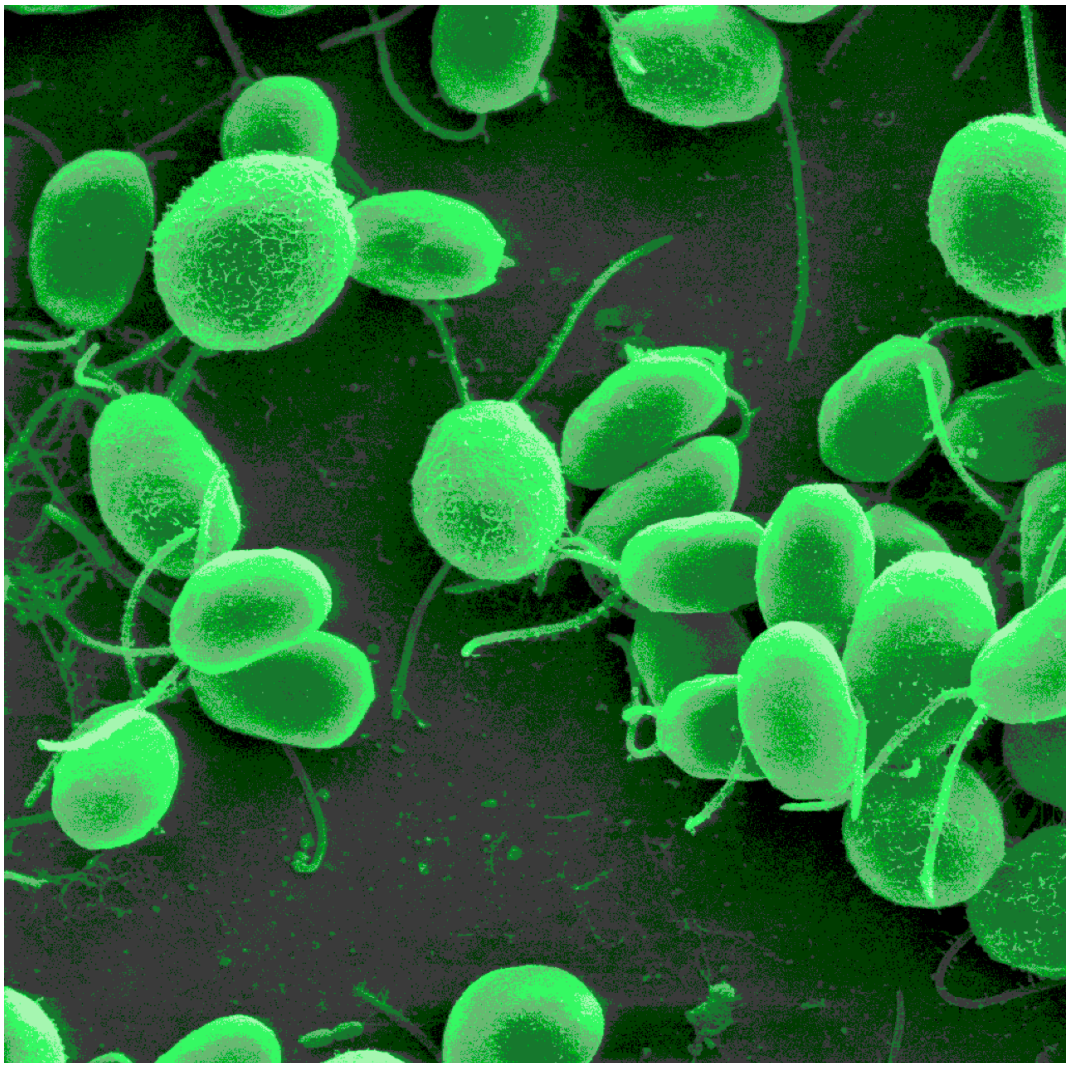
GM/GE Microorganisms:

a new global environmental disaster in the making?



GM/GE Microorganisms: a new global environmental disaster in the making?

A report by GeneWatch UK

November 2024

Cover photo: Scanning electron microscope image, showing an example of green algae (*Chlorophyta*). Adapted from: https://commons.wikimedia.org/wiki/File:Chlamydomonas6-1.jpg

GeneWatch UK
53, Milton Road, Cambridge, CB4 1XA, UK
Phone: +44 (0)330 0010507
Email: mail@genewatch.org Website: www.genewatch.org
Registered in England and Wales Company Number 03556885

Table of Contents

Executive summary

This report describes the use of genetic engineering techniques (including genome editing techniques) to create genetically modified (GM) microorganisms, including bacteria, viruses and microscopic algae and fungi. Microorganisms are ubiquitous in the environment, and many evolve in close proximity to humans, animals and plants: for example, in the gut and skin microbiomes of humans, pets, livestock and wild animals; and in plant roots and soil.

Contrary to established norms, the deliberate release of living genetically modified microorganisms, which can survive and reproduce in the environment, has recently begun, driven by commercial interests and new technological developments. Existing products are limited and do not appear to deliver on their claims, and future products, likewise, are at an early stage of development and will face many technical and other challenges. Despite much hype, there is every reason to be very sceptical of claimed future benefits. Nevertheless, GM bacteria, viruses, microalgae and fungi are already being genetically engineered for open release, with proposed applications in a wide variety of environments (e.g., in soil, freshwater and marine environments).

Large-scale releases of GM micro-organisms into the environment could take place, even if future products do not deliver on the claimed benefits. Most of the examples discussed in this report involve living GM organisms (GMOs), which can reproduce and spread in the environment, surviving for multiple generations (perhaps indefinitely). This risks creating a form of 'living pollution' that cannot be contained, controlled, or recalled if anything goes wrong. In some cases (such as the idea of 'self-spreading vaccines'), widespread dispersal is intentional.

Although only a tiny fraction of the multiple species of microbes that exist have been genetically modified with the intention of open release, they already represent species that inhabit a wide range of habitats. These include several species of marine microalgae; bacteria that inhabit soils and freshwater habitats; fungi and bacteria that infect plants and animals, including many species of insects; and viruses that infect humans and animals. These GM microorganisms can be spread through a variety of mechanisms, such as sewage, insects, dust storms and rain, and interact with the communities of microbes in human and animal guts and on skin. Uncontrolled spread of GM microorganisms could therefore pollute all ecosystems: rivers, lakes, oceans, farmland, forests, grasslands, gardens, parks and nature reserves. Allowing open releases of GM microorganisms into the environment risks permanently (and negatively) altering these complex ecosystems.

It is impossible to predict the consequences of such releases as GM microorganisms interact and evolve with their environment, spreading new genetic constructs into other organisms. Within the human gut, for example, the introduction of new genetic variants can alter metabolism, the breakdown of drugs, and resistance against pathogens. Novel genetic constructs are easily transferred from one microbe to another and can spread unwanted traits, such as antibiotic resistance. A particular concern is the potential creation of novel pathogens as microbes evolve.

The need for a precautionary approach is enshrined in global environmental treaties such as the Cartagena Protocol on Biosafety to the UN Biological Convention on Biodiversity (CBD), and the Rio Declaration. This means that where there is a threat of serious or irreversible damage, lack of scientific certainty about the impacts shall not be used as a reason for postponing measures to prevent environmental degradation. This leads to the conclusion that GM microorganisms (including gene edited microorganisms) should not be deliberately

released into the environment, due to the inability to predict and/or manage future adverse effects on human and animal health and the environment.

In addition, 'contained use' applications of GM microorganisms (including gene edited microorganisms) should be properly contained and this requires more scrutiny as more potential applications are developed on a larger scale.

1. Introduction

Microorganisms (also called 'microbes') are microscopic organisms that include bacteria, protozoa, some algae and fungi (known as microalgae and microfungi) and viruses (despite not being considered living organisms). They live almost everywhere in our planet, from land to marine and freshwater ecosystems, as well as in complex communities inside and outside plants and animals, including humans. Across environments they play pivotal roles in ecosystem functions, as well as in the health and functioning of their diverse hosts. Microorganisms have also been long harnessed by people across cultures for conducting other important functions, such as fermentation of foods, and more recently, industrialised processes such as bioremediation and production of chemicals and medicines.

Today, they are increasingly becoming targets of the genetic modification and 'synthetic biology' community, spawning the latest era of promises to address all manner of environmental and even health challenges within the spheres of crop and livestock agriculture, climate and conservation, as well as human health and 'lifestyle' products, such as probiotics, which target the communities of micro-organisms (known as a 'microbiome') living in the human gut. Headlines such as "Can microbes save the planet" in Nature in 2023 ('Can Microbes Save the Planet?', 2023), regarding the Audacious Project's 'Engineering of the microbiome with CRISPR to Improve our Climate and Health', exemplify the efforts to market this shift or expansion in interest from genetically modified organism (GMO) developers as the latest GMO revolution, in some cases using new genetic engineering methods (of which CRISPR is one example).

Genetic modification is the genetic engineering of living organisms to alter their DNA in a laboratory, resulting in the creation of a genetically modified organism (GMO). New genetic engineering techniques such as genome editing tools (including CRISPR), that are used to perform 'targeted' modifications of genomes, are increasingly used by developers. The increasing complexity and range of genetic modifications, or changes, that new techniques are being tasked to perform are often now described as 'synthetic biology', though GM and synthetic biology are both classes of genetic engineering approaches (often overlapping) used to develop GMOs.

The development of genetically modified (GM) microorganisms is commonly framed as being part of a sustainable solution, e.g., as 'nature-based', 'climate-friendly' or as part of 'regenerative agriculture', and for viruses such as those aimed at 'vaccinating' wildlife populations, as an integrated approach to addressing the connections between the environment and human diseases. 'Biologicals', or 'bio-inputs', that may include either GM or non-GM microorganisms, are increasingly being sold as a "farm of the future" package, alongside other corporate products under a corporate reframing of regenerative farming, to include products such as GMOs, digital technologies and carbon and/or nitrogen markets.

However, fundamental questions remain regarding the ability of GM microbes to progress beyond the limited utility of GMO crops to date, in addressing societal and environmental ills.

This report provides a summary of state of play with regard to GM microorganisms (including microalgae), defined here to include genome edited (GE) organisms, in line with the

definition of a GMO under the Cartagena Protocol of the UN Biological Convention on Biodiversity (CBD), and regulations in the vast majority of countries in the world. As detailed below, there are a wide range of actors, both commercial and governmental, involved in GM microbe applications, though how successful this latest GM push will be remains highly questionable. Less questionable, is that the development of GM microorganisms for environmental release poses fundamental challenges to the ability to assess risk, with basic biological characteristics such as rapid evolutionary potential, multi-generational transfer of genetic material, and self-spreading nature, raising huge degrees of uncertainty. Environmental releases of GM microorganisms thus also challenge the principle of precaution, a principle or approach at the heart of both international conventions such as the UN Convention for Biological Diversity (CBD) and other national and regional regulatory bodies, including the EU. Any prospect of large-scale microorganism product releases brings the potential for GM microorganisms to exist and spread to all spheres of our environment, in rain, in sewage, and in soils. This could irreversibly pollute all ecosystems, e.g., rivers, lakes, oceans, farmland, grasslands, gardens, parks and nature reserves. As with 'forever chemicals' (Box A), GM microbes may result in a form of genetic pollution that will be impossible to remove. Such products, risks and implications are detailed further below.

Box A: 'Forever chemicals' as an example of irreversible environmental damage

PFAS (Per- and polyfluorinated alkyl substances), also known as 'Forever Chemicals', are a large chemical family of over 10,000 highly persistent chemicals that don't occur in nature. PFAS are the most persistent synthetic chemicals to date, they hardly degrade in the natural environment and have been found in the blood and breastmilk of people and wildlife all around the world (ChemTrust., n.d.). PFAS are used in fast-food packaging, non-stick pans, and some textiles, cosmetics and electronics (e.g., smartphones).

In 2024, the New York Times published an investigation of PFAS spread onto U.S. farmland via contaminated sewage, used as fertilizer (Tabouchi, 2024). Contamination of sewage with PFAS can occur via industrial wastewater and perhaps also household sewage. Although the extent of the problem is not fully known, fertilizer sludge is thought to have been used on about a fifth of all U.S. farmland (nearly 70 million acres), as well as for landscaping, golf courses and forest land. Concentrations of PFAS in sewage sludge are not regulated but high concentrations have been linked to cancer, birth defects and other health risks. The U.S. Environmental Protection Agency (EPA) has only recently begun to take action, by slashing the level of PFAS allowed in drinking water to near zero and stating that there is no safe level of PFAS in humans. However, some farmers allege that PFAS in sewage sludge have killed their animals, and are suing the company that provided the sludge, and the EPA.

In the UK, the Environment Agency has described the cost of dealing with PFAS problem sites as 'frightening' (Salvidge & Hosea, 2024). According to the Agency, there could be more than 10,000 locations in England contaminated with PFAS, but, so far, they are only taking action at four sites. Millions of pounds of estimated costs to date cover only the costs of investigations, not the costs of cleaning-up the contaminated sites.

'Forever chemicals' are only the latest example of extensive environmental damage and huge costs, caused by ignoring early warning signs of potential harm to the environment and human health. Other examples include allowing lead as an additive in petrol and asbestos in buildings (EEA, 2013).

2. A shift towards microbes

To date, the genetically modified (GM) crop industry has failed in its long held promises to address agricultural challenges, evidenced in the plateauing of GM crop adoption rates and

the failure to produce novel, useful traits that meet the promises of BigAg (ISAAA, 2018). Until now, the vast majority of GM crops remain herbicide-tolerant crops, and/or those designed to kill certain pests (Bt crops), over three decades since they were commercialised. Countries that have widely adopted GM crops have often experienced adverse economic, ecological and social impacts as a result of GMO adoption (GeneWatch UK, 2022; Sirinathsinghji, 2022), with smallholder farmers bearing a particular brunt of the failures and limitations, including the development of resistant pests and weeds. Extractive financial systems that promote economic policies tying countries into GMO production systems, e.g., to increase foreign exchange from exports (CCJ et al., 2024), however, remain powerful forces that prop up a limited, and increasingly failing, scientific and agricultural paradigm.

As the GMO industry is forced to address the declining utility of its products, a dearth in novel crop or animal traits ready for market remains a problem. This may be, at least in part, an underlying factor behind an increased focus on novel microbial products. In recent years, BigAg giants have been buying up biologicals companies, consolidating their market share in the field. Microbes, also included within the more market-friendly term 'biologicals', are being claimed by developers to be projected to make up an increasing proportion of crop protection revenues (FoE, 2023), spurred on by enthusiasm for the purported potential of genome editing to increase the development of useful products. A few applications are already making their way to market or the fields, including a GM (genome edited) soil microbe developed by PivotBio in the US (see Section 3.1.1 *Biofertilisers*). Another factor behind a shift towards microbes is the increasing ease with which databases can be generated to identify and sequence microbial species, useful traits, facilitating huge data mining projects that also raise important questions regarding biopiracy (see Section 5), and the incentives to profit from the selling on of database information.

The genetic engineering of microbes is not new, but represents a conceptual, business and technical shift in the approach to agricultural technologies and beyond, with in an increasing focus being added to a broader range of potential applications, such as carbon-capture and environmental clean-up. The rising popularity of new genetic engineering tools such as genome editing also appears to be encouraging this effort (see Box B). Indeed, the first experiments of genetic engineering to ever be performed in the laboratory involved the engineering of modified microorganisms, with the first GMO being a bacterial species that was developed in 1973 (Cohen et al., 1973). Commercial production of insulin was introduced in the early 1980's in contained use facilities. However, releasing live GM microorganisms into the wild has been largely considered too controversial. With regard to self-replicating GM viruses, only one field trial release in Spain in 2000 has been performed but nothing has been released since (see Lentzos et al., 2022a).

Box B: Genetic engineering methods

Genetic engineering, or genetic modification (GM), involves altering DNA inside the cells of an organism, to change the genetic make-up of the organism (its genome), creating a genetically modified organism (GMO). Creating a GMO includes multiple steps, beginning with choosing the DNA to insert or remove, in the hope of achieving the desired effect. There are a variety of ways of inserting new DNA into a host genome, including using methods derived from bacteria or viruses to carry the DNA into the cell. Early GM techniques often involved transferring DNA from other organisms (known as 'transgenes') – this is called transgenesis. New DNA is often inserted randomly into the target organism using these techniques. More recently, artificially synthesised DNA has also been inserted into GMOs.

Genome editing techniques (also known as gene editing) are a newer set of genetic engineering techniques, which use chemicals called enzymes to cut the DNA of the target organism in a specified (targeted) location. The cell's repair mechanisms are then used to seek to make the desired genetic change. One of the most publicised genome editing

techniques is known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats).

The targeted nature of genome editing techniques is sometimes given as justification for deregulation of these new GM techniques. GM proponents regularly claim that such targeted changes, some of which do not intend to insert genetic material but instead generate smaller mutations in a desired sequence, are akin to natural mutations and thus safe.

However, accompanying the 'targeted change', if indeed it is achieved, are also well-established unintended changes that are also generated, including at the target site of interest, as well as elsewhere, in 'off-target' locations. Such limitations are well recognised in the biomedical field, with regard to their potential use to address human disease (Ledford, 2020; National Academy of Medicine (U.S.) et al., 2020), due to the potential risks associated with unintended genetic changes that could lead to illnesses such as cancers.

