In Dialogue with Macauley Greene

iGEM CONCORDIA

ASTROYEAST 2020

Macauley Green
PhD Candidate Astropharmacy and Astromedicine
Univeristy of Nottingham
July 2020

Interviewed by iGEM Concordia Montreal
(Paula Gomez, Hajar El Mouddene, Lancia Lefebvre)

SUMMARY KEYWORDS
microgravity, bacteria, genes, yeast, bacterial, archaea, space, regulated, upregulated, horizontal gene transfer, antibiotic, stress responses, affected, organism, pathways

Macauley 02:28
We're trying to branch out with connections because I think we're the first seven students to study AstroPharmacy, there were seven in the cohort, and it's also quite new for us at the moment as well.

iGEM Concordia 02:43
Okay, you are the first cohort

Macauley 02:45
Yeah, and our supervisor Phil, he's got affiliations with Lynn Rothschild at NASA Ames. We worked a bit with her and two corporations, that's when Ames expanded the cohort in the next couple years for our PhD students. We're like the guinea pigs at the moment.

iGEM Concordia 05:07
I wanted to start off with sharing a link and giving you a quick tour of our software. I wanted to know, let's say for the purposes of your research, whether you'd be interested in using this web application, and if it would make it a little bit easier for you in terms of the research process. We only have yeast right now. But bacteria is going to be coming up soon. We're gonna have a whole bunch of organisms and species.

Macauley 06:21
I like the name AstroYeast. Everytime I'm talking about my research to someone, I put Astro at the start and they seem more interested straight away. The database is definitely something that would be useful to me now. I've just written a big review paper on bacteria and immune cells in microgravity. I'm hoping to get that published in the next couple of months. Would it be helpful? Something like that?

iGEM Concordia 07:24
That's really cool. Yeah, definitely send me a link once it's out. And the database, what you're seeing now is not the final one, we're working on having more filters. Right now, you can only put in the gene name and the status up or down. But after you can be putting the organism name or the species and a whole bunch of other factors that you can use to do the search. So basically, this is specifically for differential expression analysis.

Let's jump right into my first question, so let's say your dream literature review for your study... and you are interested in looking for information for a specific gene, what are some of the things that you are looking to know?

Macauley 09:13
One the first things obviously is if it's upregulated or downregulated, but the other main issue is, trying to find out if it's upregulated or downregulated across all different types of microgravity because especially with immune cells, a lot of the genes that are upregulated or downregulated in simulated microgravity, you have a lot of different book regulations compared to true microgravity when it's been sent up to the ISS. One of the major findings found during my literature review was a math report and so far is in simulated microgravity using a Clinostat, like the HARV or RCCS, there's 900 genes that are differentially regulated. But when you go into true microgravity, there's 1600 genes that are differentially regulated. A lot of the issues that researchers tend to find this is, a lot of research doesn't stipulate what type of microgravity, the upregulation or downregulation foundings or something like that could be really useful.

Macauley 13:47
I'll try to get the paper I found it on. Yeah, basically there was a study in 2020, it's given me a lot to look at, at the course of my PhD. They did a volcano plot of the genes that are differentially regulated, upregulated and downregulated, in microgravity in the US space flights to compare it to the microgravity simulations.

[shares paper]

Figure 2A is the genes upregulated and downregulated in spaceflight and the ones on the right for figure 3B, are the ones that are in the ground control using a RCCS (Rotary Cell Culture System) or a HARV (High Aspect Ratio Vessel). You can see that there's quite a big difference in the differential genes between them. One of the things I struggle with when I was writing my review paper was I couldn't find any sources that actually told you if there's any difference between them, so it won't tell me if the two are from the same one or if one upregulated in space or if one is downregulated in simulated microgravity.

iGEM Concordia 15:49
Do you think that could be because you don't have the radiation factor. If you go up, you don't isolate for that normally. Do you think we're getting more of a true microgravity effect here on Earth and we would be getting it in space?

