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Vincent: This is TWIM, This Week in Microbiology, episode 169, recorded January 18th 2018. Hi everybody, I am Vincent Racaniello and you are listening to the podcast that explores unseen life on Earth. Joining me today from Small Things Considered, Elio Schaechter.

Elio: Hello there, nice to be with you.

Vincent: Welcome back, happy new year.

Elio: Thank you, same to you.

Vincent: You’ve been away a while but we are happy that you are back.

Elio: Thank you, it’s nice to be back.

Vincent: Did you miss us?

Elio: Oh boy, did I miss you. My first thought. (laughs)

Vincent: Also joining us from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello, it is a bright and sunny day here in the north country.

Vincent: What is your temperature, how many degrees below zero?

Michele: You know, we had a little bit of a warming trend. I think it was going to be in the mid-twenties today and currently it is 29 degrees Fahrenheit.

Vincent: Also joining us from Charleston, South Carolina, Michael Schmidt.

Michael: Where it is a beautiful sunny 43 degrees with a chance of snow. Again. Again.

Vincent: Well, that’s winter for you.

Michael: That’s winter for us.
Vincent: I have a person in my lab that is going to Auburn next week and she said that they closed the university today because it was cold.

Michele: (laughs)

Michael: I can understand that.

Vincent: Can you imagine? (laughs) No snow, just like 34 degrees, not even freezing.

Michael: Yeah, they close it for the cold.

Vincent: Maybe they don’t have heat in the rooms, I don’t know. Maybe.

Michele: What’s it like in New York City?

Vincent: This morning it was below freezing. I do Celsius, so it was like minus seven Celsius. Currently it is minus one. It is sunny, it’s just really cold, and we had a little more snow the other day. It has been a tough winter because we had this arctic air coming through. Oh well, that’s weather.

Michele: Speaking of arctic and antarctic air.

Vincent: We are gonna talk about antarctic air today. That’s a good one, Michele, very good.

Michael: In the winter, in the winter.

Vincent: The winter, that’s right. After we do our papers for you we are going to give away a book. I forgot what it even is. Disinfection, right. (laughs)


Vincent: A brand new book. So at the end of our discussion we will give that away. We had 7 entrants, so we will give one of those lucky seven an amazing book on disinfection. First we have a snippet and a paper for you and Michael is back with copper.

Michael: I’m back with copper. The snippet will actually reacquaint us with the concept of nutritional immunity that we covered about a year ago in TWIM 141 with Jennifer Bomberger who talked with us about virus deregulation of traditional immunity. In today’s paper it is copper import in E. coli by the yersiniabactin metallophore system, and it was by Koh, Robinson, Bandara, Rogers, and Henderson. And it was published in September of 2017 in Nature Chemical Biology. And today’s story is a riff on this broad concept of nutritional immunity which is a process by which the host organism sequesters trace minerals in an effort to limit pathogenicity during an infection. So circulating concentrations of minerals such as iron and zinc decline rapidly and dramatically during inflammation associated with infection.

Elio: Isn’t iron the classic, I mean--

Michael: Iron is the classic one involved in it, but I think in terms of how the host sequesters metals, I think this story with copper will begin to show us how important metals are to the microbial cell in terms of being able to do many important things. And they effectively function as coordination molecules in transport of metals into the cell. But today’s story is a bit different where we learn about the role that this copper, which is an essential
micro trace element, and its integral role as co-factor in the function of many of these redox enzymes that the microbes have--

Elio: At high concentrations or at medium concentrations it is highly toxic.

Michael: That’s right. And that is the cool part of this paper. It is this tour de force between toxic and what it needs, and it is really a neat story, they lay it out beautifully. So I am going to try to take us through this fairly quickly so I apologize since it is only a snippet but I think you will get the gist of things as we go through. First the nutrient part. In E. coli there is one copper based enzyme that you might imagine would be important as a virulence factor, and that is the amine oxidase and it goes by the abbreviation TYNA, which is a primary amine oxidase that catalyzes the breakdown of aromatic over aliphatic amines and enable it to deaminate molecules such as phenethylamine to phenethylaldehyde and the other waste products, ammonia and peroxide. Copper is required for that enzyme’s activity.

So the advantage that copper provides is that it serves as a necessary metal co-factor making those enzymes work, which in turn enables the cell to degrade amines like amino acids which is in essence how E. coli can utilize all the protein that is surrounding it in this infection process and in this case the authors grow their initial cultures in lysogeny broth, or luria broth depending on which one you attribute it to. Then they switch out to a minimal medium in which they can begin to dissect it.

The toxin part of the story is of course what got me interested in this paper and I will get to the toxin issue in a few minutes, but in the end, the authors are very much interested in this because urinary tract infections are the second most common bacterial infection in humans, counting for 8.6 million physician visits each year and over a million hospitalizations. An acute uncomplicated cystitis or this inflammation of the bladder caused by E. coli is responsible for one of the most common indications for prescribing antimicrobials and what I don’t think most people appreciate is that in otherwise healthy women, before they reach their 32nd birthday at least half of the women will have experienced at least one urinary tract infection. And so that is just phenomenal, there is a great discrimination between E. coli UTIs in women and until the male-female ratio gets into this age 60 or so-

Elio: It goes with the anatomy, doesn’t it?

