

# Fermentation, but make it precise: using microbial cell factories to make our food

**Karson Bateman**

(Wisconsin Lutheran College)

**Yong-Su Jin** (Food Science and Human Nutrition, University of Illinois, Urbana-Champaign, Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana-Champaign and Centre for Precision

Fermentation and Sustainability (PreFerS), Illinois Advanced Research Center in Singapore)

**Michael J. Miller** (Food Science and Human Nutrition, University of Illinois, Urbana-Champaign, Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana-Champaign and Centre for Precision

Fermentation and Sustainability (PreFerS), Illinois Advanced Research Center in Singapore)

Like age-old fermentation, which is the use of food-safe microorganisms to convert an ingredient into a product with different sensory and nutritional properties through microbial metabolism, precision fermentation promises to improve the appeal of foods for generations to come. Precision fermentation harnesses engineered microorganisms to produce specific molecules through carefully designed modifications of their metabolic pathways. In the food industry, this includes both using engineered food-fermenting microorganisms to make fermented foods with new sensory and nutritional properties and using engineered microorganisms to manufacture food ingredients that are scarce or require unsustainable production methods. Enzymes and metabolic pathways that are integral to cheesemaking, expensive flavor determinants in hopped beer, one of the industries' favorite oils, and the blood-red iron-containing proteins that make meat alternatives craveable can all be produced in sustainable and affordable ways because of precision fermentation. Thanks to rapidly advancing precise genome editing tools, the future of the food industry can rely on microbial cell factories to keep consumers fed and satisfied.

## A new twist on an old favorite

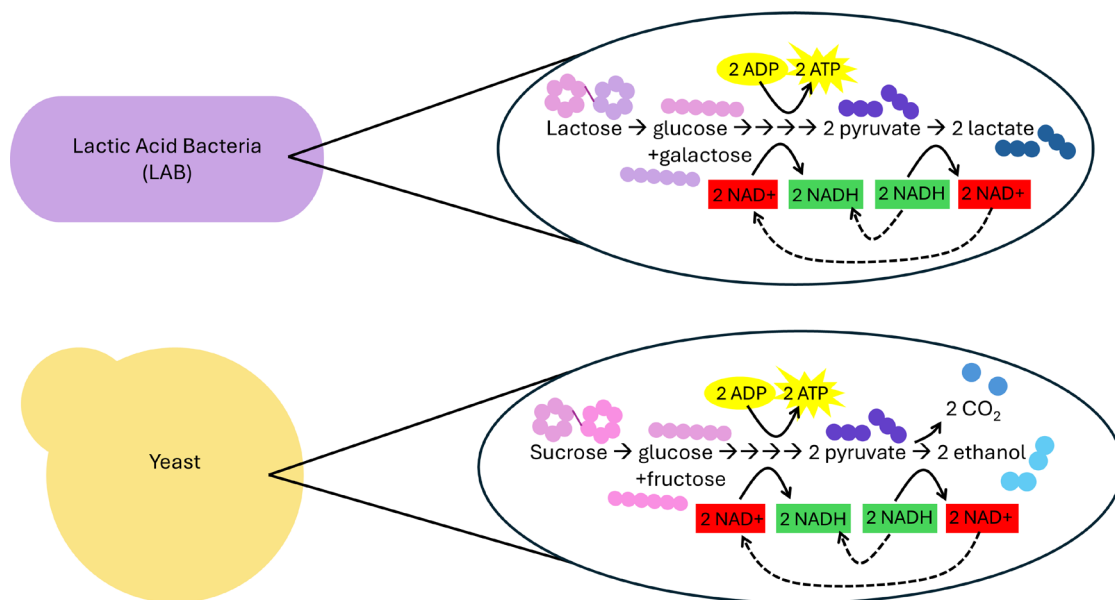
Fermented foods are seeing a resurgence of interest. As consumers are turning away from ultra-processed and high-calorie treats, they are returning to fermentation, which is a millennia-old technology.

