This Week in Microbiology

With Vincent Racaniello, Michael Schmidt, and Michele Swanson

Episode 190: Exosomes in your nose and in your gut

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Vincent: This is TWIM, This Week in Microbiology, episode 190, recorded on November 29th, 2018. I'm Vincent Racaniello and you are listening to the podcast that explores unseen life on Earth. Joining me today from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello!

Vincent: How is winter treating you out there?

Michele: It has arrived, my bicycle ride is not nearly as pleasant these days.

Vincent: Chilly, huh?

Michele: Yeah, cold.

Vincent: Do you have snow on the ground?

Michele: Not really, a little decorative stuck here and there to a bush.

Michael: You didn't get the Thanksgiving blizzard?

Michele: It didn't last, it didn't stick.

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

Michael: Hello, everyone!

Vincent: Where it is a balmy 80F.

Michael: No no no, right now it is sunny and 55 degrees.

Vincent: Alright. Well, here it is chilly, it is 4C, but it is sunny although it is really windy. My colleague next door said he went to rake his leaves and then he went inside and a minute later he came out and they were all back.

Michele: Some neighborhood kids had messed it all up (laughter)

Vincent: When it's windy you can't do leaf moving, it just doesn't pay. It's frustrating. The wind just says "ha ha ha."

Michael: Laughs right in your face.

Vincent: In an effort to make ASM's podcasts the best they can be, TWIM wants to learn more about you, the listeners of TWIM. Please take 5 minutes to answer this short 12 question survey. Surveys help us collect the data that we need to seek sponsorship, which allows us to grow the podcast. The survey is at ASM.org/twimpoll. It is only 12 questions, you can answer them really quickly. They are really easy, they're all about you, you don't have to solve any problems, you don't have to say what an exosome is, for example.

Michele: Yeah, while we are chatting about the weather they could do the podcast poll. That would be great.

Vincent: Thanks for your time, thanks for listening, subscribing, and helping us improve the show. We do appreciate the support. Today we are gonna talk about exosomes. It was kind of accidental because Michael picked the snippet which has to do with exosomes, and we were originally gonna have Elio on and he had a paper and he realized he couldn't make it. I just picked something that turns out to involve exosomes and now we have two exosome papers but they are very different, right Michael?

Michael: Yeah, and they give us an idea of what is actually going on with these wonderful, as Michele said in our pre show, this blebs that are coming off of our eukaryotic cells.

Michele: And prokaryotic, our bacteria.

Vincent: They make them, too?

Michele: Yeah, yeah yeah.

Vincent: Are they called exosomes?

Michele: Yeah. You can call them different things like little vesicles but yeah. Legionella sheds them and Neisseria is famous for shedding a lot of LPS rich blebs that then trigger a lot of inflammation that can contribute to disease.

Michael: So the warning up front is we are going to have a little immunology in today's TWIM.

Vincent: Well here is a lovely review article in Infection and Immunity called "Membrane vesicle release in bacteria, eukaryotes, and archaea, a conserved yet underappreciated aspect of microbial life."

Michele: Who are the authors on that review?

Vincent: Nocera, Mueller, Stephan, Hing, Seifert, Han, Lin, Amiji, Libermann, and Bleier.

Michele: Yeah, Brad studied the delivery of antigens to T cells through blebs from salmonella.

Vincent: I will put that in the show notes.

Michele: Efficient packaging devices to concentrate and deliver antigen.

Michael: I'll start with our snippet today, and the reason I picked it is we are moving into cold and flu season, so I thought the snippet could provide us some perspective into how valuable our noses are in protecting us from the constant assault from the things we talk about in each episode in This Week in Microbiology and This Week in Virology. So the paper for our consideration in this snippet is titled "Exosome swarms eliminate airway pathogens and provide passive epithelial immunoprotection through nitric oxide." This comes to up through the Bleier group out of the Department of Otolaryngology from the Massachusetts Eye and Ear Infirmary, and the lead author is Angela Nocera and she coauthored this paper along with SK Mueller. And so it is open access I believe and it is appearing in the Journal of Allergy and Clinical Immunology.

So here is the story. When the cells at the front of our nose detect a bacterium, typically one of the outer surface elements for example in the Gram negatives it is likely gonna be LPS, this serves to toggle from an off state to an on state a receptor called Toll like receptor IV which in the jargon of immunologists is abbreviated TLR4, which is a germ line encoded pattern recognition receptor of the host, or a PRR.

If you will, they alert the equivalent of our body's smoke detector, where they are triggered secondary to exposure to the microbial structure motifs, which are known by the immunologist and microbiologists as pathogen associated molecular patterns or PAMPs. So this interaction or toggling of the Toll like receptor IV results in this case the release of antimicrobial peptides, and like the metaphoric smoke detectors in the ceilings of our homes where when smoke particles block the alpha particles being emitted from the americium that is in every ionizing type smoke detector, the normally ionized oxygen and nitrogen molecules are quenched by the smoke, and the electrons that are normally free to generate a current fail to supply a sufficient current to the circuit and the receptor horn of our smoke detector goes off. We all rush out of bed before the flames harm us.

Here in our nose it is no less complicated than an ionizing smoke detector, but it is as elegant as the ionizing smoke detector. And what happens is we see the release of billions of exosomes which are these tiny, in this particular case, 30-150 nm fluid filled sacs that are released from the eukaryote cells lining our nasal passage. Second there is that toggle switch in the nasal cell being switched from an on state, or excuse me, from an off state to an on state. And these exosomes come out in a swarm and they are thrust into our mucus layer, killing bacteria as they go, and serving then to activate our innate immune system or if you will arming our nasal cells that have not yet seen the PAMPs. So the intruders have not yet gotten back there but these exosomes get into the back of our nasal passages and get our cells ready for the onslaught that is coming in our airway.

Michele: So Michael, are you telling us that our snot is full of these exosomes?

Michael: When there is sufficient detector, and that could, I've often wondered about mucus and the characteristics of mucus when we are sick, but it changes an I really got curious about it, so I have to go off and visit my otolaryngologist friends and ask them to give me a 10 minute lesson on mucus and snot.

Michele: Because then we could feel proud of it because it is a part of our natural host defenses responding.

