This Week in Microbiology

With Vincent Racaniello, Elio Schaechter, Michael Schmidt, Michele Swanson

Episode 179: Viable but not culturable

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Vincent: This is TWIM, This Week in Microbiology, episode 179, recorded on June 21st 2018. 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: Well hello there, how are you?

Vincent: It was very good to see you in person!

Elio: It was lovely, it was lovely indeed!

Vincent: We had a nice little gathering. Also joining us from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello! Our time in Atlanta went too fast.

Vincent: It did. It is over before you know it. And from Charleston, South Carolina, Michael Schmidt.

Michael: Hello, everyone!

Vincent: I thought we had a good TWIM at ASM.

Michael: It was fascinating!

Elio: We were in vivo!

Vincent: In vivo, I thought it was the best one we did in vivo so far.

Elio: I figured, that's right.

Vincent: We are getting better. Not that the guests are not good, but we're just getting better at it.

Elio: It's fun to have a live audience in front of you.

Vincent: It's a lot of fun, yeah.

Elio: I thought it was stimulating. Lots of people.

Vincent: They had lots of people and they stayed and I like interacting with them, exactly. So thank you for coming, those of you who were there.

Michele: I also really appreciated that we had a little time afterward to talk one on one with a number of the people who attend. So it gives me a much richer idea of who we are talking with and who we are reaching.

Vincent: For sure, otherwise you don't know, right?

Michele: Right.

Vincent: You record, you release, who knows who is listening.

Michele: And it was energizing for me, so yes, I echo the thank you for everybody who participated.

Vincent: So today we have our usual science for you but I first want to tell everyone, I don't remember if I mentioned this on our last episode but we now are having transcripts made. These are being done by Sarah Morgan who is a recent PhD (actually I am a grad student!) from the University of Pittsburgh, and she offered to do it and so she is now working her way back.

Elio: Wow!

Vincent: I think there are five or so transcripts of TWIM and you can now read them if you can't listen, and they are searchable by Google.

Michael: Oh no! Searchable!

Michele: That's great.

Vincent: So I have them on a web page and as a PDF, so there are two forms. You can download a PDF or you can go to a web page, and of course the web page is searchable by Google so now it will be in the index of the world's knowledge. (laughs) Which I think is good, because of course podcasts can't be searchable because they are audio and Google can't make its way into those files. So thank you, Sarah, for doing that. We appreciate it. We have a snippet today that was suggested by listener Frank who writes:

Should be of interest to either or both TWIM and TWIV. What does it mean? (laughs) Best Regards…

Michael: That's a good question.

Elio: We won, we won.

Vincent: What does it mean. The paper is published in Nature Communications, it is open access, everyone can see it. It is called "Taxon specific aerosolization of bacteria and viruses in an experimental ocean atmosphere mesocosm." And the first two authors who contributed equally are Jennifer Michaud and Luke Thompson and then we have a whole slew of recognizable people here. We have Farooq Azam, we have Rob Knight, and the last author is Kimberly Prather and they come from UC San Diego.

Elio: All over the campus.

Vincent: University of Southern Mississippi, the National Oceanic and Atmospheric Administration, the Venter institute, Scripps Institute of Oceanography and here is one I just have to pronounce. Istituto Nazionale di Oceanografia e di Geofisica.

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Michele: Ooof.

Elio: Francesca Malfatti.

Vincent: (laughs)

Elio: She is a very nice person, I know her.

Michele: And smart.

Elio: Damn right.

Vincent: So this is all about the bacteria and the viruses that are in the ocean and how they get into the air, because as you know there is a lot of bacteria and viruses in the oceans and they go into the air, the surface of the ocean aerosolizes and that goes up into the atmosphere. The atmosphere contains between 6 x 10^4 and 1 x 10^7 cells per cubic meter bacterial cells, and huge numbers of viruses. These come from Earth of course an organism but from the ocean as well. And the spray that is generated is called sea spray aerosol, SSA. This can throw up bacteria and viruses and the bacteria have been documented to travel as far as eleven thousand kilometers from days to weeks.

Michele: That's amazing.

Vincent: It's amazing and again, they are aerosolized from the surface of the ocean and these are important because they can influence the climate, they can seed clouds, induce ice nucleation, they can impact air quality, they can seed new areas, and as the authors say we don't really know very much about this whole process.

Elio: But they're certainly frequent fliers, huh.

Vincent: (laughs) They are.

Michael: Well no, they're more than frequent fliers. They stay aloft because that's what air resonance time means is they stay aloft for days to weeks.

Vincent: Amazing, amazing. And of course, they also fall from the skies and studies have been done recently quantifying the viruses that fall from the skies, huge numbers are falling on an hourly basis and of course many of them aerosolize from the surface of the ocean. So that's what this paper is about, they want to investigate this.

Elio: You want to add that the bugs in the clouds participate in ice formation and cloud formation.

Vincent: For sure, totally.

Elio: So they effect the climate.

Vincent: Yeah. And so the SSA, this is made as bubbles burst on the sea surface.

Elio: Can you say what SSA means?

Vincent: SSA stands for sea spray aerosol. You can't buy it, you have to go to the ocean to get it.

Michele: (laughs)

Vincent: And it is caused by the bursting of bubbles on the surface of the ocean which is called the sea surface microlayer, that is the top 1000 microns of the ocean.

Elio: It's like the ocean is sneezing.

Michael: Yeah, that's exactly it! And if you have ever gone to the beach and parked your car overnight you can come out the next morning and you can feel the fine scum, if it is not dew it's dried onto the surface and this is the bacteria along with some of the other debris that have settled on to your car overnight.

Michele: And salt.

Michael: And salt!

Vincent: For sure. So this problem, what's in the surface and what goes off, it hasn't been studied much. There are a few studies where it looks like using cultivation it looks like not every bacteria in the ocean gets aerosolized. It looks like it is specific to certain taxons and so it's one of the things they want to study here. But of course, studying it in the ocean is really hard, right? Although as I'll say later, it's hard to duplicate the ocean, but you can't just go out and sample the same place over and over because the boat moves around and it is very hard to do the weather and so forth. So what they have done is they have made a mesocosm and they have studied it. This mesocosm is amazing, I'm gonna go--

Michael: Not anything ordinary.

Vincent: It is a tank, it is a big tank, it is a wave channel, basically. And it is one meter deep, 33 meters long, half a meter wide and one meter deep. And the water is about 0.6 meters. And this is filled with 13,000 gallons, sorry, 13,000 liters of ocean water collected off the Scripps pier in La Jolla.

Michele: Wow.

Vincent: Which Elio must know.

Elio: Of course.