Fermentation can be defined as the use of food-safe microorganisms to convert a substrate (ingredient) into a product with different sensory properties through enzymatic and metabolic action. For example, people around the world have been making wine for thousands of years by allowing the naturally abundant yeasts on the skin of fruits to turn juice into a complex, sometimes carbonated, alcoholic beverage. Similarly, yogurt, aged cheeses, beer, sourdough bread, and kimchi are tasty products of microbial metabolism. Not only do these fermented favorites taste different and have more complex nutrient profiles than their unfermented counterparts, but the promotion of “good” microorganisms creates a competitive environment where pathogenic microorganisms are much less likely to survive and cause illness. No wonder humans have been using microorganisms to improve their food for millennia!

These familiar fermentation processes entirely depend on the native biochemical processes of the fermenting microorganisms, which we harness to do our

food processing. For example, lactic acid bacteria (LAB) make lactic acid for the same reason yeast makes ethanol and carbon dioxide. They get energy in the form of ATP from the reactions (called glycolysis) that convert glucose into pyruvate, and then they restore their redox state (aka NAD<sup>+</sup>/NADH ratio) by reducing pyruvate into lactic acid or ethanol (see Figure 1). These are the most basic metabolic pathways that fermentation microorganisms use. There is a myriad of ways that microorganisms make ATP and restore redox states through enzymatic reactions. These biochemical pathways can result in essential molecules for cell survival or simply byproducts of an otherwise necessary metabolic process. We observe and appreciate primary and secondary metabolites from these cellular processes to transform the flavor, texture, and shelf life of our foods.

Metabolic pathways vary between species (and even between strains) of microorganisms. Humans have used these differences in our taste buds' advantage by identifying, isolating, purposefully adapting, and applying optimal strains to make desired products. This is one of the reasons that the beer brewed by your favorite company tastes different from the beer brewed by your least-favorite company—they're using different yeasts. Mutations that change the genes and, therefore, enzymes and metabolic products of an organism occur naturally, and over the centuries, we have selectively bred the strains with metabolites that we think make



**Figure 1. Simplified diagram of basic metabolism in LAB and yeast.** Circles represent carbon atoms in labeled molecules. The four arrows between glucose and pyruvate represent the entire process of glycolysis.

the best food products. We have also, more recently, developed tools to purposefully, safely, and precisely edit the genes in microorganisms to produce metabolites that are beneficial to humans. This is what we call precision fermentation.

Precision fermentation is the use of engineered microorganisms to produce *specific* molecules through enzymatic and metabolic action. In the food industry, this includes both (1) using engineered fermentation microorganisms to make fermented foods with improved sensory and enhanced nutritional properties and (2) using engineered microorganisms to produce food ingredients that are scarce or sourced unsustainably or unethically. This technology enables companies to offer consumers more safe, flavorful, and sustainable food options. What follows is a handful of examples to illustrate how precision fermentation already affects your food and its increasing importance in producing food for a growing population.

