This Week in Microbiology

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

Episode 189: Salmonella BonJovi

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(music)

Vincent:: This is TWIM, This Week in Microbiology episode 189 recorded on November 8th, 2018. I'm Vincent Racaniello and you are listening to the podcast that explores unseen life on Earth. Joining me today from Small Things Considered, Elio Schaecter.

Elio: Hello there, how are ya?

Vincent: I'm well, how are you?

Elio: Middling, middling well.

Vincent: Good to hear you again. Also joining us from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello!

Vincent: Welcome back, we missed you for one podcast there. And from Charleston, South Carolina, Michael Schmidt.

Michael: Hello everyone!

Vincent: People complain on TWIV that I spend too long introducing people, so I just made it quick, see? I can do it (laughs)

Elio: Oh no (laughs)

Vincent: People can complain about anything, right?

Michael: They try.

Michele: That's a democracy.

Vincent: On TWIV they mostly complain about the weather. But I always say you can't do anything about it, so why complain? It's funny, isn't it. All right. Before we go do some science here, I have a little request for all of our listeners. Some of you may know or if you don't you are going to learn that we do have a YouTube channel, youtube.com/profvrr. Youtube.com/profvrr. That's me, Professor Vrr, that's my YouTube channel where I have videos of my lectures, select TWIV TWIM TWIP episodes,

short videos, and other things. And I learned recently that YouTube is giving out educational grants for educational content. I would like to apply for one but you need 25,000 subscribers, and at the moment I have made a plea on all the possible places I can at the moment we have 16,600 subscribers.

Elio: That's very good.

Michele: That's terrific.

Michael: You have an additional 42, you're up to 16,642.

Vincent: All right. So subscribe if you haven't already, it's free.

Elio: Is it clear how to subscribe?

Vincent: Yeah, just go to youtube.com/profvrr there is a big red subscribe button right there on the front page. I'll tell you exactly where it is, it is located…

Michael: In the upper right hand corner.

Vincent: I don't see it since it's my channel so I'm not gonna see a subscribe. Have you subscribed, Michael?

Michael: I have indeed, and if you click the bell next to it what will happen is any time Vincent posts new content, whether it be from TWIV, TWIM, TWIP, whatever podcast he has, when you click that little bell any time there is a new podcast posted, it will show up on Vincent's channel and you will then be able to know that there is something new for you to listen to.

Vincent: So do that and if we get to 25,000 I will apply for support.

Michele: And not only do that but tell all the people in your group, your fellow students, labmates, friends and neighbors to also do the same.

Vincent: Yes. Let's go viral. So thanks for your support. Alright, today we have something a little different from Elio.

Elio: Yes. Let me tell you, I'm gonna talk about a report that was put out by the Kew Royal Botanic Garden, very important institution in England, about the state of the world's fungi 2018. So instead of talking about an article, which we normally do, this time I'm gonna give you a small summary of what this report says and see if you find it interesting because fungi are mighty important, so. Okay. We're gonna play a little game here. In addition to listening, those of you who are not driving or performing an experiment may want to keep a tally as I mention the item, check off yes I knew this or no I didn't. And then let us know what you think, if you think you are up on the state of the world's fungi, so much the better. If you learn something from this, even better. Okay.

Michele: Can we play along, can I play along?

Elio: Yes, you can!

Michael: There's gonna be a test later, Michele.

Elio: Just for us! Okay. By coincidence, in today's Small Things Considered, not today when TWIM appears but the one in time, anyhow, in today's Small Things Considered Roberto Kolter, coblogger, wrote a piece about just that. He wrote a very lovely piece. So this is a list, it's a laundry list if you wish, but written extremely well with a great deal of thought and beautiful pictures. It is worth going through. It is called the State of the World's Fungi 2018, if you google that you will get to the Royal Botanical Gardens site.

Let me summarize first what they talk about. They talk about new species being discovered, the usefulness of fungi in food production and bioremediation, by the way you don't have to check this off because this is just general, this is just a summary of what is in the article. Classifying fungi for large scale genome sequencing, the importance of fungal-plant interactions, both in promoting plant health and causing plant disease, the effects of climate change on fungal ecology, catalog on the state of the world's fungi and conservation efforts aimed at protecting endangered species.

So that's what this is about. I'm not gonna present it in the same order, just because. So the first thing I want to tell you about is that in research, fungi appear very very prominently. You know that yeast is the E. coli of the eukaryotic world. It's the most studied organism other than humans and a tremendous amount is known about it. In fact, since 1910, a quarter of the Nobel prizes in medicine were awarded for work based on yeast, so that tells you. And yeast is of course kind of a little substitute for humans because 7% of yeast genes can be replaced by human genes. So you know, it is a great organism. Okay. So with that in mind, lets go over a list of items. Let's start with recreational uses, okay. About 200 species of fungi are thought to be hallucinogenic (laughter)

Michael: The important part!

Elio: We don't have the exact number but that's about right. In Mexico there is the highest recorded number, they have 76 species of which 46 are in the genome psilocybin. I'm gonna add something that is not quite in the report, psilocybin which is the active principle of the magic mushrooms is now increasingly popular in research, in medical research. It is used for treatment of drug addiction, depression, and it seems to have a wonderful effect and there is effort to remove it from the totally forbidden class of drugs to the ones that can be used under supervision, etc. So a lot of research is gonna go into it. Anyhow. Let's go on to--

Michele: Wait, can we anticipate then medical mushrooms?