Michael: It goes with the anatomy. And so UTIs, and that is where this group of workers are in at Washington University in St. Louis, they are all associated with looking at urinary tract infection. So we now infected hosts control the chemical composition of different anatomical environments to limit the microbial growth and prominent among these changes is withholding metal ions. So you can take a look at TWIM 141 and learn about the canonical version with iron restriction and the way the microbes get around this is with the production of siderophores, things that can scavenge metals. If you will, they ying and the yang between the host and the pathogen. In the uropathogenic E. coli, the prominent virulence factor associated with this urinary tract infection is yersiniabactin. And as its name implies, yersiniabactin, or they abbreviate it YBT, along with enterobactin which comes from E. coli and the other enterics, collectively harvest the iron being stingily withheld by the host.

Michele: You can think of them as super strong magnets.

Michael: That’s it. It’s really magnets.

Michele: They pull the iron to the bacterial cell.
Michael: And they need it for everything. They need it to make DNA, they need it to make all sorts of things. And when an uncomplicated genome associated with these organisms that cause UTIs, and they compare the rectal E. coli to the urinary tract E. coli, they learn that the urinary strain is more likely to carry this thirty kilobase yersinia high pathogenicity island, and we have not talked much on TWIM about pathogenicity islands but they are really accessory genes that the microbes pick up and they carry because it confers a selective advantage to them in this particular niche. The YPT production, or this yersiniabactin, its production is coordinated by this sophisticated multi operon yersinia high pathogenicity island that these uropathogenic E. coli have acquired either through a phage or through conjugation with another organism in which it comes along.

Elio: By the way, this brings up my favorite subject which is horizontal gene transfer because the very existence of pathogenicity islands indicates a very important function in virulence but also are transmitted from bacterium to bacterium horizontally, which I think is one of the great findings of the end of the twentieth century.

Michele: And their prevalence in the urinary tract E. coli predicts then that there are fitness advantages coded on this particular island for the urinary tract.

Michael: The bottom line to this story is that it is this yersiniabactin that confers one of these advantages because it not only enables it to scrounge iron from the stingy host but it also enables it to get copper which is important in all sorts of redox balanced enzymes in the cell in addition to the amine enzyme that I have already introduced you to. So earlier--

Elio: Another question. Copper is both essential and toxic.

Michael: At the same time.

Elio: How does the cell know to regulate the amount, because too much is bad, too little is no good.

Michael: They don’t have that whole story yet, and so I am going to jump to the toxic part. So recall that when copper ions get inside the cell, the copper ion participates as a catalytic metal in a component of the Fenton reaction where copper +1 is oxidized to copper +2.

Elio: What is the name of the reaction again?

Michael: The Fenton, you probably remember it using iron. And so the Fenton reaction is this extreme oxidative force and what it does is the metals, be it iron 2 going to iron 3 or copper 1 going to copper 2, that oxidation of the metal converts the endogenously produced hydrogen peroxide and all metabolism produces hydrogen peroxide as a consequence of dumping the waste electrons as quickly as they can and hydrogen peroxide is a common waste product of metabolism. What the Fenton reaction does is it converts hydrogen peroxide, H2O2, into one part singlet hydroxy ion and one part hydroxyl. They in turn wreak havoc in the cell by destroying nucleic acid, proteins, membrane proteins, the membrane, and they just bleach everything. It’s just havoc. And so that is effectively how when copper ions get in the cell they do their dirty deed.

And the story that these authors take us on is they show us first and foremost that the yersiniabactin binds the metal and they first give us some background. A recent study by one of the authors that was published in May of 2016 in Infection and Immunity, so that is a free download because it is a year later is that cells lacking this yersiniabactin in an experimental urinary tract model had a marked virulence defect. That is, those cells that didn’t have this pathogenicity island with this particular molecule were not able to compete. They weren’t as virulent as the wild type. And my suspicion is that even though when they were put in this model but they grew
them in minimal media and they were able to restore this trait, if you will, of the cell being able to grow vigorously. My suspicion is that there was enough iron being provided through the minimal medium because you always have plenty of iron in minimal medium, and copper sometimes is a contaminant associated with iron compounds because they sort of go hand in glove in terms of where you find copper and where you find iron is that it was probably getting enough iron and iron is needed in higher concentrations.

So in this study they use the same strain, UTI 89. So in addition to having the yersiniabactin pathogenicity island, urinary tract 89 the strain also has enterobactin and salmonella siderophores, salmochelin. These strains are typically grown in lysogeny broth, they are happy and it all goes together. The two last pieces of previous data that you need to know before we walk through the paper is that previous data has shown that there are 15-fold more copper 2 YBT complexes than iron 3 YBT complex in the infection models. So it seems that this YBT molecule--

Elio: Would you explain that? It’s a little bit murky.

Michael: My suspicion is that the affinity of the yersiniabactin is greater for copper than it is for iron, or the two strains enterobactin and the two other siderophores that the strain has, enterobactin and salmochelin, have a higher affinity for iron than they do the copper. I didn’t have time to look up all the equilibrium kinetics of those three siderophores to know which had a greater affinity for what. So there is a lot going on and since this was only a snippet I don’t want to go down that particular rabbit hole, because I am already giving you a lot of background that you need to appreciate the elegance of their study.

And the second fact that you have to remember is that for many years it was thought that E. coli lacked a copper import system, which prompted the authors to pose the question of whether or not these uropathogenic E. coli could even import copper with this yersiniabactin complex, and was it then the nutritional source of copper during infections. Now the jump that these authors made from the time of their I&I paper is they began to use quantitative mass spectroscopy in order to do some of the experiments.

