This Week in Microbiology

With Vincent Racaniello and Elio Schaechter

Episode 166: Dark fermentation

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Vincent: You are listening to TWIM, This Week in Microbiology, this is episode 166 recorded on November 30th, 2017. Hi everybody, I am Vincent Racaniello and you are listening to the podcast that explores unseen life on Earth. Joining me today from Small Things Considered, Elio Schaechter.

Elio: Well hello there, how are you?

Vincent: I'm well, how are you doing?

Elio: Okay. Hanging in there, ready for the holidays. I live in California, some of the listeners may know, but I'm going east in Boston and Washington DC for family reasons. I'm going to go to my old haunts, I used to work at Tufts University and Medical School, so they asked me to come and give a talk and give a talk based on materials from the blog. Call it tales from the blog.

Vincent: Tales from the blog, that's cool.

Elio: It's a little different than talking about research.

Vincent: Yeah.

Elio: But it's fun.

Vincent: That should be fun, enjoy that.

Elio: Oh, yeah, I'm sure it will.

Vincent: Well, Elio, today it's just you and me.

Elio: Hey, we better be good, we better be good.

Vincent: Yeah, we always have good support, so let's see how you and I, we're gonna do a snippet and a paper and some email. This was actually a paper that was picked by Michael Schmidt, too bad he's not here.

Elio: Too bad he can't be here.

Vincent: To participate. This is a paper published in PNAS, the Proceedings of the National Academy of Sciences, the title is "Contemporary H3N2 influenza viruses have a glycosylation site that alters binding of antibodies elicited by egg-adapted vaccine strains."

Elio: It's quite a mouthful, isn't it?

Vincent: It's quite a mouthful, for sure. And we have, let's see, we have the first author here, Seth Zost and the last author, the PI, is Scott Hensley. And this comes from the University of Pennsylvania, the University of Chicago, and the University of Rochester.

Elio: That's quite a team.

Vincent: So we thought we would talk about the flu vaccine because we are coming into the flu season right now, and influenza virus can be a serious illness, that's why we vaccinate against it.

Elio: It is a fascinating illness.

Vincent: Yes, it is fascinating. We'll get into some of that here. We update the vaccine routinely, not every year but we look at what strains are circulating and try to match the vaccine with it on a yearly basis, and some years it changes and some years it doesn't. And this paper is all about a story back in 2014, 2015. During that season, the vaccine did not work very well, even though we had thought we had guessed. So the way this works is in January you have to start guessing which strains you are going to pick to put in the vaccine which is going to be released in August of that year.

Elio: Don't they look for how it operates elsewhere, in the southern hemisphere, with influenza happening earlier?

Vincent: That's right. So for the northern hemisphere, they start in January, because their winter is coming in the fall, and for the southern hemisphere it's six months, so.

Elio: It comes in the summer.

Vincent: Yeah, in August, actually, that's right, and people start getting immunized in August, September and the flu season goes to about May in the northern hemisphere. And it's the opposite in the southern hemisphere.

Elio: Don't be shy about explaining the background to this, because this is not This Week in Virology, this is This Week in Microbiology, not all our listeners are excellent virologists, okay.

Vincent: That's right. Okay, so we have, so flu is a disease that occurs every year, in temperate climates it occurs on a seasonal basis, so the northern and southern hemisphere have winters at different times so you have different flu seasons. It occurs every year, and we make a vaccine, we try and match the vaccine to whatever strains are circulating. So back in 2014, the match was not good and the vaccine was not very protective, and this paper is all about trying to understand why that is. What happened was back in 2014, one of the components of the vaccine, the vaccine has three or four different viruses in it, one of them is called H3N2. That's a way of designating a strain of influenza virus. H3N2, back in 2014 apparently the strain changed, and what happened is these viruses have glycoproteins in their membranes that they use for various functions and the main one is the hemaglutinin or the HA. And that strain in 2014 of H3N2--

Elio: The hemaglutinin helps the virus stick, doesn't it?

Vincent: The HA helps the virus stick to cells and get into cells, absolutely. And when you vaccinate people against flu, most of the antibodies that protect you are directed against this HA protein. It's a very important one.

Elio: In other words, it keeps the virus from sticking to the cell.

Vincent: Yeah. So the virus changed in 2014, it was a single amino acid change in the HA that we think was responsible for the poor vaccine efficacy. So a couple of years later, we updated the vaccine to include this new strain, but it still didn't protect very well. In the 2016 2017 flu season, the efficacy was still terrible. So what's going on here?

