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White Paper

Fermentation for future food systems – What are the opportunities for New Zealand?

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Executive Summary

Fermentation is one of the oldest methods of preserving and enhancing food.

Today, it has expanded to industrialised processes, yielding products with novel formats and benefits. It can also contribute to new agrifood production systems that are more diverse and sustainable. The potential of animal-free proteins to be obtained from fermentation has attracted the most attention from manufacturers and entrepreneurs and venture capital.

There are three principal categories of fermentation:

Traditional as for beer and yoghurt; *Biomass* for single cell protein, fungi mycelium and mycoprotein; and *Precision* whereby microbes are selected and modifiedor genetically engineered to act as 'cell factories'. The latter is a rich vein for research and commercial innovation, with many start-ups and food conglomerates investing heavily.

This biotech is being adopted internationally.

For instance, it is part of Singapore's 'green vision' to secure food supply and enable a circular bio-economy. The UK is building synthetic biology research centres and accelerating the scale-up and translation of biomanufacturing applications. In Australia, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) has a national synthetic biology roadmap for commercial and economic opportunities. And in New Zealand, the MPI initiative "Fit for a Better World – Accelerating Our Economic Potential" identified fermentation as part of the alternative protein solution to productivity and mitigating disruption of our traditional farming systems.

New Zealand should invest in future fermentation.

We need to develop new tools and capabilities aligned to local issues, while maintaining our international reputation as an innovator and trusted food producer. From such research, intellectual property (IP) for precision fermentation could be licensed globally. From its commercialised production, New Zealand-centric substrates, raw materials, and optimised microorganisms could provide valuable differentiation.

There are hurdles to using genetic modification for food systems in New Zealand.

Innovative methods involving genetic engineering (GE) and genetically modified organisms (GMO) can potentially achieve outcomes faster than non-GMO methods, but these approaches are currently restricted by legislation. Various reviews of these standards are either being conducted or recommended. Given that microbes are part of the natural world, we also need to respect kaitiakitanga of Māori and ensure sensitive progression, co-design of applications and equitable sharing of benefits.

Science and innovation to transform fermentation is already underway.

A large scale research programme to advance food fermentation methods (Endeavour, 2017-2022) was praised by the Ministry of Business, Innovation and Employment (MBIE) and industry partners. Its capabilities, facilities, activities and success stories are described at www.agresearch.co.nz/fermented-foods/. The work serves as a foundation for coordinated R&D in this area.

The next step could be establishing an enduring national capability, through a *Future Fermentation Science and Technology Enabling Platform* **(FFstep).**

This collaboration of government agencies, researchers, commercialisation enablers, industry early-adopters and Māori agri-businesses would provide a science, technology and innovation eco-environment to:

- Expand the science and broaden the commercial reach of the non-GMO approaches already demonstrated in Accelerated Microbial Evolution MBIE Endeavour programme
- Co-develop a permanent biobank of indigenous microorganisms and their applications to benefit Māori communities and New Zealand
- Identify and promote future fermentation targets for New Zealand specific needs
- Invest in scaling up of food grade fermentation facilities and the associated expertise
- Ensure New Zealand has science and technology capabilities that can be readily accessed by the existing industry and newcomers who see the opportunities of future fermentation to produce new ingredients and foods in a sustainable manner.

New Zealand research institutes, universities and businesses have the science knowledge, research skills and reputation to join the microbial biotechnology global revolution and support a transformation of the agrifood industry.

1 Introduction

Fermentation is one of the oldest and most efficient methods of preserving and enhancing food. Most regions of the world have their own fermented food traditions, including our Māori mara kai and kānga pirau, for example.

Today, the role of fermentation has expanded far beyond its historical usage, to a much broader range of new processes and products. Industrialised fermentation can serve to extend shelf-life, enrich flavour, aroma and texture, enhance nutritional benefits (e.g., microbially-produced vitamins and essential amino acids; better digestibility of macronutrients) and reduce antinutrients. It can also contribute to new agrifood production systems that are more diverse and sustainable, with less impact on the environment.

Whilst many applications are possible, the theme of animal-free proteins has attracted the most attention of governments, private investment and entrepreneur innovators. It joins plant-based proteins and cellular agriculture as the third technological pillar of the alternative protein revolution (2). There is concomitant and parallel development of functional fat ingredients using yeast and fungi fermentation (3).

In November 2020, the World Economic Forum flagged fermentation as a key global innovation area: Fermentation presents an opportunity to fundamentally change the way the world eats and improve global human and environmental health and the economy (3).

Fermentation is being seen as a powerful technological platform for taking future food and ingredient production to the next level. It combines the wisdom of traditional food preservation, the learnings from biofuels, the precision fermentation pioneered by biopharmaceuticals, and the break-through of the plant-based industry.

These advanced biotech-based technologies are being adopted internationally. For instance they are seen as part of the Singapore government's 'green vision' and the Singapore Food Story to secure food supply, enable a circular bio-economy, and improve sustainability (4). The UK has invested significantly in initiatives to build new synthetic biology research centres and accelerate the scale-up and translation of biomanufacturing applications, including food-grade precision fermentation (5). National roadmaps for protein production and synthetic biology, which serve to identify commercial and economic opportunities for Australia, have been developed by CSIRO (6, 7). In New Zealand, as part of the MPI Roadmap "Fit for a Better World – Accelerating Our Economic Potential" (8), fermentation is being touted as part of the 'alt-protein' solution (9).

Local research on the fermentation revolution is well underway. In 2017, AgResearch, Massey University, the Riddet Institute and Callaghan Innovation received MBIE Endeavour funding for a 5-year programme to develop science and technologies enabling very rapid improvement of traditional food fermentation, particularly with dairy and meat. These new tools led to success at negotiating regulatory agency approval for a novel 'evolved' bacteria strain.

The MBIE Endeavour programme 'Accelerated Microbial Evolution' and its high-throughput screening tools for identifying desirable functional traits have been applied to selected industrial microbes for commercial applications.

Conversations have commenced with government agencies and the industries regarding the science and infrastructure needs of New Zealand. The partners (including Māori entities) recognise that Accelerated Microbial Evolution and related technologies for product innovation have potential beyond the scope of the original MBIE programme and should be continued.

New Zealand needs to develop knowledge and capability in fermentation to remain a competitive international food producing nation. To differentiate New Zealand production, combinations of New Zealand-centric substrates and thereupon optimised microorganisms and industrial processes are required. This should be underpinned by validated understanding of how microbial metabolism can be harnessed to convert raw materials to desirable products.

1.1 Scope

The objectives of this white paper are to:

- Provide an overview of the current landscape in both science development and industry innovation.
- Examine and describe the science gaps, needs and opportunities for New Zealand.
- Identify national and international collaborations.
- Outline the steps to position New Zealand as a science and technology hub in this area of the agrifood sector.

Out of scope for the current discussion are:

- Cell culture-based methods and technologies for alternative food production, such as cultured meat.
- Non-food applications for fermentation, including compounds for medical treatment purposes.
- Conversion of substrates by ex vivo enzyme catalysis.
- Economic feasibility assessment.
- Funding requirements to develop science and infrastructure.

1.2 Food fermentation–past and present

The history of fermentation dates to at least 10,000 BCE for the preservation of camel, cattle and sheep milk. Reference to fermented alcoholic beverages can be found as far back as 7,000 BCE. These fermentations were likely spontaneous due to natural microorganisms present in the substrate and a conducive temperate environment. The traditions of fermentation developed to preserve food, increase its storage time, and minimise waste. This was especially important when food supplies were insecure, and families relied on effective storage to survive lean periods (winter, drought) or long distance transportation. In that era, the fermentation process was not well understood. It is only in more recent times that people have studied the impact of microbes on food systems and the mechanisms by which these systems can be harnessed for greater purpose.

When applied to food systems, the term 'fermentation' typically relates to any instance where microorganisms (bacteria, yeast or mould) grow on an edible substrate, usually under anaerobic (oxygen-free) conditions. Carbohydrates are converted to alcohol or organic acids, while enzymes produced and released also convert proteins and fats to free fatty acids, amino acids, peptides and other small molecules. Most consumers are familiar with yogurt, cheese, wine, beer, cider and kombucha; however, there are many more products that rely on fermentation. Depending on the microorganisms added or naturally present in the raw material, various metabolic by-products are generated that contribute to the flavour, structure, safety, or general health-related aspects associated with fermented products. For example, acetogens (*Acetobacter*) produce acetic acid (vinegar), *Streptococcus thermophilus* and *Lactobacillus delbrueckii* produce lactic acid (yogurt), yeast (*Saccharomyces*) produces ethanol (alcoholic beverages) as well as carbon dioxide which is important in baking bread.

The advantages of preservation are demonstrated in cured salami production. Labile fresh meats and fats are protected through the combined actions of microbial lactic acidification, bacteriostatic metabolites, and water loss. Many such products also benefit from the growth of penicillium moulds.

The diversity in flavour production achievable through fermentation is best exemplified by the variation in cheese. Many cheeses are produced through similar methodologies with their distinctions being the microorganisms present and time used to ferment the cheese.

New Zealand was the first place to develop a continuous fermentation process pioneered by Morton Coutts for brewing beer. Continuous fermentation allows the brew to flow from tank to tank, fermenting under pressure, and never contacting the atmosphere, even when bottled. The world's first exclusively continuous fermenting brewery began its operation at Palmerston North in 1957 (11).

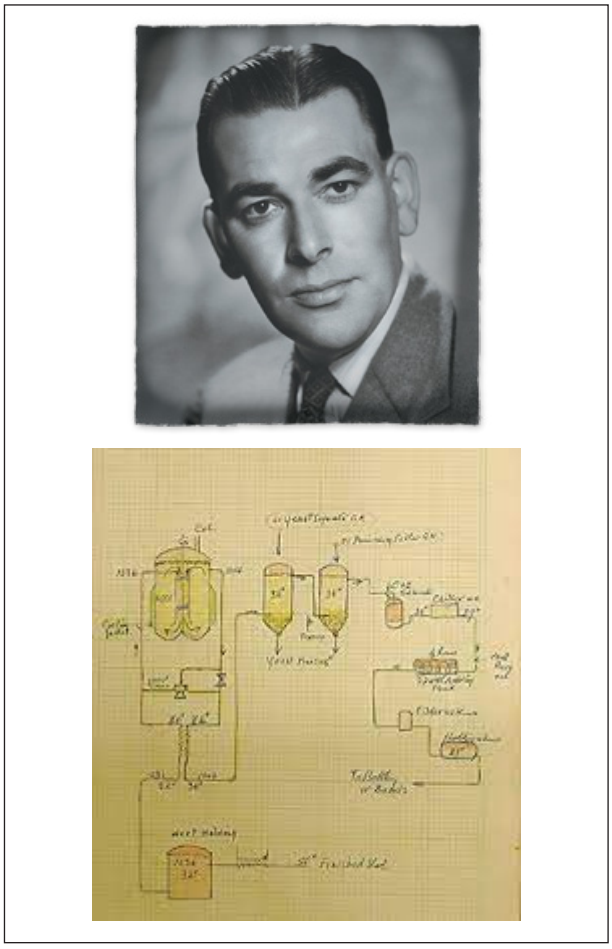


Figure 1. Morton Coutts, father of continuous fermentation and a drawing of the continuous fermentation system, patented in 1956.

As technology progresses, new uses for microbial fermentation have emerged. Previously only thought of in the context described above, where microbes are added to a food material to produce a desired end-product, now fermentation technology is involved in biofuels, waste recycling (biodegradation), and environmental clean-up (bioremediation).

1.3 Traditional Māori practice and knowledge

Early Māori were hunters, gatherers and cultivators who brought plant foods with them to Aotearoa (12). Fermentation was used as a means of food preservation and enabled storage in pātaka (storehouse) and rua kumara (underground pits). The fermentation process for food such as crayfish and fish was traditionally known as mara kai. After European arrival, this was extended to other foods such as kānga (corn), with the fermented product known as kānga wai or kānga pirau. This was made by placing the kai in a basket or kete under very slow-running water for days or weeks until the kōpiro (inner flesh) settled to the bottom of the kete. Kūmara could also be fermented if they started to rot and were called kōtero.

2 Fermentation Technologies

There are at least three principal categories of fermentation technology being used to produce foods or specific compounds that can be used as food ingredients or processing aids (Figure 2).

1. Traditional fermentation: Applicable to artisan and industrial production of dairy, meat and vegetable products, alcoholic beverages, bread, etc. Intact live microorganisms (bacteria, yeast, or fungi) are used to convert energy substrates such as sugars in primary food materials into other compounds (e.g., organic acids, gases or alcohol) that contribute flavours, modified texture, or nutrients to the original food.
2. Biomass fermentation: Filamentous fungi, yeast, microalgae or bacteria are used to efficiently produce large quantities of biomass from simple low-cost substrates. Microbial protein is sometimes referred to as single cell protein (SCP), although some of the producing microbes, such as filamentous fungi or filamentous algae, may be multicellular. Thus, the microorganisms themselves serve as either the predominant ingredient of a food product or one of several primary ingredients in a blend.
3. Precision fermentation: Microbes are selected, modified or engineered to act as 'cell factories' to produce specific molecules that can be used as functional ingredients. A microbial chassis is optimised by metabolic engineering to introduce the genetic coding for the purpose of producing a target compound. The compound is used to improve sensory characteristics and functional attributes of new foods.

While Traditional and Biomass fermentations can find successes through exploring the functional traits of microbes without intentional genetic modification, Precision fermentation relies on genetically engineering (GE) microbes to produce specific and customised (recombinant) molecules that can serve as new food ingredients. GE is defined as the introduction or removal of DNA from an organism by means other than natural processes of horizontal gene transfer. Common methods include the introduction of self-replicating DNA molecules such as plasmids that harbour new and desirable genes. Alternatively, target genes can be integrated, modified or deleted in the microbial hosts' chromosome. The latter option has gained world-wide attention with the discovery of CRISPR-Cas gene editing technologies that enable precise and scar-less gene edition, making it impossible to determine whether a genetic change occurred naturally or as a result of GE techniques.

Governments are beginning to recognise that legislation needs to catch up with innovation. The 2022 UK Genetic Technology (Precision Breeding) Bill will take certain techniques like CRISPR-Cas out from under the umbrella of genetically modified organisms (GMO) rules – acknowledging that the resulting organisms could have occurred through conventional breeding methods. Until New Zealand legislation and standards are updated (discussed in 'Safety and Regulatory'), most new strains for Precision fermentation would be classified as GMO, although their derived products are usually separated from the host microorganisms through further purification processing.

