Steve Lewis 00:10
Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its trending applications in the life sciences. I'm your host, Steve Lewis. And as you may remember from past episodes, I'm super interested in the world of beer and all things fermentation. And while today's guest Dr. John Leech also enjoys beer, he has found a passion in the science of fermented foods. He is currently working as a technologist at Teagasc in County Cork, Ireland, where he is exploring the microbiome inside all of us and the ways that we can use it to engineer a healthier future. We hope you enjoy our conversation. We begin by asking John about his evolving interests in science and how he found his way to his current work.

John Leech, PhD 01:01
I originally started off studying marine biology when I left school. Mostly what I focused on zoology and animals. It's always been an enormous passion of mine. I then went on to study a master's in evolutionary biology. And at the end of that master's, we had to do a research thesis. I was lucky enough to end up with Professor Emma Teeling in UCD. She's a world-leading expert on bats and aging. And Emma was doing a lot of fantastic, really exciting research into why bats live such a long time, many of these bats live 40-plus years. So, they're exceptional animals in that sense, and unlocking the secrets as to why they can live such a long time. And as part of my master's thesis, there was a small side project looking at the microbiome of bats, because microbiome science had emerged as very important in terms of health. That's kind of what got me first aware of the microbiome and microbiology in the first place, but it sparked my interest. So after that then I followed, I pursued that area of interest, and I joined Teagasc in 2016 to do my PhD in fermented foods. Fermented foods being a very promising field of research at the time for ways to improve microbiome health.

Steve Lewis 02:23
I'm very intrigued by the microbiome diversity meets health and longevity conversation. So, before we get down to that more granular level, I just like to kind of explore a little bit about your perspectives, and perhaps your learnings, through your studies in these areas at the kind of landscape higher level, if that sounds okay.

John Leech, PhD 02:49
Of course. So again, this goes back to my master's. One of the first things I learned was how the microbiome changes as we, first of all, as we're born, so we're born with really an absence of a microbiome in our gut. And then as we get older as our diets change and if we've pets in the house, and all sorts of other factors, who we live with, starts to shape our microbiome from a very young age. Our mother's breast milk is extremely important for this as well. Lots of different fibers are oligosaccharides that are contained in all mammalian breast milk. But human breast milk is particularly complex. And these oligosaccharides, we call them prebiotics, they're like a food for the microbes that are already there, they encourage different species, and they shape the ecosystem. By the time we reach the age of three or four, we reach an adult level of microbiome diversity and that can be hundreds of different species living within our gut. And that stays at that level of diversity throughout our life, when we get sick, or things like that happen. It can fluctuate a little bit and decrease and come back up, then once we get into better health. But then, towards the end of our life, we see that diversity decline again, as we get frail, as we start to experience chronic diseases.

Steve Lewis 04:09
That's a very fascinating overview of developmental biology and scope of the microbiome. And I'm curious, what do you think about the juxtaposition of fermented foods are some of the oldest,

right, established food sources with a lot of different benefits. Beer and bread making are two of the very oldest and of course, you know, fermentation often does a lot of different beneficial things to what we ingest from a just pure process perspective, right? So, the alcohol production in fermentation, at least for a microbial process, it allows for, you know, ingestion of things that we might not otherwise be able to ingest because of perhaps, infection potential or otherwise. So, I'm curious for that juxtaposition of some of the oldest technology, if you will, versus today, you're a technologist, you're an expert in bioinformatics. What do you think about that juxtaposition where you're working at the cutting edge, but it is with one of our oldest harnessed technologies?