Elio: Yes, or no, medical psilocybin. The reason for not giving you a mushroom is because although you may think you've identified you may misidentify and it may have something else in it, so it's not a good idea. In fact I would say please, if you are interested in recreational uses, try to really know what you are doing. The best thing to do would be in fact to get a hold of the chemical and use that, because swallowing mushrooms indiscriminately is a good path to a trip to the emergency room.

Michael: Or the coroner.

Elio: That's right.

Vincent: Or a trip. (laughter)

Elio: A final trip. So be careful, okay? Anyhow. We can go on with that, let me go take on the subject of food. Mushrooms are of course the best expression of food produced by fungus, the global market of edible mushroom is skyrocketing. It is now 42 billion dollars in the US per year. About 350 species are routinely collected and eaten. There is a big difference between cultivation and eating because the number of cultivated mushrooms in the supermarket today is really also skyrocketing. In my local regular supermarket there are five different kinds of mushrooms. And they are white buttons, cremini, and portobellos. Now, don't say I never taught you anything, cremini and portobellos are the same thing as the white button mushroom, only they have let them crow, they've been allowed to grow further. So remember that, same species, Agaricus bisporus. Shiitake, which is very common now, oyster mushrooms of which there are many varieties. There is one called the giant trumpet, see if you can find it in your supermarket. Very cheap to grow, very easy to grow, and it is quite delicious. Then there is the woodears which are mainly for consistency, sort of chewy, and then enokis, enokies are those little things that look like spaghetti with a cap.

Vincent: Elio, which is the one my grandmother used to cut up?

Elio: Eh, white buttons.

Vincent: Really?

Elio: Yeah.

Vincent: So they can be quite large, right?

Elio: Sure. Oh yeah, they can be quite large. If they let them grow far enough to make spores, you see them on the underneath on the gills, underneath the cap, that's what makes a cremini. If you wait long enough it makes portobellos.

Vincent: Portobello, right, love 'em.

Elio: Portobello, beautiful port. Anyhow, in food we should also mention that brewers and bakers yeast in the making of breads and essentially all if not most alcoholic drinks. So much for fungi being our friends, they also participate in the ripening of cheese, many cheeses are ripened by that. In addition, they are used in making miso and tempeh, soy material which is solidified, that is done by fermentation by mold, molds make food coloring, soy sauce, etc. So great uses of fungi in foods.

Industrial products. Fungi have contributed to the production of a large number of essential compounds, like antibiotics, statins, a statin to combat high cholesterol is there, not essential but they are important, and compounds like cyclosporin used in tissue, in organ transplants to lower the immune response. They also are used, and this is less known, in modifying steroids chemically. They

may not make the steroid backbone but they contribute to the modification. To give you an idea, in vaccines, in therapeutic proteins, about 15 to 20% of all are made in yeast. Then molds are used in making vitamin B12 and a whole lot of enzymes. Before I go into enzymes, I found a cute one. There is a platform chemical, a platform chemical is a chemical that is used to make plastics in various ways. It is called itaconic acid. And guess where you find it? You find it in Lego toys. (laughter)

Michael: So fungi are responsible for Legos?

Elio: Indeed! Indeed.

Michael: That is something, that's going on the list. That's going on the list, that is something I did not know.

Elio: Good, okay. In addition, the mycelia, the filaments that mushrooms, that fungi made molds and mushrooms alike, a hairlike network of cells, they can be grown in labs to make lab grown meats. Look out for that, because apparently it is very very good and it is a substitute for meat. It doesn't look like a chop but it can be made to look like anything and it can be very good.

Michele: So it has the chewy texture that people are used to with meat?

Elio: I guess. I guess. It probably depends on the binder you put in, you have to put in some binders and I imagine you can tamper with that. But look out for non cow made meat made by fungi. In addition, they can be used to make the scaffolds for 3D organ printing. That is, if you want to make an artificial organ, you need to put the cells on something and the scaffold can be made out of the mycelium of fungi. There is a huge number of uses like that. They are used, people are talking about using them to make biofabricated leather, imagine that.

So anyhow. Enzymes. There is a whole lot of enzymes which are made by molds, one of them you may not have heard of but you know the consequence of it, when you have sweet, when you eat or drink soft drinks which are artificially sweetened, you are likely to imbibe high fructose corn syrup. What is that? That is starch from corn which has been modified into fructose by an enzyme called glucoamylase, from the species aspergillus. I mention aspergillus because it is a species of mold which is extremely common, perhaps the most common mold in our environment and it is used in a tremendous number of different industrial productions. One use that you may not have heard of is that certain cellulases from a thermophilic fungus, this is good because you can use the enzyme at high temperatures, are used to trim the fine cotton threads on the surface of cotton fabric. This makes for a smoother feel of the cotton.

Michele: Like acid wash jeans or stone wash jeans?

Elio: No, I don't think, maybe. I wouldn't know. But the threads apparently interfere with the feel, the fine feel of cotton, and they can be removed with this enzyme. The of course enzyme from yeast, from molds, are used to remove stains from fabric surfaces, and that is very helpful because it allows you to wash your clothes at a lower temperature, which is very good for the environment. And they are added to detergents. They also can be used, are used to turn crop waste into bioethanol. This is important because if you want to make ethanol out of corn, you use the corn ears and the rest you

throw away. But the rest is cellulose, which is perfectly good carbohydrate which can be broken down by enzymes made by fungi into something which can be fermented into ethanol, etc etc. So there is lots more of that, I don't think I want to go into much on that.

