**Application of Systems and Synthetic Biology to Improve Fermented Foods**

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The principle of food fermentation is to use carbohydrates or protein present in the food as a substrate for microorganisms to produce organic acids and ethanol through anaerobic, aerobic, or a combination of both respiration pathways.This process is accompanied by the synthesis of ATP. The food industry benefits greatly from it, as the byproducts of food fermentation contain B-vitamins, a variety of flavors, antimicrobial agents, oils, and fatty acids.

In this presentation, Jeroen introduces three aspects of systems biology that are applied in the food fermentation industry. The first is the use of products resulting from lactic acid bacteria fermentation, such as lactic acid and antifungal compounds, to prevent food spoilage and prolong the shelf life of the food. However, during the aerobic growth of *Lb. plantarum*, temporary growth delays can occur in the early stages of its life cycle. By comparing transcription profiles before and after the delay, researchers found that all related reactions are CO2-dependent. They then hypothesized that flushing out the CO2 would arrest the growth of the strain, and this condition would trigger CO2-producing reactions, leading to resumed growth. By flushing out 1% CO2 compared with the air control group, the test identified that flushing out CO2 improves the fermentation process. Protective cultures (LAB) are cultures composed of multiple fermentation products that can be sprayed on fresh fruits to keep them fresh longer.

The second aspect involves using metabolic networks to optimize fermentation processes in order to improve diacetyl production. Diacetyl is a major flavor component in butter and can be produced from citrate, lactose, or glucose by *Lactococcus lactis*. The production process requires oxygen. Pyruvate is the main reaction product of glucose/lactose or citrate, and the pyruvate reaction has two pathways. One pathway produces α-acetolactate, which is then followed by the production of acetoin or diacetyl. The other pathway produces lactate. To improve the production of diacetyl, the researchers cut off the pathway for producing lactate by using an ldh-mutant strain. Then, they further optimized the process by eliminating the pathway to acetoin, which ultimately led to up to 75% conversion to diacetyl.

The third aspect involves the use of a DBTL strategy for the production of tailor-made microbial oils that can replace traditional edible oils and reduce CO2 footprint. The first step is Design, where the chosen strains are *C.oleaginosus* and *Y.lipolytica*. Using omics technologies, researchers identified and validated candidate genes that regulate the production of fatty acids and modified the strains accordingly.The second step is Build, where the modified genes are assembled to create the engineered strains. The third step involves conducting experiments and analyzing the outcomes. In this case, factors affecting lipid accumulation include C/N ratio, temperature, pH, and dissolved O2 concentration. Usually, these factors are combined and can affect each other, making it time-consuming and labor-intensive to study them one at a time. To overcome this, researchers used Response Surface Methodology (RSM), which helps decide which points in the feasible region to experiment with in order to make the most accurate predictions. Once the researcher has defined the problem, experimental domain, and response, RSM provides alternative experimental strategies and criteria for evaluating them. (Luis et al., 2009)The finalized optimal combination was found to be C/N 175 at 30°C. Another important finding is that at low temperatures (15°C), polyunsaturated fatty acids increased, while in the high-temperature group (35°C), longer chained fatty acids increased.The fourth step is Learn, where we can curate and further validate our gene expression model. These findings about microbial oils show that we can design oils with different structures as requested by customers to replace traditional oils like palm oil. We can also optimize production and cut costs by cultivating strains on waste streams.

References

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