Steve Lewis 00:09
Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its trending applications in life sciences. I'm Steve Lewis, and when I'm not hosting this podcast or doing other parts of my job at Thermo Fisher Scientific, I'm often pursuing my side passion of home brewing. I've loved learning about this world and the science behind it. So, this week, I'm especially excited to welcome Kelly Tretter to our program. Kelly is a Senior Quality Integration Manager at New Belgium Brewing, and she's been working as a scientist in the brewing world for over 30 years. With Dr. Gabriel Alves joining us as well, we have a fascinating conversation to share with you today. We begin by asking Kelly about her career history and how she arrived at her current role with New Belgium.

Kelly Tretter 01:05
It 100% is a serendipitous career. I did not intend to be a brewing microbiologist. I went to school at Colorado State University, and I really wanted to be a genetic counselor. And when I graduated, I got a job at Coors in the microbiology lab as a temp. It was a handwritten resume or application. I got told after the fact that I got hired—and this is nice and it's funny—because I had nice handwriting on the application, and I was short enough to fit under all of the pipework, because there's lots of pipes in the bowels of Coors. And that was like “Okay, great. My penmanship actually like paid off.” I was a, just, I was a shift worker. I was working overnights walking all throughout Coors with an Igloo cooler under each arm and a 70% ethanol bottle. And my job was to go get sterile samples of the process every four hours, so we worked 12-hour shifts. So, we would go walk around and collect our samples, we would come back to the lab, process them, mostly looking for bacteria that spoiled beer. I got to know the brewery really, really well and then eventually moved up in the lab. I got onto a day shift, started doing more analyses of the organisms; not just testing for the organisms, but identification of them. And then I got an opportunity, way later, maybe halfway through my career at Coors, to go, I was in grad school already at CU Denver. And Coors was doing work with one of the only professors that was actually working on beer-spoiling organisms. Coors sent me to go work with Dr. Barry Ziola at the University of Saskatchewan to actually do my project work. So, I had already done the coursework. They sent me up to Saskatchewan to go work with him to do, to learn how to do PCR, which is where I first learned industry-wise, not just school, but actually how to apply it. And then did research on a novel method to detect and speciate the smallest beer spoiling organism called *Pediococcus*. So, I spent my six months in Saskatoon working on that and learning PCR, and then came back to Colorado and resumed my career at Coors for about six or seven more years and then New Belgium came calling to come back to Fort Collins, and work at like my favorite brewery. It was a super easy, “Yes!” I have now been at New Belgium for 15 years, I just passed my 15-year mark, basically in the same capacity for 1313 of those years as the senior microbiologist in charge of yeast propagation and looking for the beer-spoiling bacteria and applying PCR to our industry. And then the last couple of years, we have been, we're out of capacity at our breweries. So now I travel around the country, and I make our beer at other sites that are not Fort Collins and Asheville. And then we have also ventured into the beyond beer space. So, seltzers, hard teas, hard nectars, hard juices, things like that. So, I travel around, and I go make those at third-party sites.

Dr. Gabriel Alves 04:45
That is so much fun. What an impressive background. Tell us a little bit about your experience and the challenges throughout these processes in both breweries.

Kelly Tretter 04:56
Coors was an amazing place to work and when I was there, back in the ‘90s, there was only one main yeast strain. It was the Coors lager that they used for Coors Light, Coors Banquet.

They only had one yeast strain. And it was the historical one that they had been using. So, one of the main differences when it came to New Belgium, one year, early on in my career, I think I counted that we were using 23 unique yeast strains within one year to make all of the different things that we were going to make. So, that in and of itself, just yeast management, was more complex. And making Coors Banquet and Coors Light, if you can imagine and if, if you drink light lagers, there's not much in there to hide any flaws. So, we had to be spot-on to make that beer consistently over time and the millions of barrels that they would make a year and they didn't have a pasteurizer it was all sterile filtration. So, if there was a flaw, it would, it would shine through, there was no place to hide it. So, not disparaging, if folks don't like light lagers just because it's a style, that's totally cool. You don't have the light lagers. But don't bash Coors and the bigger breweries on quality because they absolutely have thorough and excessive quality systems in place to be able to brew a light lager on a regular basis, year after year, and have it taste the same all the way across the country.