The documented range of unintended changes to edited organisms at the molecular level has steadily accumulated as a result of the widespread interest in these technologies, but in the sphere of environmental applications such as GMOs, risks of genome editing errors are often dismissed. Unintended changes can range from small-scale mutations to large-scale mutations, and even wholescale structural chromosomal damage/loss (e.g. Biswas et al., 2020; GeneWatch UK, 2021; Kawall, 2019; Koller & Cieslak, 2023; Kosicki et al., 2018; Norris et al., 2020; Ono et al., 2015, 2019; Papathanasiou et al., 2021; Smits et al., 2019; Tuladhar et al., 2019; Zhu et al., 2017).

Moreover, genome editing is facilitating a push for deeper genetic interventions, for example, the modification of multiple, even dozens of genes in one organism.

The conceptual shift in interest and needs is moving towards an increasing acceptance of the use of self-spreading technologies, including within wild ecosystems. Such shifts in risk acceptance were recently described with regard to microorganisms, and specifically viruses, as an 'erosion of norms', where a minority of scientists/developers ignore decades of common consensus that regarded such releases as being too controversial (Lentzos et al., 2022). Alongside other technologies such as gene drives, there is also now the increasing deployment of genetic engineering machinery itself into the wild, moving the 'lab to the field' (Simon et al., 2018) (Box C).

Box C: Using microbes as delivery agents of genetic engineering machinery 'transfers the lab to the field'

An increasing trend across GM technologies has been described as 'environmental genetic engineering' (Heinemann & Walker, 2019; Sirinathsinghji, 2019) or 'transferring the laboratory to the field' (Simon et al., 2018). This includes the development of gene drives (a method of seeking to spread genetic changes through an entire species), e.g., in insects, as well as some non-GM technologies, such as RNAi sprays (used to attempt block gene functions in pests, for example). As discussed below, GM microorganisms are also being developed for genetically modifying organisms in the wild. This trend is extremely controversial, entirely removing the ability to assess the GMO prior to release and the ability to ensure against potential unintended effects or impacts. It is effectively an open-air genetic engineering experimental approach, designed to occur in the wild. The emergence of such applications has thus become a recent focus of the UN Convention for Biological Diversity (CBD), which sets international regulations and guidelines on environmental biotechnologies due to the inherent risks associated (e.g. CBD multidisciplinary ad hoc technical expert group, 2024).

Microorganisms present a method for delivery of genetic engineering machinery such as CRISPR systems as well as a target for modification, e.g., microbiome communities in the gut of people and animals. Several strategies are being deployed for this, using plasmids (small circular pieces of DNA, usually found in bacteria), viruses or bacteria to deliver genetic engineering machinery directly to other microbes. While viruses are easily modified and are highly efficient at infecting and transforming cells, some are limited by target host range, vectors for infecting hosts, e.g., sucking insects, and the limited size of genetic material that they can carry. However, Jennifer Doudna, who won the Nobel Prize for the co-invention of the popular genome editing tool CRISPR, and is co-leader of the Audacious Project's microbiome work (see Section 3.1.3 *Feed Additives and Digestive Microbiomes in agriculture*) has published a method that bypasses the use of a microorganism to express the GM tools, instead directly delivering them to perform 'microbial community engineering' via DNA plasmids (Rubin et al., 2021).

The genetic engineering of organisms directly in the wild marks a significant departure from classic GMO applications, increasing the depth of intervention and adding yet more layers of complexity with regard to risk. It potentially exposes both target and non-target species over vast scales, for example via the use of sprays, or insect vectors (a vector is a living organism that can transmit a micro-organism elsewhere, as often happens with pathogens that cause disease). With such applications, the spread of both the CRISPR-carrying GM microbe being applied, and the spread of any resultant genetically modified organism, perhaps indefinitely, over multiple future generations, needs to be considered.

The exposure of non-target organisms to genome editing machinery in the wild has recently been predicted to have widespread unintended effects (Hoepers et al., 2024). When applied outdoors using typical delivery methods for pest and disease control applications, such as fumigation, irrigation or fertilization (e.g., direct application to soil), potential off-target effects of unintended 'editing' on various potentially exposed organisms, including humans were detected. Predicted unintended changes occurred within genetic sequences involved in development of nervous and respiratory systems and metabolic function. Key knowledge gaps such as the genomes of all potentially exposed organisms are not currently known, increasing uncertainties regarding the ability to rule out unintended effects on non-target organisms.

In the US, where most GMOs, including genome edited organisms are deregulated, genome edited microbes by Pivot Bio have already been commercialised and applied to crop fields. This commercialisation could set a precedent that raises international concerns about the unleashing of edited microbes across the globe. Bayer (formerly Monsanto), closed a deal with Gingko Bioworks in 2022 to work on their products targeting 'nitrogen optimisation', 'carbon sequestration' and 'next-generation crop protection' (Bayer, 2022). More recently, Syngenta announced a collaboration with Gingko Bioworks to 'guide rational strain engineering strategies' to develop microbial strains for producing 'biologicals' (Gingko Bioworks, n.d.).

Beyond agriculture, other industries are also heavily investing in microorganisms. Two industrial giants, Novozymes and Chr. Hansen from Denmark, recently merged to form Novonesis, describing itself as a "leading global biosolutions company", with "enzymes, functional proteins and microorganisms" being "tiny agents of change" (Novonesis, n.d.-b), and providing "a biosolution for (almost) everything". They are marketing the company as an opportunity to transform businesses through biology. The company is offering contained use applications to existing industries, with the stated aim of increasing their sustainability. Products pertain to a wide range of applications from production of bioethanol, post-combustion carbon capture from power stations, proteins for livestock farming, or enzymes for cleaning agents, textile preservation and other uses, as well as environmental release applications in the form of food products, e.g., using bacteria that prevent foods such as

sausages from rotting, creating ‘exciting new tastes’ for cheeses and other fermented dairy products, and infant formula milk. While Novonesis already sell non-GM products, the two parent companies have been working on genetically modified (GM) products for many years, with some commercialised already for contained use ethanol production applications (Novonesis, n.d.-a). In 2024, a new consortium funded by the Bill and Melinda Gates Foundation (BMGF) and the Novo Nordisk Foundation, the latter of which is a controlling shareholder in Novonesis and Novo Nordisk pharma company, formed to work on alternative protein production as a meat replacement. By working on production of acetate from carbon dioxide (CO_2), they hope to bypass the sugar requirements currently needed as feedstock for any microorganism-based production system, to ‘de-couple food production from land use’. The CEO of Novo Nordisk Foundation recently stated that their work represents the first step in a “novel bioeconomy providing a more sustainable, safe and stable food production, reducing the strain on nature's resources in multiple ways”, for the purported benefit of “low- and middle-income countries” (‘Novo Nordisk Foundation: CO_2 as a Sustainable Raw Material in Our Future Food Production’, 2023). However, the business practices of these large foundations challenge the very notion that they are driven by altruistic motives. In some cases, an alternative interpretation would be aiding the greenwashing of environmentally destructive industries, such as industrial farming. One latest controversy is that the prohibitive costs of Novo Nordisk’s medicines are pricing out patients in low- and middle-income countries, including essential insulin medicines for diabetes patients. Novo Nordisk is one of three giants that controls the insulin market, most recently challenged in September 2024 by Medicines Sans Frontiers for the harmful consequences of their approach to medical access, or lack thereof (Medicins San Fronriers, 2024). The practices of such powerful players at the forefront of promoting such technological solutions for the benefits of the global majority from which they regularly profit, warrants careful scrutiny of these latest microbial based solutions to agriculture, health and other applications.

Alongside increased interest from the private sector in deploying GM microorganisms, is an unsurprising push for relaxation of GMO regulations for microorganisms, including those considered first generation GMOs (e.g., those designed to carry foreign genetic material). It is of particular concern that living GM micro-organisms (until now, used to produce chemicals in ‘contained use’ facilities) are being considered for deliberate open release into the environment. Moreover, the deregulation of genome editing in various countries gives a green light to a number of microbial applications that may now receive little, if any, scrutiny and oversight. Current regulatory discussions within regions such as the EU, suggest that a relaxation of GMO laws governing micro-organisms is indeed potentially underway. New draft laws in the EU currently exclude certain genome edited plants (described as derived from New Genomic Techniques, NGTs) from risk assessment under the GMO legislation. However, Novonesis have their eyes on extending this exclusion of GMOs developed via new genome editing GMO techniques to microorganisms (Dal Bello et al., 2024), proclaiming that “*Microorganisms derived from NGTs have the potentials of becoming an important contribution to achieve the ambitious targets set by the European ‘Green Deal’ and ‘Farm to Fork’ policies. To encourage the development of NGT-derived microorganisms, the current EU regulatory framework should be adapted*”. The CEO of Novonesis recently described current regulation as a roadblock: "*We still experience regulation that is based on the past with fossil-based solutions and chemicals. This is delaying the more sustainable solutions of the future. In Europe, if we develop a new biological microorganism to replace a chemical fertilizer, we have to wait up to 8 years before we can sell it.*" Novozymes, one of Novonesis’s parent companies, is also a member of three prominent lobby groups working within the EU, EuropeBio, Fefana and Amfep (Info’OGM, 2024). Chr. Hansen are members of Fefana and Amfep (InfoOGM, 2024).

Despite the deregulatory push, amidst years of hype surrounding a new era of synthetic biology, spurred on by new genetic engineering tools such as genome editing CRISPR

systems becoming hugely popular amongst researchers and developers, there appear to also be financial cracks appearing within the synbio sector. Several flagship companies have faltered this year in the synbio space, including Ginkgo Bioworks (Science, 2024) (see Section 5). The company is said to be laying off significant percentages of staff, having failed to deliver on products, instead selling platforms that utilise microorganism databases for others to develop products. Such difficulties raise pertinent questions regarding whether GM microorganisms can progress beyond the limited trait development for GM crops to date, or whether they will succumb to the same reductionist paradigm of genetics that fails to address complexities that challenge efficacy and safety of GM technologies as a whole. It also raises interrelated questions regarding the drive for huge microbial databases, and what the endgame really is, drawing in additional questions and concerns regarding biopiracy and biosecurity risks that accompany such data accumulation (see Section 5). Questions therefore remain about whether all of these efforts will merely result in the commercialisation of non-GM strains, as product failures amass.

Potential spheres of applications are discussed below, bearing in mind that commercial claims and hype often fail to materialise into commercialised GM products.

3. Spheres of applications

GM microorganisms are being researched for a wide variety of applications, taking advantage of the variety of roles that microorganisms play in ecosystems and health, as well as in manufacturing applications where microbes can be used to synthesise compounds (e.g., artificial ingredients), or metabolise and break down compounds (e.g., pollutants).

The focus of this report is particularly on living GM organisms intended for deliberate release into the environment, because of the significant threats they pose to ecosystems (see Section 6). Much research is also focused on the potential contained use of genetically modified microorganisms to produce a wide range of biological and chemical substances (e.g., food ingredients, industrial and medical products) for use in the food, feed or pharmaceutical industries. Many such applications already exist: for the purposes of this report such products are relevant only to the question of whether 'contained use' (in facilities called 'bioreactors') is properly contained (see Section 6.5 Contained Use. How contained?). This will increase in importance if a wider range of products is produced on a larger scale than is the case today. In some cases, it is unclear whether products are destined for 'contained use' or open release and/or whether the product contains living micro-organisms or only dead ones.

A recent horizon-scanning study of scientific as well as grey literature (databases, regulatory authorities, websites, other reports e.g. by OECD) for potential environmental release applications of GMOs (excluding plants and insects) outside agriculture, reveals a wide array of applications under development using GM bacteria, fungi and microalgae (Miklau et al., 2024). There appears to be significant interest in microorganisms, identifying them as taking up the largest share of research and application-orientated research when compared with applications for fish and terrestrial animals (when combined with microalgae applications). Applications identified focus on bioremediation (34 in total) and biocontrol (31 in total); and for microalgae, which were assessed separately, biofuels production was the major focus identified. Biofuels could be produced in contained use, but in some cases may be aimed at future production in open ponds (see Section 3.2.3 *Biofuels*). Four microalgal species field trial applications were identified. In terms of genome editing, three environmental applications were recorded as having been approved already (in Brazil), none for algae.

Separately, another study has looked at potential applications of GM viruses (Eckerstorfer et al., 2024). This recent horizon-scanning study on GM viruses for agricultural, veterinary, and

nature-conservation purposes highlights the interest in the field (although many applications are yet to be commercialised). Plant and animal viruses have been used for a long time as genetic engineering tools, to introduce new DNA into cells. However, some new viral tools facilitate the genetic engineering of organisms outside the lab, in the open environment, which raises major new concerns (see Box C). GM vaccines may also be used in veterinary medicines. A number of new GM viral tools have been developed for use in genetic engineering applications and 3 GM vaccine agents are regarded as at or near market. Relevant publications also exist in relation to using GM viruses against plant pathogens or as biological control agents to control pests.

GM microbes may also be developed for use in medical applications, such as vaccines and therapies based on bacteria (e.g., probiotics) and bacterial viruses (called phages) (see Section 4). Some of these applications (e.g., probiotics) may be used as foods rather than as medicines, with weaker regulatory requirements (Section 3.3.1 *Probiotics*).

It is worth noting limitations in the ability to track all products that are close to market, approved or even commercialised following approvals in many cases. Approvals are not always indicative of environmental release. Scientific literature also may not capture many products that are not necessarily published prior to marketisation. Whether some applications are envisaged for open or contained use is also not clear. Moreover, for genome editing specifically, differences in regulatory frameworks that continue to emerge or remain under discussion for genome editing and other new GM techniques, makes tracking products being approved for environmental release difficult. These issues raise immediate transparency concerns with regard to public access to information and ability of independent stakeholders to assess risks and implications, let alone the lack of ability for authorities to assess potential impacts. As noted in a recent report by Friends of the Earth US (FoE, 2023), tracking what is indeed on the market in the US is extremely difficult despite efforts to research regulatory filings, without a means, for example, to perform a general search of GM microbes going through the regulatory process.

Some examples of potential products are discussed in more detail below.

3.1 Agricultural applications

3.1.1 *Biofertilisers*

Microbial life is essential to maintaining healthy soils and thus food production and wider ecosystem health, performing a whole plethora of functions from carbon and nutrient cycling; support for plant growth, stress-tolerance (e.g. to drought), defence and communication; nitrogen-fixation; and degradation of nutrients and/or pollutants.

Mainstream recognition of the importance of soil microbial biodiversity for agricultural sustainability has come, in part, from witnessing the consequences of industrialised farming systems increasing soil erosion and reducing soil biodiversity via excessive tilling, the use of fertilizers, and monocropping that destroys organic matter and increases soil erosion. Chemical pesticides are known to also kill or impact soil microorganisms and organisms. The weedkiller glyphosate, for example, has been linked to adverse impacts on soils. Nitrogen fertilizers have been shown to reduce the role of nitrogen-fixing bacteria, a key nutrient for crop/plant growth, alter soil structure and alter carbon cycling (D. Chen et al., 2015; Kidd et al., 2017; Lehmann & Kleber, 2015).

It is estimated that there are billions of microbial cells in a single gram of soil (these include bacteria, archaea, fungi, protists and their respective viruses). Archaea are another group of microorganisms that are similar to, but distinct from, bacteria. Protists are a diverse

collection of microorganisms that do not fit into animal, plant, bacteria or fungi groups. The majority of soil microorganisms are yet to be studied or understood due to various complexities including the sheer number of species and their high levels of interactions with other species, as well as technical difficulties such as inability to isolate and culture them in the laboratory (Jansson et al., 2023). Moreover, microbial communities necessarily vary depending on soil type, geography and environmental variables such as weather conditions. As summarised by Sessitsch et al., (2023), "The soil microbiome, the environmental parameters, as well as the physiology of plants all determine which microorganisms are transferred to and establish within and upon plants."

BigAg players are attempting to take advantage of their own part in contributing to declining soil health with an array of technologies that either directly, or indirectly, address the adverse impacts associated with this decline. This includes the development of GM (and non-GM) microbes for applying to soils, as well as attempts to address stagnating yields via more indirect means, such as modifying plant microbiomes to influence plant growth. Aside from any proclaimed environmental benefits of microbial products is an economic incentive to replace synthetic fertilizers. As reported recently in the UK's Financial Times, the main driver of interest in microbial fertilizers is the rising costs of synthetic fertilizers.

GM soil microbes have recently hit the markets, with PivotBio leading the way in the U.S. PivotBio is a start-up launched in 2021 born out of the University of California. Raising $430 million in venture capital funds, the company embarked on GM bacteria intended for use as a nitrogen biofertilizer. Being the first product commercialised for soil organisms, PivotBio's product raises critical questions regarding the underlying aims of the application compared the claims being made, as well as efficacy and biosafety risks and an overall lack of transparency that prevents public or independent oversight of the technology (see Box D).