In agriculture and in the environment, there is a really a tremendous fungi have tremendous importance. The main one which is not emphasized in this article as much as I would have liked to see, it is mentioned but not highlighted as much, is that fungi are the great recyclers. If it were not for fungi, things that decay in the world would not decay. Things that which are dead remain dead and if it were not for the fungi, if you were to go for a walk in the woods, you would have to take a chainsaw along because you couldn't make your way. So obviously this means that they participate in a major way in the cycle of carbon in nature. They do release carbon in the form of CO2 by the decomposition of plants. That sounds like a thing that is not desirable if you think that too much carbon is a bad idea, but they make up for it by allowing plants to grow better and therefore absorb CO2 or use CO2 for photosynthesis.

So it is a mixed bag, they increase the amount of carbon dioxide and decrease it at the same time. One item that I didn't know about is that the enzymes from a mold are added to animal feed and they increase the weight of cattle by up to 13%. I didn't know that. So they save food supplement. The big thing of course is that fungi make a relationship with the roots of trees, the mycorrhizal relationship. 90% of all plants have mycorrhizal relationships. And this is done by only about 2% of all fungal species, so these are specialized fungi that undertake mycorrhizal relationships. Just to remind you, they provide the plant with water and minerals, especially phosphorus from the soil. So they are like drinking straws because they extend the reach of a tree by a huge factor. Can be a factor size, they count it, and if you wish it can be even thought of as the size of the whole land mass because mycorrhizobe fungi make an osmosis with other species. So the term for that, they get it from photosynthesis. So the plants receive water and nutrients and the fungi receive sugars. In addition, they are, they increase resistance to pests and disease. Mycorrhizobe fungi and endophytes, that is fungi that grow inside the tissue, in some cases allow plants to withstand dryness. And they also, as I said, they protect plants from certain parasites so they are kind of like body guards, not just a drinking straw for the tree but they are also body guards. So by increasing the growth of trees, of course they decrease the amount of carbon dioxide in the atmosphere. Were you gonna say something, Michele?

Michele: No, I'm just being humbled by the number of no's I've got (laughter)

Elio: So fungi degrade pollutants like pesticides, dyes, explosives, and they can make heavy metals so they can even maybe thought to be able to clean up toxic waste contaminated with radioactive material or probably chlorinated biphenol chemicals. So they are useful in that way.

Michael: And the phanerochaetes species are really especially good at that because they effectively make these molecular grenades and the explosive in the grenade is, if you will, radical oxygen, and as the phanerochaetes throw out these radical oxygen grenades they can actually bust up lignin. And lignin of course is what holds the tree vertical. So it's one of the most refractory natural chemicals on our planet and the fungi just liquify it because they throw these tremendous grenades at this very, to use Dickson's phrase, chicken wire chemistry and it is just remarkable.

Elio: Yeah, that's right. So they work in that sense, they also, there is something which you may not know. They change the structure of soil by formation of soil aggregates and pores, which enhances the growth of plants. So they are really the tillers of the land, as well as everything else. All those are good things. How about bad things? Well there are plenty of them. Fungal diseases are a worldwide threat to ecosystems. They are, I'll give an example. Until 2010, the disease myrtle rust was known as a problem in plantations of plants introduced into South America. However it was detected in Australia which has the potential of infecting a thousand different native species, including eucalyptus and the paperback tree, melaleuca. And of course the number of plant diseases which are caused by fungi is enormous. I'll just rattle a few names. Dochell disease, ash dieback, dogwood anthracnose, I've never heard of that one, beech bark disease, white pine blister rust. So are these threats? Yeah.

Michael: If you are looking at this report online, they have interactive maps showing specifically the ash dieback in Europe and it is really quite remarkable. You click on 1992 and you see most of Europe is green. There is just one country with orange, which is indicating that the ash dieback has actually taken place. And as you walk through 1992 to present day, which was 2017, you are gonna be shocked. The amount of dieback that has occurred across Europe, just from looking at this interactive map. They have done a tremendous job with this website and you can also download each of these as individual chapters if you want to put it on your tablet as you are riding the subway to work if you want something to read. So you can actually read it if you lose your connection going underground. It is really quite fascinating.

Elio: It is, that's right.

Michael: How they laid this out and made it so approachable so you can digest it with time.

Elio: Yes. Now the question is, are fungal threats emerging? And the answer is that global warming may have a big impact on this, because climate change opens new regions for fungal pathogens. One study they say indicates that fungi are moving towards the poles following their plant hosts at a rate of 6 or 7 kilometers a year as the Earth warms. Of course the plant monocultures, which is increasing, industrial farmers increasingly use one species, one of potatoes, one of green beans, and that of course makes them more susceptible to disease. So disease in plants, not so much in animals, though in animals it is certainly important as well, in immune compromised people, fungi are the main threat. But in plants it is bigger and it is important.

