MIND FOUNDATION

Magic Yeasts and How to Make Them Produce Psilocybin

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J: Welcome, Nick. Thank you so much for coming! In your lab at The Novo Nordisk Foundation Center for Biosustainability, you have mainly worked on projects related to sustainability. What made you interested in psilocybin?

N: The research center where I worked is the Center for Biosustainability – A core focus of the center is to develop bio-based processes to replace non-renewable synthetic chemistry. I have always been interested in mental health, and particularly how we treat mental health, or rather how badly we treat mental health today. I was also always interested in the potential of psilocybin.

J: Psilocybin is not a mainstream medication yet; it is actually still quite far from it. At the same time, your research topic sounded like a great idea; was it really so easy to pursue?

N: At the time, it was undoubtedly a bit of a risk, and the first time I pitched the idea, I got laughed at! Eventually, what swayed people was showing them the clinical data. About two years ago, there was a lot of excellent clinical data starting to come out that showed the potential of these molecules, and I think that was what really swayed the people at the center to kind of say: 'Okay, let's give this a go.' Thankfully, it has spun out into a company, and now, a year later, the psychedelics industry is becoming a promising one.

J: And just a little bit over one year later, you have already published your results! That went pretty quickly.

N: It was indeed fast! Even by our standards, it did go very quickly, and the time it took to go from idea to proof of concept was only about four months. It took a while to put everything into publication form, but the research went surprisingly well. Thankfully there was a bunch of other researchers who helped a lot with this project which made the whole thing go faster.

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J: That sounds great! Could you then summarize what you found in your study and how difficult it was to obtain these results?

N: Our research's primary goal was to identify how *Psilocybe* mushrooms produce psilocybin and then figure out how to replicate that process in *Saccharomyces cerevisiae,* also known as brewer's yeast. The biosynthetic pathway to produce psilocybin was discovered a couple of years ago, but notably, not all of the genes were found. Since biosynthesis is a process requiring a concerted action of genes involved in synthesis, transport, and regulation it is necessary to classify them all. The main genes identified were the ones that catalyzed the critical reactions, but there was a couple that were left out and still needed to be found. We had to dig a little bit into the genome of *Psilocybe cubensis* to find the missing genes. We then introduced them into yeast by transferring the genetic material.

We were able to demonstrate the production of psilocybin, which was a good start, but a lot of the work was focused on figuring out how to produce more psilocybin. Basically, the way that we did that was to work on the native yeast metabolism. Essentially how this works is that the genes that we transferred from *Psilocybe cubensis* work together with the genes that could transform tryptophan into psilocybin. Tryptophan is an essential amino acid, but it is also an amino acid that yeast produces endogenously, and actually, most species do. The most challenging part of the work was increasing tryptophan production – so playing around with the native yeast metabolism to increase the rate of tryptophan production, which subsequently led to more psilocybin production.

The last thing we did was to play around with the genetic code of the *Psilocybe* genes a little bit so that we could divert production towards some of the other intermediates and derivatives of the pathway. For example, we showed that we could make a small tweak and produce *aeruginascin* instead of psilocybin, and we showed that by introducing entirely foreign genes, we could make molecules that did not yet exist in nature.

J: Other studies, however, successfully demonstrated the production of psilocybin in *E. coli*.1 Could you explain why did you chose brewer's yeast and what was the advantage of it?

N: I think, first, it is good to explain why it is a good idea to produce psilocybin in living cells in the first place. Today, apart from our method, there are two main ways of getting psilocybin – either you have to grow the mushroom, or you have to synthesize it chemically. When it comes to the mushroom, if you have seen what a mushroom looks like, it is not that easy to grow, and the psilocybin accumulates in tiny quantities in the mushroom.

J: Psilocybin is also found not evenly distributed across the different parts of the mushroom, so I imagine that extracting psilocybin from large quantities of *Psilocybe cubensis* must be tricky.