Miklau et al. (2024, Supplementary Tables 21 to 24) identify two GM microbe-based biofertiliser products close to market in Brazil – one attempting to improve ammonium fixation (using the bacteria *Klebsiella variícola*, originally identified in plants, but which can also cause disease in humans and animals), and one using a subspecies of the soil bacteria *Bacillus thuringiensis* (*Bt)* for soil conditioning. They also identify three more applications at the research stage: two using the nitrogen-fixing soil bacteria *Azotobacter vinelandii* to enhance nitrogen fixation and ammonia release; and one using cyanobacteria (commonly known as 'blue-green algae', although they are not algae) of the *Anabaena* species to attempt to increase resistance against abiotic stress (stress caused by non-living factors such as drought, salinity, heat and cold). *Anabaena* are nitrogen-fixing plankton, commonly associated with aquatic ferns. A further three papers describe applications of GM microorganisms in basic research on biofertilisers, according to Miklau et al. (2024): one using the nitrogen-fixing soil bacteria *Sinorhizobium meliloti* to increase the efficiency of plant roots; one using the bacteria *Mariprofundus ferrooxydans*, found in hydrothermal vents, to seek to enable plants to make more use of iron in the soil; and one using *E. coli* bacteria (found in the human gut) to alter quorum sensing (a cell-to-cell communication system that exists widely in the microbiome).

A multiyear Bill and Melinda Gates Foundation (BMGF) funded project founded in 2012, involving a number of universities within the UK, EU and US, is also working on interactions with plants and microbes to improve nitrogen fixation. It is working on a number of methods, including investigating the use of GM microorganisms (as well as GM plants and other non-GM methods). This project was originally targeted at the African continent only, and was originally named the Engineering Nitrogen Symbiosis for Africa initiative, though it has since been recoined as the Nutrient Symbioses in Agriculture (ENSA), expanding its work to beyond Nitrogen fixation and aims now to roll out its work globally (ENSA, 2023).

Box D: Pivot Bio case study: obscuring product and performance

Pivot Bio is one of the lead flagship companies being promoted as a successful synthetic biology company that is translating the promises of microbial engineering to commercial products. The synthetic biology industry is indeed reaching crunch time in terms of producing products after years of hype regarding its ability to revolutionise various spheres of biotechnology applications to health and environment. However, how true are the claims of success for Pivot Bio?

What is PROVEN 40?

The product was developed by isolating a microbial strain, which was later genome edited to alter its nitrogen fixing properties for corn cultivation. This bacterial species naturally performs nitrogen fixing, but only when it senses that nitrogen levels are low in the soil. If nitrogen levels are high, then the bacteria turn off the process. As stated in PivotBio's papers, the *K. variicola* 137 strain was isolated from corn (maize) roots in US and edited to modify the nitrogen fixation pathway to 'de-couple' regulation from the presence or absence of exogenous nitrogen (Bloch et al., 2020). This was reportedly achieved by changing the genetic regulatory elements that control the gene expression of a gene involved in nitrogen fixation, such that it is permanently on. The genome editing involved insertion of a new promoter sequence into the bacterial strain. While described as 'non-transgenic' by developers, the product does indeed involve insertion of genetic material from a different gene within the same strain. The company's study reporting the edit, fails to describe the methods used to edit the microbe, only referencing their patent (Bloch et al., 2019).

Pivot Bio appears to be the first company to release a genome edited soil microbe into the environment globally, following a pilot launch in 2022. It has since been reportedly applied to 3 million hectares of US farmlands, with the company claiming it has saved 16 500 tons of synthetic nitrogen fertilizer across over 800 000 acres (AFN, 2023).

Does it do what it says on the tin?

However, a closer look at the data gives a less clear cut picture of 'success' or even the underlying rationale of the product. While it is sold as a means of reducing external nitrogen fertilizer use by providing an alternative source of nitrogen for crop plants, the product appears to be designed for high nitrogen soils. Indeed a recent Pivot Bio publication states that the product is designed for cereal crops in **nitrogen-rich soil** where the same strain of non-GM conventional bacteria would normally switch off nitrogen fixation. Diazotrophs are bacteria and archaea that fix atmospheric nitrogen. Their paper states that "*Free living crop-associated diazotrophs capable of providing nitrogen at agriculturally relevant levels as observed by Van Deynze et al. and Ladha et al. indicate that this nitrogen source could be developed and optimized for modern agriculture. However, any microbe identified as providing BNF for cereal crops* ***will need to be gene-edited to function in the nitrogen-rich soil conditions*** *which would normally suppress nitrogen fixation use conventional species for low N soils*" (Wen et al., 2021).

When looking at the trial data (Bloch et al., 2020), further questions are raised about the applicability and evidence of PROVEN 40 working, particularly in low nitrogen soils where it would be relevant as a product. Indeed, trials were done with standard application of the farmer inputs, such that nitrogen fertilizer was supposedly still applied. Indeed the concluding sentence of their Bloch et al., (2020) paper reveals the reality of the limitations of their product in meeting its supposed unique selling point- the transition away from synthetic fertilizer use, stating: "*Designing bacteria that fix nitrogen in the presence of exogenously*

fertilizer is a first step toward developing strains that can replace synthetic fertilizers in cereal crop production."

Moreover, clear yield data is not provided, with no information on how much yield increased, just claiming instead that 71 % and 74 % of farms across two seasons had higher yields. While field trials were conducted in three locations, yield data was only provided for one. Puerto Rico, a historical testing ground for BigAg, showed no yield improvements, while in Illinois, yield was not measured. Indeed, Pivot Bio in their paper state that "*because yield data correlated with plot location rather than fertilizer treatment, we assumed significant nitrogen mobilisation across the field and therefore averaged the yield data across plots and nitrogen treatments".*

Broader questions regarding the suitability of such an approach is also warranted, considering its intended use for industrialised commodity crop systems that regularly over apply nitrogen fertilizers. Indeed, a recent UK study showed that 77 % of nitrogen fertilizer is lost and thus applications could be significantly cut down (Rathbone & Ullah, 2023). Environmental impact assessments on the sustainability of the manufacturing process that requires water, sugar and nutrient inputs, should also be taken into consideration when assessing the overall efficacy and suitability of such products.

Biosafety risks

Despite the open release of PROVEN 40 into millions of hectares of US farmland, very little biosafety testing has been conducted on the product. It appears that there is no information on molecular characterisation for potential unintended changes that are associated with any genetic engineering process. Environmental persistence appears only to have been assessed for efficacy testing, with the company promoting persistence at 12-week post application as a positive indicator of the product working, though little data exists beyond that. Information regarding the risks of horizontal gene transfer to non-target organisms is lacking (see Section 6.2 Horizontal gene transfer). With regard to potential toxicity, data was not shared in either of their two studies, though they claim that GLP compliant studies were performed. Larger questions remain about potential disruption to nitrogen cycles, either directly, or via the unintended transfer of the trait to other non-target organisms. Environmental issues are discussed further in Section 6.

3.1.2 *Biocontrol of agricultural pests and pathogens*

The term biocontrol (short for biological control) includes the use of biological agents to control unwanted insects, weeds, or diseases. This section covers the potential use of GM microbes as biopesticides (biological agents which kill pests or pathogens), as well as a more complicated idea called paratransgenesis, which involves genetically engineering microbes that infect pests, to seek change the ability of the pest to reproduce or spread disease. This section focuses on agricultural applications, including pests and pathogens that infect plants and livestock, applications related to human diseases that do not originate in agricultural settings are considered in Section 4.

Biopesticides are living microorganisms to act as killing agent to target agricultural pests or pathogens (infectious micro-organisms that can cause diseases). The use of microorganisms is not a new practice, for example the bacterial species such as *Baccilus thuringiensis* (Bt) has been on the market for decades, while fungal agents are also on the market, though they are not as widely used in industrial farming systems.

Concerns around tighter regulation on chemical farming inputs and potential incoming bans on certain pesticide products in regions such as the EU is reportedly driving the the BigAg

industry to look for alternative products. As recently reported in the UK's Financial Times, "*Billions of dollars' worth of existing products are likely to be banned in Europe, says Corteva's Beudot, adding: "That is driving innovation*."

"*Pesticide companies are focusing more on biopesticides and shifting their investments away from chemical pesticides. The pesticide industry is about $56 billion worth, with biopesticides accounting for only $2–3 billion. On the other hand, biopesticides in the future are likely to overtake chemical pesticides. This shift is thought to be the result of the rising demand of customers for chemical-free foods and the increased legalization of synthetic pesticides in some parts of the world. Furthermore, many biopesticides are potentially less expensive to develop and commercialize*" (Financial Times, 2023).

Miklau et al. (2024, Supplementary Tables 20 to 25) identify one application close to market in Brazil: the use of GM *E. coli* bacteria to seek to suppress *salmonella* species in chickens. This application, by the company Folium Science, is discussed further below. Miklau et al. (2024) also identify 17 further GM microbe agricultural biocontrol products in application-orientated research, and a further 17 at the basic research stage. These potential applications utilise a wide range of micro-fungi and bacteria, most commonly found in soil, and which often infect pests, such as insects and locusts. A further 6 advanced research applications are described, attempting to use the freshwater algae *Chlamydomonas reinhardtii* and the fresh and marine water algae *Nannochloropsis oculata* for disease control, focused on production of vaccines and anti-virals for shrimps, and antimicrobials and antibiotics for other species. In the separate category of 'paratransgenesis', Miklau et al. (2024) identify a further application at the application-orientated research stage: attempting to use GM microbes commonly found in plants and insect guts (*Pantoea agglomerans)* for control of the glassy winged sharpshooter, a leafhopper pest that spreads harmful bacteria to grapevines. At the basic research stage, Miklau et al. (2024) find 3 papers describing attempts to use paratransgenesis for the control of aphids, nematodes and other pests, utilising GM bacteria that are known to infect these pests.

Eckerstorfer et al. (2024, Table S4) identify 7 potential applications and 36 relevant publications for biocontrol using GM viruses, including insect pests and invasive alien species. However, Eckerstorfer et al. (2024) highlight that much of this research is at the early stage and most did not in fact use GM viruses (for example, two field trials they list tested non-GM viruses). One single R&D-stage study identified describes the insertion of a toxin gene into *Chilo iridescent virus* (CIV), with the aim of controlling a wide range of pest insects, such as weevils. An approach that Eckerstorfer et al. (2024) describe as particularly elaborate involves the potential use of bacteriophages (viruses that infect and replicate within bacteria and archaea) to protect or cure plants and crops from bacterial pathogens. This research, based at Wageningen University in the Netherlands, involves the potential use of GM bacteriophages to protect olive trees and vines from a bacterial plant pathogen, *Xylella fastidiosa*. It requires the use of a GM phage-delivery bacterium (PDB), as well as a GM phage, and considerable uncertainties exist regarding the long-term stability and interactions of the GM PDB and the GM bacteriophages in natural environments.

Potential applications of bacteriophages in humans are discussed in Section 4.2 Anti-microbial treatments for human health/veterinary applications, including proposals to use them as a tool to tackle antibiotic resistance. Most of these projects do not use genetic engineering, but some research includes investigating GM phages. Concerns about antimicrobial resistance are also leading to policy changes within the sphere of agriculture that is also driving agricultural research into the use of phages. For example, the EU has recently restricted the use of antibiotics that are important for human health to be used on livestock. Innovate UK for example, as part of Agrifood Africa Connect Project, advocates for pursuing the use of phages for livestock, crops and food for the control of infections as

well as human disease transmission (PHG Foundation, n.d.), although the possible potential role of genetic engineering in some of these applications is not discussed.

Eckerstorfer et al. (2024) also identify the potential use of GM citrus viruses to protect trees against bacterial diseases (using Citrus tristeza virus, CTV; Citrus yellow vein clearing virus, CYVCV; and Citrus psorosis virus, CPsV). Most are at the basic research stage, but attempts to use a GM virus called CTV to develop GM citrus with resistance to citrus greening disease (also known as Huanglongbing, HLB), caused by bacteria, is at a later stage of R&D, and was risk-assessed by the US Department of Agriculture (USDA) for field trials in Florida in 2020. The GM virus expresses a variety of antimicrobial proteins (called defensins) in the citrus trees. Eckerstorfer et al. (2024) identify potential adverse effects that could occur due to evolutionary changes in the virus and/or its spread to other plants, as well as loss of efficacy due to the development of resistance or genetic instability of the transgenes in the GM virus.

A particularly controversial application that is emerging is the concept of environmental genetic engineering applications, that have been described as one of a variety of emerging technologies that 'transfer the lab to the field' (See Box C and Section 4.3.2 *Self-spreading viral vaccines to prevent pathogen spill over from animals to people*). Such applications involve the genetic engineering of microbes to express genetic engineering machinery such as CRISPR to modify other organisms, such as plants or pests/pathogens, in their native, open environment. One company, Folium Science has developed what it calls a Guided Biotics® platform that deploys bacteria to either target gut pathogens in farm animals, or crop pathogens, in the form of a 'spray-on CRISPR' (Folium Science, n.d.). The company's first product targets *Salmonella* infection in poultry, involving an engineered *E.coli* strain that has been altered to carry a CRISPR genome editing tool, to target Salmonella in the chicken gut by cutting its DNA in order to disable the microbe. This product appears to be already in the process of market approvals, with Brazil recently determining it as not being a GMO, despite the product being a transgenic microorganism that carries the transgenes encoding for CRISPR. The website has announced potential product launches for 2025. In 2024, they were also awarded a UK government grant to also target the *Campylobacter* pathogen in poultry, described as a challenge to the longevity of antibiotics.

US Defence funded projects have also worked on the use of insects to deliver GM viruses to crops in order to perform rapid in field modification in the event of potential crop failures - these GM viruses are termed horizontal environmental genetic alteration agents (HEGAAs). These include the use of insects to deliver GM viruses expressing genetic engineering machinery, with the aim of rapidly modifying crops in response to a biocontrol threat (a project called 'Insect Allies'). The reliability of approaches such as HEGAAs has been questioned however, considering the complexities involved in involving three different species – a virus, insect vector and plant in the process. Moreover, the ability to edit a gene to alter complex traits is highly questionable, compared to the ease with which a gene could be edited, or more accurately, destroyed in order to damage or kill crops.

A 2016 report published in the Netherlands lists several GM microbial biocontrol agents that it states were by then already registered for use in the USA (Scheepmaker et al., 2016). One product is based on a strain of the soil bacteria *Agrobacterium radiobacter* (NOGALL), and was also registered in Australia. It is aimed at stopping Crown Gall disease in trees (the pathogen *Agrobacterium tumefaciens*) with antibiotic-producing bacteria. However, Chemla et al. (2024) report that the genetically engineered version of this product was never used commercially as such, but was registered because it was deployed in the field outside of a testing area. Scheepmaker et al (2016) also list two products based on GM strains of *Bacillus thuringiensis (Bt)* (Crymax WDG/WP, Lepinox WEG/G bioinsecticide). These two *Bt* products use genetically engineered versions of this common insecticide to kill pests, however it is unclear whether any GM versions of Lepinox are actually on the market (a non-

GM product is widely available). In contrast, Crymax is explicitly marketed as a genetically engineered product by the US company Certis Bio (Certis Bio, n.d.). According to Scheepmaker et al (2016), one further US-approved product is based on a strain of *Pseudomonas fluorescens*, found in soil and water, and was initially aimed at reducing frost damage but later used to suppress fireblight, a disease of pear and apple trees (Frostban B, changed name to BlightBan A506). However, it is again unclear whether the current BlightBan A506 product (marketed by US company NuFarm) uses GM bacteria or not (NuFarm, n.d.).

Bayer and the flailing flagship synthetic biology company Ginkgo Bioworks entered a multiyear collaboration in early 2024 that includes crop protection products alongside carbon sequestration (see Section 3.2.1 *Carbon capture*) and nitrogen fixation (see Section 3.1.1 *Biofertilisers*). Bayer have entered a number of R&D partnerships with microbial specialists, with others including Novozymes (now Novogenesis), and AlphaBio control. Ginkgo have also entered a partnership with Brazilian company Vitales to develop new biocontrol methods for soybeans, which are widely cultivated in Brazil where GM soybeans have been widely adopted into vast monoculture systems (Ginkgo Bioworks, 2024).

Within the EU, a French company, Amoéba, has sought approval for a fungicide product that involves the use of GM amoeba for treating mildews and rusts. The product is however, to be applied with dead organisms, which potentially reduces many biosafety risks associated with the release of live organisms. Regulatory approval was granted by the US EPA in 2022 and has been sought within the EU.

Corteva has already commercialised non-GM microbial products and has agreements with other microbial product producers such as STI Biotechnologie to licence their products that aim to improve soil conditions. It is worth noting that various projects seem to extend to researching both non-GM and GM applications, with many project announcements not specifying either way.

3.1.3 *Feed Additives and Digestive Microbiomes in agriculture*

Modification of the microbiome is being applied to both human health and lifestyle applications (see Section 4 and Section 3.3.1 *Probiotics*) as well to livestock. One of the most prominent examples in the field of agriculture is the Audacious project's 'Engineering of the microbiome with CRISPR to Improve our Climate and Health', being co-led by Jennifer Doudna, one of the inventors of CRISPR genome editing technologies. Their project includes the development of technologies to genome edit the microbiome of cows in order to reduce methane production, as methane is a gas that contributes to global warming. The Director of the host organisation, the Innovative Genomics Institute (IGI), Brad Ringeisen, recently stated that: "*The vision is to get to a point where an oral treatment delivered to a young calf could provide a lifetime of low emissions. To meet the scale of global agriculture, a solution needs to be simple and affordable for it to create the impact we need*." (Murdoch, 2023). Significant publicity accompanied the funding of this project, but the research is at an early stage. Some researchers have argued that CRISPR technology could change microbes which produce methane from feed in the cattle's digestive system (known as methanogens) into microbes that produce different chemicals (acetates), and, thereby, reduce the methane that is produced. However, others have noted that trials attempting to increase acetate production have had a poor success rate, and that many alternative options are already being investigated, including the use of various non-GM supplements in feed (Nylén and Brady, 2024).