So they can do everything quantitatively with the mass spec and follow things along. And so using quantitative mass spec, they found that this uropathogen can quantitatively convert copper 2 in this yersiniabactin complex during growth in low copper concentrations. Then they cleverly used radioactive copper 64 radio labeling and they found that the FYUA YPTPQ import system, so you have the outer membrane protein which is FYUA and then the inner membrane system YPTPQ effectively get together and collectively they bring the copper in and then release the copper slowly into the cytoplasm.

Elio: Maybe that is the answer of how copper can be good and bad is if you bring it in and release it slowly you have a mechanism for ensuring it is the right low amount.

Michael: Or they are segregating it and they don’t have it completely worked out, there are other membrane proteins involved, but they are beginning to show us glimpses of things. Their first set of experiments confers that this copper yersiniabactin complex forms during low copper concentrations, and it is a really cool experiment in that what they have done is they take these urinary tract 89 cells that have been grown in minimal broth and they ask the question, how much copper can you buy?

And so they show us a nice graph showing us the total YPT and then how much copper is bound to it. And so the cell is making much more YPT than it actually needs and you could make the argument that really copper is just a secondary actor and it is really making all this YPT for maybe iron, but the next aspect of their experiment is when they up the concentration of copper in the medium by supplementing it with copper sulfate. They then see a significant increase in the amount of YPT made and more importantly every molecule of copper they add
to that medium is sucked up by this complex. It is stoichiometric. You put five micromolars in, you get five micromolars bound to this complex! It is phenomenal.

And then they ask the question, is it toxic? And it had no effect whatsoever on the viability, none whatsoever. The viable counts look equivalent. You can’t even see the error bar. So that was a really cool experiment that they did to show that this yersiniabactin may really want the copper and it makes enough of it to actually protect that cell. And what this showed me is that the cell does not like copper at all. It wants to keep it in this straight jacket molecule locked up tight and it was just remarkable. The third piece of data that they showed out of that--

Michele: So it’s kind of like a buffer, acting like a buffer for the cell?

Michael: Exactly. Exactly. So there is a lot of cool concepts that they introduce the reader to and you can begin to see the remarkable aspects of the mechanism of how the cell protects itself. Remember, this is all related back to a pathogenic model. So this then sets up the hypothesis that the pathogenic bacteria may be able to benefit from being able to access copper from the copper 2 YPT complex to support its nutritional demands during infection. One of the things pathogens have to constantly defend themselves against is all these redox insults that are being manifested by white blood cells. They are getting superoxide thrown at them, they are getting all of these things, and all of them are metal dependent enzymes.

So we now transition to their second set of experiments that show us that this uropathogenic E. coli dissociates the copper 2 YPT complex and then it can regenerate the YPT without any penalty. So normally, some cells bring metals in and they destroy the carrier in order to get the metal off of the carrier. What that experiment shows us is that doesn’t happen. They go through nice detail using another K12 typical lab strain and the experiment is that they take two strains, they grow them to a relatively high density, 0.8 optical density units, and I sort of object when people just write an OD so I put into the show notes a handy dandy cheat sheet of converting OD 0.8 to CFUs/mL, so that is about 6.4 x 10^8 and one of the molecular biology companies has out there a handy dandy conversion to figure out what OD in E. coli mean in terms of cells per mL.

Michele: Depending on the growth media, right?

Michael: Right. And they are using the standard lysogeny broth. So it is a kosher way of looking at them. So what they do is they grow them in to relatively high density. They resuspend them into a minimal medium and they incubate them for two hours at which time they ask the question of whether or not E. coli expressing this pathogenicity island operon are capable of dissociating copper YPT and iron YPT complexes. And the answer was yes! They did this cleverly with stable isotope C13 and they made the complexes and since they were using a mass spec, they could tell if it was getting in and whether the metal was free at the end.

They then take us to the bread and butter experiment where they address whether the copper 2 complex and dissociation required the outer membrane components recall is FYUA and then the inner membrane compartments are YPTPQ companions. Again they use their LCMSMS to measure the concentration of the YPT complexes and they did a clever thing where they overproduced the membrane component the two independent membrane components and they had mutant cells in which they made the outer membrane one and the inner membrane one and you could tell where things were going.

And they tell us that in order to measure YPT in E. coli they had to do this ectopically where they express these outer membrane proteins FRUA and then the inner membrane one YPTPQ and the two proteins were expressed and this mutant UTI 89 delta YPTA which is then transcriptionally silent. So they are doing a lot of sexy genetics and in this mutant they exogenously supply the YPT complex with copper, the YPT complex with iron, or the YPT complex with gallium, and gallium is very similar in size and shape and function to iron but you
cannot reduce gallium at physiological pH, the pH isn’t right. So gallium if it is bound to it will never come off. I love the way they organized their figure, it was just easy to follow along and they tell us that in the mutant exogenously supplied with YPT complex with copper, iron, or gallium it remained extracellular and never got in. So they needed either the membrane piece or both membrane pieces in order to get in. In their next mutant, so you’re just going in their figure. The first one is the mutant with everything taken away and the next one is you are adding the outer membrane one and that will allow the cell to grab the YPT complex with whatever metal it has and transfer it from the outer membrane to the periplasm.