Steve Lewis 06:27
A lot of our listeners, of course, they're going to be scientists who may not have so much insight into brewing. So, I'd love to take an opportunity just to draw a couple of parallels there. One specifically, just talking about the consistency batch after batch, that's very similar, I would say to some of at least in the U.S., the FDA CGMP requirements, the Current Good Manufacturing Practices, where you aim for that kind of consistency. The other thing to think about is, you know, there are some parallels with biologics development as well. So, in a lot of biologics development, you're seeking a molecule specifically from the big fermenter. Whereas beer, the liquid actually is the product. Of course, there are many other nuances there, right? You have genetic engineering, scale up, purification techniques actually overlap quite a bit. So, I just for some of the listeners on here, I wanted to kind of share that there's a lot of parallels to the drug development industry and the beer production industry. And we're lucky to have Kelly here because quality I think, spans through pretty consistently, especially from a microbio perspective. So, I'd love to hear, Kelly, some of your thoughts on perhaps some of the parallels there or even some of the perils, right, of some of the contaminations that can happen as well.

Kelly Tretter 08:01
Oh, yeah, we're really lucky. We actually have an analytical chemist who comes from pharma. So, brings like method development on all sorts of, they just did a paper on hop taint. All the fires last year in California, they got asked to develop a method to determine whether or not the hops damaged by smoke and by fire and smoke were going to be able to be salvaged and be able to be used. So, they actually developed a brand-new novel method to help hop growers out on whether or not they could save their hops. From the micro side, we mostly, and this is also how we use PCR, beer is naturally antimicrobial, the pH is too low. If we're doing our processes, right, there shouldn't be any oxygen introduction. Hops are naturally antimicrobial and normal beer ABV is also, does not let pathogenic organisms grow. So, what we're concerned about are organisms that can withstand the bitterness of the hops and still proliferate and grow in alcohol, but it's not pathogens. We are strictly talking about beer-spoiling organisms, that their metabolic byproducts are off flavors. Lactic acid, where it's not supposed to be, you know, any sort of anaerobic lactic acid bacteria. Specifically, there's also a couple different species of a genus called *Pediococcus* that produces diacetyl that smells like butter. That flavor threshold was pretty low. So, if you have that, in your beer, your customers are going to, they're going to smell it. It's also a byproduct of just natural fermentation, but that can get eaten up by yeast. So, mostly we're thinking about diacetyl as an off flavor and excess lactic acid. I am a huge fan of PCR and Coors was an early adopter of real-time PCR. So, real-time PCR was a very powerful and very quick method to speciate beer-spoiling organisms. And the reason I cared about species and not just genus is that through the years, we've proven through spiking studies of

different matrices, different recipes, of what species of a particular organism actually makes a difference. If you have product on hold for a bacterium, but you don't actually know if it's going to harm your beer, if it is going to live and produce these off flavors, you're wasting money. You're wasting time and money if you don't actually know that because all species are not created equal. So, PCR is quicker and it's more powerful to be able to get a species like *Lactobacillus brevis* or *Pediococcis damnosis* and not just the genus that just leaves you like, that's *Pedio* but I don't know if it's going to do anything. So, having that in conjunction with traditional plating you, I've never been able to and never wanted to use PCR instead of, but it is a tool to use in addition to very traditional microbiological mediums, looking for those same beer spoiling organisms, but also looking for hygienic aerobic organisms as well.

