINAL SOURCE ARTICLE

Clemmons, B. A., Martino, C., Powers, J. B., Campagna, S. R., Voy, B. H., Donohoe, D. R., et al. 2019. Rumen bacteria and serum metabolites predictive of feed efficiency phenotypes in beef cattle. *Sci. Rep.* 9:19265. doi: 10.1038/s41598-019-55978-y

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CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

CATHERINE, AGE: 15

I love music and singing, I play the violin and guitar, and I also enjoy writing! I am part of a highland dance troupe and volunteer with children at local kids clubs and guides. I enjoy attending youth events at my church and doing fitness. I hoped that by reviewing these articles I could learn about new and interesting stuff!

HARRISON, AGE: 11

I love playing sports, such as hockey and going running and chasing my dog! I also love discovering new things, but not new foods! Because I currently go to primary school I am excited to start my new secondary school and try lots of new subjects. My favorite subject at the minute is math.

JOSHUA, AGE: 15

I have been very interested in science from a young age and am fascinated by the wonders of the world. I like to know as much as I can about everything to be honest

I am a bit of a nerd! I am interested in biology and chemistry and play rugby for my school.

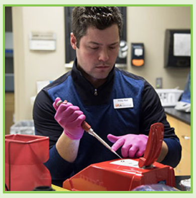
MEGAN, AGE: 15

Hi, I am Megan, my hobbies include musical theater, baking, and surfing (only during summer though!). I got involved with Frontiers for Young Minds as I really want to learn more about science and the world around me, I through reading these articles would be a good start!

AUTHORS

BROOKE A. CLEMMONS

Brooke A. Clemmons is a Ph.D. student in Animal Science in Knoxville, TN. She grew up in cities, but always loved farm animals. Brooke currently works with 132 cows to understand how different environmental things affect how cows use food. She specifically focuses on how different factors like pregnancy and genetics affect the microbes that live in the guts of cows, and how we can use those factors to make animals grow better and healthier.



PHILLIP R. MYER

Phillip R. Myer is an Assistant Professor at the University of Tennessee Department of Animal Science. He is interested in how microbes in beef cattle impact feed efficiency. Phillip is also interested in how beef cattle can interact with their microbes. His research group uses DNA technologies to help investigate these interactions. He hopes his research can help inform, improve, and be part of the solution to future global protein and food demand. *pmyer@utk.edu

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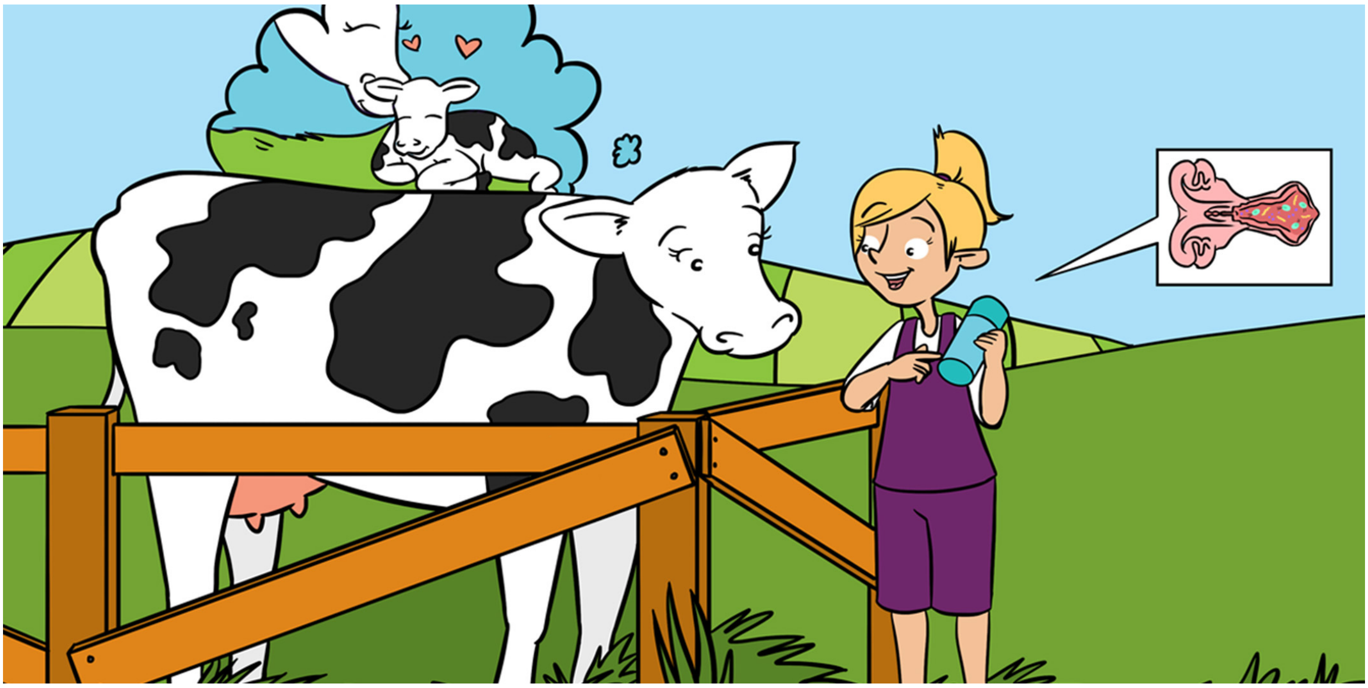

BACTERIA IN THE REPRODUCTIVE TRACT: THEY ARE NOT ALL BAD!

Taylor B. Seay, Brooke A. Clemmons, Phillip R. Myer* and Kyle J. McLean*

Department of Animal Science, University of Tennessee, Knoxville, TN, United States

YOUNG REVIEWERS:

ETHAN
AGE: 14

NETTRA
AGE: 16

Bacteria in the reproductive tract were previously thought to be "bad" and it was believed that they decreased the chances of successful pregnancies. However, healthy bacteria have been found in the reproductive tracts of humans and cattle! These "good" bacteria help maintain a healthy reproductive tract by reducing the ability of "bad" bacteria to grow, helping immune cells to function properly, and maintaining an appropriate pH in the environment. Researchers have found that differences in the bacterial communities of the uterus can influence whether cattle get pregnant and give birth to healthy calves. Future studies may evaluate how we can modify these bacterial communities to create the best environment in the reproductive tract for better reproductive success.

INTRODUCTION

The world population is quickly increasing, from 7.7 billion people today to an estimated 10 billion people within the next 30 years. This growing number of people leads to an increase in the amount of food needed to feed everyone. The agricultural and livestock industries are very important to the food supply and they are faced with the challenge of producing enough food with limited resources. To overcome this challenge, scientists are evaluating factors that can affect food production. Animal science researchers are evaluating the bacterial communities in the reproductive tract and their role in the successful reproduction of animals that contribute to the food supply.

PATHOGEN

A microorganism that is commonly harmful and can cause disease.

Bacteria are commonly thought to be "bad," due to the many types of **pathogenic** bacteria that can cause various illnesses. However, there are also "good" bacteria present in the environment and the bodies of humans and animals, which help to maintain healthy and productive bodies! Helpful bacteria are found in many organs of the body, with most of them located in the digestive tract. These gastrointestinal bacteria help to break down the foods humans and animals eat. The products that bacteria produce from digesting foods can be used by humans or animals for growth or for maintaining normal body functions. The importance of bacteria in the digestive tract led scientists to explore bacteria in other body parts, such as the reproductive tract, and to ask how these bacteria may influence reproductive health and breeding success.

ARE THERE HEALTHY BACTERIA IN THE REPRODUCTIVE TRACT?

FEMALE REPRODUCTIVE TRACT

Organ system that functions to produce new offspring in females. Consists of the vagina, cervix, uterus, oviduct, and ovaries.

Many years ago, scientists believed that the **female reproductive tract** did not contain any bacteria unless there was an infection present. The presence of bacteria in the reproductive tract, especially during pregnancy, was thought to be bad for fetal growth and the health of the uterus. However, as scientists developed new technologies to detect and identify more bacteria, they found that there is a community of healthy bacteria in the reproductive tract!

BACTERIAL DIVERSITY

Measurement of the number of different bacterial species and how genetically different the bacteria are in a sample. A sample with a higher number of different species has a greater diversity.

In humans, there is a very low **bacterial diversity** of bacteria in the healthy reproductive tract. The low diversity is due to a single genus, named *Lactobacillus*, accounting for the majority of the reproductive tract bacteria. The bacterial communities of the reproductive tract in cattle, however, are very different from humans in this respect. There is high bacterial diversity in the cattle reproductive tract, meaning there are many different types and species of bacteria present. The bacteria in the cow vagina are more diverse than that of the uterus, with many bacteria that are similar to those found in the gastrointestinal tract (Figure 1). The high diversity of bacteria in the vagina and the similarities to gastrointestinal tract bacteria are believed to be due to the opening of the vagina to the external environment, which allows bacteria to enter and grow, while the uterus is more internal, with limited access to external bacteria sources [1].

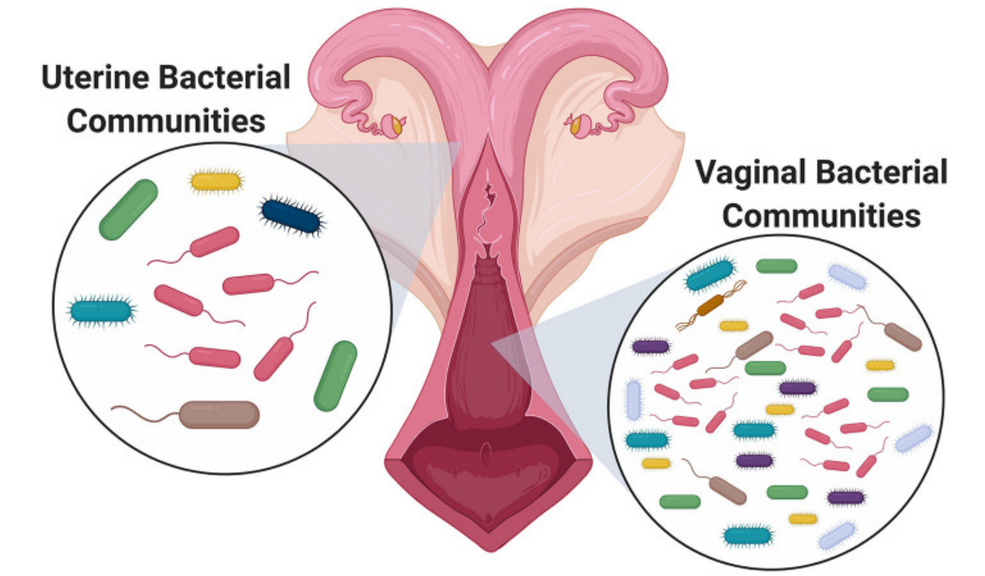

Figure 1

Figure 1

The cow vagina has a greater number of bacteria, and a greater bacterial diversity, than the uterus. Some bacterial species are shared between the uterus and vagina, but in different abundances (Image created using BioRender).

HOW DO BACTERIA HELP KEEP THE REPRODUCTIVE TRACT HEALTHY?

Just like humans and animals, bacteria must consume "food" that supplies them with energy and nutrients to survive. The process of converting food to energy and nutrients is called **metabolism**. Metabolism includes reactions that occur inside cells to produce many different molecules and compounds necessary for the functions that keep us—and bacteria—alive. Bacteria can consume "food" in the form of various substances that are produced by the cells of their host's reproductive tract or that enter the reproductive tract from the bloodstream. The bacteria consume these substances to create energy and other molecules that allow them to survive and function. Bacteria release other products of metabolism that they do not need, which can help support functions of the host, including maintaining a healthy reproductive tract (Figure 2).

METABOLISM

Chemical reactions that breakdown substances, such as food, into other molecules needed for normal body functions.

The products that bacteria release into their environments help to maintain a healthy reproductive tract by supporting healthy bacterial communities, inhibiting pathogen growth, maintaining the proper acidity of the environment, and helping the cells of the immune system to function properly. For example, *Lactobacillus* species of bacteria help to keep the human reproductive tract healthy by metabolizing the sugar glucose to produce energy and **lactic acid**. The bacteria use the energy for their survival, while the lactic acid is released into their environment. The lactic acid released lowers the pH of the reproductive tract, making the environment more acidic, which prevents pathogenic bacteria from growing [2]. *Lactobacillus* species have been shown to be in low abundance in the cattle reproductive tract [3], however, other bacteria are present that can perform similar functions. Additional types of bacteria can

LACTIC ACID

A chemical formed when sugars are broken down for energy in the absence of oxygen. Lactic acid is produced by the metabolism of several bacterial species.

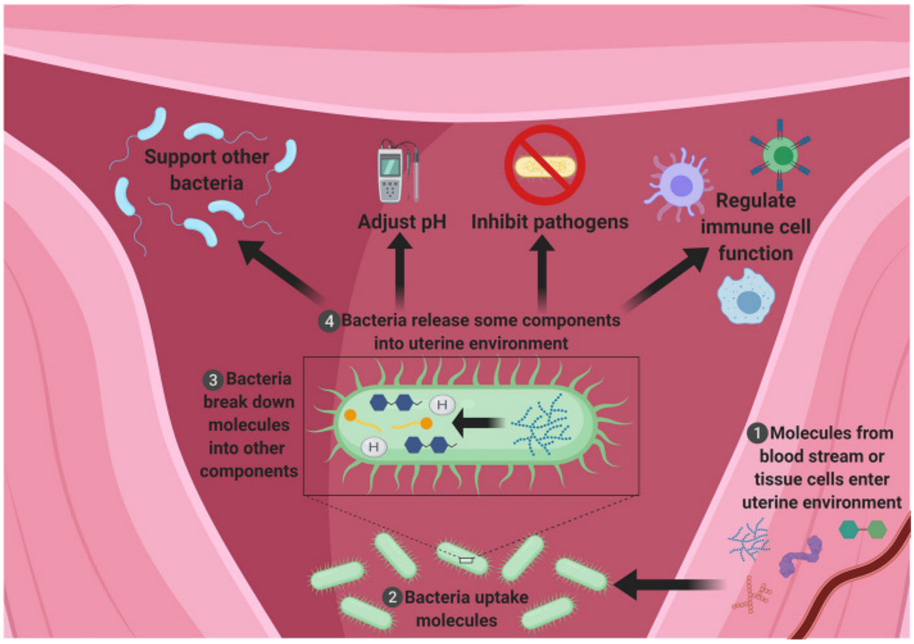

Figure 2

Figure 2

Bacteria contribute to a healthy environment in the reproductive tract by taking in molecules and breaking them down through metabolic processes. Bacteria's metabolism provides them with the components they need to survive. Other components are released into the surrounding environment, such as that of the reproductive tract. These products can benefit the reproductive tract environment by supporting the functions of other bacteria, adjusting the pH of the environment, and regulating the functions of immune cells (Image created using BioRender).

produce products that regulate the activity of the immune cells. These immunes can help an animal fight off pathogens or create an environment in the uterus that helps support pregnancy development. Good bacteria can also inhibit the growth of pathogenic bacteria by producing anti-bacterial compounds, or by out-competing pathogens by consuming the resources those pathogens need to grow. All these functions of bacteria in the reproductive tract are necessary for a healthy environment that can allow for a pregnancy to develop.