Vincent: You must have been to the Scripps pier, right?

Elio: Well you can't really get into it but you can see it.

Vincent: So 13,000 liters, they filter it a bit to remove the grazing zooplankton and they put it in their tank and then at one end of the tank they have a machine that rotates a paddle and this makes waves, slow pulse waves. The waves break halfway through the channel and there there are ports that can sample the aerosols that are made. And they basically ink, they have this thing for 34 days or so and they sample aerosols constantly. They sample the top thousand microns and so forth of this water and they see what is going on with the bacteria and the viruses. They do metagenomic studies, they basically take their samples and they sequence them. So it's not culture based, it is metagenomic based. And they want to see what is there, it is thirty four day and they sample it several times. They have nutrient induced blooms, I guess they are adding nutrients at different times, right?

Michael: They say exogenous organic matter was not added.

Vincent: I guess they are natural blooms. Oh, here we go, bloom conditions were initiated by addition of F2 medium supplemented with sodium metasilicate. It is a common marine medium for algae cultivation that provides nutrients. So they induce these algal blooms and they are sampling it and so you can see what happens when algae bloom to the bacteria and the viruses not only in the top part of this water but in the aerosols that are produced by the waves that break bubbles that they are producing.

So this is an artificial system that is inside but it is the way that they can do this. And you are looking at the microbes and the viruses that came with those 13,000 liters of sea water. They find some interesting things which we will talk about and one of them is not every bacteria in the water gets aerosolized and bacteria are more enriched in the aerosol than viruses. Kind of interesting, two of the interesting things. Alright, so this is Pacific seawater, 34 days of phytoplankton blooms, they have this wave going and they collect the aerosols. What they do is they count cells, they count viruses by microscopy, epifluorescence microscopy, and then they do metagenomic sequencing analysis.

And now, a few things to tell you, first they can count the bacteria in this SSML, again it is an SSML stands for sea surface microlayer. So think of it as the top part of the ocean, and then the SSA is the aerosols that form. So when they see bacteria increasing in number in the SSML, they don't immediately get released into the aerosols, something else is controlling that. Virus particle concentrations that in the oceans are much higher than bacteria--

Elio: About tenfold, isn't it?

Vincent: About 5 to 25 times greater than bacteria.

Elio: And there are mainly phages, right?

Vincent: Mainly phages, right. And here there is only tenfold. But the average concentration of viruses in bacteria in their mesocosm was about the same but bacteria were preferentially aerosolized compared to viruses, which I found surprising because viruses are so small, you would think they would easily aerosolized, but there is something more than size as they say.

Elio: Counter intuitive.

Vincent: Counter intuitive.

Michael: Well they are not affected by the same gravitational forces as the bacteria is. They are that much smaller.

Vincent: Yeah.

Elio: But that would make them easier to aerosolize, you would think.

Vincent: You would think, but there must be other chemical, physical and chemical properties that are governing that, right? That's the interesting part of this paper that lots of curious observations. So then they take six time points and they take this, they take the bulk sea water, they take the surface and they take the aerosol and they extract the DNA and they sequence it so they get bacterial and viral metagenomic studies and so what do they find?

So they find a lot of alpha proteobacteria, flavobacteria, and gamma proteobacteria, these are both abundant in the seawater, in the top of the seawater, and in the aerosols. But not every bacteria in the sea water gets into the aerosol. That's the title of this is taxon specific aerosolization and this is the basis for that. The sequence analysis shows that there are far more different kinds of bacteria in the water than what gets aerosolized. They also find, we will go into that in a little bit, they also find things you don't normally find in sea water. They find an avian strain of E. coli and a novel strain of legionella.

Michele: Yeah, how about that.

Vincent: Yeah Michele, what did you think about that?

Michele: I thought a lot of things.

Vincent: Legionella drancourtii.

Michele: I mean, again they are finding the DNA so we don't know whether the legionella are surviving although they did say that it looked like it became more abundant over the course of this experiment so it is possible that under these culture conditions legionella was growing in their tank.

Vincent: So it might have been present in the ocean to begin with but in low amounts.

Elio: I must say, the whole thing is it depends a lot on that, but I want to believe that the tank is a real--

Michael: And it is an open system, too.

Michele: And I don't see what temperature the tank was, ambient San Diego temperature (laughs)

Vincent: 70 degrees Fahrenheit, right?

Michele: Yeah.

Michael: The water is probably closer to 50 degrees Fahrenheit.

Vincent: Yeah, but here, in this 13,000 liters would rapidly get up to ambient temperatures.

Michael: Oh, ambient temperature, you're probably right.

Vincent: So that is another thing is it is not as cool as the ocean. It is not as refreshed, right? So 13,000 liters seems like a lot but the ocean is going to be mixing billions and billions of liters so there is a lot of difference, but this is what you can do.

Michele: And they point out temperature also affects the aerosolization, so.

Vincent: It does, yeah. So anyway, these two interesting bacteria, avian E. coli and Legionella drancourtii, they say is consistent with increasing evidence for enteric contaminants in coastal marine waters.

Elio: Funny that you mentioned the temperature of the mesocosm. I don't think I, I'm looking.

Vincent: Keep looking. Most common viruses were phages, podoviradae and myoviridae, these are tailed icosahedral bacteriophages, they are very common. But they don't see a lot of different ones because they say the viral databases are not as well developed as the bacterial one.

Michael: That makes sense.

Vincent: There's just fewer sequences in the database. So then they look at bacterial aerosolization, they calculate an aerosolization factor, it is a ratio of the species that are in the aerosol divided by the species either in the bulk water or in that surface water, and they get this factor. So you can calculate it. And they say you would expect, if aerosolization was random you should see a Gaussian distribution of species in their graphs, but they don't. They see this spindle shaped distribution when you compare the aerosolization factors and they say this means non random patterns.

So which ones are being aerosolized? So, here are some interesting examples. These are bacteria with high aerosolization values. Rotococcus erythropolis, Cutibacterium acnes and Methylbacterium radiotolerans. On the other hand, flavobacterium indicum, Cellulophaga lytica and Erythrobacter litoralis had low factors. Now this doesn't mean anything to most of you and to me (laughs) to say these things. So what does it mean? They say basically genomes within the same class or order tend to have the same aerosolization profiles, although there are some exceptions, they are rare cases where a species departs from other members of its class. They say we don't know why this is true.

Michael: Could it be due to surfactants that the microbe is making?

Elio: They kind of say they do.

Vincent: Well, they speculate later on, I'll tell you that now. Surface, cell surface modifications, yes, so that could be. Also, C. kroppenstedtii lacks mycolic acids, it is missing hydrophobic cell envelope components and maybe that explains its reduced aerosolization. So the character of the bacteria could influence this hydrophobicity, right?