So when it goes from the outer membrane to the periplasm, it is inside, it is effectively sequestered. So they can precipitate it down and find it as opposed to remaining in the medium. And it just literally lines up and then finally the third mutant coexpressing A and YPTPQ, the copper was taken from the medium and cells while increasing the metal that was free and then you got the free YPT back where the YPT returned back into the environment, showing us that the cell was recycling the siderophore. So it just was a really, and to a redox lover like me, to get reacquainted with how siderophores are able to carry out their task, the gallium experiment was sort of like the icing or whipped cream on the cake.

Michele: That was a great reagent to have.

Michael: Oh, it was just fantastic. And overall without going into all the gory details of the remaining experiments where they effectively demonstrate what you expect, the copper that is transported in is indeed the nutritional source of copper and that nutritional source is this copper yersiniabactin complex, and they finish things off with a neat model showing us that this UPC, this uropathogenic E. coli can use this YPT system to acquire the copper to support the copper dependent enzyme activity. It is a really cool model showing the copper binding to the YPT, that then gets stuck to the cell and is only brought in when the cell effectively has the right set of circumstances, and it is a tightly regulated operon based on the concentration of copper so it doesn’t get too high, and they still have a little black box around the YPTPQ reduction and how it makes certain going back to what Elio said, how do we control how much copper is actually brought into that cell?

And I guess that is where we say stay tuned and hope they figure that out because that of course will give us new drug targets. That is where you back to how do you deal with this in a urinary tract infection where you may not necessarily want to use antibiotics to treat everything. You may want to effectively lie to the cell and steal its copper or control its iron akin to what the host does with nutritional immunity. So the snippet is one worth reading, it is real easy to get through, and--

Vincent: Michael, could you control copper locally like that? You don’t want to take all your copper away to treat a urinary tract infection.

Michael: No.

Vincent: Could you limit it just in the bladder?

Michael: In fact, funny you should ask, there are studies being done presently as I was reading around and was how I stumbled into it. There are copper nanoparticles out there now doing--

Vincent: (laughs)

Michael: Doing effectively, they are looking at copper nanoparticles to do toxicity and at the same time they are looking at copper sinks to effectively suck up the copper. So I think you could figure out how you could chelate it to something, because the bladder is amenable to depositing something in to steal the metals away to starve the cell.
Vincent: You could imagine putting something in and inflating it so it stays in there and then you take it out when it is done or maybe it dissolves or something like that.

Michele: So you can naturally pass it.

Vincent: Maybe, yeah.

Michael: Yeah.

Vincent: It just has to stay long enough, obviously, to work. That’s interesting. Neat.

Michele: Lovely use of genetics to take it step by step through the mechanism.

Vincent: Yeah, I liked that. I like when they do that.

Michael: Michele asked for a mechanism paper, so I went hunting.

Vincent: When Michele says jump, Michael says how high.

Michele: (laughs)

Michael: She’s our president-elect!

Vincent: That’s right.

Michele: Oh, please.

Vincent: Are you going to be president this summer, is that when you get inaugurated?

Michele: July 1 would be the normal, yes.

Vincent: Nice, very good. Alright, thank you, Michael.

Michael: Thank you.

Vincent: We have another paper which will be in tune with the weather here.

Michael: The weather across the country.

Vincent: Across the country, last week on one day it was below zero in every state, including Hawaii, I guess up on the mountain there it was also freezing. So this paper will play into that coldness. It is a letter published in Nature called “Atmospheric trace gases support primary production in Antarctic desert surface soil.” So you think of Antarctica, you probably think snow and ice but it is not all snow and ice there. It happens to be the coldest and driest climate on Earth, but there is a terrestrial ecosystem, some parts have large areas of uncovered rock. There is very limited resources, limited carbon, nitrogen, water and there is lots of UV, in fact that is one of the places on Earth where the UV is crazy high because there is not a lot of coverage for it. And of course there are freeze-thaw cycles, it freezes and thaws and freezes and thaws. No trees, no shrubs in these areas that are desert, but there are plants, there are nematodes there are insects and snails, mites, spiders, birds, other things. And there are bacteria and fungi.
Michele: I think tardigrades, too. Am I saying it right?

Vincent: Tardigrades.

Michael: They gotta be there, they’re too cool not to be.

Michele: They mention them specifically in this paper, I remember that.

Vincent: So this is all about the microbial communities in this terrestrial ecosystem and people have previously studied the desert soils. They are rich microbial communities in these soils but many of the microbes are dormant and whatever energy they can make they use for maintenance, to stay alive not to grow. They are not worried about dividing, they are worried about staying alive. The real question is where do they get the energy for this? There is not a lot of carbon in the soil, not a lot of primary producers, so these ecosystems down there in Antarctica have a very low abundance of cyanobacteria which would be used to make photosynthesis to make materials that other animals could use and other microbes, but they are not there. So here they set out to find how these ecosystems, how these microbial communities are supported, and they use the combination of metagenomics doing sequencing of soil samples and they do some biochemistry. They do some biochemical experiments.

Michele: It was a great combination of those two approaches.

Vincent: It is nice. The metagenomics is great but when they then take it a step further as you will see it is very cool. In this paper they study soil samples from a few locations which as far as I can tell seem to be on the coast of Antarctica. They have some pictures in the supplementary data. By the way, if you want to get there, you can’t fly there, you have to take a boat from South America and it takes a while. Dickson Despommier’s wife just went on a vacation and you fly to South America and you take a boat and it takes a couple of days to get to Antarctica. If you are a tourist you stay on the boat, you can’t traipse around (laughs)

Michele: Hey Vincent, did you already say who did this work and where they are?