Michael: It is! And this then takes us down to the path where we are going to get in to-- so we are talking about nasal exosomes, and these things are tetraspanin enriched microvesicles that are secreted in almost all body fluids, and Vincent is gonna talk to us about another exosome that is in a different body location that has different behaviors. So stay tuned as Elio would say. So here in the human nose, these nasal mucosa derived exosomes are filled with immunodefensive proteins. And again, they are nothing more than blebs that are coming off of our eukaryotic cells that have responded to this insult of whether it be LPS, and they are gonna use LPS a lot in our story, and they make these immunodefensive proteins or antimicrobial peptides in addition to nitric oxide, which we know is bacteriocidal. And it also upregulates nitric oxide synthase, which makes the nitric oxide that then this whole nasal mucosa derived exosome product actually sneaks down with the mucus carriage and it does intraepithelial protein transfer that is a hallmark behavior of these exosomes, which have this remarkable trans membrane 4 superfamily type canonical structure that you can learn more about.

So in this fascinating manuscript, the authors ask the question: what contribution do these nasal derived exosomes do to effectively act like our smoke detector for our innate immune system? They hypothesize that in inhalation of a pathogen, expressing a TLR4 stimulating PAMP, the epithelial cells in the front of our nose or the anterior nasal mucosa released the swarm of exosomes. And they are talking in the billions of exosomes that are released. Which then exert a direct antimicrobial response within our mucus environment. So I will take you through their data here in a second, but they then secondarily hypothesize that this population of exosomes is then simultaneously swept backwards into the nose through this mucociliary clearance, where our cilia are actually sweeping this population of billions of exosomes back, where the exosome then donates its immunoprotective cargo, and they are gonna show us that it can actually move nucleic acid in addition to protein to these naive epithelial cells that then switch them on to defend against the advancing pathogen. So it sounds pretty neat, and so I was reading this and I said, this is really cool.

And that is what the paper takes us through, something that is neat, that is actually occurring. Now this group is uniquely positioned to have access to human nasal mucosal derived exosomes in the fact of where they are. They are in an otolaryngology department, they do surgery so they can get access to these tissues, and the first portion of their paper is a very elegant definitive series of experiments where they show us that they can indeed isolate human nasal mucosal exosomes, and I'm not gonna burden you with all the methods but their methods are beautifully written and the first figure effectively demonstrates equivalently that they are indeed isolating exosomes from the tissue. They're doing what they say. So their data that go into this beautiful story of what the exosome is doing is really pretty remarkable. So the first key piece of their data is LPS or this PAMP stimulation increases both basal exosome secretion, so we are gonna make more exosomes, and secondly, the nitric oxide expression by a factor of 2. And they showed this in a really clever experiment that just simply by

increasing the concentration that they are adding, the numbers of exosome present increased. And they showed it in a beautiful dose dependent manner. The dose they used, we need to point out, because they are actually gonna do these experiments in live patients, was non toxic.

Michele: And it was the dose of the TLR4 receptor ligand LPS that they were increasing. It's a lovely experiment.

Michael: Yes! It was so cool to see that. The next aspect was the fact that the antimicrobial components, the nitric oxide synthase, increased by a factor of 2. So we saw more of the enzyme, so they showed us the expression of the gene, and then more importantly the expression of the gene product. So we now know that the exosome is going to make this antimicrobial enzyme that will make more of this antimicrobial factor, so the next aspect of their story was whether or not the TLR stimulated nasal mucosa exosomes conferred a direct antipseudomonal activity equivalent to a surface acting antibiotic that they used, colistin. Pseudomonas is a pathogen that we are constantly inhaling that it appears from their data that we are quite easily able to dispatch with this TLR stimulated system. So they took their TLR4 stimulated nasal exosomes and what they did is the exosomes that were activated were shown to significantly decrease the number of viable Pseudomonas in comparison to exosomes that were untreated. And colistin was still much better at whacking down the concentration of Pseudomonas, but it was significant over the untreated control.

Michele: And just to remind us what colistin is?

Michael: It's a surface acting antibiotic that is specifically targeted against Gram negatives.

Michele: So that's their positive control. But still, the exosomes are during…

Michael: And colistin doesn't go systemic. It is effectively excluded. So the final aspect to their immediate story is that these TLR4 stimulated exosomes donate their immunoprotective cargo to naive epithelial cells through this mucociliary clearance mechanism, thereby conferring passive immunity to if you will the recipient epithelial cells that are further back in our noses. So here they explored the capability and the kinetics of the exosome transferring protein and nucleic acid to naive nasal epithelial cells with a hypothesis that this canonical behavior of an exosome having the ability to enter epithelially transport, and they showed it that this was through mucociliary flow, and the way they were able to confirm this was they restricted the time of their studies to less than 30 minutes which is the transit time from the front of our nose to the back of our nose. And that is roughly the maximal intranasal residence time of suspended particulates within nasal mucosa before being removed by the mucociliary carriage.

And here in this beautiful set of figures they show us how the stuff moves from the exosome to the analogous cells and the demonstrate that it enhanced nitric oxide production in first primary cell culture. And they do do these experiments both in primary cell cultures and ultimately in vivo in patients. And so they show the proteins are moving by using a particular dye that will pick up and stain the proteins, and they have beautiful fluorescent microscopic images that you can see. Then they show that the exosomes are actually moving nucleic acid, and the final aspect of this series of experiments was that the LPS activated exosomes were significantly able to induce more nitric oxide

release from these naive epithelial cells. And it was just absolutely incredible. You don't need any fancy statistics, you just look at the figure and say yep, it's up.

So the final piece to their story is that they showed that these nasal activated exosomes express multiple innate immune proteins so again, the whole story is that the exosome is activating our innate immune system and the way they showed this is they were able to visualize relevant immune and inflammatory pathways were indeed activated. That's effectively the final figure of their story. And their findings ultimately because they did this in vivo with three patients provides us a novel approach to understanding how our innate immune surveillance and defense mechanisms of our upper airway are really indeed working. So unlike the smoke detector that simply serves to wake us up, here in our nose when our nose detects a foreign invader, not only does it sound the alarm it literally turns on the water to put out the fire by liberating this swarm of these billions of activated nasal mucosa derived exosomes.

They quickly move through our nasal passage nearly doubling the levels of nitric oxide synthase as well as nitric oxide that is being able to be dumped into the system. And these swarms of angry exosomes, not only exhibit dramatic direct microbiocidal activity, but because they are proactively arming the posterior naive epithelial cells with these immunoprotective proteins because of the fact that the exosome has these unique 4 trans membrane protein regions, it is absolutely remarkable that they are able to defend the back of our nose from the invading microbes before they get there. Because the microbe is sort of stuck in the advancing mucus blanket but the mucocilliary carriage is moving the billions of activated exosomes at a much more rapid clip. So the key here is that the exosome is very much like Superman. It is faster than a speeding bullet, or the mucus carrying the Pseudomonas. So that's the story.