Michele: A classic example is Mycobacterium tuberculosis. Which can be aerosolized and persist in these little airborne droplets for a long time.

Elio: That's a good point, that's how it used to be spread.

Michele: It's the mycolic acid that confers that.

Vincent: That's right, they say an unusually waxy coat which may--in the end this kroppenstedtii has no mycolic acids which may be the reason it doesn't aerosolize. They say basically do some experiments, folks. Figure out why this is true (laughs)

Michael: We have given you the survey, now you all have to get to work.

Vincent: They give you the survey. Then the viruses that they looked at, viruses don't aerosolize as efficiently as bacteria as I mentioned. Even though they are present in the upper layer of the open at about the same levels.

Michele: Do you think they are bound to larger things?

Vincent: They could be, could be they are stuck.

Michele: Rather than them just floating by themselves.

Vincent: Yeah, could be.

Michael: Particulates.

Vincent: Particulates. And they say the size isn't the only contributor because it doesn't correlate with the size of the bacteria, the viruses. And also they see differential transfer of viral genomes into the aerosol, just like the bacteria not every virus is transferred. And here is some of the patterns they see. Non enveloped viruses are less enriched in the aerosol compared to lipid envelope species. So most of these phages are non enveloped but there are some enveloped viruses that they do detect, including eukaryotic viruses.

Elio: It is remarkable, isn't it.

Vincent: Yeah, I guess that's consistent with what Michele mentioned before about the waxy outer coat, so lipids have something to do with this. But exactly what, we don't know.

Elio: I was talking about finding eukaryotic viruses.

Vincent: Oh, yeah, they find a few, not too many eukaryotic viruses, right. But I think that may be...

Elio: Zooplankton is eukaryotic so they must have viruses.

Vincent: They do but again, I think the databases are not big enough to be able to do this.

Michael: And it depends on how they are filtered, excluding data that may not treat anything and it just the heap of dead data at the bottom of the pile that is, it doesn't go in to any thing.

Vincent: The other thing they find which is interesting is that some genomes are constitutively aerosolized. And others are intermittently aerosolized. So in other words, some of them are always in the aerosolized fraction and others are only now and then, which mostly I believe correlates with them being present at higher numbers in the surface of the ocean.

Elio: Some of our listeners may be confused by the term constitutive, all it means is steadily.

Michael: Yes.

Vincent: All the time, right.

Elio: Even if it's not needed.

Michael: You could probably do an experiment looking for isolating RNA from the same community at the same time and asking what genes are being expressed at that particular time if you're harvesting, and then compare and contrast, and if it is indeed a differential expression it should correlate with that transient evolution into the air column.

Vincent: Yeah. So I can imagine going forward, taking this mesocosm and make it sterile and then introduce individual bacteria and see which aerosolize, which cannot, and then do some comparative studies to try and figure it out. It would be great if you took one that did aerosolize and did a transposon mutagenesis and found ones that did not and see what genes are involved, right?

Michael: (laughs) You certainly could do that.

Vincent: But it's a big--

Michele: That's mind boggling considering the volume of their sample.

Vincent: It's big, it's a big petri dish here.

Michael: It's more than that because i have some experience aerosolizing bacteria when we were doing our air studies and the mere kinetic of taking a microbe out of the liquid environment and forcing them into an air environment, you lose like two to four logs of viability to the population when you do that phase transition, and it--

Elio: What bacteria are you talking about?

Michael: We were using bacteria and fungi, principally we used Serratia because we could detect it fairly easily because of the color change, and we were using some common--

Elio: Serratia makes red colonies on the agar, by the way.

Michael: That is indeed true at particular temperatures. We were also using some colored fungi that, we weren't using any of the nasty aspergillus we were using a common penicillin.

Elio: It may be that few bugs were sensitive and other bugs are less sensitive.

Michele: And as kind of a prelude to our second paper, Michael, was that all culture based?

Michael: It was all cultured, it was all culture based and that is indeed the prelude to our next paper.

Michele: It's entirely possible that they went into VBNC mode.

Michael: I think they do. I think they do indeed, and again we should point out that this particular paper is all nucleic acid based. We don't know whether or not any of these microbes in the air column were able to either grow colonies on a plate or whether or not they could have the potential to cause an infection in an animal or a plant or whatever it may be.

Michele: Right.

Vincent: Absolutely.

Michele: We know the DNA survived.

Vincent: Yes.

Michael: That's all we know.

Vincent: So this paper basically establishes the baseline. it says things like more bacteria than viruses are aerosolized. Only certain kinds of bacteria and viruses are aerosolized, and to answer Frank who said what does it mean, well, those taxa that are enhanced in the aerosol, they could have a bigger influence on climate, the

could have a bigger influence when they are spread, so you want to know why they are there. And I will read the last sentence of the paper which is often in most papers I find the last sentence or two really tells you what the authors are thinking. Elucidating the specific aerosolization methods of pathogens either from environmental reservoirs or from hosts themselves may form the basis of therapeutics that target aerosolization by interaction with surface features or inhibition of relevant biosynthetic pathways. Interesting statement, isn't it?

Michael: It is indeed.

Michele: Yeah.

Vincent: You think they're trying to inhibit aerosolization in the ocean?

Michael: No. That would be a fool's errand at the level of technology we currently have.

Vincent: For sure.

Michele: Wow. But that's exactly what my lab is thinking about now because Legionella is a water borne human pathogen, we get it by inhaling aerosols and there is very little mechanistic work on what allows one type of Legionella survive aerosolization and therefore be at risk to humans versus others that are common in the environment but not often seen in patients. So I would like to get at that question, too. So I was really glad that you chose this paper.

Vincent: Yeah, this is of interest to you then.

Michele: Yeah. But we will be working on a smaller scale.

Vincent: Yeah of course, you're not going to have 13,000 liters.

Michele: No.

Vincent: So the Legionella is typically aerosolized in a shower head, right?

Michele: Showers or...

Michael: Cooling towers.

Michele: Whirlpools or, yes, cooling towers, which are parts are elevated to cool air.

Vincent: So Michele, could you imagine if we could figure out how to prevent aerosolization in a cooling tower that we would have like a compound that we could add that would prevent it? Does that make sense?

Michele: It's possible but I think the more immediate step would be that we could screen for strains that we know survive aerosolization and then that would send an alert to the building operator whereas this one will not survive aerosolization so we would have a higher threshold of tolerance.

Elio: Given that the surface property of the bug may be involved in aerosolization, how about adding some detergent and seeing if it makes a difference?