N: Exactly! If we are thinking about commercial applications, we would need massive amounts of mushroom biomass. It would be challenging to extract, and the complexity of this process makes it very difficult for pharmaceutical applications. If we want to use psilocybin in humans, it needs to be very pure, and

the production needs to be very consistent. On top of that, most pharmaceutical drugs are derived from non-renewable resources, including psilocybin. Synthetic psilocybin is derived from benzene, which is a fossil fuel. It is also very environmentally hazardous and the chemical synthesis methods generate toxic waste, so there is undoubtedly an urgent need to develop a more sustainable way, which leads us to production in microorganisms.

And why yeast instead of *E. coli*? That's an excellent question; in my mind, there are two reasons – the first is that yeast is the world's oldest example of domestication. A thousand years ago, humans started domesticating yeast to produce beer, and fast forward to today; we have this organism bred essentially to thrive in an industrial environment – it does exceptionally well in beer fermentation and ethanol production. That is because it has evolved over all these years to tolerate the very extreme conditions of industrial-scale fermentation.

Still, importantly for psilocybin, the other main reason is that yeast is a very close relative of *Psilocybe*mush-rooms from an evolutionary point of view. They are both from the same kingdom – they are both fungi, so this makes a crucial difference if you want to express foreign genes in an organism; it helps if they are already closely related, and for *Psilocybe* this is very important. Hydroxylation, which is the key enzymatic step, does not work in bacteria such as *E. coli*. *E. coli* is actually missing an organelle – an essential piece of machinery that would allow this enzyme to work. Hence, reconstruction of the entire pathway is virtually impossible in bacteria. Other reports have successfully produced psilocybin in *E. coli*, but essentially, they had to circumvent this problem by feeding their strain a synthetic compound. From a cost point of view and from an environmental point of view, it is not really feasible. Our study has used sugar as a starting substrate, so it is very sustainable, and we can achieve a much lower cost of production, and our process is almost CO2 neutral.

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J: The sustainability of the process is definitely a significant advantage over other methods. You have also fine-tuned the yeast metabolic pathway, but it is probably not optimized yet for large scale production. What exactly would you have to improve to make it optimal for production on an industrial scale?

N: The main challenge is to increase the amount of sugar that gets converted into psilocybin. Beyond that, to reach commercial-scale production satisfactory enough to be used for human clinical trials, we need to scale the process from one liter to thousands of liters. The fermentation development is critical, and of course, the other issue that we need to look into is how to efficiently purify psilocybin from the fermentation broth.

J: How far is the work, do you think, from achieving a production protocol available to be used industrially?

N: This work is what we are doing at Octarine right now. It is already feasible to produce small amounts of psilocybin, but to produce the quantities needed for human clinical trials and eventually commercial applications, there is still a little way to go. Still, the biggest hurdle is the regulatory approval, so we can have a production process ready considerably faster than the regulatory bodies will give the authorization to use it.

J: You have used the method of introducing foreign pieces of DNA into the yeast genome. This has been done already with gene-editing techniques like CRISPR all over the world, and it is not a new thing to do, but there is always a risk involved in the process of playing around with genomes of living organisms, which may produce some unwanted effects. Have you observed any of these?

N: Indeed, it can be a big issue. Not only expressing foreign genes in heterologous hosts [an organism naturally lacking the gene of interest] but also just producing molecules which the organism has not encountered before. In our case, thankfully, we did not see any of these issues. That is another good reason for using yeast – it tolerates foreign gene expression very well and it typically does well when faced with a foreign metabolite. The natural host often produces these molecules for a very good reason, so there has to be a reason why mushrooms produce psilocybin. Often the produced compound acts as a deterrent, which means it is a toxin to microorganisms. This is the case with, for example, cannabinoids, which we also work with. Cannabis produces cannabinoids primarily as an antimicrobial, so if you are trying to produce it in a microorganism, you are going to come across toxicity effects. Thankfully we have not seen any issues so far in the psychedelics we are working with.