So that's more or less my take, but let me read you the conclusions so then you get the feel from their voice rather than mine. They say, despite early recognition of the importance of fungi for human wellbeing and economic evidence of human uses of fungi for food, drink, and medicines going back at least 6,000 years, historically they have remained in the shadow when compared with research on plants and animals and bacteria. In fact, many of the early writings on fungi assumed they were simple or lower plants. It was not until detailed work on fungal features including the cell wall, incidentally the cell wall is just like condensed chitin, just like the exoskeleton of insects. Anyhow. It wasn't until works on fungi features including cell walls, methods for digesting and storing food and DNA, it became apparent that in fact they were a kingdom of their own right closer to animals than plants. It says here that most fungi have cell walls composed primarily of chitin, the substance is also found in the exoskeleton of insects and the shells of crabs and lobsters.

The realization that fungi are closer to animals than plants is however only one of the remarkable facts to emerge in the past decades. It is now becoming apparent that this organism which you often cannot see with the naked eye expend a vast part of the life cycle underground and inside plants and animals. They are found to be in incredibly important processes. This includes global cycling of nutrients, carbon sequestration, and prevention of desertification in some drought prone regions of the world. Fungi underpin products and processes that we heavily rely on in aspects of everyday life from critical drugs like statins to synthesis of biofuel, cleaning the environment through bioremediation. Some like species of aspergillus have multiple uses as diverse as making antibiotics, the synthesis of third generation contraceptive pills, and cheese production. The global market in edible mushrooms is also huge and increasing. That's pretty much it. So you can get a lot of pleasure from reading this because it is extremely well written, like Michael pointed out, the graphics are amazing and it is just a pleasure to the mind and a pleasure to the eye.

Michael: So there are ten chapters, folks. This will keep you busy for a while and they are absolutely fascinating.

Vincent: I would say it is gorgeous, also.

Michele: It is.

Vincent: Beautifully drawn.

Michael: It's like National Geographic except for fungi.

Michele: And so well organized. If you are interested in reading about useful fungi, you click on that and then you get another beautiful graphic that lists about 15 different products you can click on each of those, like washing powders or leather processing and learn more about that. So you need not feel like you have to go from page 1 to 200 or whatever. You can really go in and follow your interests. It is beautifully done, what a great public service.

Elio: Indeed, I think that is a good way to put it, and I hope the listeners will understand why we departed from our usual format to highlight this remarkable piece of writing.

Vincent: I have to say I probably knew 10% of what you said. Most of it was new to me.

Elio: That much!

Vincent: Maybe less. (laughter)

Michele: I didn't appreciate that the Kew Botanical Gardens had such a large collection. On their website, they say they have more than a million different specimens of fungi in their collection and that collection has been visited by the likes of Charles Darwin and Beatrice Potter.

Elio: Yeah Beatrice Potter was a mycologist on the side. She illustrated mushrooms beautifully. She was a very good painter. Anyhow, the Kew Botanical Gardens is indeed one of the world's center for mycology studies. You are very right. The other one is at Harvard.

Vincent: I would guess that most people don't know much about fungi except mushrooms, right, from what they eat. So this is great, it is gonna teach a lot of people.

Michael: They have a chapter on edible mushrooms, too.

Elio: Absolutely. (laughs)

Michele: And another chapter on the many contributions that the Chinese have made to this field, because there is a long history of the Chinese using fungi in medicine, food, and other applications, so they get a special highlight here, as well.

Vincent: Well folks, dive in to stateoftheworldsfungi.org. It's free, go look at it after you subscribe to my youtube channel. (laughter)

Michael: Shameless plug.

Vincent: Thank you, Elio.

Elio: My pleasure, I really enjoyed it.

Vincent: And of course, you are also an aficionado of fungi, right?

Elio: I am indeed. I once wrote a book called "In the Company of Mushrooms."

Vincent: That's right, it is still on Amazon, you can buy it.

Michele: I think we need to link to that.

Vincent: You think it's out of press?

Elio: It's out of press but they have used copies. I don't get any royalties, so that's fine.

Vincent: Yeah, you can find lots of them. In the company of… yeah, there's plenty of used ones. That's the beauty--

Elio: It sold fairly well when it came out so it is not surprising there are used copies.

Vincent: Beautiful, thank you Elio.

(music)

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(music)

Vincent: Alright, on to a paper, and from this, we will hear about this from Michael.

Michael: Something completely different, the paper we are gonna talk about today is "Regulatory evolution drives evasion of host inflammasomes by Salmonella typhimurium." This paper appeared on the 23rd of October 2018 and it is an open access contribution that appeared in Cell Reports. So the story is--

Elio: Explain what that means for a second, not every listener may know what open access means.

Michael: Open access means that it is available for anyone listening for public download. So the authors or their grants have paid the publisher all the cost associated with bringing the paper to the journal, and there are real costs associated with publishing a paper, and they have opted to pay that fee so that anyone who wishes to read their manuscript may do so. So it is available, all you need is an internet connection, you can get into it and download it and it will be in the show notes so that you can see the beautiful figures that they have in this particular paper. The paper was authored by Illyas, Mulder, Little, Elhenawy, Banda, Perez-Morales, Tsai, Chau, Bustamante, and Coombes, who are from two institutions. The first group is from the Michael Degroot Institute for Infectious Disease Research at McMaster University in Hamilton, Ontario, which is in Canada, and the others are from the Departmento de Microbiologica Molecular Institute de Biotechnologica at the Universidad Internationale de Mexico.

Vincent: Elio's laughing at you.

Elio: You're getting, half the world is smiling right now.

