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Fermentation for future food systems

Precision fermentation can complement the scope and applications of traditional fermentation

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The growing human population and global warming pose an impending threat for global food security (Linder, 2019). This has prompted a critical re-examination of the food supply chain from producers to consumers in order to increase the overall efficiency of food production, storage and transport. Much research in plant science consequently aims to increase production with new, high-yield crop, fruit and vegetable varieties better adapted to changing climatic conditions. Yet, there is also much room for improving food safety by minimising food losses and recycling waste, valorising by-products, improving nutritional value and increasing storage time. This is where fermentation comes in as a cost-efficient, versatile and proven technology that extends the shelf life of food products and enhances their nutritional content. Moreover, there is enormous potential in fermentation to further increase efficiency and product range and even create new food products from non-food biomass.

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In a broader sense, fermentation can be defined as the cultivation of microorganisms such as bacteria, yeasts and fungi to break down complex molecules into simpler ones, notably organic acids, alcohols or esters. In a practical sense, it is one of the oldest food processing technologies to increase storage life along with cooking, smoking or air-drying: fermentation was already fully industrialised for producing beer and bread millennia ago in ancient Mesopotamia and Egypt. It is also an elegant and simple technology as these microorganisms do most of the work without much human involvement.

Louis Pasteur's discovery that microorganisms cause fermentation laid the basis for further improvement of the technology from traditional spontaneous fermentation to the use of defined starter cultures. Fermentation is now widely used to produce alcoholic beverages, bread and pastry, dairy products, pickled vegetables, soy sauce and so on. More recent advances based on genomics and synthetic biology include precision and biomass fermentation to produce specific compounds for the food and chemical industry or medicinal use. This is not the limit though: when combined with genomics, fermentation has even greater potential for creating novel foods and other products.

Untapped potential of an old technology

Such further improvements require a much better understanding of the microbes involved in fermentation processes and their metabolic capabilities and their interactions. For instance, the industrial robustness of lactic acid bacteria (LAB)—commonly used in cheese and yoghurt production—is due to differential expression of stress response genes that protect against environmental stresses (Smid & Hugenholtz, 2010). Identifying these genes and analysing their interaction with lactic acid production could help to determine the environmental factors that LAB respond to and use this knowledge to induce overproduction of lactic acid. To this end, genomics and metagenomics studies have used high-throughput sequencing (HTS) to analyse the microbial communities in fermented food so as to identify desirable strains and characteristics. This would not only help to increase efficiency and production but also selection of strains that generate specific metabolites for a more complex flavour or texture profile. By way of example, genomics research has identified genes involved in proteolysis and amino acid conversion in LAB as a basis for generating specific flavour molecules from amino acids (Smid & Hugenholtz, 2010).

Even though fermentation was originally applied to preserve food through overproduction of acid or alcohol—the intoxicating effect of the latter was a welcome side effect—there are still inherent risks involved, in particular when using traditional or spontaneous fermentation. Pathogenic microorganisms or harmful metabolites can spoil the final product and present health risks for consumers. The application of genomic analysis would therefore help to increase safety by early detection of harmful microbes. Lastly, the combination of genomics and synthetic biology to rationally design desirable characteristics holds great promise for using non-food biomass to generate wholly new food products that are safe, wholesome and appealing to consumers (Figure 1).

Refining traditional fermentation

With the advent of refrigeration, extended shelf life was no longer the main reason for fermenting food, yet these products remain hugely popular owing to their complex taste, texture and consumer demand for natural

