CASE STUDY

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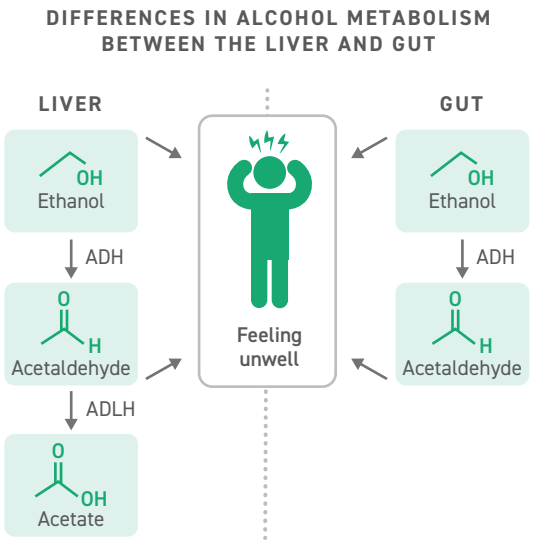
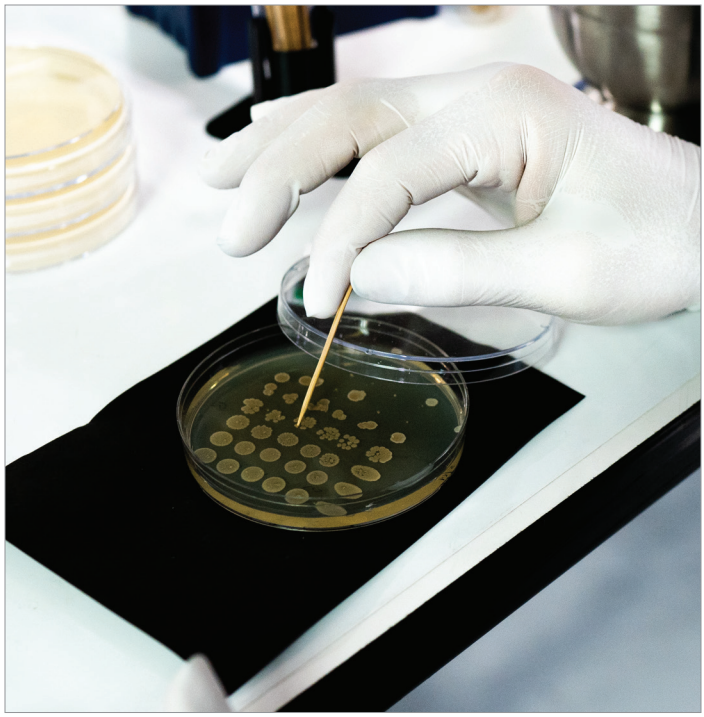
TWIST BIOSCIENCE

# Designing the World's First Engineered Probiotic

Feeling your best the day after drinking isn't guaranteed, but the scientists at ZBiotics believe that synthetic biology can help. With a passion for genetic engineering, the ZBiotics team set out to create a safe and effective probiotic that would help people enjoy a few social beverages without fear of feeling rough the next morning. To do this, they needed to be the first to engineer a probiotic, prove it was safe, and bring it to market. Twist's absolute precision in DNA synthesis was a key part of this journey.




*ADH: Alcohol dehydrogenase*
*ALDH: Acetaldehyde dehydrogenase*



People aren't always thinking about their next day when they start drinking alcohol, but sometimes (especially as we age out of our 20s) even a few responsible drinks with friends or colleagues can lead to a rough morning. Water, food, and an assortment of home remedies may help reduce the next day's malaise. At best, these efforts are only able to treat the symptoms, and often, time is the only effective remedy. But Bay Area-based synthetic biology start-up ZBiotics is using synthetic biology to go after the root cause of these symptoms, and help alcohol enjoyers worry less about suffering the next day's consequences.

"We set out to develop a probiotic that helps the body break down acetaldehyde," states Dr. Zack Abbott, CEO, and founder of ZBiotics. "Acetaldehyde is a highly toxic compound produced as a byproduct of alcohol breakdown in the body. Research points to acetaldehyde as the culprit behind much of that next-day misery you feel."

"The liver takes care of roughly 80–90% of ethanol metabolism," Zack explains. "There, acetaldehyde is rapidly converted into innocuous acetate and water. In the liver, this two-step process is so fast that little to no acetaldehyde accumulates.

"However, the remaining 10–20% of ethanol is broken down into acetaldehyde in the gut by our microbiome, where it's subsequently absorbed into the bloodstream. Evidence suggests that it is along this journey to the liver where acetaldehyde does its damage by reacting with various proteins, nucleic acids, cell membrane parts, and signaling molecules."

The human microbiome doesn't naturally produce large amounts of acetaldehyde dehydrogenase, the enzyme responsible for breaking down acetaldehyde. This is why the chemical is able to accumulate in the gut and enter the bloodstream, ultimately to be metabolized by the liver. Equally, this is why supplementing the microbiome with a probiotic is a promising strategy.

ZBiotics researchers created a probiotic that alcohol enjoyers can consume before a drink. On its natural passage through your body, the probiotic breaks down acetaldehyde created by your microbiome.

## Creating a Probiotic that Breaks Down Acetaldehyde

For their probiotic, the ZBiotics team selected the nearly ubiquitous, gut-friendly bacterium *Bacillus subtilis*.

