Genome Insider S2 Episode 3: Better Living Through Bioenergy

ALISON: Hey! I'm Alison Takemura, and this is Genome Insider, a podcast of the US Department of Energy Joint Genome Institute, or JGI.

Today, we're throwing open the proverbial doors to a sister organization that the JGI works closely with: the Joint Bioenergy Institute, or JBEI. They're also funded by the Department of Energy, just like JGI. And their mission is to develop bioproducts and advanced biofuels, the kind that replace gasoline, diesel, and jet fuels. They patent their breakthroughs and transfer these technologies to companies, who can work on getting products to market.

To make the first iterations of these biofuels and bioproducts, JBEI scientists work with plants and microorganisms. And JGI helps interpret and modify these organisms' genetic blueprints. JGI and JBEI are like siblings with complementary strengths.

Today, we'll dive into how two JBEI scientists, with JGI's help, are working with a biofuel-producing bacterium. But before we get to the bacterium, let's get to know the humans of the story.

STEVE: I'm Steve Singer. And I'm the director of microbial and enzyme discovery at the Joint BioEnergy Institute.

Steve Singer, director of microbial and enzyme discovery at the Joint BioEnergy Institute (JBEI), a U.S. Department of Energy (DOE) Bioenergy Research Center. (Courtesy of Steve Singer)

ALISON: Fun fact about Steve: he partly attributes his interest in bioenergy to when then-President George W. Bush mentioned the biofuels hydrogen and ethanol in a State of the Union address in 2006. Let me play you the clip. Here, he's talking about creating a more sustainable energy future.

PRESIDENT GEORGE W. BUSH: We will increase our research in better, better batteries for hybrid and electric cars, and in pollution-free cars that run on hydrogen. We'll also fund additional research in cutting-edge methods of producing ethanol, not just from corn, but from wood chips and stalks, or switchgrass.

ALISON: We just did an episode on switchgrass! OK, so that's Steve and one of his inspirations. Now, let's meet his colleague, Aindrila.

AINDRILA: I'm Aindrila Mukhopadhyay. And I'm the vice president of biofuels and bioproducts division of JBEI, and I also lead host engineering at JBEI.

ALISON: Aindrila and Steve are two of the original team of scientists that founded JBEI in 2007.

AINDRILA: Yes, We're part of the original band, I think.

ALISON: And they joined JBEI to help solve a problem. As George W. Bush put it, the world has a damaging addiction to oil. Burning fossil fuels is altering the world's climate, which is a problem that's bigger than we can easily comprehend. So, something that I think about to get a handle on this, is how millions of people's lives will be upended, if we continue down this path.

Climate change is already causing sea level rise, more powerful storm surges, and transforming land into desert. The World Bank predicts that these worsening conditions will force millions of people around the world to leave their homes, especially if they live in the most vulnerable places: Sub-Saharan Africa, South Asia, and Latin America. World Bank experts think that, by 2050, climate change will force 143 million people to migrate. One hundred and forty-three million people. That's such a big number, and it's hard to grasp, so, let me break it down: it's like 2 out of every 5 people in the US. That is how powerful and life-altering climate change is.

So, JBEI's Steve and Aindrila are working to avert the worst of climate change by finding bioenergy alternatives to fossil fuels.

AINDRILA: To me an opportunity of a lifetime to work on a challenge that affects every single human being on our planet.

Aindrila Mukhopadhyay, the vice president for biofuels and bioproducts, and director of host engineering at JBEI. (Marilyn Chung)

Time will tell, you know, how large my wedge of the impact will be, but for a scientist, it is such an amazing opportunity to apply their knowledge or their skill towards a problem that affects so many people.

ALISON: So, getting energy from plants is part of the solution. And you could say, Aindrila and Steve work on different pieces of the bioenergy pipeline. Steve works upstream, going out into the environment and looking for organisms. Then bringing them back to the lab, and trying to understand them, how they can break down biomaterial, like plants, and make biofuel. He sometimes even artificially evolves strains.

Aindrila, on the other hand, works further downstream. She engineers well-studied microbes to try to make them better at their job of making biofuel and bioproducts.

Now, one of the main organisms that Steve and Aindrila research is a bacterial workhorse that you might not have heard of: Pseudomonas putida.

Pseudomonas putida — which I'll call putida for short — is shaped like a stubby caterpillar, covered in bristles of little hair-like appendages called pili, with a tuft of flagella, which are like tails, at one end. It lives in diverse places — in soil, water, and associated with plants.

The word ‘putida,’ comes from Latin and means foul and putrid. One of the first places putida was isolated from was manure, so.. I guess the name is a reflection of the habitats that it favors.