Miklau et al. (2024, Table 20) identify one example of the freshwater and marine algae *Nannochloropsis oculata* being genetically modified to produce a fish growth hormone, for use as a feed additive. This application is at the applied research stage.

3.1.4 *Veterinary use*

Eckerstorfer et al. (2024, Table S2) identify several veterinary applications of GM viral vaccines. At the basic research and R&D stage, these include vaccines for use in cattle, pigs, poultry, horses, dogs, cats and fish. A few of these applications have reached the stage of field trials: 2 vaccines against Porcine reproductive and respiratory syndrome virus (PRRSV) in pigs; and 3 vaccines against Avian infectious bronchitis virus, Infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV) in poultry.

Applications of GM vaccines to wild animals are included in Section 3.2.4 *Applications in wild animals*).

3.2 Conservation and Climate Mitigation

Potential applications in the area of conservation and climate mitigation include the use of GM micro-organisms for carbon capture, bioremediation (clean-up of contaminated land and other habitats), or production of biofuels (as alternatives to fossil fuels). There are also some applications aimed at vaccinating wild animals or controlling non-agricultural pests.

Some applications for bioremediation, carbon capture, or biofuels production could be applied in contained use systems. Many, however, may be applied to open systems (for example, by being released into land, freshwater or marine ecosystems, or, in the case of biofuels, produced in open ponds). It remains unclear at this time, how many applications are envisaged for open release (Miklau et al., 2024). Nevertheless, the scale of production needed to make these applications economically worthwhile suggests that open releases may be envisaged in many cases.

A project aimed at reducing methane production in cows (also a greenhouse gas) is not included here but is discussed above in Section 3.1.3 *Feed Additives and Digestive Microbiomes in agriculture*.

3.2.1 *Carbon capture*

Carbon capture technology aims to capture the greenhouse gas carbon dioxide (CO_2) before it reaches the atmosphere, in order to store it and prevent it contributing to climate change. Some researchers are investigating the possible use of GM microbes in carbon capture (also known as carbon sequestration). Microorganisms mediate carbon cycles in many different processes and ecosystems, generating interest for the potential to modify them as a climate mitigation strategy. Soil microbes are well known for mediating carbon cycling processes, but other microbial ecosystems are also becoming targets for climate mitigation.

Miklau et al. (2024, Table 20) list one carbon capture application at the advanced research stage, utilising freshwater algae of the *Chlorella* species, and one at the basic research stage utilising the green algae *Chlamydomonas reinhardtii*, widely found in soils and freshwater. Both projects use genetic engineering to seek to improve the efficiency of these micro-algae in removing carbon dioxide (CO_2) from the atmosphere.

Loam Bio, an Australian start-up founded in 2019 is developing fungal seed products to increase soil carbon storage, raising over $50 million in the seed round, with the biggest donations coming from the Silicon Valley venture capital fund TIME Ventures. Their website

states: "*Our technology helps plants take CO_2 from the atmosphere and transform it into the most stable forms of soil carbon*" (Loam Bio, 2024). The founder is reportedly betting on microbial technology to mitigate the effects that decades of industrialised farming have had on the planet.

Although a much more distant prospect, researchers from the Tara Oceans Consortium are also investigating the use of viruses to engineer the ocean microbiome toward better carbon capture. Ohio State News reports, "*the team has now revealed which viruses have a role in carbon metabolism and are using this information in newly developed community metabolic models to help predict how using viruses to engineer the ocean microbiome toward better carbon capture would look*" (Caldwell, 2024). This research relies on vast databases of marine viruses, discussed further in Section 5. It is unclear whether the aim is to use GM viruses as the proposed means to engineer the ocean microbiome.

3.2.2 *Bioremediation*

Various applications are being researched for bioremediation and environmental restoration projects. As summarised by Miklau et al. (2024), applications under R&D include the use of microalgae, particularly for marine purposes and including waste water treatments, fungal and yeast products for the removal of toxins such as heavy metals from soils, wastewater treatment, and the use of cyanobacteria for inorganic waste removal (phosphate, nitrate, ammonium and nitrite) from shrimp aquaculture. Microbes are also being genetically engineered to degrade plastic waste (Schneier et al., 2024). These are not discussed in further detail here, since the current assumption is that, if they are successfully developed, these will be used in contained use applications only (degrading shredded plastic in bioreactors). However, possible open use applications could emerge in future.

Miklau et al. (2024, Tables 21 to 25) identify two applications at the market-development stage, using genetically modified versions of the bacteria *Pseudomonas fluorescens* (found in soil and water) to attempt to clean-up naphthalene (found in fossil fuels and used in chemical production) and the herbicide atrazine. A further 25 applications are at the applied research stage and 8 at the stage of basic research, aimed at removing a wide range of contaminants from industrial agriculture, oil and chemical production. At the applied research stage, 10 applications use *Pseudomonadales* species (mainly the soil bacteria *Pseudomonas putida*) and 8 use the human gut bacteria *E. coli*.; other species of bacteria utilized include the fast-growing freshwater cyanobacteria *Synechococcus elongatus* and *Synechocystis*, *Bacillus subtilis* (found in soil, human and animal guts and marine sponges), *Sphingomonas paucimobilis* (found in soil, drinking water and plants), *Saccharomyces cerevisiae* (yeast used in winemaking, brewing and baking) and *Sphingobium japonicum* (originally identified in contaminated soil in Japan). The applications at the basic research stage use some of the same microorganisms and, in addition, the yeast *Trichosporon oleaginosus* (found widely in tropical environments), soil bacteria of the *Arthrobacter* species, and the common mould (fungi) *Aspergillus niger* (found throughout the environment, including inside buildings).

Miklau et al. (2024, Table 20) also identify 3 bioremediation applications using the green algae *Chlamydomonas reinhardtii* (widely found in soil and freshwater) at the applied research stage: these create GM microalgae intended to remove the herbicide penoxsulam (used on lawns, rice fields and cereal crops), the heavy metal cadmium, and the poison cyanide. At the basic research stage, two further projects genetically modify the same algae to seek to absorb copper and nickel.

3.2.3 *Biofuels*

Biofuels are fuels that are produced from plants, trees or agricultural waste, rather than from fossil fuels (coal, gas and oil). As biofuels use plants or trees that are grown today, the carbon that is released by burning them can in theory be replaced by re-planting, creating a system that is more sustainable. In contrast, burning fossil fuels releases carbon (as carbon dioxide, CO_2) that cannot be replaced. However, the use of biofuels has often been controversial as, in practice, they are not always produced or used sustainably. One major area of research is the potential use of microalgae (most algae other than seaweeds fall into this category).

Much research using GM microorganisms, particularly GM microalgae, is focused on biofuels, mainly seeking to increase the oil content of such fuels. To date, this has not led to successful commercial biofuel production. If successful, biofuels using GM microbes may be made in contained use bioreactors. In such cases, the main issue of concern of relevance to this report is whether contained use is properly contained (see Section 6.5 Contained Use. How contained?), especially given that biofuels will require much larger-scale production than food ingredients or pharmaceuticals. However, it is currently unclear whether future biofuels utilising GM microorganisms will be produced in contained use or open ponds. Microalgae, in particular, are likely to be grown in open ponds, as this is expected to be less costly than contained use.

Miklau et al. (2024, Table 20) identify numerous studies aiming to develop GM microalgae for biofuels production, mainly to increase the oil content of the microalgae, but also to modify other properties, including for use in hydrogen production. They list 3 potential applications at the market development stage utilising the freshwater green algae *Scenedesmus dimorphus* and *Acutodesmus dimorphus*, the marine and freshwater algae *Nannochloropsis oceanica*, and the green algae *Prototheca moriformis* (found in freshwater and sewage), aiming at altering the composition of the biofuel. An addition application at this stage is an attempt to create a GM strain of the marine algae *Chaetoceros gracilis* which would have a reduced chance of survival if it leaked from an open biofuels production site (known as 'biocontainment'). Miklau et al. (2024) identify a further 6 potential applications at the advanced research stage: six using GM *Chlamydomonas reinhardtii* (a green algae found widely in soils and freshwater), one using GM *Phaeodactylum tricornutum* (a marine algae), and one using unnamed algal species to seek to increase production of hydrogen from water. A further 11 examples are identified as at the basic research stage: 13 use *Chlamydomonas reinhardtii*, one uses *Fistulifera solaris* (a marine algae originally found in a Japanese mangrove swamp), one uses the marine algae *Phaeodactylum tricornutum*, and one attempts to increase growth in the marine algae *Nannochloropsis salina*.

3.2.4 *Applications in wild animals*

The release of live viruses into the environment has in general, been considered too controversial due to the lack of controllability, high capacity for spread and potential for viral evolution following release (Lentzos et al., 2022a). Only one field trial release of a live virus into nature has ever been conducted, in Spain in the late 1980s, with the purpose of protecting rabbits, though the vaccine was never approved.

Eckerstorfer et al. (2024, Table S2) identify one application at the basic research stage for a GM viral vaccine against *Baculovirus* in sea lions. They also discuss in detail a field trial authorised in Australia for the use of a GM vaccine to protect Tasmanian devils against a communicable facial cancer called devil facial tumour disease (DFTD). This project uses a replication-defective viral vector, modified to express antigens against these tumours.

‘Replication-defective’ means that the GM virus cannot replicate or cause a disease. Nevertheless, GM viral particles could be excreted or shed from the vaccinated animal. There is also some uncertainty about the stability of the GM virus and how it might spread and evolve.

Eckerstorfer et al. (2024) also identify a review of biological control strategies for immunocontraception (the use of an animal's immune system to prevent it from producing offspring) of wild pests, such as rodents, rabbits, cane toads and carp in Australia, some of which involved the potential use of GM vaccines. However, major challenges arose and these approaches appear to have been abandoned.

Research on self-spreading viral vaccines to prevent pathogen spill over from animals to people is discussed separately in Section 4.3.2 *Self-spreading viral vaccines to prevent pathogen spill over from animals to people*.

3.3 Food applications

3.3.1 *Probiotics*

Probiotics can be defined as “live microorganisms” that confer benefits to health. The ‘discovery’ in Western science of the role of our symbiotic relationship with microorganisms in mediating aspects of human health and behaviour has opened up huge interests in research, medical, consumer health, and agricultural fields. Such concepts are not necessarily completely new outside of ‘Modern’ biomedical thinking, e.g., the term and concept of ‘kokoro’ in Yoruba medicine. Most, if not all, cultures seem to have examples of probiotic foods in their traditional cuisine, including foods such buttermilk, sauerkraut, kefir, fermented soya (miso), fermented millet, fermented sorghum, pulque (a fermented probiotic beverage from Mexico), that were consumed to maintain good health. Indeed, one of the common probiotics researched today is the fungus *Saccharomyces boulardii,* which grows on the outside of lychee skins. It was first isolated when scientists noted people consuming the skin to protect themselves from cholera. It is also used to treat *Candida* infections.

Within the biotech industry, numerous companies have emerged in recent years purporting to sell personalised lifestyle products that aim to test people’s gut microbiomes to detect “abnormal” microbiomes that may be associated with disease. Such companies are benefitting from an unregulated space where clinical validity and utility is not required for marketisation due to these products being sold not as medicines, but instead, as ‘lifestyle’ products. This industry is facing criticism for potential consumer harms that can arise due to the lack of evidence of their tests’ utility that may lead to harms such as recommendations for treatments/actions that may have adverse impacts on a patient based on inadequate evidence (Hoffmann et al., 2024). Hoffman et al., (2024) call for regulatory updates that can protect against questionable practices and harms, but also warn that there are significant challenges to being able to adequately assess the potential efficacy of assessing microbiome health, which also has implications for GM probiotics that are also in the pipeline (see below). For example, current knowledge around gut microbiomes is still evolving, with no consensus on what constitutes a healthy human microbiome and a lack of reference databases that can be used to gauge what is healthy and what is not. This is at least in part due to the properties of bacteria, which are highly genetically diverse, fast evolving, and differ by geography and population. Moreover, the links between the microbiome and disease have been recently questioned by experts in the field of colorectal cancers with implications for microbiome-mediated disease research in general. Studies report inconsistencies in microbiome signatures associated with disease, raising questions regarding the analytical tools and methods involved. As stated in a recent *Nature Medicine* article “*These problems are not exclusive to CRC* [colorectal cancer] *studies, they are*

pervasive throughout the fields of microbiome and metagenomics research" (Jiao et al., 2024).

A new avenue for GM microbes is the industry of consumer health products such as probiotic bacteria that are believed to mediate certain aspects of human health (and other species including mammals and even plants). The potential goals of applications may be to improve microbes' tolerance to stress during food production, promote survival in the gut, improve probiotic function, modify disease, or aid medical diagnosis by engineering bacteria to produce biosensors for detecting diseases (Liu et al., 2023).

Zbiotics is the first company in the world to produce and sell genetically engineered probiotic products. The California-based company began by marketing a probiotic drink aimed at reducing hangovers. It uses a genetically modified form of the bacteria *Bacillus subtilis* (found in soil, water, associated with plants, and in human guts) to breakdown the chemical acetaldehyde (a product of drinking alchohol) in the human gut (Naidu et al., 2019). More recently, the company has begun marketing a second product, aimed at turning sugar into fibre in the human gut, using a different genetic modification of the same bacteria. Zbiotics notes on its website that its products are 'FDA compliant' (meaning that they comply with the legal requirements of the US Food and Drug Administration), but also states, "*FDA compliance is not FDA approval*", which is not required because their products are classed as functional foods not drugs (Zbiotics, n.d.). No process for assessing environmental risks is mentioned (see Section 6).

Recently, genome editing techniques such as CRISPR have begun to be developed in order to create genetically engineered probiotics (Liu et al., 2023). Many of these have potential medical applications (discussed in Section 4) but they could also be used to create so-called functional foods (with weaker regulatory requirements). Bacterial species that have been gene edited (at an early stage of research) include *Lactobacillus* species (lactic acid bacteria, which constitute a significant component of the human and animal gut microbiota), *Bacillus* species, which are ubiquitous in nature (such as *Bacillus subtilis*, found in soil and the gastrointestinal tract of ruminants, humans and marine sponges), some yeast species (including brewer's yeast, *Saccharomyces cerevisiae*), *Bacteroides* species (abundant in the human gut) and the gut bacteria *E.coli*.

Research appears to be at early stages but there are suggestions that for example, genetically engineered probiotics could be used for protecting against food borne pathogens (Cruz et al., 2022), and also for antimicrobial treatments described further below (see Section 4.2 Anti-microbial treatments for human health/veterinary applications).

3.3.2 *Food additives*

GM microorganisms are already used in 'contained use' to produce a variety of food additives.

Live genome edited microbes are being commercialised for fermentation processes, including in alcohol production. Live GM yeast has been commercialised in the US by the Berkeley Yeast company, which sells its yeast as allowing for improved taste qualities, e.g., fruity flavoured beers, at a cheaper price due to the GM yeast being responsible for the fruity flavour without the need to add real fruit to the recipe (Berkeley Yeast, n.d.).

Genome edited yeast strains for rice wine production have similarly been patented for rice wine production in China, though they do not yet appear to be commercialised yet. Moreover, at least for this product, only dead, rather than live yeast would be present in the final product. Patents filed in China, according to the horizon-scanning documents published

by the European Food Safety Authority (EFSA) (Ballester et al., 2023), all involve the use of dead rather than live microbes, posing potentially lower risks in terms of spread and controllability.

4. Medical applications

Some medical products are manufactured using GM bacteria in contained use. The first of these was synthetic insulin, approved as a treatment for diabetes in 1982. It is produced using GM bacteria that have had the human gene for insulin inserted into them. Since then, numerous other products have been developed. However, recently, attention has turned to the possibility of using live GM bacteria and viruses as medicines. Potential applications are discussed below.

4.1 GM bacteria as potential medicines

Applications are being developed that would presumably be aimed at the medical market, with numerous clinical trials using GM bacteria already being conducted. A review from (Ma et al., 2022) lists nine such trials. Five of these trials were conducted by the U.S. based company Synlogic, and are aimed at introducing genetically modified bacteria that may mediate disease, in collaboration with Roche, in what they describe as 'precision genetic engineering', i.e., genome editing, although the process also involves the insertion of transgenes. For example, products are targeted at diseases that result in excessive build-up of toxic metabolites, which the GM bacteria are designed to remove, e.g., gout and phenylketonuria. Other potential applications (at a very early stage of research) include delivering CRISPR in the gut to eliminate anti-biotic resistant bacteria, sensing and then degrading pro-inflammatory chemicals, and regulating metabolism to prevent obesity (Liu et al., 2023).

Unlike products marketed as lifestyle products, the fact that these products are aimed at the medical field, means that they have to undergo clinical testing for safety and efficacy. In February this year, Synlogic announced that they were discontinuing trials for a lead product (Labafenogene marselecobac (SYNB1934)) for the rare genetic disorder phenylketonuria (PKU) for failing to show efficacy in phase III trials (Synlogic, n.d.). The product is an *E.coli* strain engineered to carry two enzymes to digest phenylalanine, a harmful chemical which builds up in patients with PKU. The company is now intending to slash 90% of its workforce, and the President and Chief Executive Officer are stepping down. This drug had already received Fast Track and Orphan Drug designation in the US, which aims to speed up market approval for medicines for rare diseases. While the company states that the phase III trial was not discontinued due to safety issues, the previous phase II trial had four out of 9 participants discontinuing the trial, based on adverse events that included gastrointestinal problems with symptoms including nausea, abdominal pain, diarrhoea, abdominal distension and flatulence (Vockley et al., 2023).