Michele: Right, right.

Michael: Well the old fermentation microbiologists will tell you the easiest way to prevent foam in a fermenter, remember that fermenters spin, they have anti foaming agents and one of the easiest anti foaming agents that most folks have running around in their labs is you can use spent lipstick and lipstick will actually collapse a foam very very easily, and you can tell as to how good your bar's dishwasher is by virtue of the fact of when you order a beer whether or not it comes out with a real foam head or whether or not there is no foam left on the beer by the time it gets to your table, it tells you whether or not they are rinsing the soap suds away from that glass or if there was lipstick on the glass.

Elio: Good point.

Vincent: That's good.

Michael: You can see how well it is actually collapsed that foam because that is after all the first step--

Elio: Next time I am in a bar, I'll think of you.

Vincent: (laughs)

Michael: I learned that from my PhD adviser who was trained in the old days of fermentation microbiology and he told me the trick and we were at a bar.

Elio: It's plenty, I run a lot of chemostats and in chemostats that is absolutely essential so I did exactly what you are saying.

Vincent: Nice. That's a good tip, I notice that, Michael, beers are not all foamy, especially in the UK. Now we are gonna get emails from our--

Michael: Oh god, emails.

Vincent: But that's good. I will think of you, too, the next time I am in a bar.

Michael: Or having a beer.

Vincent: Yeah.

Michele: I am having this image of Michael's lab where everyone is walking around wearing lipstick. I'm very impressed.

Vincent: (laughs)

Michael: It was very impressive. It was very impressive. They were all carrying magnets at the same time because the lab was also working on magnetotactic bacteria.

Vincent: So Michael, just one more point here, so if you are wearing lipstick and drinking beer, the foam is likely to go away quicker, right?

Michael: Yes.

Vincent: That's really an interesting study, I will have to make that at some point in the future.

Michael: You just have to watch. You just have to, and it works really dramatically with Guinness. Because Guinness gets that nice tight foam head.

Vincent: Yeah. Thank you. So that is our snippet, thank you Frank for sending that, it is very interesting and thought provoking, I like it.

Elio: It was.

Michele: And ambitious, for sure.

Vincent: Extremely. And the next time you go to La Jolla Pier, or the next time I go and look at it, I will think of this study.

Michele: I guess we should say that there is no evidence that the microbes that are aerosolized from the sea are a risk to people.

Vincent: That's right.

Michele: So enjoy your summer holiday frolicking in the surf.

Michael: The sand and surf, yes.

Vincent: One last thought I just had, I would like them to do this with Atlantic ocean water and see if the results are similar.

Michael: Oh, that would be cool.

Vincent: They did it with Pacific water because Scripps is right there.

Michael: Are you just looking for a reason to go to the Jersey Shore and collect water?

Vincent: No, I'm not going to do this, this is not my thing, although it would be interesting to know what viruses are in it, but I think someone with an oceanographic bent should do it.

Michael: Chris Kellogg's listening, we'll put it next on her list of things to do.

Vincent: Well Chris likes the corals, which are deeper. But she might be interested in this. Alright, so that is our snippet. We now have a paper which is from Michael.

Michael: And the paper appeared in the 17 April 2018 issue of MBio and it was entitled "Viable but nonculturable Listeria monocytogenes and Salmonella enterica Serovar Thompson induced by chlorine stress remain infectious." And it was authored by Highmore, Warner, Rothwell, Wilks, and Keevil, and it comes to us from the University of Southampton in the UK. I first stumbled across this manuscript as it was a featured story reported by the Guardian newspaper on May 26 and I happened to be flying to England on that day and as often happens they have newspapers on planes and this was one of the stories I stumbled upon a day late.

And when you read the story, and I will put the story link in, and the Guardian is one of these newspapers that I don't believe has a paywall and you can actually see the whole story, you will appreciate why it is worthy of our attention here on TWIM and how it dovetails nicely into our discussion, it works into our last snippet.

In full disclosure I know the senior author very well, that is Bill Keevil and I value many of his varied contributions to microbiology. He is published on antimicrobial copper, legionella, and he is well known in the UK food safety world for his many contributions to the United Kingdom. And so the story in the Guardian newspaper concerned the chlorine washing of foods, specifically the controversial practice of cleaning used by many US poultry producers, that simply wash the chickens before they are slaughtered with a dilute concentration of bleach, typically between 20 and 50 parts per million. To give you an idea of the real concentration or how dilute that bleach actually is, it is effectively 25 microliters of household bleach per liter of water. So 25 microliters into a liter is for 20 ppm and 64 is for 50 ppm, so you can see it is not going to actually do much and it specifically is used by the US poultry industry because we grow our poultry in a more confined space and the washing is freeing the birds of contaminants such as salmonella and campylobacter, and so the Guardian reports on the data here in this particular manuscript that argues that this practice would not likely remove all the contaminants and in fact may give us a false sense of hope that the birds are actually cleaner than they truly are.

Currently chlorine washed chickens are barred from entry into EU on animal welfare grounds because the animals are washed while they are still alive, and so it has become a contentious issue for opponents of liberal trade deals with the US post Brexit. So here is where the Guardian brings in the work of Highmore and colleagues. It begins by telling us that previous studies with similar findings have been dismissed by the US poultry industry as producing laboratory only results with no relevance to the real world. So what Highmore and his colleagues do is they actually bring this all into reality and introduce us to this notion that viable but non culturable bacteria can indeed still remain very infectious and that this viability where we are asking the microbe to form a colony on a plate may be giving us a false signal.

So here is the story. Many of you recall that one of the easy triggers to send a microbe into this VBNC state is any form of environmental stress. One easy way to push the microbe into this viable but non culturable state is you can starve them, you can expose them to a little bit of UV light, and of course you can expose them to strong oxidants such as sodium hypochlorite which can trigger this condition. That has been known for many many years. And the routine way that the food industry determines whether or not a food product is safe to consume is to simply grind up the material, whether it be lettuce, chicken, hamburger, and ask whether or not you can recover a live microbial colony that you are concerned with on a petri plate. And of course, we all know there are colorimetric agars out there for E. coli 015787, salmonella, even for listeria now. But what should happen if the food that is routinely processed such that not all of the microbes are killed but some are rendered or transformed into this VBNC state?

Well, this is precisely what the food industry for the most part uses to assess for the presence of bacteria. They use a viable count asking for colonies to show up on a plate. So here is where Highmore comes into play. And they demonstrate when the sanitizer chlorine is used on produce, and so they specifically were looking at spinach. The pathogens that are routinely contaminating our greens remain albeit in a VBNAC state. They did this in a very clever way by asking if the common roundworm, C. elegans, were to eat one of these VBNC microbes what would happen?