Elio: How unique is this? In most antigenic change that takes place with influenza viruses year after year, I know it's an RNA virus and RNA viruses like to mutate fast. Is this unique among RNA viruses or what's the story?

Vincent: So it is unique in the sense that it's a virus that causes global infections every year and it also undergoes variation every year. There are other viruses that do this similar thing, for example HIV in an individual person undergoes enormous antigenic variation during the course of infection. And there are other viruses that can vary as well, but I think a nice contrast would be measles virus, which is also an RNA virus, which as far as we know it doesn't undergo any significant antigenic variation. The vaccine that was developed against measles in the 1960s is still working today.

Elio: Right. Does this have to do with the segmented genome?

Vincent: In part. So when you have a major strain emerging every 20 or 30 years of influenza, that is because the segmented genome of the virus allows for what we call re-assortment. When two different influenza viruses infect the cell, all the different RNA segments can mix together and the viruses that come out can have combinations of segments and so if you have an animal and a human influenza virus infecting a host, you can imagine new strains come out and periodically those will spread globally. But the year to year—yeah, go ahead.

Elio: How common is the segmented genomes among RNA viruses? Is it something unique to this kind of virus? Or do you find it elsewhere? And by segmented we mean that the genome is not in one molecule, it is in several molecules, right?

Vincent: That's correct. It's in several pieces and it's not unique, there are other viruses that have segmented genomes. There can be several segments, there are even heliobacteriophages with segmented RNA genomes.

Elio: No kidding, I didn't know that! Neat. Learn something every day.

Vincent: So there are influenza viruses that have single stranded segmented genomes, there are viruses with double stranded segmented genomes like the reoviruses, so there are plenty of them out there, for sure.

Elio: And this leads to a sort of possibility of recombinance like in chromosomes, right? In higher cell having chromosomes.

Vincent: Yeah, if two different influenza viruses infect a cell, they can mix.

Elio: Sort of Mendelian, right?

Vincent: Yeah, and the offspring will have a mixture of the two, exactly.

Elio: Right, right.

Vincent: So that's one of the reasons why we have a lot of variation with influenza virus. But the year to year variation is due to single amino acid changes mainly in the HA glycoprotein, and that's what happened in 2014. A single amino acid change that caused a mismatch between the vaccine and the circulating virus. The conundrum here is we did update the vaccine in 2016 but it still did not protect, so that's the puzzle that we are going to address here. As background, two things we need to know. First of all, the single amino acid change that occurred in the H3N2 virus in that 2014 season, single amino acid change, introduced a new glycosylation site in the HA.

Elio: Uh huh, right.

Vincent: So a glycosylation site is an amino acid where enzymes in the cell will attach chains of sugars. In terms of influenza virus, these sugars can actually block sites on the HA to which antibodies will bind. Where antibodies bind on the HA are called antigenic sites, and so the idea is this new sugar site blocks some crucial antigenic sites on the HA. The other part of this puzzle which is really interesting is most of the flu vaccines we make in this country are grown in chicken eggs.

Elio: These are fertile eggs, right?

Vincent: Yeah, they're fertilized 10-12 day old embryonated chicken eggs. You inject them with influenza virus, it grows very well, and then you can harvest the amniotic fluid.

Elio: Where do they grow, in the embryo or in the amniotic fluid?

Vincent: They grow in the allantoic membrane. There are cells in the membrane, they grow in that, yeah. You get very nice production, it's very easy to purify the virus. So that's one of the reasons that eggs are used, and they've been used for many years. It's kind of an old technology. But it turns out that if you take an H3N2 influenza virus with this mutation that causes the addition of a glycosylation site, and you grow it in eggs, the mutation goes away. You lose the mutation so you no longer have a glycosylation site. And so this is probably why the vaccine did not protect in 2016 because it had lost the mutation that was in there to try and match it to the circulating strain. Is that clear, Elio?

Elio: Yeah, but you might repeat it. Say it again.

Vincent: So remember, in 2014 the H3N2 virus changed, it acquired a glycosylation site. So two years later we say okay, let's put this new virus in the vaccine.

Elio: And what does that do, why did the glycosylation site matter?

Vincent: That's the subject of the paper, we're gonna get into that.

Elio: Right, okay.