Macauley 16:16
That's one of the things we're trying to look into because one of the people in our cohorts and engineering students, he's building a Cubesat for us but he is wanting to look into, 'Is it worth it to block the radiation and isolate the sample?' Tricky thing to do because what you are doing in the HARV, in the Clinostat, it's LSMMG, which is the Low Shear Modelled Microgravity. The LSMMG still applies some fault onto the cells, whereas in the true space you get in the net zero, you get no actual force on the cell because of the lack of gravity, whether the LSMMG is still getting some fault applied to the cell. So that could be a little difference. The interesting thing is that there's not many differences in the actual phenotype, the overall result of this, even though there are different genes, upregulated and downregulated, from what I've seen, the phenotype seems to be the same between the two anywhere. It'd be interesting to look into something like that.

iGEM Concordia 17:35
If we would think of bacteria let's say, what are you generally seeing the changes are in terms of genes overall, and like pathways and phenotypes?

Macauley 17:45
Yeah, so there's a lot of increased biofilm formation. The bacteria form a different shape of biofilm as well. So instead of forming these nice monolayers they form a mushroom structure, it's like a column structure. It's mushrooming out a little bit and there's increased bacterial reproduction. And there's one or two bacteria that actually developed an antibiotic efflux pump in space as well. The one thing one of my supervisors tries to drill into my head is its antibiotic tolerance, because once the bacteria come back down to Earth they are losing this resistance to the antibiotics. It's a phenotypical tolerance while it's up there and as soon as you get back down to normal gravity, you are losing quite a lot of these behaviors that they are picking up in the space environment.

iGEM Concordia 18:57
What you see when they're in spaceflight, they sort of change and then when you come back to Earth they change back, that's what you're saying?

Macauley 19:05
Yeah, it's a lot slower. I think there was a study with simulated microgravity with *E.coli*,

they threw it into space and it develops eight different antibiotic resistances and then even after 100 reproduction cycles back down on Earth and in normal gravity, two of the antibiotic resistances still remained but it lost six of them. So some of the changes are staying but quite a lot of them are going back in normal gravity, which is why I said it keeps telling me it's a tolerance not a resistance.

iGEM Concordia
Are there any specific pathways which you have found affected in microgravity?

Macauley 21:00
Yeah, so the major genetic change I've been finding… one of the major pathways that was all due to microgravity is the global Hfq, which is the regulator, it's a RNA binding chaperone protein whose activity regulates bacterial protein expression via small bacterial RNAs. A lot of people find that it seems to have altered expression levels in microgravity.

Then another one that's been shown to be dysregulated is the ferric uptake regulator. It's homologous, which is the zinc uptake regulator and the manganese uptake regulator. It's in some simulated microgravity stress responses. So Fur (Ferric Uptake Regulator) is a transcription factor and it represses siderophore synthesis in pathogens by utilizing iron as a corepressor. Quite a lot of the microgravity response genes have been found to be clusters of operons and upstream quite lightly as clusters of operons being like a FUR binding site.

iGEM Concordia 23:11
Let's say in terms of the pathways that you looked at. Would you say that these responses to microgravity are specific to it? Or are they a more generalized stress response? Because one of the things we're trying to do is figure out are these microgravity specific responses, or are these just generalized stress responses?

Macauley 23:33
Hfq and Fur are generic stress responses. The interesting one with Fur is the genes it is regulating, they are found operons of genes that tend to only be microgravity stress specific, with the ferric octet regulator. The global Hfq gene is a generic stress response. You get that quite a lot due to the plethora of stresses to the bacterial cells. But the ferric uptake regulator is quite an interesting one. I think at least half of the microgravity stress responses that have been found in clusters are operons and this Fur regulator has been upstream of them as well. I'm trying to make a connection through that, but those papers are from about two between 2000 to 2010. Since then, there has not been as much research into it. Developed by someone called Wilson. Wilson did a lot of bacterial work in microgravity between 2000 and 2005 to 2010. He's quite a massive source.

iGEM Concordia 24:52
Now you're seeing all these different phenotypic and genetic changes in these bacteria, if bacteria were to be used to biomanufacture pharmaceuticals in space, do you see that these changes would be obstacles for that process?