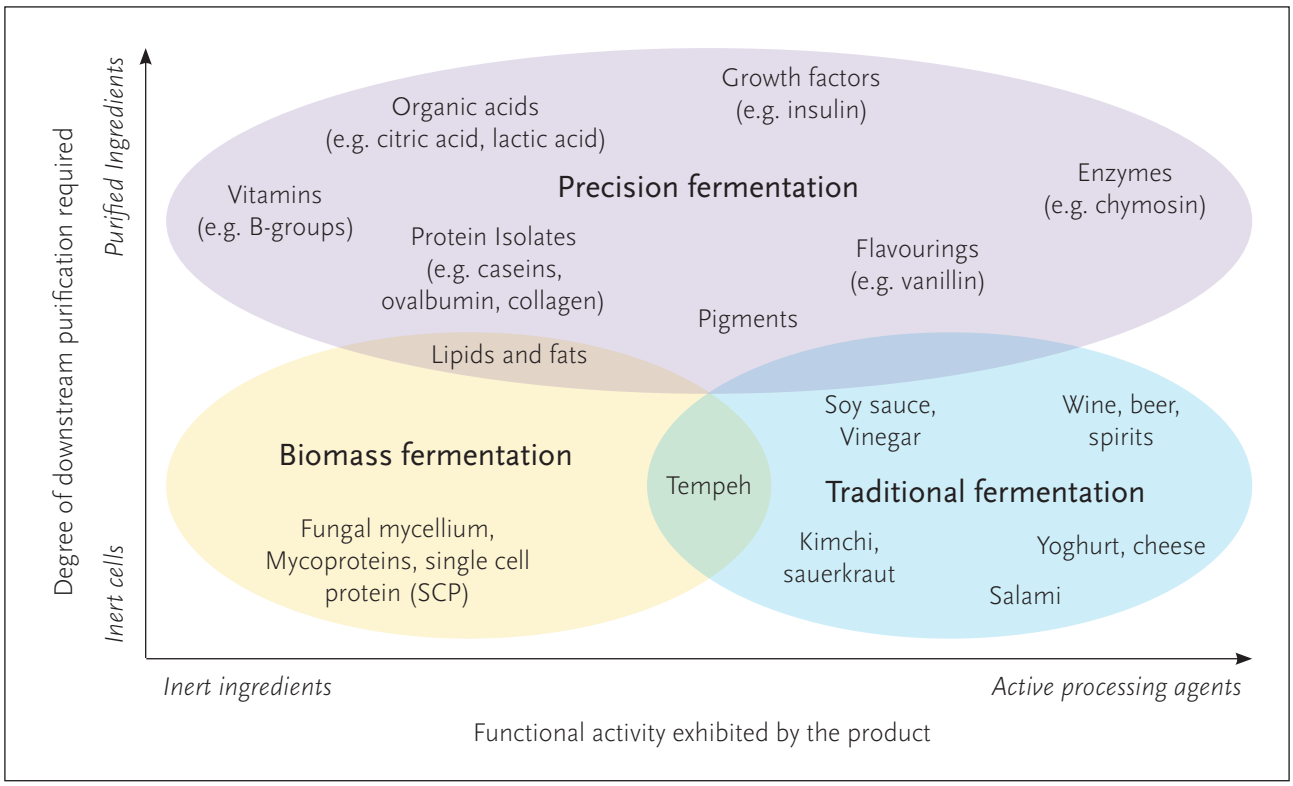


Figure 2. Divergence and overlap of the principal fermentation technologies and examples of the foods, ingredients and compounds produced (modified from (1)).

3 Opportunities for New Zealand

New Zealand is a minor player in the *existing* global commerce of precision fermentation, which includes flavouring compounds, vitamins and specialty enzymes. For example, production of chymosin B from the filamentous fungus *Trichoderma reesei* engineered with calf DNA has long been a substitute for rennet in cheese coagulation. A current trend in innovation is replicating the familiar proteins and fats of animal origin such as meat and milk. Formulators use these to provide sensory qualities and functional attributes that cannot be achieved with solely plant-based biomaterials or cell-culture-based approaches.

More than 80% of the new companies pursuing fermentation-enabled applications have formed in the past five years. This rapid growth, the novel products being conceived, and the niche markets created are described below in 'Emerging Landscape'. As a consequence some traditional industries are threatened (see 'Challenges and Risks'). And despite the pace, troublesome gaps in research knowledge are still being identified (see 'Science and Technology').

New Zealand is renowned for high quality foods produced from natural farming systems, so its strongest position for fermentation opportunities may be in developing solutions to the global demand for food.

Radical new processes are needed to meet consumer expectations for less waste, reduced pollution and cleaner, healthier environments.

This will continue, particularly with the technologies being implemented to mitigate impacts on greenhouse gas emissions, climate change, water quality, carbon footprint and animal diseases, as well as provide better welfare. However, there are risks associated with the rise of alternative ingredients and foods produced using non-animal new fermentation technologies. One example could be our dairy ingredient industry, which prospers from exporting high quality whey protein powders and infant formula. Disruption of the whey trade would not only affect New Zealand export revenue, but also the whole dairy and its ingredient processing industry, as whey is a by-product of cheese making. Alternative uses of those products, of potentially lower value, will have to be found, for them not to become an environment burden to the New Zealand dairy industry.

Risks are balanced by opportunities to use fermentation technologies to develop unique advantages in this area for the benefits of the New Zealand people as well as contributing to a better planet. A compilation of Strengths, Weaknesses, Opportunities and Threats (SWOT; Table 1) highlights the current environment around future fermentation for an alternative agriculture food production system in New Zealand.

Table 1. Strengths, weaknesses, opportunities, and threats of New Zealand being involved in the future fermented foods pipeline.

Strengths	Weaknesses
There is a driver for change, New Zealand exports are heavily dependent on meat and dairy – we need to diversify	Current regulatory environment in New Zealand, limiting the GMO pathway/commercial set up
Low-value fermentable feedstock from land-based production	Lack of integrated innovation eco-system between government, research organisations and commercial end-users
New Zealand indigenous microbes remain to be discovered	Size of government and private investment in science discovery and innovation is limited
Strong traditional basis (e.g., cheese, dairy, wine) in fermentation	Late starter, doing catch up
Strong track-record of food safety	Lack of investment in scaling up facilities, expertise, capabilities, capital money, regulation and regulatory support
Microbial science, from soil to human	Limited pilot and manufacturing capacity in food fermentation
Food science and omics analytics capability	Lack of co-ordinated biobank resource for research and commercial applications
Non-GMO capabilities that can be re-purposed	Distance to/from key markets (an issue for commodities), cannot compete on price
New Zealand's clean green image can be leveraged	

Opportunities	Threats
Add/extract new values from by-products as part of the circular bioeconomy drive	New Zealand being seen as low-tech, difficult to attract international investment
Science and technologies established in the MBIE Endeavour Programme, including ability to continually improve or extend opportunities through each new generation of microbes	Lack of diversity from its traditional pastural based agrifood systems – risk to New Zealand economy
Niche products or services that can be produced locally, reduce the reliance on import	Loss of R&D capabilities
Create protectable IP which can be licensed	Trained graduates look offshore
Value return on indigenous fauna and flora resources	International capability not interested in coming to New Zealand
Functionalise plant biomaterials and produce new food products	Need to balance IP vs manufacturing if all production becomes off-shore (e.g., no job creation in New Zealand)
Ability to create ultimate nutrition foods	Loss of key export revenue of ingredients and formulated products (e.g., whey protein powders, infant formula, etc.) where/when new and better ingredients can be produced offshore
Leverage with other bioproducts, help reduce carbon footprint and GHG emission and add to carbon zero story	

3.1 Adding new value to the circular bio-economy

Increasingly, consumers are demanding ethically-mindful products with fully-considered environmental impacts. Fermentation technologies can help to reduce agricultural waste or create value from waste. Across the New Zealand horticultural and forestry sectors, a large volume of waste biomass is produced. These are the leafy or woody vegetative parts of plants, which are usually left over after harvesting crops for edible tissues such as their fruits or seeds. Some of it may be useful as low- or no-cost feedstock materials for fermentation, by converting it to a useful nutrient media (such as sugars) that microbes can re-assemble into specific ingredients for food applications and other marketable products. For example, sugars derived from the processing of lignocellulosic residues can be used for mycoprotein production (53). Towards that goal, Australia is focusing on its sugar cane industry, particularly the waste stream, as part of CSIRO's Synthetic Biology Future Science Platform.

Interests are currently being discussed and explored with New Zealand industries including forestry, pastoral, wine and mushroom. We are already composting some waste streams (e.g., grape marc), producing and extracting high value products using fermentation can provide additional applications and value, with minimal impact on the environment. Forestry waste and the residual biomass remaining after protein extraction from pasture might be used as low-cost feedstock. Fermentation may offer ways to generate and extract high value compounds from other co-products or by-product streams from dairy, meat, fisheries etc.

3.2 Strain discovery and improvement

New Zealand as an island nation harbours a wide range of recognised unique plant and animal life. Bioprospecting for indigenous microbes with novel biological phenotypes (traits) and activities presents a significant opportunity for New Zealand in the food sector and beyond. We acknowledge the kaitiakitanga of Māori people, as discussed in 'Indigenous Flora and Fauna'.

Some iwi-guided bioprospecting activities have already been carried out and others such as rumen microbiome are ongoing. One key challenge before beginning any bioprospecting is the clear definition of the targeted trait or metabolite. Otherwise, the activity can slip into random collection of uncharacterised microbes and will be of limited value.

Examples of targets relevant to "New Zealand Inc." would be the degradation of lignocellulose or hemicellulose (linked to 'Adding New Value to the Circular Bio-Economy', and 'Functionalising Plant Food'). These are cell wall structure compounds in plants. Microbe strains that can effectively breakdown these highly structured molecules are not currently available for industrial scaling and processes.

Bioprospecting for microbes in the rumen of New Zealand livestock – which have developed to degrade cellulose and hemicelluloses of forage plants – could result in novel strains that efficiently break down and metabolise plant materials from forestry and food crop waste streams. Accelerated Evolution can then be applied to further enhance specific metabolic activities of lignocellulose and hemicellulose degrading enzymes and enzyme complexes, thereby increasing commercial feasibility. However, the rumen is a strictly anaerobic environment and isolated novel microbes may therefore be oxygen intolerant. To overcome this practical limitation for upscaled industrial processes in the forestry industry, candidate strains can be genetically engineered to introduce genes coding for oxygen tolerance enzymes and proteins such as catalase, cytochromes or haem-containing proteins.

Another example of a bioprospecting target is the breakdown or bioconversion of the keratin- and collagen-rich hair, feathers, nails, horns, hooves, scales, and wool from animal industries. The feather waste stream from poultry production is a recognised burden in New Zealand and therefore an opportunity to transform this low-value substrate. Keratinase activities have been reported for a wide range of microbes. Novel strains with either improved keratinase activity or the ability to grow preferentially in the industrial low-value substrate would be valuable candidates. Both Accelerated Evolution and GE approaches can then be employed to either enhance keratinase activity or drive the bioconversion of metabolic energy into a different, high-value compound or applications. These could include nutrient-rich hydrolysate feeds, novel fertilisers or food chain supplements from keratinous wastes, formulation of liquid and solid detergents, or green leather processing.

3.3 Indigenous flora and fauna

New Zealand retains large areas of land and sea that are almost untouched by human influence. These are likely a reservoir of novel bacteria with unique phenotypes found nowhere else in the world. This reservoir should also be recognised as including traditional Māori fermented foods that are created by spontaneous natural resources.

Māori have an intimate and interconnected relationship with the natural world and its resources and see themselves as part of the ecosystems (41). Indigenous organisms including microbes are under kaitiakitanga of Māori so there is both opportunity and risk associated with their discovery and characterisation. For example, the values and beliefs underpinning how Māori view the living and non-living world, which include whakapapa (the genealogy) and

tapu (protected and sacred) (43), may influence the acceptable use of new microbes. It is therefore imperative that any research and applications respect these values as we explore and potentially improve on the natural resources that nurture and sustain the future generations. Unease about genetic modification is not unique to Māori and relates to the long reaching effects that this technology can have on tikanga. Māori are also concerned with negative impacts on the tino rangtiratanga, all living organisms, food, rongoa practices, health and any intellectual property rights. By following tikanga Māori guidelines, through appropriate consultation and not relying solely on 'selected' Māori experts who reinterpret tikanga to align with this technology, we might be able to move forward for the benefit of all (52).

As part of the MBIE Endeavour research programme, a new partnership with Wakatū was established. Under their guidance, kawakawa plant samples were systematically collected in a remote area of the South Island near Nelson. The scientists' aims were to isolate novel strains of lactic acid bacteria and to begin a curated collection of New Zealand indigenous microbes. This task was successful and new strains are stored in a temporary biobank at AgResearch. Although detailed characterisation of these New Zealand strains is still required, initial phylogenetic classification by 16S rRNA gene sequencing has already demonstrated a low level of DNA sequence similarity to other known bacterial strains, giving confidence that unique indigenous strains can be readily isolated from the New Zealand biosphere. These strains, when found to possess beneficial traits, can then be fully characterised, patented and commercialised. Ownership and IP management of this new bioresource needs to be carefully defined together with the Māori partners.

It may be possible to improve the desired traits of indigenous strains through the Accelerated Microbial Evolution Technology, to further enhance the utility of New Zealand indigenous resources.

3.4 Functionalising plant food

A preliminary fermentation step can increase the techno-functionality and nutritional value of plant proteins. Compared to animal proteins, the properties of plant proteins are often more difficult to be realised due to the differences in their molecular structures (54). Microbes can be used to modify (e.g., open up) the structures to increase protein solubility and bioavailability, and hence the nutritional value of plant proteins. Sometimes the sensory properties can also be enhanced through the release of metabolites associated with aroma and flavours. Sourdough is a classic example of employing lactic acid bacteria fermentation to achieve a distinct flavour and texture signature of a bread. Similar fermentation methods can be used to functionalise other plant proteins (55) and lift the sensory and nutritional quality of plant protein-based foods.

New Zealand has strong plant breeding capability, experience, and knowledge, particularly for forage, cereal and legume crops. There could be new opportunities in coupling fermentation and plant breeding programmes to target and develop useful microbe traits. Functionalising all of the plant materials (the protein and fibre) could reduce the carbon footprint of production and obtain maximum economic benefits.

These or other crops might be optimised or diversified to develop cheaper and more sustainable substrates. Years of research geared toward making crops more amenable to fermentation for biofuels might be leveraged to target protein ingredient outcomes.

The opportunities for New Zealand in this area have been included as part of the MPI "Accelerating Protein Diversification Science Plan" (9).

3.5 High value ingredients

One of the key advantages of precision fermentation is the ability to generate products that are otherwise scarce in nature, such as bioactive proteins lactoferrin, IgG, and IgA. These proteins are present at very low concentrations in milk and are generally heat labile which means active levels in heat treated milk are even lower and large volumes of milk or whey streams must be processed to extract commercially viable amounts. To date lactoferrin is the most developed of the minor proteins and is used as an ingredient in infant formula, as tablets and in a range of other delivery methods. As proteins like lactoferrin are not human identical and there has been a drive to humanise infant formula type products, precision fermentation could provide a means to achieve this in the future and therefore be disruptive to the existing manufacturing routes.

Other targets from high value dairy ingredient space could be a range of minor proteins (e.g., anti-aging compounds in dairy) yet to be commercially exploited.

Another area could be healthy lipids such as the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) commonly used in dietary supplements. These are found in oily fish such as herring, anchovies, sardines, mackerel and tuna, which are threatened by overfishing. Production of these specific lipids from algae is more sustainable and less likely to be contaminated by pollutants like mercury, however concentrations of EPA and DHA are lower than in fish oils. Production of EPA and DHA using microbes with improved efficacies and precision fermentation could produce higher yields of EPA and DHA, as well as allowing fine-tuning the fatty acid profiles of the oils, without exploiting marine sources.

The ability to produce animal-identical compounds using precision fermentation may become more attractive than extracting out of animal sources, particularly for higher value applications such as supplements and personal care products, for instance collagens. Traditional methods for recovering collagen (e.g., gelatine, collagen peptides) using chemicals and heat, result in changes to the structure and functionality of the protein. This is particularly important when considering their use in applications like cosmeceuticals, wound healing or biomedical devices where the collagen's structure and function needs to impart a therapeutic effect which requires high biocompatibility, low immunogenicity and appropriate material properties. In such cases, precision fermentation could allow the production of designer collagens that retain the original structures and associated functionalities.

4 Emerging Landscape

New companies pursuing fermentation-enabled applications span the globe, operating in at least 24 countries (Figure 3). The largest concentration of companies is in the United States, followed by Israel, Spain and Germany (3).

In addition to the start-ups, the global industry leaders in biotechnology such as DuPont, Danone, ADM and Nestle are also making notable investments into this area. Chr. Hansen announced the launch of a 'culture kit' specifically designed for the fermentation of plant proteins (13). ADM recently formed a partnership with the Asia Sustainable Foods Platform, a company wholly-owned by Temasek, in Singapore, to provide precision fermentation consulting and technology development.