Vincent: And so we grew up the vaccine in eggs but the problem is when you grow that virus in eggs the glycosylation site is lost. So now those are the background informations, and the question that you have is the right one. What does this do? Why does this make the vaccine not work well? So what they did in this paper is they constructed two influenza viruses, two H3N2 influenza viruses. One that has the extra glycosylation site in the HA, and one that doesn't have it. So these are two otherwise identical viruses, one with the extra glycosylation site, one that lacks it. They did a number of very straightforward experiments with these two viruses, and we'll go through that and then we'll make the conclusion.

First thing, they have a panel, 26 different human monoclonal antibodies that were raised against an H3N2 virus that does not have the extra glycosylation site. And so a monoclonal antibody is binding to a very short sequence of amino acids, 8 or 12 amino acids on the HA, and they simply asked, we take our two viruses, how do these antibodies bind to them? So again, the antibodies are made against a virus that lacks the glycosylation site. Well, they found that most of these antibodies bound the virus without the glycosylation site. And that makes sense because they were raised against it, but very few of the antibodies bound the virus that had the glycosylation site. So that sort of suggests right away that this glycosylation site is blocking the site that the antibodies are binding to.

Elio: Keeping it from binding.

Vincent: Exactly.

Elio: Do you think this is a clever thing of the virus to do, to mutate that way?

Vincent: Absolutely. It's one of the things, yeah, you can imagine that the virus can either change one of the amino acids that the antibody is binding to to escape, or it could simply add a glycosylation site which will shield the amino acid to which the antibody binds. So either strategy works, and it's very clever, yeah. So next they infected ferrets, which is a model for influenza, with these two viruses.

Elio: Ferrets are kind of like, oh, what's a ferret, actually.

Vincent: A ferret is, is it a rodent? Let's see.

Elio: I think it's a rodent.

Vincent: A ferret is domesticated, it's sort of like a weasel.

Elio: Oh yes, a long, long--

Vincent: It's a very long animal.

Elio: Flexible.

Vincent: It's a furry animal, you can bend them, they have a long-ish nose, and they are a good model for influenza. Some people have them as pets, actually. They have very sharp teeth, you have to be careful. They are mustelids. They are in the family mustelidae.

Elio: Are they rodents, in fact?

Vincent: No, I don't believe they are rodents, no.

Elio: Okay.

Vincent: Mustelidae, if you would like to know what that is, is a family of carnivorous mammals including weasels, badgers, otters, mink, and ferrets. Those guys.

Elio: Now I know, I've learned something.

Vincent: So ferrets are used to study flu, so that's why they use them here. They infect them with these two viruses, one have the glycosylation site, the other does not, and then they take the antibodies, they take serums of this viruses and they ask which virus they can bind to.

Elio: They are from the ferrets?

Vincent: Yes, from the ferrets, right. So if you take antibodies from ferrets who were infected with the virus with the extra glycosylation site, they recognize that virus way better than viruses that don't have the glycosylation site. So it's kind of a similar conclusion from the human studies except in this case we are infecting animals directly. So the idea here is that putting a sugar on the HA blocks an antigenic site which is probably a major one. When you infect animals, either humans or ferrets, with viruses that don't have a glycosylation site on the HA, most of the antibodies are directed against this site. When you put the sugar on, it blocks it. SO that's why a vaccine that doesn't have the sugar, most of the antibodies you make will be against this antigenic site, and then if the circulating strain is hiding that site you won't be protected. So that is the basis of this. Now, they did do some studies in humans, they took serum from humans who were immunized during the 2016 influenza season, and the most of them got vaccine grown in eggs, so their antibodies did not react well with the circulating virus with the glycosylation site. But some sera reacted very strongly with H3N2 viruses that had the glycosylation site. It turns out that those individuals received a vaccine that was not grown in eggs. It was grown in insect cells.

Elio: Baculovirus, right?

Vincent: That's right, baculovirus. In insect cells, you actually just produce the HA protein, so there is no opportunity for the mutation to revert, so these vaccines have the glycosylation site in the HA. And so they are quite protected, those individuals.

Elio: The baculovirus cell, the insect cells can be grown on an industrial scale, obviously.

Vincent: Yeah, absolutely. Now, the insect grown flu vaccines comprises only a small fraction of the vaccines that are grown.

Elio: The reason being?

Vincent: To grow cells in a fermenter, it's expensive and it's difficult to grow hundreds of thousands of liters of cells, and eggs of course are simple. You have a prepackaged animal (laughs), right.

Elio: Drill a hole in the shell and you've got it.