And their hypothesis was that if the chlorine was effective you would see no or a limited effect on the worm. But if the viable microbes remained, that is the microbe that wasn't able to form a colony, could somehow still infect this animal or worm, they would effectively see an effect on the lifespan of the worm. And recall we have already discussed using C. elegans as a method for detecting bacteria and it was all based on age, we did that a couple of TWIMs ago, if I am remembering right. So they used two indicator strains, two common food borne pathogens, Listeria monocytogenes and Salmonella Thompson.

And food borne diseases present consistent but frequently preventable threats to public health and are responsible for an estimated 2.2 million deaths worldwide on an annual basis. Highmore talks about the issue in the United Kingdom where each year one million people will suffer a food borne illness with 500 deaths. Here in the US the CDC estimates that 48 million people in the US will get sick with 128,000 of them being hospitalized, and approximately 3,000 folks will die from a food borne disease each year in the US.

Michele: And Michael, if I could just interject, you mentioned the CDC, they maintain a terrific website that lists the current food borne outbreaks, their investigations that are currently ongoing, and then you can also look back in previous years and you can search it either by year or by pathogen. So if you are curious about what kinds of food different pathogens can contaminate it is a great way to look at that. You can look at the epidemiology. It is a wonderful resource for learning or I use it quite a lot for teaching.

Michael: It is fantastic, I was just coming coming to that. It is called the food borne disease outbreak and surveillance system, or FDOS, and we will put into the show notes the most recent publication that was in Emerging Pathogens and of course, we all know the most recent outbreak that we have had. We have all in the United States been for the last four months scared to death to eat romaine lettuce, where 5 people have died and 197 have fallen ill as a result of E. coli infections linked to romaine lettuce.

So there is ample evidence illustrating that lettuce and spinach provide an effective transmission vehicle to help these pathogens literally move from farm to table. Despite our lack of understanding in how to culture or switch a VNBC microbe to a culturable variant, it does seem that our own systems, our human cells, while we are unaware of how to change them back to an organism that can cause problems for us, our cells seem to know how to do it automatically. And in fact, in their introduction they point out that listeria has been found, VBNC listeria have been found that were avirulent when exposed to human adenocarcinoma cells but were resuscitated back to their virulent form by just simply passing them through an embryonated chicken egg and they regained or relearned how to be virulent in human adenoma carcinoma cells.

And similar results have also been observed with the other marker strain that these folks used and that was Salmonella Thompson, where the VBNCs were induced by UV radiation. So this is an old observation that VBNC can actually cause illness. We already discussed how chlorine is widely used to decontaminate fresh produce, both food borne pathogens and spoilage bacteria. And in fact, those of you that are fans of the prepackaged ready to eat salad mixes that literally are thought to open the box and put on the table, we know that occasionally they cause problems and in fact that is what caused the problem with the recent romaine lettuce scare.

Vincent: Michael, can you explain the viability assay, exactly what do they do? I know culturing is clear, they culture it on a plate, but how do they measure viability?

Michael: Okay. The way they measure direct viability is the two indicator strains that they have have a plasmid that expresses GFP. So the cells glow in the dark.

Vincent: If they are viable.

Michael: As long as if they are--yes Elio?

Elio: Is it a little bit of a stretch to see that the bug makes GFP, is that the same thing as saying it is alive?

Michael: Well, what they do is when they harvest it, and this is going deep into the methods, when they harvest it they spin them down, they incubate them under static conditions for a day, and then they ask the question,

are they still alive? And so it is an overnight assay, if you will, to assess in their direct viability assay whether or not they still are alive.

Vincent: Okay.

Michele: So many people will know that GFP is fairly stable as a protein so unless they are using a variant that is less stable, so...

Michael: Without getting too much into the weeds of the intact membrane in order to provide the energy in order to fluoresce the protein. And if you look at the method itself there is, where is the method, so in the method you specifically look and under the VBNC visualization of samples, the sample is first resuspend in a ml of PBS and to aid visualization of the samples the cells are given R2 broth which is nothing more than a little bit of peptone, yeast extract, some glucose, some starch, some phosphate buffer, and then there is pipemidic acid at a concentration of 10 micrograms per ml. And pipemidic acid is a quinolone and the suspension is incubated at 18 hours at 22 degrees in darkness. And so the suspension is then concentrated and this is a standard assay that they have used, it is the modification of the method that is pretty much standard for direct viable count measurements.

Vincent: They haven't invented anything is what you're saying, right?

Michael: No.

Vincent: So Elio, your comment. Would it be addressed by the finding that if they feed these chlorine treated non culturable but viable bacteria to worms, the worms die.

Elio: Well, yes and no. I mean, these are two separate tests. They come up with the same conclusion. Does it mean that is exactly the same measurement? No it doesn't, it is possible but it is very difficult to do this. It is not, I am not blaming them for not coming up with--

Michael: It's not trivial, they first teach us how to visualize the pathogen and they show us that the two pathogens in question can indeed adhere to the spinach phylloplane.

Vincent: This is after bleach treatment, right?

Michael: No, it is before. They first show us that the pathogens will stick to lettuce, or stick to spinach in this particular case.

Vincent: Looks like they are making a biofilm, right?

Michael: They are making a biofilm. And you look at the uninoculated control spinach leaves and the inoculated and they can see the unevenness indicative of the biofilm growth. Next they demonstrate how easy it is to transform microbes from culturable, where you can form a colony on a plate into a VBNC state. And this gets us into the weeds.

Vincent: That's the chlorine, there.

Michael: That's the chlorine there. And they show in a series of figures where they first incubate listeria or salmonella and chlorinated water. And they use a progression of concentrations of bleach from 0 to 15 parts per million and they first learn that it is 12 parts per million that is the triggering concentration for listeria, and at 3 parts per million is the triggering concentration for salmonella. When you look at their figure for their

VBNC or their direct viable count, you can see that the concentration of direct viable count cells effectively remain static across the concentrations used while the cells that are able to form a colony on a plate collapse very quickly.

Vincent: So let me understand this, so if you treat it with chlorine they can still adhere to lettuce.

Michael: Yes.

Vincent: That's what the gray bars are on that figure, right?

Michael: Yes, they are still adhering but they are not dead.

Vincent: And I guess they looked at that by the fluorescence that we showed in the first figure, right?

Michael: Yes.

Vincent: Okay, got it.

Michael: So the next series of figures that they do is they now adhere it to the lettuce or excuse me I have lettuce on the--

Michele: Spinach.