Michele: It's neat, these vesicles are basically packed full of bioactive molecules so they are delivery mechanism and a transport mechanism. They deliver it right to the site and then transport back so that we have broader coverage and broader elevating a barrier to whatever onslaught was just sensed. Very cool.

Vincent: I have one word for you listeners: Exosomes. (laughter) Both of you recognize that little…

Michele: Plastics.

Vincent: Elio once said it for something else, I can't remember, but exosomes are something we learned about not too long ago and they are really amazing and turning out to be really important in many different ways.

Michele: Kind of an ancient secretory system, ancient way to pack a lot of molecules and then deliver it in a bolus.

Vincent: Yes.

Michael: And the innate immune system is relatively old evolutionarily, but the adaptive arm of the immune system, the one that the TL4 is responding to, is evolutionarily new. So here you have the two working together, and I should also point out that in their manuscript they have this beautiful

graphical abstract that really walks you through what is actually going on in this very simple 3 panel figure that just shows you the TLR4 mediated exosome release, the mucociliary transport, and the antimicrobial effect, and the epithelial protein transfer. So it is really pretty cool.

Vincent: Thank you, Michael.

Michael: You're welcome.

Vincent: Just to fill in a little bit more on exosomes, they are basically nanovesicles, 50 to 100 nm in diameter, they have a lipid bilayer and they can contain lots of different things from the cytosol. Nucleic acids, proteins. They come from a compartment in the cell which is associated with late endosomes and this is called multivesicular bodies, and they of course go, they ferry their cargo from cell to cell. And they can change what happens, you heard just now how they have immune effects. As we will see in a moment they can have metabolic and broader effects, as well. And these are in all three domains of life, as we said earlier. Bacteria, archaea, and eukaryotes.

Among the eukaryotes a very recently discovered exosomes is in plants, hasn't been discovered too many years ago, but plants make exosomes and when you eat plants, which many of us do, whether it be lettuce, not lettuce with E. coli of course, but other kinds of lettuce, peppers, anything. Any plant, you are eating exosomes. And that is the topic of our paper today which is an article from Cell Host and Microbe, it is called "Plant derived exosomal microRNAs shape the gut microbiota." And this comes from a whole lot of authors, the first is, let's see, the lead authors. Yun Teng and Yi Ren. The first two authors, they contributed equally. And the lead contact is Huang-Ge Zhang. From the VA medical center in Louisville, University of Louisville, the People's Hospital of Nanjing Medical University, MD Anderson Cancer Center at University of Texas, Translational Genomics Research Institute in Phoenix, and University of Louisville School of Medicine.

Now we have talked many times here on TWIM about how diet can influence composition of the microbes in your gut, and if you go to a different place, let's say you live in Ann Arbor, Michigan and you go to Beijing for a month, your gut microbiome is going to change. That is in part because you are eating food with different microbes but it is also because, as we will see in today's paper, you are eating exosomes from plants that can modulate your gut microbiome. So the idea of having a healthy diet is probably linked to what we are going to talk about today. So this is all about plants, and plants have lots of exosomes of the same kind that Michael was just talking about. And the question here is whether these exosomes can affect gut bacteria and how they do so. And there has been some previous work on this, we know that eating plants changed the gut microbiome.

We know that a pathway in your cells called the aero hydrocarbon receptor pathway, this is a pathway that is activated when you are exposed to halogenated and polycyclated aromatic hydrocarbons. You have a response that tries to take care of those because those can be nasty things, and that is activated by eating certain things, and a cytokine called IL-22 is induced by this AHR, these aryl hydrocarbon receptor pathways, and this is a beneficial cytokine. It suppresses inflammation and so the question in this paper is whether these plant, what they call them are plant derived exosome like nanoparticles, ELNs. A lot of abbreviations in this paper that constantly threw me for a loop because I never remember from one minute to the other oh that's what AHR is and then on the next page I can't remember it any more.

Michael: That's one thing I would like to compliment the Journal of Allergy and Clinical Immunology, they had a box with all of the abbreviations in a gigantic font that you could read and I would have appreciated that same giant box with all these abbreviations as well. I had your same problem as well, Vincent.

Vincent: There is no box here, I'm scrolling through the copious stuff at the end of the paper.

Michele: We're looking at you, immunologists (laughs)

Michael: Yes, we are indeed!

Vincent: There's a lot of metabolism in this paper that has abbreviations, too.

Michele: That's true.

Vincent: So in this paper we are going to talk about ELNs, which are exosome-like nanoparticles. The main plant that they use is ginger, which is not too common here in the US I guess. But in certain Asian countries they love their ginger.

Michael: You don't actually eat it like lettuce, it's sort of a condiment, isn't it?

Vincent: Yeah.

Michele: I love cooking with ginger, ginger scones, yeah.

Vincent: You can have ginger tea, of course. So they make exosomes from ginger and they are called ginger ELNs or GELNs. And they are gonna be making exosomes from a few other plants, as well, and see what happens. So let's go through the experiments a little bit. These are edible nanoparticles, you can eat them. You do. When you eat plants you are eating billions and billions of plant derived nanoparticles of all sorts. And just like the ones in your nose, they have some effects. So they can purify these from plants and they can sequence them and see what's in them. The first thing they found was that ginger exosomes contain lots of micro RNAs. These are small RNAs produced by cells that are involved in regulating gene expression. So each 21 nucleotide micro RNA has some target mRNA somewhere in the cell. It is gonna bind to it and it will be degraded and it wont be translated any longer. So they found micro RNAs that could be mapped 109 different plant species, but they also found that some of these plant micro RNAs, again these are in the plant exosomes, they could potentially bind bacterial mRNAs.

Michele: Are they fairly short in general?

Vincent: The micros are 21 nucleotides.

Michele: They could just by chance bind a lot of different places.

Vincent: Yeah. So there are lots of what we call off targets which are not really intended targets. If you are doing any kind of editing with either these microRNAs or with CRISPR you are always gonna have off target effects. As you said, they will bind sequences just because they are so short, there is a likelihood there are gonna be other sequences in the genome and they're gonna hit them. So one of the first things they do is say okay, so if these are eaten, is the RNA gonna be stable or is it gonna be all degraded in the gut and we don't even need to study this because if it is all degraded then it is useless. They do some work in mice where they feed them different kinds of exosomes and they find that, for example, grapefruit exosomes go to the liver.