Vincent: You've got it. Drill a hole in the egg and you infect and then some days later you harvest, it's really easy. You don't need fermenters. So it's a matter of convenience and cost, but first of all the conclusion here is that the virus does change and some times the vaccine, even if you make a vaccine to match it, by growing it in eggs you are losing the difference. Growing it in eggs was the culprit here, we lost the glycosylation site mutation. Why it does that in eggs is a good question, I don't know why you would lose that glycosylation site.

Elio: I wonder if it is specific to the species that makes the egg, do duck eggs do the same thing as chicken eggs?

Vincent: That's a good question, yeah. No one has checked.

Elio: Because you could make duck eggs, you could make a lot of duck eggs, if you wanted.

Vincent: Interestingly, Elio, there is a version of the flu vaccine that is grown in cells in culture.

Elio: Human cells?

Vincent: These are dog cells, actually.

Elio: Dog cells, I see.

Vincent: Even there the mutation reverts, you lose the glycosylation site mutation. It's something to do with growing in cells and in eggs. Exactly what, we don't know. But I think the take home message here is we should probably work on getting non egg ways to produce flu vaccine to avoid this happening in the future.

Elio: I bet you a lot of clever people are thinking of how to do that.

Vincent: Yes, a lot of people work on this because flu is a big deal and there is a lot of money to work on it, so a lot of people are interested in it. I did my PhD on flu and maybe I should have stayed with it (laughs)

Elio: You know, there is always the possibility of a strain arising which is even worse than what we have, like the Spanish flu strain, right? Did they say something about that?

Vincent: So the problem is that in addition to this year to year change in the influenza virus, every twenty to thirty to forty years is a major change such that nobody has any protection against the virus. And this can cause huge outbreaks globally. We call them pandemics. It's currently very hard to respond to them because it takes 6-9 months to make a brand new flu vaccine. And so the insect vaccine, the vaccine grown in insect cells is appealing because you could make that very quickly. You could probably make it in two months.

Elio: Why is that?

Vincent: Well, if you have a new strain arise, you would get the sequence of the HA gene, you could then put that in a baculovirus, infect an insect cell, and right away you have HA protein which is the basis of that vaccine. So it is a lot less involved than growing it in eggs.

Elio: How about that. I didn't think of that.

Vincent: The alternative is, of course, to have what we call a universal flu vaccine.

Elio: Yeah, I was going to ask you about that, too. Why don't we have one?

Vincent: Many people are working on it. I've heard many many talks on it, there are a number of different approaches that are being used and they look promising so I would guess that maybe in 10 years or so you wont have to be immunized every year, you will be immunized maybe once in your lifetime or maybe once every ten years but, more importantly, if a new strain arises you will be protected, and that is really the important part of it.

Elio: Right. But the problem must be that among the conserved antigens of influenza, none of them are so powerful to give the immune response. Is that the problem?

Vincent: No, people have identified conserved sequences in the HA protein.

Elio: That all strains have them?

Vincent: Yeah. And they are broadly cross protective. The key is to induce them. That's not so easy, because in people who are infected with influenza virus, these antibodies are very rare.

Elio: Could you make a strain that has, I'm not quite sure I understand what the problem is. If you make a vaccine, it has those proteins.

Vincent: It does, but most of your immune response is directed against the variable antigens, not the conserved ones. So the key would be to figure out how to bias the vaccine response to these conserved antigens, and that's what people are working on.

Elio: I'll tell you, I'm ready, I'm tired of getting a vaccine of dubious efficiency year after year.

Vincent: It is, yes. You're absolutely right. There's even some evidence that getting the flu vaccine every year is not a good idea. So it's difficult. We do need some improvement in this vaccine. Meanwhile, if not every year, you should get it frequently.

Elio: One other interesting thing in the paper that made me feel a little bit cheerful is that old age seems to be a protective factor, the vaccines seem to be less effective among younger people than among older people and the reason given is that the older people have had much more experience with the kinds of virus in their lifetime. Is that right?

Vincent: It depends, it really seems to be what the first strain is that you're infected with. So there's this phenomenon called original antigenic sin which is no matter which strain of flu you are infected with, you tend to make the strongest response to the very first one that you saw in your lifetime. This can affect the age group for susceptibility, so it's pretty complicated. That has to be taken into consideration in a universal vaccine as well. It's really interesting stuff.

Elio: It sure is, I gotta tell you, I find it fascinating because there is a counter intuitive aspect to influenza. It doesn't obey the rules. It is an exceptional virus.

Vincent: For sure. And we don't talk about that much here on TWIM, so it's probably a good idea.

Elio: It's high time we did.

Vincent: Yeah, because our listeners I'm sure are interested.