Investment in the fermentation technology sector has skyrocketed in the last two years, albeit from a small base. In 2021, this reached US$1.7 billion (Figure 4). Part of this is driven by the government-related investors around the world (such as Israel, Singapore, Europe, UK and USA) recognising the potential of fermentation technologies in the development of sustainable protein and consequently rewarding multiple companies with grant funding.

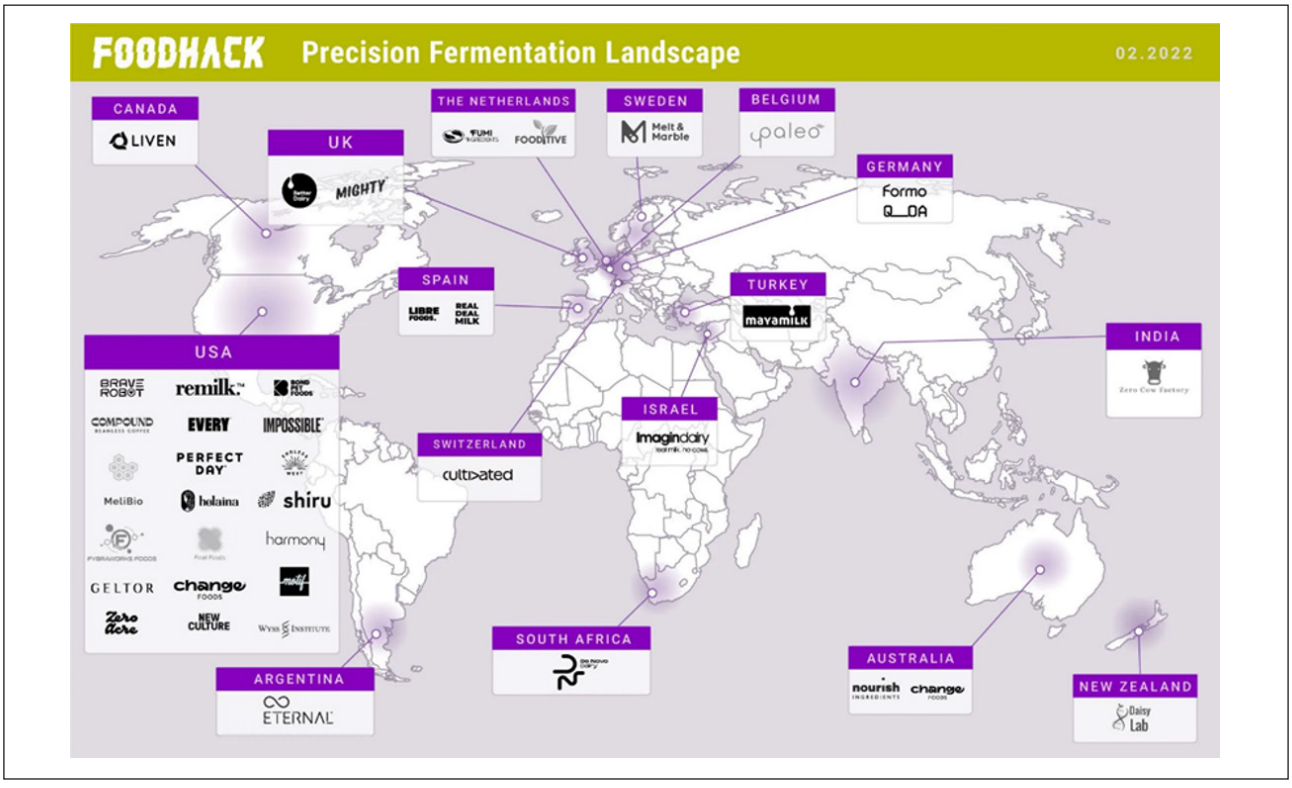


Figure 3. Global landscape of emerging companies in the development of fermentation-enabled applications to replace animal-based food and ingredients. (c) FoodHack.global, for HackVentures Ltd 2022.

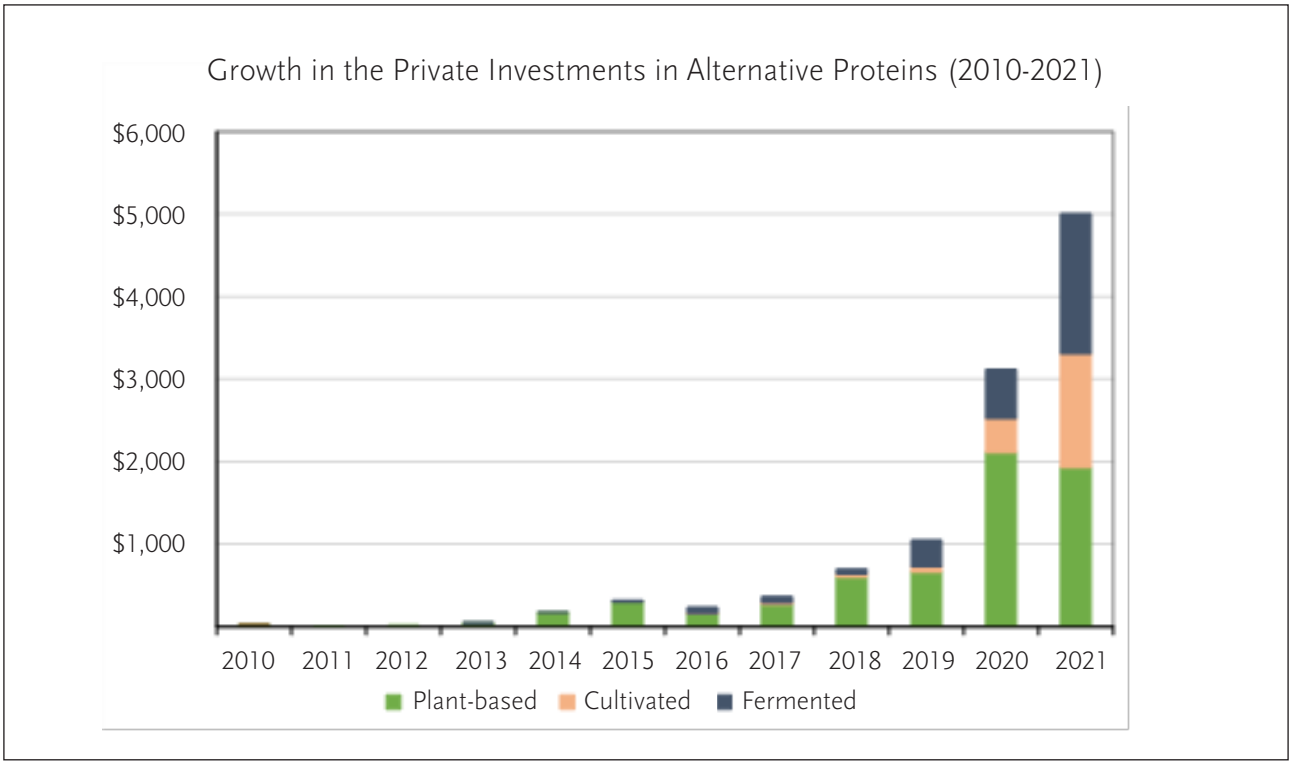


Figure 4. Growth in private investments (US$ millions) in the alternative production of mimicking animal-based foods. Data from the GFI report (3).

While investment capital in fermentation and other alternative protein industries has grown at an impressive rate, it remains a small fraction of global food production systems. Much more investment will be needed to enable the continuing development of critical R&D, scale production, and bring down costs to better compete with conventionally produced animal-based food products. Some of that opportunity turns on leveraging existing IP. See Appendix I for preliminary analysis of the patent landscape.

4.1 Meat substitutes and analogues

Products purporting to mimic meat are common in the plant-based sector. They rely on extrusion and other physical processes to create the desirable structure of strands and fibres. In contrast, biomass fermentation can inherently achieve a filamentous texture.

The first commercial meat mimic by fermentation was Quorn™, which is sold as a cooking ingredient and as a meat substitute included in pre-packaged meals. It uses the mycoprotein derived from the *Fusarium venenatum* fungus as an ingredient (14). The fungus is grown in continually oxygenated water in large fermenters. Glucose and fixed nitrogen are added as nutrients for the fungus. Vitamins and minerals are also added to improve the nutritional value. The resulting mycoprotein is then extracted and heat-treated. The commercial operation started in UK in 1985 and entered the US market in 2002. In 2021, KFC Singapore rolled out Quorn-based Zero Chicken Burger at most KFC Singapore locations.

New products have emerged using the same principle of biomass fermentation (see Table 2 and Appendix I). For example, in 2020 Prime Roots released Koji Bacon using batch fermentation of *Aspergillus oryzae*. Koji, often called "Japanese mould" is traditional in Asian cuisines to give dishes an umami flavour through popular fermented products such as miso and soy sauce. Koji's mycelium (root structure) grows in microscopic fibres that can be processed and mixed with other plant and fungi derived ingredients to mimic the dense fibrous texture of animal-based foods. Their range of products has expanded to sliceable hams, turkey, and salami.

In 2022 the start-up company Nature's Fynd launched meatless breakfast patties produced by biomass fermentation of *Fusarium flavolapis* (discovered in a Yellowstone hot spring) into Whole Foods Market stores across the USA. Other companies experimenting with mycelium-based fermentation to produce whole-cut steak and chicken mimics are Meati Foods (USA), The Better Meat Co. (USA), and Mycovation (Singapore).

MycoTechnology is the most active company in patenting mushroom mycelium-based fermentation through a technology called FermentIQ™. It has been reported that feedstocks of pea and rice protein fermented with shiitake mushroom mycelium improve flavour, aroma, digestibility, anti-nutrient content, and application functionality.

Impossible Foods is mimicking some of the taste and appearance of meat by using precision fermentation technology to engineer a soybean leghemoglobin (legH) gene into yeast (*Pichia pastoris*) and produces large quantities of this protein via fermentation. The Impossible Burger is available in the New Zealand supermarket Countdown.

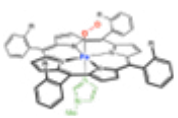
Similarly, Motif FoodWorks released HEMAMI™, a GE yeast-derived haem protein that is identical to bovine myoglobin. It is available to plant-based food manufacturers to give their products the flavour and aroma of meat.

These new technologies may deliver desirable sensory and functional properties in animal-free alternatives. If so, our meat industry could be at risk of substitution of the manufacturing beef that it exports, as well as the carcass co-products that are currently used for pet foods and diverse ingredients.

Bond Pet Foods developed the world's first animal-free pet food using precision fermentation technology and filed the patent in 2020. A recombinant chicken protein is produced by engineering genes into yeast using precision fermentation.

Geltor is developing intact Type XXI collagen protein from yeasts containing chicken genes as an alternative to collagen derived from animal rendering. Note that this is different from the Type I collagen that comprises the bulk of the current animal industry, and so may have special use cases.

Table 2. Examples of meat analogues and meat substitute products produced using biomass fermentation or precision fermentation technologies for one or more ingredients.

Monde Nissin Corporation (Previously Marlow Foods) Biomass fermentation using *Fusarium venenatum* fungus to produce mycoprotein.	**Nature's Fynd (California, USA)** 'Fy' protein produced using *Fusarium flavolapis*, first identified in geothermal springs in Yellowstone National Park.	**Prime Roots** Batch fermentation of *Aspergillus oryzae* to make koji, a type of fungi.	**Goodside Foods** Meatless crumbles, produced by MycoTechnology mushroom mycelium-based fermentation technology.
			
ENOUGH (3FBIO) (Glasgow, UK) Grow fungi using the naturally-occurring sugars in grains to produce ABUNDA mycoprotein.	**Impossible Burger** Expression of soy leghemoglobin (legH) in GE yeast *Pichia pastoris*. legH is then added to plant-based burger to help it look like 'red meat'.	**Motif FoodWorks** Expression of myoglobin (GEMAMI™) in a GE yeast strain.	**Bond Pet Food and Hill's Pet Nutrition** Using precision fermentation to produce recombinant chicken protein GE yeast. The protein is used in a prototype pet food.
			

4.2 Production of dairy ingredients

Products that leverage the appeal and utility of milk fill the plant-based sector. They are typically extracts or 'juices' of grains, pulses and nuts, plus adjuncts to aid functionality and palatability. In contrast, the production of fermented animal-free dairy foods is achieved by engineering the genes of key animal proteins into microbes. These are usually bovine but can be from other mammals. The idea of using precision fermentation to produce casein and whey proteins, which then form the base to combine with minerals, sugars, fats and flavours to achieve the composition of milk and dairy powder ingredients, is gaining attraction worldwide (see Table 3).

2020 saw the first commercial release of a dairy product made through precision fermentation in Perfect Day's Brave Robot ice cream. It comprises recombinant whey protein in a sugar and vegetable oil base. In 2021, that whey became available in other products and brands, including cream cheese and whey protein powder. The latter is now available in Singapore and Hong Kong, making Perfect Day the first to export an animal-free dairy protein outside the USA.

Starbucks began trialling Perfect Day's milk at select locations, and Hong Kong's Igloo Dessert Bar launched Asia's first-ever animal-free ice cream, using Perfect Day's dairy proteins. A lactose-free cream cheese alternative is also being developed by General Mills (Bolt Cultr Brand), using the animal-free dairy proteins from Perfect Day via microbial fermentation.

While the focus of Perfect Day to-date is on the production of whey proteins, the Israel start-up Remilk claims to have produced casein proteins using microbial precision fermentation (Appendix I). Other start-ups, such as Change Foods and NEW CULTURE, are also investing in precision fermentation technologies to produce dairy proteins aiming for cheese and yoghurt mimic products. The type of protein is rarely described, perhaps to retain flexibility as their business develops. No commercial products have reached markets yet. Finally, 2021 start-up Fermify claims to have engineered yeast to produce all four casein types (alpha 1, alpha 2, beta kappa), with an aim to create ingredients for vegan cheese.

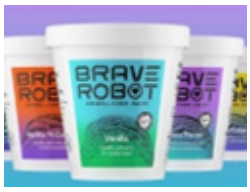
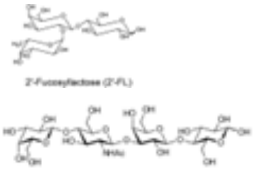
Cheese substitutes can also be developed and formulated with proteins sourced from biomass fermentation. For example, Sophie's Bionutrients has patents pending to generate protein flour from microalgae grown in bioreactors (see Appendix I) and is collaborating with Ingredion to formulate vegan-friendly cheese products. Similarly, Corbion/Purac expanded its fermentation capability to include microalgae and in 2019 partnered with Nestlé to create 'plant' based proteins and nutrients (no reports of products yet).

Human milk oligosaccharides (HMO) molecules are another group of compounds in milk to be produced using precision fermentation (15). Chr Hansen and DSM are leading the innovation in this field with a wide range of HMOs that match their natural counterparts in terms of structure and function, manufactured via microbial fermentation using various strains. The products go through multistep purification and isolation processes, and are claimed to not contain GMO microbe cells once purified (16).

> Precision fermentation could disrupt ingredients for infant nutrition, by producing 'milk molecules' that are functionally and immunologically similar to those in breast milk. In theory, this could 'humanise' infant formula products to a degree not possible using milk from. animal sources.

Founded in 2019, Helaina, a USA start-up, is making proteins with the aim of immune-equivalency to the proteins in breast milk as well as bio-active properties that span benefits beyond immunity. The company has raised US$20M in November 2021 to begin its manufacturing and commercialisation process (17).

Table 3. Examples of development in the production of dairy products using precision fermentation or biomass fermentation.