John Leech, PhD 05:25
Yeah, it's very, very exciting. Fermentation evidence goes back at least 9,000 years to China with the first fermented foods. And then, as you mentioned, bread and beer from Egypt four and a half thousand years ago. But we have genes that we share with other apes that actually allow us to consume and tolerate alcohol. And there's many other animals that enjoy fermented foods too. And there's monkeys that will drop fruits onto the ground, and then leave them for a couple of days and come back, because it's okay for us now living in the western world where we like unlimited access to calories, but alcohol is quite energy rich. And when food scarcity is an issue, then they were a very useful source of calories. So, there's many animals that have evolved an appetite for fermented foods, so it's not unique to us. But obviously, we harness the power in a very controlled way as far as 9,000 years ago. It wasn't till Louis Pasteur just over 150 years ago that we really understood what was going on. Ferment, the word, comes from the word fervor. It's Latin, which means boil, because before we knew about microbes, we'd see all the bubbles in our kimchi or sauerkraut or kombucha. We kind of compared that to boiling, I guess, and before we knew about the microbes. It's exciting now to be working on, you know, a technology that is that old. And it's also very exciting because one thing that's happened in Western societies, and this is another thing very related to health and longevity, is that what we find is when we look at Western, industrialized microbiomes, they're far less diverse. And as we mentioned earlier, that's a big biomarker of health. And it's in the western society as well that we've moved away from fermented foods. So, a lot of the, what we say are more akin to a hunter-gatherer lifestyle, people who eat, you know, far less processed foods have far less sanitation, they generally, they have to ferment their foods. Fermentation was initially a form of preserving foods. We've got the luxury of refrigeration and food processing and additives to keep our foods safe and not going off too quickly. But, we're now starting to see some of the health, well, we've seen the health consequences of the westernized lifestyle for a long time. But now we're starting to see that signature in the microbiome too, where we have, as I said, moved away from fermented foods, from foods containing live microbes or produced by microbes to foods that we're producing on an industrial scale, which really are not good for a cell. As I said, try not to go off on too many tangents there. I probably did. But it is very exciting to harness these ancient technologies.

Steve Lewis 08:11
Absolutely. And not a tangent at all. I think very, very relevant to the overall conversation. People are fermenting all kinds of stuff with better understandings of the fermentable sugars, the oligosaccharides as you mentioned. In your world of genomics, can you describe some of the methods that are progressing in the field right now?

John Leech, PhD 08:35
Yeah, for sure. So, lots of different progress is made on a lot of fronts. In terms of sequencing, that's where most of, almost all of my data comes from. The sequencing methods are getting much better. Extracting DNA from different food sources is improving. We've worked on some of

the protocols, ourselves. Amplification, the library preparation, the steps that you need to do before sequencing. The length of the reads, the reads are, how many base pairs of DNA are contained within each read, they're getting longer and longer, which is improving the accuracy and the insights you can get from studying these things in the microbiomes in such a way, but the huge progress has been made as well and bioinformatics from the software that is being used to gather these insights from. So, it's evolving on every front. And nearly as soon as you've published something, some of your methods are almost out of date already. So, it's just, it's huge progress, it's a really rapidly evolving field, rapidly moving. And as I said, things, technology on all fronts and the sequencing, the molecular biology front, and particularly the bioinformatics front are just really getting better and better.

Steve Lewis 09:50
One of the gaps that I know there are in a number of fields is what does the baseline model look like? If somebody were just, let's say, came out and they will live as healthy, and I'm doing air quotes, as healthy as possible, let's say into their 40s. What would you expect is that diversity of strains that you mentioned, does that still hold today in 2024? Is that the primary thesis of microbiome and health or has that expanded even beyond just species diversity?

John Leech, PhD 10:33
Yes. So, now there's a lot more, when I started studying this after my master's or during my masters, diversity was the key biomarker for health. And it's still probably the strongest. But now, with these molecular biological techniques and bioinformatics we're able to now see very important species and what they can do. So *Bifidobacterium*, or Bif we shorten it to, we know that's very important for producing a lot of molecules that are extremely important for health have anti-inflammatory properties. They break down fibers that we, that we otherwise can't access, or access to nutrients within certain foods, the microbiome makes these much more bioavailable. Once we're feeding our microbiome the right thing, it's not breaking down the mucus layer in our gut and causing all sorts of health issues and such. So, biodiversity is still a massive biomarker, but we're now looking at strains and species that on their own are exerting health benefits on us too.

Steve Lewis 11:35
Wow. What does that look like? Do you explore the interaction between the individual species and the microbiome environment that you're looking at?

John Leech, PhD 11:46
There's a lot of models for that at the moment. So, trying to replicate the human digestive system. And it's extremely complex. Obviously, the best model we have is putting it into real people. But because it's such a complex ecosystem, it's very difficult to untangle all the noise, to find out what's actually going on. Fermented foods actually lend themselves very well to being a good model ecosystem for looking at species interaction, cross feeding, for example. So, if one microbe produces a health-promoting compound, it might not be able to produce that in a particular matrix like milk, unless there's another species there that produces something else from the milk that then feeds the microbe that's producing the resulting health benefit. So, to give you an example, if we take milk kefir, again, there could be species in there that produce a particular health-promoting compound, maybe lowering cholesterol or something. But that species can't do that in the milk on its own. It needs other species there first to access some of the nutrients in the milk, break them down into something that's usable by that probiotic, if you want to call it that. So yeah, fermented foods are a good way of looking at that. But there are other models, as I said, where we get a lot of human fecal samples, make a human fecal slurry,

we put that into a device that tries to replicate the human gastrointestinal tract, and then feed that different nutrients and see what effect that has on the ecosystem and what metabolites are being produced.