Macauley 25:17
You'd have to make sure that the bacteria won't develop any sort of virulence or potential things like that. You see if you're using them in space, a lot of the problems are due to horizontal gene transfer as well, because NASA have got bacterial accessibility limits for all their launches. So everything doesn't have to be sterile when launching stuff up. On the space flights, in the air pre-flight you are allowed up to 300 colony forming units per meter cubed on the surface, up to 500 colony forming units per meter squared. In the water, you're allowed up to 200 colony forming units per ml. It might not be as much of a problem if the bacteria themselves that you are use are developing virulence, but unless you're going to keep it in an airtight place you won't need to try and avoid the horizontal gene transfer from any potential normal bacteria transfer.

iGEM Concordia
But besides the virulence, it's all these other pathways are affected and it's growing, but it's not typically growing as it should be, and things aren't functioning the way they're supposed to be. Would you imagine that also this would affect the biomanufacturing aspect of it?

Macauley 27:12
It depends how the bacteria are going to manufacture the pharmaceuticals as well. There's quite a few other things, such as cell membranes become more fluid in space. If there's any process that involves transport across the cell membrane, then something like that needs to be adjusted for various reasons.And then, obviously because biofilms are forming easier in space, and they're forming column and canopy structures. Would you still be able to produce what you need to if they do end up forming a biofilm?

iGEM Concordia 28:03
And to go back to this sterility question. We've heard this a lot throughout our meetings is that there's this whole sterility issue that is happening on the ISS and we're wondering, how do you address the need for this one, you're working with bacteria in space conditions.

Macauley 28:26
The bacterial accessibility limits, slightly different for the overall, area in general. If you could keep it contained, if you can keep it somewhere completely isolated and contained, I don't think the bacterial acceptability limits will affect the sort of the

payload that you're putting in. I imagine, I don't know if they have it now, but I imagine they would be able to quarantine a section off of an ISS or another side of the Space Station in the future, or a shuttle. Like a bacterial working zone where they can sterilize themselves before they go in and out of the place. So if you're keeping it quarantined to one place, I don't see it been as much of an issue, it's just the issue that it needs separate air flow and everything, because obviously they have been circulated around the shuttle to begin with, you need some sort of infrastructure there for that, which is more of a engineering problem.

iGEM Concordia 29:30
Let's say everything is worked out in terms of understanding the bacteria responses to microgravity, if there was a way to make them more resilient. Is there a way you think that you can make bacteria more resilient to microgravity stress?

Macauley 29:57
Yeah. I think if we can come up with a microgravity genome because there will be some commonality between all the bacteria cells. There will be some commonality there to build on. Every bacteria will have its individual response and some slight differences. But if you can get to the best set of genes that are changing.And then bacteria are quite easy to genetically engineer compared to other things. You can do genetic knockouts and everything. If you can find out which genes have been affected, then genetically engineer to almost change the genome for some genetically engineered bacteria to be resistant to those particular genes that normally do get affected, then it might be a way to do it that way.

iGEM Concordia 30:57
One of the questions that we got is “Why use yeast and not something else?” From what I'm seeing, let's say from NASA they're also using yeast to bioproduce food because it's already like an organism that we consume. We have baker's yeast. This fact that bacteria has this virulence problem once it's up in microgravity, does it make it an unsuitable organism to produce food versus let's say something like yeast that doesn't have this virulence problem?

Macauley 31:32
I wouldn't say it makes it simple, but I think it's just less predictable than yeast is. So there's a lot more differences that can happen to bacteria, like I've said, the horizontal gene transfer through anything that’s in the system. Archaea are really good at surviving in space being extremophiles. Sometimes they get little bit of Haloarchaea problems in the water even though there's no non-pathogenic archaea at the moment, but found in microgravity is one form of archaea that can develop an antibiotic efflux pump. Obviously, archaea and bacteria can perform horizontal gene transfer, if the archaea developed the

antibiotic efflux pump and then transferred it to the bacteria present then you've got an issue there as well. Whereas with yeast, it's not going to gain any pathogenic tendencies from the environment or the surrounding organisms.

iGEM Concordia 32:52
Let's say you were designing a microgravity experiment. What would you use as a control in the lab?