Perfect Day - and subsidiaries Expression of bovine β- lactoglobulin in *Trichoderma reesei (Hypocrea jecorina)* Its subsidiary brand products:	**Urgent Company - Modern Kitchen** Animal- and lactose-free cream cheese	**Urgent Company - Brave Robot** Animal-free, vegan, lactose-free ice cream	**Urgent Company California Performance** Animal-free whey protein powder
			
Bold Cultr Foods by General Mills Lactose-free cream cheese alternative using the animal-free dairy proteins from Perfect Day via microbial fermentation	**Nature's Fynd** Animal-free cream cheese formulated using biomass fermentation produced 'Fy' (fungi) protein, and other plant-based ingredients	**Chr Hansen and DSM** Human milk Oligosaccharides, produced using genetically modified *Escherichia coli (E.coli)* strains	**Sophies Bionutrients** Cheese made from biomass fermentation using microalgae
			

Although outside the scope of this report, it is worth mentioning that two other start-ups, TurtleTree in Singapore and BIOMILQ in USA, are working on mammary cell culture-based technologies to produce milk comprising protein, lipid, and oligosaccharide components at concentrations that mimic and/or are substantially similar to human breast milk (see Appendix I).

Here in Australasia, several start-ups have emerged and raised the investment to begin developing dairy ingredients, primarily proteins, using precision fermentation (Table 4). They are in the early stage and aim to release products from 2023. Nourish Ingredients is following a different path, with their focus on animal-free fats, primarily for the plant-based meat analogue market (see below).

Table 4. Start-ups founded in Australia and New Zealand aiming to develop precision fermentation technologies for producing dairy proteins, lipids and other compounds.

Eden Brew, Australia (2020) Caseins and whey proteins. Prototype testing at CSIRO's Food Innovation Centre in Victoria, and expecting to launch in 2023. Backed by CSIRO and Australian dairy co-operative Norco. www.foodingredientsfirst.com/news/casein-without-the-cow-eden-brews-precision-fermentation-milk-to-launch-in-australia.html	**Change Foods, Australia/US (2019)** Casein and whey proteins, lipids and aromatic compounds. The first in Australia to work on cheese production and aim to launch their first (precision fermentation cheese) product in 2023.	**All G Foods, Australia (2020)** Casein and whey proteins. Established an alternative dairy brand called "MilkCELL", partly funded (AU$5 m) by the Australian government's Clean Energy Finance Corporation (CEFC). allgfoods.com/our-brands/#milkcell
Nourish Ingredients (2019) Animal fats. Create the molecular structure of animal fats by using GE yeasts to enhance the flavour and taste of plant-based meat mimics. Partly funded by CSIRO's Main Sequence Ventures. Nourishing.io/science/	**Daisy Lab, New Zealand (2021)** Casein protein. Producing lab-grown casein by the end of 2022 and entering market around 2024. www.daisylab.co.nz	

4.3 Other protein foods

The development of other protein foods such as chicken, eggs and seafood using fermentation-enabled technology has also gathered pace.

The EVERY Company (formerly Clara Foods) has developed precision fermentation technology to produce animal-free egg white protein (see Appendix I), which was launched in March 2022 and is debuting in a high-value specialty food (gourmet macaroons).

4.4 Fats and oils

Although the prime drive is to replace animal proteins, microbial fermentation technologies are also being exploited to produce fats and oils. These can be included in plant-based products to help replicate the flavour, texture and mouthfeel sensory experiences of conventional animal foods.

Nourish Ingredients (Australia) has prototyped a GE fermentation process that creates lipids with structures that mimic animal fats, without the use of palm or coconut oils (Table 4). Swedish university start-up Melt & Marble has recently scored its first seed funding to make meat-mimic lipids using precision fermentation of GE yeasts, purportedly with tailor-made fat structures. Cultivated Biosciences (USA) is developing a functional fat ingredient from oleaginous yeast that can be used as a high-fat component of plant-based dairy formulations. Their advertising states that the yeast is not GMO. Several other start-ups in Europe are also working to create bioidentical fats via precision fermentation.

4.5 Research and development

The science and research communities have also been active in driving new discovery. This is facilitated by a global shift in government funding and private investments towards agriculture and food systems that have the potential to reduce environmental impact and improve sustainability (see 'Environmental Impact'). Several of the large research and technology programmes that have been established through government-private partnerships are listed in Table 5, including the New Zealand MBIE Endeavour Research Programme.

Recently, in cooperation with Food Valley Netherlands, Wageningen University and Research (WUR) purchased The RoboLector, an automated platform for the highly parallel real-time optimisation of cell culture fermentations, that includes robotic liquid handling and microfluidics. It enables high throughput selection of microbe strains and processing conditions, and therefore increases the success rate to develop proteins from fermentation. The equipment is available for shared use by other businesses and institutes. It might also be a valuable tool for New Zealand projects.

The Singapore government aims to produce 30% of its nutritional needs locally by 2030, up from less than 10% currently. In 2019 the local public sector agency A*STAR set up the Singapore Institute of Food and Biotechnology Innovation (SIFBI) to support the country's food innovation ecosystem. Its key science capabilities and research activities include strain engineering, biotransformation and food processing engineering. In 2021, SIFBI partnered with Temasek Asia Sustainable Foods Platform to provide start-ups with tailored infrastructure for scaling up the production of alternative proteins via extrusion and fermentation technologies.

Table 5. Examples of fermentation-focussed programmes in several leading international research organisations

Organisation	Future Fermentation related research programme
CSIRO, Australia	Part of its Future Protein Mission to create new Australian protein products and ingredients that earn an additional $10 billion in revenue by 2030. www.csiro.au/en/about/challenges-missions/future-protein-mission Synthetic Biology Future Science Platform to position Australia in one of the fastest growing areas of modern science. research.csiro.au/synthetic-biology-fsp/ Working with Eden Brew to create animal-free dairy proteins using yeast. Eden-brew backed by CSIRO Norco Main Sequence.
Wageningen University, Netherland	Fermentation technology for sustainable chemicals and food ingredients Expertises/Fermentation-technology-sustainable-chemicals-food-ingredients.htm B-12 Insight, developing plant-based meat alternatives with meat-associated vitamins and flavours by use of fermentation. Food-biobased-research B-TWELVE-Insight-1.htm Alternative Protein project, created in 2020 and supported by the Good Food Institute (GFI), is a student initiative to encourage the development of alternative protein sources, including plant-based analogues, biomass and precision fermentation tabledebates.org/blog/introducing-wageningen-alternative-protein-project
Singapore Institute of Food and Biotechnology Innovation (SIFBI) A*Star, Singapore	Strain engineering – develop and apply synthetic biology and metabolic engineering approaches for the engineering of biological systems including microbial cell factory. Biotransformation – optimise the physiology of microorganisms by controlling growth conditions and engineering bioreactor configurations. www.a-star.edu.sg/sifbi
The Fraunhofer-Gesellschaft, Germany	EU2020 Smart Protein Consortium (Smartproteinproject.eu) Screening different fungi for their ability to ferment by-products from baking / pasta products
Institute of Agrifood Research and Technology (IRTA), Spain	EU2020 ProFuture Consortium (www.pro-future.eu) Focusing on boosting the production and use of microalgae protein-rich ingredients in food and feed
New Zealand collaboration	MBIE Endeavour Research Programme. Accelerated Evolution: A step-change in food fermentation (2017 – 2022) www.agresearch.co.nz/fermented-foods/

5 Science and Technology

Fermentation has its roots in traditional food preservation and more recently in the production of natural products, enzymes, therapeutics, pharmaceuticals, and other consumer goods.

Despite similar approaches, the use of fermentation technologies for mainstream agriculture and food production will require unprecedented scale, new science discovery and innovation, and co-development of suitable infrastructure. Figure 5 depicts the key steps that need to be considered for both R&D and commercialisation.

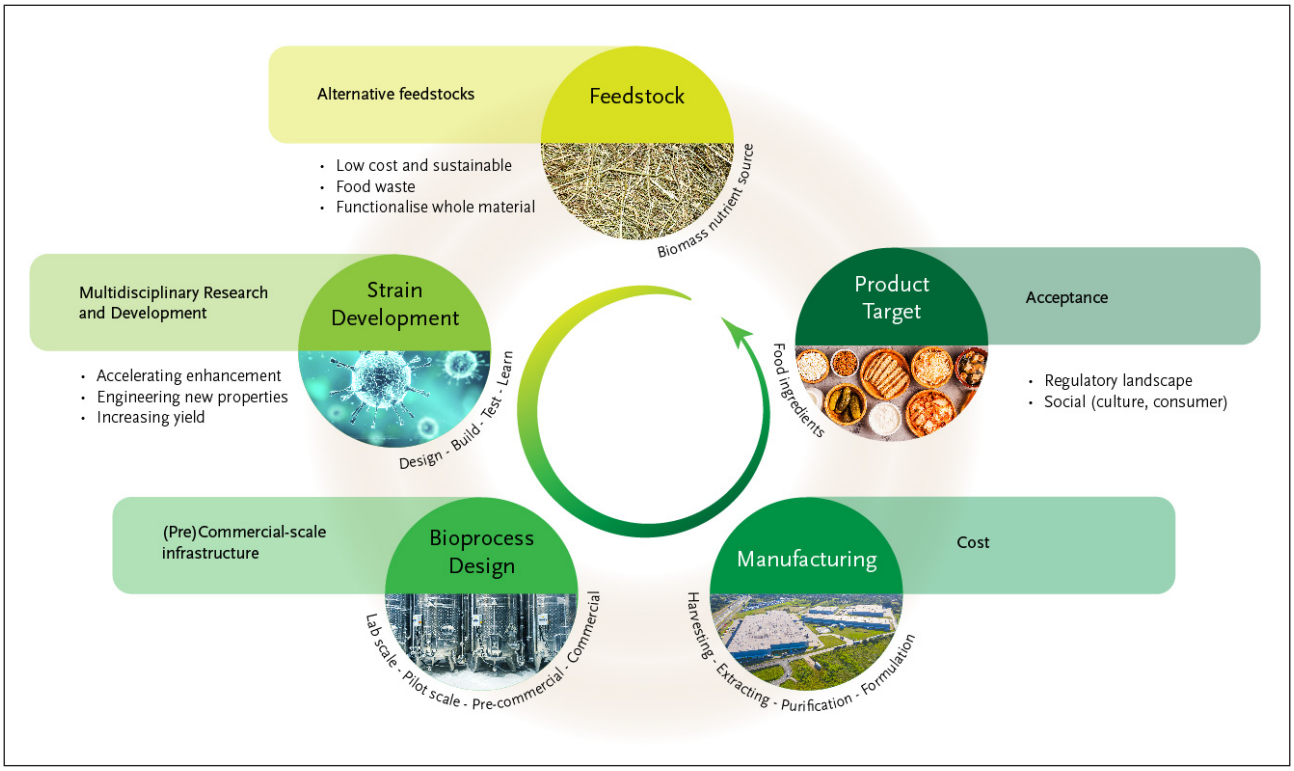


Figure 5. Key steps to be considered in the development of future fermentation technologies leading to large scale production of foods and ingredients.

5.1 Feedstock selection

A feedstock is required to support microorganisms' growth. It can be as simple as glucose or lactose sugars to provide energy and carbon, plus a source of nitrogen to sustain amino acids. Its cost is a major factor for most fermentation processes, and optimising feedstocks can gain both economic and sustainability advantages.

The choice of feedstock, the type of fermentation and the desired composition of end products are mutually contingent. Dependencies include availability, volume and what alternative value might otherwise be obtained. Some examples of fermentation goals illustrate how feedstock influences and constrains the desired outcomes:

- Modify a plentiful raw material to increase its value
  Make sauerkraut from cabbage
- Generate an extractable by-product
  Produce fuel ethanol from corn
  Produce specialised or bulk proteins from the co-product steams of food processors
- Create a complex high value material from simple substrates
  Grow fungal mycelia or SCP edible bacteria from nutrient broth
- Create 'food for cells' through a two-stage process
  Crude diverse feedstocks are used to first generate a semi-purified, industrial sugar stream that is subsequently fed to a second fermenter growing cultured cells. Alternatively, in a consortium of microorganisms, a preliminary fermentation by one transforms the feedstock into a form that can be used as an input for the next.
- Degrade a low-quality and undesirable materials, or use non biological feedstocks
  Targeted composting to dispose of wool carpet waste
  Rumen-like degradation of the ligno-cellulosic leftovers of leafy and woody plants.
  Plastic waste is not only degraded but turned into edible protein (18).
  Hydrogenotrophic microbes, which include methanogenic archaea, are fermented in tanks and fed an inorganic mix of carbon dioxide, oxygen, minerals, water, and nitrogen.
  The result is a protein-rich flour.

As feedstocks tend to be low concentration materials, the logistics of concentrating, transporting, processing and storing will add expense, especially if the sources are widely dispersed. Co-locating the fermentation production alongside these critical inputs could improve efficiency. This might apply to a food-processing plant, a winery, a timber mill or co-location of bioreactors beside existing dairy capacity. Companies already operating such businesses will have substantial advantage and influence over the quality and cost of co-product inputs to the fermentation. There would be additional long-term benefits for waste management.

There are challenges, however. Some biomass waste streams are already siphoned off, such as feed for ruminants or energy generation. Diverting to future fermentation would need to be more attractive than the current model and continue to be desirable even if other high value uses for the material were discovered (e.g., bioactives extracted from grape marc). As a waste product, the composition of the material may not be a priority for the parent industry, so specifications for its subsequent fermentation would need to be flexible and resilient.

If the feedstock is not a waste- or side-stream, then it must be grown somewhere. Quantity of production is not the only metric of desirability. For example, if a plant produces particularly useful proteins or sugars or other substates, then it may be more profitable to ferment than one that is more efficient on tonnage per acre. Before repurposing agricultural capacity towards a new and large-scale fermentation industry, various constraints and alternative costs of feedstocks should be considered:

- Land utilisation
  Will biomass substitute for or commandeer traditional land uses? Can it utilise low-demand land?
- Social impact
  Will current farms and communities be displaced? Do we have the necessary new farming skills?
- Infrastructure
  Can the biomass be collected, moved and stored efficiently? What needs to be built? What might be repurposed?
- Environment, sustainability, climate, wastes
  Is shifting to biomass production an improvement over status quo?

5.2 Strain development

Microbial cells have long been the favourite model for production of recombinant proteins due to their relative ease of genetic accessibility, less complex cell physiology and ability to perform in up-scaled vat-type factory systems. Within bacteria, a major differentiation exists between Gram-negative and Gram-positive cells. The Gram-negative strains, such as *Escherichia coli*, are very well understood from a physiological and genetic perspective and serve as easy and amenable models for genetic engineering. However, their endogenous endotoxins such as antigenic lipopolysaccharide (LPS) are a serious human health concern that may limit their use in commercial fermentation to the production of feed additives (19).

Gram-positive cells, which lack endotoxins, are a phylogenetically diverse group that includes lactic acid bacteria and are generally recognised as safe (GRAS), with uses already as probiotics or food fermentation strains. The genetics of these strains do tend to be less labile, making (stable) changes to their genetic blueprint more challenging. Over the last two decades, extensive research on the GRAS microbe *Lactococcus lactis* has yielded a collection of genetic tools and recombination technologies (20). Today, L. lactis is a major food fermentation strain with significant economic value (21). It is heavily used in the food-grade biotechnological production of important molecules, such as nisin (22), flavouring additives, sweeteners compounds, etc. (Refer Table 6).

Table 6. Lactococcus lactis can be an efficient cell factory, as shown by examples of industrial enzymes and compounds produced from various strains (reproduced from (20).