J: Right. You have also achieved a stable transformation of the cells, which means that the foreign genetic material is integrated forever.

N: Exactly! This is another excellent reason for using yeast. It is very easy to integrate straight into the genome and integrate stably, so essentially the genes are in there without any selection pressure or anything to force them to stay there.

J: Usually antibiotic resistance genes are transferred together with genes of interest, so that when an antibiotic is introduced into the growth medium the cells are continuously forced to express the foreign genes. You have managed to integrate your genes of interest without any antibiotic selection?

N: Yes, and that is important for large scale production, you do not want to have antibiotics or any sort of selection pressure, both from a cost point of view – antibiotics are expensive – but also from a human safety point of view – you do not want antibiotics in your mixture, and you also do not want any activation of the antibiotic resistance genes.

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J: Next to all those impressive achievements, you have managed to do another thing – to synthesize a novel molecule that has not existed in nature before, *N-acetyl-4-hydroxytryptamine*. Could you tell us about this compound and if you are going to investigate it in more detail in the future?

N: We are not only interested in producing psilocybin; we are also interested in the range of other molecules that *Psilocybe* mushrooms produce. We were inspired by the fascinating findings in the research on cannabinoids, which shows that some of the minor cannabinoids have new or improved functions. For example, CBD seems to be very effective at treating epilepsy, but one of the minor cannabinoids, CBDv, potentially could be even better. The amount of evidence is growing, and it suggests that the same could be true for *Psilocybe* mushrooms, so we are, of course, very interested in all of the derivatives and all the intermediates that the *Psilocybe* mushroom produces.

Going beyond that, one of the things that we are very interested in, especially at Octarine, is how we can use biological machinery to create molecules that do not exist in nature. At least in theory, enzymes should be vastly superior to synthetic chemistry. They should be able to catalyze reactions that synthetic chemistry cannot do, do it much more efficiently, and under much more favorable conditions. The trick here is figuring out how you can ‘convince’ an enzyme to catalyze a reaction it does not normally do. That is a part of the core technology that we are developing at Octarine, but in this paper, we just wanted to demonstrate the potential of natural enzymes to be coaxed into catalyzing reactions, which they do not normally do. That was an example with this molecule N-acetyl-4-hydroxytryptamine, which is essentially half psilocybin and half melatonin. In general, we are very interested in investigating the properties not only of novel molecules that are produced

in our platform but also the properties of some of the minor tryptamines that *Psilocybe* mushrooms and other psychedelic organisms produce.

J: Melatonin is a sleep hormone found in the human brain and psilocybin acts on serotonin receptors. Do you think this new type of tryptamine could also have some psychoactive effects in humans?

N: I would say it is too early to speculate. This work is more about a production proof of concept, but what I can say is that, in general, the idea is that we are creating new molecules in the hope of creating a better version of psilocybin or a molecule that has a different therapeutic effect. This is essentially the work that is going on right now – we try to figure out what these molecules do and whether they could be interesting from a therapeutic point of view.

J: It seems like there is a whole new generation of compounds waiting to be discovered, which is very exciting! Now, imagine somebody gives you an unlimited grant for Octarine, for any research project. What would it be?

N: This is a very good question and what comes to my mind is that in nature, there are thousands and thousands of interesting molecules that have interesting therapeutic effects, and we have been exploiting these natural sources since, well, forever.

J: That is true in the indigenous context, but in Western medicine they have been relatively neglected.

N: Exactly, and I think part of the reason is that there is a big gap that exists between understanding what these molecules do and figuring out how we can produce them. There are plenty of examples of molecules that we know exist in nature, and we even know that they have remarkable therapeutic properties, but we do not have the tools yet to produce them in quantities suitable for pharmaceutical application. For a very long time we had no idea how the natural organism produced them. It was only in the last few years that we actually figured out how cannabis makes cannabinoids and how *Psilocybe* mushrooms make psilocybin. However, for the thousands of other molecules, we have little idea how they are produced, and we have no idea which enzymes and which genes are responsible. I think this is a bottleneck in developing natural molecules for therapeutic applications.