Elio: Yeah, because if we call it This Week in Microbiology, that should include viruses.

Vincent: Absolutely. For sure.

Elio: Okay, let's do that.

Vincent: If you'd like to hear more about the process of selecting the flu vaccine every year, let me direct you to the episode of Meet the Microbiologist #70, and Julie Wolf's guest on that episode is Stacey Shultz-Cherry. She is a member of the WHO committee that selects the vaccine every year. It's a long process starting for the northern hemisphere in January. In that episode of Meet the Microbiologist she talks all about it and it's much more than we can talk about here. So check that out, Meet the Microbiologist episode 70. You can go to ASM.org/mtm and look for episode 70 and we will have a link in the show notes. Alright, do you have a paper for us, Elio?

Elio: I have a paper. The paper is about a very very different subject, the subject being a heat loving bacterium that is found in the ocean called Thermotoga maritima and this is the first time ever that we have discussed a paper from a journal called Waste Management. Well, you know something I thought we should correct that, because waste management is far from being less than many things. It is very biologically a challenging thing and a very important one. So the article, the title is "Biohydrogen production from hyperthermophilic anaerobic digestion of fruit and vegetable wastes in seawater:", there's a colon there, "Simplification of the culture medium of Thermotoga maritima."

Before we go into that, the authors are a very interesting mixture, the first author is Rafika Saidi, then Pierre Pol Liebgott, Hana Gannoun, Lamia Ben Gaida, Baligh Miladi, Moktar Hamdi, Hassib Bouallagui, and Richard Auria. And this is a combination of people from two universities in Tunisia, one is Universite de Carthage, that's one that we've heard about in history. The other one is Universite de Tunis El Manarand the other people are from France from the Aix Marseille Universite in Toulon which is in Marseille, but anyhow, whatever. This is a consortium of people who did something extraordinarily simple and extraordinarily clever.

So here is the story. First of all, I have to introduce you to Thermotoga maritima because it is really really an interesting bug. The reason I got into this at all is because I was looking at Thermotoga. Thermotoga is a, the name thermos means heat and toga means it has a toga like cover, it has an envelope on it which is quite unique and in fact under the electron microscope what is revealed is that the bacterial cytoplasm looks like a regular bacterium but the cell envelope is distended at the poles. It has therefore a periplasm, the space between the inner membrane and the outer membrane, in the negatives, it is kind of the equivalent of that. And that is full of enzymes, so this guy is a bacterium which is full of hydrolithic enzymes. They break down catechins and other things and in large amounts. So a self contained factory.

Anyhow, thermotoga likes heat, and it grows to temperatures up to 90+ degrees. So where do you find that? You find that in places where there are maritime bacteriums, so you find it in places where there are hot vents and other hot sources of water. This was found in an island which has a wonderful name. It's an island off Sicily in Italy called Vulcano! So why is it called Vulcano? It is called Vulcano because the volcano there, there actually is a volcano, but the volcano was supposed to, according to the Romans, the volcano was the crater which was the chimney of Vulcan's forge. You remember Vulcan was a god that made things, he was a smith, and he had a forge, and therefore it was thought by the Romans that this crater is simply the chimney of the volcano. So an interesting story. The person who discovered this among others is Karl O. Stetter, a German microbiologist of note, a very notable guy who is also a character. And the reason I say he is a character is because he has a video or a Vimeo I guess, if we can find it on YouTube, put that in the show notes, and in it he describes the discovery of this organism and he is very lively, he stipulates a lot, he's like, he could be Italian rather than German.

Vincent: (laughs) Hey Elio, I noticed that one of the species is called Thermotoga neapolitana.

Elio: That's right, that's right. There's a whole lot of thermotogas and they're all similar in that they are all thermophilic and maritime. Anyhow. Here is the story. Thermotoga maritima among others makes a lot of hydrogen. I wont go in to how they do it but they do it by reduction of oxidized material and, sorry, the oxidation of reduced material, I had it backwards. One of the products of fermentation is hydrogen. Before I tell you more about it, let me tell you another thing which is really weird about the phylum thermotoga. And this is what is unusual about it. Unusual among bacteria is upwards of 24% of its genes are similar to archaeal genes, so 24% of the genome is archaea! Well, we find archaeal genes in bacteria, we find bacterial genes in archaea, but never in such amounts. So what does it mean? Who knows. The interesting thing is that in the same place where they isolated Thermotoga maritima from the volcano, also isolated a very high temperature loving archaea called Pyrococcus furiosis. Pyro means it's very hot.