Steve Lewis 13:14
The amount of complexity is just insane to think about.

John Leech, PhD 13:18
Yeah, a lot of people working on it, a lot of progress being made. But again, just the complexity of it makes it very difficult to really, and everybody's microbiome is different, and lots of different people have healthy microbiomes that don't look anything like each other. That's why diversity kind of has stood head and shoulders above everything else. We know that that's more important. Some people have some of these microbes that we know to be very good for health, but other healthy people don't have them. So again, it's just trying to untangle this, and people have different responses, responders and non-responders to certain interventions.

Steve Lewis 13:56
How much do you explore that in your bioinformatics work?

John Leech, PhD 14:01
That would require a lot of modeling, and that is something that other people are working on. There is also for cancer drugs, there are some cancer drugs are more effective. There's been studies shown that some of the cancer drugs are more effective after the administration of a probiotic so that your microbiome has a role to play, if you like, in how responsive somebody is to particular medical interventions. And that's something again, really in its infancy, to see, you know, can we improve the efficacy of drugs that are already there by altering the microbiome?

Steve Lewis 14:37
To your point, right, about the idea of a baseline model, you introduced the concept of, you know, you're not just upregulating and downregulating different reactions from species to species but also from species to vector, right, in that whether it's the gut or what have you, so I, my mind is just kind of blown with how complicated all of these components are and how you even extrapolate some of the useful tidbits that you've already mentioned so far. How do you go about experimental design? Are you like, I know a little bit about this species, I want somebody to have, I don't know, less of bloat in their gut. Therefore, I'm going to take this cohort of humanity, maybe it's one specific population, and you're just using that as a control? Tell me a bit about your experimental design because it seems really challenging in my mind.

John Leech, PhD 15:42
The easiest way to get into this would be maybe some plate assays first. So, you could have cell line assays grown in a petri dish, and you might introduce some microbes to that and see what effect that has. One of the most studied at the moment, I guess, is intestinal permeability. You don't want your gut to be too permeable or pathogens can grasp that barrier and make you quite sick. So, they'll put microbes or probiotics, or maybe metabolites derived from particular bacteria onto these cell lines and see what effect they have. Cancer cell lines as well. So, there's a lot of study going on kombucha metabolites, things that are produced when you make kombucha. What effect that can have on cancer cell lines or inflammation, or oxidative stress, different things like that, then you can step that up to animal models. So, mice are usually one of the first animal models you go to, and you see what effect either fecal transplants might have, or really cool interesting studies are when they display or transplant microbes from lean mice into obese mice. They don't alter the diet of the obese mice, that they're still eating the things that

made them obese in the first place. But then those mice become much leaner, just simply because they've now got the microbiome of the lean mice. And then you go to human studies.

Steve Lewis 17:04
I would love that because I'm a big fan of cheeseburgers and other.

John Leech, PhD 17:10
If we could eat what we like.

Steve Lewis 17:13
Would, I would continue to eat a lot more of if I had that xenograft into me.

John Leech, PhD 17:21
I say go make a lot of us very happy and a lot less stressed about what we eat, right?

Steve Lewis 17:25
For sure.

Steve Lewis 17:30
We hope you're enjoying this episode of Speaking of Mol Bio. We wanted to take a quick moment to remind you about the Invitrogen School of Molecular Biology. It's a great educational hub for molecular biology with rich and reliable technical content designed for new and experienced molecular biologists alike. Check it out today at thermofisher.com forward slash I S M B. And now back to the episode.

Steve Lewis 18:01
I'd like to talk a little bit more about a list of common foods or maybe some of the more obscure fermented foods that people don't think about. Are there any that you of course, you know, recognizing that you're not giving medical advice? Are there any that you might be able to anecdotally recommend or that you might take yourself?