Steve and Aindrila chose to work with putida because of how the microbe breaks down plants.

AINDRILA: So, a plant biomass is, simply put, composed of the woody part and the sugary part.

ALISON: In the early years of JBEI, the team had focused on converting the sugary part into biofuels, which could be done by the model microbes they used, like E. coli and engineered yeast.

AINDRILA: But now we had to turn our attention to the woody part.

ALISON: And that was because they wanted to get the most out of the plant. But the microbes they had worked with couldn’t handle the woody part. So, Aindrila and Steve looked to a more versatile microbe: Pseudomonas putida.

Pseudomonas putida. (JGI Genome Portal)

AINDRILA: Pseudomonas putida, was a really ideal candidate. It wasn’t like a recently discovered organism with this one unique trait that was desirable but nothing else was known about it. It was one of those organisms that could eat or catabolize, the woody part of the plant. And yet, a lot was known about it because it had served as a model system for other biologists.

ALISON: Other biologists had studied putida because it could remediate toxins in the ground and colonize plant roots. And they had already sequenced putida’s genome and developed genetic tools that could be used to alter putida’s genetic code. Putida had even been engineered to degrade crude oil back in the 1960s. And it made headlines, when it was the first organism to be patented in the world. At the time, it wasn’t like you could just patent something that was alive. But in the Supreme Court case of Diamond v Chakrabarty, the Supreme Court decided you could patent a genetically altered organism.

Putida’s strong scientific foundation meant that Aindrila and Steve could start experimenting with putida quickly.

One of the first challenges that they threw putida into was a dangerous chemical bath.

STEVE: In the process of converting biomass, we need to chemically pretreat it. And so we chemically pretreat it with a pretty unique class of chemicals called ionic liquids. And they are salts made up of organic compounds that despite they’re like, like salt, like you know, table salt, which is solid and melts at —

ALISON: — about 1500 degrees Fahrenheit —

STEVE: — this actually melts at or near room temperature. So they're liquids, but they're salt.

ALISON: These unusual properties mean that they can readily break down and fractionate, or separate, biomass.

STEVE: In fact, they're quite good at fractionating the sugar from the lignin, the woody part of the biomass. And the problem is, you know, if they're good at breaking down plant biomass, they're also good at breaking down microbial biomass. So microbes aren't happy when they are incubated with these ionic liquids.

ALISON: Would you be happy if your bath were toxic to you? Pseudomonas putida, though, can be a ridiculously tolerant microbe. One study found it could grow in a mixture that was 50% water and 50% toluene, an aromatic solvent that's used in paint thinner.

The putida model strain that Steve started using is called KT2440; the "KT" part is named after Kenneth Timmis, who characterized the strain. And this strain, well it didn't love the ionic liquids. It actually didn't grow too well.

But Steve and his team thought maybe it could evolve to be OK with this harsh environment. So, they grew putida for many generations in media containing ionic liquids

STEVE: We adapted Pseudomonas putida to grow in the presence of some of these ionic liquids. And then we show that they develop tolerance to these ionic liquids.

ALISON: They identified mutants adapted to living in up to 8 percent ionic liquid by volume. And together with a JBEI group at UC San Diego, they sequenced these adaptive mutants to find out how the putida was doing it. They discovered that the adapted putida strains relied on membrane pumps. And, when ionic liquids got into the cells, which would be bad, causing an internal commotion, these pumps would pump ionic molecules back out, keeping the microbes happily intact.

So, while Steve has been working on understanding and evolving putida, Aindrila has a more hands-on approach. She and her team engineer putida. Here's Aindrila: she has a fascinating point to make that gives context to her work.

AINDRILA: You know, if you were to Google, right, X compound, and synthetic biology or engineered and microbe, you will find dozens, if not hundreds of examples of efforts that people have made to show that a particular fuel or product or compound can be made in a microbe from a renewable carbon source. So why aren't we surrounded in our daily lives by bio-made compounds? Why has this not happened?

Yeast make ethanol through fermentation. Here, a new yeast cell, artificially colored in gold, is emerging. (Juergen Berger, Max Planck Institute for Developmental Biology, and Maria

Langegger, Friedrich Miescher Laboratory of the Max Planck Society, Germany)

ALISON: Wow, uh. Microbial ecology grad school did not prepare me for that question. I honestly don't know. So, Aindrila told me. She said most of these compounds couldn't be produced in large quantities. Only ones that are, make it to market. So what separates compounds we can easily scale up from those that we can't?