Michael: Big surprise!

Vincent: Whereas the ginger exosomes stay in the intestine and they can look at that in mice obviously by looking in the tissues but also in the fecal pellets.

Michele: That was the first big surprise of the paper, that these little exosomes would themselves home to different places.

Vincent: They home, and the homing is, I think we will see later it has to do in part with the lipid composition of the exosomes. These plant exosomes, by the way, are different from the exosomes from mammalian cells. So the mammalian exosomes are rich in phospholipids, rich in sphingomyelin, but these ginger exosomes, they have 90% phospholipids.

Michael: That makes sense because the exosomes are being blebbed off of the parent cell. And so mammalian cells will look very similar to mammalian cells in terms of their composition. In plant cells, the exosomes from the plants will look very similar to the plants.

Vincent: The enxt thing they did was feed mice ginger exosomes for a week and then analyze their intestinal fecal microbiome by doing a 16S ribosomal RNA sequencing and analysis of the sequences. And what they find is that you get after this treatment, feeding ginger exosomes, purified exosomes, you get an increase in lactobacillaceae and bacteriodes S24-7 and a decrease in clostridiaceae.

Michele: That must have been exciting right from the start because in general we associate the lactobacillus with healthy, probiotic foods.

Vincent: The clostridium with not so healthy.

Michele: With danger.

Michael: This is really very intriguing because we have had enough microbiome papers and one of the things we have always been scratching our heads with is how might we modulate the gut to get the good bacteria, to keep them there?

Vincent: Right. In the end they say this looks like a good way to do that.

Michael: Change your diet just a little bit.

Vincent: I predict they will start selling plant nanoparticles in capsules, right?

Michael: Yeah, just like fish oil.

Michele: Ginger capsules are available but also I'm sure many of our listeners know that ginger has a long history not only of being an important cooking ingredient in Asia, but in China it is also a part of their traditional medicine. So we are actually now taking modern techniques to understand what is it about ginger that could impart benefits to our health.

Vincent: They do a range of feeding mice these ginger exosomes and including the dose that would be relevant to humans, and they always see the same increase and decrease. With the higher doses of treatment there is no problems with the mice, as far as they can tell. There is no increased liver enzymes and so forth. So they seem to be okay and it is equally effective in male and female mice. So then they took exosomes from a couple of different plant sources: ginger, grapefruit, and carrot. I have to say that most of the papers I read for all the podcasts never mention these things, so.

Michael: Grapefruits and carrots, I was getting hungry when I was reading this paper.

Michele: I'll just add I corresponded with the first author, Yun Teng, and he said that they have their lab really focused on using edible plants as a source of potential therapeutics for tumor and immune regulation, so every day in the lab the smell of various edible plants pervades the space. Grapefruit, lemon, mushroom, ginger, and they all just thoroughly enjoyed those fragrances.

Vincent: I'm sure, I'm sure. So what they do here is they make exosomes and then they extract the RNA to see if it is the RNA that is having this effect, right. And they encapsulate the RNAs in what they call nanovectors which are made from lipids derived from these ginger exosomes. And then they finally feed those to mice, they put it into their stomach with a tube. And they also have similar change in the composition of the gut microbiome as when you feed the exosomes. So it looks like the RNA is actually what is happening, what is affecting that change. This is interesting. All of a sudden in the middle of this paragraph about mice, they have a human study.

Michele: Yeah.

Vincent: To determine whether our findings can be translated into clinical application, stool samples of healthy subjects after being given ginger exosomes for a week were analyzed.

Michele: These were 58 healthy volunteers that they were able to incorporate into their study.

Vincent: I guess feeding ginger extract is not a particularly dangerous thing to do. Anyway, they saw similar increases in lactobacilli, bacteroides, and a decrease in the clostridiaceae and ruminococcaceae. What is a ruminococcaceae., Michael?

Michael: Ruminococcus is a good example, they are common anaerobes that are associated with the rumens of cows and any other ruminant.

Vincent: We must have some in our guts then.

Michael: We do, indeed.

Vincent: Can they be dangerous?

Michael: No. Well, if they perforate the sterile space they will do what all good gram negatives do and give you sepsis, but for the most part, they are good bacteria.

Vincent: So next they say, is this ginger RNA actually getting into the bacteria? Because if it is then they could pursue that. So they label these exosomes with fluorescent dyes and then they do microscopy. And you can see that the dye gets into the bacteria, it is being taken up, they can do microscopy, flow cytometry, then they say, well, which bacteria are taking up these particular exosomes so again, they use labeled, and they take the labeled bacteria that are a product of this previous experiment and they can sequence them and see what they are. And they see that 31% of the fluorescent bacteria that were given this ginger exosomes are lactobacillaceae, so that is interesting. A lot of them are being taken up by those specific bacteria. Then they want to know whether you can preferentially target bacteria. They want to know if you can say okay, I'm going to give you this exosome from a plant and it's gonna go to these specific bacteria. So they take turmeric, which I only know as a spice. We often cook with turmeric, and I love the name, turmeric.

Michele: And it comes from a root, it's related to ginger.

Vincent: They say it is the same family as ginger, garlic, and grapefruit. So they gave mice garlic, turmeric, and grapefruit exosomes, and they did 16s ribosomal sequencing on their fecal samples, and they find that the bacteria that take up the ginger exosomes, all three types of exosomes, garlic, turmeric, and grapefruit, are taken up by bacteriodales S247. Turmeric is taken up by lactobacillaeceae, but the garlic and grapefruit derived exosomes are taken up by ruminococcaceae. So that is really interesting, exosomes from different plants target different bacteria. So you could imagine right now that you could use this, okay we want to target lactobacillaceae, we're gonna use ginger.

Michael: It's gonna really help us understand how best to develop the ideal microbiome as we begin to understand the relationships to health and wellness of what a good microbiome is, and you know that there are companies out there that are isolating, they are paying good money for stool donors, for stool transplants, and they are trying to come up with the ideal stool. And you can well imagine that if the some of the more successful stool transplants come from a loved one living in the same home because they are eating the same materials, they are living the same life style, and it is only because they were unfortunate had an aggressive course of antimicrobials and developed C. diff that they need the transplant. But if you could imagine how we might be able to modulate the gut, we may be eliminating the need for transplant, or we may be able to help a family member become a better stool donor.