John Leech, PhD 18:23
Yeah, well, when I started studying this, I began to produce a lot of it at home. So, when I was studying zoology, it was very easy for me to really engage with the research animals all around me. And I used to keep a lot of pets of all sorts, frogs, mantids, everything. So, with the food I started producing everything I could. I started producing kombucha, water kefir, milk kefir, sauerkraut, kimchi, tepache. One of the best studied will be milk kefir and there is some health benefits from milk kefir. But something really important with fermented foods is, I'll give you an example. We studied milk kefir from around the world. We took lots of different samples of lots of different people. We never find two milk kefirs that have the exact same microbiome. And it is the microbes in these foods that changed that milk into something else, giving it the health benefits that it has that milk doesn't have. So, just because my milk kefir might be able to lower cholesterol, the one you're producing in your house might not, and if mine is doing it this month, when the milk composition changes later on in the year when the weather is warmer, and the grass changes my milk kefir might lose that benefit. So, it's very difficult to say that any particular fermented food is having particular health benefits. There was a really cool study from Stanford University and from the Sonnenberg group that looked at just general fermented food consumption. There's eight or nine different fermented foods people were consuming, and they were buying it in the shops as well, like pickle juice, kombucha. And what they found was that the consumption of all of these fermented foods, they compared it to a fiber diet. And fiber is another very well-studied intervention in microbiome science. And the more fiber people eat, the

higher the microbiome diversity tends to become, the healthier the outcomes. So, it was really one of the champions of microbiomes and health. And when they compared the fiber diet to the fermented food diet, or fermented food diet actually outcompeted us. There, people had higher microbiome diversity at the end of the study, their inflammatory markers were decreased. Whereas with the fiber, it was really interesting. If you had low microbiome diversity to begin with, then your inflammation actually increased under fiber diets, whereas if he had a moderate or high level of diversity to begin with, then you did have the health benefit of your inflammation going down. But in the fermented food arm of that study, in all cases, of the microbiome diversity from low to high your inflammation markers went down. So, I think, again, without giving medical advice, but a diversity of fermented food consumption seems to be very good. Specific fermented foods can be good. But those effects can be very specific to a particular community that is very subject to change over time. It's one thing we've been working to do for the last couple years is to capture, if you like, like a photograph, we find fermented food microbiome, that we have shown in a lab to have a health benefit. And then using, you know, bioprocessing and innovation, we can keep recreating this in a very scientific, measured way so that we get the same composition, the same product, again and again. As you mentioned earlier, kombucha production and the inconsistencies of getting that right each time you're going to have a slightly different product. And over time, it could be a vastly different product. So that's one thing we're putting a lot of attention on is to find these health benefits, capture them, and then make them repeatable and scalable.

Steve Lewis 22:02
How are you moving toward that repeatable process?

John Leech, PhD 22:06
I won't give you too many specific examples right now, because of patents that are still pending, and also that we can't publish on them yet either. So, but let's just say, we found a particular health benefit of a food, we sampled maybe 20, or 30, of these different foods and they all had slightly different microbiomes. We examined these foods for health benefits, and we isolated all the microbes in each of these foods and we put these back together and made the food again, tested again, it still had the health benefits. From an industrial point of view, you don't want something so complex, it's hard to scale. So, we tried to simplify this community until the health benefit was lost. And when it was lost, we put back in that last microbe to bring back to health benefits. So, using this iterative approach, we got the community as simple as possible without losing the health benefits. And then we ended up with a product that we could produce as many times as we want as long as we had the starter strains that we were using, and we combined them in the right way. What's important to note there as well is it wasn't just one microbe that was able to do this, it was a community that was required in order to get this health benefit from that particular food.

Steve Lewis 23:26
The way that you're talking alludes to the idea that there, the starter material, right, like the consistency of the beginning microbes that you're using, enables the consistency, perhaps of production. And I think in my world for, like, brewing that there's like two or three big yeast producers, right? And they're kind of trying to do almost, not similar, but the idea that you can brew more consistent beer styles by having consistent starter yeast. But there's considerations like drift. How much does genetic drift play into the processes that you're talking about and is that a big risk or something that because it's a symbiotic relationship maybe it's predictable?

John Leech, PhD 24:23

Yeah, I guess we'd have to be doing it for long enough to find out if that's an issue. It hasn't gotten to commercial scale yet. We've got it at bench scale. And that's part of my new role now back with Teagasc is to keep scaling up these products. So, I guess I'll find out soon if genetic drift becomes something that, you know, diminishes the health benefits of these foods. I guess until you're doing it on a commercial scale again, and again, and again, it's not something you'll find out by making it a handful of times for clinical trials in a lab. It hasn't been a factor so far.

Steve Lewis 24:57
Talking about some of your technical areas of interest, sequencing, I would assume, is one of them. Are there any other molecular biology techniques that might not be directly related to specifically you in your work, but across this landscape?