HOW ARE BACTERIA IN THE REPRODUCTIVE TRACT RELATED TO SUCCESSFUL BREEDING AND PREGNANCY?

As the agricultural industry is trying to produce more efficient cattle to feed the growing world population, it is important to have cows become pregnant as easily and quickly as possible. However, if the bacterial communities in the reproductive tract are altered, creating an unfavorable environment for pregnancy, the cow may not be able to get pregnant or produce a healthy calf (Figure 3). The majority of previous research evaluated pathogenic bacteria and how they reduce the ability of an animal to become pregnant. It is known that cows are more likely to have infections and high abundance of pathogenic bacteria in the uterus after giving birth to a calf, which leads to difficulty when trying to breed the cow again. However, recent research has begun to evaluate the relationship between the healthy bacterial communities in the reproductive tract and an animal's ability to establish a successful pregnancy.

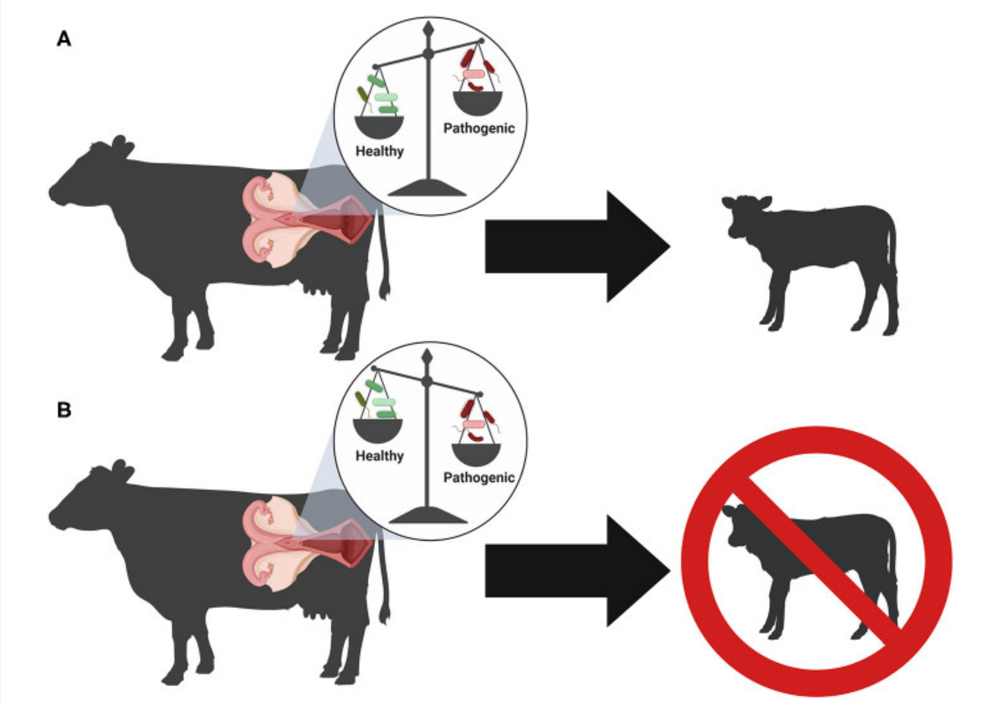

Figure 3

Figure 3

Bacterial communities present in the uterus prior to breeding can affect reproductive outcomes. **(A)** Cows with balanced bacterial communities in the uterus have a better chance of getting pregnant and producing offspring. These cows have more healthy bacteria (green) that contribute to a better uterine environment. Pathogenic bacteria (red) can be present in healthy animals at low abundances. **(B)** Cows with altered bacterial communities in the uterus are less likely to become pregnant. These cows have a greater abundance of pathogenic bacteria (without infection) in the uterine environment, which does not support pregnancy development (Image created using BioRender).

Scientists evaluated bacterial communities in the reproductive tracts of cows prior to breeding, to determine whether bacteria were related to pregnancy outcomes. Cows that became pregnant had a distinctly different composition of bacterial communities in the uterus prior to breeding in the uterus than cows that did not become pregnant. Specifically, cows that did not become pregnant had more pathogenic bacteria, although no infection was present [4]. These data suggest that differences in uterine bacterial communities can influence a cow's reproductive success (Figure 3). The ability of bacteria to influence reproductive success is likely due to their effects on the reproductive tract environment, by controlling factors that are important for pregnancy development. Further studies will evaluate the role of the uterine bacteria during pregnancy and how they may impact fetal growth, health, and development.

HOW CAN WE IMPROVE BREEDING SUCCESS WITH REPRODUCTIVE TRACT BACTERIA?

Since a cow's bacterial communities have proven to influence the reproductive tract environment and pregnancy development, studies are also evaluating methods to modify the composition of the bacterial communities. By altering the kinds of bacteria present in these communities, we could potentially create a healthy reproductive tract environment that is the best for supporting pregnancy. Studies have tested **probiotics** for this purpose. Probiotics are live bacteria, such as *Lactobacillus*, that are known to have health benefits for the host.

PROBIOTICS

Live bacteria that can be added to an environment for health benefits. Probiotics can alter the bacteria present in the community and change the metabolic products that are released.

Probiotics can be added to the reproductive tract and contribute to the animal's reproductive environment. Although *Lactobacillus* has a naturally low abundance in the reproductive tract of cattle, scientists have begun to test the use of *Lactobacillus* as a probiotic in cows. Results have been positive for treating infections, controlling immune system responses, and improving the number of animals that become pregnant [5]. Further studies are needed to evaluate the use of probiotics in breeding when infection is not present, to see how probiotics regulate the normal balance of bacterial communities in cattle.

CONCLUSION

Bacteria are not all bad, as there are many different types of bacteria that perform important functions to benefit the host. Bacterial communities are essential to the host's health and performance, due to their contributions to the various environments throughout the body. Although the reproductive tract was once believed to be bacteria-free, research has now uncovered the importance of healthy bacteria in reproductive health and success. There are many diverse bacteria, including *Lactobacillus*, that live in the reproductive tracts of cattle and benefit the reproductive tract environment. *Lactobacillus* is well-understood for its function in the reproductive tract and has been the most studied regarding probiotics in the reproductive tracts of cattle and humans. Further studies are needed to determine how and if other bacteria can be used as probiotics. As we come to further understand the roles of bacteria in the reproductive tract, we may be able to use these good bacteria to improve breeding outcomes, which would help to produce more cattle to feed the ever-increasing population of the world.

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CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

ETHAN, AGE: 14
Hi! My name is Ethan. At my regional science fair, I won Best Junior Project, Best Biology Project, Gold Medal, and a qualification to the Canada-Wide Science Fair, where I achieved a bronze medal. I have also been a finalist at the InspoScience Canada IRIC. Besides, I enjoy debating, having won the title of national champion and second speaker this past year, and public speaking. I am an avid writer, being published for my poems, short stories, and argumentative articles.

NETTRA, AGE: 16
She is a young scholar studying second year Higher Secondary schooling. Her fascination with Biology began to develop at a young age, led her to conduct simple experiments at home and shows keen interest in molecular biology. Inspired by her doctor parents, she set her goal to become a doctor (ophthalmologist) herself and serve the scientific community. Her dream is to explore underwater creatures by snorkeling the Great Barrier Reef. She reads more of scientific fictions and spends her spare time with baking cakes and cookies.

AUTHORS

TAYLOR B. SEAY

Taylor B. Seay grew up in East Tennessee with a passion for working with animals. She received her bachelor's and master's degrees from the University of Tennessee-Knoxville Department of Animal Science, where she is currently a Ph.D. student and Graduate Research Assistant. Her research focuses on evaluating the reproductive tract and rumen bacterial communities of beef heifers. She is interested in the factors affecting these microorganisms and how they can influence reproductive efficiency in beef cattle.

BROOKE A. CLEMMONS

Brooke A. Clemmons is Ph.D. student in Animal Science in Knoxville, TN. She grew up in cities, but always loved farm animals. Brooke currently works with cattle to understand how different environmental factors affect how cows use food. She specifically focuses on how different things, like pregnancy and genetics, affect the microbes that live in the guts of cows, and how we can use those to make animals grow better and healthier.

PHILLIP R. MYER

Phillip R. Myer is an Assistant Professor at the University of Tennessee Department of Animal Science. He is interested in how microbes in beef cattle impact feed efficiency. Phillip is also interested in how beef cattle can interact with their microbes. His research group uses DNA technologies to help investigate these interactions. He hopes his research can help inform, improve, and be part of the solution to future global protein and food demand. *pmyer@utk.edu

KYLE J. MCLEAN

Kyle J. McLean grew up in southeastern Montana where he developed a passion for science and animals. He received an Associate's of Science degree at Eastern Wyoming College. Then, he went to Stillwater, OK where he received a Bachelor of Science and Master of Science degrees from Oklahoma State University. Finally, Dr. McLean received a Ph.D. in Animal Science from North Dakota State University. Upon graduation, he spent 2 years as a post-doctoral research associate at the University of Kentucky before coming to the University of Tennessee where he is conducting research in cattle reproduction. *kmclea10@utk.edu

frontiers FOR YOUNG MINDS

BIODIVERSITY
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GETTING BIG COWS BY FEEDING THEIR TINY MICROBES

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YOUNG REVIEWERS:


MADDIE
AGE: 15


MATÍAS
AGE: 14


SADIE
AGE: 15


SEBASTIAN
AGE: 10


TACY
AGE: 13

Cattle are used for many purposes, but today beef cattle are mainly raised to provide people with meat and many other useful products. Did you know that leather, soap, gelatin, and even medicines come from beef animals? Besides, cattle can thrive on low-quality land not suitable for building houses or growing plants for people to eat. More importantly, they eat foods that other animals cannot use up. Cows rely on plants as their main source of food and they can digest tough plant parts because they have a team of microbes in their gut. The microbes in this tiny team (called the microbiome) provide cattle with about 70% of the energy they need. As the microbe team is very important for cattle to grow and get nutrients from food, scientists are working on strategies to get bigger, healthier cows while, at the same time, caring for our planet.

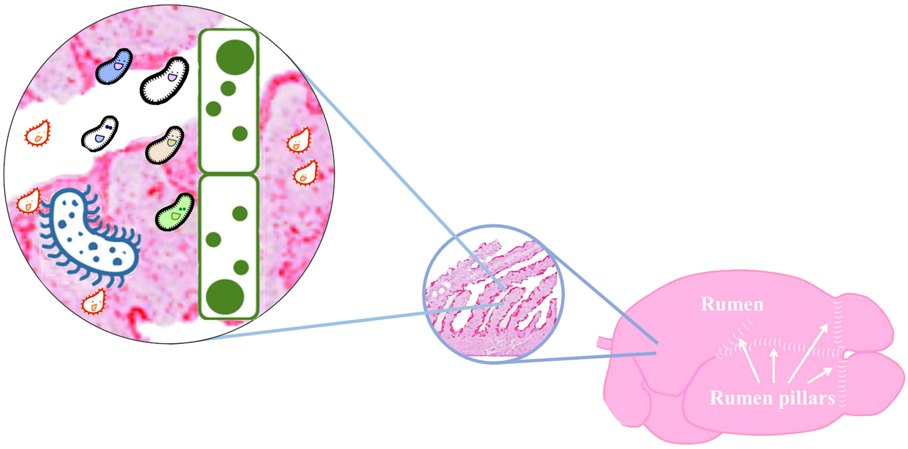

Figure 1

Figure 1

Microbes of different types and sizes exist in the rumen of cattle (left). Prokaryotes, including archaea and bacteria, are smaller than eukaryotes, which include protozoa and fungi. In the middle figure, can you recognize the small papillae, shaped like fingers, where the microbes live? The entire rumen, including the hand-like pillars that mix and squish the food, is shown on the right.

DID YOU KNOW THAT COWS ARE RUMINANTS?

Humans get many useful products from beef cattle. Not only do they provide us with a source of meat, but also leather, soap, gelatin and medicines come from cattle. Cows also eat foods that other animals cannot use up and live in land that is not suitable for building houses or growing plants for people to eat.

Cows are **ruminants**, which means they have a **rumen**. The rumen is the first part of a cow's four-part stomach. The rumen helps cows to break down tough materials like those found in plants, and the smaller, broken-down pieces are easier for the cows to use for food. Imagine having hands and tiny fingers inside your stomach. The hands, called pillars, squeeze the food up and down and all around in the rumen until it is squished into little pieces, while the fingers, called papillae, help to take nutrients from the food (Figure 1, right). Now imagine yourself repeating this process for 8 h a day! That is a lot of work! Cows are not the only ruminants—goats, sheep, and even giraffes and moose are ruminants too [1]! Wild ruminants, such as elk and caribous living in grasslands help to prevent a dangerous rise in greenhouse gasses in earth's atmosphere. Animals trim the grass constantly, helping the grass to grow taller and healthier. Their roots become longer, and the grasses can take up more of the greenhouse gas carbon dioxide from the atmosphere. Thus, wild, and grazing domestic ruminants protect the diversity of our native plants and animals, which are foods for humans.

RUMINANTS

An animal that eats plant-based foods and breaks them down using the rumen.

RUMEN

The main compartment of the special stomach of the cows, which is divided into other three.

DID YOU KNOW THAT MICROBES CAN HELP COWS USE FOOD?

The rumen is a special organ because it is home to millions of tiny, microscopic organisms called **microbes**. All the microorganisms together are called the cows' **microbiome**. Cows can eat plants as their

MICROBES

Tiny forms of life, including bacteria, fungi, and protozoa.

MICROBIOME

Entire habitat, including the microbes (bacteria, archaea, fungi, protozoa), their genomes (i.e., genes), and the surrounding environmental conditions.