Steve Lewis 11:40
And to your point about the importance of getting down to the species level, I would not be surprised to find out that you may have been interviewed for this book, but I, wanting to get into brewing sour beers, I got a book called 'American Sour Beers' and I was trying to figure out what specific strain of bacteria I wanted to use, specifically *Lactobacillus*. And the example that came to mind immediately when you were speaking, is there's a species I'm gonna butcher the name, but it's *Lactobacillus*, I think it's *delbrueckii*, something like that.

Kelly Tretter 12:22
Oh *delbrueckii*, you got it.

Steve Lewis 12:24
So, what stuck out to me about that strain is it's extremely sensitive to hops. And so, it won't grow out of control and basically continue to sour once in the presence of some of the hop-like lupulins or what have you.

Kelly Tretter 12:42
Yeah, that is one of the main ones, and folks getting started in souring need to pay attention to. So, most of these are sensitive to hops, you actually have to propagate your *Lactobacillus* in an unhoped, or very, very lightly hopped wort, or a growth medium, or it's not going to do anything. Picking which *Lactobacillus* species you're going to use is actually really important. And then *Pedio* species can also sour, you get to pick based on what it is you're trying to achieve. They both are self-limiting also, it's like they die off. As they as they reduce the pH, they actually kill themselves with the like reduction in the pH. So, it's self-limiting in that way as well.

Steve Lewis 13:31
And steering it toward, you know, quality considerations and molecular biology, right? Sharing organisms, I have to imagine are really important for quality assurance and in the brewing environments, right?

Kelly Tretter 13:45
Yes, and unless it's coming in on raw materials, we would be mostly concerned about molds. While we could kill it in the boil, we wouldn't be able to control like the spores. We do a lot of testing of raw materials. So, at the dilution at which they would be dosed into our beers, so we can understand if there is a risk of using a particular spice like anything cinnamon, lemongrass, lemon peel, anything like that.

Dr. Gabriel Alves 14:19

I'm very interested in the, on the workflow actually the job. Let's pick for like a New Belgium beer. I really liked the Fat Tire. Tell us how the quality control of that beer from the raw material to the end of the line. How does it happen?

Kelly Tretter 14:37
It's extensive.

Dr. Gabriel Alves 14:39
I'll bet.

Kelly Tretter 14:39
I'll start the story with that. Any raw materials, hops, malt, anything coming in like that we would get C of As from the vendors. We can't control micro or things like that, but we can control the quality of those products coming in. So, we do keep track of that, once all of those materials and then yeast. What we do pay a lot of attention to is yeast. So, we have the ability to just purchase very small quantities from a yeast vendor, test it in our own labs, and then grow it up, propagate it to the volumes that we need to. And every single step of the way gets tested, not only with the traditional plating but we also use PCR for that. And so, we're looking for the bacteria that I spoke of earlier. But we're also looking for wild yeast, especially *Brettanomyces*. So, we can choose to trust or not trust vendors, once we buy something new, and run it through that battery of tests, the plates and PCR, they get slanted. And then they go into our own library. So, since we're controlling the quality from the very, very beginning, putting it on slants, and then going into propagation for the brewery, we don't need to rely on any other labs to tell us if it is usable or not usable.

Steve Lewis 16:08
We hope you're enjoying this episode of Speaking of Mol Bio, we wanted to take a quick moment to tell 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/ismb. And now back to our conversation.

Dr. Gabriel Alves 16:43
A quick question there, aren't you concerned that the yeast, over time, as you keep passing them and growing the population, that yeast might change its behavior and maybe change the pattern, the behavior inside the tank?