## A champion for cheese

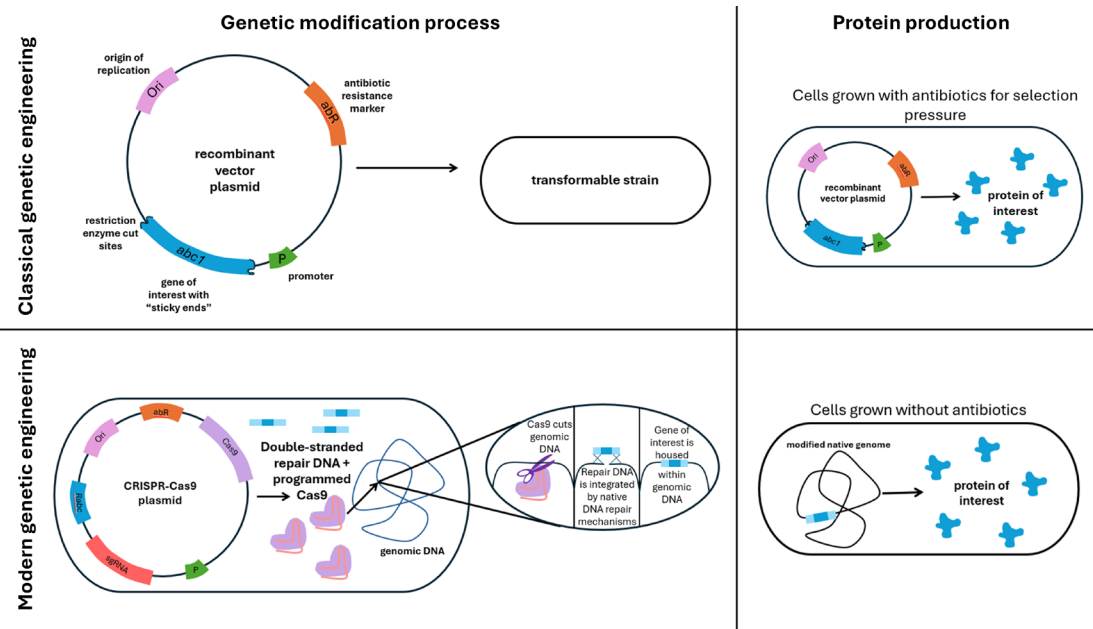
Chymosin is the classic example of a food ingredient produced through microbial cell factories thanks to precision fermentation. Casein describes a family of phosphoproteins that makes up most of the protein content of cow's milk. In milk, casein forms micelles with hydrophilic  $\kappa$ -casein molecules facing outward. Chymosin, aka rennin, is an enzyme that cuts  $\kappa$ -casein, thus removing the hydrophilic  $\kappa$ -casein tails, which enables the now hydrophobic micelles to coagulate and form a gel that we know as cheese curds. Chymosin

was originally identified and harvested during livestock butchering as rennet, a mixture of enzymes from the lining of the 4<sup>th</sup> stomach of unweaned ruminant animals. To have enough chymosin to support today's cheese industry would require an unsustainable number of calves to be sacrificed.

However, thanks to precision fermentation concepts, genetic engineers were able to clone the gene for bovine chymosin into microorganisms (originally, in lab strains of *E. coli* and more commonly now, in the filamentous fungi *Aspergillus niger*). So instead of sacrificing calves, we can make chymosin by harnessing microbial cell factories. First, the gene for bovine chymosin was identified, then it was inserted into a plasmid and given to microbial cells. The cells then use their inherent protein production and secretion systems to make chymosin that is structurally and functionally identical—or even superior—to that found in ruminants. This is not only cost-effective, but much more sustainable than harvesting chymosin from calf stomachs. This was one of the first FDA-approved uses of biotechnology, and the field has greatly advanced since its approval in 1990.

## An assortment of advancements

Since Jennifer Doudna and Emmanuelle Charpentier first identified the CRISPR-Cas9 bacterial adaptive immune system as a programmable genome manipulation tool in 2011, we have seen a massive increase in the tools available for precise genome editing. By using systems innate to microbial defense and replication, such as the CRISPR-Cas9 system, homology-driven repair, TALENs,



**Figure 2. Simplified diagram of the difference between classical and modern genetic engineering.** The top row represents classical genetic engineering using a recombinant vector plasmid with an antibiotic resistance marker for selection pressure. The bottom row represents CRISPR-Cas9-mediated genetic engineering resulting in a stable modification of the native genome.

and more recent innovations like base editors and prime editors, we can change the metabolic processes of microorganisms without leaving a “scar”. We can induce microorganisms to express heterologous genes, like in the case of chymosin, but the genes can be inserted directly into the native genome without using antibiotic resistance markers (see Figure 2). We can also change the expression levels of, or entirely delete, endogenous genes to change metabolic pathways without affecting surrounding genes.

There are many food companies, both established and start-ups, that have filed patents and received FDA Generally Recognized as Safe (GRAS) notices for their uses of precision fermentation. The advancements made possible by precision fermentation can be categorized into the two main branches of the science: making improved fermented foods and making food ingredients using microbial cell factories.

## A better bitter

Brewers are experimenting with new ways to keep their customers *hoppy*. Hops, one of the four traditional ingredients in beer, is an incredibly water-demanding crop to grow. As IPAs and other hop-flavored beers maintain their popularity and simultaneously water and arable land become more protected resources, a major conflict has arisen. How can beer companies justify spending so much water and land on a crop that does

not even get eaten? Well, they might not have to. Brewer’s yeast (*Saccharomyces cerevisiae*) has been engineered to produce flavor molecules that make beer taste “hoppy” without adding hops!