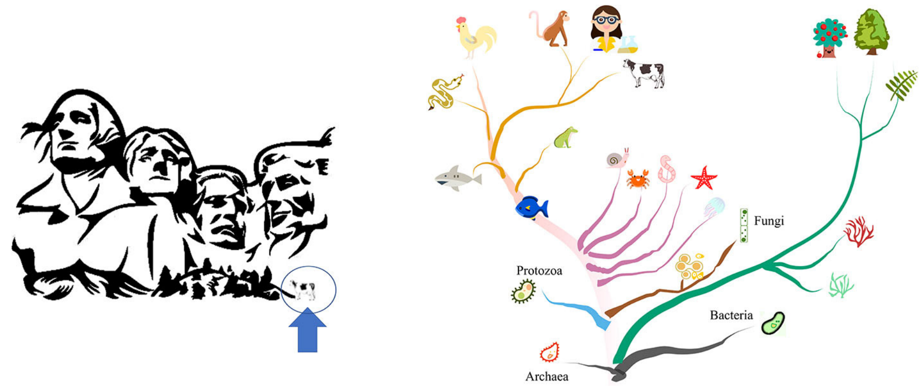

Figure 2

Figure 2

Cows are about 1,000 times larger than their microbes. For comparison, if Mount Rushmore were a cow, the small cow in the right corner of the mountain would be a bacterium. On the right side of the figure, you can see where archaea, bacteria, protozoa, fungi, cows, and humans are on the tree of life. The bottom of the tree includes less complex organisms and the top branches include those more complex.

main food source because the rumen houses many different microbes that break the plants down into usable energy for the cows. Scientists have recently become aware that the microbiome is necessary for cattle to grow and be healthy [2], and scientists have been trying to help this microbe team to be more efficient [3]. More efficient microbes could mean healthy, bigger cows that eat less food. Although the scientists have worked hard to achieve this aim while increasing the environmental benefits, the results have not been as good. Scientists now know that there is much more information we need to piece together before we can support the cows' microbiome in its mission: getting as much energy as possible from less food, so cows can grow better and be happy.

DID YOU KNOW THAT THERE ARE DIFFERENT KINDS OF MICROBES THAT DO DIFFERENT JOBS?

Cows are around 1,000 times larger than the microbes living in their rumens (Figure 2, left). The cow microbiome is made up of squads of different kinds of microbes: bacteria, protozoa, fungi, and archaea. The types of microbes all have different abilities and there are many things that affect their behavior. Although cows and their microbes belong to different kingdoms of life (Figure 2, right), they collaborate, and both the cows and the microbes obtain positive results from this teamwork.

Bacteria and archaea are a lot smaller than fungi and protozoa (Figure 1, left). Bacteria can eat many different things and they produce several kinds of nutrients for the cow. Archaea are fewer and smaller, and they do not do as many different things as bacteria do. Fungi are good at breaking into tough plant walls. Protozoa can hold on to foods and store them longer than other microbes, so they can release energy when needed, to provide fast resources for the cow. To give you an idea of how many microbes of each type are present, if our microbiome team had 20 members, 10 would be in the bacteria squad, eight on the protozoa crew, and fungi and archaea would be represented by one participant each.

Beef cows can eat many kinds and parts of plants. For most of their lives, cows graze, meaning they eat grass. But as cows get older, they can eat grains. Grains, like cereal, come from the seeds of plants. Grains are not as hard to break down as grass, so the microbes can digest them faster than they digest grass. We know that diet is a big force driving the composition of the microbiome team [4]. If their preferred food is not present in the cow's diet, microbes will not grow and the members on the team will change. For instance, some bacteria can only live and grow when cattle eat mostly grains, but others are more adaptable and can survive [4]. Archaea are often found when cows are eating more plants. The protozoa squad does not change as much because of the diet, while fungi really do not like grains and will sometimes die off when cattle eat more grain.

Although microbes do a lot of important things for cows, they can also do things that are not as helpful. Some microbes produce methane, which is a gas that contributes to global warming and is also a waste product for cattle, preventing them from growing to their full potential. Some microbes might not make energy from certain foods as well as other microbes can. Scientists want to understand the helpful effects of the cows' microbiome while also making sure their microbes release less methane.

DID YOU KNOW WE CAN HELP MICROBES HELP COWS?

There are things that scientists and farmers can do to help the microbiome team do their best to grow happy, healthy cows. Like we mentioned before, cattle eat mainly grass when they are young, but they are usually fed more grain as they grow older. Grains are used not only to feed cattle, but also to produce food or fuels for humans. Grain leftover from other uses is a great food for cattle because protein and other nutrients contained in the grain can be broken down by the microbiome team. The broken-down grain can then be used by animals and help them to grow and be healthy. When this diet change from grass to grain happens, the microbiome helps cows to successfully pass from digesting plants and their mother's milk to eating grains. Of course, the microbiome must change to keep young cows healthy. Remember what happened when your mom or dad tried to feed you a new food for the first time? Maybe you felt that the texture was weird, maybe you even cried. Cows do not cry, but they get stressed when their food is changed. For this reason, letting cows try new foods early in life helps their microbiomes get used to breaking down different kinds of food. When the microbiome is adjusted to the new food, the cow can grow faster and be healthier.

Scientists have created special foods or medicines that can be fed to cows to limit the growth of microbes that produce things that may be a waste for the cow, like methane. However, the positive effects of the special foods or medicines do not usually last very long. Scientists are trying to find longer-lasting, inexpensive ways to help microbes help cows use food better [5]. For example, do you like the smell of rosemary or thyme? The aromas of these spices are contained in the essential oils that come from these plants, which can eliminate particular microbes producing waste in the gut of the cows. Scientists are trying to learn if providing these oils to cows can help them to use their food better, because they may help to stop the activities of the bad microbes.

ARE THERE ANY OTHER WAYS THAT SCIENTISTS ARE WORKING TO HELP ANIMALS AND THE ENVIRONMENT?

As we have shown, a cow's microbiome is very important for helping the cow to grow, because the microbes participate in the breakdown of the foods that the cow eats, providing the cow with nutrients. Diet and other factors in the cow's environment can change how well the microbiome does its job, but we still need to find out exactly how this happens so that we can make beef production the best it can be. Researchers are trying to determine whether some microbes make different nutrients that might help cows to eat less but gain more weight. This type of knowledge will help to improve livestock farming. Knowledge about a cow's microbiome can also help us care for the environment, because we can maintain or help cow's growth, while decreasing the wasteful products released in the atmosphere and in the environment.

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YOUNG REVIEWERS

MADDIE, AGE: 15
My name is Maddie. I am 15 years old, and live near San Francisco.

MATÍAS, AGE: 14
I am a 14 years old who loves science, programming, Vikings, mythology, Jiu-Jitsu, rock and roll, singing, and playing drums.

SADIE, AGE: 15
Sadie is active in volleyball, track, and band. She enjoys science and mathematics and is looking forward to Biology and Anatomy & Physiology coursework. In her free time, she is training and competing in rodeo events including pole bending and team roping with her horses; Autumn, Calypso, and Coco.

SEBASTIAN, AGE: 10

I like sports, reading, math, and animals.

TACY, AGE: 13

Hi I am Tacy. I am 13, and live near San Francisco. I like drawing, videogames, and playing guitar.

AUTHORS

BROOKE A. CLEMMONS

I am a Ph.D. student in Animal Science in Knoxville, TN. I grew up in cities, but always loved farm animals. I currently work with cows, and study how different things in their environments affect how cows use food. I specifically focus on how things like pregnancy and genetics affect the microbes that live in the gut of cows, and how we can use those microbes to make animals grow better and be healthier. *Brooke.Clemmons@tamuc.edu

EMMA HERNANDEZ-SANABRIA

I am a vet and I love cows and the bacteria living in our guts. My passion for studying both of these things took me to the University of Aberdeen (in Scotland) and The University of Alberta (in Canada) for post-graduate studies. I decided to continue seeing the world and I moved to Belgium, where I now work as a Post-doctoral Fellow at the Flemish Institute of Biotechnology (VIB) in Leuven. I use simulations of our gut environment to see how our microbiome changes in health and disease. *emma.hernandezsanabria@kuleuven.vib.be

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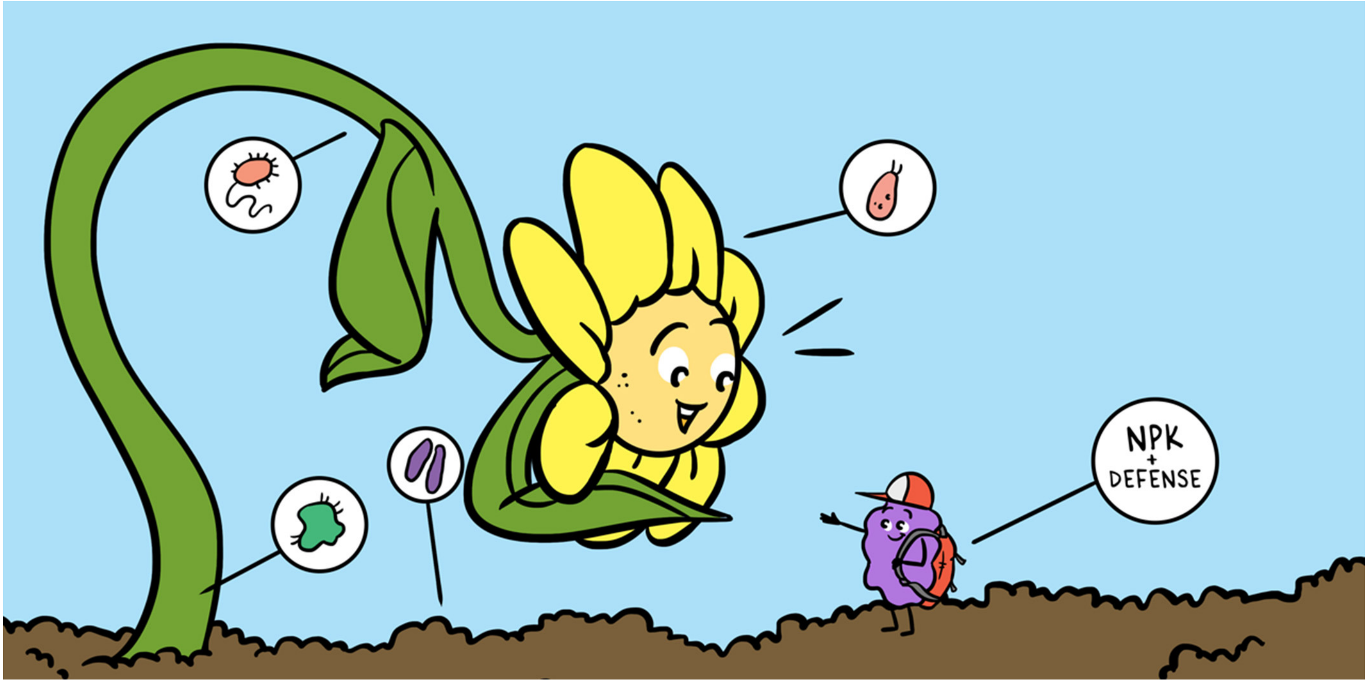

RESCUE RANGERS: HOW BACTERIA CAN SUPPORT PLANTS

Rocío Hernández-León* and Yunuen Tapia-Torres

Escuela Nacional de Estudios Superiores, Unidad Morelia, Universidad Nacional Autónoma de México, Mexico City, Mexico

YOUNG REVIEWER:

KONSTANTIN
AGE: 14

Plants, in addition to being very beautiful, provide us with oxygen and food, among other things. In their ecosystems, plants coexist with various types of bacteria, some of which are friendly and others that are the plant's enemies. Friendly bacteria can help plants grow by helping the plants to obtain nutrients such as phosphorous and nitrogen, or by defending the plants from other microbes that can make them sick. Our goal in the lab is to find the bacteria that can help plants grow and defend them from enemy attacks, understand how those bacteria work, and join forces with the bacteria to make agricultural plants produce enough food for everyone's table. In this article, we discuss four bacteria we have identified that can help and defend some plants.

RELATIONSHIPS BETWEEN PLANTS AND BACTERIA

Plants are a very important part of our lives. They provide us with oxygen, wood, medicines, and foods, but they do not do it alone. Plants share the soil habitat with many microorganisms, such as

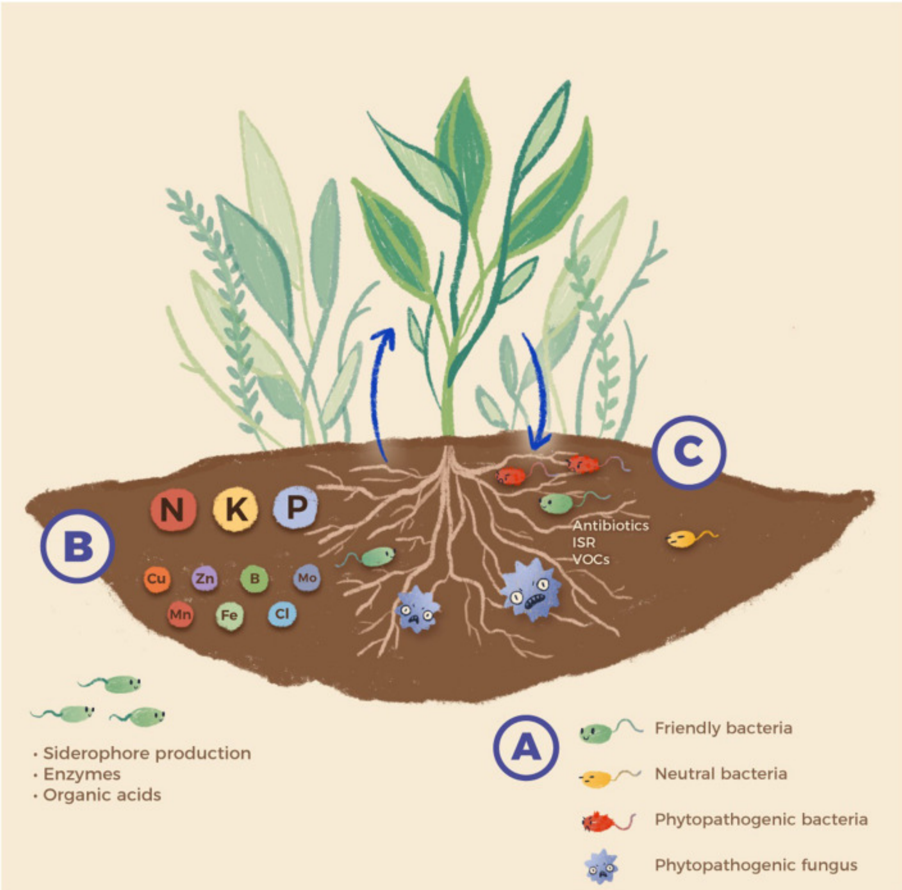

Figure 1

Figure 1

(A) Relationships between bacteria and plants can be helpful to the plant, harmful to the plant, or neutral. **(B)** Through the production of enzymes and acids, friendly bacteria can help plants obtain necessary nutrients from the soil, including nitrogen (N) and phosphorus (P). Other important nutrients for plants are potassium (K), copper (Cu), zinc (Zn): boron (B), molybdenum (Mo), manganese (Mn), iron (Fe), chlorine (Cl). **(C)** Bacteria can defend plants from harmful pathogens through a process called induced systemic resistance (ISR), which is like a vaccination for the plant, or by producing antibiotics or volatile organic compounds (VOCs).

fungi, small worms called nematodes, and bacteria. Bacteria are tiny living things that we cannot see with the naked eye. Various types of bacteria look different from each other and play different roles in the ecosystem. What kind of relationships exists between soil bacteria and plants?