Michael: It's a problem with my Chicago accent. From Cuernavaca, Morales, Mexico. So it is a fascinating story and the story in brief is bacterial pathogens tune their gene expression based on the niche or the location they find themselves in. The story today is gonna describe the identification of a very clever immune evasion strategy that evolved in salmonella enterica serovar typhimurium, which I am just gonna refer to now forevermore as salmonella typhimurium or just typhimurium, that couples repression of its flagellar motility once inside the host.

Recall that strains of salmonella that are infectious in humans often become facultative intracellular parasites. And so intuitively, it makes sense that they turn off motility, because once you are inside, where do you need to go? And we are gonna describe how these authors worked through this remarkable observation that once inside the cell, by turning off motility, they effectively lie to the eukaryotic cell so that it doesn't turn on all the immune activation pieces-parts that Michele studies in her lab, don't you work on inflammasomes as well, Michele, with Legionella?

Michele: That's right, I wont take the punchline but we made similar observations in Legionella.

Michael: So the story has four parts. First we are gonna learn about the horizontal acquisition of the regulatory element, SSB. SSRB. Which is nothing more than a transcriptional factor that finds the right piece of DNA, activates it, or turns it off for transcription, and this is part of a two component regulatory system that many of you are familiar with. And the second piece is they begin to dissect how this regulatory molecule divergently regulates flagellar motility in the typhimurium versus the ancestral strain bongori. Salmonella bongori is presumably one of the ancestral strains of the Salmonella that become facultative intracellular parasites of mammals.

Vincent: Isn't that a rock star, bongori?

Michael: I think it is Bon Jovi and he's from Jersey so you'll probably get notes from him. (laughter) The third piece of the story as they were able to beautifully show an evolved SSB binding region. So this is where the transcriptional factor binds in the nucleic acid. Upstream of the flagellar operon, that then drives the repression of typhimurium once it is inside the cell. And then finally, the fourth piece of their story is that this motility repression then physically promotes the evasion of the host inflammasome activation during the infectious process once the salmonella is inside the cell. If you studied any immunology, you know that once one of our immune cells effectively has engulfed bacterium, it turns on a whole suite of genes and then that consequence is it gets rid of the bacterium, but it does this through this beautiful cascade of immune regulatory molecules and that is effectively how it clears the infection.

The hallmark of a salmonella infection is they live happily ever after inside the immune cells, and this is the story of how they are able to do this. So now as background, if you are unfamiliar with salmonella, you need to know that the evolution of salmonella as a pathogen has been shaped prominently by genomic evolution via horizontal gene transfer. Now, the horizontal gene transfer effectively was accomplished by the acquisition of pathogenicity islands that we have talked about in the past. And these pathogenicity islands are differentially distributed in different strains in serovars. And if you really want, if you have trouble sleeping, you may want to learn about the taxonomy of salmonella. Because it is long and complicated and I'll put a specific short piece in the show notes about the CDC talking about how the naming conventions of salmonella have evolved with time. But the authors of this manuscript tell us that the acquisition of the pathogenicity island #2 in salmonella followed the divergence of salmonella typhimurium from salmonella bongori. Elio asked me a question via email before this paper, why bongori? Well, bongori simply because that is if you will the evolutionary great great great grandfather of typhimurium.

Elio: Today it affects many reptiles and invertebrates, right? Or not invertebrates, non mammals.

Michael: Yeah, and the principal difference is that typhimurium has the ability to survive and replicate with immune cells, whereas bongori does not. And so the principal difference is one, the typhimurium is capable of being a facultative intracellular parasite while bongori is not. So this then, by acquisition of this pathogenicity island, enables salmonella by virtue of the fact of acquiring this pathogenicity island to exploit this new intracellular niche, because nothing is gonna attack it once it is inside the attacking cell of our system. Second, when salmonella typhimurium acquired the pathogenicity island, it got for free this two component regulatory system that the authors are investigating. And if you recall, the two component regulatory system is the hallmark of this canonical system is one molecule adds a high energy phosphate to the other molecule and when that molecule acquires a high energy

phosphate or loses the high energy phosophate, it effectively acquired the ability to modify transcription. It either modifies it up or in some cases it can modify it down, depending on what its specific function is gonna be.

So we are gonna talk principally about the B-component of this 2 component regulatory system, and it tells the cell if you will when it came in from the cold. So that's what this regulatory system that was on the pathogenicity island does. It effectively says okay, you are now inside, you can do other things. One of the curious things about this regulatory system is that under acidic conditions, the sensor kinase adds a high energy phosphate to the SSRB response regulator, if you will, activating it so that it can now bind to the proper regulatory regions of the target genes to regulate their expression directly or through counter silencing of protein systems called HNS's. And as an aside, I don't believe that we have ever talked about these nucleoid associated protein, which just has the abbreviation of H-NS, which is able to recognize and bind AT rich horizontally acquired genes, which are typically the hallmark of pathogenicity islands. Pathogenicity islands in general have different G+C ratios than their host, and often they are more AT rich, and so--

Elio: That's how they were originally found.

Michael: That's how they were discovered, right. And this H-NS system, and after I read this, I said we probably should look for a paper that talks about these nucleoid associated proteins that can silence expression.

Michele: But in general it is a way for the bacterium to protect itself from foreign DNA to shut it off.

Michael: Correct.

Michele: Wrap it up, shut it off.

Elio: For the non microbiologists, this protein is vaguely something like histones in eukaryotic cells.