Michael: I haven't eaten lunch yet, so I am thinking of salad. So they adhere it to the spinach and here when the spinach, they subject them to the chlorinated wash, not surprisingly it takes more chlorine to trigger the VBNC state.

Vincent: Why is it not surprisingly on spinach?

Michael: Because the microbes are forming a biofilm and they form these extrapolysaccharide matrices and they can actually shield themselves from the chlorine atom that is coming in to inactivate it. And so here listeria is not triggered into being a VBNC state until 50 parts per million, and salmonella is being triggered at 100 parts per million, and the salmonella is able to form a more robust biofilm that can actually serve to protect it.

Vincent: Michael, would it be a reasonable experiment to take some of these and see if it has become culturable now? Take some of these leaves?

Michael: Off of the leaves?

Vincent: Yeah, grind them up and see if you can.

Michael: That's effectively what they are doing.

Vincent: And they are still not culturable, right?

Michael: They are still not culturable by virtue of the fact that they don't see any colonies.

Vincent: Got it.

Michael: When you look at the Salmonella Thompson, that is figure 5, since this is in MBio it's open access, when you look at the Salmonella Thompson plot you see the black bars are this ones that form colonies on a plate and as the concentration of chlorine increases the black bars go down. But what you see for the gray bars indicating that there is indeed some death associated with the viable but non culturable bacteria you see there is a diminution in the number of viable but non culturable bacteria.

Elio: There is another way, however, they could have done something else. The non culturable bugs are supposed to be alive by definition, the definition is they can resuscitate. They can come back to life. Not by putting it on regular media but by different manipulations. They could have done that and shown that the bug was actually alive, not just by stain but actually by making colonies. So I don't know what in the case of these particular bugs, I don't know what, how you resuscitate them, but there has to be a way because otherwise--

Michael: This is the next experiment.

Elio: No, wait a minute. Doing it with the worm is one thing, using something in a petri dish is another thing. I don't think it is quite the same thing.

Michael: You're partially on target because what they are using to culture the bacteria are selective and differential media, and we know that selective and differential media are very harsh conditions and it will always kill some fraction of a population. Again, they are measuring viability per mL as a function of exposure, so the selective media that they are using to know it is salmonella, to know it is listeria, are harsh media. So you will lose some bacteria. So if we follow Elio's logic, we should have tried to grow them on something very luxuriant in which we would get lots of different kinds of bacteria because spinach is of course not sterile and you would effectively have gotten the epiphytes associated with the spinach leaves.

Elio: Good point.

Michael: And so by using the selective and differential media they know that it is listeria on the listeria plate and they know it is salmonella on the salmonella plate by virtue of the selective and differential media that they used. So that is why I think they tried this gold standard experiment that I am suggesting to effectively try to rescue the VBNC listeria and salmonella by using the C. elegans worm, how long does the worm live assay.

Michele: Michael, before you go on to that, you mentioned biofilms were one of the protective forces of the bacteria while they are growing on the spinach, but they also pointed out because they did the microscopy that they could see the bacteria kind of burrowing down into cell junctions on the leaf.

Michael: Yes.

Michele: So they think the location in the leaf, too, might have conferred some protection from the bleach.

Michael: That is the other aspect that is very important. They were, in addition to doing colony counts and the direct measurement under the microscope looking for the green fluorescence protein, they were also looking at what was going on on the spinach itself. So when the same experiment, now we have the same paradigm, we have chlorine washed cells plus and minus spinach plus and minus putting them into the nematodes. And so now what we can see going on is--

Vincent: The nematodes die.

Michael: The nematodes die.

Vincent: But they don't culture them out of the worms. We don't actually know, we are assuming they are dying because they are multiplying, right, but we don't know.

Michael: Yes, we don't know.

Michele: Figure 7 addresses that a bit, but go ahead, Michael.

Michael: I was going to say, that is where they bring the salmonella images are extremely impressive in that when you look at the microscopy that they present in addition to the death kinetics, where they clearly articulate that the microbes can actually inactivate and my notes are doing funny things…

Michele: Figure 6 is very clear because it shows that the kill curve, if you will, of the worms is identical whether they were using culturable bacteria or viable but non culturable bacteria. The kinetics were no different so that was their main mathematical argument.

Michael: Yes.

Vincent: I guess you could also say, Michele, that the photomicrographs of the worms, that one worm on top is full of green fluorescence so there must have been some multiplication there, right?

Michael: Yes.

Michele: Right. And I guess the worm specialist was able to see that the bacteria had actually invaded kind of systemic, which you would need viable bacteria for that.

Michael: And the fact that it, when C. elegans is grown on E. coli it has a maximum lifespan of approximately 22 days. When they were exposed to either culturable or VBNC listeria, they died by day 16 with no statistically significant difference between either of the groups. When the same experiment was conducted using culturable salmonella, or the VBNC salmonella, all the worms that ingested the culturable salmonella were dead by day 13, and the VBNC salmonella were all killed, all of the worms, by day 15. Now this was statistically different. So again, showing that the worm wasn't quite perfect, the listeria were, there was no appreciable difference between the two of them but listeria and salmonella behave very differently and recall from the biology of listeria and the biology of salmonella, they behave very differently when in their normal infection cycle of eukaryotic cells.

Michele: But from a food safety standpoint, I wouldn't draw any distinctions.

Michael: No, no, no, no, no.

Michele: I would be concerned both about salmonella and listeria.

Michael: Yeah, and I was impressed and which really sold me on this particular assay is that the fact that the organism was still able to get into the C. elegans and that the C. elegans had a significant difference in its life expectancy. So when you put this all together they show that a very small concentration of chlorine can induce a VBNC state, and the routine concentration of chlorine that is used in the US to, if you will, prepare our prepared salads for us is about 90 parts per million.

And so we know what will trip listeria, we know what will trip salmonella, and E. coli is probably something similar in terms of concentration. Secondly, we know that this nematode assay may suggest whether or not there are potentially virulent microbes associated with produce, but the data do not support whether or not

the microbes will behave like this in people. We just know that it will be able to result in an infection in the nematode. However, there is evidence in the literature sufficient to suggest that the use of chlorine to decontaminate fresh produce is not only ineffective but actually permits virulent food borne pathogens to reach the public undetected. I mean, the most recent romaine lettuce excursion effectively showed us that. And so I think what this tells us overall is that VBNC is a much more common behavior by microbes than originally thought, and that the practices that we are using to keep our food safe, our monitoring efforts may actually be giving us a false sense of security. We may not know when something is heavily contaminated.

Vincent: So basically chlorine is useless.

Michael: Pretty much.