AINDRILA: The compounds that we have managed to scale up are in reality compounds that these organisms make naturally, natively; it is part of their metabolism. In most of these cases, the example is also of a compound where the production of the compound is done under a condition where it is necessary for that organism to make that compound. Otherwise, it cannot live.

ALISON: You know a good example: ethanol, the key ingredient in beer, wine, and other alcoholic beverages. Ethanol is made by yeast during a particular growth mode called fermentation. And during fermentation, yeast breathe out ethanol like we breathe out carbon dioxide. You just— you can't stop it.

AINDRILA: Without making ethanol under those conditions, the yeast typically cannot live.

ALISON: So this particular metabolic wiring means that as yeast grow in culture, they make more ethanol, in very high amounts and in a very reliable way.

AINDRILA: There are no trade-offs here between growth and production; it is one and the same thing.

ALISON: This is the same idea that Aindrila and her team are applying to putida. They want to get it to produce a desired biocompound as it grows. Do that, and you've got a potentially more marketable product.

Another way to picture this approach is to imagine you've just discovered how much you love the color blue, and you really want to have a blue houseplant. So, on a whim, you engineer a plant that makes blue leaves.

AINDRILA: But you know, it doesn't need to make blue leaves. So after a few cuttings, or after a few seed-to-plant cycles, it will lose its ability to make blue leaves, and you'll be so sad because you lost your blue plant. But now, what if you made it so that having that blue leaf is necessary because it's on a different planet, and the sun shines different, and you need blue leaf to get energy? You have changed the relationship of that modification with its life; it is no longer a decoration, it is necessary. So you can go through any number of cuttings or seed-to-plant, it will not lose its blue leaf, because that's how it lives. You have changed the plant; you have changed its metabolic wiring. It has to make blue leaf to live.

ALISON: That's an example of a plant making a blue pigment.

Watch bioengineered indigoidine diffuse through water, illustrating its vibrant hue. (Marilyn Chung)

In Aindrila's work, she decided to try to engineer putida, to also make a blue pigment. This experiment would be a stepping stone, allowing her team to confirm their engineering approach, so that they could ultimately make better biofuels. To engineer putida, she and her team turned to a computational technique developed in 2017 by a group in Germany. By taking a model of a microbe's metabolism, this technique could determine what genes a scientist should knock out in order to have a desired compound coupled to the microbe's growth.

AINDRILA: And it was demonstrated bioinformatically but never implemented. And we kind of ran with that, and tried to figure out well, if you wanted to implement it, what more do you need to do in your computation?

ALISON: So Aindrila and her team took the genomic model that they had of putida's inner workings.

And, they decided to try to rewire putida's metabolism so that it could easily make this blue pigment, called indigoidine. It's like indigo, and it produces a vivid, almost bottomless blue. It's incredible. With it, the scientists would literally be able to see how well their metabolic modeling and rewiring worked. The bluer the bacterial culture, the better.

AINDRILA: And a postdoctoral researcher in my group, Deepanwita Banerjee, took the task of doing some of this modeling to tell us what could be the genes one would have to remove in order to make the production of a final metabolite paired with the growth of that organism.

ALISON: Deepanwita predicted that there were 14 genes responsible for 16 reactions that, if removed from putida's metabolism, would allow the production of indigoidine to be paired with putida's growth.

AINDRILA: Basically meant we would have to delete 14 genes all at once. Not one at a time in separate strains, but in the same genome, 14 separate edits would have to be made. This had not really been tried before, this was a big ask.

Research assistant Andrew Lau, project scientist Thomas Eng, and postdoctoral researcher Deepanwita Banerjee (left to right) were the hands-on team that engineered P. putida to produce indigoidine. (Courtesy of Aindrila Mukhopadhyay)

ALISON: But Thomas Eng, a research scientist and senior member of Aindrila's team, was up to the task. He used the popular technique of CRISPR interference to target and stop all these genes.

AINDRILA: And so in one step, he was able to interfere with all of these reactions.

ALISON: And the experiment worked. It worked really well. The engineered putida produced lots of indigoidine, churning it out as it grew — just as Aindrila had hoped.

Biology can be tricky, though, and Aindrila wasn't about to go shouting their results from the rooftops yet. First, Aindrila wanted to make sure they had knocked down the right genes and done nothing else to the organism, and that that alone had been enough to get this successful result.

AINDRILA: So for that, we turned to JGI. We sent up these strains to JGI for some characterization, to see if these genes had indeed been taken down. And so it was a very useful resource for us to have to be able to validate what had actually happened in the cell. We collaborated with another team that studies proteins to do the same.