Michael: Yes! Yes, yes yes. And it is sort of, if you will, the histone analogue. So the take home then is that collectively, the genes turned on by the activated transcription factor SSRB by the acidic conditions contribute then to the overall collective fitness of the typhimurium once inside the host. The third bit of background we need to know before we move into the deep end of the paper is that many of the genes outside of the pathogenicity island, so this is like genes in the normal chromosome, that are controlled by this same transcription factor that is turning things on in the pathogenicity island or turning things off in the pathogenicity island have equivalent genes in the evolutionary ancestor that they are studying, which is the salmonella bongori. And recall that the principle difference between bongori and the typhimurium is the absence of this pathogenicity island. So our journey is gonna have four steps. The first two portions of our story concern comparative transcriptomics and what they learn.
The second piece of our journey specifically looks at the functional classes of the genes that are turned on or turned off in typhimurium and bongori. And then the ultimately demonstrate that this transcription system mediates the repression of the flagellar base motility, serving to limit the inflammasome activation during intracellular infection of macrophages. And they actually do the full scale animal activity. So the first stop on our journey--

Elio: You can do all of this in 10 minutes, right?

Michael: I'm gonna try, I'm gonna try.

Michael: So they do the traditional things, they isolate total RNA, but thee clever thing they needed to do is rather than worrying about SSRA and SSRB, they just cloned SSRB and they put it in to a strain of salmonella typhimurium and then also into a strain of bongori. So they have this active transcription factor that is consistently turned on, and then they of course have the isogenic control that is lacking it. So they isolate total RNA from vector and the strains that they have modified, and they identify about 331 genes that are regulated by this molecule, which is about 6% of the genome of typhimurium and typhimurium has about 5,500 genes in total. So 331 genes were upregulated and they tell you what upregulated means, which is operationally transcription is up two fold. And then 224 genes were downregulated, and again, downregulated is greater than two fold down. Of those genes with altered regulation in salmonella typhimurium, 62% of the upregulated genes and 87% of the downregulated genes had orthologs in bongori.

And this is interesting considering that the B system, this transcription regulatory system, only came to typhimurium with the pathogenicity island. So they were most interested in the orthologic genes that was then going to be controlled by this transcription system. And they were most interested in the ones where there was discordant regulation between the two species, where typhimurium was up, bongori had to be down. And so here is where they make their fundamental hypothesis that this was likely an outcome of regulatory evolution, principally driven by fitness. So there were 20 genes that were upregulated in typhimurium but downregulated in bongori, and 14 genes downregulated in typhimurium but upregulated in bongori. As you might guess, they next assign the genes to functional classes. And what they learned is they had to do with motility. So the next section of their paper describes how this transcription complex divergently regulates flagellar motility in typhimurium and bongori.

Now, here is where you get really impressed with their figures. Their figures are quite striking and while we know gene expression is but step 1 in our regulatory story, the proof is often in measuring the activity of the result in gene product or here, they are actually looking for motility. And so they show you motility assays in their figure. And this is the most curious case, or curious observation, and here the discordant effect of the transcription factor on motility was confirmed by bacterial swimming assays in which the transcription factor SSRB significantly decreased swimming in typhimurium but increased it in bongori. And when you look at the photograph of the swimming assay in the top portion of the figure, and then they present the control without the SSRB in typhimurium, the swimming is quite rapid. And then with the SSRB it is very much lower and it is significant out to 4 stars, so that means 0.001, so it was indeed significant. And then the bongori is literally flipped. So the swimming assays correlated beautifully with the significant decrease in the number of flagella present on typhimurium and a significant increase in the number of flagella on bongori.

Michele: And Michael, if I could just interject, I was able to correspond with our first author, B. Ilyas, and she said that this was one of the most exciting moments in the project. She was working on this collecting pellets of bacterial cells to make the RNA and do the large transcriptional profile, but they noticed that in the pellets they either got a really tight bacterial pellet or a really loose one.

Elio: Aha!

Michele: And it was attributed to whether or not they expressed the SSRB and whether or not it was typhimurium or bongori. So just by looking at their cell pellets, they knew that they had a distinct difference and then they confirmed that by doing the molecular analysis that you just described.

Elio: That is funny.

Michael: This is so elegant because normally when you think about transcription stories, they go from RNA, they show you a bunch of Western blots with the proteins going up or down, but here we are actually showing motility and it really makes sense. You don't need to be motile once you are inside the cell.

Michele: It's expensive.

Michael: It's expensive! And so you can understand the evolution of how this all came about. And so that then takes us to the next component of their study where they begin to understand and look at what this regulatory complex is doing vis a vis the flagellar complex, and so they go and you have to have a little digression to learn about flagellar gene expression in salmonella, and there is a hierarchy of three different promoter classes followed by the apparatus and chemotaxis genes through class 2 and class 3 promoters, but the bottom line again by confirming it via RNA sequencing, they show that the B-dependent repression of all levels of the motility hierarchy in salmonella typhimurium. They do this beautifully by RT quantitative PCR and it is again a very striking figure, bongori is up, typhimurium is down. It is crystal clear, you just look at their figure and all the typhimurium is going negative, all the bongori is going positive.

They remind us of previous work that has gone on before by Earheart and Durst that the flagellar promoter system is a hotspot for transcriptional input into motility regulatory cascades and it is almost similar to what we recall from the regulatory cascade when you think about sporulation, there is a specific timing to it.