Michele: By modulating the diet once we understand.

Michael: So, you know, as Vincent said earlier, exosomes.

Vincent: So far we found that the numbers of lactobacillaceae increase in mice given ginger exosomes, and these are preferentially taken up by the same bacteria in the gut. And then they do some culture experiments where they show that culturing one particular lactobacillus, Lactobacillus rhamnosus, which they abbreviate LGG, its growth is promoted by ginger exosomes. However, grapefruit derived exosomes reduced the growth of LGG. And then they looked at a variety of other lactobacillus species. They get an indication that it is actually the metabolic products of these bacteria that are treated that can inhibit other bacteria. For example, you treat LGG with ginger exosomes, the supernatant will inhibit listeria, E. coli, bacillus fragilis, but not itself.

So they decided to focus on LGG, Lactobacillus rhamnosus and its regulation by ginger exosomes, so the rest of the paper is mostly on that. And the first question is how are the exosomes targeted to particular bacteria? As I mentioned earlier, it has to do with the lipid profiles. They do lipid analysis of these and they do experiments where they deplete particular lipids and show that certain lipids are needed for targeting. So the summary there is that PA lipids, phosphotidic acid containing lipids, play a role in targeting the exosomes to particular bacteria. In particular, these lipids can also influence the migration of exosomes to the liver, which we mentioned is a property of grapefruit exosomes. It all has to do with the lipid composition of the membranes. So they are slightly different among all the different plants.

So how is this working, this change in the growth of the bacteria? Their idea is somehow the exosomes, the micro RNAs are modifying gene expression of the bacteria. SO they add these ginger exosomes to a bacteria. They give them to mice, and then they take out the florescent bacteria that have taken up the exosomes, and then they do gene profiling. They sequence MRNAs in these bacteria and they compare the ones that have taken up ginger exosomes with the ones that have taken up controls and see what is different. As you might guess, there are a lot of mRNAs that are different. 249 LGG mRNAs are reduced in cells treated with ginger exosomes. And they have a table showing a whole list of them and one of the top ones is the transcriptional repressor LEX-A.

Michael: Oh, my.

Vincent: Can either Michele or Michael tell us why LEX-A is important?

Michele: I can talk a little about that. We have our bacterial cells have a system where they can detect DNA damage and that activates a protein called REC-A to cleave LEX-A which is normally keeping off this whole protective response pathway, which is called the SOS pathway. So when there is DNA damage, the LEX A repressor gets cut, comes off the DNA, and then the cell turns on a whole bank of genes and pathways that arrest cell cycle so the bacteria stop growing. They also begin to repair their DNA. So in its simplest form, that is the response. But it is a very broad and widely distributed regulatory pathway in bacteria and each one uses it a little bit differently, but nevertheless it is an interesting target because it does have a known regulatory impact.

Michael: You know it’s a big deal because it has its own page in wiki. (laughter) On wikipedia.

Vincent: That’s right. SO basically one of these micro RNAs in the ginger exosome can potentially target LEX A mRNA and in fact, they show that treating the lactobacillus with this particular micro RNA reduces LEX A expression, and these bacteria grow faster and that may in part be responsible for the

enhancement of the lactobacillaceae in the gut of mice fed ginger exosomes, to work all the way back to the beginning there. The next series of experiments address whether these ginger exosomes or ginger exosomal RNAs can have an effect on the biology of the host. Here they are looking at colitis in mice, which is induced by feeding mice DSS, dextransulfate sodium. It compromises the barrier of the gut and bacteria come out of the gut and go into other tissues, into circulation.

Michele: You end up with inflammation in the gut, the colitis inflammation.

Michael: It's a fairly well defined model system that folks have been using to study the immunology of Crohn's and the immunology of colitis. So this is a well established model for which there is a lot of data in the open literature and a lot of immunological understanding.

Michele: And tools.

Michael: Absolutely, tools, too.

Vincent: So they can give mice DMichele:, hey will develop colitis, and then if they give them ginger exosomal RNAs they get protection against DSS induced mouse colitis. So somehow, these ginger RNAs are reducing the damage caused by DSS. They do some studies using germ free mice, which show that you have to have the lactobacillus in the gut for this protection to work. If you give germ free mice these ginger exosomal RNAs, it doesn't protect them against colitis.

Michele: I bet that was a great day in the lab when they realized this really is a connection between the food, the microbiome, and the host health.

Vincent: That's right.

Michael: Along those lines, when I was a young child and had an upset stomach which was likely due to an inflammatory condition in my stomach, my mother used to give us flat ginger ale. Now, granted, it didn't have any of these exosomes, but I wonder if that is how the lore of homeopathic remedies were passed down, is that previously before there was commercial ginger ale, people actually made a ginger extract that they gave.

Michele: Ginger tea.

Michael: Ginger tea, to effectively do the same thing. So what we used to do a hundred years ago, before the advent of modern everything, there may be a lot of wisdom in that traditional or folklore type medicine that we have for a lot of us, we have forgotten why we do what we do.

Michele: I think some ginger sodas are made with real ginger extract, not all of them but some. Craft sodas are.

Michael: Yes.

Vincent: Now when mice get colitis by feeding them DSS if you look in their intestines, what are increased are a number of what we call pro inflammatory cytokines and chemokines. These are immune proteins that are in part responsible for the inflammation typical of this colitis, include tumor necrosis factor alpha, IL1 beta, and in contrast, there is another cytokine called IL22 which is protective. It modulates the inflammation, and what they find is that in mice where they feed them DSS to induce the colitis and then they give them the ginger exosome RNAs, it reduces the amount of tumor necrosis factor alpha and IL1 beta, and increases the production of IL22. And if you do the experiment in germ free mice, remember where the exosomal RNAs are not protective against colitis, you do not see an induction of IL22. So this is really interesting, because IL22 of course is produced by the cells in the gut, but not by the bacteria, so we are feeding, we are giving exosomal RNA to the mice, it is taken up by bacteria and somehow this is influencing the production of cytokines in the gut.

Michael: In the same way we saw in the last paper that we discussed except that was in the nose and this is in the gut.

Vincent: That's right.

Michele: IL22 in particular in the gut tightens up the barrier so if you think of the cells that line our gut as a cobblestone, it pulls them in tight next to one another so things can't leak through. That's how it increases the barrier function so that you can't have bacteria and other things leaking out of the lumen of the gut and into space where they don't belong.