Anyhow, make something out of it. The fact that these two organisms are isolated probably in vicinity, but the idea from various types of arguments is that these archaeal genes were acquired by horizontal gene transfer. It is one of the great discoveries of genetics and evolution that a lot of genes can be transferred horizontally between existing species and therefore make a mess out of things like the tree of life, because the tree of life is probably a more bush of life.

Vincent: (laughs)

Elio: There's a lot of branches coming across, anyhow. This is interesting and somewhat perplexing and can't think of what to make of it because nobody can. We don't know what it really means. But it does tell you that these are interesting bugs, in fact, fascinating bugs. Okay. So here's the story. The first thing that it does, thermotoga, can be fed vegetable waste. Vegetable waste can be broken down, a tremendous amount of the vegetable material which is grown for human or animal consumption goes to waste. Parts of fruit, most of the stems of plants, and all of that goes into a waste place and the waste place is a mess, we have to deal with it on a large scale, it is expensive and difficult. But thermotoga can chew on a lot of the pectins and celluloses and so forth and break them down. So here is an incredible story—and convert it to hydrogen, but I didn't say that hydrogen is potentially a very important fuel because, first of all, when you burn hydrogen you get more energy than what you get from anything else. More than from methane, more than from hydrocarbons. So it is really, although it sounds like it is dangerous because it is so flammable, in reality it is very easy to handle and there are more industrial concerns to making cars that are driven by hydrogen and not electricity.

Vincent: As long as we don't make dirigibles with them, with hydrogen.

Elio: That's right, as long as we don't make dirigibles. Like the Hindenburg, right. So you have to know what you are doing but the technology is there, apparently that's not the problem. The problem is that the source of hydrogen is relatively expensive, I think that's the main problem. And so making hydrogen biologically is a big deal. So here is the story. You start with vegetable waste in the waste place. You grow thermotoga on it. It decomposes the material into fermentable sugars and the fermentable sugars become hydrogen. So I like to think that this is the equivalent of having a slaughter house where you bring in a pig or a cow and use everything except the squeal. It is a very efficient mechanism.

So this is important. The reason it is not used is yet on a large scale, one of the hitches is that when you take the vegetable material you have to add a whole bunch of salt to it, minerals, because it is not rich in minerals and the bacteria need it. So the paper begins with something extraordinarily simple. These guys thought of something just about as clever as can be, namely instead of making a mass of minerals which is expensive, use sea water! The sea water except for nitrogen and sulfur has all the necessary salts that you need. And so they show that the production of hydrogen from vegetable waste using sea water is at least as good if not better, slightly better, than that of using expensive artificial mixers and minerals.

Vincent: So they have to add nitrogen?

Elio: They have to add nitrogen and sulfur, yeah. But, you know, that can be done, and it certainly is easier than what they had done before. And by the way, there is the whole literature about what minerals to use, it's not like people haven't studied it, and it all can be bypassed by just using seawater.

Vincent: Amazing. That's amazing.

Elio: It is amazing. And you know, I love this paper for a couple of reasons. First of all, it is just lovely to see that the bacterium can be the breakdown agent for the substrate, it can then convert the substrate into hydrogen, I mean all of that is contained in the cell of a single bacterium. But I also like the fact that the simplicity of this

paper tells you that you can do certain things in a laboratory which I imagine are not as well endowed as some in the developed world. So I imagine that here the laboratories are fine, but you know, it takes somebody with the ingenuity that is required to cope with, perhaps, I don't know, but perhaps more difficult situations. I can't imagine that in Tunisia there is the money in science which exists in other countries, in developed countries. But whatever it is, I'm not making a point of that so much as to say that cleverness is a universal quality that is not limited to the noble halls of Columbia University and the like.

Vincent: (laughs) For sure.

Elio: So that was a fine paper.

Vincent: Elio, a couple questions. Now, what do you get in the end besides hydrogen, anything else?

Elio: It didn't say, I was thinking though, what if the proteins of thermotoga are useful? You could harvest the thermotoga. They don't do that, I don't think anybody has done that, but you could harvest, see if by harvesting the thermotoga, which is probably there in large amounts.

Vincent: Yeah, because it's growing on the substrates, right, yeah.

Elio: Bacterial proteins tend to be more interesting for various purposes than vegetable proteins. So who knows! You could make thermotoga protein out of it.

Vincent: Yeah, that could be a problem if you have a lot of thermotoga, then you have to get rid of it if you can't use it.

Elio: Right, but maybe you could convert it to something else.