Their next set of experiments specifically look at trying to understand how this transcriptional complex is interacting with the various gene products, and although they found that they looked at one specific regulator and that is the HIL-D, which is a transcriptional regulator shared by typhimurium and bongori that is known to activate the flagellar gene complex, FLHDC, and although they found that to purify HIL-D bound equally well the typhimurium and bongori FLHDC promoters, the interaction did not affect the binding of this global regulatory factor SSRB to typhimurium or bongori, vice versa, thus ruling out competitive inhibition by this transcriptional activator.

And if you know a little bit about transcriptional activators, they often don't work by competitive inhibition. They are typically binding some place else, and that is effectively where they take us in the next component of the paper, where they beautifully dissect and they did this by good old gel shift, and that is where you add a protein to some nucleic acid and when the protein binds, you see a shift in the mobility on a gel based on the binding of the transcriptional regulatory protein on to the nucleic acid. And so they slice and dice without going into detail giving you the landmarks you can read that in

the paper. And so they were able to dissect precisely where this factor SSRBC was specifically binding. So that effectively, they have now identified it, so now it is on to the behavior that this B dependent repression of flagella gene expression limits inflammasome activation.

Michele: But it is also worth pointing out from that analysis that it was beautifully, aesthetically pleasing that the direct binding of the regulatory protein was at the top of that regulatory cascade for the flagella operon. It was the master regulator, FLHD. So that shuts off the entire class 1 class 2 class 3 cascade of flagella genes.

Michael: And getting back to Michele's earlier comment about the expense of expressing motility in the wrong spot, by doing this upstream you effectively shut down and literally if you will save lots of money in terms of energy for the cell. And so then this then takes us to the whole notion of how the underlying hypothesis the authors had was that the selected driver, if you will, behind all of this evolution of this beautiful molecular biology that they have shown us so far was simply to repress motility which in turn was to limit caspase 1 activation and the release of IO1-beta. So how did they prove this?

Michele: And to bring the listeners up to speed, that is an innate immune barrier, so all macrophages for example have that if they recognize minute quantities of flagella they turn on this fever producing and cell death pathway.

Michael: And it is quite specific and those of you who know that flagella, there are more than one flavor often in salmonella and they can switch the flagellin protein type that is used to make flagella and they do that using a very elegant system of phase variation in salmonella typhimurium. So what they now have done is now we go into mice. So we are asking does this really work in an animal situation? So mice were either infected with typhimurium carrying strain that did not produce SSRB or ones that did that expressed a luciferase transcriptional fusion that showed the typhimurium with the SSRB+ regulatory element significantly reduce luciferase activity compared with typhimurium lacking this regulatory molecule. They then demonstrated that similar to their in vitro results, the fly C was more abundant in macrophages infected with typhimurium lacking SSRB and was undetectable in cells infected with SSRB+ constructs within them. And they have the bongori controls as well in their figure, and it is really quite elegant to see how well they are able to do it.

So we are coming to the last experiments, so you are probably fatigued, but I encourage you to take a look at what they have done because it is a tremendously elegant story. They next wanted to ask the question, the effect of these fly C levels on inflammasome activation, and this was tested in bone marrow derived macrophages using secretion of caspase 1 and the pro inflammatory cytokines IL1 beta as readouts following infection with either typhimurium and bongori. And here again, the SSRB- typhimurium triggered tremendous amounts of caspase 1 processing, enriching for the active 20 kd subunits suggesting that the inflammasome is on, significantly increases the secretion of IL1-beta, and the ones that had SSRB were off.

And bongori was flipped, and the activity was dependent on both the fly C and SSRB because deletion of fly C in the SSRB- typhimurium or expression of the SSRB in the fly C+ typhimurium prevented caspase processing and significantly inhibited IL1-beta release from the bone marrow infected macrophages. In contrast, the bongori was the opposite. And then they did the complementary

experiments treating bone marrow macrophages with caspase 1 inhibitors, which fully prohibited caspase 1 processing in IL1-beta release in the infected cells, and all told, their data supports their overarching hypothesis and they were able to identify these regulatory circuits that repress flagellin in typhimurium by using a horizontally acquired regulatory 2 component system that becomes highly active in this intracellular setting.

They make the final argument that this evolved regulatory circuit limits inflammasome activation and dampens the host cell response from consuming the salmonella typhimurium. So summing up, they walk us through a variety of experiments that demonstrate how the mammalian immune system imposes a selective pressure on intracellular bacteria to repress the expression of immune activating proteins, ultimately compromising host defenses. They end though with a very provocative notion, with antibiotic resistance on the rise and bacteria increasingly being linked to acute and chronic diseases, a thorough accounting on how bacterial immune evasions may ultimately guide us in our thoughts of new paradigms in developing antimicrobial treatments is a must. So I thought this was a cool paper when I glanced at it and I thought it was something that you all might enjoy.

Vincent: Fortunately for salmonella, downregulated flagella being intracellular doesn't matter, as we said, it's good otherwise.

Michael: It's in!

Vincent: Otherwise if you needed it you wouldn't be able to do that.

Michael: No.

Michele: I agree, this is a lovely addition to a large literature that shows bacteria shut off flagella both for economy reasons but also to avoid the innate immune system.

Elio: Some of this was known before, some of the important conclusions were totally novel.