Kelly Tretter 16:58
Oh yes, and that is a great point. Fermentation is not a gentle process. Fermentation is actually a very stressful process on yeast. And it's, there's all sorts of pressures on yeast that are going to genetically modify it over time. What New Belgium does is that we have more than one generation of yeast out at a time. So, we'll start a new culture in the lab. When that fermentation is done, and it gets harvested that's generation one. So, we will keep track of the yeast that was started on X day, generation one, and then use that for 5, 6, 7, 8, 9, 10 generations, as long as fermentations are true to brand, and it is as expected. And all of the analytics, the analytical chemistry parts, also true to brand or as expected or true to target and there is no micro. Any step along the way we could have cut that yeast, if a fermentation went long, if the analytical chemist told us that something is out of whack and it's showing stress markers, ethyl acetates, other types of things, acid aldehyde, that's showing that the yeast is done, we can just cut it out of the system, but we have multiple to choose from at any point in time.

Dr. Gabriel Alves 18:20
Right, and then you control the quality of the raw materials you mix everything, now it's boiling. Is that the system from now on is entirely closed and you'll just collect samples?

Kelly Tretter 18:33
It is, yes, it is entirely closed. We have multiple laboratories. We have our microbiology lab, we have analytical chemists, and we have sensory scientists. So, that fermentation is done, it gets its cooling, it's to facilitate the flocculation of the yeast so that we can get more we can get the yeast off and reuse it. And then we'll send the beer portion on to its next step. So, all along the way, we will get a micro check. Either our brewers or our microbiologist will go to the brewery or go out into the brewery and go collect sterile, actually sterile samples, to bring back to the lab and process. And then our analytical chemists will go get samples, and they don't need to be sterile, but they're looking for all of the physical aspects; is, did it reach ABV, the residual extract the apparent extract how much sugar is left in it. The pH is really important. As it goes on farther, we also have other specs and targets for esters. The esters that the wonderful fruity aromas that are produced during fermentation. We have specs and targets associated with what is true to the brand. So, our analytical chemists are looking at those as well at different segments, different stages as the beer moves through the process. And then once it gets closer to being done, our sensory scientists, we have panels. And they're not just ‘Go. No go.’ They could be true to brand, there could be people that are very, very good at smelling diacetyl, for example. So, our sensory scientists test the panel, they actually will spike our beers with these off flavors, and to be counted as a statistically relevant data points you need to participate in a panel and you need to prove that you can detect, whether aromatically or by taste, these up to a certain percentage of these off flavors. It's pretty, it's pretty extensive.

Steve Lewis 20:47
New Belgium, I think, recently got acquired, is that right? In the past couple of years? What are some of the interesting things that come along with being bought up by an international group that's able to have some of those more broad distribution lanes and things like that?

Kelly Tretter 21:07
We, so yes, you are absolutely, you did your research. We were purchased, early in the pandemic or right before the pandemic started, by Kirin. So, a large Japanese conglomerate. New Belgium by that point was 100%, employee owned. It was tough. Coming to New Belgium, one of the things that drew me and drew other people was employee ownership. I wasn't, so, while I was sad about the loss of that, I was also excited being a scientist and being purchased by Kirin. I must say there aren't many places that are still doing true brewing research. Kirin is one of them. They cared about quality; they have amazing R&D labs. Kirin bought us for a reason: they saw what New Belgium could do for their like U.S. portfolio.

Steve Lewis 22:07
That's amazing. I know we're running out of time. But with some of the remaining minutes, I wanted to take a moment to talk about some of the technological advances in brewing as well. Enzymes are now, and even at large scales, have been used for quite some time, but they're gaining popularity even in the home brew space. Very curious. One, what's your favorite enzyme? Whether it's a part of brewing or not, I'm sure you have an opinion. And then two, what do you what do you see on the horizon?