"*B. subtilis* is everywhere. It is found in soils, our food, and as a transient member of our microbiome. It has been intentionally used in food fermentation by humans for centuries and is arguably one of the safest bacteria on the planet for human consumption," explains Zack.

However, *B. subtilis* presented a challenge: it can't break down acetaldehyde because its genome doesn't contain the naturally occurring acetaldehyde dehydrogenase enzyme. However, the species does have a long history as a model lab organism and comes with a well-established set of protocols for its engineering. To give it acetaldehyde metabolizing properties, the team turned to synthetic DNA.

Their plan was to synthesize a gene encoding the acetaldehyde dehydrogenase enzyme and insert it into the *B. subtilis* genome under the control of a constitutive promoter, a type of genetic switch that is always turned on. This, in turn, would produce a probiotic that is a miniature acetaldehyde dehydrogenase factory.

## Engineered for Safe Consumption

To get their probiotic to market, the Zbiotics team took special care to ensure it was safe for both consumers and the environment. This was largely uncharted territory, as no engineered probiotic had ever been taken to market before.

ZBiotics received approval from an independent panel of food toxicologists following a battery of safety studies that spanned two years, including *in vitro* and *in vivo* testing. In line with regulatory guidance, they also self-affirmed the product as generally recognized as safe (GRAS). The team also had to ensure adherence to all of the food-safety regulations typical for probiotics.

"Regarding the safety of the product, it was important for us to prove that the probiotic was free of any allergens, toxic proteins, mobile genetic elements, and antibiotic resistance genes," Zack discussed.

"Also, we wanted to make sure our bacteria would have minimal impact or disruption to your native microbial ecosystem. We opted to use bacteria that were non-colonizing and simply transited through the gut before being excreted.

"As for the engineering, it was essential to show the synthetic gene had no adverse effects when expressed in the gut and had no increased chance of horizontal gene transfer."

Horizontal gene transfer is a process where bacteria share genetic information, often using small "mobile" circles of DNA called plasmids. In designing their new probiotic, it was important for the ZBiotics team to consider the effect their new gene may have if transferred to other species, either in the gut or in the environment.

First and foremost, to minimize any risks associated with gene transfer, ZBiotics chose a gene that was extremely common and therefore unlikely to create any risks or undue advantages if it was transferred to another species. The acetaldehyde dehydrogenase gene is, if nothing else, common: As much as 70% of all life on earth contains an acetaldehyde dehydrogenase gene in its genome, including many bacteria in your natural gut microbiome. In addition, the research team carefully designed the synthetic gene to avoid coding for mobile elements and used next generation sequencing to ensure that it had faithfully been inserted into *B. subtilis* genome.

"It is impossible to guarantee that a gene will stay put, but these efforts at least ensure the synthetic gene is no more likely than any other piece of DNA to jump from one organism to another. And if it does jump, it's highly likely that the organism already has this gene, or has at least seen this gene before, and would thus gain no benefit or disadvantage" states Zack.

It was also essential that the new gene performed as expected, carrying out its sole function and eliciting no unanticipated side effects. To be sure it performed as it was designed, the gene's DNA sequence would need to be synthesized with perfect accuracy, which is why ZBiotics turned to Twist Bioscience for help.

Twist Bioscience is the market leader in DNA synthesis. "Working with Twist really simplified the engineering process for us. We didn't have to extract DNA from another organism. Instead, we could synthesize the exact gene sequences we needed," states Zack. "Twist also verifies the synthesis is 100% accurate with next-generation sequencing, so our team could be absolutely sure we were working with the right DNA."

The resulting product of these efforts is *B. subtilis* ZB183—a novel probiotic bacterial strain identical to the parent strain but for one difference: the robust production of acetaldehyde dehydrogenase.

Thousands of customers have now taken ZBiotics, and their testimonials are compelling.

"I have recently been reminded that as I get older, I feel the effects of just a few drinks even more the next day," remarked one customer, echoing the larger trend that this probiotic, born of synthetic biology, helps them not sacrifice their healthy morning routines and activities even after having a social night out. They continue, "I was introduced to Zbiotics by a friend and now I make sure to have one anytime I know I'll have more than a few drinks. It helps me be confident that I will remain productive the day after date night or going out with friends."

## Proudly GMO


ZBiotics is the first genetically modified probiotic to hit the market. Instead of endlessly searching for a bacteria that was safe for consumption that could also naturally reliably and efficiently break down acetaldehyde, the team gave this ability to a bacteria already known to be completely safe.

If you look at the ZBiotics website, bottle, and box, you will see "proudly GMO" in large visible text. As a company, they believe that genuinely transparent labeling of products that contain genetically modified organisms is the only way to assuage the stigma around GMOs.

"The reality is that GMOs are common-place in the American food industry. Corn, soy, sugar beet, canola oil, and lab-derived meats are all engineered, but this fact is shrouded in mystery as they're unlabeled. Companies have lobbied against GMO labeling in attempts to limit public concern, but the secrecy was dishonest and has led to misinformation and mistrust," explains Zack.

Flying in the face of convention, ZBiotics' bold stance on GMO transparency means every customer knows what they are consuming. If anyone is interested in learning about GMOs, ZBiotics offers a wealth of essays and resources on their website.

"We are also inviting others to help us develop a clear labeling vocabulary made for genetic engineering companies who care about their customers, who value transparency, and who want the best for this technology and for the planet," Zack closes. ■