Michele: Yeah, the conceptual framework had been established, but they provided some additional molecular detail and discussed it in an evolutionary framework. But for example, Camplylobacter jejuni and Helicobacter pylorii, they have actually altered the architecture of their flagellin molecules so that it can't be recognized by Toll-like receptors. So we know that there is a lot of selective pressure on the flagellin system, either to shut it off or change the structure. The other interesting thing, and this gets a bit more sophisticated, but Brad Cookson's lab and others have been studying the populations of salmonella in a mouse model, and they find that there is what they call a bi-stability wired into the genetic circuitry that controls whether or not they are making flagella, so the majority of the population will be off, but a minority is on.

And the idea is, and this is backed up by a lot of animal work, that a little bit of inflammation may actually benefit salmonella pathogenesis in the GI tract. Now if every single cell is alerting the immune system to its presence, then the infection is cleared. But what they have shown is there is this bistability so that a subpopulation does express flagellin, you get a bit of inflammation, and that seems to benefit the whole population. It is a really interesting experimental model to understand host pathogen interactions but also as this group did to understand evolution. Michael, were you

going to mention that they discussed this in context of a new especially virulent strain of salmonella typhimurium that is beginning to look more like typhi and cause systemic disease?

Michael: I wasn't gonna do that simply for lack of time. I knew I was gonna run out of time if I do it. But now that you've mentioned it, go ahead and expand on it.

Michele: The authors in their discussion point out that there is a particular clade of non-typhi salmonella, so typhimurium that usually stays in the GI tract, but is now being found to cause life threatening bloodstream infections and when they go in and do careful analysis they find that more invasive salmonella typhimurium expresses less flagellin. So again, it has gotten better at hiding itself from this innate immune barrier and is able then to cause not only GI disease but get into other tissues and cause terrible disease.

Elio: Let me ask you a question from far out. Couldn't something analogous happen with your O antigens external to the surface of O negatives? They are also a signal that, here I am, a visiting card, or a neon light like the authors say.

Michele: So there is evidence that some pathogens will modify their LPS to make it less easily recognized.

Michael: And in fact, bacteroides, one of our predominant gut microbes, has a relatively low level of annoyance to our immune system. Its O antigen systems are not as bad as some of the others. This selection is going on probably longer than humans can talk, so.

Michele: I'd like to tell everyone more about the PhD student who led this project, Bushra Ilyas. She, I am happy to share, said that TWIM has been fueling her passion for microbiology since she was an undergraduate at the University of Toronto. So while she was working in research labs she would listen to these episodes and increase her knowledge base and get inspiration. So she was an undergrad at University of Toronto, majored in molecular genetics and microbiology, and she did a thesis project will Will Nevar, so he also is a salmonella pathogenesis scientist, and that is where she first became interested in pathogen evolution.

So for her PhD at McMasters, she joined Brian Coombes' lab, and we heard about their lovely work today. She said this research project extended earlier work from Suzanne Osborne and Brian Tounema, who had discovered that SSRB, this regulatory protein, was controlling other genes. She credits David Mulder for conceiving of this strategy to exploit the differences between typhimurium and bongori to understand the impact of regulation by SSRB. She also wanted to credit their collaborator, Victor Bustamante, and his trainees in Cuernavaca, Mexico, who did a lot of the molecular biology including the gel shifts that identified FLHD as the binding site for SSRB. But reflecting back on the whole study she says it is easier to forget about the frequent failures that accompany the few successes that actually go into the paper, so she wanted to share what for her has been the biggest lesson so far so that junior researchers can follow this advice. That is to tap the expertise of people around you. She said that there were numerous experiments where she could have spent 2 or 3 weeks troubleshooting all by herself, but instead thanks to the diverse expertise in the Coombe's lab and also elsewhere and his willingness to collaborate, she learned that by asking for assistance early on, you can really accelerate the project and learn a lot more. So she says it is an

honor to share this success with all her co-authors and she is taking pride in having a large part of her lab on the paper with her.

Elio: Very nice. I think this illustrates a principle, I think especially when you start out but even later in life, when an experiment doesn't work, you say there must be something wrong that I did, let me try again. That's not right, you should really think about what it is that caused the failure and consult with people who know better. This is very important.

Michele: And science is very collaborative. We have this cartoon image of scientists just working solo in their laboratory with test tubes, but it is a really people intensive business.

Vincent: It must be nice when someone who has been inspired by TWIM gets their paper done on it, right? (laughter)

Michele: She was really excited.

Michael: So you called her and told her we had picked her paper?

Vincent: And then she fainted.

Michele: I actually emailed her and then she was responding right away and was really generous and thoughtful in talking about her own path.

Vincent: Thank you, Michele.

Elio: Lovely story all around.

Vincent: Alright, that is TWIM 189, you probably listen on a phone or a tablet using an app. There are many apps. Whatever app you use, please subscribe. That way you get every episode and it helps get our subscription numbers up, so we would appreciate if you do that. If you really like what we do, consider supporting us financially. You can go to microbe.tv/contribute. We have a number of ways you can do that including a subscription over at Patreon. And finally, we love getting your questions and comments, didn't have time to get to them today, but next time, TWIM@microbe.tv. TWIM today has been Michele Swanson from the University of Michigan, thank you Michele.

Michele: Thank you.

Vincent: Elio Schaechter who is at Small Things Considered, thanks Elio.

Elio: My pleasure.

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

Michael: Thanks, everyone.

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

(music)

Transcribed by Sarah Morgan