Macauley 33:04
Yes. When I'm doing experiments at the moment I've got the RCCS for things called the 4D, which is a 2D Clinostat, it's got four rotational points on it. So stick to the ground controls to this. I'll explain it in immune cells. It's what I'm doing at the moment so it's easier to relate to for me. For the apparatuses we've got two metal glass discs that you touch on to the Clinostat and it rotates it around for microgravity. So all I'll do for the controls to keep everything the same, I'll just take these discs off of the apparatus, I'll just put them in the incubator horizontal instead so they're not moving. That way you're getting the full effects of gravity. And you're not changing any variable, even stuff such as the cell culture, plastic and sometimes effects, especially cell attachment and things you need to make sure all your materials are the same.I keep the time points the same, I keep all the media the same. And then if I'm looking at stuff such as cytokines released, I'll also want to control were just media only with no cells or bacteria in it all, as well and do three repeats of media only in the microgravity and just three repeats of media only in the normal gravity conditions. Then I'll just do all my analysis after that, such as ELISA for the cytokines or flow cytometry and stuff like that.

[time signatures shift]

iGEM Concordia 01:22
Why the choice for a 2D Clinostat, why not 3D? That's something we're looking at, and we were trying to get access to one but it seems it's a very rare find. And so we've decided maybe we can 3D print our own so I mean, do the other any differences between 2D and 3D Clinostat in terms of the results that you're getting?

Macaulay 01:52
No, so we use the RCCS (Rotary Cell Culture System) by Synthecon, because it's probably the most used one out of all the applications and it's also a monetary issue as well. Some are funded by the EPSRC, which is the Engineering and Physical Science Research Council. We have a finite source of money and to be honest, the only reason I'm using RCCS is for two reasons. One, Synthecon originally produced them for NASA with the HARV and now

that there's room for commercial use, and we already had a few in our university because people were using them for brain tumor investigations. And the attachments that are needed are only about 1- 2000 pounds, whereas one was looking at getting a 3D Clinostat or even looking at the Random Positioning Machine at one point, about 30,000 pounds, so it's not with us being a startup group. It's not economically viable at the moment. So our lead supervisor, he's got quite a large research group, he's the director of pharmacy research at our university but with only seven PhD students for the Astropharmacy cohort, there's not a huge pot of funding for that at the moment. And out of the seven of us on the only one that's using it full time. So if you spent a lot of the budget just on one student, it'd be unfair to the other six at the moment.

iGEM Concordia 03:27
We're looking at 3D printing our 3D Clinostat. What kind of simulated microgravity do you work with?

Macauley 03:45
Yeah, it's the LSMMG, so it's the low shear modelled microgravity. We don't use the disposable vessels, we use an autoclavable one so we can just keep cleaning them.

iGEM Concordia 05:32
My next question, when you did a review, you found differences. But in terms of your own experiments, I don't know how far along you are with collecting data, but have you already seen some differences with simulated microgravity versus spaceflight experiments in terms of the genes you're looking at?

Macauley 05:56
I've not actually had the chance to get any usable and simulated microgravity data yet. Well I've been doing the PhD for a year now, I have lost six months because of the Coronavirus. In the first month, you do a lot of training, so I've done everything with my ground based data, such as cell surface markers like CD206, which is mannose and calprotectin cell surface markers and other ones such as CD11C, and then I've got all my data for ELISA for cytokines and others just finished up about a week before our university got close for Coronavirus as of literally about to start having fun with the microgravity setup.

iGEM Concordia 06:51
We're also having to do our experiment over two years now because our labs are closed. So this year we're doing the software part. But next year we're going to get to do some lab work, hopefully if the university decides to open.