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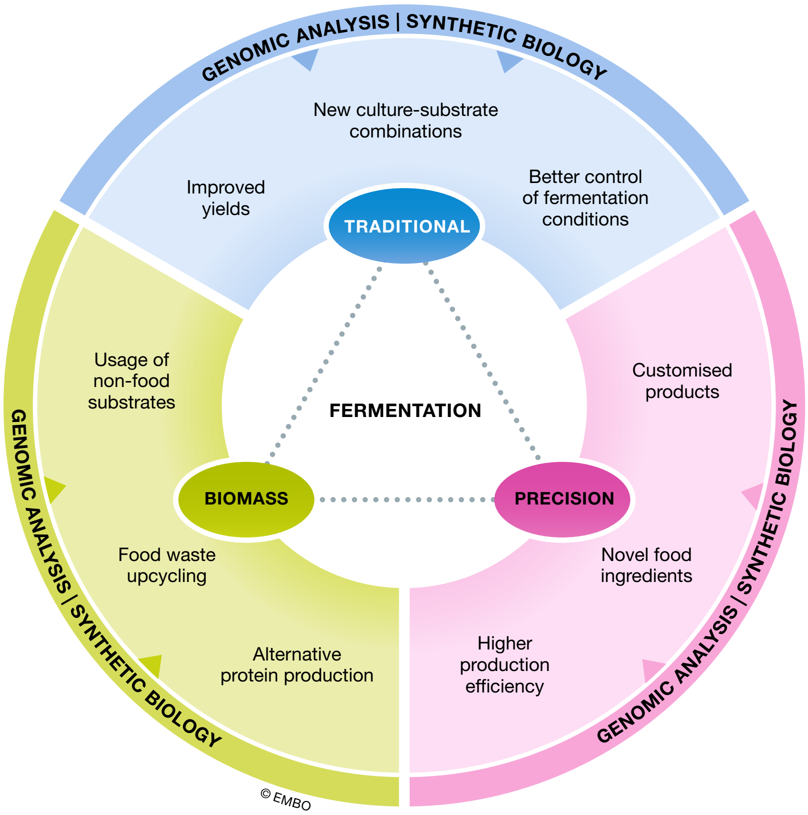

Figure 1. The potential of genomics and synthetic biology to further improve applications of fermentation.

products with potential health benefits. Fermented food products still occupy whole supermarket shelves: beer and wine, kimchi, cheese, yoghurt, soy sauce, lemonade, and so on. These are all produced by traditional fermentation that either uses microorganisms naturally present on the substrate—wild yeasts that grow on grapes for instance—or controlled fermentation with a starter culture, or through so-called backslopping by using a small amount of a previous fermentate to inoculate fresh substrate. Some of the desired outcomes include probiotics, phytochemicals, short-chain fatty acids, peptides, vitamins and amino acids with antihypertensive, blood glucose-lowering, anticancer and anti-obesity properties (Leroy & De Vuyst, 2014; Chai *et al*, 2020). As sugars are converted into other metabolites, fermented foods are also considered low-caloric.

Moreover, fermentation creates a complex taste and texture profile from amino acid and lipid degradation, extracellular polysaccharide production or mycelium growth. For instance, tempeh production starts with adding *Rhizopus oligosporus* to soybeans to break down antinutritional factors while increasing nutritional content. The fungal biomass remains in the final product and contributes to its flavour and texture. Similarly, different microorganisms also create the complex texture, taste and smell experience of a Stilton cheese or a Riesling wine.

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Large-scale production of fermented foods commonly employs the use of starter cultures: a collection of one or more microorganisms—such as *Saccharomyces*, *Lactobacillus, Lactococcus, Streptococcus* or *Enterococcus*—to inoculate the substrate. Starter cultures enable control over and predictability of the otherwise random nature of fermentation to improve food safety and standardisation. Primarily, starter cultures provide the food substrates with the necessary microorganisms required for propagation and colonisation even before fermentation begins. LAB for instance rapidly increase acidity and thereby inhibit undesirable microbes from growing. Similarly, *Saccharomyces* produces alcohol that inhibits other microorganisms. Starter cultures also confer important functions such as bacteriophage immunity, exopolysaccharide formation or amino acid biosynthesis, which prevent spoilage and improve flavour and texture of the fermented products. Again, better knowledge of the metabolic capabilities of the organisms involved would help to improve any of these aspects.

Despite the advantages of using starter cultures, many artisanal and small producers still prefer spontaneous fermentation by microbes from the environment. The presence of multiple species and strains has further potential for symbiotic utilisation of different metabolic pathways, which results in improved sensorial properties. Furthermore, indigenous microbiomes have a highly diverse gene pool along with adept metabolic functions which can provide beneficial effects. For instance, the bacterial populations collected from artisanal doenjang, a fermented soybean paste, are more complex than those collected from its commercial counterparts (Nam *et al,* 2012).

However, an undefined starter composition introduces large variabilities to the fermentation process. The final composition of the microbiota heavily depends on the environmental conditions, such as temperature, pH or salinity, and it can result in batch-to-batch variations when conditions change. Spontaneous fermentation inevitably results in inconsistent quality and can lead to the growth of undesirable or even pathogenic microorganisms. It is therefore crucial to refine both spontaneous and inoculated fermentation to increase predictability and safety.