The Audacious Project's 'Engineering of the microbiome with CRISPR to Improve our Climate and Health' is also working on editing microbes to prevent asthma (as well as for livestock applications, see Section 3.1.3 *Feed Additives and Digestive Microbiomes in agriculture*). In a recent interview, Jennifer Doudna and her partner Jill Banfield describe the application of 'precision genome editing' to microbial communities, including to mediate asthma in children (Murdoch, 2023). The project states that it aims to "*pursue this finding to develop screening protocols to identify newborns at high risk for asthma and allergy based on the presence of this and other microbial molecules in their stool, as well as interventions that could reduce infants' risk, either through therapies that reduce levels of these compounds or by promoting early life gut microbiomes that prevent production of such compounds*" (Weiler, 2019).

It appears these projects are at the early stages based on purportedly 'clear microbial targets to pursue', including bacteria that produce a specific molecule that they identified in previous research to correlate with asthma incidence in babies. Potentially, this approach could utilise Doudna's recent work on delivering CRISPR directly to gut microbes in their native gut environment, as a form of environmental genetic engineering. However, questions remain about a strategy to target single species of bacteria, when full understanding of how microbes impact asthma is incomplete. Moreover, questions remain about the suitability of such an approach to address health disparities, as the project purports (see Box E).

Box E: (Un)suitability of a techno-fix approach to systemic health inequalities

The Innovative Genomics Institute states that the work on microbiome engineering would most impact racialised communities, or as they put it, 'People of colour' (in the US), due to disproportionate levels of asthma in these communities (Murdoch, 2023). However, attempting to address a single disease with this technofix approach fails to address the structural and systemic root causes of health inequities that impact minoritised and marginalised communities unequally. The identification of bacterial abnormalities raises questions as to why racialised communities are indeed more affected, as microbiome composition is obviously an environmental and not biological component of people. Racialised and marginalised communities are well known to suffer health disparities, and the private sector and those with profit motives often promote their work as a public good to address such disparities. Asthma in particular, has been linked to structural racism that negatively impacts health. As noted by Martinez et al., (2021) structural racism "*is embedded across multiple levels, including the economic, educational, health care and judicial systems, which manifest in inequity in the physical and social environment*".

Such claims and weaponisation of race politics need to be carefully scrutinised, not just for this project, but as a whole, lest we risk reinvigorating the racist biological essentialism that has been a major historical component of Western biomedical thinking. Following the failure of the human genome project to find evidence of genetic races, some scientists began to re-focus on inequities as causes of ill-health, identifying racism itself as a health risk factor by forcing people into adverse socio-economic conditions (including mass incarceration of racialised peoples) that promote ill health and increase stress.

Social inequalities more broadly, also termed social determinants of health, including poverty and living environment, are well established indicators of respiratory illness including asthma. Structural and systemic forces shape overall health of populations, and for asthma specifically, housing conditions and exposure to pollution, access to healthcare, early life trauma, are all linked to increased incidence of asthma (Gold & Wright, 2005; Pearlman et al., 2006; Redmond et al., 2022).

4.2 Anti-microbial treatments for human health/veterinary applications

The threat of antibiotic resistance, relevant to both the medical and agricultural spheres, is spurring investigations into alternative forms of antimicrobial technologies. One such technology is the utilisation of bacteriophages, which are viruses that reside in bacteria, to target and kill bacterial pathogens. They kill bacterial hosts by infecting them and replicating inside, eventually bursting (lysing) and killing their hosts.

Bacteriophages (which are often just called 'phages') receive little public attention despite their ubiquitous abundance in nature, being present wherever bacteria are, including within humans and other species, as well as the wider environment. Studies estimate that there are 250 million phages per millilitre of surface water, and over a billion are estimated to be

present in one gram of certain soil types. It has been estimated that around 31 billion enter the human body every day from the gut, where they support microbial life (Faltus, 2024).

The use of phages for medical purposes is not strictly new, and has been practiced at a very limited scale in a handful of countries. At present, 6 treatment clinics have emerged globally (the US, the UK, the Republic of Georgia, Poland and Belgium) offering phage therapy for alleviating infectious disease (Tang et al., 2019). The rationale is that phages can be used to infect and kill pathogenic bacteria, and that safety issues are limited due to their host specificity, i.e., they will not infect, and thus kill, non-target bacterial species. Another potential application is their use as bacterial detection systems, by being engineered to carry fluorescent or luminescent genes such that they will produce a signal when infecting a bacterial species of interest, with the idea that they could be used to diagnose specific bacterial infections (Mitsunaka et al., 2022).

The UK is funding certain projects on GM phage applications, though this research appears to be in the early stages, with a limited number of applications focusing on genetically engineered versions. A small UK company called NexaBiome is involved in research on GM phages (NexaBiome, 2023). There appears to be a lack of consensus amongst researchers regarding both efficacy and safety, e.g., regarding host specificity.

Research studies have included 'proof of principle' experiments where phages have been engineered to express genome editors that were able to modify mouse microbiomes *in vivo*. The research team were able to target and edit (and purposefully not kill) certain bacteria in the mice (Conroy, 2024). This is another environmental genetic engineering application (see also Box C and Section 4.3.2 *Self-spreading viral vaccines to prevent pathogen spill over from animals to people*). This work raises serious questions about the unknown consequences of eventually using such applications in people or animals.

Potential agricultural applications of GM phages are discussed in Section 3.1.2 *Biocontrol of agricultural pests and pathogens*.

4.3 GM viruses and vaccines

A few applications using GM viruses as human and veterinary vaccines have been developed. These GM viruses are intended not to spread or replicate in the environment, although whether this is always the case in practice is yet to be determined. In contrast, some research is focused on creating GM viruses that are intended to spread in the environment, with the aim of preventing the spread of animal diseases into people. These self-spreading vaccines raise significant concerns.

4.3.1 *GM viruses as vaccines*

Vaccines stimulate the immune system of humans or animals to recognise a pathogen, leading to an improved ability to fight off a current or future infection. There are lots of different types of vaccines. Some (called 'attenuated' vaccines) use live, weakened form of the virus or bacteria that causes the disease; others use inactivated forms of these microbes, or fragments of them. A variety of other methods can also be used to deliver toxins, proteins or other biological materials to stimulate the immune system in the desired way.

Viral vector vaccines use a non-pathogenic virus to deliver pathogen genes into the body, to stimulate an immune response. Virus-based vectors are recombinant virus genomes from non-pathogenic viruses, that are genetically manipulated to express protective antigens from other, pathogenic (i.e., disease-causing), viruses. Recombinant genomes are formed by

bringing together genetic material from several sources, creating DNA sequences that would not otherwise be found in the genome. Viral vector vaccines are designed to be 'replication-defective' (meaning that, at least in theory, the GM virus cannot replicate or cause a disease). Zuber et al. (2021) note that several veterinary and human viral vector vaccines have been licensed (for Japanese encephalitis, dengue and Ebola), with more in the pipeline. However, they also note that there is still limited experience about their efficacy and safety in humans, and list 11 issues of critical importance, including the potential for recombination with wild-type pathogenic strains, and questions regarding the genetic stability of replicating recombinant viruses outside the laboratory.

Another approach is the genetic attenuation of live vaccines. This means using GM viruses, bacteria and other micro-organisms to attempt to create attenuated vaccine strains that can replicate the pattern of natural infection (and generate immunity) without causing disease or other side effects (Zuber et al., 2021). A new polio vaccine, using two GM strains of polio virus, is the first example. It is believed to be less likely to mutate into the infectious form of polio than the non-GM vaccine currently in use: however, it only protects against one strain of polio (Gadye, 2023).

GM viruses as veterinary vaccines are discussed in Section 3.1.4 *Veterinary use*. Their use in wild animals is discussed in Section 3.2.4 *Applications in wild animals*, but applications for use in wild animals intended to stop the spread of diseases into humans are discussed further below.

4.3.2 *Self-spreading viral vaccines to prevent pathogen spill over from animals to people*

A highly controversial application involving the use of genetically modified viruses to act as self-spreading vaccines is under way, with the aim of releasing them into wild populations of disease-carrying vector species (Lentzos et al., 2022a; Murphy et al., 2016; Nuismer et al., 2016; Nuismer & Bull, 2020). Such viruses would be engineered to produce an immune response in a host animal population. A few projects are reportedly underway, including those targeting bats that carry the Rabies virus, apes that carry Ebola virus, and rodents that carry Lassa fever. The University of Glasgow has a lab working on targeting bats, while UC Davis California, alongside the Vaccine Group, a UK-based company, have collaborated and received funding from the US military research arm DARPA under the PREEMPT project, to work on Ebola and Lassa fever projects (*PREEMPT Project*, n.d.). Such work is being promoted under the "One Health" banner of integrating human and environmental health in recognition of the interconnectedness between people and their environment. This concept is not new, but has gained recognition since the COVID pandemic. However, technology developers appear to have got on board with a mainstreamed approach that dilutes the holistic concept, focusing on pathogen data collection to the benefit of leading biotech countries and potentially entrenching global health inequities further (Ramakrishnan, 2023) by focusing on techno-fix approaches such as this.

These projects have drawn acute biosafety, ethical and political concerns and drawn attention from policy makers and biosafety scientists, alongside other GM virus applications (Lentzos et al., 2022a, 2022b).

A recent assessment performed by the synthetic biology working group at the Convention for Biological Diversity (CBD), where international regulations and guidelines are set on biotechnologies such as GMOs, that may pose risk to biodiversity, (and human health), reported numerous potential risks with the use of 'self-spreading vaccines'.

Their report concluded that: "*Despite technical feasibility, ethical, ecological and regulatory concerns surround this approach, releasing genetically engineered organisms with*

contagious self-spreading capabilities into the environment introduces substantial challenges in risk assessment, monitoring long-term effects and mitigating harm, especially with evolving dimensions that test current knowledge limits. The complexity with the recombinant vector raises concerns such as unknown evolution and virulence risk upon release. Further concerns involve the possibility of the viral vector co-opting the immunogenic insert expanding its ecological niche or hosts. Issues extend to the biology, ecology and population dynamic of hosts as well as potential vaccine transmission to other species, including humans" (CBD multidisciplinary ad hoc technical expert group, 2024).

4.3.3 *Biocontrol of mosquitoes*

Miklau et al. (2024, Table 23) list one example of application-orientated research which involves using GM *Beauveria bassania*, a soil fungus and insect parasite, to produce a substance toxic to mosquitoes.

As described in Section 3.1.2 *Biocontrol of agricultural pests and pathogens* (which deals with agricultural pests), paratransgenesis involves genetically engineering microbes that infect pests, to seek change the ability of the pest to reproduce or spread disease. Outside the field of agriculture, this approach has also been explored for mosquitoes, to seek to prevent mosquito-borne diseases, such as dengue and malaria. Miklau et al. (2024) list one basic research study which proposes genetically engineering the bacteria *Wolbachia pipiensis* as a potential biocontrol agent for mosquitoes. However, infection of mosquitoes with non-GM *Wolbachia* has already shown significant potential for controlling mosquito-borne diseases (by reducing mosquito populations or disease transmission) (Minwuyelet et al., 2023) and it is unclear to what extent GM approaches will now be pursued.

Semi-field testing in Burkina Faso of a GM fungus engineered to carry a spider venom was also conducted and published in 2019 by US scientists (Lovett et al., 2019). Concerns were subsequently raised by civil society organisations, regarding not just biosafety risks and efficacy questions, but also the legality of the so-called semi-open release of the live GM fungus (ACB, 2020). No new public information appears to exist on this application to indicate if this project is still active.

4.4 Skin microbiome/topical skin products

A narrow interest in skin microbiome products from the US military, for example, has fuelled research into the use of skin microbes to repel mosquitoes, with project starting in 2020 and receiving a second round of funding in 2022. As stated on the DARPA website: "*The ReVector program aims to precisely, safely, and efficiently reduce mosquito attraction and biting, and, subsequently, to help maintain the health of military personnel operating in disease-endemic regions*." (DARPA, 2022).

4.5 Bioweapons

Biological weapons (bioweapons) are living organisms that are released deliberately to cause harm to humans, animals or plants, by causing disease or producing toxins. GM microorganisms, including viruses, bacteria or fungi, could be deliberately created to be used as weapons in this way: for example, by making a pathogen more harmful or easier to spread. The UN's Biological Weapons Convention effectively prohibits the development, production, acquisition, transfer, stockpiling and use of biological and toxin weapons by governments worldwide. However, even if the treaty is adhered to, there are concerns about the 'dual-use' of the scientific and technological methods developed to create GM microbes, e.g., by terrorist groups, in order to cause harm. This is discussed further in Section 6.7 Biosecurity Risks.

5. Microbial databases, biopiracy and biosecurity

Major questions remain regarding the potential for GM microbes to be successfully developed into marketable products on a broad scale. In some cases, efforts may be more about big data, and/or deployment of conventional microbes under the guise of a genetic technology revolution, to restore the reputation and life span of destructive industrial practices, including biopiracy. Biosecurity threats could also arise from this approach.

Human knowledge regarding the role of microbes, especially how individual species operate and behave in various ecosystems, or what traits they exhibit, remains far from complete. The rise in big data, computational and sequencing technologies is now giving the GM and synbio industry belief that, with enough data, (plus the advancing ease of laboratory manipulation of DNA with techniques such as genome editing), such technological advances will drive a revolution in 'biologicals'. As discussed in Section 3, GM 'biologicals' encompass using engineered microbes to manufacture a whole manner of products in contained use, e.g., food ingredients, medical drugs, as well as living GM microorganisms to be released into the environment, e.g., soil microbes to improve for nitrogen fixation to reduce the need for synthetic fertilizers.

However, having information and data is not sufficient for developing a *useful* GM microorganism that a) can be easily manufactured at scale; b) functions as intended and; c) functions without unintended effects that compromise efficacy. Safety is also key, but perhaps not the first priority for most product developers, especially outside the realm of medical applications. Moreover, GE engineering processes, both old style transgenics and newer genome editing techniques (see Box B) are associated with unintended effects that potentially lead to bottlenecks in developing successful products (e.g., see ENSSER, 2021; GeneWatch UK, 2021).

One company that exemplifies the rush for data is Ginkgo Bioworks, one of the 'flagship' synbio companies founded in 2008, and recently reported to be suffering serious financial difficulties and forced to get rid of a third of its staff in 2024 (Science, 2024).

In 2008, the year of its founding, an article in the pro-GMO Gates-funded media outlet, Alliance for Science, entitled "Ginkgo Bioworks: Restoring pride to GMOs", claimed that their USD$100 million partnership with Bayer would unleash a new era of nitrogen-fixing microbes for crops like maize (Alliance for Science, 2018). The article claims that the company, at that time, was performing 40% of the world's 3D printing of DNA, designed to be inserted into microbes to make novel GMOs.

However, there have been no commercially significant products to emerge from Ginkgo Bioworks, for either agriculture or any other sphere, and live GM microbe products appear to be specifically lacking. An article in Technology Review from 2021, detailed this problem (Regalado, 2021), reporting that "*it is surprising that 13 years after it was founded, Ginkgo can't name a single significant product that is manufactured and sold using its organisms*." The CEO was quoted as saying, "*I am not a product company, and I have no desire to be a product company*."

Indeed, with regard to microbial products for environmental release, from their website it appears that the company is no longer discussing the development of transgenic GMO microbes with foreign DNA inserts – the standard form of GM techniques used for developing the GMOs that have been commercialised to date. Nor is it clear that they are deploying genome editing techniques for modifying organisms for environmental release, the so-called flagship GM technique purported to allow for speeding up product development and the generation of useful traits. Instead, the current methods used involve 'random

mutagenesis', and 'rational engineering approaches', the meaning of which seems to be deliberately obscure.

Gingko Bioworks has, however, generated and acquired numerous databases and platforms for identifying microbial candidates since its founding. For example, in 2022, the company acquired Bayer's Ag Biological R&D division, including a library of 200,000 microbial strains (Ginkgo Bioworks, n.d.). More recently, in 2024 they also acquired AgBiome and its microbial strain library with 8,000 and 500 million sequences. The company now states that their large microbial sequence library provides them with a unique ability to provide other companies with product development head-starts by identifying strains with beneficial traits, e.g., nitrogen fixation, and then providing assistance with further engineering to 'optimise' the beneficial traits with either random mutagenesis or 'rational engineering approaches' (it is unclear what this means), manufacturing optimisation, as well as formulation optimisation, e.g., packaging the products into liquid suspensions such as foliar sprays, or dry formulations such as seed treatments. The website claims to have identified over 1,600 candidate nitrogen-fixing strains from analysing the genetic data (by computer). The company then aims to give manufacturing licences to companies while retaining ownership of any intellectual property protections involved. This begs an important question regarding whether any engineering process will improve on the naturally occurring beneficial traits already present in the strain libraries at all. Indeed, the company offers services in testing wild type strains, *or* 'optimising further' with genetic engineering approaches.

