EXCLUSIVE INTERVIEW

MICROBIOME MOVEMENT
HUMAN NUTRITION

November 7-9 , 2023 | Livingston, NJ

GenoWrite

Zack Abbott
Founder, Chief Executive Officer
ZBiotics

Introduction:

The Microbiome Movement — Human Nutrition Summit is filled with amazing talks covering the latest in biotic research. Genetic engineering stands tall among the many discussions about making probiotics safe and effective.

The two-word term brings about feelings of skepticism and fear. Discussions abound about genetically modified organisms (GMO), particularly as misinformation about their safety pervades discussions on the matter. Despite the concerns that GMO-based discussions bring to the table, researchers continue to advocate and ensure their safe consumption.

Such efforts have also taken place in the probiotics sphere, especially with microbes being a common playground for genetic engineering experiments. ZBiotics stands among the first companies to develop a genetically engineered probiotic for health.

> *I was fascinated with all the microbes that live within and on us, working at least in part to help us with many fundamental aspects of our health.*

To learn more about their pioneering work, GenoWrite had the great pleasure of interviewing Zachary Abbott, founder of ZBiotics.

Read this interview to learn more about the genetically engineered probiotic ZBiotics produced.

What is genetic engineering?

At its core, genetic engineering alters the DNA makeup of a living organism. It can be as simple as changing a single building block of DNA called a nucleotide or be as complicated as changing an entire region of DNA. In recent decades, we've heard about gene editing technologies such as CRISPR-Cas to correct mutations that cause a myriad of genetic diseases.

Bacteria can also edit their genomes, the makeup of genes that make the bacteria what they are. One mechanism by which bacteria can modify their genome is via natural competence and homologous recombination. Natural competence is the ability of bacteria to take up and transport DNA fragments from their environment into the cell. Now, if that exogenous DNA fragment has some level of similarity to the bacteria's own genome (i.e. some homology), the bacteria will swap in (i.e. recombine) that exogenous DNA and replace a stretch of its chromosomal DNA: hence "homologous recombination". Researchers can leverage this natural process of competence and homologous recombination to guide bacteria to integrate stretches of DNA into its genome. With it, scientists can make bacteria express many characteristics of interest.

We've heard that genetic engineering can do a world of good through gene therapies, and homologous recombination is one such approach that you described. But what inspired you to dive into genetic engineering and eventually ZBiotics?

My love for genetically engineering microbes began as far back as my PhD years at the University of Michigan. There, I was studying a family of genes in *Legionella pneumophila*, the pathogen that causes Legionnaires' disease. Specifically, I was studying genes that allowed the bacteria to persist in water systems that could be reservoirs for human infection.

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During my PhD work, I began thinking about ways to engineer beneficial microbes to express genes that could be useful for human health. I was fascinated with all the microbes that live within and on us, working at least in part to help us with many fundamental aspects of our health. We know that the human microbiota executes many essential functions, from keeping our immune systems working to helping us absorb any remaining nutrients from our food. But could we engineer good bacteria to help people live fuller, more healthy lives?

Answering this question has been my primary driver in founding ZBiotics and developing genetically engineered probiotics.

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Tell us more about the probiotic you developed. What is it, and how does it help people?

We at ZBiotics have been working with a microbe called *Bacillus subtilis.* We considered the microbe an ideal probiotic candidate for two reasons:

Ubiquity/Safety: *B. subtilis* is found everywhere in the environment, from soil to the surface of many of the plants we eat to our kitchen countertops. Because of this, the microbe regularly passes through our bodies, and indeed it is one of the safest microbes on the planet in terms of human health.

Easy to engineer: *B. subtilis* is naturally competent and regularly performs homologous recombination. This makes it easy for us to insert and remove genes via genetic engineering.

In *B. subtilis*, we saw an opportunity to use this technology to address a pressing problem that comes with drinking: acetaldehyde build up in the gut. Most of the alcohol we drink is absorbed from our stomach and gut into the bloodstream before making its way into the liver. There, our liver breaks down alcohol into acetaldehyde and then into acetate. However, a small amount of the alcohol we drink is broken down directly in the gut – in large part by our microbiome – before the alcohol is absorbed into the bloodstream. Unlike our livers, however, our gut microbes can break down alcohol into acetaldehyde but are far less capable of converting the acetaldehyde into acetate. This gut-derived acetaldehyde then gets absorbed into our bloodstream and wreaks havoc throughout the body, causing a lot of misery that you might feel after enjoying a few drinks.