Industrial type and products	Applications/functions	***Lactococcus lactis*** strain
Compounds		
Lactic acid	Preservative, flavouring, polylactic acid, plastic, emulsifier, moisturiser	All strains
Acetoin/diacetyl	Flavouring	CRL264
l-alanine	Sweetener	AlaDH+LDH–
Linalool	Flavouring	NZ9000
Germacrene D	Antimicrobial, insecticidal, pheromones	NZ9000
Hyaluronic acid	Cosmetics, medical	NZ9020
Vitamins		
Folate (B11)	Health supplements	NZ9000
Riboflavin (B12)	Health supplements	NZ9000
Biofuels		
Ethanol	Energy source	CS4435
Peptides		
Bacteriocin	Anti-microbial, preservative	NZ9000
Brazzein	Sweetener	Not specified
Mabinlin II	Sweetener	Not specified
Nisin Z	Food preservative	F44
Enzymes		
ß-Cyclodextrin glucanotransferase	Starch degradation	NZ9000
Coumarate CoA ligase (4CL)	Metabolic engineering	FI9974
Alcohol acyltransferase (SAAT)	Metabolic engineering	NZ9000
Linalool/nerolidol synthase (FaNES)	Metabolic engineering	NZ9000
Bile salt hydrolase (BSH)	Intestinal metabolism, probiotics	NZ3900
Acid urease	Urea hydrolysis	N/S

N/S not specified

The use of microbial cell factories to generate protein drugs and enzymes, high-value metabolites and food ingredients, as well as SCP biomass, usually requires genetic engineering and results in GMOs. Whether the whole cells or just their purified products (e.g., recombinant proteins) are used in the respective applications determines their regulatory status. For example, although GMOs are prohibited in New Zealand, at least 70 recombinant food ingredients, manufactured by GMOs overseas, are approved for commercial use and human consumption in New Zealand (personal communication MPI).

Depending on the application, eukaryotic microbes (typically budding fungi like yeasts and to a lesser extent microalgae) or prokaryotic bacteria might be the preferred factory vehicle. A crucial determinant is the requirement for post-translational modifications such as glycosylation. These organisms have distinct differences in their form and degree of glycosylation. The downstream functionality and digestibility of a fermented protein may depend on the correct modification patterns. Another aspect to consider is potential allergenicity of recombinant proteins (23), but this can be assessed using well established immunological methods such as RAST or ELISA.

Filamentous fungi have not played a major role in the production of food ingredients. Lately however, *Trichoderma reesei* has been recognised for its secretory and post-translational abilities (24). The high-yield expression and correct modification patterns remain a challenge, in particular when trying to minimise the physiological stress exerted on the production host by strong gene promoters (25).

In contrast, budding fungi are ubiquitous as recombinant cell factories. More than 6,000 scientific articles have been published since 2000 on *Pichia pastoris* alone. *P. pastoris* has been the subject of extensive genetic manipulation and metabolic pathway engineering with the aim to increase secretion efficiency (26) making it an ideal model organism for the development of recombinant food proteins and ingredients. Another important budding yeast for biotechnological applications is *Saccharomyces cerevisiae* (>86,000 scientific publications since 2000). Genetic engineering efforts have, for example, resulted in strains with increased mannoprotein secretion, which is used in the wine industry to reduce wine hazing (27). Other applications include synthesis of patatin lipase, chymosin B and preprogaline B proteases for use in milk processing or conversion of lactose to ethanol in cheese whey permeate.

A key aspect to consider is optimising the yield of strains when grown in reactor vessels in economically and environmentally viable growth media. Such fermentations can be static, batch or continuous, depending on the compounds produced and whether these can be secreted into the reactor vessel. Emerging concepts such as consortium-based fermentation strategies and multi-step fermentations will be an exciting area to follow over the coming years and present a notable area of capability development.

Several excellent reviews have been published recently, that provide an overview of the field, albeit through the lens of specific applications such a biofuel (28), collagen expression and modification (29), economically viable growth media (30), optically pure lactic acid (31) and terpenoid production in yeasts (32).

Genetic engineering

GE involves inserting new genes or modifying or removing genes. The aim is to change the phenotype of an organism in a way that is beneficial for a given application. Several methods are available (see Appendix II).

Single gene insertion is the most amenable modification, often introducing a new ability by redirecting the host organism's metabolic flux towards a new protein, enzyme or metabolite. This is particularly effective with xenobiotic compounds that are not natively part of the phenotypic makeup of the host and therefore are unlikely to interfere with its overall metabolism. The energetic strain of production remains a consideration. In a similar manner, the targeted modification of a single gene might serve to alter enzyme reaction kinetics or the substrate specificity. Examples for such genetic modification of strains include nisin peptide production (33) and caseins.

Recombinant casein production is a fascinating area and already widely commercialised. Early expression was achieved in bacterial hosts such as *E. coli* (34) with relevant literature going back to at least the 1980s. Other recombinant caseins, such as the antimicrobial polymeric protein beta casein-E 50-52 that has promise for enhancing safety in preservative-free foods, have been made by expression in prokaryotic hosts (35). Most commercial casein products rely on recombinant expression in the more challenging model of eukaryotic hosts, such as wheat germ for recombinant human casein protein.

Metabolic pathway engineering is much more complex. Aspects include modelling metabolic fluxes and predicting their changes, genomic stability of production hosts, enzyme engineering, substrate conversion efficiencies, etc. (36). Several recent review articles cover in *silico* modelling (37), design of prokaryotic cell factories (38) and yeast expression systems (39).

Non-GMO strain improvement

Random mutagenesis is not GE, although similar outcomes can sometimes be achieved. Mutagenesis employs physical or chemical mutators that affect the host cell DNA molecule either directly or facilitate errors in DNA repair mechanisms (40). The resulting variants are not considered GMO and can be directly used in the manufacture of foods and ingredients (41). Non-GMO methods are an inefficient way to change the phenotype of an organism, as the random nature of DNA modification does not allow for targeted adjustments of the metabolic network, nor the introduction of truly new capability (i.e., not already present in the host's genetics).

This non-GMO approach underpins the Accelerated Microbial Evolution Technology. Following mutagenesis, we use customised, high throughput assays to characterise the phenotypic traits of tens of thousands of variant bacteria. Their improved functionalities are then applied to meat and milk fermentation. Refer to MBIE Endeavour programme 2017-2022 at www.agresearch.co.nz/fermented-foods/.

The new Accelerated Microbial Evolution Technology is the only capability in New Zealand and Australia to advance microbial functional trait selection. The successfully evolved strains are being fast-tracked to commercial applications, in partnership with our industries.

There is a potential to apply this technology to select and modify microorganisms for fermenting plant materials, with a caveat that the functional traits that can be delivered by industrialised bacteria are not well developed to metabolise plant lignin-cellulose-based structures. Some rumen bacteria contribute to the breakdown of recalcitrant hemicellulose, but protozoa and fungi are better suited to degrading those materials. This presents both challenges and opportunities for science and technology innovation.

5.3 Bioprocess design and manufacture

The booming industry of microbe-produced animal proteins and other ingredients suitable for food applications draws heavily on the first wave of technology pioneered for food enzymes. Continuous innovations allow more precise genetic selection techniques that increase protein yield and tailor protein modifications and structural conformations.

The old and new industries have processing steps in common, such as growing the organism to large numbers in bioreactor tanks to express specific metabolites (protein for example) or to create cell biomass as the product itself. The degree of downstream separation, purification and concentration will depend on the intended end use. These process stages and techniques already exist, but the challenge for a transformational fermentation pipeline is to do them at much larger scale with purity and safety.

Process flow

Figure 6 shows a generalised process flow diagram representative of a variety of fermentation systems. Wrapped around this are environmental and regulatory control envelopes to maintain the safety and integrity of the microorganisms and of the products of fermentation (application dependent).

Critical steps include maintaining a stock pure culture and being able to grow that aseptically to sufficient cell density to inoculate the fermenter vessel. The degree of asepsis required depends on the source and stability of the cellular culture. This will also influence the preparation of feedstock, any additional nutrient requirements, and the heat treatment requirements to sterilise the feed and the fermentation vessel.

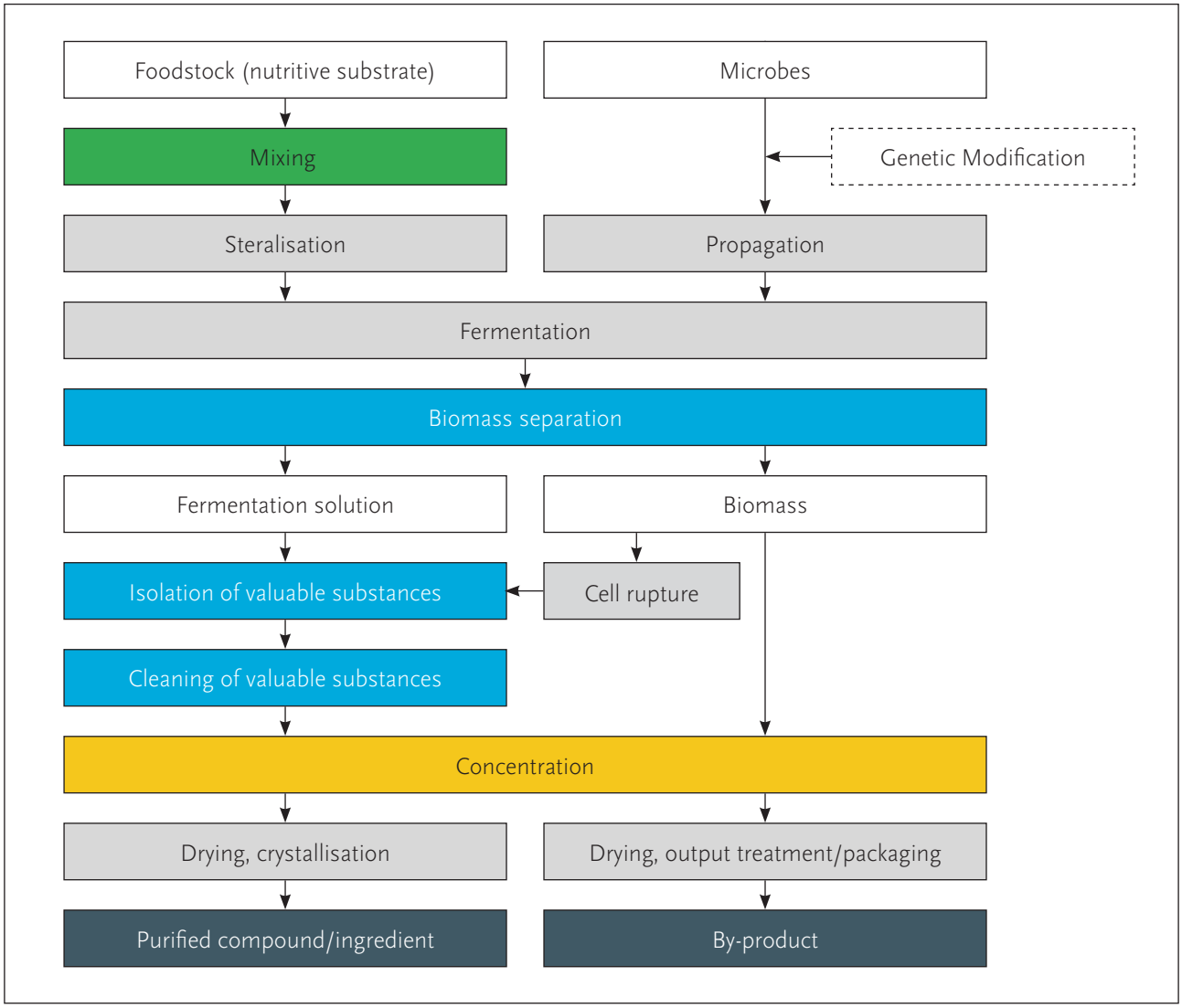


Figure 6. Generic operations flow diagram of a fermentation process with possible adjacent technologies (adapted from (42)).

Following fermentation, the biomass is separated from the media. If the biomass is the intended product, it is usually concentrated and dried. If the valued product(s) is a component of only the fermentation solution (i.e., it has been secreted by the cells), it is separated, purified and concentrated then dried or otherwise stabilised. If the valued components are internal to the cell, the cells must first be lysed. This is common when large protein molecules are being expressed in GE microbes, as they are more likely to accumulate within the host rather than be secreted into the culture media. It can be technically challenging and expensive to eliminate the cellular debris. With GE microbes, some additional quality control checks are made for strain DNA, mycotoxins and pathogens such as salmonella.

In most cases the extraction and concentration steps are membrane processes such as ultrafiltration and nanofiltration. Specific proteins could require some form of chromatographic separation.

The most common fermentation system for food applications is a batch reactor. The organisms and nutrients are held in the vessel and not removed until a certain reaction time has elapsed. An alternative, continuous fermentation, is used for brewing beer (e.g., Morton Coutts, Figure 1) but is rare for other applications. Batch reactors can be aerated, whereby filtered air or oxygen is sparged-in to maintain an aerobic state while exhaust gases are removed. Small additions of nutrients or precursors can be made over time. The pH and temperature may be controlled to maintain a certain phase of cell growth and biological reactions. Specially designed stirrers provide mixing.

Hurdles

Fermentation is the oldest biotechnology, but up-scaling its application to produce alternative proteins will initially be disincentivised by the low cost of established products. This is particularly relevant when the purpose is to produce structural and storage proteins (e.g., bulk protein intended for formulation in animal-free foods)

where the inclusion level is high and needs to be cheap. Over time this will change by targeting the required protein (or other metabolite) functionality required right at the outset and then applying an array of existing process optimising options at each step. Mass production of the major food proteins such as milk caseins and whey is expected to become affordable with technology improvements (43), as has been modelled on bacterial production of whey a-lactalbumin (44).

The choice of microorganism and its genetic improvement will always be crucial for optimising the yield and purity, as well as the post-translation modifications that are important to achieving desirable techno-functional properties. New bioprocess designs are necessary for new strains being used by fermentation-derived alternative protein products to achieve the taste and texture needed to function as true analogues of animal products.

6 Challenges and Risks

A 2020 survey of the New Zealand biotechnology sector identified access to capital as by far the most significant constraint on research and commercialisation activities, followed by the current state of GMO regulations. Access to experienced staff and access to research data are also considered significant constraints (46). These challenges would be similar for the uptake of fermentation technologies, particularly where novel production of large or complex food molecules is involved and for microbial-based precision fermentation with GMO strains.

Another hurdle for the emerging fermentation sector is scaling up production. Innovation by start-up companies is only a first step and while some of the existing processing technologies and facilities can be potentially adapted or re-purposed, new fit-for-purpose production systems will need to be designed and built.

A corollary to the how and where issues of large-scale fermentation is the question of who. Who will own and control the means of production – the massive stainless-steel capital, the distribution rights and networks? The history of global food manufacturing trends towards consolidation, with a few mega players (Nestlé, General Mills, Cargill, DuPont, etc.) buying up and becoming responsible for vast production. This seems a likely fate for fermentation methods and other alternative food resources. Within New Zealand, will the sector have the scale, clout and points-of-differentiation to participate in new markets under these terms?

Another consideration is whether a new fermentation industry will create 'reputational risk' for existing New Zealand industries. As discussed earlier, GMO use could affect public sentiment and the confidence of consumers who are buying our current exports. The new sector will not have the long history of food safety regulation and rigorous enforcement that our overseas markets take for granted. The country has a valuable pedigree of clean and green and producing the highest quality products from the best ingredients. This has fared well for competitive advantage but may not be realised for technology-derived products unless something unique (e.g., our indigenous flora and fauna) is being included. Alternative proteins produced in bulk as a food ingredient (sometimes referred to as 'nutritional sand') will have no country-of-origin provenance or a differentiated New Zealand-ness to help offset their cost of transport overseas.

Substantial investment is pouring into fermentation ventures, as discussed in 'Emerging Landscape'. It is important to not equate that with the immediate potential of fermentation. Much of the money is speculation driven by the new companies' estimates about market size, breakthrough methods and imminent returns. No doubt there is optimism and hyperbole in play. An example of the difficulty in translating promises to outcomes is the nascent cultured meat 'industry'. With each new investment call and IPO comes assurance that meaningful yields are just a few years away, yet the businesses repeatedly miss product launch deadlines. For more than 50 such declarations, the predicted date of a product debut has been compared back to the date of its prediction (47). Thus far little in the way of scalable production has eventuated.