Macauley 07:08
One thing that comes up a lot is that this issue of microgravity versus altered gravity. If you look at the moon, or if you're at the ISS or on Mars, there's all different microgravity conditions, right? This is a factor that you take into account in your experiments. Have you seen it? Have you seen it being taken account for when you're looking at the other studies for your review?

iGEM Concordia 41:32
It's something we're going to potentially look at in the future because the overall theme of our project is preparing for life on Mars. It's based off of the big NASA document and also the film The Martian. The only ones I've seen in the literature is they do test it for the takeoff gravity as well. The increase in gravity that the rockets are putting on the payloads and what they're carrying, because I've seen it a lot with immune cells, not always with bacteria cells, a lot of these changes occur within seconds. Even though they're only in the extra gravity like the 4-5 g for a little bit that can have an end impact on the gene expression of the cells that the rockets are carrying.

iGEM Concordia 08:52
If you would be designing an experiment that you are going to ship as a payload to the ISS.
What are some considerations that are necessary with respect to how you design the project? You know, we've heard a couple of things like sterility. What are some things that you have to take into consideration for the project design?

Macauley 09:18
We just put an application to use the FLUMIAS that's been sent out there, which is the fluorescent microscope.First of all its the payload restrictions, so the size and the capacity they can carry. So for example, with the FLUMIAS experiments, you can only send up 50 mL of one liquid and 4-5 mL of a different liquid, so you'd send 50 mL of your cell media, and then you send 4-5 mL of your adjuvants, and your additional nutrients and there's stuff like PMS which differentiate the cells. And the main things, the biological one is the access time before the launch. I think It depends what company you go with, I think Virgin Atlantic or SpaceX were advertised and you can access the payload up to 48 hours to 72 hours before, but with some of the ones that NASA sends, sometimes the latest you can access your payload is three weeks before launch. You need something in there that's going to be, especially with bacteria or immune cells, you need it to be in a state where it's going to be able to survive in a pail of nothing added to it for like three to four weeks. You need senescent bacteria or senescent immune cells which can just self sustain themselves for that amount of time.

iGEM Concordia 11:05
We spoke to CUBES (Center for Utilization of Biological Engineering in Space) and

they're producing pharmaceuticals. They argue for plants as a chassis for bioproduction in space, because plants consume CO2 and they produce and contribute to psychological benefits for the astronauts. They don't require sterility as much. And they help with menu fatigue because astronauts can consume fresh food. So what are your thoughts on this and arguments for using bacteria instead?

Macauley 12:05
I imagine bacteria are probably easiest to keep alive, you're gonna need less overall nutrients and equipment. They're gonna take up a lot less space, especially if you're cramped in. The ISS it's not as bad because there's a little bit more space and being cramped in a small shuttle. The other space bridges they are building for this space flight to Mars where you can dock for a few days, and which is going to be like a quarter the size of the ISS or something there, size is definitely an issue. But I think the mental health aspect is quite a good thing because there have been a few studies that microgravity upsets the biome in your stomach and that's linked to your mental health. Your stomach can give you detrimental effects to your mental health. Something that can benefit in that way would be a positive thing, but it is subjected to the astronaut isn't it.

If i was up there I don't think plants would make a difference to me. If it is just a few leaps up there, it's not going to be a massive mental health boost to me. When you're definitely limited by how much you can take up, I think bacteria makes more sense. Because less nutrients you're taking on less space. In the end, you can send more up to produce more of what you need.

iGEM Concordia 15:00
From the studies you saw, what is a good enough threshold to say, oh, there was a difference between my control and my experimental group? What would it be? How much? How much of a full change? Does it have to be to be considered? There was a difference between the control and the experimental for any given gene?

Macauley 15:34
There's no commonly accepted bond. It's quite subjective compared to the research. The issue is that a lot of people when they're publishing the results, they'll decide it to fit the narrative that they want it to. I've not seen any commonly accepted values, which is the issue with a lot of things like the immunology stuff I've been doing, there's no commonly accepted definite protocols or any results, things like that. So I wouldn't be able to tell you which one it would be. But for them to get published, I mean, there has to be at least some minimum right for them to be able to say, oh, there was a difference. They just base it off statistical significance or they don't. It depends on the journalists, and if you publish it in Nature, and you get a peer reviewer

who knows about the field, they will be strict about it and they will send you something back saying no, this is what we think.