Vincent: I didn’t, I forgot to say that. It is a wonderful consortium from the Australian Center for Astrobiology, from the Monash University, University of Queensland, Wairakei Research Center and the Australian Antarctic Division of the Department of Sustainability, Environment, Water and Population and Communities. The first author is Mukan Ji, Chris Greening, and the last author is Belinda C. Ferrari. I got so excited about the paper I forgot to say who did it.

Michele: I know, but what reminded me is that of course the continent of Australia is close to the continent of Antarctica.

Vincent: Close as in a couple of days away, right.

Michele: Closer than us, yeah.

Michael: The other thing that I think most folks may not instantly appreciate is that Antarctica is about the third of the size of Asia and Asia is the largest continent.

Vincent: Yeah, it’s big. And we don’t think about it very much, right?

Michael: No, Antarctica is huge.
Vincent: It’s huge, but it is out of sight, out of mind pretty much, but there is some interesting stuff going on down there. So they have soil samples, the first sample is from a place called Robinson Ridge, they get some surface soil and they analyze it, they show that there is very low carbon, nitrogen, and water in it. They extract the nucleic acid, they sequence it, and they make a shotgun genome from the soil, 264 million bases which contains after analysis 298,233 genes that they can pick up. The microbial communities are mainly actinobacteria, chloroflexi proteobacteria, acidobacteria, and then two candidate phyla which are called AD3 and WPS2. The only prototrophs they identified were cyanobacteria phototrophs or cyanobacteria but very low abundance, only 0.28% of all the reads.

Elio: It’s fair to say that this spectrum of bacteria is kind of unusual, right? This is not what you find in other soils.

Vincent: This is not your usual soil microbiome for sure. This is because it is an unusual ecosystem, it is very harsh, this is apparently adapted to be able to exist there.

Elio: This serves a model for Martian studies, doesn’t it?

Vincent: Probably.

Michael: It does.

Vincent: Can anyone comment on chloroflexi? I’m not familiar with this collection.

Michael: I think it is a gliding bacterium if I’m remembering right, and it actually has chlorophyll.

Vincent: Okay. So now they have 280 thousand genes, what are they? They want to know what is the metabolic potential. You can analyze your genes and say what are they encoding and what do they do. They constructed 23 genomes from all of their data. All of these 23 encoded terminal oxidase genes which are needed to do aerobic respiration and they also have genes to oxidize organic carbon compounds. In these actinobacteria WPS2 and AD3 they found genes for carbon dioxide fixation by the Calvin cycle. Remember this is a light independent cycle that converts carbon dioxide to glucose and one of the enzymes of this cycle is rubisco. Rabulose 1,5 biphosphate carboxylase.

Michael: The most abundant enzyme on planet Earth.

Vincent: Is that right?

Michael: It is indeed.

Michele: It’s thought to be--

Elio: Well, well, well--

Michele: I don’t know if that takes in all the microbes that we are studying but (laughs)

Michael: That’s probably true, but remember, we have a lot of blue green algae on Earth.

Michele: Right, right.

Vincent: So this is--
Michele: It’s how plants capture CO2 and convert it into sugars.

Vincent: So that’s the key enzyme there to capture carbon dioxide and add it to the first compound, rabulose 1,5 biphosphate, and this enzyme is also known to support growth of actinobacteria using hydrogen as a source of energy. So genes encoding this enzyme were found in many of these microbes. They also found enzymes for aerobic respiration of molecular hydrogen and carbon monoxide, and these are very high affinity enzymes that can scavenge these gases which are present at trace levels in the atmosphere, so we have high affinity enzymes that can grab them. They also found genes encoding enzymes using methane as a carbon source, genes for proteins that are involved in ammonia oxidation, nitrogen cycling, and tolerance of low temperatures as it gets pretty cold down there.

Elio: But they did not find enzymes for photosynthesis.

Vincent: They do not, that’s right.

Elio: And that is a key point because if there were, what is the big deal? They would just be dealing with photosynthetic bacteria. But this is not the case, so this is what the big news is. They are not dealing with photosynthesis but something else.

Michele: What could it be?

Vincent: Right, so that is the question, no photosynthetic genes because there is not a lot of light, and so how are these microbial cells surviving? Right, that is the key question.

Michael: What are they eating is the question at hand, when everybody goes home for dinner, what’s for dinner?

Vincent: And so that is the theory here based on what genes they found in these organisms, they suggest that the surface soil microbes in Antarctica can scavenge hydrogen carbon dioxide and carbon monoxide from the atmosphere to get their energy and their carbon.

Michele: Amazing.

Elio: What is amazing is not only that this is a new metabolism but that it works on such a low concentration of gases.

Vincent: Very low.

Elio: There is not a lot of hydrogen, there is not a lot of carbon monoxide, carbon dioxide doesn’t count because that is what is being fixated.

Michael: It sort of violates Henry’s Law which says gas does never want to go back into liquid and since all the chemistry of the cell is effectively aqueous, it is really remarkable that it is able to concentrate these gases which are at low concentration out in the atmosphere into making more cells.

Elio: Exactly, exactly.

Michele: They do what they can.

Michael: They do what they can.
Michele: With not a lot of glucose.