These challenges, which may deter entrepreneurs and early adopters, mean there is an even greater need for Government support of early-stage R&D that will underpin science and technology innovation, commercial application development and changes in policy to establish a new era of bioproducts. (Refer to discussion of the FFstep platform)

6.1 Environmental impact and Life Cycle Assessment

Global growth of alternative proteins using non-animal sources and fermentation technologies is partly a response to issues of climate change, environment sustainability and animal welfare. Manufacturers are responding to those consumers who are wary of the environmental impact of conventional foods and want to make a conscientious choice. But merely being animal-free does not guarantee that a new food will have good environmental credentials.

Life cycle assessment (LCA), when done rigorously, can demonstrate the holistic and systemic impacts of products and processes. Two LCA describing fungi fermentation of biomass and whey protein have been released recently, with the caveat that they are sponsored by manufacturers.

Nature's Fynd claims that their production of Fy proteins uses 99% less land and 87% less water and emits 99% less greenhouse gases than conventional beef production (presumably referring to Northern hemisphere intensive management). Perfect Day claims that their whey protein production reduces blue water consumption by at least 96% and up to 99%, and non-renewable energy use by at least 29% and up to 60%, compared to dairying (41). This assessment extended a previous analysis showing that Perfect Day's version of whey protein could be produced with up to 97% less greenhouse gas emissions.

> A 2022 article in Nature journal reported that "substituting 20% of per-capita ruminant meat consumption with fermentation-derived microbial proteins globally by 2050 (on a protein basis) offsets future increases in global pasture area, cutting annual deforestation and related CO2 emissions roughly in half, while also lowering methane emissions" (1).

An independent LCA was conducted by the University of Helsinki to compare ovalbumin produced using engineered *Trichoderma reesei* culture with an equivalent functional unit of dried chicken egg white protein produced in Finland, Germany and Poland (45). The study showed that the fermentation production reduced most agriculture-associated impacts, such as global warming and land use. The areas of increased effect were related to industrial inputs, such as electricity use and glucose consumption. Switching to low-carbon energy sources could be essential for large scale fermentation to maintain and reduce its environmental footprint.

Given the diversity of fermentation scenarios, further LCA are required to corroborate these benefits, particularly under New Zealand conditions and compared to our efficient traditional farm management systems. Nevertheless, early assessments suggest that fermentation technology can offer an environmentally friendly food production alternative, albeit capital and processing intensive.

6.2 Safety and regulatory

The safety risks associated with evolved or engineered microbes are low, particularly when the microbes themselves are removed from the end-use ingredients or are inactivated through processing (e.g., heat). Whether GMO or not, single-cell proteins sourced from bacteria, fungi and microalgae can have other specific food safety hazards that include toxins, allergens and high ribonucleic acid (RNA) content (48).

Feedstocks may introduce allergens, anti-nutrients and thermally induced carcinogens that are different to the traditional protein or food sources. Monitoring for biological and chemical hazards in the feedstock and managing these during fermentation (e.g., mitigation or suppression) needs to be part of the choice of microbes and fermentation design.

There are hurdles to using genetic modification for food systems in New Zealand. The use of GE technologies and GMO are currently restricted by the 1996 Hazardous Substances and New Organisms (HSNO) Act. Recently, the New Zealand Productivity Commission 2021 identified that modern technological advances are not adequately accommodated and recommended a full review of GE regulation to ensure it is fit for purpose. The 2021 Proposal P1055 by FSANZ (Food Standards Australia New Zealand) is considering whether to revise the Food Standards Code and expand the opportunities for precision fermentation.

Internationally, new microbes may need to comply with the US Food, Drug and Cosmetic Act, including possible assessment for generally recognised as safe (GRAS) status. New Zealand and Australia have recognised and are using the term GRAS under the umbrella of (food) additives and flavouring. This is stipulated in the current FSANZ code. A proposed revision of the Code seeks to modernise this legislation by expanding GRAS designation to include microbes, while acknowledging that GRAS status does not equate to legislative approval for use in food. Further concerns are highlighted in the proposal where it is recognised that the pathways to GRAS status have been abused in the USA and elsewhere. Careful implementation planning is required in New Zealand before GRAS approval is granted (49). Proteins or ingredients produced using microbes (fungi, bacteria, yeast) as a biomass and then re-structured to mimic traditional dairy or meat products are likely to be regulated as novel foods in Australasia under FSANZ regulations.

Motif FoodWorks, The EVERY Company, and Nature's Fynd all received a 'no questions' letter from the U.S. Food and Drug Administration (USFDA), implying that their new products are GRAS. These include a haem protein and a soluble egg protein produced by yeast, and a fungi-derived protein (3). In similar approvals, two oligosaccharides found in human milk, 2'-fucosyllactose (2-FL) and lacto-N-neotetraose (LNnT) produced by microbial fermentation, have been certified by FSANZ for addition to infant formula.

<u>Consumer acceptance of GMO</u>

The topics of GE and GMO can be a polarising. Yet malnutrition still rings in as one of the leading factors for poor health and death across the world. The promise of these technologies to expand and diversify the means of food production is swaying the public, manufacturers and governments to rethink old attitudes.

A recent study in the UK revealed substantial consumer acceptance of dairy products derived from precision fermentation, seeing 78% of consumers as probably or definitely likely to try such a product, with 70% probably or definitely likely to buy, substantially higher than previous research has found for cultivated meat products (50).

A 2022 Spanish study (51) found that public resistance to GMO crops rests on two main factors: the belief that GMOs are ineffective, and emotional concerns. The study also suggests that scarce data about the long-term effects of consuming GMO crops, paired with the potential of toxicity, allergies, and gene transfer, is making some people hesitant about their safety.

Given the decades of success with GE microbes as a tool to produce enzymes for manufacturing and various compounds for medicines, their extension into food proteins and ingredients might be a manageable leap for consumers. Policy makers will need emphasise the potential benefits of achieving better environmental, sustainability, and health nutrition outcomes compared to traditional animal farming systems.

6.3 Māori perspectives and cultural considerations

Much GM work for plants and crops has already been carried out on iwi land, so the concepts are familiar. This is not tantamount to acceptance. Since 1989, Māori have been putting forward submissions regarding the intellectual property laws and the consistent theme is that until the Wai262 Native Flora and Fauna Claim is resolved there should be a halt to all decision making. What has been established is that any patenting that comes from the use of indigenous organisms is an infringement of the kaitiaki rights given by the Treaty of Waitangi.

The Waitangi Tribunal report into Wai262 released in 2011 identified the 'burden of colonisation'. The current laws allow commercialisation of indigenous organisms and there is no protection against the use of Māori mātauranga without consent or acknowledgement. To respect the Treaty, we need to ensure that iwi and hapū have authority over taonga and guarantee tino rangatiratanga. This means we need to be equal partners, support rongoā and traditional knowledge. We must acknowledge the concerns voiced by Māori about the "cultural and spiritual concerns with the alteration of life forms" (52).

There are considerations needed in the Māori approach towards technology for food production including tikanga, especially if the products are of significance to Māori. The growing of a natural resource using new technologies to improve the product will require an understanding of how the resource may have cultural significance to Māori and how it will benefit Māori. Māori also are concerned with how we feed our own people first before feeding the world.

> "Genes are a part of the whakapapa relationship as animal or plant life. For Māori, a gene has mauri that continues to exist ex-situ (when taken from its original place). The same perspective is carried over to issues of replication, trans-genetic engineering and cloning. Hence to alter the genes or genetic material is to alter the blood of ancestors, altering the whakapapa relationship by changing or introducing 'new blood.'"
>
> From "Māori and the patenting of life form inventions: An information paper produced by the Patenting of Life Forms Focus Group for the Ministry of Commerce", Ministry of Economic Development, February 1999.

The technology used to help Māori advance food production needs to acknowledge the differences between commercial versus whanau whenua. This means that what may be considered commercial practice may not be accepted by Māori when it does not give effect to whanau whenua. Protection of New Zealand resources and benefit sharing for all needs to be considered at the outset of discovering Māori cultural products and indigenous plants for commercial gain. We may also need to consider how we incorporate maramataka (for the daily food-associated activities like planting, harvesting, and fishing) within the food technologies adopted. Because Māori interests are aligned with principles of kaitiakitanga reciprocity (tau utuutu between humans and the environment), the natural resource should also enjoy benefits (for instance in terms of sustainability) (41). Through this, the resource then cares for the people.

7 Positioning New Zealand Science and Innovation

The non-GMO Accelerated Microbial Evolution Technology from the MBIE Endeavour Research is a considerable advancement. This puts the programme's collaborators in a strong position to exert science thought leadership and facilitate the innovation and development of future fermentation technologies. Their aim is to help drive diversification that preserves New Zealand's reputation as a high-quality food production nation.

Government science policy agencies, funding resources and researchers from other Crown Research Institutes and Universities, Māori groups, the national Food Innovation Network (NZFIN) and industries (traditional + upcoming SMEs) need to work together to achieve this vision.

7.1 Future Fermentation Science and Technology Enabling Platform (FFstep)

A new, enduring national Platform (Figure 7) would enable, support and promote microbial strain development. It could pool and coordinate resources around a full suite of tools, establish and maintain culture collections, state-of-the-art microbial improvement and fermentation performance testing, 'omics analytics resources, and lab-to-pilot scale prototyping feasibility assessment. The Platform would facilitate creating and retaining fundamental science knowledge and capability within New Zealand. It would also advocate for infrastructure that can be readily accessed by the existing industry and newcomers who see the opportunities of future fermentation technologies to produce new ingredients and foods in a sustainable manner.

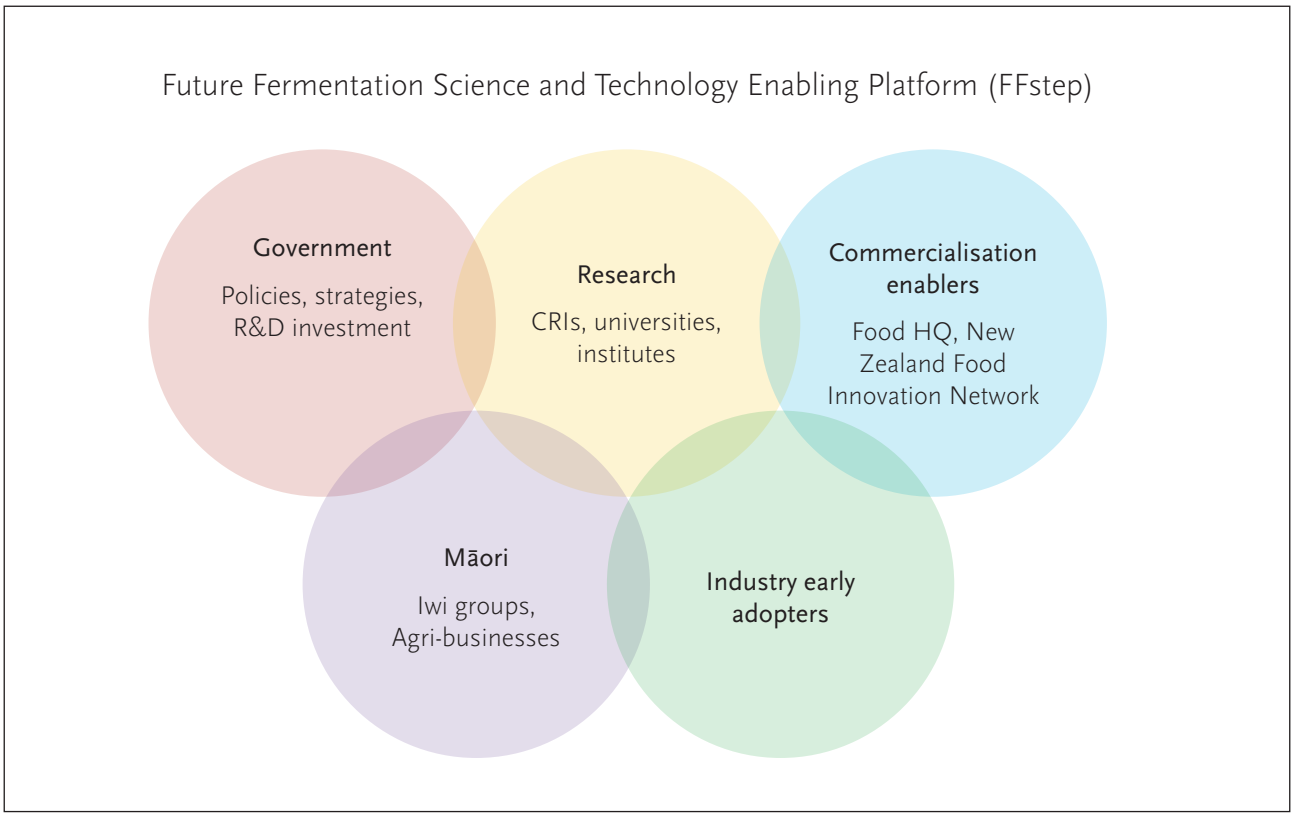


Figure 7. FFstep is envisioned as a national platform supporting research and industry collaboration.

Many of the required capabilities and resources already exist and are distributed across research agencies, often well-aligned with the country's agricultural production systems. **FFstep** is about leading the development of cutting-edge science and building the connections to accelerate commercialisation. There would be advantages for fermentation businesses in having a consistent interface to access science providers.

National partnership platforms and centres can help coordinate and advance the interests of industry, government, Māori and research organisations. Several already exist, including the New Zealand Agricultural Greenhouse Gas Research Centre (NZAGGRC, nzagrc.org.nz) and the New Zealand Food Safety Science and Research Centre (NZFSSRC, nzfssrc.org.nz). While these organisations have headquarters, they are primarily virtual. Their effectiveness derives from the strengths of the participants. They play prominent roles in support of New Zealand industries and national policy making. Over time they have become internationally recognised voices and standard-bearers.

Several science disciplines are key to advancing new fermentation technologies and would underpin **FFstep**.

1. Microbial biotechnology

This includes microbiome expertise encompassing soil, forage, animal, food (including food safety) and human domains. The Platform would:

- Continue to promote and extend the non-GMO Accelerated Evolution Technology, as the national unique capability, develop new microbes and demonstrate their applications
- Through co-design of applications, partner with Māori businesses to discover and develop unique microbes from the New Zealand eco-environment and their potential to benefit land, environment and people
- Develop new functional microbes by leveraging knowledge in ruminant microbiomes; and expertise in forage; endophytes (fungi/bacteria interaction etc.)
- Build New Zealand expertise in the microbes other than bacteria (e.g., fungi; yeast; microalgae, etc.) through collaboration with other researchers in New Zealand (see 'Collaboration')
- Develop and apply metabolic engineering approaches to deliver better technological solutions for bio catalysis and microbial cell factory development, and to ensure we are technologically ready if GMO technologies are being adopted in New Zealand.

2. Food processing, quality and safety

Food science and engineering are the foundation of getting new foods with the taste and quality that meet consumers' need. New Zealand's food science community has a long history of working with traditional dairy and meat sectors, where they provide expertise in bio-processing design, risk assessment and mitigation to achieve functional properties in ingredients and foods. This knowledge is readily applicable to fermentation. Areas for extension include prototype manufacturing processes for microbial-based new products, coupled with tailoring techno-functional properties of protein, lipid, and carbohydrate ingredients for food applications.

3. Chemistry, molecular biochemistry and 'omics

Proteomics and metabolomics deliver a broad range of specialised measurements to the industry. Those results are becoming ever more useful as part of complex and dynamic 'big data' organised through the emerging disciplines of Systems Biology and E-Research platforms, bioinformatics and artificial intelligence. These will provide knowledge into metabolic tailoring of microbes. Such insights will help to identify desirable phenotype targets that can be produced precisely and at speed.

Institutes and universities have valuable databases, established from years of research on traditional animal-based foods, that relate to identification and generation of flavour compounds, taste, and health benefits. These can also serve for developing microbes and bio-processing design of fermentation-derived ingredients and ultimately the formulation of new products with differentiated taste and health benefits.