Three main types of relationships exist between plants and bacteria (Figure 1A). Some bacteria can be harmful to plants and attack them, causing them to rot, or they might make the plants sick or cause them to dry up. The relationship between these harmful bacteria and plants is a negative relationship, and these bacteria are called **phytopathogens**. However, there are also bacteria that can be plant-friendly, helping plants or defending them from phytopathogenic bacteria, fungi, and viruses. These relationships are positive. In return for this friendship, plants feed bacteria with carbohydrates, vitamins, and organic acids, among other things. Plants also provide a home for the helpful bacteria, allowing the bacteria to live near the plants or even within them. If the bacteria do not attack the plant but do not help it either, the relationship is neutral.

PHYTOPATHOGEN

An organism that makes plants sick.

SOME HELPFUL BACTERIA HELP PLANTS GET NUTRIENTS

Plants grow in the soil and cannot move around, meaning they are **sessile**. Sessile organisms cannot go looking for the **nutrients** they need to grow. However, bacteria can help plants to obtain the proper nutrients (Figure 1B). For example, plants need phosphorus to grow, but much of the phosphorus in the soil is trapped in **molecules** that plants cannot break down. In this case, the plant's bacterial friends show up to help, releasing molecules like special acids, or proteins called **enzymes** that help break down these phosphorus-containing molecules and release the phosphorus so plants can use it [1].

Nitrogen is another important nutrient for plants. Bacteria can help plants to obtain nitrogen by releasing other enzymes. But there's a problem, because the nitrogen-freeing enzymes are destroyed by oxygen. To get around this problem, bacteria help plants to form a protected fort called a **nodule** on the plant's roots. This nodule protects the bacteria and their enzymes, and, inside the nodule, nitrogen is made available for the plant.

SESSILE

The inability to move from place to place.

NUTRIENTS

Chemicals from which energy is obtained to perform a function.

MOLECULES

Two or more atoms united. Is the smallest unit of a substance.

ENZYMES

Molecules that regulate chemical reactions.

NODULE

Round or oval structure made of plant cells.

BACTERIA TO THE RESCUE!

Beneficial bacteria can defend plants from the attacks of phytopathogens (Figure 1C). One of the ways that they do this is called **induced systemic resistance**, which is something like a vaccine that protects plants against fungi, bacteria, and viruses. The bacteria provide resistance by being in contact with the roots of the plant, which activates the plant's defense system and prepares it for a battle. Induced systemic resistance involves signals that are carried through the plant from the roots, or signals that travel through the air, alerting the parts of the plant that have not yet been attacked [2]. Thanks to those signals, when plant pathogens arrive, the plants are ready for the invasion and can better defend themselves.

Another thing that bacteria can do to defend plants is to release substances that chase away the plant's strongest enemies. These substances are called **antibiotics**, and they are similar to the medicines we might take when we have certain infections. These bacteria-produced antibiotics have funny names like phenazine, pyrrolnitrin, or zwittermycin. Some of these antibiotics are launched against enemy bacteria and others against dangerous fungi. The antibiotics can be released as **volatile organic compounds**, which are compounds that at room temperature are in the gaseous state or can easily become a gas. Release of volatile organic compounds is a highly effective mechanism that bacteria can use to quickly help a plant, because gases can spread easily and quickly reach all parts of the plant. **Siderophores** are also molecules that bacteria produce and release when elements such as iron is not enough in the soil,

INDUCED SYSTEMIC RESISTENCE

It is one of the mechanisms that plants have developed to defend themselves against phytopathogens.

ANTIBIOTICS

Substances that destroy microorganisms.

VOLATILE ORGANIC COMPOUNDS

Molecules released as gases.

SIDEROPHORES

Small molecules that bind to iron and make it easier for the plants to use it.

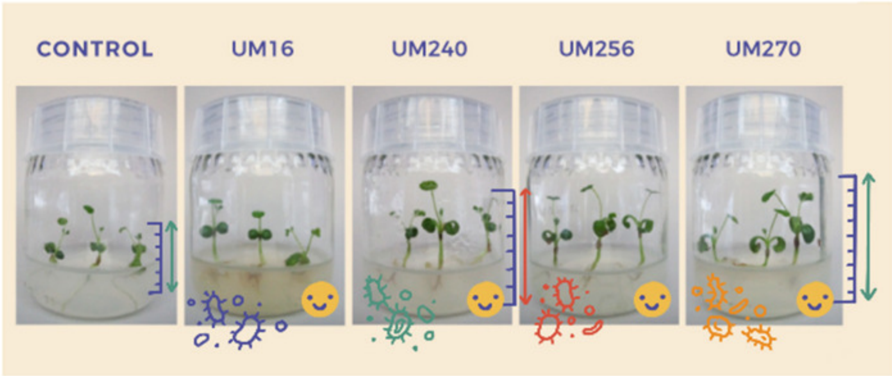

Figure 2

Figure 2

The control flask (left) contains a *Medicago truncatula* plant growing without any added bacteria. The remaining flasks contain the plant and the indicated friendly bacteria. The arrows show that the plants grew better in the presence of the bacteria UM240 and UM270. The color of the arrows has no meaning.

these molecules can give a hug to the little iron that is there, forming a siderophore-iron complex in which iron can be transported more easily and used by plants.

CREATION OF A BACTERIAL RESCUE FORCE

In the laboratory, we looked for the best "rescue rangers," to form an army of bacteria that can help make the plants that feed us stronger. To do this, we first recruited many bacteria from the soil under different plants. Then we had the bacteria fight against many fungi that cause damage to those plants, and we selected those bacteria that did not allow fungi to grow. We will tell you about four bacteria that are part of our team of rescuers. These bacteria are called UM16, UM240, UM256, and UM270 [3].

These four helpful bacteria belong to the genus *Pseudomonas*. We put each bacteria together with a plant called barrelclover inside a flask. After 15 days, we could see that the plants were bigger when they were accompanied by the bacteria than when they grew alone (Figure 2). We also grew the bacteria in a glass bottle located inside the flask, so they couldn't touch the plants. We saw that the plants were still bigger when the bacteria were present, even if they were not touching the plant. This means that the bacteria released volatile organic compounds that helped the plants to grow.

Another test that these rescuers passed was the ability to fight against a phytopathogenic fungus that scientist call *Botrytis cinerea.* This fungus sickens crops like tomatoes, strawberries, and grapes, causing decreased food production. To perform this test, the bacteria and the fungus were both added to a petri dish (Figure 3A). When the bacteria and the fungus were together in the dish, the bacteria prevented the growth of the fungus. We also put the fungus and bacteria into petri dishes that were divided up so that the bacteria and fungi could not touch; they could only communicate through a

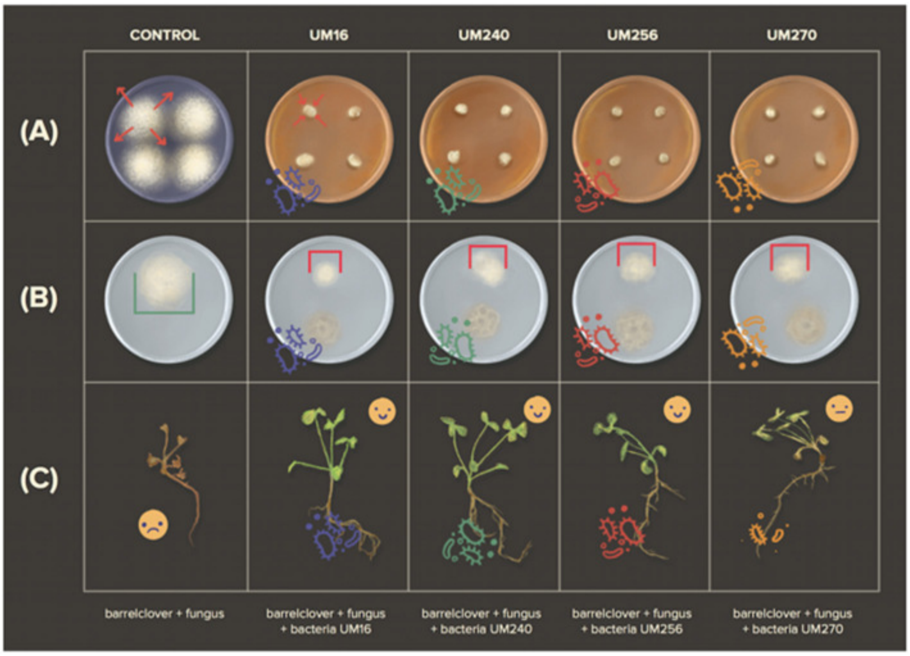

Figure 3

Figure 3

(A) When the bacteria were grown in the same chamber of a petri dish with the fungus, the bacteria reduced the growth of the fungus compared to the control (far left). **(B)** When the bacteria and fungus were grown in separate chambers, the growth of the fungus was still less than the control, indicating that the substance that reduces fungal growth is a volatile organic compound. The arrows and brackets show the differences in the growth of the fungus. **(C)** Barrelclover infected with fungus had fewer signs of disease when grown in the presence of the bacteria.

small window. Even when they could not touch, the fungus did not grow as large as when it was grown alone, which tells that volatile organic compounds produced by the bacteria inhibited the growth of the fungus (Figure 3B).

Once the bacteria won the battle against fungus, we had to know if they could protect an actual plant. We put the fungus in contact with barrelclover and saw that the fungus turned the plant brown and killed it (Figure 3C). When the plant was accompanied by UM16, UM240, UM256, or UM270, the fungus did not make the plant as sick as it got in the presence of the fungus alone. This means that our bacterial rescuers managed to defend the plant from the evil phytopathogenic fungus and keep the plant alive.

NEXT STEP: FORMING A BACTERIA SUPERSQUAD!

Now our bacterial rescue rangers are ready to go out into the field to protect plants and help farmers produce enough food for the world's growing population. We are still studying these bacteria, because each of them has a unique mechanism for helping plants. As we have already seen, these four bacteria are good rescuers on their own, but imagine if they all combined their superpower to form a squad—they would be invincible! Remember, all kinds of bacteria, both friendly and harmful, live together in the soil. The more microbial diversity there is in the soil, the greater the ability to fight against phytopathogens. To have healthy food, we need healthy soil—and the help of our friendly bacterial rescuers!

ORIGINAL SOURCE ARTICLE

Hernández-León, R., Rojas-Solís, D., Contreras-Pérez, M., del Carmen Orozco-Mosqueda, M., Macías-Rodríguez, L. I., Reyes-de la Cruz, H., et al. 2015. Characterization of the antifungal and plant growth-promoting effects of diffusible and volatile organic compounds produced by Pseudomonas fluorescens strains. *Biol. Control* 81:83–92. doi: 10.1016/j.biocontrol.2014.11.011

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YOUNG REVIEWER

KONSTANTIN, AGE: 14

Hi I am Konstantin, your nearby Young Mind! I am from Rousse, Bulgaria and since I was little I had questions like: what's the point in recycling etc. Now, as an adolescent, I really got into ecology and decided to help bring awareness of some of the problems in our world has like the air pollution, species extinction and deforestation. If I, an ordinary student, can make a difference you can too-so what are you waiting for my young reader!

AUTHORS

ROCÍO HERNÁNDEZ-LEÓN

Rocío Hernández-León is deeply in love with plants. That is why she has dedicated herself to investigating the abilities that bacteria have to help them grow. With a master's degree and Ph.D. in experimental biology, she seeks to find in the laboratory the most powerful bacteria for this important task, with as and goal to produce food in a safe, nutritious, and sufficient way. *r.hl83@hotmail.com

YUNUEN TAPIA-TORRES

Yunuen Tapia-Torres is a soil scientist who loves the complexity of the bacterial world. Her research at the Escuela Nacional de Estudios Superiores, Morelia, UNAM focuses on understanding the importance of bacteria on the transformation of molecules containing carbon, nitrogen and phosphorus in soil. She is working to strengthen research in the area of soil biogeochemistry in Mexico, and thereby guarantee fertile soil for the future. ytapia@enesmorelia.unam.mx

BIODIVERSITY
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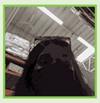
WHO FEEDS THE PLANTS? MICROBES!

Aline Lacaze**, Antoine Zboralski and David L. Joly

Biology Department, Université de Moncton, Moncton, NB, Canada

YOUNG REVIEWERS:



MEHA
AGE: 15

NIVEDITA
AGE: 14

SHREEYA
AGE: 11

SHRIYA
AGE: 13

Plants get their food from the soil they grow in. But this food is only available thanks to a great diversity of microbes, especially bacteria and fungi, that can chemically and mechanically transform materials in the soil into nutrients. These soil microbes have received a lot of attention from scientists all around the world, because these organisms could improve the way we grow our food and could make agriculture more sustainable. In this article, we explore some of the main mechanisms used by these microbes to feed plants.

INTRODUCTION

Beneath the surface of the Earth lives a tremendous diversity of **microbes**, including many species of bacteria and fungi. These organisms vary in shape and size, and most of them are not even as thick as a hair. We cannot see soil microbes with our eyes, but they play a fundamental role in our lives: they feed the plants that we grow for food. In other words, soil microbes are essential for us to live on this planet. Soil microbes have evolved many different ways to help plants grow, such as breaking down rocks, recycling dead matter,

MICROBES

Tiny living organisms such as bacteria or fungi, also called microorganisms. Some microbes cause plant diseases, but others are helpful.

and establishing cooperative relationships with plants. Let us see how these tiny organisms carry out all these tasks.

A TINY TASTE FOR ROCKS

Plants cannot directly live on rocks because the nutrients in rocks are not available to them. The nutrients plants need are found in the soil and include elements like iron and potassium, or small molecules like phosphate or nitrate. These nutrients are the building blocks required for plants to grow, just like the food we eat is used to fuel our bodies.

In the beginning of their evolution, plants existed only as algae living in oceans. At least 420 million years ago, they started to settle on the land [1]. At that time, the land had just been formed from volcanic activity, meaning it was mainly rocky, and no soils were present to help plants grow. Luckily microbes were already present on the land and these microbes had the ability to get nutrients from rocks, through a process called **rock weathering**. The microbes made the nutrients previously trapped within rocks available for the new terrestrial plants, allowing these plants to thrive on land and helping to form soils for future generations of plants!

ROCK WEATHERING

the mechanical and chemical breakdown of rocks.