The lab of Jay Keasling, a prominent metabolic engineer, has used CRISPR-Cas9 to construct a monoterpene biosynthesis pathway in brewer’s yeast. Monoterpenes are the class of flavor molecules that are traditionally added to beer by hops. Through iterative improvement, the Keasling lab was able to engineer a strain of brewer’s yeast that contributed the primary flavor of hops to brewed beer more consistently and sustainably than when hops are added. The first author of the seminal paper, Charles Denby, co-founded the successful company Berkeley Yeast, which provides genetically edited strains to beer brewers nationwide. An important way that metabolic engineers keep their strains safe is by starting with a GRAS brewer’s yeast and adding genes from GRAS organisms to amend its functions, such as the flavor determinants in hops. By combining two safe organisms, labs and companies alike have the best chance of gaining consumer acceptance of their genetic engineering pursuits. In beer production, yeast cells are almost always filtered out before the final product is consumed. This gives innovators the advantage of not having to label their products as genetically engineered, thus avoiding consumer pushback. If consumers knew that a beer they love is made possible by precision fermentation, done safely, perhaps there wouldn’t be so much pushback in the first place.

## Another option for oil

Fermented foods are not the only products that benefit from precision fermentation! Many food ingredients are costly to extract from their natural source, but we can use precision fermentation to make microbial cell factories that produce these ingredients more sustainably and affordably. Like chymosin, harvesting palm oil, which is beloved across industries for its melting properties, demands environmental sacrifice. Deforestation and unsustainable practices have spoiled the reputation of many markets, including the palm oil industry, inspiring many start-up companies to investigate precision fermentation as a solution. Although economic viability is still in question, many companies have engineered oleaginous yeasts to produce oils with similar chemical profiles as palm oil. By starting with strains that inherently produce and store high proportions of lipids, genetic engineers can rewire metabolic pathways to result in a desired lipid profile. Palm oil alternatives have yet to gain approval for use in food products but are certainly on the radar as a promising, sustainable use of precision fermentation.

With a growing population and increased demand for sustainable, affordable foods, it is no surprise that ingredients like palm oil are increasingly produced through precision fermentation by microbial factories. Another successful and popular example of this concept is Impossible Foods' production of soy leghemoglobin.

## An unconventional umami

Hemoglobin is the red, iron-containing protein found in animal muscles that is responsible for the red color and meaty flavor of animal meat. For people who crave these characteristics but do not want to eat animal meat, Impossible Foods has used precision fermentation to provide a solution. The company makes a vegetable-based patty and adds soy leghemoglobin, which is functionally similar to animal hemoglobin (red and tasty), to make it "impossibly" like animal meat. Soy leghemoglobin, however, is only found in small concentrations in the nodes of soybean roots, so harvesting enough to sustain their company would be impossible. Enter *Pichia pastoris* (aka *Komagataella phaffii*), a methylotrophic yeast that can be (and has been) engineered to overproduce both its native heme and heterologous soy leghemoglobin. While the exact metabolic pathway that Impossible Foods uses is proprietary, their use of *P. pastoris* to produce soy

leghemoglobin is a fundamental triumph for precision fermentation in the food industry.

## A positive prediction

Every month, more start-ups and GRAS proposals enter the stage. Researchers are perfecting genome editing tools, making new strains, and experimenting with fermentations to make valuable or even new-to-nature products. Because of their work, we have yeast strains that use the inedible parts of corn as a sugar source and filamentous fungi strains that can produce proteins that are functionally comparable to those in milk and eggs.

The field of precision fermentation is not just concerned with the production of genetically engineered strains but also with the downstream processing and approval processes that will get these foods and food ingredients onto the plates of consumers. It is important that companies continue to use precision fermentation in a safe way, like using GRAS genes in GRAS organisms, and that these new foods and ingredients are continually held to high standards for approval.