Kelly Tretter 22:44
I uh, so I'm, we are using enzymes. So full, full transparency, because it is a tool that breweries can use now. So, Juice Force does use amylase. It's not in a very high dosage, but what we're

finding that it does is that it actually can make our fermentations more consistent. And what we're also finding is a glucose-to-maltose ratio for yeast health and finishing fermentations is actually really important. So, what the amylase is doing is allowing us to be more repeatable and more reproducible with our large batch fermentations across those five sites that I was talking about. So, there's not, I can't extrapolate or talk too much just because it's not my area of expertise, but it has proved, it is helpful in this particular brand. And then we also, Fat Tire, Gabriel you brought up Fat Tire. We did just change the recipe. The United, the United States does know this, and it's almost like new Coke, some people love it. Some people say, say on our social media. "Why did you ever change that? Why did you change that?" So, I'm going to take the opportunity to say we changed it because Fat Tire was the first carbon neutral beer certified in the United States. So, moving farther into sustainability, we tried to make the recipe more sustainable. The idea was to use some raw barley. Unprocessed, the barley hasn't been turned into malt yet, and you do need some enzymes to help that process. So, our new Fat Tire is all to try and make it more, even more sustainable than it already was with ingredients. And then we do use some enzymes in that process as well.

Steve Lewis 24:42
Very interesting. So, you had mentioned previously that you had been interested in becoming a genetic counselor, loose parallel there. I'm curious about hearing a bit about maybe some of the other techniques like genetic sequencing or even on the analytical chemistry side, like mass spec, what other scientific methodologies do you see and instruments as well?

Kelly Tretter 25:12
Right? As far as sequencing goes, would, I actually, I actually requested to purchase a benchtop one about four years ago, to help out with our foeder program. So, the foeders, the big oak casks in the back, that have just microbiological soup in them. And those are, that's what makes all of our sour beers. So, I had thought, ‘Oh, DNA sequencing is, would be a really great tool to help that program.’ I got, I got denied. So, if we do any DNA sequencing, we actually send out. Many, many labs have it, but PCR if the kit that we are using. If we find something but it's not identifiable, we'll actually send that organism out to go have somebody else sequence it so that we can add it to our library. And then we'll record what the melting, like the melting curve was, and what the peak was and what that looks like from PCR, get a sequence, and get an identification from an outside lab, and then we can add it to the library. So, if we ever get another unknown again, but the melt curves present in the same way, we will know what the organism actually was. Just recently, the micro lab after I was already out of it, they did just get one of the pretty small sequencers. I can't remember who and it—it doesn't matter. But to kind of do a foray into DNA sequencing in the lab. I don't know how many base pairs it can do. I think it's on the smaller side because it was much more affordable. So, I don't have an update, I haven't talked to the lab recently if they're using it and what they think about it. I think it's an amazing technique. I wish I could have bought one. But we're also lucky that we have a lot of labs, including CSU that we can go to for that. As far as analytical chemistry goes, though, it's an amazing lab. They have HPLC, they have multiple GCs. They have a GC mass spec with an olfactory port on it. They don't use it as much anymore. But when we were starting up our Asheville facility, they did use it to match our flavor profiles, our aromatic profiles, between Fort Collins and Asheville. And while it sounds super cool, it's also very time consuming to have somebody sit at the port and sniff the port for seven hours as those compounds come off, come off of the column. So, it was very helpful for a period of time, but we actually don't use it as much anymore. But the other GCs, absolutely they get used on a daily basis. We also have an ICP. So, we're interested in doing lots of metal analyses of our worts, of our wort matrices, because that's going to optimize yeast growth and yeast health.

Steve Lewis 28:15
Amazing. And yeah, the wort being sugar water, fermentable sugar water.

Kelly Tretter 28:20
Yes, with the malt and the sugar.

Steve Lewis 28:22
For our listeners. And while I'm on my trend of really smooth segues, I know Gabriel had another question.

Dr. Gabriel Alves 28:31
Yes. Well, it's a two-part question. To start. The first part is more academic. I teach part time at Western Michigan University. Western Michigan University partnered with Kalamazoo Valley Community College in creating this brewmaster's degree, which is pretty interesting. What are some other courses that folks that are aspiring are looking for this career? Besides brew-master's in microbiology? What are some specialties that folks could go for?