How do microbes perform rock weathering? They make specialized proteins and acids that can affect the first layers of rocks through chemical reactions. These reactions cause the release of tiny components of rocks that can then be taken up by microbes and plants (Figure 1). For instance, the bacterium *Bacillus subtilis* can release manganese from rocks in a form that is easy for plants to take up [2]. The fungus *Talaromyces flavus* is another example. This organism uses acid to alter rocks and extract magnesium and iron. Fungi have long, tubular structures called **hyphae** that they use to grow and to absorb nutrients. *Talaromyces flavus* uses the pressure from the tips of its hyphae to break rocks and reach interesting minerals [3]. This fungus grows into small cracks and then pushes to expand them. Both fungi and bacteria also produce small molecules that can bind iron and help to facilitate rock weathering. Many types of microbes can alter rocks, and those pioneering species were essential to the formation of soils and the ultimate creation of complex land-based ecosystems.

HYPHAE

Long, branching, tube-like structures of fungi that are used by the fungi to grow and find nutrients.

MICROBIAL SCAVENGERS: GUARDIANS OF THE SOILS

In the eighteenth century, the French chemist Antoine Lavoisier formulated the law of conservation of mass, as follows: "Nothing is lost, nothing is created, everything is transformed." This principle also applies to living organisms and is illustrated by microbes called **saprobionts** (from the Greek words *sapros*: rotting and *biôn*: living). Saprobionts can recycle elements by decomposing dead matter like

SAPROBIONTS

Any organism, especially a fungus or bacterium, that lives and feeds on dead matter.

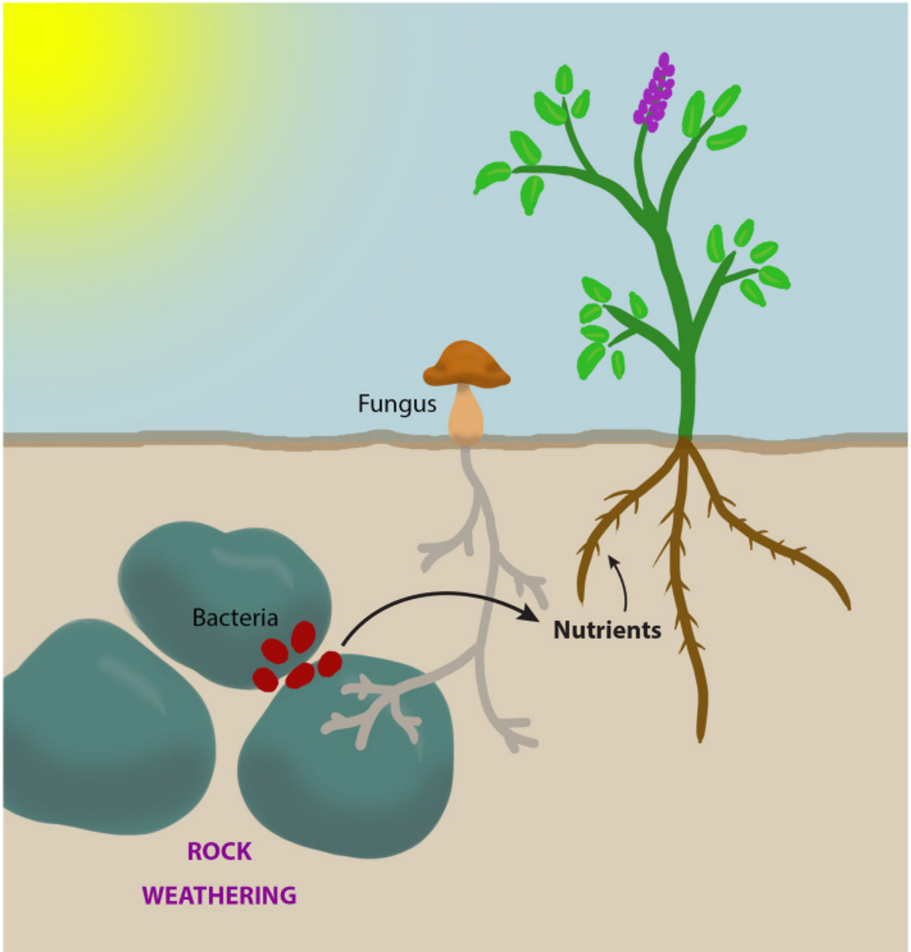


Figure 1

Figure 1

Both bacteria and fungi help to feed plants by breaking down rocks to make the nutrients trapped within the rocks available to nearby plant roots. This process is called rock weathering. Microbes that perform rock weathering enrich soils with nutrients and help crops to grow better.

plant debris, and then taking up the resulting nutrients [4]. This process allows the saprobionts to get nutrients like sugars that they could not find otherwise. Plants are efficient sugar factories functioning on solar power. They combine molecules of carbon dioxide, using light as an energy source, in the process called **photosynthesis**. When plants die, the sugars they made can be used again by other organisms. Over time, more and more dead plant material accumulates on the ground and is recycled by saprobionts to form soils.

PHOTOSYNTHESIS

The process by which plants use sunlight to make sugars from carbon dioxide.

Saprobionts include an astonishing diversity of fungal and bacterial species and can live in a wide range of places. In farm soils, saprobionts are essential for transforming compost into forms of nutrients that can be used by crops (Figure 2). Without microbial saprobionts, compost would not be a good way to feed plants, because plants cannot directly collect nutrients from dead matter. In the absence of saprobionts, crops would not grow as well, and less food would be produced.

When extreme weather events occur, such as droughts caused by climate change, soil saprobionts can be affected and may not be as efficient at recycling dead matter. But if the saprobiont population is

Figure 2

Saprobionts are soil bacteria and fungi that help to feed plants by decomposing dead material in the soil, such as compost. These microbes make the nutrients in the dead matter available for plants to absorb through their roots.

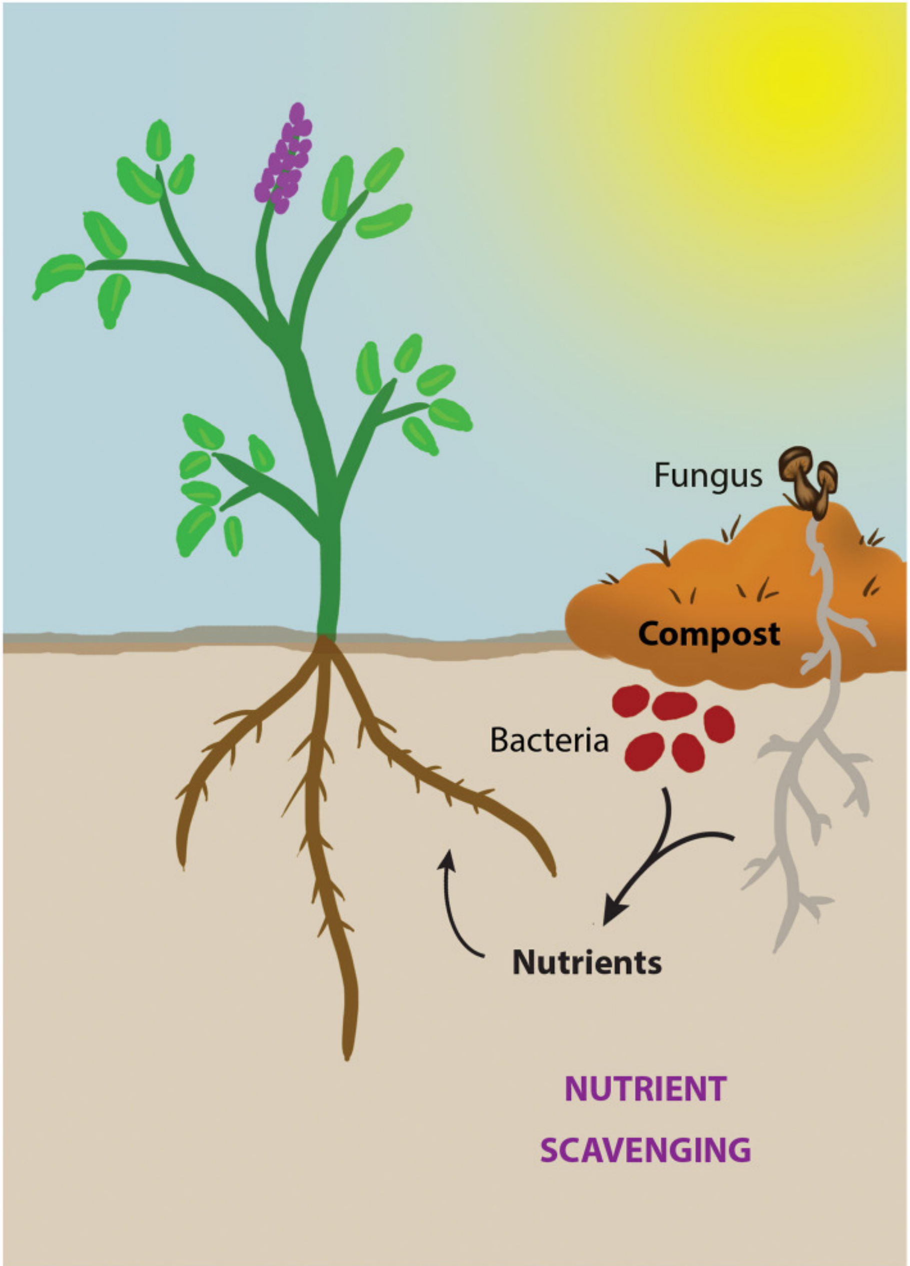


Figure 2

diverse enough, some species will not be as impacted as others by the extreme weather and will continue to degrade dead matter. So, it is important for soils to maintain a diverse population of saprobionts, so that matter can always be decomposed to feed other organisms and to keep nutrients flowing through ecosystems, supporting the crops we need to live.

HIDDEN ROOT MARRIAGES

The areas of the soil right next to plant roots are hotspots of microbial diversity. Some bacterial and fungal species find shelter inside the

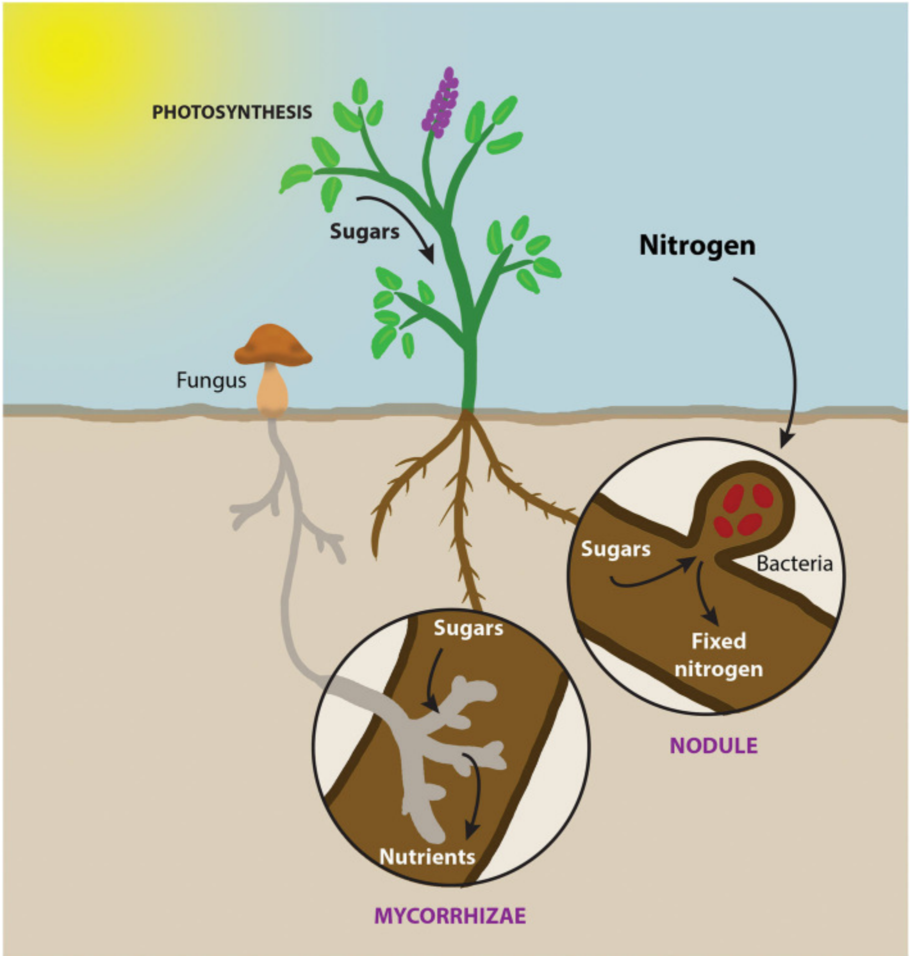


Figure 3

Figure 3

Both bacteria and fungi can form symbiotic relationships with plant roots, which feed the plants and benefit the microbes. Plants called legumes create nodules on their roots, in which certain bacteria can live and provide the plants with a form of nitrogen usable by plants. Mycorrhizae are structures created when plant roots form relationships with fungal hyphae. The hyphae transport nutrients from the soil to the plant roots. These symbioses greatly improve plant growth and health.

roots and get access to the sugars from photosynthesis, in exchange for providing nutrients and other services to the plant. This is called **symbiosis**: a relationship in which both organisms benefit, primarily by exchanging nutrients (Figure 3).

SYMBIOSIS

A close relationship between two organisms of different kinds which benefits both organisms.

NODULE

A swelling on the root of a leguminous plant that contains bacteria able to collect nitrogen from the air to give it to the plants.

Legumes are a group of plants that includes crops like soybeans, peanuts, and peas. Legumes can establish a symbiotic relationship with bacteria of the genus *Rhizobia*. The legumes produce a new organ on their root surface for the bacteria to live in, called a **nodule**. Inside nodules, *Rhizobia* reproduce and are protected from environmental stresses. The bacteria also get some plant sugars, which they use to grow and to convert nitrogen from the air into ammonia, through chemical processes [5]. Ammonia is a source of nitrogen that the plants can use. Nitrogen is important for living organisms because it is an essential component of many biological molecules, such as DNA and proteins. Thus, plants benefit from the nitrogen provided by the bacteria in their root nodules. If *Rhizobia* species are present in the soil and nodulation occurs, farmers do not need to add as much additional nitrogen to the soil in the form of fertilizer as usual.

MYCORRHIZAE

An association of a fungus and a plant in which the fungus lives within or on the outside of the plant's roots forming a symbiotic relationship.