> *You ingest this engineered probiotic bacteria before you drink alcohol, and it's able to help your body break down gut-derived acetaldehyde.*

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That's where *B. subtilis* engineering comes in. We used *B. subtilis's* ability to take in foreign DNA by introducing a gene into its genome that encodes an enzyme called acetaldehyde dehydrogenase (ALDH). This enzyme breaks down acetaldehyde and is the same enzyme your liver uses. By engineering a probiotic bacteria you can eat to express it in the gut, we can move this function of the liver into the gut, where the acetaldehyde is initially being formed.

So the idea is that you ingest this engineered probiotic bacteria before you drink alcohol, and it's able to help your body break down gut-derived acetaldehyde as it forms before it can be absorbed into the bloodstream to wreak havoc throughout the body. And then you're able to wake up the next day feeling better than you would've otherwise felt and able to get on with your day and maintain all your healthy habits and routines.

What did you do to make sure your probiotic was safe to consume?

We did a lot of testing to ensure our probiotic's safety. We began with a *B. subtilis* strain known for its safety, PY79, and modified it with a single gene encoding the ALDH. We already knew that ALDH was extremely common in humans, and so we were really just combining two things known to be safe and common in the human body. There was no reasonable expectation that it would be unsafe. But then of course we wanted to validate its safety, and so, we conducted a barrage of safety tests. We sequenced and analyzed s genome to verify that there were no genes predicted to encode toxic, pathogenic, or allergenic proteins. We also made sure the bacteria was devoid of mobile genetic elements and antibiotic resistance genes.

After confirming the genomic safety, we then conducted lab tests to make sure that the microbe wasn't cytotoxic (i.e. able to kill cells) or hemolytic (able to rupture blood cells). Lastly, we confirmed the bacteria caused absolutely no harm in *in vivo* animal studies.

What challenges did you encounter as you scaled up the production of your probiotic?

I think our biggest challenge with scaling up production was in finding a partner to work with who could do the large scale bacterial fermentation. When we launched our product in August 2019, we were the world's first genetically engineered probiotic of any kind and of any use to go to market. A lot of the partners we spoke with had done contract manufacturing with a menu of bacterial strains that they had already worked with before. Convincing them to work with a strain that they hadn't worked with before was challenging.

Finding ways to scale up the fermentation of the bacteria was also challenging. The microbe could grow extremely quickly, which caused technical challenges like ensuring that there was enough air, that the bacteria didn't get too hot, and that the bacteria didn't foam too much in the bioreactor. Scaling up the fermentation process can also affect the life cycle of the bacteria, which was also a challenge we had to work through with process optimizations.

Even so, the fast doubling times made manufacturing the probiotic more efficient. We also chose to work with *B. subtilis* because it can form an endospore: a tough, non-reproductive structure that remains dormant until favorable conditions for growth reemerge. That meant the bacteria could naturally be shelf- stable for years, and also tolerate huge swings in temperature (e.g. think of a hot mailbox in Arizona or a freezing porch in Alaska) and the acidic environment of your stomach without issue.

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As you talk about genetic engineering in your upcoming summit talk, what are you hoping your listeners will take away from your talk?

Ever since I started ZBiotics, I have been passionate about sharing all the incredible things that we can do with genetic engineering. With probiotics, I saw the endless possibilities of engineering a function that would help our bodies stay healthy or improve our well-being. Our first product – a probiotic genetically engineered to help you feel better the day after drinking – is a very exciting proof-of-concept that's easy to understand and provides a clear and visceral benefit for the person using it. But that's just one example, and I'm excited about the potential for genetically engineered probiotics to solve all kinds of challenges in completely novel and ownable ways. I'm excited about the potential for genetically engineered probiotics to solve all kinds of challenges in completely novel and ownable ways.

Just as important to me is public perception towards genetic engineering and correcting misconceptions. There's a lot of misinformation about genetically modified organisms (GMOs) in the public. Interestingly, I believe this negative public perception is actually being largely created and perpetuated by the industry, which erroneously assumes that people will reject a GMO on principle.

That is not true.

There is a huge opportunity to use genetic engineering to make safe, beneficial, and exciting products that people want.

Responsible use of the technology, and transparent communication with customers (for instance, every bottle and box of ZBiotics says "proudly GMO") results in new positive associations with a beneficial application of a modern technology..

That's why I love talking about the work I do with my team at ZBiotics. Sharing the possibilities of genetic engineering and the safety of probiotics through summits like the Microbiome Movement - Human Nutrition Summit will pave the way for genetic engineering across many industries.

Don't miss Zack's session at the Microbiome Movement - Human Nutrition Summit:

"The Benefits, Strategy & Challenges of Using Genetic Engineering for Product Development"

9.30 AM | Wednesday, November 8

VIEW THE AGENDA

> *I'm excited about the potential for genetically engineered probiotics to solve all kinds of challenges in completely novel and ownable ways.*