ALISON: That other team was Chris Petzold's functional genomics group at JBEI. And to scale up production of indigoidine, Aindrila also worked with another organization at Berkeley Lab, the Advanced Biofuels and Bioproducts Process Development Unit, or ABPDU. They work with outside scientists and biotech companies to help make their production lines more efficient. Some of those companies' products are delicious: like vegan egg alternatives, and milk from plants; and some of these products are stylish, like leather made with mushrooms. Check out our show notes to find out more.

Alright, back to Aindrila's work on engineering putida to make the blue pigment indigoidine. Thankfully, it was all completed before the pandemic.

AINDRILA: If that had not been the case, then it would have been a different story.

ALISON: So, I asked Aindrila if, given her team's success, if we'd be seeing biobased indigoidine on the shelves and in our blue jeans, next week, and she gently encouraged me to hold my horses.

While I can't expect that to happen just yet, they have submitted a patent for the experimental approach that they've developed to engineer an organism to produce a bioproduct. And that, in turn, will help pave the way for companies to license their technology. What's neat is that this technique can work with, not just indigoidine, but many kinds of metabolites. Aindrila's team is looking to engineer putida to make other chemicals, like polymer precursors and solvents, that can help offset fossil fuels. Because you know what they're used in? Practically everything: including engineering, construction, plastics, footwear, textiles, dry cleaning, ink, paint, adhesives, cosmetics, toys, and pharmaceuticals. Aindrila is also really keen on making bio-based jet fuels.

But research is like a spiral, not a straight line. It bends back on itself. Steve has even gone back to the beginning — looking for new strains of putida that could have different genes that solve different metabolic problems.

STEVE: In the world and the environment, there are, you know, an infinite number of related Pseudomonas putida strains, that we potentially could mine for capabilities that KT 2440, this strain, does not have.

ALISON: For example, strain KT 2440 can't metabolize xylose, which is a sugar found in plants.

So, Steve and his team went looking for other strains that could. Leading the charge was a then-high school student, now UC Berkeley undergrad, named Taqwa Tofaha.

Taqwa Tofaha, then-high school student and current UC Berkeley undergraduate student, who did the iCLEM summer internship program with JBEI in 2018. Taqwa helped discover a new isolate of P. putida that can metabolize the plant sugar xylose. (Peter DaSilva)

She had joined Steve's lab as part of a summer internship program that JBEI runs, called the Introductory College Level Experience in Microbiology. And at the end of it, she still wanted more. More microbiology! More putida! So she stayed on in the lab, and went looking for new putida strains, in the hope that one might be able to metabolize xylose.

STEVE: And so when, you know, Taqwa, started working in my laboratory as a high school student, she and Miri Park, who's my postdoc, and Bonnie Fong, who is a research associate, we did sort of a round of isolations, where we isolated Pseudomonas strains from soil, from bark, from like, the bay shore, and so, surprisingly enough, we got about 30 or 40 strains, but only five of them grew in liquid culture.

ALISON: Sigh, such is biology.

STEVE: And so, we sent those strains to JGI, to sequence them. We knew they metabolized xylose, and we had some idea of the pathway. But we were really surprised because part of the pathway is known in other organisms, but there, there is one gene that is very novel, that is not seen in this pathway in other organisms.

ALISON: At least, this gene isn't seen in microorganisms. The gene appears to be very similar to one found in plants!

STEVE: Now, that's interesting, because Pseudomonas putida is often found associated with plants in the rhizosphere.

ALISON: The rhizosphere is the zone around the roots.

STEVE: So, that that was sort of food for thought that maybe, that plants and microbes are exchanging genetic information. And so this sort of variant of the pathway, suggested that Pseudomonas actually had found a way to incorporate xylose metabolism into its genome in a pretty unique way.

ALISON: Putida got a gene from a different kingdom of organisms. That's like us getting a gene from putida! The next step is to domesticate this new putida strain for research and make it genetically malleable.

STEVE: My postdoc, Rahul Gauttam, has spent a few months figuring out how to get DNA into these environmental isolates of Pseudomonas putida. And recently, he succeeded.

ALISON: Which means that Aindrila and her team could engineer this new putida strain like the one that they did before, except this time maybe to make a biosolvent or a jet fuel. So, Steve and Aindrila have the research spiral going. Steve's work informs Aindrila's, and Aindrila's informs Steve's. Their work together and with JBEI as a whole, is creating a new world, one where we're not dependent on fossil fuels. Instead, we're harnessing the power of plants, of putida, of biology!

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Dan Udwary and me. That’s it for now. See ya next time!