Another symbiosis commonly found in ecosystems is established between plant roots and fungi. The structures formed from this type of symbiosis are called **mycorrhizae** (from the Greek words *mýkēs:* fungi and *rhiza:* roots). More than 80% of land plants participate in this type of symbiosis [6]. Mycorrhizae are fascinating in their diversity and complexity, and new things are regularly being discovered about them. Basically, nutrients from the soil are transported to plant roots through the fungal hyphae. The hyphae can explore a greater volume of soil and can reach more distant soil areas than the plant's roots can, which increases the amount of nutrients available to the plants. Without mycorrhizae, plants would not be able to collect as many nutrients from the soil and would not grow as well. In return, plants share their sugars with the symbiotic fungi. Some mycorrhizal fungi can also weather rocks, as described earlier, to provide nutrients like phosphorus to their host plants. In crop fields, mycorrhization is critical for increasing the quantity of food that can be produced without the use of synthetic fertilizer.

WHAT HAVE YOU LEARNED?

So, now you know that soil microbes are critical in plant feeding in many ways. Although researchers have learned much about the important beneficial relationships between plants and microbes, many questions remain unanswered, such as, "Are bacteria and fungi always the main plant feeders in soil?"; "Which kinds of microbes are the best for promoting plant growth and health?"; and "How will climate change impact soil microbes?" These relationships are critical for building the kinds of soils that can sustain our agriculture. Also, microbial communities need to be diverse to face soil disturbances, such as those caused by climate change. It is important for scientists to better understand the complexity of plant feeding by microbes, so that we can face agricultural challenges.

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YOUNG REVIEWERS

MEHA, AGE: 15
Hey, I am a sophomore in high school, and looking forward to a career in medicine. My hobbies include drawing, tennis, and just hanging out with friends! I also love to volunteer and give back to my community. I am excited to be a part of Frontiers for Young Minds, as I want my peers and other students to be able to access these great scientific accomplishments made every day.

NIVEDITA, AGE: 14
Hi I am Nivedita, my pronouns are she/her/hers, and I am excited to start this year off! A little about me, I love listening to music in my free time (Frank Ocean is a favorite ☺) and I like to draw when I can. I like hanging out my friends, and my favorite subject is chemistry!

SHREEYA, AGE: 11
Hi my name is Shreeya. I live with my sister and my parents. In my free time I like to walk with friends, play board games, and doing karate. During this time, I have been keeping myself busy by talking with my friends, reading Harry Potter books, and finishing a 3D Hogwarts Puzzle.

SHRIYA, AGE: 13
Hi, my name is Shriya. I live in the U.S. I am in eighth grade, and my favorite subjects

are science and math. In my free time, I like to dance and do art. I just started working with Frontiers for Young Minds, and am very excited to continue!

AUTHORS

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I graduated with a master's degree in plant genetics in France, and I am now pursuing a Ph.D. at the Université de Moncton, New Brunswick, Canada. My research focuses on the late blight disease affecting potato plants. In the future, I would like to participate even more in science popularization. *aline.lacaze@hotmail.fr

ANTOINE ZBORALSKI

I am a Ph.D. student at the Université de Moncton, New Brunswick, Canada. I study underground plant-microbe interactions for improving plant health, which could make the way we grow our food more sustainable. I try to popularize my research to get people to better understand science. I am also an environmentalist involved in the local community to reduce our collective environmental footprint.

DAVID L. JOLY

I am an associate professor at the Université de Moncton, New Brunswick, Canada. Research in my group revolves around plants and their associated microbes, and we use molecular biology tools and genomics to understand the interactions between those organisms.

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WHAT ARE THE SMALL LUMPS I SEE ON SOME PLANT ROOTS?

Fede Berckx * ***and Katharina Pawlowski***

Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm, Sweden

YOUNG REVIEWERS:


ELLE
AGE: 11


JACK
AGE: 13


SIRI
AGE: 12


SOPHIA
AGE: 15

Microorganisms, such as bacteria and fungi, can live together with plants in a tight relationship where they help each other to provide nutrients. Some groups of bacteria can turn nitrogen, an essential element, from a gas in the atmosphere into a form that plants can use. In return, plants provide carbon to the bacteria. This special relationship is called symbiosis. Researchers try to understand why some plants interact with these helpful bacteria and others do not. They do this by looking at how the symbiotic relationship was formed and how it evolved over a long period of time of several million years. If we can use symbiotic microorganisms to provide plants with nutrients instead of using fertilizers, we could reduce the negative effects caused by greenhouse gasses associated with their production.

IT IS ALL ABOUT NITROGEN

NODULE

A special organ formed on the roots of plants, in which certain bacteria can live and exchange nutrients with the plant.

If you have ever dug up a clover plant you might have seen small, pink or yellow lumps on the plant's roots (Figure 1). These are structures—we call them **nodules**—that are made by the plant. Nodules

Figure 1

Figure 1

Diversity of nodules on various plants. The plants are shown on the left and their nodules are shown on the right. **(A)** *Coriaria* sp. (tutu). **(B)** *Discaria trinervis* (chacay). **(C)** *Pisum sativum* (garden pea). Photo credit: F. Berckx.

NITROGEN

The most common chemical element in the atmosphere.

are essential for helping the plant get **nitrogen** from the atmosphere when there is not enough nitrogen present in the soil. Yes, structures of a plant found in the soil are important for getting nutrients from the atmosphere! Like humans and animals, plants need water and nutrients to grow. Like carbon, nitrogen is an important building block of any organism. It is the most common component in the atmosphere. Nitrogen gas makes up around 78% of the atmosphere. To compare, the oxygen we breathe makes up only 21% of the atmosphere. While humans and animals breathe in nitrogen gas, we cannot directly use the nitrogen in the air. So, we rely on food sources for getting our nitrogen supply. Getting enough nitrogen is usually not an issue for people or animals, but it can be a problem for plants, that do not eat other organisms.

So where do plants get their nitrogen from? Well, they use their roots to take up nitrogen from the soil. In modern agriculture, nitrogen and other nutrients are mostly supplied by man-made fertilizers. Nitrogen-containing fertilizer was invented roughly 100 years ago by two people: Fritz Haber and Carl Bosch. Both received the Nobel Prize for their work. Unfortunately, we are now learning about the negative effects on the environment by using this fertilizer. For instance, during the production, a lot of CO_2 is released into the atmosphere. The fertilizers can also pollute aquatic ecosystems such

Figure 2

After a volcanic eruption, the volcanic ash that covers the soil is low in nitrogen (N). Nodule-forming plants can settle in this harsh environment, and with the help of their bacterial friends, they can get nitrogen from the atmosphere. As the soil becomes richer in ammonia (NH_3), more plants can begin to grow in the area.

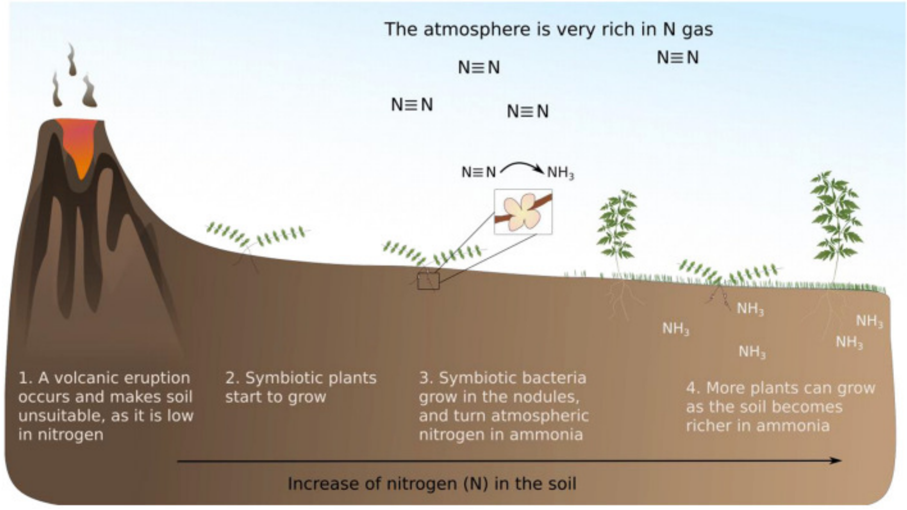

Figure 2

as lakes when too much nitrogen enters. Plants have been grown as crops for much longer than fertilizers have been in use. Not all plants are grown by farmers. So how do those plants get nitrogen? And, can we learn anything these plants to make agriculture more environmentally friendly?

MICROORGANISM

Tiny organisms like bacteria, archaea and fungi, but also very small animals or algae which can only be seen with a microscope.

NITROGEN-FIXING BACTERIA

A type of bacteria that can take up nitrogen from the atmosphere and convert it into a form that plants can use, such as ammonia. Some archaea are also able to fix nitrogen.

SYMBIOSIS

A relationship between two organisms from different species live closely together and both profit from the relationship.

LEGUME

Plants that belong to the pea family, which also includes beans and clover. Most of them are symbiotic.

BACTERIA IN NODULES CAN HELP PLANTS GET NITROGEN

The soil contains some nitrogen that is available to plants. Part of this nitrogen comes from decay of organisms, for example. Soil also contains a lot of **microorganisms** which are super small organisms like fungi, bacteria, or tiny animals like water bears. Certain bacteria in the soil, called diazotrophs, can turn nitrogen gas from the atmosphere into ammonia, which is a form of nitrogen that plants can use (Figure 2). This process is called nitrogen fixation, and the bacteria that can do it are called **nitrogen-fixing bacteria**. Some diazotrophs form a close relationship with plants through a process called root nodule symbiosis. **Symbiosis** means that two, or more, organisms live and interact with each other in a close relationship, in which both organisms profit. The plant forms nodules of various shapes, colors, and sizes on their roots (Figure 1). If we think about all plants that exist, only a small group is able to make nodules. The bacteria live inside the root nodules where they trade nutrients with the plants. The bacteria provide nitrogen and the plant provides sugars from photosynthesis. Not all plants can make nodules, so this type of symbiosis is restricted to only certain plants. For examples, clover, pea, bean, and peanut plants are all part of the same plant family which we call **legumes** [1]. But there are also some non-legume plants that can make root nodules [2].

Every plant that can make nodules shares a great-great-great grandparent who lived 100 million years ago. This means that they are all related to each other and that their ancestor could make nodules [3]. But since that time, many plants have lost the ability to make nodules. How do these plants get nitrogen? They must rely on nitrogen available in the soil. Plants that make nodules are often found in harsher environments, like on the edges of beaches, or they are the first plants to grow in the ash after a volcanic eruption. Thanks to their bacterial friends, they can grow in these harsh environments and, over time, make the soil richer in nitrogen. Enriching the soil with nitrogen benefits plants without nodules, which generally arrive later (Figure 2).

Symbiosis seems like a big advantage, so why did many plants lose this ability? This is puzzling to researchers. Some think that perhaps it was because of "cheaters" in the soil: bacteria that could pretend to be beneficial but did not provide enough nitrogen to plants. Also, since symbiosis requires energy, if the soil is already rich enough in nitrogen, it might be better for plants *not* to invest in symbiotic relationships.

PLANTS AND BACTERIA USE CHEMICALS TO FIND EACH OTHER

How do host plants and bacteria find each other? To communicate with other organisms, plants release chemicals into the soil. These chemicals tell bacteria which plants are growing nearby. If the plants are the right kind, the bacteria will release chemicals too, telling the plants that friendly nitrogen-fixing bacteria are nearby. In some cases, only specific types of bacteria will interact with certain plants, and the details of plant-bacteria communication are still not well-understood for some plants. Scientists *do* know that the way plants communicate with their friendly bacteria is very similar to the way plants communicate with certain types of fungi that also help plants to obtain nutrients like nitrogen and phosphorus [4]. Symbioses with these fungi are much more common than nodule symbiosis. Researchers estimate that the symbiotic relationship with fungi emerged 500 million years ago, when plants started to grow on the land. This was long before bacterial symbiosis began. The first dinosaurs only appeared around 240 million years ago. Around 100 million years ago, the ancient ancestor plant that first performed symbiosis with bacteria probably borrowed the genes involved in communicating with fungi and used them to communicate with nitrogen-fixing bacteria. Over time, many descendants of this ancestor plant lost the ability to communicate with bacteria but kept the ability to communicate with fungi.

HOW CAN WE USE THIS KNOWLEDGE TO PROTECT THE PLANET?

Although modern agriculture provides us with much of the food that we need, researchers have found that agriculture has a negative impact on our planet. The production of fertilizers releases greenhouse gasses, which enhance global warming. But at the same time, fertilizers help us to produce enough plants to satisfy the global demand for food, so we must balance the greenhouse gas emissions with the benefits that fertilizers provide to crops. Nitrogen-fixing bacteria in plant nodules can help us because they provide nitrogen to plants and decrease the need for fertilizers. One way that farmers already make use of this symbiosis is to plant legumes in their fields in rotation with other crops. The legumes naturally increase the amount of nitrogen that is available in the soil. Some researchers are trying to modify the genes of crop plants so they have their own tools to fix nitrogen, without the need for bacteria. Other researchers are trying to understand why many plants lost the ability to form symbiotic relationships with bacteria and whether it is possible to re-introduce this ability into certain crop plants. By harnessing or improving the natural abilities of crop plants to obtain their own nutrients, hopefully we can decrease the negative impacts of agriculture on the environment and so many wild species while still growing enough food to feed the human population.

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YOUNG REVIEWERS

ELLE, AGE: 11

My name is Elle, and I just turned 12 years old. I love cats, and wish I had one of my own. I love to dance, write, sing, read, and draw. I would like to become a lawyer when I am older. My favorite subjects in school have always been ELA and history. I have participated in the science fair all of my life, and I enjoy watching videos and reading articles to better understand the world around me. Fashion is a passion of mine too.

JACK, AGE: 13

My name is Jack. I am interested in coding, programming, and cybersecurity. I participate in science and math competitions like Science Olympiad and Math League. I am an avid basketball player. I love to travel and have visited 4 of the 7 continents so far.

SIRI, AGE: 12

My name is Siri. I am in the sixth grade. Some things that I enjoy doing are reading, soccer, science, computer science, cooking, and baking. I like to read a series called Warrior Cats, and novels, non-fiction, and scientific novels. I played piano for a few years, but then changed to violin. I love animals and have two dogs and six chickens, and am getting ready to adopt two kittens. I love learning more about animals and plants and have my own succulents. I am trying to learn how to propagate it is clippings.

SOPHIA, AGE: 15

My name is Sophia. I am on the pre-med pathway in high school. I compete in Science Olympiad, Quiz Bowl, and the Science Fair. I have a love for spelling. I won my school spelling bee multiple years and competed in the Scripps national spelling bee. For relaxation, I enjoy doing art projects and baking culinary treats for my friends and family. I also love to travel. One of my favorite places is Tokyo.

AUTHORS

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Fede is a doctoral candidate at Stockholm University in the lab of Katharina Pawlowski. She received her master's degree in biology at Ghent University in Belgium. She is interested in the interactions between various organisms in their natural environments, with a particular interest in microbial interactions.