Kelly Tretter 29:09
There are, so in our current world, there are so many different choices, as opposed to zero choices when I graduated. One of the more popular things to do, and it sounds similar to maybe what has started in Michigan, Colorado State University has a fermentation science program. It's a degree that you actually enter in, and it is a degree program. Part of fermentation science is brewing science. So, we have helped fund and I think so has Anheuser Busch. They have actually two pilot breweries on campus at Colorado State where the students who can actually get their hands on brewing, valves, steam, and also automation so that they could be prepared for old-school, small-scale breweries, but they can also learn automation and be able to come to a brewery like ours. That's probably one of the more popular ways to go is a program like that. But there are all sorts of accreditations. There is Heriot-Watt, it's in Scotland, but you could do things online, super intensive programs. There's the internet, oh, my gosh, acronyms IBD, International Brewers and Distillers, you can take exams and get certified in that. There are even just classes to take on QA and the microbiology of beer, if it's not part of the bigger fermentation science. You can just take individual coursework at certain colleges that are now available all over the country.

Dr. Gabriel Alves 30:46
You mentioned chemists too. Chemistry, yeah, chemistry, a lot of analytical chemistry. Yes, that is very interesting.

Kelly Tretter 30:55
Yeah, the thing that's really cool, and I can, I only know the most about Colorado State, is that since they're in our, in our backyard they have asked for feedback through the years on how can they make their program very transferable; have the skills be transferrable to come work in a brewery or in a lab. So, they have purchased instrumentation that somebody could graduate and then come right into our lab, and know how to use, or have had experience using in their college classes.

Dr. Gabriel Alves 31:28
Thank you so much for the second part where my questions were a little bit a professional. So, you've graduated and now you're looking for a job. What are some of the openings and opportunities in the brewing industry?

Kelly Tretter 31:42
It's yeah, if you have a couple of different avenues. It would be helpful to decide if you if you can, I know when you're young, you want to do everything. Going in a lab-specific direction or going in a brewery-specific direction. And the reason I say it's, it's more powerful to pick a direction, in that at least at New Belgium, Coors wasn't as particular, they, they would hire general scientists. Lucky for me, they hired a general scientist. We at New Belgium tend to hire more classically trained microbiologists for an entry-level microbiology position. Or a classically trained analytical chemist to work in the analytical chemistry lab. Or we look for people that have brewing experience from other breweries, even if they're not classically trained, they have hands-on knowledge of the brewing process, sterile sampling, brewery layout, the different phases of brewing or the different like parts of brewing. And then we also hire internally folks that don't have any experience, train them ourselves. I do have to say the majority of folks have been classically trained scientists in their discipline.

Dr. Gabriel Alves 33:04
Molecular biology, then, is involved from beginning to end, which is very exciting. What do you have to say, for those young scientists, especially for women? What do you have to tell them? What is your message to them?

Kelly Tretter 33:18
That is such a good question. It's actually one of my favorite questions. No one in the early days knew, including myself, and my early days, of course, knew you could be a brewing microbiologist, it just wasn't something that people realize number one didn't realize that as much science is needed to go into making beer. And number two, actually, that you could be a scientist at a brewery, not just apply the science to the brewing part of it. Even New Belgium has done some data gathering. And we even don't have a lot of women were well representative in the labs were not well represented in brewing. And so, I think that there's a couple of things I personally would love to talk to women in STEM that are already in STEM programs, maybe already taking some science classes, and letting them know what opportunities there are that these jobs even exist. It's a kick-ass industry. Are there ways to improve it? Absolutely. By doing things like this and getting the word out, sharing my experience, and if I can just get more women interested in just even just knowing that it's a really cool career, that it’s even an option. Let's just even just start there.

Steve Lewis 34:48
That was Kelly Tretter, senior quality integration manager at New Belgium Brewing. Thanks so much to Kelly and Dr. Gabriel Alves for joining us this episode. We love sharing our conversations with great people. This episode was produced by Matt Ferris, Sarah Briganti, and Matthew Stock. Cheers and good science!