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J: What exactly would be the first big step to improve this situation?

N: In my opinion, the main problem is speed – we have methods in place for elucidating biosynthetic pathways, but they are incredibly laborious and time-consuming. There have been considerable advances in obtaining increasingly large data sets on cellular processes. Perhaps using things like machine learning or artificial intelligence to take some of the guesswork out of the interpretation of this data could be a significant improvement. We make some good guesses about what we think is going on, but then we spend a very long time testing it, and far too often… we are wrong.

J: So, computer modeling could definitely speed up the process.

N: I think so. Taking the human aspect out of it and improving our methods of figuring things out would definitely improve our best guesses on which genes are involved. It would be a giant leap in the field, I think.

J: I will keep my fingers crossed for the developments in Octarine. My last question relates to what you said before about the first time you pitched your idea to others and it was met with… not the most fertile ground. Do you have any hints for researchers and students about how to push for this kind of research?

N: I think that, at least in the research community, the stigma around these molecules is rapidly decreasing. In my opinion, it is because we can look at the clinical data and it is fairly black and white that these molecules hold a lot of promise, so it is not the research community that needs to worry about stigma; it is the public perception which I think is a more significant issue. In general, a part of how I got into the field was looking at the clinical data and being convinced that there is something interesting there. I always think that a good place to start is to dive deep into the data and see for yourself the potential of these molecules.

One of the great things about this field, as well as with cannabinoids, is that it brings together people from entirely different backgrounds. I could never imagine that I would be working with all different kinds of researchers, but that just shows how promising these industries are and that it requires collaboration from so many different backgrounds. In my case, the background is yeast and microbiology. I have spent my career making biofuels and trying to decrease fossil fuel emissions, so it is fascinating how different experiences can be relevant. The field is developing thanks to people applying the skills they have picked up in one area and figuring out how it can be applied to the psychedelics industry. I think that would be my advice – look at the skills you have and try to see how they can be applied to this new industry because I really believe that there is so much opportunity for development in the ecosystem.

J: Thank you so much for this advice! One piece of good news that I could share with you is that the Central Institute of Mental Health (ZI) Mannheim have just recently announced the start of a psilocybin-depression study lead by Prof. Dr. Gerhard Gründer. The MIND Foundation participates in this research and our clinical partner OVID supports with therapists at the second study center in Berlin – and both are thus providing additional financial resources for the study. It is the first study of this kind with the prospect of being funded by German governmental funds.

N: I think that in the world, the momentum is very rapidly building, and I read about this the other day. It is a huge moment, since public funding is being used for psychedelic research. One thing that was really encouraging for us is that one of our investors is the Danish government.

J: That is amazing!

N: The Danish government has a venture fund and they are one of our biggest investors. For us, that is a hugely positive sign for industry in terms of overcoming the stigma – the fact that public institutions and even governments are getting behind this kind of research. Even the FDA has a lot of positive things to say about psychedelics and they are very pragmatic about this, so indeed, times are changing and I have no doubt that five years from now, this will not be an issue anymore and it will become clear that psychedelics can be used for therapeutic benefit.

J: As soon as the pharmaceutical industry recognizes the benefits?

N: Essentially, that is what we have seen with cannabinoids.

J: That is true; the therapeutic use of cannabinoids became mainstream so quickly.

N: It is essentially mainstream by now, right? The majority of people agree that cannabis is therapeutic and it is a pharmaceutical drug now, so seeing how that has changed in such a short amount of time makes me really think that psychedelics will closely follow.

J: It makes me optimistic as well, so fingers crossed for that and thank you so much for the talk!

N: Thanks, I appreciated the time.