The financial struggles of the company may explain why it seems to have, in recent years, moved more towards selling its data services, and promoting their unique capacity for using Artificial Intelligence (AI) - the latest technological hype that has become intertwined with synbio, with the aim of generating novel genetic sequences for GMOs and proteins (ACB et al., 2024). The theory goes, that inputting data in AI models allows for training and improving of models, such that the information provided by these databases can spur on the development of novel sequences for new GMOs or proteins. Ginkgo Bioworks is thus selling itself as uniquely placed to lead in the AI field with the huge amounts of microbial data it has collected. Indeed, this year they launched a collaboration with Google Cloud, which Google describe as for '*building a next-generation AI platform for biological engineering and biosecurity. Ginkgo is pioneering new large language models for biological engineering applications, powered by Google Cloud's Vertex AI platform*." Google is one of the major players in the AI field, including with regard to synthetic biology, with a subsidiary called DeepMind claiming to now be able to predict protein folding arrangements, and structures of DNA, RNA and other molecules.

A Californian company, Biome Makers seems to be leading the charge on soil microbial databases, claiming to have 24 million strains in what is claimed to be the largest soil microbe database in the world (EuroFruit, 2024). The database reportedly contains information on microbes sampled from across 56 countries over the last decade. The company then sells a platform of an 'intelligence-based' service that samples farmer fields to 'diagnose' and recommend interventions for particular soil conditions, e.g., related to nutrient cycling, health, and biodiversity, with the added assistance of AI to help sieve through the information with its BeCrop digital platform. CropLife, a notorious pesticide and GMO industry lobby group, promotes Biome Makers as having analysed 201 crop types for their soil microbial profiles, "uncovering microbial ecosystems" (Nix, 2024). BeCrop is further claimed to support "product development". Whilst they do not engineer microbes themselves, the company is collaborating with major GMO BigAg players who are themselves working on GM biologicals, such as Bayer (formally Monsanto) (e.g., see Section 3.1.2 *Biocontrol of agricultural pests and pathogens*), who appear to have access to their database information.

Other databases being gathered include a US government funded soil virus database containing “2,953 previously sequenced soil metagenomes and composed of 616,935 uncultivated viral genomes and 38,508 unique viral operational taxonomic units” (Graham et al., 2024), and other company owned databases include Loam Bio’s library of almost 2,000 microbial strains and identified those that help plants store carbon in the soil (ABC News, 2021).

Symborg, a Spanish based company that produces microbial (non-GM) products, acquired by Corteva in 2023, also has a 10,000 strain library that it states is “*selected from different areas of the planet (forests, deserts, frozen areas, sea beds…), obtained through targeted microbial extractions. Depending on the needs of the market and farmers, candidate substances are tested. The result is an active substance that will become a biotechnological solution*.”(*Symborg*, n.d.). Corteva also has a collaboration with PacBio to utilise its sequencing platforms to allow for large-scale sequencing of plant and microbial DNA, to drive “*implementation of seed product development tools like CRISPR-Cas gene editing, and cutting-edge crop protection solutions*” (PR Newswire, 2023).

5.1 Database biopiracy risks

The growth of digital databases raises important questions regarding potential biopiracy - the appropriation of genetic resources through exploitative practices such as patents and intellectual property rights. In recent years, discussions and regulations around biopiracy have expanded to consider digital sequence information, and not just physical samples of organisms. Biopiracy was a long-held colonial conquest that allowed colonial powers to usurp entire economies through unregulated capture and transport of seeds, for example the British transport of rubber from the Americas to Asia that set the stage for the eventual demise of the Amazonian rubber boom (Chee & Hammond, 2016).

For microorganisms, the digital sequence information (DSI) of a strain allows for laboratories to synthesise the strain themselves without a physical sample, allowing a virtual version of colonial era theft and appropriation. Moreover, the growth of databases with thousands or even millions of strains or species, as raised above, is key for the development of novel products, and “*act as a sort of roadmap and resource pool, enabling scientists to compare sequences on a computer screen and identify pertinent variations*.” (Chee & Hammond, 2016). Many microbial databases generated by companies are not open access but restricted to business partners that include corporations such as Bayer. However, others are open access.

The generation of microbial databases, that appear to include the sampling of farmer fields, raises urgent questions regarding whether they are applied to non-GM or GM microbe applications. Digital sequence information (DSI) regulation has been a particularly important issue for many countries, often in the Global South where biodiversity tends to be high, and moreover, where the experience of colonial theft, and appropriation has had lasting impacts on both economies and biodiversity. Indeed, it has become one of the most controversial and heated discussions under the UN Convention of Biological Diversity where regulations around DSI are under development and a clear divide between biotechnology friendly countries and those who wish to protect their genetic resources and surrounding knowledge linked to it, from extraction. One such concern is databases being “open access” without any form of benefits sharing obligation that should arise from the utilisation of this information, back to original custodians. At a meeting of the UN Convention on Biological Diversity in November 2024, ministers agreed on a global levy on products made using genetic data (DSI) from nature, in the form of a fund that companies will be expected to pay into (Greenfield & Weston, 2024; Mundy, 2024).

Examples of open access microbial databases also include a new marine database of around 315 million genes and microbes including bacteria, viruses and fungi, set up by researchers in Saudi Arabia. Samples were collected from various regions including the Arctic, Indian, Southern, Atlantic and Pacific oceans and the Mediterranean sea (Wong, 2024). Marine bioprospecting is also a huge focus of multinational corporations, with three companies alone - BASF, IFF and DuPont filing over 900 patents on deep sea organism sequences, the majority of which come from bacterial species (Zhivkoplias et al., 2024). A new treaty (the UN High Seas Treaty) deems genetic resources obtained from international waters to be common property. Once enforced, "*it will regulate the use of high seas' genomes in both physical and digital form, arguably making it the strongest international agreement yet to counter the threat of biopiracy*" (Heffernan, 2023). However, the Treaty is far from being fully implemented, and only covers international waters.

5.2 Database biosecurity risks

The rise of microbial databases raises biosecurity risks with regard to the ability to use the information collated in databases for dual-use modification of pathogens for bioweapon applications (see Section 6.7 Biosecurity Risks). The ability to modify viruses is an obvious and long-standing biosecurity concern. It is now, however, further exacerbated by technical advances in genetic engineering as well as advances in other fields such as AI, where information in databases could be deployed to train AI models to accelerate the development of novel pathogens.

Indeed, the company Ginkgo Bioworks has a long history of connections and funding from the U.S. military research arm, DARPA, whose remit is national security, not health or agriculture. The ex-VP of business development up until 2022 at the company, who also served as and head of innovation at Concentric by Ginkgo focusing on 'applying the tools of synthetic biology to outpace infectious diseases', was also the Program Manager at DARPA where she 'leveraged the tools of synthetic biology and gene editing to enhance biosecurity, support the domestic bioeconomy, and thwart biothreats.' (*DARPA Forward*, n.d.).

6. Risks and Uncertainties

The potential applications described in Section 3 may never deliver useful applications to the market-place. However, they could still result in large-scale releases of GM microorganisms into the environment. In most of the examples discussed, these are living GMOs, which can reproduce and spread in the environment, surviving for multiple generations (perhaps indefinitely) in order to deliver the claimed applications. This risks creating a form of 'living pollution' that cannot be contained, controlled, or recalled if anything goes wrong. In some cases (such as the idea of 'self-spreading vaccines'), widespread dispersal is intentional.

Although only a tiny fraction of the multiple species of microbes that exist have been genetically modified, they already represent species that inhabit a wide range of habitats. These include several species of marine microalgae; bacteria that inhabit soils and freshwater habitats; fungi and bacteria that infect plants and animals, including many species of insects; and viruses that infect humans and animals. Uncontrolled spread of GM microorganisms could therefore pollute all ecosystems: rivers, lakes, oceans, farmland, forests, grasslands, gardens, parks and nature reserves.

Microorganisms are ubiquitous in the environment, and many evolve in close proximity to humans, animals and plants: for example, in the gut and skin microbiomes of humans, pets, livestock and wild animals. Novel genetic constructs are easily transferred from one microbe to another and can spread unwanted traits, such as antibiotic resistance. A particular concern is the potential creation of novel pathogens as microbes evolve. Lack of knowledge

of vast numbers of species, and limited understanding of how to test for possible harms, means that even supposedly harmless environmental microorganisms can produce unexpected toxins (Miller et al., 2018). Some bacterial species can also promote the development and exacerbation of allergic inflammation (Nordengrün et al., 2018).

This section considers the issues of uncontrolled spread, transfer of genetic material (via horizontal gene transfer, HGT) and questions regarding how GM microorganisms and their ecosystems will evolve. Complexities are then discussed, as well as the question of whether 'contained use' applications can be properly contained.

6.1 Uncontrolled Spread

"*Once a persisting transmissible GMO is released (whether intentionally, legally, or otherwise), it is unlikely that it could be completely removed from the environment*." (Lentzos et al., 2022).

One of the central risks of GM microorganisms is their capacity for rapid replication, spread and persistence. This opens the door to a form of 'living pollution' that may lead to unpredictable exposures, as well as unintended spread, including transboundary movements (i.e., between countries). As with 'forever chemicals' (Box A) that freely move and persist in the environment, exposure to GM microbes could well be widespread and long-term. While measures are being designed to seek to contain spread, e.g., at the molecular level (known as 'biological containment'), such molecular designs remain under development, and will introduce more genetic modifications with their own risks and complexities (Ke et al., 2021). Much of the uncertainty and lack of knowledge around applications such as the GM viruses known as HEGAAs (horizontal environmental genetic alteration agents), intended to move genetic engineering from the lab to the field (Box C and Section 4.3.2 *Self-spreading viral vaccines to prevent pathogen spill over from animals to people*), centre around the lack of understanding of uncontrolled spread, persistence and gene flow (Pfeifer et al., 2022).

Microbes in general have a high capacity for spread due to their particular properties including their small size and replication capabilities. With farm applications, dispersal from soil/plant microbiomes has been reported to occur both locally within a field or site, but also further afield, across different environments. Dispersal routes include air, leaf litter, pollen, seeds, insects, or soil-associated animals or fungi. Moreover, dispersed microorganisms have been shown to establish both transiently, and over the long-term, with even transient invaders capable of causing shifts in microbial communities (Sessitsch et al., 2023). Plant seeds and pollen are also vehicles of microbiota transmission.

Airborne transport of plant-associated microbiomes, including those that reside in the soil and spread to plant leaves, are well characterised for foliar (leaf) plant pathogens. Similarly, water droplets are another important vehicle for microbial movement, with several pathogens detected in both rain and snow samples, and rain is also a key reservoir for leaf (phlyosphere) microbiota, e.g., for tomatoes (Mechan Llontop et al., 2021). Transport of microbes by air and rain is discussed further in Box G.

Critical knowledge gaps also exist with regard to potential persistence of certain microbes within plant systems, with, for example, a lack of knowledge around the persistence of bacteriophages that reside in plant bacteria (Eckerstorfer et al., 2024).

Food/feed and digestive microbiome applications are also relevant to unintended spread. Foods consumed by people can get contaminated with cattle gut microbes, as occurs with *E.coli* outbreaks, for example, from lettuce crops that have been sprayed with manure. Microbes could also be potentially transmitted via milk (Lyons et al., 2020), and may be

transferred via a number of routes, including faecal transplants which are performed to restore gut microbial composition (Gupta et al., 2016). Moreover, animal species that are co-housed have been shown to transmit microbes between them, e.g., goats and pigs (T. Zhang et al., 2022). Oral and gut microbiomes have also been shown to be shared by people who co-habit together, including but not limited to, mother to infant gut transmission (Valles-Colomer et al., 2023). As such, applications involving live microbes that target human microbiomes as food, can be considered open release applications. The release of sewage into open environments also raises a very real risk of GM microbes that were not even intended for environmental release, being dumped into rivers and marine environments in particular. Genetic elements such as antibiotic resistance genes, can spread through wastewater treatment sites and rivers (Cai et al., 2014; Mao et al., 2015; L. Zhang et al., 2024), and bacterial aerosols can spread in landfill sites (Cyprowski et al., 2019).

Microbiomes are also present on skin, where they play an important role in protecting humans and animals from diseases (Box F), and can be transmitted from one person (or animal) to another.

Box F: Skin microbiomes

Human skin is home to millions of bacteria, fungi and viruses that compose the skin microbiota. Skin microbes have essential roles in protecting against invading pathogens, developing the immune system and breaking down natural products (Byrd et al., 2018). The composition of skin microbial communities is still poorly understood: they are shaped by interactions between different microbes and the human host. They appear to play a role in skin disorders such as acne, eczema and psoriasis, as well as wound infections. Bacteria called *Staphylococcus aureus* are a common cause of skin infections, and can sometimes spread through the blood stream and infect other parts of the body. These bacteria can also evolve resistance to antibiotics. A recent catalogue of skin microorganisms found that more than half were novel species, which have yet to be categorised or understood (Li et al., 2023).

Direct contact between people may transmit skin microbiomes from person to person (Neckovic et al., 2020). Similarly, the skin microbiomes of farm workers in intensive livestock farming may be affected by microbes from the animals they work with (Peng & Biswas, 2020), including antibiotic resistance genes (Chen et al., 2024).

The skin microbiomes of other species are even less well understood than those of humans, but may be vitally important.

Only a few studies have been conducted on the skin microbiomes of non-human mammals, such as dogs, cats, mice, squirrels, raccoons, cattle, pigs, cows, kangaroos, horses and sheep (Ross et al., 2019). Microbial communities are typically more diverse on healthy skin, and there is evidence that microbial community composition affects several skin conditions in mammals, including diseases that cause lameness in cattle and sheep. The skin microbiota of several fish species, eels, and cetaceans (such as humpback whales, dolphins, and killer whales) have been sampled, but understanding is at a very early stage. There have been very few studies of the skin microbiome of reptiles, such as lizards and snakes, focusing on some microorganisms that are known to cause reptile skin diseases. Birds also have complex microbial communities on their skin, and it is thought these may possibly play a role in social communication, by emitting volatile chemicals (Engel et al., 2020). Evidence suggests that vertebrates share an evolutionary pattern with their skin microbiome, suggesting that they co-evolve (known as phylosymbiosis) (Ross et al., 2019). Phylosymbiosis has been studied in more depth in insects, but understanding is still at a very early stage (Qin et al., 2023).

Amphibians, such as frogs and toads, breathe through their skin, which they need to keep moist in order to survive. Their skin microbiomes are vital for their health, protecting them from pathogens and aiding in development, immune system training, and their ability to reproduce (Kueneman et al., 2022). Amphibians all over the world are rapidly declining for multiple reasons including fungal diseases and other factors (e.g., habitat loss, susceptibility to pesticides). The antifungal capacities of many bacteria living on amphibian skin are important in disease prevention (Rebollar et al., 2020).

GM microbes, such as viruses, may be present in inoculated animals for several weeks or even months (Eckerstorfer et al., 2024) facilitating spread and persistence and creating viral reservoirs for onward transmission, with likely 'shedding' of the viruses into the environment, potentially affecting non-target organisms such as farm animals or people. Similarly, inoculated plants may serve as a GM microbe reservoir. GM microbes could also be spread by insects or other species (such as shrimps) which are the target of proposed applications. This includes humans who consume GM probiotics.

Knowing the limits of host species, e.g., for viruses, is very difficult, with the potential for spill-over events to non-target species (when a pathogen jumps from one species to another). Unintended impacts of the genetic engineering process or design may alter host range. Moreover, the premise that certain viruses such as bacteriophages are host specific, even if true, does not fully remove the potential for unintended impacts on non-target organisms. Bacterial communities are interconnected. As stated by Tanaka et al. (2024), "*If a microorganism, whose growth links to a target bacterium, has growth linkage with another microorganism, the addition of a phage infecting the target bacterium will cause change in the population of both bacteria by cascading effect*." Experiments assessing host specificity in more complex artificial systems with multiple bacteria suggest that specific phages that have previously been shown to have non-target effects on bacterial species may indeed do this via this indirect mechanism of altering species interactions, rather than by the direct infection of non-target organism.

Spread and persistence are also dependent on various factors including fitness of the microbe, which cannot be tested in the lab due to environmental mediators. For example, De Leij (1998) raises uncertainties regarding the ability to ensure against unwanted spread or persistence following release. While there is potential for modifications to reduce fitness/survival, etc., enhanced survival has been observed in some cases and thus uncertainties are significant with regard to making assumptions on persistence and spread. Moreover, while some microbes, such as bacteriophages, are thought to be host-specific, they are also, at the same time, being modified to broaden their host range or replication capacity, e.g., by modifying genes encoding for receptors that bind to target bacteria (such as tail spike proteins), for the purposes of their application. It should be remembered that it is not a single generation of GM microbes that will be released into the environment: open release applications depend on multiple generations of the microbe to survive. The potential for evolutionary change to alter these properties is also a heightened uncertainty with microbes that is difficult to ensure against prior to release.

The mode of application may also exacerbate spread. For example, intended applications that are designed as sprays for crop fields may increase air-borne spread, or unintended exposure in neighbouring farms. Just as the weedkiller dicamba has caused significant damage to neighbouring crops as a result of farmers applying it to dicamba-tolerant GMO crops, 'genetic rain' (Box G) in the form of GM microbes may also lead to unwanted exposure that is near, if not entirely, impossible to control against.