But the trouble with Astropharmacy and Astrobiology is there's a lot the journals are quite new, there are not that many peer reviewers in the field.I've seen a lot of journals get aware. I've seen a lot of papers with Excel graphs and stuff like that. I've seen someone draw macrophages with smiley faces on them to show that they're happy and stuff like that, that you wouldn't expect. Because it's the issue at the moment. There's no gold standard for the research at the moment. There's quite a lot of, I don't say, dodgy journals because getting it published is still good. But there's a lot of journals out there that I wouldn't apply to, I'd want it to a higher standard such as Nature. The only one Nature has is NPJ.Microgravity.

I think the best thing to do would be try and find out who peer review some of their papers and maybe drop them an email and see what they found acceptableWith the review that I've published, I'm not even aiming to publish it in a microgravity journal, I'm going for a standard immunology journal and trying to expand out from that way to get a wider audience. Just because there's not that many Astro journals out there at the moment of good enough quality to give you decent results and papers.

iGEM Concordia 18:53
This complicates things for us a little bit. Because in our database as you saw, we had an upregulated status, downregulated status and so far the way we did it, we said, okay, everything with an adjusted p value that is less than point five is significant. So it's either categorized as upregulated or downregulated. Anything above we're saying that we cannot be confident, so we're not categorizing it as up or down. But, I've seen some papers, right that for logFC, they consider the hundred and 50% change. So that's 1.5 fold changes, sort of like good enough, right? It's like a 2% increase, or like a 50% increase between your controlled experimental but again, like this is not like all papers, but I've seen it. Like I've read it in a couple of ones. But then we decided, first of all, do you think this is like a good compromise? Considering the situation and the fact that we don't have any standards, because we want to keep it flexible, we don't want to exclude anything based on some random threshold for Fold Change. And we thought, at least the p value, there's a consensus on it, right?

Macauley 20:16
Now think of it, that's a good thing to do. Because someone's got to draw a line for the values. As long as you make it clear. On the website, where you're driving these values are upregulated and downregulated, then it's up to the people who use it to decide if to agree with that or not.

Macauley 21:25
I think this is why a lot of papers will use a heat map to give you just give you a visual representation of how much genes changed on that as well.You can see the heat map of all the genes so you can just compare them. From what I'm doing my background research, I'm not as much interested in the actual number value. It's up to see if I can see the dark blue where that's down regulated by quite a lot or I can see the orangey red and I know that's upregulated by quite a lot straightaway.

iGEM Concordia 22:12
That makes sense. Basically, you want to just know the genes up or down, and then you look at the heat map to see in relation to the other genes how much is it regulated?

Macauley 22:26
Yeah, that's exactly right. In immunology especially, they usually present a big set of data. So when we're looking at genetic pathways, there's some like the NFKB pathway, for instance, there's about between 30 to 40 genes. When the microgravity study on that laid out 30 genes aren't next to each other and they had two rows for simulated microgravity and ground control. And if you just simulated microgravity in space flight, and if you just see the differences in red and differences in blue, so you could look at it straight away and see the differences between true spaceflight and simulated things without actually getting into the numbers and working out yourself.

iGEM Concordia 23:20
One thing we're trying to do in terms of our genetics plan is we're trying to do our literature research as we're building our database. Find out which genes are up or down regulated and we're gonna pick 10 and we wanted them to be like very reliable genes. We're going to pick genes with high Fold Change, and also a very low p value. We wanted to create a stress reporter for microgravity-induced stress, right? After that we want to test them. We're going to use that to test our resistance strains that we're going to develop.

Originally, a thought is what we can do is use directed evolution to get our resistant strains. But then also there was the idea of, oh, maybe we can do this with CRISPR and genetic engineering. What are your thoughts on those and let's say if you were doing this in bacteria, and you wanted to make them resistant bacteria, how would you do it?

Macauley 25:05
When you put a plasmid in, it incorporates the plasmid DNA into its actual DNA. My supervisor does this for a different process, he introduces a firefly luciferase to make basic glow-in-the-dark bacteria. And you can use that by introducing the firefly luciferase into the bacteria. You can do toxicity tests for how effective antibiotics are

on this thing. So obviously the more it glows, the less that are being killed off by antibiotics. So all he does is use the vector of plasmid and puts in the gene of choice. He also uses PCR to multiply up and puts it into the bacteria then he tests the bacteria using the genetic test to make sure it's been incorporated, by putting on some sort of gene that can be visualized. Like the Firefly luciferase, if the bacteria responds under a UV light and emits light, then he knows it's there. So the way we do it is we probably just use a vector or plasmids put in the gene of choice.And then we replicated the bacteria for that work and testing. We'll test them before replication to make sure the gene of choice is in the genome.

iGEM Concordia
If you wanted to make them resistant, what would you do?