Vincent: And so these gases can permeate the cell membrane so that is one of the properties they have that makes them available to these bacteria. So next they do some experiments to show that their theory might be correct. First they extract RNA from the soil and they do reverse transcriptase PCR and show that the genes encoding rubisco and the high affinity hydrogenase and carbon monoxide hydrogenases are expressed in the soil, they can see that the RNA is there by amplifying it as DNA. And they also use gas chromatography to show that the soil communities can aerobically oxidize atmospheric hydrogen and carbon monoxide.

Next they did a cool experiment, they have C14 labeled CO2. Now there’s a compound you have to be careful with. C14 labeled, they take their soil samples, they add the C14 CO2 to them, they incubate it and they can measure the uptake of carbon because it is isotopically labeled by uptake into the microbial communities. And they find low uptake and they tried this with soil from several different sites and the amount of uptake is variable. But if they added hydrogen they could stimulate the uptake of the labeled carbon dioxide so that is consistent with their idea. And if they shine light on it it doesn’t matter. Again, there is no photosynthesis going on.

Michele: So I was in touch with the first two authors, Mukan Ji and Chris Greening, and they both said that this was the most exciting part of this three or four year long project when they actually did the enzymatic studies of the soil and found before their eyes these cool reactions happening.

Vincent: So did they take that back somewhere else and back to Australia and do that or did they do that in situ in Antarctica?

Michele: So they brought the soil samples back to labs, the gas chromatography experiments were actually lead by Carlo Carere and Matt Stott and they are at the GNS Science Institute in New Zealand. So they did that. Professor John Beardall who is described as an esteemed algal physiologist, he is the one that conducted the carbon fixation experiments. So both of them pointed out that this project is really the fruition of a terrific collaboration between a variety of people at different institutions that all had expertise and they brought it together and really brought these soil samples to life in this paper.

Vincent: Cool. They looked at the metagenomics, they did some carbon fixation studies with samples from other sites, a couple of sites throughout Antarctica. So they get an idea that this happens not just at one place but in multiple places in Antarctica there are microbial communities that can oxidize trace atmospheric gases. They did some calculations which are interesting. They figure that oxidation of hydrogen and carbon monoxide could sustain microbial communities from 5 to 8 x 10^7 bacteria per gram of soil.

Elio: Not bad.

Vincent: Not too bad, not shabby.

Michele: Wow.

Vincent: Just breathing, just taking air. Again, they feel that the primary producers which are doing this, taking atmospheric gases are actinobacteria AD3 and WPS2 phyla and again, they make biomass from atmospheric hydrogen carbon dioxide and carbon monoxide. They propose the names for these two phyla, Candidatus Eremiobacteraeota for the desert bacterial phylum and Candidatus Dormibacteraeota for the dormant bacterial phylum, for WPS2 and AD3. They are kind of cute names, right, for those two.
So basically, as Elio implied before, these extreme ecosystems, physically extreme and chemically deprived sites, they have microbial communities that have been shaped by the ability of things to live there. They are dormant heterotrophic aerobic bacteria that oxidize atmospheric gases and there are not many phototrophs because there is not much water and nutrient availability is very dark and there is a lot of radiation, that is the other part that is a problem.

There is some evidence from other studies, I thought this was cool, that wind can bring microbes from other less hostile sites and then selection only brings out the ones that can scavenge trace gases, this might be a general mechanism of survival. One thing they brought up which I though was really interesting, there are some parts of Antarctica where there are lots of phototrophs and the soil is moist, so it does support photosynthesis, and these include hypolithons. These are phototrophic microbes that live underneath rocks. The rocks protect them from UV light, but the rocks are translucent so the bacteria can get the light that they need to do photosynthesis.

Michele: Wow.

Vincent: How about specialization, isn’t that amazing?

Michael: So they are filtering out the bad so they get the good.

Vincent: Yeah. You don’t want UV light, right.

Michael: They are probably eating the red spectrum of the energy scale, that is where a lot of bacterial photosynthesis is, down in the red zone.

Vincent: So they end up by saying most ecosystems are driven by solar or geologically derived energy and in these Antarctic soils it is by atmospheric trace gases.

Elio: Incredible. Incredible.

Vincent: Isn’t that amazing?

Elio: This is like a grand alternative to photosynthesis.

Michele: Yeah.

Elio: Discovering a mechanism that is equivalent to photosynthesis because it uses atmospheric gases, it is just amazing.

Michele: They found that these reactions too happen at even -12 degrees.

Vincent: That’s right, yeah.

Elio: Oh my God.

Michele: So the possibility then, your imagination just goes wild, we talked about Mars earlier. Maybe this is how life got its start in extreme planets.
Vincent: We should point out that these bacteria are just getting by, they are not dividing crazily, but they are existing and presumably at some period they do divide and so forth, but most of the time they are just maintaining themselves.

Michele: The other exciting implication is could we now use these pathways to generate energy from gases as a new energy source like solar or wind.

Michael: You could imagine putting them into smokestacks to take out the waste CO, effectively filter out the carbon monoxide so that we are dealing with it and at the same time fixing CO2. You kill the proverbial two birds with the one system.

Elio: I was wondering in terms of future work, it would be nice to know what these bugs look like. It is not easy to do but since you have specific genes you can make specific probes and you could look under the microscope of things that light up with certain probes. I am very interested in what these bugs would look like. I imagine they are very small, most of those bugs are very tiny.

Vincent: (laughs) I bet they are.

Michele: Interesting.

Michael: We don’t even know if they are Gram negatives, that is they have an outer membrane and a periplasm and an inner membrane--

Elio: I don’t think we learned anything about the bugs themselves, do they.