Vincent: Yeah. And maybe the other thing is in this paper they did this in a bioreactor, a closed system, but if this were actually to be used, you would do it in some huge facility of some kind.

Elio: You would have a huge fermentation tank, that is right. The thing is that minerals is the key here because you don't have to add a lot. Certainly to add some thermotoga but it probably grows very fast because it likes high temperatures and it makes a lot of it very fast just from seawater.

Vincent: And then the end result, you have a lot of hydrogen produced which as you said you can use for various things, but you have to be very careful that it doesn't blow up the facility, right?

Elio: I know, but I thought it would be a problem, it turns out it is not. It was a problem before we recognized it. I don't think, making cars with a hydrogen tank sounds very dangerous. What if there's an accident and the tank explodes? But apparently it is not a problem. I haven't run into that. Maybe in the years they've solved that flammability issue of hydrogen. But it is a very desirable fuel. It makes no CO_2, by the way.

Vincent: That's good.

Elio: You burn it and you make water, which is terrific. If the energy requirements of the world were met by burning hydrogen, we would not have the problems of global warming. It's as important as that.

Vincent: You can make water from it, you mix it with oxygen, you get water. And then as a consequence it generates electricity if you do it in a fuel cell, so that could be interesting.

Elio: That's right. I think cars are designed to use fuel cells, fuel cells are very adaptable, in a way. Even on a primitive level, I mean, it's a lot of development here, but maybe our children would see a hydrogen driven world in terms of energy production.

Vincent: Yeah. So I've just searched PubMed for thermotoga and there are 1,700 articles published.

Elio: Yes, sir.

Vincent: (laughs)

Elio: Most microbiologists have never heard of it.

Vincent: It is interesting. It sounds, to me, very promising. I hope it proceeds. Sometimes these discoveries, they sound great but no one picks it up.

Elio: Not everybody reads waste management journals.

Vincent: Right.

Elio: But they are germs.

Vincent: Very nice. So you were looking up thermotoga and that's how you found this?

Elio: That's right, exactly. Thermotoga, I knew what thermotoga was, let me see, actually I was thinking of writing a piece for the blog and I did write this up for the blog, on Small Things Considered. So I looked up on PubMed, I looked for thermotoga and this article popped out. My first reaction was what, vegetable waste, who cares? And then I was like wait a minute, wait a minute, and it turned out to be interesting.

Vincent: Yeah, very nice. Alright, thank you, Elio.

Elio: Sure, my pleasure.

Vincent: I have a couple of emails, let me read them and we'll wrap it up. One is from Kayla:

Dear Microbe-crew,

It is currently 9 degrees C in Cheongju in South Korea.

I have been listening to Twim for over 2 years now.

I am from Cork in Ireland, I graduated from University college cork a little over 2 years ago with a Microbiology degree and started working in a lab in Cork.

Last month I decided to pack up and move to South Korea for a year as an English teacher to explore the world a little.

Your podcasts have been keeping me in touch with the microbe world while I have been over here. They have even inspired me to attempt to start up a podcast of my own with a Biologist who is also working over here as an English teacher.

One of the most Important things your podcast has taught me is that a scientist can also be political. I find myself paying more attention to political affairs and thinking what can I do?

I just wanted to email in to say keep up the fantastic work, education is the most powerful tool on this earth and the twix podcasts are a wonderful resource of education.

Kind Regards,

Kayla

Elio: Hey, thank you! Lovely.

Vincent: Very nice.

Elio: Lovely thoughts.

Vincent: Good luck with your podcast, there. We have an email from Yousef who writes:

Dear Microbial explorers,

I'm writing this letter to thank you for your inquisitive podcasts and to let you know just how much they can make an impact. Let me briefly tell you about my story so far. During my teens, I was not an ideal student as I'd constantly skip school in favour of video games and skateboarding. I was far from an A grade student constantly balancing on the edge of failure. Near the end of college (UK College), I had already failed biology and on a trajectory for unimpressive grades that wouldn't see me to University. About 6 months before finals my grandfather gave me a book he said was "fascinating but a load of garbage near the end", the book was Bill Bryson's Short history of nearly everything. Having no particular interest in books I unamusingly gave the first page a quick glance, I was hooked! I spent the next few days entranced by the storytelling of science, from the big bang to, my favourite part apparently "garbage", evolution and the origin of life. Bill Bryson had single-handedly both ignited my fascination for science and given me a goal to pursue, to become a scientist.