Box G: Genetic rain

Because of their small size and large numbers, microbes can be easily spread to distant habitats, including by the wind and rain. Strong winds lift dust particles, with microorganisms attached, up high in the atmosphere and can transport them for long distances. When it rains, many of these microbes return to earth. If GM microbes are released into the environment, this raises the concern that the novel genetic constructs they contain (including antibiotic resistance genes, for example) could return to earth as 'genetic rain'.

It is well-known that airborne viruses, bacteria, and fungal pathogens have the capability to cause disease in plants, animals, and humans over multiple distances - from near range to continental in scale (Dillon & Dillon, 2020). For example, long-distance atmospheric infectious disease dispersions (LDD), on the wind, have been shown to play a crucial role in the spread of plant pathogens. This can happen in several steps, or in a single step over long distances: for example, Asian soybean rust is thought to have been transported from South to North America, across the Caribbean, by Hurricane Ivan in 2004. Dust and sandstorm events, in particular, inject substantial quantities of foreign microorganisms into global ecosystems, with the ability to impact distant environments (Behzad et al., 2018).

Peter et al. (2024) explore how microorganisms can be transported long distances in the air and enter lakes as rain. They find that viable bacteria associated with Saharan dust can reach a high mountain lake in the Swiss Alps. In this study, rain events with Atlantic or continental origins were dominated by different bacteria from those associated with Saharan dust. Dust from the Sahara also transported nutrients and organic carbon, which might support the growth of the transported microbes. Similarly, Yahya et al. (2019) find that airborne microbes, carried to the Red Sea by dust, may potentially have a large impact on human health and on the Red Sea ecosystem. Rain drops, wind speed and direction are the main contributing factors to the deposition of airborne microbes. Viruses require a host to survive during transport and, thus, are typically associated with bacteria attached to particulate matter. Reche et al. (2018) find that deposition rates of bacteria are significantly higher during rain events and Saharan dust intrusions, whereas, in this study, in Spain's Sierra Nevada mountains, rainfall did not significantly influence virus deposition. Viruses were associated with smaller particles, so they could stay longer in the atmosphere and, consequently, be dispersed over greater differences.

Microbes that are transported in rain and snow can then impact on local ecosystems. For example, snow-derived bacteria can be deposited onto Arctic soils and some of these bacteria can colonise the soil (Malard & Pearce, 2022).

6.2 Horizontal gene transfer

Horizontal gene transfer (HGT) is the movement of genetic material from one organism to another, that is separate from the sexual transfer of genetic material from parents to offspring. This phenomenon raises the risk that altered genetic material may transfer from GM microbes to non-target organisms, including the potential jumping of genetic material into completely unrelated species. This concern is widely discussed, for example, with regard to the spread of antibiotic resistance genes from species to species which may threaten long term efficacy of medical and veterinary treatments.

HGT is long recognised as a 'pillar of bacterial evolution', alongside mutations and other factors (Arnold et al., 2022). However, it is also not exclusive to microorganisms and has gained increasing recognition as a phenomenon that can occur in eukaryotic organisms (organisms whose cells have a membrane-bound nucleus), such as fungi, plants and

animals. As such, HGT has been described as a ‘major force driving evolution’ in both single cell prokaryotes (organisms without a nucleus, i.e., bacteria and archaea) and eukaryotic organisms such as plants and animals, and the phenomenon behind the concept of a ‘web of life’ or ‘network of life’ (Qiu, 2005). Bacteria can use horizontal gene transfer (HGT) to adapt rapidly to unstable environments through the acquisition of new functions. Indeed, some consider that without taking HGT into account, ‘it is impossible to describe the evolution of microbial communities’. HGT is increasingly being detected in previously unexpected scenarios, including for example, the recent study finding the transfer of plant genes to sucking insects (Xia et al., 2021).

For microbiome applications such as probiotics, or those intended for animals such as cows and poultry, the gut is a well-known hotspot of HGT to other resident microbes in the gut. Indeed, a 2024 study found HGT occurring between gut microbes in patients who received faecal transplants as a medical treatment for obesity (Behling et al., 2024), prompting the authors to conclude that due to persistence of the transferred genes, “*HGT is relevant to the long-term modulation of the human gut microbiome*”. HGT occurs at high frequency within individual human guts, and comparison across human populations reveals that industrialized (and urban) lifestyles are associated with higher HGT rates (Groussin et al., 2021). Mammalian gut bacteria have experienced frequent HGT events over millions of years of evolution, however, the authors of this global study suggest that particularly high rates of HGT in the human gut may be a recent development in human history linked to industrialization. They suggest that this could contribute to low-grade chronic intestinal inflammation in healthy individuals and to the higher incidence of inflammation-associated diseases in the industrialized world. The human gut microbiome is a complex community with a vast network of microbe–host interactions and horizontal gene transfer (HGT) in the microbiome has profound consequences for human health and disease. HGT can occur in the gut via various mechanisms in bacteria, including via bacterial viruses, known as bacteriophages, described by some as ‘the most abundant gene-transfer particles in the human microbiome’ (Borodovich et al., 2022). Although less studied, there is also evidence for HGT in the human skin microbiome, suggesting a role in the evolution and adaptation of bacteria within the skin environment (Li et al., 2023).

HGT is thought to have profound implications for health and disease. For example, it may promote the transfer of virulence factors that determine how pathogenic bacteria are. What impacts any transfer of altered traits to other microbial communities in the gut will be very hard to predict, given the incomplete understanding of gut microbial composition and function/s. Beyond the gut, GM microbes that are consumed by people or animals may end up in the open environment, or waste water systems, providing another opportunity for HGT to occur. HGT has been associated with the transfer of antibiotics resistance genes in waste water treatment plants for example (Brown et al., 2024). In places where raw sewage is being dumped into the open environment, the opportunities for HGT only increase.

In the open environment, other ecological niches are also well known hotspots for HGT, including, for example the rhizosphere. This is the region of soil close to plant roots where many microbes reside, and where HGT may occur between bacteria, fungi and plants (Ku et al., 2021). Above ground, insects and other organisms are exposed to plant associated microbes, another potential source of HGT. A recent intriguing example is the whitefly acquisition of two genes from bacteria, both involved in nitrogen metabolism (Yang et al., 2024). The acquisition of these genes is now thought to underly the ability of this global pest to be able to consume a wide variety of plant species, as well possibly explain the increase in whitefly outbreaks following crop fertilizer treatments. Indeed, bacteria-derived HGT is thought to be common in insects (Husnik & McCutcheon, 2018; Li et al., 2022).

HGT raises the risk of transfer not only of engineered traits, but also of unintended mutations that may arise in a GM microbe, to other organisms. It may have a variety of implications

depending on the trait transferred, the nature of the unintended mutations, and the behaviour of the transferred DNA within the context of the genome and biology of the non-target organism and its environment. For example, virulence factors, or metabolic traits may increase pathogenicity of microbes, or offer selective advantage to particular microbial species, resulting in shifts in community composition, with potential impacts on the functions that such microbiome communities mediate, e.g., the mediation of human health by gut microbes (Borodovich et al., 2022; Dapa et al., 2023).

All of these factors are also subject to evolutionary changes in open release systems (see Section 6.3 Evolutionary dimensions below), further complicating the ability to predict with sufficient certainty, what the risks and likelihoods of HGT really are.

The main concern of this report is the deliberate release of living GM microbes into the environment. However, the risks of HGT are not restricted to live microbes alone. DNA from dead organisms can survive numerous environments, from human/animal digestion to open environments in soils for example. The use of dead GM microorganisms in foods for example thus does not ensure against the unwanted spread of engineered DNA to non-target species (see Section 6.5 Contained Use. How contained?).

6.3 Evolutionary dimensions

The open release of GM microorganisms raises significant concerns regarding any subsequent evolutionary changes to the microbe, changing, e.g., its persistence, pathogenicity or host range, or leading to the evolution of a novel pathogen that may have adverse impacts on food crops, animals or humans. For regulatory purposes, this aspect of microbial GMOs in particular, is a major challenge to assess prior to release. There are numerous ways in which microbes can evolve. Viruses for example, in addition to the potential for mutations to arise, are able to exchange genetic material with other viruses (a process called recombination), with the potential to, for example, alter host specificity. Bacteria also commonly exchange genetic information.

Several researchers have raised concerns regarding whether the changes introduced into GM microbes will be stable over time (e.g., Eckerstorfer et al., 2024; Zuber et al., 2021). A further concern is whether pests or diseases targeted for biocontrol by GM microbes will evolve resistance (Eckerstorfer et al., 2024). This may not only limit the efficacy of the approach, but also lead to pests and diseases that are more difficult to control. A wider perspective also requires consideration of how the GM microbe and its environment will co-evolve together.

Within the human gut, the introduction of new genetic variants can alter metabolism, the breakdown of drugs, or colonization resistance against pathogens (Dapa et al., 2023). As well as the transfer of specific traits from GM microorganisms to other microorganisms (discussed in Section 6.2 Horizontal gene transfer), the whole gut ecosystem may evolve, leading to significant changes in species composition. Even if new GM microbe pathogens are not directly introduced into the environment, existing pathogens also evolve within human and animal guts, and their evolution may utilise any newly introduced traits from GM microorganisms in unpredictable ways (Didelot et al., 2016; Lauring, 2020).

The dynamic nature of mutation and recombination events in wild global viromes (viral genomes), are speculated to play a defining role in various processes such as spillover events, when a pathogen jumps from one species to another (Apari & Földvári, 2023; Lentzos et al., 2022a), adding yet another layer of complexity to risk of unintended spread and persistence raised above. Which potential non-target species may get exposed, over evolutionary time-scales, thus becomes very difficult to predict and assess.

Various assumptions are made regarding evolutionary risks by developers that are not sufficient reliable for ensuring safety. For example, claims that 'vaccines' can be genetically 'fine-tuned' to have predetermined lifetimes in order to eliminate concerns over unwanted mutations or ongoing evolution, have been challenged by evolutionary biology and biosecurity experts. Lentzos et al., (2022) highlight the uncertainties of such an approach and the lack of experimental testing to assert that GM 'finetuning' can simultaneously both maintain functional transmissibility of the virus in order for it to work, while also limiting transmissibility sufficiently in order to control it.

Evolution within the engineered sequences themselves also raises specific risks. For example, environmental engineering applications that use viruses to deliver CRISPR-based tools to organisms in the wild, may develop mutations within the genome editing tool (e.g., within a guide RNA sequence, that functions as a guide to where the DNA is intended to be cut) that may alter its target sequence, which may lead to altered function and properties in the targeted organism. This may also render the product non-functional. Thus, evolutionary dynamics are also a significant risk to the long-term efficacy of GM applications.

6.4 Complexities and ignorance

The vast majority of microbes remain uncharacterised, and many pose risks to human health in unpredictable ways, though evidence suggest some microbes present in ecosystems may be capable of infecting people. Many microbes present in the rhizosphere for example, are thought to be opportunistic human pathogens (Berg et al., 2005).

The approach to microbial applications takes an overly reductionist approach to genetics, organisms and wider ecosystems. Microbes do not live in isolation, but function in a community with their own species, and with other microbial species, with complex interactions with their hosts, and the wider ecosystem. Knowledge of complex microbial communities such as the human microbiome for example, is only beginning to be investigated in a Western scientific framing, though it is already well understood that its disruption can have profound consequences on health and disease. Vast networks of microbe-host interactions, and horizontal gene transfer (HGT) capabilities raise the potential that GM microbe applications could thus result in not just unintended impacts on single species of organisms, but in a broader shift in microbial composition. In such complex ecosystems, all species interact in the same habitat and influence each other. Reducing organisms and introduced traits to single parts and pieces in isolation, rather than as part of larger units such as holobionts (an assemblage of a host and the many other species living in or around it), will fail to recognise all potential risks (Testbiotech, 2020).

Applicable to GM microbe applications is the trend to apply such technologies at scale, at ecosystem levels (Heinemann et al., 2021), assisted by the increasing efficiency of genetic engineering techniques being developed. Both intended and unintended mutations introduced by genome editing or other genetic technologies, are not reliant on the processes of evolution, but instead can be driven by human activity, to ensure such mutations establish and spread in the environment (Heinemann et al., 2021). For HEGAA technologies, using GM viruses that aim to modify plants in situations of potential crop failures caused by abiotic stress such as drought, would require large-scale modification of crop fields, as would biofertilisers or biocontrol products. The required spread of such applications would necessitate complex understanding and controllability to ensure sufficient spread for the application to work, but sufficiently controlled transmission in order to prevent spread beyond targeted organisms/areas. Whether such a delicate balance can be achieved has raised repeated concerns (Lentzos et al., 2022b; Pfeifer et al., 2022).

6.5 Contained Use. How contained?

Careful oversight is necessary to ensure that contained use applications do indeed, remain properly contained. With a potential increase in scale of contained use applications, the risks of environmental leaks increase, warranting careful oversight of biosafety practice in preventing unintended escapes. Despite this, some industry players appear to be advocating for weakening regulations on contamination events from contained use, such as the presence of engineered DNA in food products (Lensch et al., 2024)

Contamination and escape of micro-organisms has already been documented in several cases. Novo Nordisk, for example, has documented contamination with 3 different species of bacteria in three different batches of its weight-loss drugs. Reports of malpractice add to risks and are another aspect that must be properly regulated (FDA, 2023; Reuters, 2023).

A stark example is the detection of lab derived genetically engineered antibiotic resistance genes in 6 major Chinese rivers (J. Chen et al., 2012), with authors suggesting that such synthetic plasmids, originating from biotechnology experiments or applications, may represent a source of antibiotic resistance in humans. With regard to food applications, contamination has been documented including contamination of fermented foods with live GM bacteria (D'aes et al., 2022). Antibiotic resistance genes have also been detected in food enzyme products (Fraiture et al., 2024). Currently, antibiotic resistance genes (ARGs) are commonly added as markers to plasmids used in genetic engineering (see Box B). Plasmids require a marker to ensure both the initial selection of cells that contain them and the continued propagation of plasmid-containing, antibiotic-resistant cells. However, even in supposed contained-use settings, the disposal or leakage of organisms containing ARGs creates potential vectors for the propagation of harmful antibiotic resistance into the food chain or wider environment. ARGs in engineered laboratory strains can be spread via horizontal gene transfer (HGT) to human and agricultural pathogens, even if the engineered strains have been killed prior to disposal (see Section 6.2 Horizontal gene transfer). This could be avoided by using alternative methods (e.g., Amrofell et al., 2023). The use of antibiotic resistance marker genes is therefore not necessary and poses unnecessary risks to human and animal health and the environment.

Whilst the primary concern of this report is the deliberate open release of living GM microorganisms into the environment, these examples illustrate the need to also ensure that 'contained use' applications are properly contained.

6.6 Manufacturing/Application use exposure risks

The large-scale manufacture of GM microbes for contained use, or for environmental release, raises concerns regarding potential exposure of people working in the facilities to the GM microbe. Exposure may come from multiple routes including direct or indirect contact. Human exposure to GM virus applications, such as self-spreading vaccines, or inactivated viral vaccines may also occur in the lab, e.g., via shedding from inoculated test animals (Eckerstorfer et al., 2024).

6.7 Biosecurity Risks

The potential dual-use of microorganisms as pathogens has been a consistent concern with regard to the engineering of microorganisms. 'Dual-use' refers to technologies that can be used for both civil and military purposes. Dual-use risks can arise from both state or non-state actors working in biodefence research, with the potential for accidental or malicious releases increasing with the scale of the industry and technical ease with which microorganisms may be engineered. New technical developments in genetic engineering, as

well as other fields such as AI (that is increasingly being applied to biology), along with the accumulation of microbial databases, are all factors that increase biosecurity risks even further.

The policy interests of scientific research can also exacerbate biosecurity risks, with some projects, in particular, representing a political shift towards increasingly risky microbial research, or towards research goals previously considered too controversial to pursue, facilitating further the avenues within which dual use applications could be deployed.

Indeed, countries may seek to harness the synthetic biology field for their military defence industries, and some defence funds are going directly towards synthetic biology applications. While bioweapons programs are outlawed under international treaties of the UN, the remit of defence agencies are national security and not health or conservation, or agriculture. The influence of national security priorities thus warrants careful scrutiny in how they promote the likelihood of the release of potentially pathogenic microbes into the environment, either intentionally or unintentionally, via, for example, a lab escape.

For example, public funding via the US military DARPA projects that utilise recombinant viruses for applications such as public health as well as 'crop protection' via the Insect Allies Project are a case in point (see Section 4.3.2 *Self-spreading viral vaccines to prevent pathogen spill over from animals to people* and Section 3.1.2 *Biocontrol of agricultural pests and pathogens*). Dual-use applications for self-spreading 'vaccines' have been highlighted by UN expert working groups on Synthetic Biology under the Convention for Biological diversity that sets the international regulations for environmentally applied biotechnologies (CBD reference report mAHTEG, 2024). Scientists have also questioned the Insect Allies project for the potential for dual-use applications to deliberately destroy crop fields, pointing to the far higher ease with which viruses could be spread to crop to genetically modify it in order to *destroy* a crop than *rescue* it from an external threat such as a crop pest (Reeves et al., 2018).