Vincent: So somehow the ginger exosomal RNAs are leading to this induction of IL22 which is protective. So how does that work? They do a whole host of studies involving metabolites.

Michael: Metabolomics.

Vincent: Metabolomics. This lactobacillus in particular, lactobacillus rhamnosus, LGG, it metabolizes tryptophan to an indole derivative, remember the indole ring is part of the tryptophan. One of them is called I3A, which is a ligand for the aryl hydrocarbon response pathway, which induces IL22.

Michele: Ah.

Vincent: A derivative of tryptophan made by the bacteria, I3A, is an inducer of IL22. And they go through a number of studies showing this to actually prove this in mice. And there is an alternative metabolite, indole 3 acetaldehyde, which is not protective. It does not induce IL22. They show that there is a gene, a mono oxygenase gene, in LGG. It is called YCNE. This is a key enzyme that catalyzes tryptophan to I3AM, it is the alternative metabolite of tryptophan which is not inducing IL22. Its expression is inhibited by ginger exosomal RNAs, and that is because one of the micro RNAs is probably targeting the mRNA of this mono oxygenase. So in other words, tryptophan is converted to I3A, which then is converted to IL22, because the RNAs and the exosome block the production of I3AM, which is the metabolite which is not inducing IL22. So the exosomal RNA is having a direct effect on the metabolism of the bacteria, which in turn can result in the cytokine changes in the gut.

Michele: If I could just interject, Vincent said that they did a series of experiments, the next block of experiments, this paper is packed.

Michael: It's a block!

Michele: There are 25 authors, 25 scientists contributed to this heroic effort and it has something for everyone. Molecular biology, biochemistry, histology, clinical trials. It's amazing.

Vincent: And I'm leaving a lot out because it would take us 3 hours. I3A, which is the inducer, they do a series of experiments to show that is in fact what is working. They add it to lymphocytes from the colon of mice to show that IL22 is induced and so forth.

Michele: They're very thorough, no stone unturned.

Vincent: It's really remarkable. So the protective role of these ginger exosomes is via induction of IL22 which as Michele said helps keep the barrier intact, and that is induced by a metabolite of the lactobacillus bacteria. Just really amazing. Just a few more things here. This is a very interesting one (laughs). So these lactobacillus, the LGG. Normally if you treat mice with DSS and give them colitis, these LGGs would migrate into the bloodstream, as Michael noted earlier. But when you give mice these ginger exosomes, the LGG do not migrate, or they migrate less frequently into the bloodstream. SO what's going on here? This is remarkable. And they can do labeling studies where they label the exosomes, feed them to mice, and look at LGG bacteria and they can see that they stay in the lumen and confirm all of this. Turns out that one of the microRNAs in these exosomes targets a pilus specific protein called SPAC. So it is downregulated. And this reduces the motility of the bacteria, basically. I'm skipping over a lot of experiments, here.

Michele: Some of our listeners may have heard of pili, these appendages that come off of bacteria and they mediate cell to cell contact or movement across surfaces. So it's amazing that they found one of the microRNAs in the ginger exosomes is directly inhibiting that pilus protein which therefore blocks migration.

Vincent: Right. So they can show that they make the specific micro RNA, they show it knocks down that particular pilus protein mRNA, they can show that that reduces migration into the blood.

Michael: Significantly reduced! I mean, it's just not a cause and effect, it was significant.

Vincent: It's very obvious, and there's less pilus protein on the surface. So again, you just feed mice this ginger exosome RNA, one of the micro RNAs in the exosome is downregulating this pilus proten in bacteria so that they stay in the gut. That's why they don't go into the liver even if there is colitis. It's amazing, right? So there is a whole bunch of different things going on here, but those are all the experiments that I want to talk about. This paper basically gives you some idea of what happens, why eating certain plants is good for you, essentially. Right?

Michael: You could spend an entire semester just dissecting this paper.

Vincent: Oh yeah. And talk about experiments that you could do next. We've talked about how the exosome composition restricts where the exosomes go. WE talked about how the micro RNAs effect particular bacteria, their metabolism, their motility via effecting bacterial mRNAs. So they end up by

talking a lot about using these exosomes as kind of probiotics to deliver orally micro RNAs to target gut bacteria for different things. Here we saw how they could target them for colitis in mice, but you could imagine if you have a dysbiotic gut, you might be able to target particular bacteria and inhibit them and amplify others. Obviously we have to do a lot more analysis to understand how to target them, but it is all doable.

Michael: In fact, if you think about it, you've all been to seminars hearing about silencing RNAs, and the big issue is how do we deliver them intact? Well, here we have mother nature's designed delivery vehicle, and we are just talking about the exosomes from plants that are working in our gut, but there are similarly exosomes that we can actually dispense into our bloodstream to get these things to other tissues. So as Elio would say, stay tuned. There's gonna be more about exosomes and how to get some of these silencing and activating RNAs out to effect change in our human condition.

Vincent: I wanna make two final points here. One is that they make the note that our gut is usually healthy, and that's probably because in part of what we are eating here in the plant material. They say through localized, so in our gut particular bacteria are close to the epithelium and the creation of stable plants help create a stable reservoir for microorganisms to persist in the face of rapidly changing conditions in the gut through localized immune facilitated and adherence dependent exosome selection, the host can maintain the stability of a diverse community of microbial symbionts. And then their last sentence, the concept that food derived exosomes are selectively taken up by the gut microbiome and host cells is relatively new in the field of gut physiology and health and we are just beginning to define the individual steps in this process.

Michele: They've certainly shown the way. It's exciting but it's also daunting. We only talked about a couple of the microRNAs found in one food, just ginger. And so, wow, there's a lot more to discover and then you could imagine trying to make just the right recipe to push the microbiome one direction or another, depending on the health.

Vincent: I guess like we said earlier, you recognize the beneficial value of certain things that you eat or drink but you don't really know why and now we are beginning to understand that.

Michael: We're getting the why.

Michele: So let me tell you a bit more about one of the two lead authors, Yun Teng, he is a PhD MD who is an assistant professor at the James Brown Cancer Center at the University of Louisville. So in the Zhang lab there, their goal is to use food as a source to identify new therapeutic nanovesicles that they could use to modulate the immune response for either tumors or different allergy type diseases. But he got his MD and his PhD at Tongji Medical College in China. He studied PhD as an immunologist.