As the rapid development of sequencing technology has considerably lowered sequencing cost and led to a rising number of published genomes in public databases, genomic studies of food microbial communities have advanced extensively. Metagenomic analysis using HTS may help to unveil the metabolic functions and parameters that affect the fermentation process such as substrate usages, enzyme production or metabolic outputs. Metabolic modelling coupled with flux balance analysis could then enable simulation of microbe growth and metabolite production in response to changes in the culturing environment (Alkema *et al*, 2016).

However, it is important to note that information from HTS reveals only the potential metabolic functions by the genes encoded, but these may not necessarily be expressed during fermentation. Metatranscriptomic analysis to complement genomic analysis reveals a more accurate picture of the metabolic activities. For instance, metabolomics, metagenomics and metatranscriptomic predicted that kimchi LAB had high metabolic capacities in a heterotrophic lactic acid fermentation process and that the fermentation was affected by bacteriophage infection (Jung *et al*, 2014). Altogether, metabolic analysis and modelling would not only help to improve yield, taste or texture in industrial-scale fermentation based on starter cultures, but could also benefit smaller, artisanal producers to avoid contamination or spoilage by suppressing the growth of unwanted microbes or metabolic functions.

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Innovation through precision fermentation

Many food additives, such as enzymes, vitamins or natural food colouring, are produced by microbial fermentation. By way of example, riboflavin or vitamin B2 is produced by fermentation with the fungus *Ashbya gossypii;* the major source of rennet for cheesemaking are genetically modified bacteria; vitamin C is now produced by a combination of fermentation with *Pantoea agglomerans* and *Aureobacterium* and further chemical synthesis. Targeted fermentation to cheaply produce large amounts of a specific compound has helped to revolutionise the food industry, but it also creates many by-products, which lowers the production efficiency and increases the difficulty of downstream purification.

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One approach to minimise by-product formation is generating synthetic cell factories where all available resources are diverted to produce the desired compounds and little else. Termed as precision fermentation or synthetic biology, the technology is now heralded as a potential substitute for traditional fermentation. At its heart is the engineering of optimised metabolic pathways and assembling the genes involved in a microbial chassis. This technology relies heavily on an extensive understanding of the microbial genomes and metabolic functions and requires a whole range of technologies such as artificial intelligence, bioinformatics, systems biology and computational biology for analysis and functional characterisation.

Starting with human insulin production by recombinant *Escherichia coli* bacteria in the 1980s, fermentation by genetically modified organisms to produce non-microbial products has been extensively used by the food industry. This is especially obvious in the protein industry, where engineered microbes now produce substances that originally came from animals, such as whey, rennet or casein. Another prominent example is Impossible Foods, which uses soy leghemoglobin produced by an engineered yeast *Pichia pastoris* to give its plant-based burger the flavour and colour of animal meat.

By pushing the boundaries of precision fermentation further, it is possible to envision future food production systems in which fermentation takes a central role to generate a wide range of food ingredients. An example is in the case of oleaginous red yeast *Rhodosporidium toruloides* which has been engineered to improve its natural synthesis of lipids and carotenoids as well as novel compounds that are industrially relevant (Park *et al*, 2018). Further engineering has now generated strains that export carotenoids from the cell for easier extraction and separation (Lee *et al*, 2016). Many start-up companies are now developing engineered microorganisms to manufacture a wide range of food compounds. These and other innovations will inevitably have an even greater impact on the food industry.

Utilising other sources

An in-depth understanding of the metabolism of the microbial community would also enable the exploitation of new substrates, in particular by-products and waste from the food industry, to create higher-value products. Research groups have demonstrated the value of fermentation of food wastes, such as okara and brewers’ spent grains, by probiotic bacteria *Bacillus subtilis,* to create new food products with increased nutritional value (Mok *et al*, 2019; Tan *et al*, 2019). The CRUST Group, a company in Singapore, collects unused and unsold bread from bakeries and restaurants as substitute for malted barley: the sugars are extracted as wort and fermented to “bread ale”. Efforts have also been made to valorise the seed of an exotic fruit, rambutan by fermenting, drying and roasting the fruit pulp and seed in a manner similar to cocoa bean processing to produce a cocoa powder-like product (Chai *et al*, 2019). These and other uses show that fermentation can become an important solution for sustainable food production by converting food waste and by-products into food products.