4. Life Cycle Assessment

New Zealand has internationally recognised expertise in LCA and has conducted many projects for industry sectors on the environmental performance of land-based agrifood systems. This is an important consideration for microbial-based technologies because of interest in them being "better for the environment". However only a few independent analyses having been published so far, and a better understanding of the potential implications on the environment is required. Assessment of ingredients and foods produced by an energy-intensive future fermentation industry will provide sound evidence and support to New Zealand-led initiatives.

7.2 R&D infrastructure

Although New Zealand has some infrastructure in scaling up microbial production for commercial manufacturing of traditional fermented foods (yoghurt, cheese, beer, etc.), this is used by a few commercial players and is currently at full capacity.

Additional infrastructure has been established across research organisations and as part of the MBIE Endeavour programme:

- Robotic Culture Colony Picker and OmniLog high-throughput screening systems for microbe strain development, which is currently the only fit-for-purpose high-tech facility in Australasia
- A small culture collection of evolved GRAS strains
- Technical and regulatory processes at Callaghan Innovation for growing meaningful quantities of novel strains
- Protocols to produce edible food prototypes at 1-10 kilogram scale at the NZFIN FoodPilot and Te Ohu Rangahau Kai pilot plants in Palmerston North for quality assessment and sensory studies
- A Physical Containment level 2 (PC2) facility within Te Ohu that can be fitted-out for trialling scaled-up fermentation processing, downstream biomass separation, purification and product fractionation.

Currently there is no sizeable capacity within the NZFIN network that can be easily used for developing new microbial-derived products at a small commercial scale. BioSouth Ltd in Lincoln facilitates contract manufacturing and is investing in the fermentation area. It has recently purchased five 250 litre bioreactors, one of which is to be installed at NZFIN FoodSouth in Christchurch. Some of the existing equipment (e.g., pilot membrane separation plant) can be repurposed to advance the future fermentation technologies.

New investment will be needed to increase the scope and speed of science and innovation. Some of the capacity will require on-going commitment beyond the typical duration of competitive research grants. For instance, a biobank storage facility for indigenous, evolved and modified microorganisms. This would act to conserve and replenish stocks of unique or valuable microbes, have a logistic system for cataloguing, data management and access, and have a distribution mechanism to supply research and product development. Coordinating or co-locating with other ad hoc microbe biobanks across New Zealand could maximise efficiency.

7.3 Collaboration

National

Active relationships are already established among AgResearch, Callaghan Innovation, Massey University, the Riddet Institute, sector-leading businesses, start-ups and Māori-owned enterprises. These are a pivotal part of **FFstep** and are necessary to ensure the successful development and adoption of new science and technologies.

The MBIE Endeavour programme was concerned only with bacteria, so new collaboration are needed for expertise in fungi, mycelium, yeast, microalgae etc. to advance the selection of microbes for further fermentation applications that of interest to New Zealand.

There is a strong willingness in the research communities and industries to work together. There is also a keen awareness in industry of rising international demand for alternatives to animal-derived foods and ingredients.

The establishment of **FFstep** will facilitate collaboration among government agencies (MPI and MBIE, MfE), research communities (CRIs, Universities and other Research Institutes), and industry enablers (Food HQ, NZFIN) to ensure that businesses are supported to innovative successfully and take their concepts through to marketable products for the benefit of New Zealand.

International

Fermentation is being touted as the next industrial revolution for the agrifood industry and its potential is stimulating changes to government policies worldwide. Investors are attracted to science and technologies that are commercially competitive (see Appendix I, where the majority of the patent applications are filed by start-up companies). **FFstep** participants will need to continue their international collaborations and strategically select new ones. This will ensure that New Zealand retains knowledge, IP and benefits and at the same time raises its reputation at the international level.

Building on the science and technology achieved through MBIE Endeavour investment, a national Future Fermentation Science and Technology Enabling Platform (FFstep) would be the next step forward in facilitating R&D consortia and collaborations. It will provide training and upskilling for the New Zealand workforce, benefit the national bioeconomy strategy for bio-based innovation, and lead as an enabler as part of the government Food and Beverage Industry Transformation Plan (ITP).

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Appendix I. A preliminary analysis of the intellectual property patent landscape

This work was conducted by Mr David Koedyk, Senior Associate at Catalyst Intellectual Property. The patent search was carried out in two parts. The first part focused on the companies listed in the Good Food Institute 2020 State of the Industry report, and the second part was based on the key words search strategy developed with the project team. The list of the patents was then screened to remove those irrelevant to the subject of this white paper.

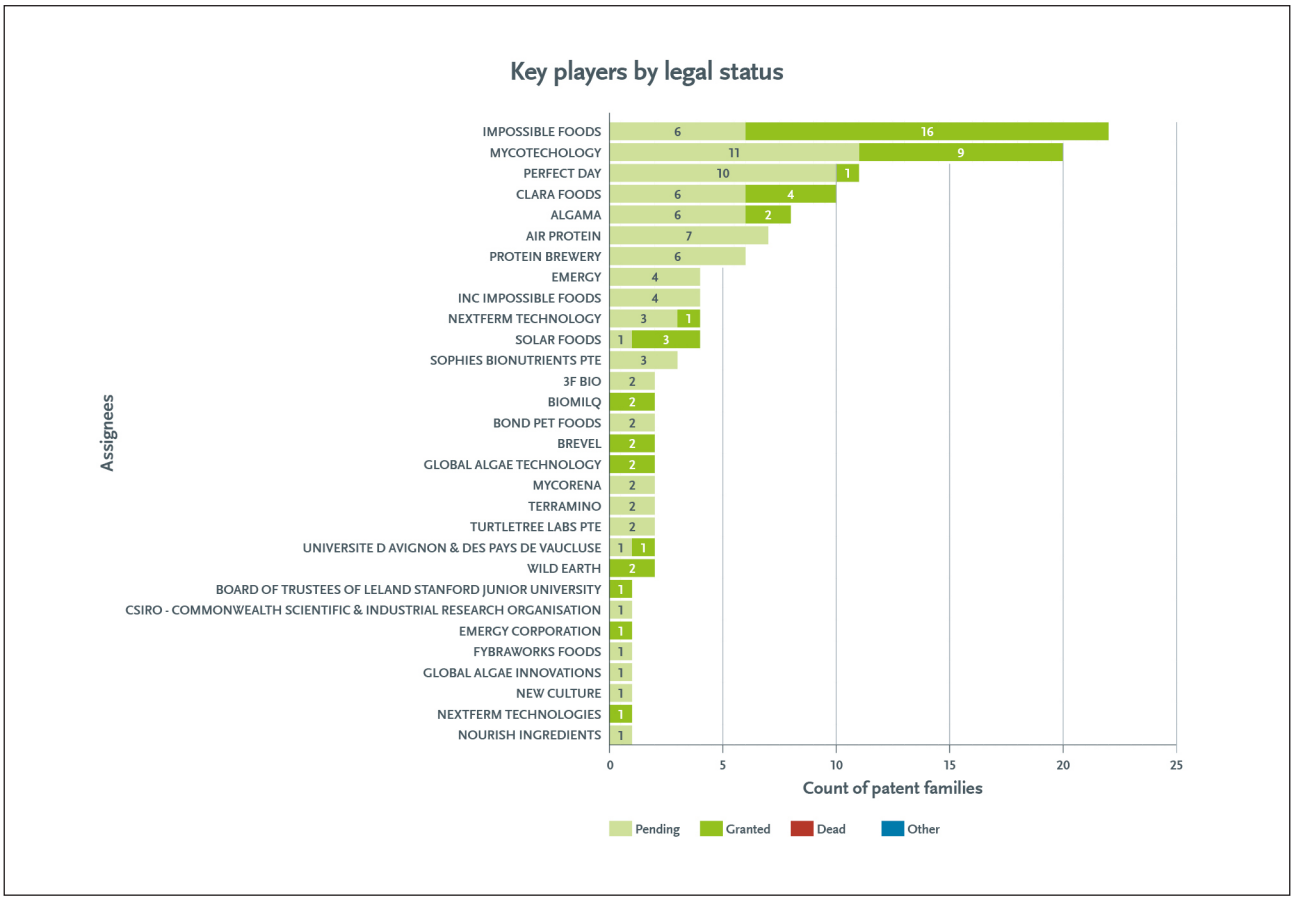

Figure I.1. Patents filed by companies who are actively engaged in the use of fermentation for non-animal food and ingredient production. The graph illustrates the top applicants in the group of patents analysed according to their legal status. All the patents, except 1, were filed since 2012, with Food Chemistry and Biotechnology being the two most active technology domains.

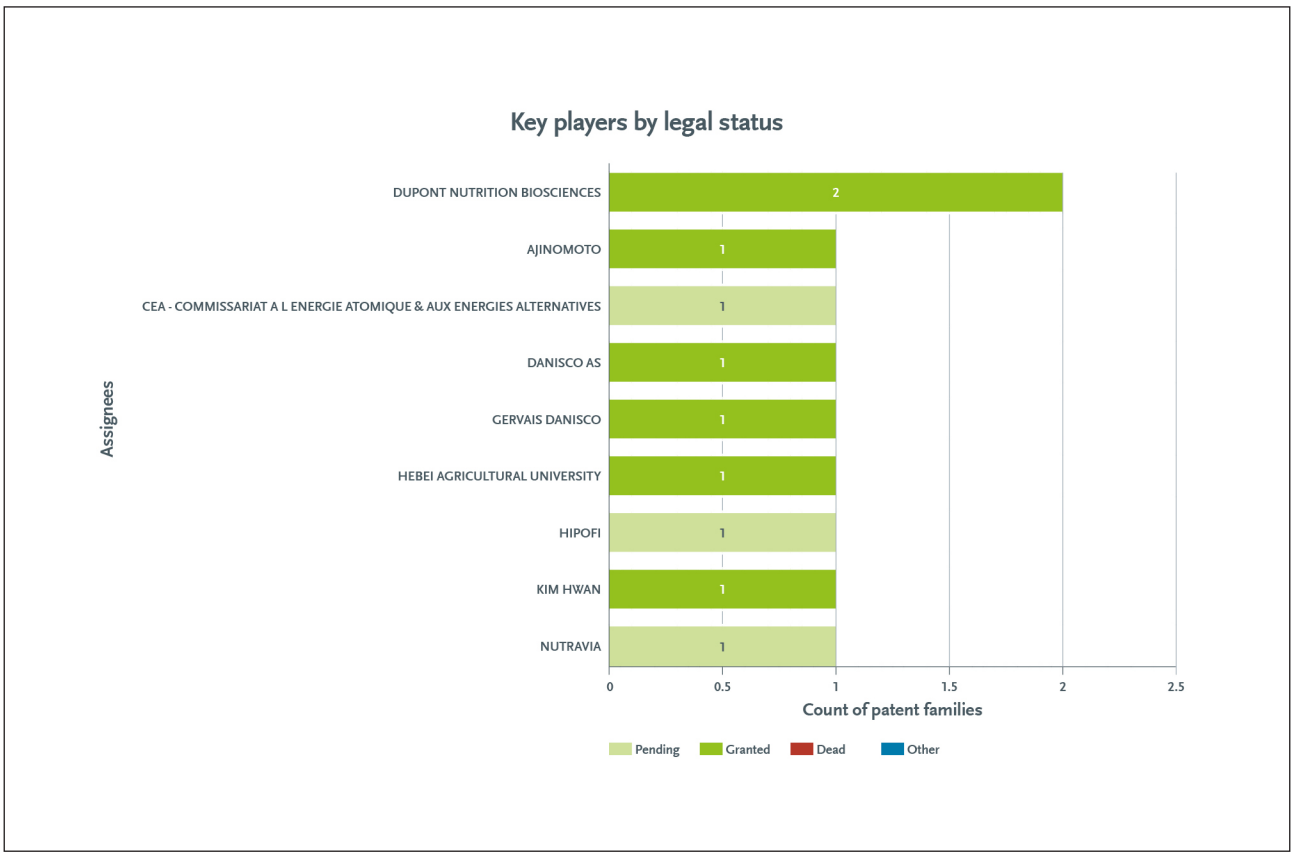

Figure I.2. Patents filed by organisations who are developing non-GMO microbial improvement technologies for food fermentation applications, between 2002 – 2020. Patents fall in the Food Chemistry and/or Biotechnology technology domains.

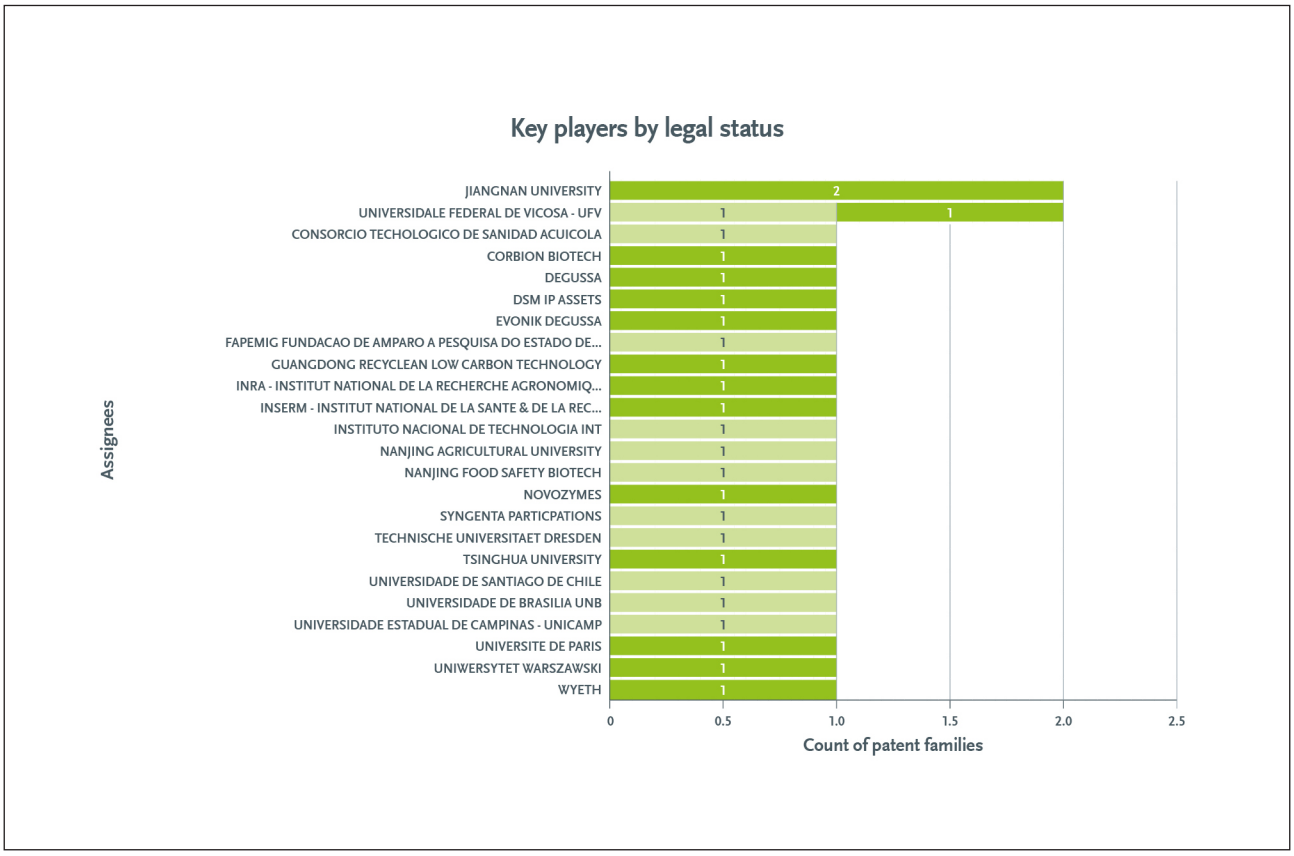

Figure I.3. Patents filed by organisations who are developing GMO microbial improvement technologies for food fermentation applications, between 2003 – 2020, with Biotechnology being the main technology domain, followed by Pharmaceuticals and Food Chemistry. Interestingly several universities are active in filling patents, in addition to DSM, Novozymes, Wyeth, etc.