The results of my finals did not complement my new found drive. All my University applications were rejected. After hours calling various Universities, I was finally accepted onto a science foundation course at Anglia Ruskin University in Cambridge. After completion of the access course, I finally entered university, the first of my family to do so, and began the study of biomedical science. Through the years I learnt much about the human body and the onset of diseases but began to become uninterested in the human centric field, that is until the microbes came along. My lectures involving infectious diseases were illuminating and incredibly thought-provoking. After successfully completing my Bachelor's degree I moved to London eventually working as a College science technician to not only fund my master's degree, setting me one step closer to my research dream, but also inspire the children of today to become the scientists of tomorrow. After a year of glorified babysitting, I was eventually accepted onto a masters degree in microbiology and infection at the University of Birmingham.

Even though I was financially destroyed (Education is damn expensive!) and living back with my mother, my time in Birmingham opened my eyes and profoundly changed my perception of microbes and life on this planet. I became entranced with host-microbe interactions specifically endosymbionts which led me to read Lynn Margules's beautiful book Microcosmos and also John Maynards Smiths and Eorz Szathmary's Major transitions in evolution (Still perplexes me today but it's nice to pretend I understand). This newfound passion for endosymbionts led me into a research project involving the fungal endosymbiont Burkholderia rhizoxinica and its possible involvement in disease progression. After completing my masters I eventually moved to Bristol with

endosymbionts constantly on my mind. Whilst working as a barista in a cafe I would spend my free time wandering the forests imagining the microbial diversity around me, listening to your podcasts and applying for PhD's.

After being invited for a PhD interview to study the transcriptomic response of Wolbachia I immediately searched through your episodes to better prepare myself and found one related, TWiP 34: Up against the Wolbachia. Within the podcast, you discussed Wolbachia in the control of malaria and filariasis and analyzed the paper entitled "Targeting the Wolbachia cell division protein FtsZ as a new approach for anti-filarial therapy", let's just say that that podcast might have been one of the biggest turning points in my life so far. I'm now 4 months into my PhD and will be flying over the Atlantic to spend the remainder of my studies at New England Biolabs under the supervision of Dr Zhiru Li and Dr Clotilde K. Carlow, the very same authors whose paper you discussed!

I am incredibly excited for the future and very lucky to be pursuing a meaningful career with a subject I find so incredibly fascinating, for this, I have to thank Bill Bryson, all of you at TWiM and my grandfather who has since passed away. Just goes to show, bad grades aren't a dead end if you find what ignites your curiosity.

Your most thankful subscriber, Yousef.

Elio: Wow, wow!

Vincent: Nice!

Elio: Lovely, lovely, and I'm glad that garbage played a role early on. That is quite a story, but it's not surprising. If somebody with this kind of intellectual curiosity finds the right thing, they get hooked, it's easy to see.

Vincent: You bet.

Elio: So I'm very glad. This is lovely.

Vincent: Good luck with that. And one quick one, Anthony writes:

On TWIM episode 163, at around 29:29, does Dr. Schmidt say "… run it through a fax machine …"?

I like to believe that my brain's not gone dull. Hopefully that is so, but in any case I'm well aware that neither my eyes nor ears function as well as they once did. If indeed Dr. Schmidt did say what he should have said, what should I be hearing?

So I wrote back and said it was FACS, fluoresence activated cell sorting, not FAX.

Elio: FACS.

Vincent: And he wrote back and said thank you, I replayed that segment maybe six times trying to figure it out. (laughs) Isn't that funny.

Elio: That is funny. It's the tricks of the language.

Vincent: FACS and fax, it sounds the same, yeah. That's English. So that is TWIM 166, you can find it at asm.org/twim. Consider donating to us to help us out, you can go to microbe.tv/contribute for different ways

you can do that. And if you have questions, comments, want to tell us your story, send them to twim@microbe.tv. Elio Schaechter can be found at the lovely blog Small Things Considered, thanks Elio.

Elio: My pleasure! I do miss our colleagues, Michael Schmidt and Michele Swanson, but you and I managed, I think. So that works. Thank you.

Vincent: It's good to mix it up. Yes, I do miss them as well, and especially their insightful comments, but I also like doing it with you. I'm Vincent Racaniello, you can find me at virology.ws, I want to thank the American Society for Microbiology for their support of TWIM and Ray Ortega for his help in production. The music you hear on TWIM is by Ronald Jenkees. You can find him at ronaldjenkees.com. Thanks for listening everyone, we will see you next time on This Week in Microbiology.

(music)

Transcribed by Sarah Morgan.