She is passionate about sustainability and issues related to food production and global change. *fede.berckx@su.se

KATHARINA PAWLOWSKI

Katharina Pawlowski is a biologist whose work is focused on symbioses between plants and nitrogen-fixing soil bacteria. She is particularly interested in the question of how these symbioses evolved. Katharina studied biology and obtained her doctorate in Germany and worked in Germany, the Netherlands and Sweden. Currently, she is a professor at Stockholm University in Sweden.

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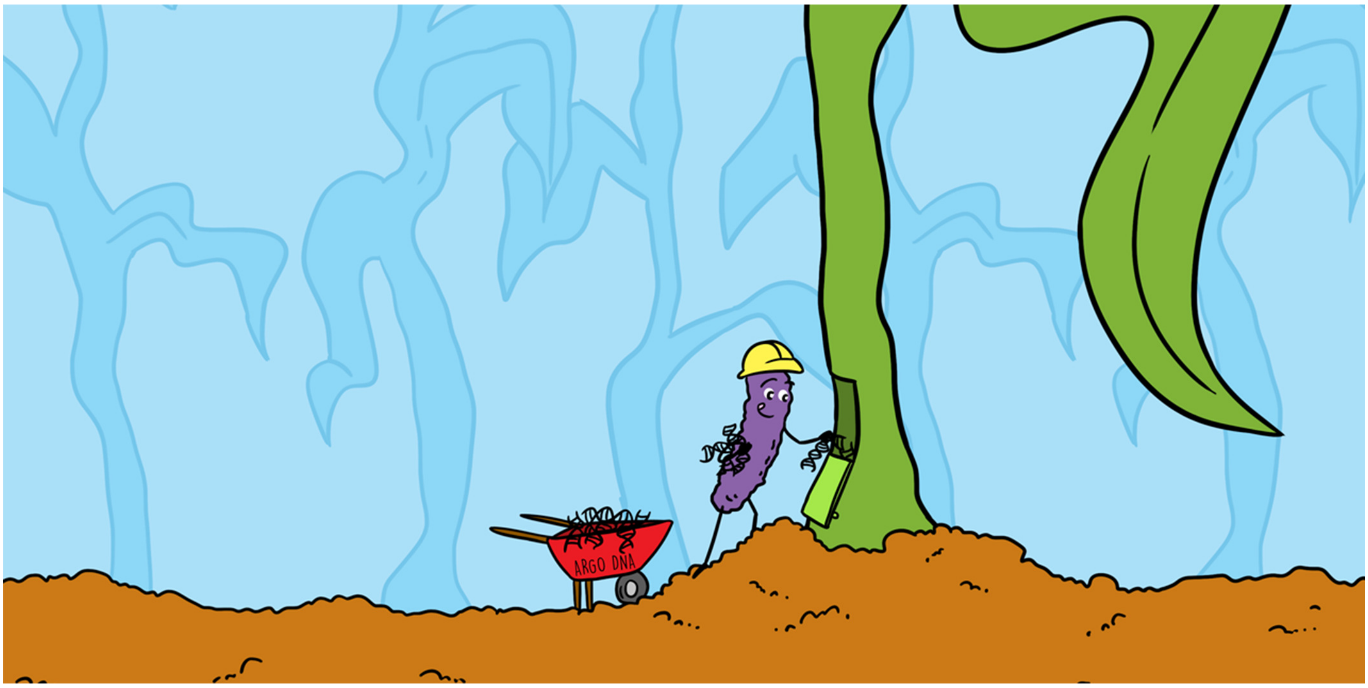

AGROBACTERIUM: SOIL MICROBE, PLANT PATHOGEN, AND NATURAL GENETIC ENGINEER

Ryan T. Weir* and Johnathan J. Dalzell*

School of Biological Sciences, Queen's University Belfast, Belfast, United Kingdom

YOUNG REVIEWERS:

MERCY SCHOOL

AGES: 10–11

Agrobacterium tumefaciens (*Agrobacterium* for short) is a single-celled microbe and natural genetic engineer. It takes part of its DNA (the blueprint instructions for life, which are found in every living thing), and inserts it into the DNA of a plant! This tricks the plant into protecting and feeding the microbe; this is great for the microbe, but not so great for the plant. The discovery that *Agrobacterium* inserts its DNA into plants shows us that genetic engineering is a natural process. Scientists can use this technique to easily introduce new DNA sequences into crop plants, which gives the crops new abilities that help them to fight off pests, grow more nutritious food, and become better at dealing with climate change! In this article, we will explore how *Agrobacterium* makes a genetically modified organism (GMO), and how this process can help us to improve crop plants and grow more food using less land and pesticides.

AGROBACTERIUM FINDS A PLANT

Agrobacterium tumefaciens (*Agrobacterium* for short) is a single-celled microbe that lives in the soil. This microbe has the ability to find a wide range of different plants by moving toward chemicals that are released from naturally occurring plant wounds. *Agrobacterium* can swim through water films in the soil to reach plants, using structures called flagella, which beat like tails. Although *Agrobacterium* does not have eyes or ears (these would be of little help in the soil anyway), it does have a number of specialized proteins that identify plant chemicals by acting like a very simple nose, which it uses to sniff out a plant. These proteins allow *Agrobacterium* to move in the right direction, toward the plant. When *Agrobacterium* realizes that it is going in the wrong direction, it flaps its flagella randomly, tumbles to point in a new direction, and swims in a straight line. This sequence of swimming and tumbling are repeated until it finds the plant.

Agrobacterium is an expert in communicating with plants, using chemicals rather than words! When the microbe contacts a plant, it releases several different chemicals, which tell the plant to make its surface "sticky." This stickiness allows *Agrobacterium* to attach to the plant and prepare to invade. At this point, the *Agrobacterium* switches on a family of *Vir* (short for "**virulence**") genes. These *Vir* genes contain the DNA instructions to make all the tools that *Agrobacterium* needs to break into the plant cell and smuggle new DNA into the plant cell's nucleus (which contains the plant's **genome**—all of its DNA instructions). A biological siege has now begun, and the stakes are huge!

VIRULENCE

The ability to infect another organism and cause disease. The word "virulence" gives the name to the *Vir* genes that help *Agrobacterium* manipulate plants.

GENOME

All the DNA instructions that an organism needs to survive and reproduce.

PLASMID

A DNA sequence that is separate from other DNA instructions in the genome. It is circular and can be copied and shared between microbes.

AGROBACTERIUM TRANSFERS DNA INTO THE PLANT

Agrobacterium has a special circular type of DNA, called a **plasmid**. The small section of DNA that *Agrobacterium* wants to transfer into the plant genome (called T-DNA, for transfer DNA), is found within the plasmid. One of the *Vir* genes that is activated when the microbe sticks to the outside of the plant makes a protein called VirD2. VirD2 functions like biological scissors, cutting the T-DNA out of the circular DNA plasmid. VirD2 then attaches to one end of the T-DNA, and drags it into the plant cell, toward the plant nucleus (like a little protein tugboat … which is also a pair of scissors). Before that can happen, *Agrobacterium* needs to break through the barrier of the plant cell wall. It does this by building a "syringe" with other virulence proteins, called VirB1 through VirB11, and VirD4. Using this protein syringe, *Agrobacterium* injects the T-DNA through the plant cell wall.

Plants have learned to protect themselves against this assault, though. When the plant discovers that *Agrobacterium* is attacking, an army of plant enzymes try to cut up the *Agrobacterium* T-DNA before it can reach the plant cell nucleus. However, *Agrobacterium* is one step ahead, having clothed the T-DNA in a protein armor made of another virulence protein, called VirE2, which prevents the plant enzymes from getting hold of the T-DNA. Once the T-DNA makes it to the plant cell nucleus, it looks for breaks in the DNA (these occur naturally) and

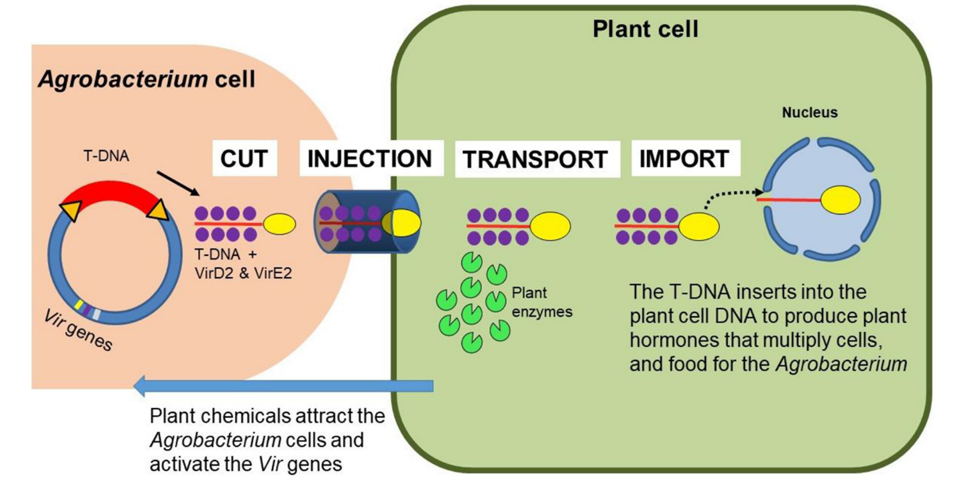

Figure 1

Agrobacterium can manipulate plant cells by inserting new DNA sequences. Chemicals from a plant wound attract *Agrobacterium* and trigger the invasion process. T-DNA is cut from the DNA plasmid in *Agrobacterium* and is injected into the plant cell. From here, the T-DNA is transported toward the plant cell nucleus, where it is imported and inserted into the plant genome (not drawn to scale; adapted from Williams and Yuan [1]).

GENETICALLY MODIFIED ORGANISM

An organism that has been beneficially modified through the addition of new DNA instructions.

CROWN GALL

The characteristic growth that can be seen on plants when they have been infected by *Agrobacterium*.

PATHOGEN

A pathogen is an organism that causes disease in another organism. *Agrobacterium* is naturally a pathogen of plants.

GENETIC ENGINEERING

The process of making known and specific changes to the DNA sequence of an organism; also called genetic modification.

inserts itself into the DNA as the plant cell repairs the DNA break. When this happens, the plant cell becomes genetically modified, as it now contains DNA instructions from another organism (the *Agrobacterium*) that will change how the plant behaves and works (Figure 1)—the plant is now a **genetically modified organism** (GMO)!

AGROBACTERIUM MANIPULATES THE PLANT

The T-DNA that *Agrobacterium* inserts into the plant genome contains instructions that will be copied into every cell that develops from this first genetically modified cell. In fact, some of the new DNA instructions stimulate the plant cell to divide and reproduce, forming large galls, which you can see as unusual growths in the plant (Figure 2). This is actually how *Agrobacterium* was first discovered—it was found to cause a plant disease called **crown gall** disease, which limits the growth and yield of crop plants. The *Agrobacterium* T-DNA triggers gall formation by changing the amount of certain plant hormones, which creates a safe environment for the microbe. On top of that, the T-DNA also codes for a recipe: instructions to make *Agrobacterium*'s favorite food. *Agrobacterium* feeds on a family of chemicals that most plants do not know how to make. When the *Agrobacterium* inserts its T-DNA instructions into the plant DNA, it is basically sharing a favorite family recipe with the plant. In summary, *Agrobacterium* invades the plant, manipulates its DNA, and inserts new instructions that tell the plant how to protect and feed it! While *Agrobacterium* benefits from this interaction, the plant does not. *Agrobacterium* is classed as a **pathogen**, because it causes disease (also known as pathology) in the plant.

AGROBACTERIUM CAN HELP US TO IMPROVE PLANTS

From what we have told you so far, you can see that **genetic engineering** is a natural process that *Agrobacterium* uses to

Figure 2

Figure 2

Agrobacterium causes crown gall disease in many plants. This picture shows the galled tissue of a mango tree, caused by infection with *Agrobacterium*. When the microbe infects a plant and inserts its DNA, this forces the plant to produce hormones that multiply the cells and gives the plant cells everything they need to feed the pathogen. *Agrobacterium* lives inside the galls.

manipulate plants. In fact, there is good evidence that many different plants have kept parts of the T-DNA after *Agrobacterium* infection [2]. Scientists can alter *Agrobacterium* T-DNA to remove all of the instructions that harm the plant, and replace them with new DNA instructions that will help the plant! Many successful plants have resulted from this process: crops that are resistant to insect pests [3]; papaya that is resistant to a devastating virus that would have destroyed farms all over Hawaii [4]; golden rice that is fortified with a chemical that we need to make vitamin A, which could prevent millions of children from going blind [5]; non-browning, healthier potatoes that reduce food waste [6]; and many others (Figure 3).

Despite the many benefits of using *Agrobacterium* to improve crop plants, some groups seek to prevent the use of genetic engineering, and even try to misinform the public about the approach. One of the most common misunderstandings about genetic engineering is the belief that changing the DNA of an organism is unnatural and therefore wrong. However, *Agrobacterium* has been modifying the DNA of plants long before humans learned how to do it. This shows us that changing DNA sequences is a natural process and part of the world around us. By using *Agrobacterium* to modify plant DNA, we are harnessing a natural process to develop crop plants that need fewer pesticides, are more nutritious, and that yield more food using less land. Using less land is a really important consideration because, if we want to avoid the destruction of natural ecosystems, we need to make sure that our farms are as productive as possible. Genetically engineered crop plants can definitely help us to grow more food from less land, meaning that more ecosystems will be protected. Also, long-term studies confirm that genetically modified crops are safe to eat [7]. Despite what some groups opposed to GMOs say, genetically modified crops are no more dangerous than any other crop we eat!

Figure 3

Figure 3

A genetically engineered tomato plant being grown in the laboratory. This picture shows a young tomato plant that has been genetically engineered. *Agrobacterium* was used to insert new DNA into small pieces of tomato plant leaf tissue. The modified cells were then encouraged to grow into a plant by changing the amounts of certain hormones available in the specialized gel that we grow the plant cells in. Roots have started to grow from this tomato plant, which will shortly be taken from this protective container and grown in soil.

CONCLUSIONS

Agrobacterium is a soil microbe, a plant pathogen, and a genetic engineer. Through understanding the biology of natural genetic modification, we can better understand the process used to develop genetically modified plants, or other kinds of GMOs. *Agrobacterium* allows us to make beneficial changes to the DNA of plants, which ultimately means we can grow more nutritious food using less land, which protects our environment. If you want to learn more about the process of making a GMO, you can watch the fantastic video by Science IRL at https://www.youtube.com/watch?v=-b_Un-lGSWo.

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CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

MERCY SCHOOL, AGES: 10–11
We are an inventor class! We love to build and create in order to find solutions to real world problems. Our students come from all over the area to attend school.