Unfortunately, no matter how safe precision fermentation is, the industry faces the steep uphill climb of overcoming consumer biases against ingredients sourced from genetically engineered microorganisms. For example, although citric acid produced by filamentous fungi is not chemically or functionally different from citric acid that comes from a lemon, the former is much more likely to be flagged by consumers as "unsafe" if they hear it comes from a genetically engineered source. This is a result of the overarching narrative surrounding genetic modification, usually inspired by genetically modified (GM) crops like insect- and herbicide-resistant corn and soybeans.

It is important to note the effect that precision fermentation could have on societal acceptance of genetic modification. If consumers are taught that their cheese, desserts, and meat alternatives are made possible by genetic modification of microorganisms, they might just change their minds about how "bad" genetic modification is—especially when the advantages that are important to that consumer are identified, such as sustainability or nutrition.

The future of fermented foods probably looks a lot like their past—flavorful and complex. In the same way that traditional food fermentation has been providing consumers with safe, delicious food for millennia, precision fermentation presents consumers with not just more options, but more affordable and sustainable options when they are shopping for food.

## Further Reading

- Precision fermentation for improving the quality, flavor, safety, and sustainability of foods, Hilgendorf et al. 2024 [10.1016/j.copbio.2024.103084](https://doi.org/10.1016/j.copbio.2024.103084)
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- Genome-edited *Saccharomyces cerevisiae* strains for improving quality, safety, and flavor of fermented foods, Lee et al. 2022 [10.1016/j.fm.2021.103971](https://doi.org/10.1016/j.fm.2021.103971)
- Production of bovine beta-lactoglobulin and hen egg ovalbumin by *Trichoderma reesei* using precision fermentation technology and testing of their techno-functional properties, Aro et al. 2023 [10.1016/j.foodres.2022.112131](https://doi.org/10.1016/j.foodres.2022.112131)
- How FDA Approved Chymosin: A Case History, Flamm et al. 1991 [10.1038/nbt0491-349](https://doi.org/10.1038/nbt0491-349)
- Website: [The promise of precision fermentation: Can it really change the game? | Roland Berger](#)
- Website: ['Precision fermentation' start-ups pursue palm oil alternatives](#)
- FAO Report on Precision Fermentation with a focus on food safety: [content](#) / news article link: [FAO Publishes Report on Food Safety Aspects of Precision Fermentation](#)
- News article: <https://www.bbc.com/news/business-67193176>

## Author information



*Karson Bateman recently graduated with an M.S. in Food Science and Human Nutrition from the University of Illinois at Urbana Champaign where she studied traditional and precision fermentation under Drs. Michael Miller and Yong-Su Jin. Her time at UIUC culminated in original research on the endolysin PlyP100, a precision fermentation product that shows promise for improving the safety of fresh cheeses. She is now on the academic staff at Wisconsin Lutheran College where she helps advise students and teaches courses, especially in the Fermentation Science department. Email: [Karson.bateman@wlc.edu](mailto:Karson.bateman@wlc.edu)*



*Yong-Su Jin is a Professor of Food Science and Human Nutrition at the University of Illinois at Urbana-Champaign, where he leads research in microbial biotechnology, synthetic biology, and precision fermentation. His work focuses on engineering yeast and lactic acid bacteria for the sustainable production of food ingredients, biofuels, and biochemicals from renewable and waste-derived resources. Dr. Jin also serves as Director of Research (Future Foods) at the Illinois Advanced Research Center at Singapore (ARCS) and leads Centre for Precision Fermentation and Sustainability (PreFerS). Email: [ysjin@illinois.edu](mailto:ysjin@illinois.edu)*



*Michael Miller is a Professor of Food Science and Human Nutrition at the University of Illinois at Urbana-Champaign where he also serves as the theme leader for the Microbiome Metabolic Engineering theme at the Institute for Genomic Biology. Dr. Miller's main research focus relates to Lactic Acid Bacteria (LAB) and their use in food and precision fermentation. Current projects include the characterization of LAB metabolites in fermented foods responsible for their health benefits to consumers and the application of bacteriophages to specifically manipulate microbial communities. [fermented@illinois.edu](mailto:fermented@illinois.edu) Email: [fermented@illinois.edu](mailto:fermented@illinois.edu)*