Utilising novel feedstock sources for fermentation can also help to meet increasing consumer preference for plant-based diets; companies are already developing alternatives to traditionally fermented dairy products such as cheese and yogurt using soy and cereals. Generally, the combination of unconventional substrates and microbial strains could give rise to novel fermented foods with enhanced nutritional content and improved organoleptic properties.

An even more sustainable strategy is biomass fermentation for protein production based on microorganisms’ abilities to rapidly multiply under optimum conditions and produce a very high protein content of more than 50% dry weight. The resulting whole-cell biomass can be served directly or blended with other food ingredients. Examples of such products include Marmite made from yeast extract, fermented bean paste and, more recently, the mycoprotein from a filamentous fungus, *Fusarium venenatum*, which is used as the base for meat analogues (Berka *et al*, 2007). The added benefit of the latter is the textural quality that mimics the fibrous structure of meat products and leads to an acceptable taste sensation. From a genomics point of view, single-cell protein production could be comparatively easily explored by high-throughput strain screening, adaptation and engineering to engineer microbial strains and cell factories for protein production.

Compared to other meat alternatives, such as cultured meat, biomass fermentation would be more efficient as it can use a much wider range of nutrients and feedstock. In fact, many complex and inexpensive feedstocks such as agricultural or industrial waste products can be valorised given the ability of many microorganisms, especially filamentous fungi, to metabolise any substrates. As such, biomass fermentation not only helps with meeting increasing demand for protein-rich food but also benefits the environment by recycling waste. In terms of nutrition and health, it generates food products with lower cholesterol, fat and sugar levels and higher fibre content. One study explored the fermentation of okara by *Rhizopus oligosporus* and demonstrated improved nutritional composition (Gupta *et al*, 2018); other studies showed that proteins produced from biomass fermentation are better accepted by consumers than plant-based meats due to their greater resemblance to animal meat (Souza Filho *et al*, 2019). Biomass fermentation is therefore an attractive alternative to supplement or substitute our dependence on animal proteins, while at the same time lowering carbon footprint and increasing nutritional value.

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Enhancing food safety

The use of fermentation to preserve food remains an important objective to date. Multiple mechanisms work together to prevent the spoilage of food which can be generally classified into two themes: competitive exclusion of spoilage microbes and the creation of an inhibitive environment with each influencing the other. Fermentative microbes release large amounts of compounds which includes lactic acid, alcohol or acetic acid, that inhibit the growth of other microbes. Fermentative microbes, on the other hand, are unaffected by these compounds and continue proliferating—a phenomenon termed amensalism.

However, improper fermentation may still present potential health hazards. Unhygienic conditions or inappropriate food handling may still lead to contamination and spoilage. Furthermore, spontaneous fermentation involving undefined indigenous microbiota carries the risk that undesirable or even pathogenic microorganisms expand and cause food-borne diseases.

The first step to ensure food safety in fermentation is to identify the presence of such microbes and prevent their propagation. Besides quality assurance, constant observation also enables early intervention at the first indication of spoilage or contamination. Metagenomics and HTS along with bioinformatics now allow quick and accurate monitoring of whole microbial communities. Metabolomics and metagenomic analysis can further detect harmful metabolites and pinpoint the microorganisms responsible for producing them.

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While it is easy to say that genome sequencing is the solution to spoilage, the reality is more complex and it requires long-term observation and continuous examination of what constitutes wanted and unwanted microbes. One way would be to simply focus on known pathogens, but that depends heavily on a profound knowledge of good and bad microbes. Another approach is to look out for virulence genes or genes with deleterious effects. Yet, the presence of genes may not necessarily translate to expression. While it is a promising approach generally, using metagenomics to ensure food safety has still a long way to go.

Approaching the problem from another angle, genomic analysis may be useful for identifying microorganisms producing, or with the potential to produce, inhibitive compounds that are safe for human consumption but restrict the growth of spoilage microbes. These notably include bacteriocins, peptidic toxins with potent antimicrobial properties. One prominent example is nisin, which is used as a food preservative to prevent bacterial contamination. Such compounds are thought to be safer than antibiotics because they are produced naturally in food and can be inactivated by trypsin and pepsin to protect the gut microbiota. Microbes encoding genes for bacteriocins are already actively identified for creating synthetic strains that secrete larger amounts of these natural preservatives or for conjugating strains from various sources into a single strain. To supplement food products with bacteriocins requires separate preparation of producer strains in industrial fermenters, followed by recovery and purification. In comparison, *in situ* production offers similar effects but at lower costs.