Michele: Ooh. Uh…

Michael: No, I think what the data shows, and if you look at the Salmonella Thompson figure, figure 5, is that the number, you are actually debulking the truly viable, the ones able to form a colony on a plate, concentration of microbes. And it is all about dose.

Vincent: So you are saying it is better than nothing, then.

Michael: It is better than nothing.

Michele: The first author, Callum Highmore, was a PhD student, this was a lot of his thesis work. He was an undergraduate in the biomedical sciences at the University of Southampton, and it was there that he became captivated by microbiology. He said he was learning a lot about different biomedical sciences but bacteria seemed so alien that he was drawn to study them in more detail. So his first study on bacteria was actually also published in MBio, and that, Michael, you referred to the work that he did looking at E. coli and klebsiella and found that they could transfer antimicrobial resistance on steel surfaces but not on copper surfaces, and so that was a 2012 paper. And then of course he turned his attention to food safety and trying to understand the microbiology on plants and our methods for trying to keep the food supply safe.

So he was really grateful to work with Bill Keevil, he said he was an excellent supervisor and was also really grateful for the assistance from his nematode tutor, William Scott who was a fellow doctoral student, also at the University of Southampton. He did admit that this project that we just talked about today really got traction when he was very close to his thesis deadline. So it was an intense period and when he finally saw these images that we talked about in Figure 7 where the fluorescent bacteria were filling the GI tract of the nematodes and getting into deeper tissues he had palpable relief knowing that this was not just an artifact, that they really had some biology.

So now that he has concluded his PhD or close to it, he is hoping to continue his training at the newly launched National Biofilm Innovation Center, which a number of universities are participating in, but it is being led by the University of Southampton. When he is not doing his research he enjoys painting, although he says that recently he has been preoccupied planning his wedding which is coming up this Halloween, October. So all the best to you! He also has some advice for junior scientists. He recognizes that it is very easy for us to get lost in our lab work or in your thesis writing, but he says don't forget to step out and enjoy yourselves.

Elio: (laughs) That's great.

Michael: And I think this paper is going to have a great effect on how we begin to try to figure out what is going on with our fresh produce and things like chickens and hamburgers when it comes to measuring whether or not they are indeed having a significant concentration of bacteria.

Michele: It also points to our need to understand the developmental biology of bacteria, especially the VBNC state so that if, for example, we had a chemical trigger that could resuscitate bacteria then people doing the food screening could first treat their samples with that inducer of cell cycle and then use the culture based methods. So I think there is a lot of research needs here.

Michael: Yeah. The food industry employs a large number of microbiologists and if the viable count business isn't working, the reason they like it so much is it is so fast. You get your results overnight, literally, so that you can release a lot of food and the lots of food are often measured by the rail car length. They use a very sophisticated statistical model to sample so much lettuce or so many hamburgers or so many chickens to determine whether or not they can actually release that to the public. We've all heard how much food is actually recalled and then destroyed based on some of these assays.

Michele: Michael, I'm puzzled because in the clinical microbiology world, they are shifting pretty dramatically to molecular methods of diagnosis rather than culture based. So what is the difference, why PCR based methods?

Michael: I think it is the cost and the fact that the diagnostic industry has not yet caught up with the food industry.

Vincent: I think also if you use the sensitive diagnostics you would find tons of microbes with everything, right? You wouldn't know what to make of it.

Michael: You couldn't separate the forest for the trees. And they are nucleic acid based, and as the Guardian story pointed out, the poultry industry or the food industry is very much concerned about how we interpret VBNC, because if they are not able to form a colony do they indeed represent a risk? And that is the study that has not been done where we know how many viable bacteria are on this piece of lettuce and how many VBNC are on this piece of lettuce and if you eat both which one will make you ill? Because that is the whole complexity of the human digestive system. So it is very big business and there is a lot of science involved in it and it is not as simple as doing a viable count like we learned as first year graduate students.

Michele: So what advice can we give our listeners? Wash your produce?

Michael: Wash your produce, because the solution to all pollution is dilution. And we know that by vigorous spinning in the salad spinner, the kinetics of water and spinning can actually displace the bulk of the microbes. If the lettuce doesn't look good, you will pitch it because a lot of the epiphytes that are growing on lettuce will damage the leaf or damage the spinach and so you can actually tell whether or not it likely has growth on it by virtue of the fact if the plant material has indeed been wounded. But for the most part, the FDA has done a remarkable job along with the USDA ensuring that the food supply, considering how much food is consumed each day in the United States, is relatively safe. We only hear occasionally of these food borne outbreaks.

Vincent: It's true.

Michael: I have no worries about buying produce or buying chickens or buying hamburger in the grocery store because we do know that the FDA and the USDA are indeed there protecting us from these unintended consequences.

Vincent: Michael, that first email is relevant to our discussion, why don't you read it.

Michael: Okay. Let me pull that up. Kevin writes:

Dear TWiM team:

I am enjoying very much TWiV (almost finished Virology 101), TWiP, and now looking at TWiM.

Every time I make bread I read the warning at the top of the flour bag that says, “flour is raw, always cook before sampling….” This prompts my correspondence.

I searched the TWiM site and didn’t see anything about the surprising (to me) flour associated E. coli outbreak last fall. I followed up on a few of the references in this article, some rather arcane food science reviews about residual water in ‘dry’ products etc etc. This reminded me of older E. coli outbreaks (sprouts I think), where I followed some obscure horticultural references that researched the ‘transmission’ or carriage of E. coli within seed capsules, mimicking vertical transmission. It would be good to learn more about these atypical or unexpected modes of transmission/spread of E coli and bacteria in general, those mechanisms that go beyond the parasitologist’s dictum (Norman Stoll) that ‘the path from the anus to the mouth is all too short.”

You know, I think he is absolutely correct, and I think the two papers that are on this TWIM first about how easy it is to aerosolize microbes from liquid to the vapor phase in concert with this whole notion of VBNC really drives home that you gotta cook your food if it is intended to be cooked because the microbes can remain if they can hold on to their water and if they can hide in something as delightful as a seed or in a sprout, and I think I have been accused of saying that in food poisoning the first thing I always suspect is the sprouts, because they have high water content and again are often washed with chlorinated water.

Michele: And we don’t put them in our salad spinners, either. They’re hard to clean.

Vincent: Yeah, very hard to clean.

Michele: With the flour outbreak, I think often that it’s the case that it is the equipment that does the processing, it’s the equipment that gets contaminated, and then it gets spread throughout the whole batch and sent to many different states?

Michael: That’s indeed what we have seen for everything from flour to peanut butter, just grinding up peanuts to make peanut butter. You effectively are putting in a dry product and because of the high oil content in will support growth of microbes and if it is not debulked periodically it will support the growth of a biofilm on the grinder that will then shed its microbes into the product mix.