AUTHORS

RYAN T. WEIR
Ryan Weir is a final year undergraduate student at Queen's University Belfast, completing a degree in microbiology. He took a year out from his studies to work with Johnathan, contributing to several research projects and scientific papers. He has experience using *Agrobacterium* to genetically engineer plants and has been developing ways to stop *Agrobacterium* from finding plants in the soil. Ryan is passionate about how genetic engineering can give crop plants the upper hand in the arms races between plants and pathogens, and he is hoping to secure a Ph.D. studentship next year, to study this further. *rweir12@qub.ac.uk

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Johnathan Dalzell is a senior lecturer in plant–parasite interactions and biochemistry programme director at Queen's University Belfast. He is interested in studying how plants interact with parasites and microbes. His research group uses *Agrobacterium* to develop genetically engineered plants that help investigate these interactions. He is also an advocate for genetically modified/engineered crops as part of the solution to global food insecurity, and he hopes to persuade everyone that this is a safe and beneficial technique. *j.dalzell@qub.ac.uk

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ONE TYPE OF SOIL BACTERIA PERFORMS TWO IMPORTANT JOBS TO HELP US PRODUCE HEALTHY FOOD

Lauren S. McKee *

Division of Glycoscience, Department of Chemistry, Royal Institute of Technology, Stockholm, Sweden

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ROHIT
AGE: 9


SWATI
AGE: 13


TANISHKAA
AGE: 8


TARUN
AGE: 13


THUVISHA
AGE: 13

Although they are small, bacteria perform some very important jobs that help us make healthy food. Food production starts with growing plants out in the field. Here, bacteria that live in the soil help plants to grow strong and tall, and they can even protect plants from some diseases. This way, the bacteria are helping the plants to stay healthy and they are helping the farmer to grow lots of food without using too much pesticide. After harvest, bacteria do even more work to make food that is tasty and good for your health, by making fermented superfoods like kimchi, kombucha, and natto. An example of a bacterium that can do both of these jobs is called *Bacillus subtilis natto*, and we have investigated what makes it such a useful and important species. We found that the *natto* bacterium produces important enzymes that can attack fungi that would cause plant disease.

WHAT IS "BIOCONTROL"?

The tiny bacteria that live in the soil can do a huge number of important jobs that help us to make nutritious and healthy food in a way that does not damage the environment. Some soil bacteria can protect crops like the soybean plant from diseases by fighting off the fungi that might cause an infection. We use the term **biocontrol** when we are using a living organism to protect our food. You can think of examples of biocontrol like using cats to keep rats away from food, or using ladybirds to eat aphids that can damage crops. Biocontrol helps us limit the growth of harmful organisms without using chemical pesticides. Pesticides can have lots of harmful effects on the environment, such as killing off the insects that pollinate plants and killing the microbes that keep plants healthy.

BIOCONTROL

The use of living organisms to control plant diseases, instead of using chemical pesticides. An example is using bacteria to control fungi that can damage plants.

In our work, we are studying how bacteria can control the growth of fungi that would cause disease in crop plants. Normally, farmers use chemical pesticides to control these fungi, but many fungi are becoming resistant to the pesticides and are no longer affected by them, so they are getting harder to control. This means that farmers use more and more pesticide, causing more ecological damage, and making resistance spread even more! Using soil bacteria for biocontrol of fungi means that farmers can use less chemical pesticide on the fields where crop plants are growing. Using less pesticide is good for the soil, the air, the water, and for the insects that pollinate our plants, so these bacteria are protecting the whole environment, as well as helping us to grow food.

THE *NATTO* BACTERIUM IS A SUPER-FERMENTER!

After we harvest food from the field, sometimes it goes through processes in a factory to make it into something that is ready to eat, and soil bacteria are often used in these processes. In fact, many of the tools that bacteria use in food production are very similar to the tools other bacterial species use in the soil to control fungi. One bacterial species—*Bacillus subtilis natto*—can do both of these things!

The *natto* bacterium is part of the *Bacillus* group of species that are found in soils all around the world. This bacterium has been used for a very long time in Japan to make a food that is also called natto. This food is made when the *natto* bacterium turns soybeans into a sticky—and stinky—food substance, through a process called **fermentation**. Natto is very popular in Japan, where it is a common snack and breakfast food, and it is eaten with mustard and soy sauce. In fact, natto has been produced as food in Japan using *Bacillus subtilis natto* fermentation since the Taisho period, just after the First World War. The *natto* bacterium is also an icon of popular culture in Japan—one of the characters in the manga comic book and anime series called *Moyasimon: Tales of Agriculture* is a cute bacterium called

FERMENTATION

A process by which microbes break down certain foods using enzymes, to turn the food into a different kind of substance. Common fermentation products include beer and yogurt.

Natto. His job on the farm is to ferment soybeans to make the natto for the human characters to eat.

It is also becoming quite fashionable to eat natto in the rest of the world, because now we know that fermented foods are very good for human health [1]. Many fermented foods and drinks are now available in shops, including kombucha tea, kimchi pickles, tempeh, and yogurt, and all of these foods can help our digestion. Natto is high in fiber and contains a lot of vitamin K, which we need to grow strong and healthy bones. By fermenting soybeans into natto, our *natto* bacteria are making a tasty snack that is great for your tummy and your whole body!

HOW DOES *NATTO* CONTROL THE FUNGI THAT WOULD CAUSE PLANT DISEASES?

Thanks to a recent project I was involved in, we now also know that our *natto* bacteria are probably making a big contribution to preventing fungal disease in some plants. The *natto* bacterium shows many of the important behaviors that we see in other plant-protecting bacteria. In particular, the *natto* bacterium can very effectively attack the cell wall that protects fungal cells [2]. Fungi, like the ones that can cause disease in soybean plants, have long, thin cells that grow quickly by extending one edge of the cell forwards in one direction, forming thin strands called filaments. These cells have a strong outer layer—called the fungal cell wall—that is partly made up of a material called **chitin** (Figure 1). Chitin is a **polysaccharide**, which is a very long molecule made up of lots of smaller sugar molecules joined together, and it is rigid and very strong. It acts like a suit of armor, protecting the fungal cell from the environment and from attack by other microbes. It also makes the fungal cell strong enough to break into plant tissue, which is how plant infection often begins.

Enzymes that can break down chitin into small pieces are therefore a very powerful weapon against fungi, because they damage the suit of armor, and let the fungal cell be exposed and killed. Plant-protecting bacteria in the soil produce enzymes called **chitinases** that can protect plants from infection with fungi. In fact, the chitinases that soil bacteria produce are one of the most important aspects of biocontrol [3]. Bacterial chitinases are also really important for the health and fertility of the whole soil ecosystem, because they break down dead fungal cells into small molecules that the bacteria can feed on. This is vital for the recycling of carbon and other elements [4], which is, after all, what drives life on Earth!

In our recent experiments, we showed that the soil bacterium *Bacillus subtilis natto* can produce enzymes that break down the chitin in fungal cell walls (Figure 2). In our lab tests, these enzymes could convert chitin polysaccharides into very small molecules called

CHITIN

A very strong polysaccharide found in the walls of fungal cells, which protects the cells from the environment and helps fungi to infect plant cells.

POLYSACCHARIDE

A long chain of sugar molecules connected by strong chemical bonds. For example, cellulose is made of long chains of the sugar glucose. Chitin is a polysaccharide made by fungi.

ENZYME

A type of protein that can catalyze a reaction, such as making or breaking the chemical bonds in carbohydrates. Enzymes that break down carbohydrates are very specific, acting only on chitin, cellulose, or some other polysaccharide.

CHITINASE

An enzyme that breaks chitin polysaccharide down into smaller pieces, called chitin-oligosaccharides.

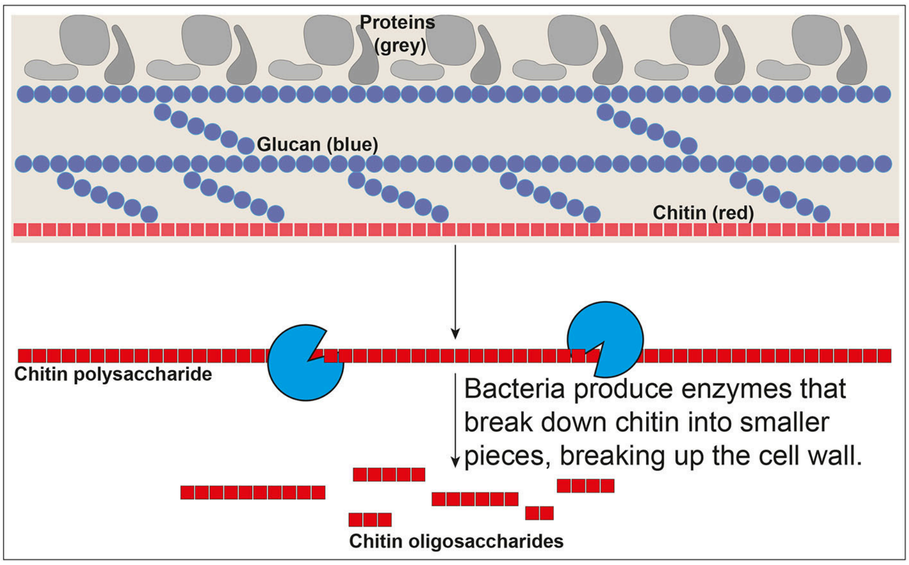

Figure 1

Figure 1

The fungal cell wall is the strong layer on the outside of a fungal cell that protects the cells from being killed. If the cell wall is broken, the fungus will die, and it will not be able to infect plants. The fungal cell wall is made of a strong polysaccharide (a long carbohydrate chain) called chitin, as well as protein and another polysaccharide called glucan. Bacteria in the soil produce enzymes that can attack chitin and break it down into smaller chunks called oligosaccharides (shorter carbohydrate chains).

OLIGOSACCHARIDE

A short chain of sugar molecules connected by strong chemical bonds. Oligosaccharides are formed when an enzyme breaks down a polysaccharide into smaller pieces.

oligosaccharides, and they could also break up big clumps of fungal cell walls when these clumps were incubated with the enzymes.

However, we were surprised to see that the *natto* bacterium does not like to feed on chitin itself, and it will not produce chitinase enzymes if chitin is the only material it can find. Instead, the *natto* bacterium needs to find a complete fungal cell wall before it will produce chitinase enzymes.

WHAT COMES NEXT IN OUR RESEARCH?

Our results tell us that the *natto* bacterium can somehow sense when there is fungus in the local environment, and start producing powerful chitinase enzymes to break down the strongest parts of the fungal cell wall. Our results also tell us that we might be able to increase biocontrol behavior in *Bacillus subtilis natto* by mixing cell walls from dead fungus into the soil. Other researchers have shown that, for other bacteria, adding only chitin to the soil can cause an increase in chitinase production [5]. But for our *natto* bacterium, the story is more complicated.

In our tests, *Bacillus subtilis natto* also produced enzymes that break down protein, which is another important component in the fungal cell wall (Figure 1). I was excited by this, because these are the same enzymes that are used in the production of natto as food! The protein-degrading enzymes have been known for a long time, but now we have seen a whole new use for them.

We are now doing some extra experiments to test whether our methods for increasing chitinase production can increase the

Figure 2

We grew cultures of *Bacillus subtilis natto* and treated them by adding either chitin, whole dead fungal cells, or fungal cell walls from dead fungus cells. Then we measured how much chitinase activity was produced by the bacterium in each treatment condition. You can clearly see that treating with chitin did not cause more chitinase activity to be produced, but treatment with fungal cells or fungal cell wall had a big impact.

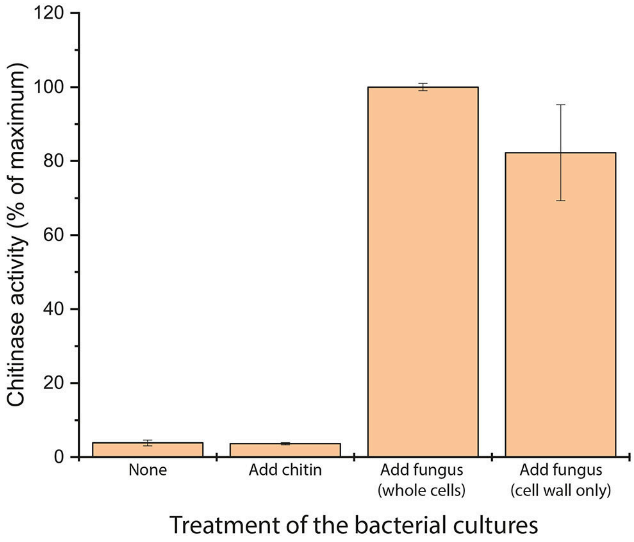

Figure 2

biocontrol ability of *Bacillus subtilis natto* and make it more effective at killing off the fungi that might harm plants. So far it looks quite promising, and we can see that several of the fungi we have tested are strongly affected by our *natto* bacterium. This means that Natto character from Moyasimon anime is actually performing two fantastic jobs on the farm and helping us in two different ways to have a good supply of healthy food!

ORIGINAL SOURCE ARTICLE

Schönbichler, A., Díaz-Moreno, S. M., Srivastava, V., and McKee, L. S. 2020. Exploring the potential for fungal antagonism and cell wall attack by *Bacillus subtilis natto*. *Front. Microbiol.* 11:521. doi: 10.3389/fmicb.2020.00521

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YOUNG REVIEWERS

KAVIN, AGE: 9
There are important and interesting things in science.

ROHIT, AGE: 9
My hobby is drawing and doing science projects. I want to do animated cartoons. My favorite sport is Cricket. I know the capitals and flags of all the countries. I also know some of the major inventions and their inventor's names.

SWATI, AGE: 13
The article was very informative. It was very interesting to learn about the *natto* bacterium. The words in the text were easy to understand. My time was spent useful during this quarantine.

TANISHKAA, AGE: 8

I have been interested in learning science since my childhood. I would like to explore every part of science. My favorite part in science is learning about the human body. I like science very much because it is very very interesting. I want science to be with me as a part of my whole life.

TARUN, AGE: 13

I am a state player in badminton. I master all the techniques in badminton. I also love to play chess. I always win when I play with my friend.

THUVISHA, AGE: 13

I like Science because it is something new and makes you discover a lot of things that are related to your daily life. It is always interesting. Without science there is nothing in the world.

AUTHOR

LAUREN S. MCKEE

Lauren grew up in the North East of England and is currently living and working in Stockholm, Sweden. She studies bacterial biochemistry and the ways that different microbial species can interact with each other and their environment. She works in the lab and teaches students about microorganisms and how they can be used in industry. She is most interested in the enzymes that bacteria produce in the soil—these are very important in natural ecosystems and are also very valuable tools for the biotechnology industry. *mckee@kth.se

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