Since 2004 he has been at the James Brown Cancer Center working with a large number of colleagues there. He describes his thought process that led to this experiment, we all appreciate now that food can change the microbiome in our gut and have important benefits, but one day the thought occurred to him that although the food is dead, its contents including stable small RNAs that we heard about today might retain biological activity. When we realize that the gut microbiome encodes more than three million genes and produces thousands of metabolites, he was really interested to see if any one of these could actually affect the microbiome and affect health. So he was looking at this cross-

kingdom regulation triggered by food. So he has 15 years experience in scientific research. We heard about some of his beautiful work today.

This is also a family affair, his wife is a neurophysiologist, she works at a hospital in the Louisville area, and they have two kids who also love science. Their son Tiger is a junior at NYU, he is majoring in psychology and pursuing a career in medicine. Their daughter Lucy is is a 6th grade student who was very interested in her dad's studies of food and impact on the good microbes in our gut. One day she said to him, I love yogurt, Daddy, you said lactobacillus is a good bacterium, but I also know that our stomach is acidic, and that would kill these good bacteria. So how do the good bacteria survive in our stomach when we eat them?

So Yun encouraged his daughter to really think about this and address it in a science fair project. So she went out and purchased three of her favorite brands of yogurt and then estimated the survival of the bacteria in acidic media. She was surprised to see that in the first 45 minutes in acidic media, the lactobacillus from the yogurt actually grow faster than when they are in media at a neutral pH. But after one hour, the growth of the lactobacillus was declined, or was reduced. So they thought about this and raised some interesting questions. Lucy is wondering if we eat yogurt on an empty stomach, then the lactobacillus in the yogurt would pass through the stomach quickly, get this acid bath, which would promote their growth. But if we eat yogurt with food, would the good bacteria then be in the stomach longer and actually inhibit their growth, or even kill the lactobacillus?

She is starting to think about not only what should we eat, but does it matter whether we eat it on a full stomach, an empty stomach, with other foods, etc. So those are questions beyond my expertise as a microbiologist, but I think it is really neat that he has been able to pursue these very molecular mechanistic studies of why certain foods have been used in Chinese medicine for as long as they had and connect it with probiotic studies and also engage his family and his young daughter in these questions.

Vincent: Nice! Love that story, love to hear the yogurt story, that's great. Thank you, Michele.

Michele: You're welcome.

Vincent: I'd like to read a couple of emails here before we wrap up, we have a little backlog, so let's get through a few. One is from Mark:

Dear TWiM-opods,

Congratulations on your successful, long running show. You have great quality and interact many ways with listeners. There is only one thing wrong with TWiM – it is a bi-weekly show. What would it take to make it a weekly event? Do you all think you could survive the fun and excitement of a weekly show?

Recently I made my annual trek from San Jose to Napa Valley, CA to pickup wine making supplies – two different primary fermentation yeasts (one for red, one for white varietals), and bacteria for secondary malolactic fermentation. This is as close as I come to doing anything microbial. Attached is a picture that might interest your listeners.

Actually, you're doing all kinds of things microbial all the time.

Michele: Very microbial. (laughs)

Vincent:

He sent two pictures of this place that he went and he says, so one of them is a picture of the actual room with the materials for fermentation and the other picture shows a copper bar in the tasting room of Moritzen winery. he host was well aware of copper's microbial properties. Strangely, he had not heard of TWiM, Dr. Michael Schmidt, or his research in using copper to limit hospital acquired infections. However he knows copper creates a safe, sterile bar surface.

The tasting room is located in the Dry Creek AVA area which is in the north western part of Sonoma Valley. Many of Mauritson's wines come from the Rockpile AVA which is further north.

Michael: That's good. It's a beautiful bar.

Vincent: I've never seen a copper bar, actually.

Michael: If you've ever been to the Hyatt next to the US capitol, they actually have a copper bar.

Vincent: I'd think it would be hard to keep it nice looking.

Michael: No it actually is not, if it is used a lot it stays clean because you are wiping it down constantly.

Vincent: Mark continues:

What is an AVA I sense your listeners asking. AVA stands for American Viticultural Area. These are distinct wine making area with unique microclimate, soils, and elevation. Each of these elements impacts the taste and character of wine.

Michele: Because it affects the yeast.

Vincent: With this long, meandering introduction I now come the my recommended listener pick of the week. It's a book dealing with practical aspects of microbiology. It is titled "From Vines to Wines" and it provides a overview of wine making spanning from where to plant though fermentation.

Its now in its 5th edition, though I have the prior one.

The weather here in Northern California is relatively cool for late summer. For about 10 days the temperature has not passed 80 degrees Farenheit. That's about 27 for those who measure in Celsius.

Bye.

Mark

Thank you Mark. Well you know for me, I love doing weekly TWIV, it's a lot of work, so my cohosts are comfortable with the biweekly show. I have some cohosts that are comfortable with a monthly show.

Michael: That'd be Dickson?

Vincent: Not Dickson, Nels Elde, I've said, why don't we go to twice a month? He said no, no, no. Justin writes:

Interviews like the one you had with Dr. Sam Sternberg are always amazing. It's so rare to grab someone who has the ability to speak about the history and future of a breakthrough technology. I would 100% read a book that details this.

Of course that is a couple of episodes ago, my interview with Sam Sternberg all about CRISPR.

Michael: Which is big in the news.

Vincent: Michael, can you take the next one?

Michael: I will. Brian writes:

Dear Elio, Michelle, Michael, and Vincent,

Thank you for the podcast.

I'm a faculty member in the Applied Math Department at the University of Waterloo in Waterloo, Ontario, Canada. I work on kinetic modelling of intracellular networks, with a current focus on bacterial genetics. I came to biology rather late in my career, and so I have significant gaps in my background. The TWIM podcast is an ideal way for me to gain broad exposure to ongoing research in microbiology.

I've especially enjoyed those occasions when you discuss papers that employ computational modelling. As you know, those tools are unfamiliar to a lot of folks working in this area. Outreach efforts like yours are valuable for helping people appreciate their potential, and also their limitations.

All the best, and thank you again for your efforts.

Brian.

Michele: That's a good point, this is a way we can recruit more computational people hopefully to our field and medicine, yeah, so many applications.

Michael: Medicine is going big because I don't know if you guys heard on this morning's NPR but in th Amazon city sweepstakes, we know that NYC and DC were big winners, but I didn't know that Nashville won 5,000 jobs and they were speculating on NPR this morning that potentially it had to do with health. Being the home of Vanderbilt University and Columbia HCA, they have a lot of healthcare

workers and big data is what Amazon does best. They may be coming to a town near you to think about big data for health. So computational efforts are sorely needed in every field in science today.