John Leech, PhD 25:17
Trying to quantify the number of microbes in it, so I look at it from bioinformatics. And when you extract DNA, amplify it up, and then I do my downstream analysis, you'll find out the relative abundance that percentages of these different microbes that are in a particular environment, but one molecular biology technique that has a real big importance is qPCR. So, actually giving a quantity. So, it's not just good enough to know what percentage of microbes might be in a particular ecosystem. But knowing how many microbes in a quantitative manner. Is there 9 billion microbes in milk kefir or far less usually in a kombucha? So, qPCR is one of the emerging technologies. It was not emerging, it's one of the utilized technologies that's quite important for microbiome science too.

Steve Lewis 26:08
Where do you see it going to enable this field more?

John Leech, PhD 26:11
I think the quantitative aspect is one of the more important things to address soon and flow cytometry and things like that. OD, measuring the optical density of a particular environment. But some environments like kombucha might lend themselves to OD and then others like the human microbiome won't. So, it's really finding a way to fully describe a microbiome, both in terms of what's there, but how many of them are there as well. I think that's really an important hurdle to overcome to really understand this.

Steve Lewis 26:52
Where are you headed? What do you see for the field and where are you working toward?

John Leech, PhD 26:59
Well, I would like to capture more of these health benefits that we're finding. I'd like to produce things like kombucha and kefir in a more consistent way. A lot of producers are having trouble with consistency with kombucha. There's problems with alcohol production, keeping these products live. When you sell them live, and they keep fermenting on the shelf, sometimes the customer gets a bit of an explosion in their face when they go to open it. So, it's really making these foods more scalable because they're much healthier alternatives. Even if some of them don't have direct health benefits they're a much healthier alternative to more modern ways we've used to preserve foods. Adding different agents to preserve them. And adverse effects that we're seeing processed foods are having on health. Fermented foods are, as I said, I think that they're really important, I think, will shed much more light on how important and how to do in the future. So, it's really digging into that, that's where my passion is, is unlocking a lot of the secrets of these foods and making them in the best, most reproducible way that we can.

Steve Lewis 28:07
It's really just an eye-opening conversation that I really appreciate that we've had today. You clearly have a disposition toward health benefits, probiotics, and prebiotics and then understanding the microbiome from a scientific and perhaps longevity perspective as well. What advice would you have for anybody who might be interested in working in this field?

John Leech, PhD 28:35
It's getting a lot of attention about microbiomes and fermented foods all around the world. There are several brilliant groups in the US working on it. A lot of their collaboration within Europe. So, I'm familiar with some of the European studies that are going on too. Asia as well. If you're passionate about it, I think you'll carve out a path, you won't have to try too hard. I found my way here accidentally, via the animals but yeah, just don't know what better advice I could give really other than follow what you're passionate about. I started making these foods and that really helped me really, I didn't know much about them. I didn't know really much fermented foods and up to 1/3 of the food that we eat is fermented and there's over 5,000 different types of chocolate and coffee. These are things that I had overlooked. So just start fermenting. I started with kombucha, and water kefir and I love making them now. There are so many different flavors you can make and so much fun you can have with them and they're really good.

Steve Lewis 29:42
Last question from my side. I'm very curious, what do you think the keys to your success have been?

John Leech, PhD 29:48
It's definitely curiosity. I got into zoology by just being super curious, mostly probably about evolution, despite doing marine biology at first. It doesn't really feel like hard work when it's like reading a book that you're really interested in. Sometimes you just don't put it down and you keep going and keep digging. And when I was doing my master's, I had this thing called tangent Tuesday, because we had very little lectures on a Tuesday. So, I'd just read a paper, and then I'd find something in that paper with a citation that caught my imagination and, like, could take me anywhere by the end of the day. I just started reading about the gain theory or something, and then ended up reading something totally different about murder rates and left handedness. And just that's kind of what got me into the microbiome as well is when I started reading about aging and the microbiome. It was my curiosity that got me into the fermented foods that I'm going to get into fermented foods and microbiome was my curiosity within that and what was known in the field just kept, as I said, I probably become a bit obsessive about things sometimes. The bioinformatics as well another huge aspect of my work was, I had no background in that really before my master's and I was curious about how to get the computer to do stuff and how the different software worked and yeah, just absolute curiosity and obsessiveness over it, I think.

Steve Lewis 31:19
That was Dr. John Leech, technologist at Teagasc in County Cork, Ireland. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti, and Matthew Stock. Join us next time for more fascinating discussion about the wide world of molecular biology. Until then, cheers, happy fermenting, and good science.