Michael: No but these are the genes for--

Elio: This is paper number one.

Vincent: Yeah. And I should say, just to end this, that there are a group of people that believe that humans can survive on air alone, and they are called Breatharians.

Michele: (laughs)

Elio: Never heard of them.

Vincent: Neither did I until I was looking stuff up.

Michael: That isn’t an add for weight loss, is it?

Elio: I’d rather have my dinner than be a Breatharian.

Vincent: Me too.

Michele: I’d like to share a bit about two of the scientists that led this project, Mukan Ji got her bachelors of science in molecular biotechnology at the University of Sydney and then she earned both her masters and PhD with Belinda Ferrari who is the senior author on this at the University of New South Wales. She wanted to share advice that collaboration is extremely important. This paper really required some collaborations with very different expertise and they were incredibly talented. She also said that you need to look at the data from many different angles. She said that the project had its origin back in 2013 and they went down a few different
avenues but finally in 2015 they got some key results and figured out where they wanted to take it. So she was encouraging junior scientists to be open minded about the data you have. So she had a very productive thesis research career, she published 8 papers and she also married and gave birth to their daughter during that time.

Vincent: Wow.

Michele: She is currently a post doc at the Institute of Tibetan Plateau Research which is within the Chinese Academy of Sciences. The other co first author is Chris Greening, he is currently a group leader and a discovery early career researcher awardee at Monash University in Australia. He got his bachelors and masters at Oxford studying heme biosynthesis enzymes in paracoccus, and then his PhD at the University of Otago in New Zealand with Gregory Cook, and there he became an expert in nickel iron hydrogenases of Mycobacterium smegmatis, which, like these microbes we talked about today, is famous for its ability to persist in low nutrient environments. He describes himself as a biochemist at heart and he had predicted based on his work with Mycobacterium smegmatis that some microbes could capture trace gases and use them as energy sources. So he was thrilled when he got an email from Belinda Ferrari who was aware of his thinking and expertise and who recruited him to join this project.

For him it was an easy sell, he was amazed that they had 22 reconstructed bacterial genomes from the Robinson ridge, and they all had genes for oxidation of trace gases in the atmosphere, hydrogen, carbon monoxide, and methane. So the most exciting moment in the gas chromatography when they saw that the soil was actually consuming these gases at really high rates and even at low temperatures because he points out for much of the nineteenth century we thought the whole continent was basically sterile and here they had evidence that that was not the case. So advice he has for junior colleagues--

Elio: Wait a minute, I have to make a comment. I was given the address, Chris Greening works at the school of the biological sciences Center for Geometric Biology at Monash. Can somebody tell me what geometric biology is?

Vincent: (laughs)

Michele: I don’t know. We will have to learn.

Elio: I don’t know, too. They invented the term which may be very useful but I wish that I could find a definition of it.

Michael: It’s probably in their mission statement.

Michele: Oh, that’s true. Look it up on the world wide web. He says to be successful he has been willing to challenge established ideas, we certainly saw that here and also to collaborate so again he wanted to give credit to their collaborators Carero and Matt Stott for the gas chromatography and John Beardsall who did the carbon fixation. He also has taken a goal-centric approach to publishing and career development and encourages other people to do that and be really proactive, and of course he says to have a massive passion for science has to underlie every thing we do.

Vincent: You got it for sure.

Michael: So according to the internet, geometric biology allows us to understand the dynamics of how living things convert energy flows into mass at all scales of biological organizations, the size and shape together the geometry of organisms ultimately determine these flows. So that is effectively what this paper did, it was geometric biology.
Vincent: That’s cool.

Elio: Wow. Learn something every day.

Vincent: Thank you, Michele.

Michele: Yeah, thank you for choosing this paper, I thought it was really just a gee whiz kind of result.

Elio: This is really an exceptional paper, there’s no question, it’s a trailblazer.

Vincent: However, I do not want to go to Antarctica.

Michele: (laughs)

Vincent: Does not inspire in me any desire to go. Especially looking at the pictures, it looks pretty barren.

Michele: But it is harder to complain about our cold spell.

Vincent: Yeah, that’s true. Let me read you some of these emails we have received. Last episode, we said we were going to give a book away. Disinfection, a brand new ASM book, got 7 emails. I will pick one at random by generating a random number. Tim writes the first one:

Hello, my name is Tim, a current high school senior in Connecticut and a new listener to TWiM. You guys are truly inspiring the next generation of scientists with what you do.

Anyways, this book would be an excellent read for me since I am planning to major in microbiology with a specialization in infectious diseases starting this Fall. As you know, one can’t research microbes if they don’t know how to kill them.

In anticipation of something new to read!

Tim D.

Michael: (laughs)

Elio: If you don’t know what?

Vincent: If you don’t know how to kill them. Anthony writes:

Please enter this email in the current TWiM book contest.

With the hygiene hypothesis in mind, for disinfection, too much of a good thing is not wonderful.

Anthony

And Anthony sends a poem by Arthur Witterman called Strictly Germproof, I will just read the first few lines, you can find the rest in the letters section.

The Antiseptic Baby and the Prophylactic Pup
Were playing in the garden when the Bunny gambolled up;

They looked upon the creature with a loathing undisguised;

It wasn’t disinfected and it wasn’t sterilised.

If you want to hear the rest, go to the letters at microbe.tv/twim.