Protoplast fusion

Protoplast fusion is not considered a GMO technology and is therefore applicable for the creation of new food of food ingredient production strains. Like Random Mutagenesis (RM), protoplast fusion mediates the random alteration of DNA. In contrast to RM, protoplast fusion enables the large-scale exchange of genetic information between two donor cells, albeit still in a random fashion. In protoplast fusion, the cell wall of both parent cells is removed, and the resulting protoplast are then merged into a single cell, resulting in a hybrid cell with two chromosomes. Recombination events then randomly create hybrid chromosomes harbouring elements of both parent cells. Respective unique phenotypes of each parent strain can be found combined in the recombinant cell (Figure II.1).

Both methods, RM and protoplast fusion, require the use of high-throughput robotics and screening capabilities to identify the desired new phenotype from a pool of thousands of random genetic candidates.

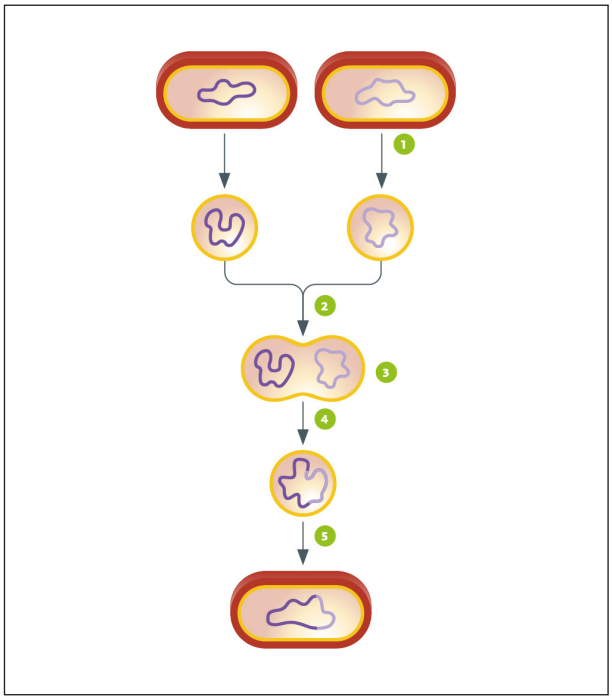

Figure II.1. Protoplast fusion. From Pearson Education, 2016

Transduction, lysogenic conversion

Another natural method of genetic modification is via bacteriophage, viruses that exclusively infect bacteria. However, due to New Zealand-specific legislation, phage is not recognised as part of the natural biome in New Zealand and resulting variant strains are considered GMO. Phage can feature either lytic or lysogenic lifestyles and transduction is associated with a lysogenic lifestyle, where the phage genome integrates into the host chromosome. During a transduction event, genetic elements from the host chromosome are packaged into the phage capsid and upon infecting a new host cell, this new genetic material then becomes part of the chromosome upon phage integration. Infrequently, these events lead to incomplete phage, resulting in a more stable integration event and permanent new genotype. A schematic overview of transduction mechanisms is shown in Figure II.2. Some phage have acquired a permanent new genetic region named the 'lysogenic conversion module' which carries bacterial (host) genetic elements as a permanent part of the phage genome without impairing the viability of the entire phage. Genetic modification of these modules (e.g., replacement of gene sets) leads to the transfer and (stable) integration of new genes into the host cell, however the modified phage is considered GMO as is the resulting transduced cell.

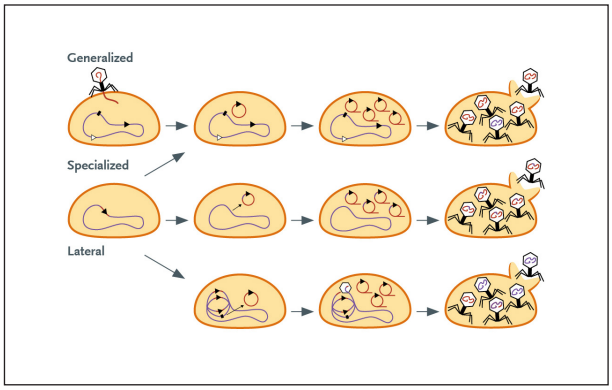

Figure II.2. Mechanisms of genetic transduction. Generalized (top), specialised (middle), and lateral transduction (bottom). The viral genome (in red) first undergoes theta replication, followed by rolling circle replication. In lateral transduction, theta replication occurs prior to prophage excision. Phage terminase initiates DNA packaging from phage pac sites (black triangles) or pseudo-pac sites (grey triangles) (56).

Transfection

In general terms, the introduction of naked DNA into a cell is called 'transfection.' This term is mostly applied when eukaryotic cells are manipulated. In case of prokaryotic cells, the same process is called 'transformation.'

The uptake of naked DNA into a cell can occur through physical (e.g., electroporation) or chemical (e.g., calcium phosphate) methods. Descriptions of the detailed methodologies can be easily found in relevant reference works. The introduction of naked DNA into any cell will result in all cases in the creation of GMOs. However, and in contrast to the methods described above, transfection enables the precise manipulation of the genetic blueprint of a cell by selectively introducing individual nucleotide changes (SNPs), the deletion of genes or the introduction of individual genes or gene operons. This allows the intelligent re-direction, termination and creation of metabolic pathways and novel enzymatic reaction.

Naked DNA carrying a single gene, or a gene set (payload genes), can be designed in different ways. The most common method to transfer DNA in a stable manner is via plasmids. Plasmids are autonomous, self-replicating DNA molecules and are either circular or linear. Plasmids carry their own DNA replication machinery, a selection marker, cryptic genes and payload genes. The advantage of plasmids is their copy number within a cell, ranging from low, to medium to high copy number plasmids that can reach up to 300 copies. This enables very high gene expression levels beyond the promoter effect (57). However, this creates a physiological and energetic strain on the host cell and without a strong selection marker, plasmids will be lost readily from a cell population. Spontaneous mutations resistant to the selection pressure also enable the loss of plasmids, creating an increasingly large subpopulation within the culture that outperforms the production strain and leads to yield loss. In food grade production systems, plasmid selection markers must also be food grade, eliminating the widespread use of antibiotic resistance genes. Instead, resistance to antimicrobial secondary compounds such as nisin or the provision of essential metabolic genes is employed to maintain plasmids (58).

Plasmids can also be used to create stable chromosomal integration of payload genes through a process called 'homologous recombination' (59). In many cases, chromosomal integration is the most desirable method to create stable genetically modified organisms that are also commercially scalable. However, chromosomal integration features several caveats, whereby the most obvious one is the loss of copy-number effects that allow high expression levels in plasmid-based systems. Some efforts have been made in the past to overcome these limitations, including promoter optimisation (60) and multiple integration of a target gene or operon (61).

A recent gene editing technology is CRISPR-Cas (clustered regularly interspaced short palindromic repeats). CRISPR structures were discovered and described in the late 1990 and early 2000s (62), but their function remained unknown until two landmark discoveries described their ability to selectively modify genomic DNA, which was awarded with the 2020 Nobel Prize. Although CRISPR-based applications are the most widely known genome editing technologies, over recent years, a range of alternative and complementary gene editing technologies has emerged (see Figure II.3).

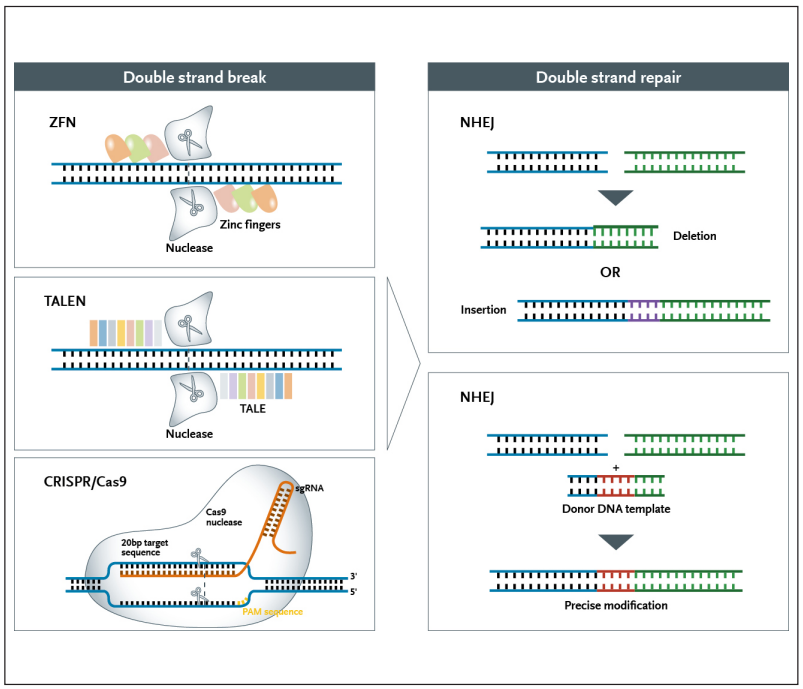

Figure II.3. Gene editing tools. Genome editing platforms and mechanisms for DSB repair with endogenous DNA. Genome editing nucleases (ZFNs, TALENs and CRISPR/Cas9) induce DSBs at targeted sites. DSBs can be repaired by NHEJ or, in the presence of donor template, by HDR. Gene disruption by targeting the locus with NHEJ leads to the formation of indels. When two DSBs target both sides of a pathogenic amplification or insertion, a therapeutic deletion of the intervening sequences can be created, leading to NHEJ gene correction. In the presence of a donor-corrected HDR template, HDR gene correction or gene addition induces a DSB at the desired locus. DSB double-stranded break, ZFN zinc-finger nuclease, TALEN transcription activator-like effector nuclease, CRISPR/Cas9 clustered regularly interspaced short palindromic repeat associated 9 nuclease, NHEJ nonhomologous end-joining, HDR homology-directed repair (63).

While CRISPR-based gene editing has seen a wide and rapid uptake in eukaryotic models, their use in prokaryotes is far less common, due to a few limiting factors. However, emerging research is beginning to develop tools and protocols to enable CRISPR-based editing in microbes, thereby enabling scarless modification of the genetic blueprint (64) (Figure II.4). From a legislative perspective, CRISPR-based technologies (and other, similar tools) will make it impossible in the future to distinguish between naturally evolved microbes and those that were genetically engineered. This will have a profound impact on how New Zealand and the world will interact with and consume GMOs.

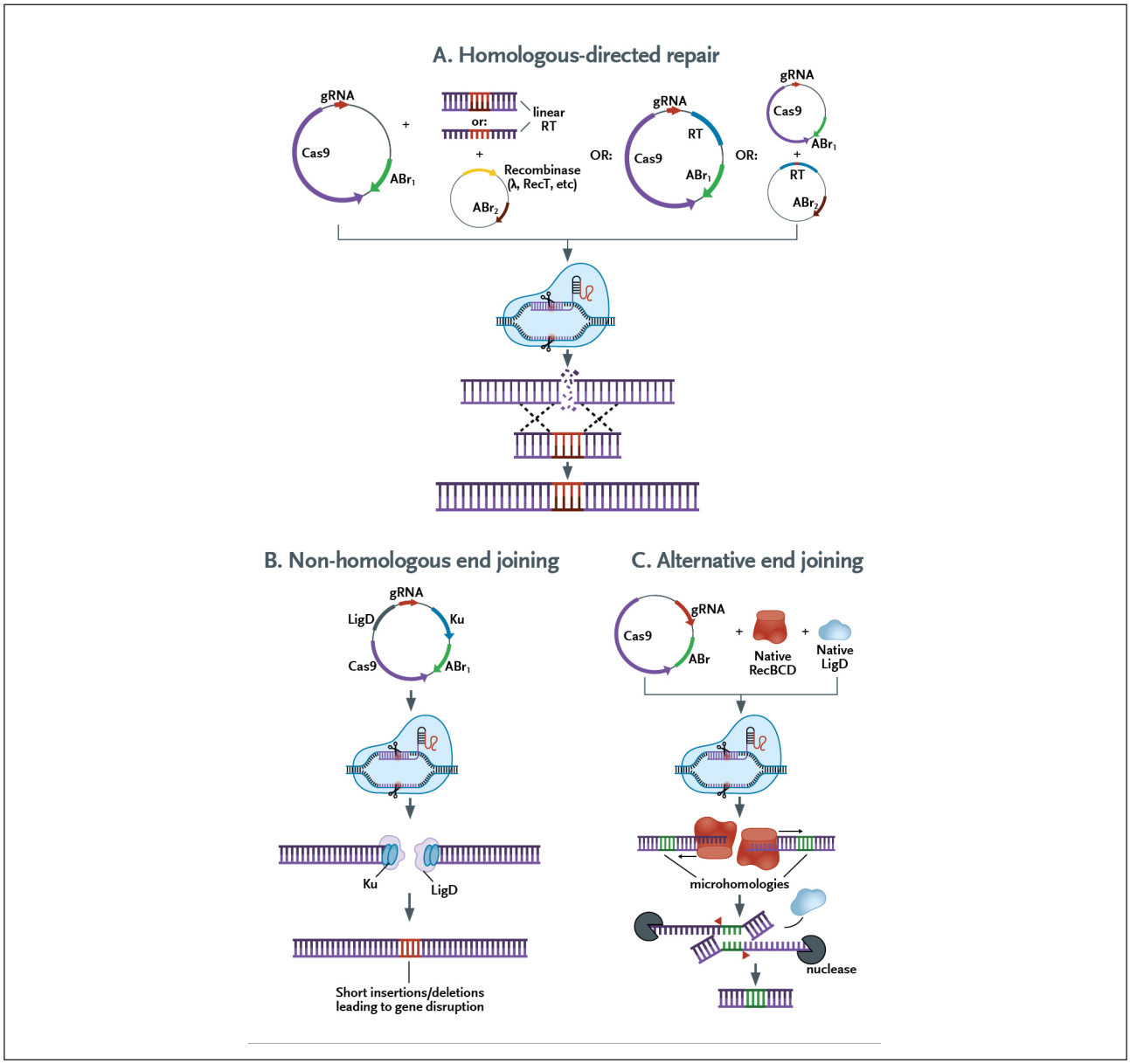

Figure II.4. Strategies used for CRISPR-Cas based genome editing in bacteria. (A) Editing via homologous recombination: Recombineering with a linear DNA template is followed by counterselection with CRISPR nucleases. A heterologous recombinase (e.g., λ red, RecT) is introduced via a plasmid (or phage) into the cell and co-transformed with the linear DNA template and CRISPR-nuclease plasmid with respective antibiotic-resistance marker (ABr). Genome editing may also be directed with a plasmid-encoded recombination template (RT) and endogenous or heterologous recombinase. The recombination template can be placed on the same plasmid encoding the CRISPR machinery for an all-in-one plasmid system, or it can be placed on a separate plasmid before transforming the CRISPR nuclease/gRNA plasmid. One-plasmid system is more streamlined, but due to its larger size it can be hard to transform, and cloning may not be possible if the gRNA can target the genome of the cloning strain. (B) Editing via the non-homologous end-joining (NHEJ) pathway. Depending on the strain, Ku and/or LigD can be encoded on the CRISPR nuclease/gRNA plasmid and transformed into the strain. (C) Alternative end joining (A-EJ) pathway can be found natively in many bacterial species with incomplete NHEJ. It does not require the introduction of foreign Ku or LigD, and instead relies in microhomology-directed repair via RecBCD, nucleases, and LigA, leading to deletions of variable sizes (depending on the location of microhomologies) at the Cas9 cut site. All strategies require plasmid curing after nuclease targeting to isolate the mutant strain, to avoid interference in pursuing downstream applications (64).

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