Protective bacteriocinogenic cultures may even serve as an alternative for long-term storage of non-fermentable foods. Importantly, the physiochemical and organoleptic properties of the food should not be affected throughout the process. In addition to selecting suitable strains to produce bacteriocins, an extensive understanding of the metabolic activities is necessary to ensure that the by-products of microbes' metabolisms will not affect the properties of the food product. Metagenomics analysis would again help to select the optimal microbe composition, environmental conditions and specific strains.

Implementation

To bring an innovation from laboratory to fork is an arduous task that requires considerable time and investment to make sure the final product is safe and accepted by consumers. Take the case of cultured meat, which is based on tissue engineering. It should be perceived as safe given that tissue engineering has been safely used in surgical implantations for decades. Yet when the technology is applied in food production, new concerns on food safety and consumer acceptance must be addressed. These hurdles that might deter the acceptance of cultured meat would be same for all other food innovations.

As with any regular food products, food safety is always a top concern for consumers and regulators. Fermented food products are no exception. While fermentation technology has been widely applied, there could be large variances across batches—or even contamination by pathogenic microbes or harmful metabolites—due to the heterogeneous nature of microbe compositions, metabolic processes and substrates. Therefore, stringent risk assessments of the final products are crucial for mitigating such risks before the new product is brought to the market.

The food risk analysis framework as proposed by the Food & Agricultural Organisation (FAO) has four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation, followed by risk management and risk communication. Novel food products can similarly be assessed under the same framework. However, given the differences between fermented foods in comparison with regular foods, additional assessments would be recommended for long-term health effects since regular consumption over extended periods can lead to gradual accumulation of metabolites in the human body.

Consumer acceptance will remain an important and major factor that determines whether a novel food innovation will eventually be successful and consumers are generally receptive towards trying new tastes and flavours. But the same cannot be said for novel foods produced by unique technologies. Traditional fermentation may have been around for millennia, but precision fermentation may hit consumer rejection. The general perception towards precision fermentation is that of an artificial process; and when synthetic biology is involved, the technology becomes negatively associated with the prejudicial impression of genetically modified (GM) foods.

> *"Traditional fermentation may have been around for millennia, but precision fermentation may hit consumer rejection."*

However, synthetic biology has been around in the food space and food processing for a long time. For instance, chymosin, a coagulant in cheesemaking, was initially obtained from the lining of calves' stomachs, but with increasing demand for cheese, calves' rennet was displaced by fermentation-produced chymosin (FPC) from genetically modified *E. coli* and yeasts. This example provides a suitable message for applications of synthetic biology in food processing: precision fermentation is a technique for producing naturally occurring products, such as enzymes and metabolites, that are not meant for direct consumption. There is fundamentally no difference in the product synthesised naturally and via GM technology, except that the latter achieves superior yield and purity. To encourage consumers to accept the products of precision fermentation would perhaps require more information and education on the process and how synthetic biology is only a means to an end.

Conclusion

Even though fermentation has been used for millennia, there is still tremendous untapped potential and endless possibilities for new applications in our current food systems—from fermentation-derived ingredients to novel protein sources and foods using unconventional feedstock. From a health perspective, fermented foods have desirable characteristics for human health owing to probiotics or diverse bioactive compounds. In addition, with biomass fermentation as an efficient alternative protein source, we can potentially lower the costs of protein production and help millions of people out of malnutrition. There are also positive environmental effects owing to the reduced demand for livestock, which helps to decrease greenhouse gas emissions and pollution while saving land, water and animal feed. Channelling the ability of microorganisms to synthesise specific molecules through precision fermentation also allows the inexpensive and large-scale production of virtually any ingredient for the needs of the food or the chemical industry.

Microorganisms show great promise as cell factories, but it is only through deploying various "omics" tools and synthetic biology that we can improve these to obtain the desired product properties and ensure predictability of an otherwise random fermentation. Through strain selection, screening and engineering, coupled with relevant risk assessments, the vast biodiversity of microorganisms can be explored to create novel and safe fermented products that can benefit human health and the environment. Though fermentation is one of the oldest technologies developed by humans, its potential for further improvement is far from limited.

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