Brian writes:

Dear cast of twim;

I enjoy hearing your program from time to time through the “tune in” app on my iPhone.

Here in North Battleford Saskatchewan Canada we have ascended out of a two week deep freeze, and are basking in -13 Celsius.

I come from an agricultural background, and was raised on a farm of “milk and honey”. So I took an interest in the program about the cultures found in the noses of dairy farmers. I also must have contracted cowpox when we milked cows by hand, since the medical people tried several times to inoculate me with the smallpox vaccine, and I never reacted. I have no scar.

That is so cool, you’re right!

Michael: That would do it!

Michele: A unique immunization.

Vincent: That would do it, how cool is that!

I have a degree in agricultural science from Guelph, specializing in honeybees. We ran 1000 hives on our family farm.

Since 1988, when my brother bought the farm from my father and myself, he has successfully run 5000 colonies annually. He is not experiencing colony collapse and winter losses. He attributes his success to the knowledge that parasitic mites resident in and on the bees are introducing viruses that cause bees to die over winter. Some beekeeping operations in the province lose 50 percent of their bees over winter-sometimes more. His winter losses have been five percent or less. He believes that by treating the mites with a miticide early in the spring (while none of the brood is capped, but all the cells are open) he can effectively eradicate most of the mites. Consequently, most of the bees, including the queen, that go into the winter have not been bitten by the mites, are not sick from viruses, and can endure the long cold months.

That is a really interesting idea.

I listen with great interest to your program when I get a chance, and marvel at how careful you are to keep it in lay terms. That language helps keep my interest, and I do learn some interesting facts almost every time.

About 15 years ago my wife became infected with herpes zoster in her eye. Sadly, no medical person offers any hope for stopping the damage. The scar has almost entirely covered her iris. Cold weather, typical here in Saskatchewan winters, and the wind aggravate the condition. I wanted to ask if there has been any
breakthrough that you could share with me so that she could receive helpful treatment. I would also enjoy hearing why she would be infected in one eye, and that it has not spread to the other eye or to me.

Thank you for considering me for the free book.

Brian.

Michele: Well, Vincent?

Vincent: The thing is you get these herpes infections in the eye because you typically touch a lesion with your finger on someone else and then you put it in your eye, so it is typically one eye that you infect. And then it doesn’t go to anyone else, it stays in that one eye because it is not easy to go from eye to eye as you might imagine. And you don’t spread it because you don’t typically, people don’t touch your eye and that is how you would spread the infection to others. Unfortunately there is nothing to be done, the damage is actually an immune reaction to infection, it is not viral damage but it is an immune reaction and there is nothing really that can be done about it. Our next one is from Jacob:

Because I never win anything,

Jake.

He’s a post doc at MIT and then Assaf writes:

Hello TWIM team,

I’ve been a TWIM listener since episode one, and shamefully it took a book contest to get me to finally write in. I work in a bioremediation company, and would love to hear you talk about the subject- maybe a special guest?

It’s been five years since I completed my PhD, and TWIM has been my journal-club, and helps me keep my passion for bacteriology.

I have a two questions for the listeners:

For those who listen while driving: does any of you have an idea for not forgetting your TWIM questions by the time you get to your destination? (laughs)
Which TWIM TWIV or TWIP episodes should I recommend on a non-scientific facebook group for podcast addicts?

Those are listener questions, so please answer Assaf.

And thank you for making my every other Sunday morning so much better. Shalom from sunny Israel where the week begins on Sunday. One more from Sophia:

Dear TWIM team

Happy new year! I never write even though I’ve been listening since 2011 (but always too long after the episode is out) but it’s a new year and unusual things happen, like Dr. Despommier being a guest (how nice!) so I thought I’d do something original too! You like to know where we listen from so greetings from Thessaloniki, Greece where the temp is 10 degrees Celsius at the moment.
Congratulations on all your podcasts. To be honest, I like how you’ve started talking about things that cross the podcast boundaries (influenza on TWIM; dengue on Immune)—this approach helps me understand things a lot! What a great idea, I hope you do it more often.

Could you talk about TB sometime? (In any podcast, actually)

Have a wonderful year and thank you for all your time and effort

Michele: Wow, she really is a devotee. She understands from one podcast to the next what we are covering.

Vincent: Very good. We had seven entries, I will do a random number generator. There’s the drumroll. And the number is 5. 5 is Jake at MIT! Who says he never wins anything. Well, here you go, Jake, you won something.

Michael: (laughs)

Michele: It’s a happy new year.

Vincent: Jake, send us your address, send it to TWIM@microbe.tv. And that is it for TWIM 169, it is at asm.org/twim, and send us your emails, twim@microbe.tv, and if you would like to help us financially you can go to microbe.tv/contribute to help us out. Michele Swanson is at the University of Michigan in Ann Arbor, thank you Michele.

Michele: Thank you, I enjoyed joining you all again.

Vincent: Elio Schaechter is at Small Things Considered, welcome back, Elio.

Elio: My pleasure, thank you.

Vincent: Good to have you. Michael Schmidt is at the Medical University of South Carolina, thank you Michael.

Michael: Thanks, everyone.

Vincent: I am Vincent Racaniello, you can find me at virology.ws, I would like to thank the American Society for Microbiology for their support of TWIM and Ray Ortega for his technical help. Music on TWIM is by Ronald Jenkees, ronaldjenkees.com. Thanks for listening everyone, we will see you next time on This Week in Microbiology.

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Transcribed by Sarah Morgan.