Macauley
For resistance, I mean the best way to do it for resistance would be some form of an antibiotic efflux pump, which you can introduce your horizontal gene transfer through a bacteria that already has it. So it transfers over that way. Or you find the gene in question and try to do a genetic knockout, introduce a new gene through the plasmid and then all you do is replicate the bacteria, put them on to some sort of agar dish or whatever medium the bacteria is growing on and introduce different concentrations of the antibiotic. And then just get rid of all dead ones and then replicate the strong ones and you know, then you've got your antibiotic resistant bacteria that are ready to use and follow up with.

If you wanted to make them resistant to microgravity, it'd be pretty much the same thing. So you'd have to find out what the stress genes are and what are activated and find some way to either modify them or block them. So with the ferric uptake regulator, for instance, the one that activates the operon, where it finds some gene downstream. If you could knock out that ferric uptake regulator gene, then the operon's not going to get activated, or you can insert a gene further down the line which blocks the operon being activated after it as well. There's two ways to do it that way, then just like the antibiotic resistance, you just give it a few life cycles in your Clinostat. And then the ones that are left alive should be the microgravity resistant bacteria. Then you could continue to replicate them in microgravity. If you brought them back to normal gravity replication that might leave the microbe a resistant phenotype. So once again, the microgravity you need to keep them alive and replicated.

iGEM Concordia 29:50
Some studies have fifth generations and 25th generations. And so if you looked at a certain genes after five generations, let's say we found that it was upregulated. But that study found that if you looked at it after 25 generations, it was down regulated. So we

found this very strange. Well, overall, it was pretty consistent. But for some genes, it was really strange. So it was not just like, oh, they were upregulated. For this generation in here, you see no effects like it was like literally upregulated here, but down here. Did you see that when you were looking at bacteria, are you seeing this generation effect?

Macauley 30:41
The other ones I've seen with generation effects adapt faster to microgravity, but once you bring them back to normal gravity, and the generation effect occurs, so the longer you put them back in normal gravity they slowly lose all the microgravity phenotypes over time. One with 100 cycles, for instance, so that they eight antibiotic resistance in space and when they brought them back there was eight upon landing, but after about 10 generations, two of them, I think that's 25 generations, the four of them went and by 100, all 106 of them had went. They weren't too sure why some are losing faster than the others and why some remained. That's the bit that needs to look into a bit more. Obviously, there's something genetic going on there, which is protecting some genes more than others.

iGEM Concordia 31:42
We like to include the generation in our database, because we felt it was important information. In case you end up finding conflicting information in another study, but the difference was oh, they only looked at five generations versus the other one looked at 25. Do you think that was a good idea to incorporate that as well?

Macauley 32:06
Yeah, it's definitely a good thing to do. Because some studies will cut off at 20 or 25 generations as well. You don't know what's gonna happen after the hundred or 200. So it's definitely a good thing. Someone might do a study after a hundred generations and find something completely different. Like, why does this happen? But if they can go back and see, 25 generations, this sort of information, it'll be a handy tool to have.

iGEM Concordia 32:36
That wraps up the questions we had prepared and I guess the interesting thing I wanted to know about was when you looked at your software, is this something that you see yourself using or other researchers using? What did you feel was missing? It's just a working example.

Macauley 33:21
I think I think on the initial table, it'd be quite handy for it to say what the type of microgravity was that has been studied. That's the thing that I definitely look for

straight away. The generations are a really good one to have and the organism and the species. But when people are writing review papers, when they put it in the tables, they'll have a separate table heading for what the microgravity was, say like HARV or space flight or 2D Clinostat and stuff like that. So it's quite handy. have that information quite firsthand.

[End]