Vincent: Interesting. Flour, huh. I hadn’t heard that one.

Michael: I did, but I knew it wouldn’t get past the TWIM test of Dr. Schaechter.

Vincent and Michele: (laughs)

Vincent: It’s one of the few pathogens that can contaminate flour. There was a big outbreak in 2016, multi state outbreak traced to a flour production facility.

Michael: And that was in the New England Journal of Medicine.

Vincent: It was! Yeah, it is, it's an EJM paper, that's right. Alright, well thank you for that. Michele, can you read the next one?

Michele: From Neeraj? She writes:

Dear TwiModulators,

I am writing to thank you for the discussing the intriguing study and the amazing discussion about the Colanic acid, that was presented in TWiM176. I hadn't heard about this Polysaccharide before, so this was a great way to get introduced to a totally remarkable molecular facet of E.coli biology. As was mentioned during the podcast, I am also totally intrigued by the idea about the age dependent level of expression for Colanic acid in mammals. Btw, do we know if there is a prominent effect on longevity of lab animals, which have been treated with antibiotics, compared to the ones that haven't? I am personally not aware of that but was wondering if it would be interesting, to see the effect on longevity of feeding Colanic acid to antibiotic treated animals. Anyways, these are just a few thoughts that come to mind! Mechanistically and functionally, the complexity of host microbiome interaction, just totally boggles the mind. Clearly, we are in the infancy of this field, but I am sure, as more and more rational scientific evidence mounts, we will see a whole different side of the human physiology. Please continue the great podcast as I am sure, you have a huge audience out there that regularly listens but only sporadically emails!

Best,

Neeraj

P.S: Thanks also for the discussion about the antiviral role of Neomycin, a widely used antibiotic

So she is a scientist, too, at SutroVax Inc. A PhD herself.

Vincent: I think there have been some studies in C. elegans of the effect of antibiotic treatment on longevity. I don't know much about it though, but there is literature.

Michele: Yeah, and of course that is a good lab model because they have a short life span. It's a little harder to do with people (laughs) or mice.

Vincent: In this paper, I think you weren't on the episode for this one, they tried to figure out why, I think C. elegans has a microbiome of E. coli only and it turns out that synthesis of colanic acid was what makes them live longer. Colanic acid produced by the E. coli.

Michael: So that's where we learned about C. elegans' lifespan, it was TWIM 176.

Vincent: Right.

Michele: Yep.

Vincent: Alright, one more, it is from Leandro who writes:

Dear Vince, Elio, Michael and Michelle

My name is Leandro Lobo, I am a professor at the medical microbiology department at the federal university of Rio de Janeiro, Brazil (universidade federal do Rio de Janeiro).

I am a big fan of podcasts and the twim, twiv, twip series have been in my devices since forever! I love your shows, and you actually inspired me to start my own microbiology podcast with a group of professors from my department. Our podcast is called “microbiando” (something like microbying in english) and much like yours, we talk about new papers and findings in microbiology and immunology. We also have a section called “microliter of news” where our students contribute with short pieces, and another one called “phylogeny of science” where we talk about famous microbiologists from the past. Our first episode was on an article describing the effect of the fruit fly microbiome on longevity, so, when I listened to twim 176, I knew I had to write you! If we are picking up similar papers, it means we are doing something right!

To my knowledge, this is the first podcast dedicated to microbiology and immunology in Portuguese. We have released 4 episodes and planning to release a new one every 15 days. Thanks for being such an inspiration!

Your fellow podcaster,

Leandro

Good for you, congratulations!

Michele: That’s great.

Vincent: We do need podcasts in science in different languages because here on TWIM we only speak in English.

Michael: And it makes it approachable for everyone and there are a lot of Portuguese speakers in Brazil.

Vincent: Brazil and Portugal, of course. Someday, there will be software that instantly translates every podcast into any language you want. That will definitely happen.

Michele: And speaking of different languages, I realized that I misspoke. Neeraj is a male scientist, I think I used the wrong pronoun. So, my apologies Neeraj.

Vincent: Thank you, you’re right, he has written before.

Michael: Back to Vincent’s comment about artificial intelligence being able to simulcast podcasts--

Vincent: I think it will, so you will be able to listen in whatever language, for sure.

Michael: I heard a statistic that in the last two years our advances have been so great in artificial intelligence as to surpass the previous 20 years of evolution in AI.

Vincent: It’s amazing. It’s going to happen and then you can listen to TWIM in any language, you just push a button.

Michele: Or take our transcripts and translate them into other languages.

Vincent: Could do that too, right.

Michele: Thank you, Sarah. (:))

Michael: Yes.

Vincent: Alright, that's TWIM 179, you can find it at Apple Podcasts, formerly called iTunes, and if you want to play it on your phone or your tablet there is usually a little app that you use on iPhones, it's called podcasts, I don't know what it is on Android but there is something for them as well. You just subscribe. Search for TWIM, make sure it is This Week in Microbiology, subscribe to get every episode as we release them, and why not do it? It's free. If you really like what we do, consider becoming a supporter. You can go to microbe.tv/contribute. We have a couple of ways that you can do that and it allows us to do many things like new kinds of shows and travelling and so forth.

Michele: And support a transcript writer and continue transcribing.

Vincent: Our transcript writer! That's right.

Michele: Yeah.

Vincent: Support our transcript writer, who we are paying modestly for each transcript, but that is where the funds go, to something like that, and we can be flexible with your support. And send your questions and comments to TWIM@microbe.tv. Elio Schaechter has left, he had an appointment, but he is at the wonderful blog Small Things Considered. If you haven't read it, check it out, it's really good. Michele Swanson is at the University of Michigan. Thank you Michele.

Michele: My pleasure.

Vincent: And your semester must be over, right? Long over.

Michele: Oh yeah, I was teaching medical students and that's over, too.

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

Michael: Thank you!

Vincent: You have a special propensity for food microbiology, don't you?

Michael: I like to eat. I think everybody likes to eat.

Vincent: And today we learned that you like to drink beer, too.

Michael: I like to drink beer occasionally.

Vincent: (laughs) And make sure your beer foam is frothy, otherwise the glass is dirty.

Michael: Otherwise the glass is dirty.

Vincent: I'm Vincent Racaniello, you can find my at virology.ws. I would like to thank the American Society for Microbiology for their support of TWIM and Ray Ortega for his help in production and Ronald Jenkees for the music you hear on the program. He is at ronaldjenkees.com. Thanks for listening everyone, we will see you next time on This Week in Microbiology.

(music)

Transcribed by Sarah Morgan.