Michele: It's a path to personalized medicine, a term that gets used a lot.

Vincent: We have another one about the Sternberg episode, Johnye writes:

Entertaining and outstanding review and historical presentation of CRISPR-Cas for a lay scientist. Dr. Sternberg's responses to your guide questions was clear, clean and CRISP. Looking forward to reading the book.

Thank you Professor R.

Best to all.

Johnye Ballenger.

Michele: Ha ha!

Michael: So the question, Vincent, is you gotta ask uncle Ray if given the recent current events with CRISPR in the news, has that podcast seen an uptick in downloads?

Vincent: Could be, that was actually a very popular one. I can actually tell you right now, I have access to the stats, and we will do this in real time, it will take me less than 30 seconds. Typically when we release TWIM we get about 3 to 4 thousand downloads, it varies, depending on the time of year. And then over the next weeks we have another 7 or 8 or 10 thousand. On release, that episode got 4 thousand downloads, which is a high one, as opposed to the previous episode which got 2,500. So let's see the number on that one, CRISPR-CAS immune system has so far been downloaded 10,000 times, which is pretty high for the recent ones. And that includes 7,600 in September when it was released, 1,300 in October, and 780 in November. So it hasn't gotten a bump from this.

Michael: They haven't discovered it yet.

Vincent: They haven't discovered it yet, but they might. Johnye is in Boston, 18C with peekaboo showers and gusts of wind. Michele, can you take Maggi's, please?

Michele: Sure. She writes:

Dear Twimmers,

I am an avid listener of both TWiM and TWiEvO and I am a huge fan. As a graduate student at The Okinawa Institute of Science and Technology (affectionately referred to as OIST), I credit your podcasts with keeping me connected to the scientific community outside our tiny isolated island, but I never expected TWiM to connect me to science happening in Okinawa! I was both surprised and elated to learn about Prof. Yu Matsuura's work on microbial symbioses in cicadas on TWiM 183. The first conference I attended as a PhD student was the Animal-Microbe Symbioses GRC that Yu talked about

in the Atlantic article included in the show notes. I learned about cicada bacterial symbionts then, but of course the role of fungus wasn't public knowledge, and I had no idea Prof. Matsuura was in the process of discovering it right down the road at Ryudai (short for University of the Ryukyus). After listening to the TWiM episode, I immediately emailed Prof. Matsuura and invited him to speak at Nerd Nite Okinawa, a monthly science communication event I facilitate. Hopefully, he will educate us about all the microbial symbionts of cicadas after his busy fall field season is completed this year. Thank you so much for helping me make a new connection all the way in Okinawa!

Thank you also for your amazing podcasts! I only wish they were released more frequently (especially TWiEvo)!

Before I sign off, I'd like to share my work, as well. I am studying symbiosis in marine microbial eukaryotes for my PhD research and the first paper from my dissertation was just published: Intra-host symbiont diversity and extended symbiont maintenance in photosymbiotic Acantharea.

The Acantharea are the incredibly beautiful heterotrophic plankton made famous by Ernst Haeckel's intricate drawings. They are rather unique both because their endosymbiotic algae are haptophytes instead of dinoflagellates, like in coral and other radiolarians, and because their elaborate skeletons are made of Strontium Sulfate. My paper demonstrates that acanthodians are also unique in the diversity of their symbionts within single hosts. Basically, they are fascinating, but still mysterious, members of the marine microbial community. Perhaps this paper would make a nice snippet in the future, if you have another symbiosis episode planned. And she smiles.

Thank you again and very best wishes from Okinawa,

Maggi

Weather here is Hot (84F), Humid (85%), and currently a little overcast.

Vincent: Thank you, Maggi. PhD candidate there in Okinawa, wow. Has anyone been to Okinawa?

Michele: I have not.

Michael: My technician grew up on Okinawa, though. His father was in the military and one of his high school classmates was an individual who rode a boat around the world.

Vincent: It's pretty cool that we could connect someone working in Okinawa to someone else's work from Okinawa. That's sometimes how it happens.

Michael: And right down the road, so to speak.

Vincent: Alright, that's TWIM 190. You can find it on any podcast player that you use to listen to podcasts. If you do, please subscribe. Search from This Week in Microbiology and subscribe so you get every episode. For us, it gives us a number of subscribers. I just told Michael and Michele how many downloads the CRISPR episode got. We know that because people subscribe and as every episode is released and it is downloaded and those numbers come back to us. It helps us a lot so please do

subscribe. If you really like what we do, consider supporting us financially. You can go over to microbe.tv/contribute, a number of ways that you can do that. Send your questions and comments to TWIM@microbe.tv.

Michele: I was gonna ask, how are you doing with your YouTube channel subscribers?

Vincent: Let's take a look, I've made a plea to increase my YouTube channel, I've got 17,170 subscribers. We are still short of 25,000. I would like to get 25,000 so I can apply for grants to make educational videos, which is what we do. So go over to YouTube and search for Vincent Racaniello or profvrr, either way it works. Subscribe, it's free, and it won't bother you. You might even like the content. Sometimes we do videos of our content like live TWIMs, like the one we did this past summer, live TWIV. I often travel and do podcasts and do video, they're there. I have my lectures in virology there. We have some of my lectures in virology translated into Spanish. We have a whole series, 45 lectures on parasitism, and a lot more. SO there's a lot of stuff there, you might like it, and get us over 25,000.

Michele: Share that plea with your friends.

Vincent: Michele Swanson is at the University of Michigan in Ann Arbor, thank you, Michele.

Michele: Thank you both.

Vincent: Michael Schmidt is at the Medical University of South Carolina.

Michael: Thanks, everyone.

Vincent: I'm Vincent Racaniello, you can find me at virology.ws. Thanks to ASM for their support of TWIM and Ronald Jenkees for his music. This episode of TWIM was edited by Ray Ortega. Thanks for listening everyone, we will see you next time on This Week in Microbiology.

The things we eat, right?

Michael: Hey, I think this was great. We covered a very challenging topic and I don't think it was too over the head for folks on the radio.

Vincent: I don't think so, I took out all of the experimental details, it's too much.

Michele: I was amazed at how you were able to pick things out of that and create a clear melody because it was just packed, my goodness.

Vincent: It was hard to read because the paragraphs go on, they don't break them up… (fade out)

Transcribed by Sarah Morgan