6.8 Monitoring, traceability and irreversibility

Monitoring of the impacts of GMOs is an essential part of regulatory oversight that facilitates the management of any potential adverse outcomes and the implementation of necessary mitigation measures in case negative impacts arise. Whilst monitoring of 'contained use' applications should be feasible (see Section 6.5 Contained Use. How contained?), provided adequate safeguards are in place, monitoring of open release applications must be regarded as impossible, given the long distances that microbes can be transported, including across national boundaries, leading to a form of living pollution which can spread far outside the intended area of application (see Section 6.1 Uncontrolled Spread). Furthermore, microorganisms raise specific challenges to monitoring their potential impacts (e.g., on human and animal health and the environment) that make it extremely difficult to manage or mitigate any release of a microbe, with no ability to recall or reverse a release. The self-spreading capabilities of microbes, as they reproduce over multiple generations and evolve in response to their environment, makes monitoring and traceability impossible. Applications such as self-spreading vaccines are being developed explicitly for hard-to-reach populations, making it even harder to track or monitor the impacts of their release.

7. Conclusions

Contrary to established norms, the deliberate release of living genetically modified microorganisms, which can survive and reproduce in the environment, has recently begun, driven by commercial interests and new technological developments. Existing products are limited and do not appear to deliver on their claims, and future products, likewise, are at an

early stage of development and will face many technical and other challenges. Despite much hype, there is reason to be very sceptical of claimed future benefits. Nevertheless, GM bacteria, viruses, microalgae and fungi are already being genetically engineered for open release, with proposed applications in a wide variety of environments (e.g., in soil, freshwater and marine environments).

Allowing open releases of GM micro-organisms into the environment risks permanently (and negatively) altering complex ecosystems. Deliberate open releases could lead to 'living pollution' of all ecosystems: rivers, oceans, farmland, forests, grasslands, gardens, parks and nature reserves. It is impossible to predict the consequences of such releases as GM microorganisms interact and evolve with their environment, spreading new genetic constructs into other organisms. These GM microorganisms will be spread through a variety of mechanisms, such as sewage, insects, and genetic rain, and interact with the communities of microbes in human and animal guts and on skin. Within the human gut, for example, the introduction of new genetic variants can alter metabolism, the breakdown of drugs, and resistance against pathogens.

The need for a precautionary approach is enshrined in global environmental treaties such as the Cartagena Protocol on Biosafety to the UN Biological Convention on Biodiversity, and the Rio Declaration. This means that where there is a threat of serious or irreversible damage, lack of scientific certainty about the impacts shall not be used as a reason for postponing measures to prevent environmental degradation. This leads to the conclusion that GM microorganisms (including gene edited microorganisms) should not be deliberately released into the environment, due to the inability to predict and/or manage future adverse effects on human and animal health and the environment.

In addition, 'contained use' applications should be properly contained and this requires more scrutiny as more potential applications are developed on a larger scale.

References

ABC News. (2021, October 21). *Australian soil carbon startup Loam Bio raises $40 million from venture capitalists*. https://www.abc.net.au/news/rural/2021-10-28/carbon-soil-research-startup-loam-bio-attracts-investors-/100564724

ACB. (2020). GM Fungi to kill Mosquitoes. Illegal experiments conducted in Burkina Faso? *African Centre for Biodiversity*. https://acbio.org.za/wp-content/uploads/2022/04/gm-fungi-kill-mosquitoes-illegal-experiments-burkina-faso.pdf

ACB, ETC Group, & TWN. (2024). *'Black Box' Biotechnology – Integration of Artificial Intelligence with Synthetic Biology. Addressing the risks, hype, and inequities underpinning generative biology*. African Centre for Biodiversity, Third World Network & ETC group. https://acbio.org.za/wp-content/uploads/2024/09/Black-Box-Biotech-AI-Synth-bio-paper_fin.pdf

AFN. (2023, April 19). *Pivot Bio pilot replaces synthetic nitrogen on nearly 1m acres of farmland*. https://agfundernews.com/breaking-pivot-bio-pilot-replaces-synthetic-nitrogen-on-nearly-1m-acres-of-farmland

Alliance for Science. (2018, June 10). *Ginkgo Bioworks: Restoring pride to GMOs*. https://allianceforscience.org/blog/2018/07/ginkgo-bioworks-restoring-pride-gmos/

Apari, P., & Földvári, G. (2023). Domestication and microbiome succession may drive pathogen spillover. *Frontiers in Microbiology*, *14*, 1102337. https://doi.org/10.3389/fmicb.2023.1102337

Arnold, B. J., Huang, I.-T., & Hanage, W. P. (2022). Horizontal gene transfer and adaptive evolution in bacteria. *Nature Reviews Microbiology*, *20*(4), 206–218. https://doi.org/10.1038/s41579-021-00650-4

Ballester, A., Roqué, M., Ricci-Cabello, I., Rotger, A., & Malih, N. (2023). Horizon scanning on microorganisms and their products obtained by new developments in biotechnology. *EFSA Supporting Publications*, *20*(12). https://doi.org/10.2903/sp.efsa.2023.EN-8503

Bayer. (2022, October 18). *Bayer and Ginkgo Bioworks close deal creating Agricultural Biologicals Powerhouse*. https://www.bayer.com/media/en-us/bayer-and-ginkgo-bioworks-close-deal-creating-agricultural-biologicals-powerhouse/

Behling, A. H., Wilson, B. C., Ho, D., Cutfield, W. S., Vatanen, T., & O'Sullivan, J. M. (2024). Horizontal gene transfer after faecal microbiota transplantation in adolescents with obesity. *Microbiome*, *12*(1), 26. https://doi.org/10.1186/s40168-024-01748-6

Berg, G., Eberl, L., & Hartmann, A. (2005). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environmental Microbiology*, *7*(11), 1673–1685. https://doi.org/10.1111/j.1462-2920.2005.00891.x

Berkeley Yeast. (n.d.). *BENEFITS Brew smarter with bioengineered yeast*. https://berkeleyyeast.com/pages/berkeley-yeast-benefits

Biswas, S., Tian, J., Li, R., Chen, X., Luo, Z., Chen, M., Zhao, X., Zhang, D., Persson, S., Yuan, Z., & Shi, J. (2020). Investigation of CRISPR/Cas9-induced SD1 rice mutants highlights the importance of molecular characterization in plant molecular breeding. *Journal of Genetics and Genomics*, S1673852720300916. https://doi.org/10.1016/j.jgg.2020.04.004

Bloch, S. E., Clark, R., Gottlieb, S. S., Wood, L. K., Shah, N., Mak, S.-M., Lorigan, J. G., Johnson, J., Davis-Richardson, A. G., Williams, L., McKellar, M., Soriano, D., Petersen, M., Horton, A., Smith, O., Wu, L., Tung, E., Broglie, R., Tamsir, A., & Temme, K. (2020). Biological nitrogen fixation in maize: Optimizing nitrogenase expression in a root-associated diazotroph. *Journal of Experimental Botany*, *71*(15), 4591–4603. https://doi.org/10.1093/jxb/eraa176

Bloch, S. E., Temme, K., Tamsir, A., Higgins, D. A., Davis-Richardson, A. G., Clark, R., & Gottlieb, S. S. (2019). *Guided microbial remodeling, a platform for the rational improvement of microbial species for agriculture* (Patent WO2020006246A1). https://patents.google.com/patent/WO2020006246A1/en?oq=+WO%2f2020%2f006246

Borodovich, T., Shkoporov, A. N., Ross, R. P., & Hill, C. (2022). Phage-mediated horizontal gene transfer and its implications for the human gut microbiome. *Gastroenterology Report*, *10*, goac012. https://doi.org/10.1093/gastro/goac012

Brown, C. L., Maile-Moskowitz, A., Lopatkin, A. J., Xia, K., Logan, L. K., Davis, B. C., Zhang, L., Vikesland, P. J., & Pruden, A. (2024). Selection and horizontal gene transfer underlie microdiversity-level heterogeneity in resistance gene fate during wastewater treatment. *Nature Communications*, *15*(1), 5412. https://doi.org/10.1038/s41467-024-49742-8

Cai, L., Ju, F., & Zhang, T. (2014). Tracking human sewage microbiome in a municipal wastewater treatment plant. *Applied Microbiology and Biotechnology*, *98*(7), 3317–3326. https://doi.org/10.1007/s00253-013-5402-z

Can microbes save the planet? (2023). *Nature Biotechnology*, *41*(6), 735–735. https://doi.org/10.1038/s41587-023-01837-1
CBD multidisciplinary ad hoc technical expert group. (2024). *CBD/SBSTTA/26/4 Synthetic Biology*. Convention for Biological Diversity.
CCJ, CCP, & TWN. (2024). *Exporting Extinction How the international financial system constrains biodiverse futures*. The Centre for Climate Justice, Climate and Community Project, Third World Network. https://twn.my/announcement/Exporting%20Extinction%20-%20English.pdf
Chee, Y. L., & Hammond, E. (2016, October 18). Gene Editing and Seed Stealing. *Project Syndicate*. https://www.project-syndicate.org/commentary/protecting-biodiversity-gene-editing-by-chee-yoke-ling-and-edward-hammond-2016-10
Chen, D., Lan, Z., Hu, S., & Bai, Y. (2015). Effects of nitrogen enrichment on belowground communities in grassland: Relative role of soil nitrogen availability vs. soil acidification. *Soil Biology and Biochemistry*, *89*, 99–108. https://doi.org/10.1016/j.soilbio.2015.06.028
Chen, J., Jin, M., Qiu, Z.-G., Guo, C., Chen, Z.-L., Shen, Z.-Q., Wang, X.-W., & Li, J.-W. (2012). A Survey of Drug Resistance *bla* Genes Originating from Synthetic Plasmid Vectors in Six Chinese Rivers. *Environmental Science & Technology*, *46*(24), 13448–13454. https://doi.org/10.1021/es302760s
Cohen, S. N., Chang, A. C. Y., Boyer, H. W., & Helling, R. B. (1973). Construction of Biologically Functional Bacterial Plasmids *In Vitro*. *Proceedings of the National Academy of Sciences*, *70*(11), 3240–3244. https://doi.org/10.1073/pnas.70.11.3240
Conroy, G. (2024). Scientists edit the genes of gut bacteria in living mice. *Nature*, *631*(8022), 720–721. https://doi.org/10.1038/d41586-024-02238-3
Cruz, K. C. P., Enekegho, L. O., & Stuart, D. T. (2022). Bioengineered Probiotics: Synthetic Biology Can Provide Live Cell Therapeutics for the Treatment of Foodborne Diseases. *Frontiers in Bioengineering and Biotechnology*, *10*, 890479. https://doi.org/10.3389/fbioe.2022.890479
Cyprowski, M., Ławniczek-Wałczyk, A., Gołofit-Szymczak, M., Frączek, K., Kozdrój, J., & Górny, R. L. (2019). Bacterial aerosols in a municipal landfill environment. *Science of The Total Environment*, *660*, 288–296. https://doi.org/10.1016/j.scitotenv.2018.12.356
D'aes, J., Fraiture, M.-A., Bogaerts, B., De Keersmaecker, S. C. J., Roosens, N. H. C. J., & Vanneste, K. (2022). Metagenomic Characterization of Multiple Genetically Modified Bacillus Contaminations in Commercial Microbial Fermentation Products. *Life*, *12*(12), 1971. https://doi.org/10.3390/life12121971
Dal Bello, F., Bocquet, L., Bru, A., Laulund, S., Machielsen, R., Raneri, M., Sewalt, V., van Peij, N., Ville, P., Volonté, F., White, Y., & Rusek, J. (2024). New Genomic Techniques applied to food cultures: A powerful contribution to innovative, safe, and sustainable food products. *FEMS Microbiology Letters*, *371*, fnae010. https://doi.org/10.1093/femsle/fnae010
DARPA. (2022, February 16). *DARPA Program to Reduce Mosquito Attraction and Biting Moves into Second Phase*. https://www.darpa.mil/news-events/2022-02-16
DARPA Forward. (n.d.). https://forward.darpa.mil/presenters/Dr_Renee_Wegrzyn
Eckerstorfer, M. F., Dolezel, M., Miklau, M., Greiter, A., Heissenberger, A., & Engelhard, M. (2024). Scanning the Horizon for Environmental Applications of Genetically Modified Viruses Reveals Challenges for Their Environmental Risk Assessment. *International Journal of Molecular Sciences*, *25*(3), 1507. https://doi.org/10.3390/ijms25031507
ENSA. (2023, August 10). *An updated name to reflect our full vision: Enabling Nutrient Symbioses in Agriculture (ENSA)*. https://www.ensa.ac.uk/news/an-updated-name-to-reflect-our-full-vision-enabling-nutrient-symbioses-in-agriculture-ensa/
ENSSER. (2021). *SCIENTIFIC CRITIQUE OF LEOPOLDINA AND EASAC STATEMENTS ON GENOME EDITED PLANTS IN THE EU*. https://ensser.org/wp-content/uploads/2021/04/Greens-EFA-GMO-Study-1.pdf
EuroFruit. (2024, March 7). *Biome Makers database surpasses 24M microorganisms*. https://www.fruitnet.com/eurofruit/biome-makers-database-surpasses-24m-microorganisms/259027.article
Faltus, T. (2024). The Medicinal Phage—Regulatory Roadmap for Phage Therapy under EU Pharmaceutical Legislation. *Viruses*, *16*(3), 443. https://doi.org/10.3390/v16030443
FDA. (2023). FDA. https://www.fda.gov/media/172377/download
Financial Times. (2023, March 19). *Microbes on the farm: A solution for climate change?* https://www.ft.com/content/71422ca3-6cc8-46c3-9f59-768a501b85f3
FoE. (2023). *Genetically Engineered Soil Microbes: Risks and Concerns*. Friends of the Earth. https://foe.org/wp-content/uploads/2023/08/GE_Microbes_Report_Final.pdf
Folium Science. (n.d.). https://foliumscience.com

Fraiture, M.-A., Gobbo, A., Guillitte, C., Marchesi, U., Verginelli, D., De Greve, J., D'aes, J., Vanneste, K., Papazova, N., & Roosens, N. H. C. (2024). Pilot market surveillance of GMM contaminations in alpha-amylase food enzyme products: A detection strategy strengthened by a newly developed qPCR method targeting a GM Bacillus licheniformis producing alpha-amylase. *Food Chemistry. Molecular Sciences*, *8*, 100186. https://doi.org/10.1016/j.fochms.2023.100186

GeneWatch UK. (2021). *On-target effects of genome editing techniques: (Un)repaired DNA damage, a hindrance to safety and development?* GeneWatch UK. http://genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/genome-editing-techniques-fin.pdf

GeneWatch UK. (2022). *Time for the end of GM/GE herbicide tolerant crops?* GeneWatch UK. http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/ht-report-fin.pdf

Gingko Bioworks. (n.d.). *Strain engineering to help you produce active ingredients at scale*. https://www.ginkgobioworks.com/offerings/ag-biologicals-discovery-development/#production-strain-engineering

Ginkgo Bioworks. (n.d.). *Harness the full breadth of microbial biotechnology for agriculture*. https://www.ginkgobioworks.com/offerings/ag-biologicals-discovery-development/

Ginkgo Bioworks. (2024, August 29). *Vitales collaborates with Ginkgo to launch advanced biocontrol products for Brazilian agriculture*. https://www.ginkgobioworks.com/2024/08/29/vitales-collaborates-with-ginkgo-to-launch-advanced-biocontrol-products-for-brazilian-agriculture/

Gold, D. R., & Wright, R. (2005). POPULATION DISPARITIES IN ASTHMA. *Annual Review of Public Health*, *26*(1), 89–113. https://doi.org/10.1146/annurev.publhealth.26.021304.144528

Graham, E. B., Camargo, A. P., Wu, R., Neches, R. Y., Nolan, M., Paez-Espino, D., Kyrpides, N. C., Jansson, J. K., McDermott, J. E., Hofmockel, K. S., the Soil Virosphere Consortium, Blanchard, J. L., Liu, X. J. A., Rodrigues, J. L. M., Freedman, Z. B., Baldrian, P., Stursova, M., DeAngelis, K. M., Lee, S., … Pietrasiak, N. (2024). A global atlas of soil viruses reveals unexplored biodiversity and potential biogeochemical impacts. *Nature Microbiology*, *9*(7), 1873–1883. https://doi.org/10.1038/s41564-024-01686-x

Gupta, S., Allen-Vercoe, E., & Petrof, E. O. (2016). Fecal microbiota transplantation: In perspective. *Therapeutic Advances in Gastroenterology*, *9*(2), 229–239. https://doi.org/10.1177/1756283X15607414

Heinemann, J. A., & Walker, S. (2019). Environmentally applied nucleic acids and proteins for purposes of engineering changes to genes and other genetic material. *Biosafety and Health*, *1*(3), 113–123. https://doi.org/10.1016/j.bsheal.2019.09.003

Hoepers, A. M., Heinemann, J. A., Zanatta, C. B., Chu, P., Hiscox, T. C., & Agapito-Tenfen, S. Z. (2024). Predicted multispecies unintended effects from outdoor genome editing. *Ecotoxicology and Environmental Safety*, *282*, 116707. https://doi.org/10.1016/j.ecoenv.2024.116707

Hoffmann, D. E., Von Rosenvinge, E. C., Roghmann, M.-C., Palumbo, F. B., McDonald, D., & Ravel, J. (2024). The DTC microbiome testing industry needs more regulation. *Science*, *383*(6688), 1176–1179. https://doi.org/10.1126/science.adk4271

Husnik, F., & McCutcheon, J. P. (2018). Functional horizontal gene transfer from bacteria to eukaryotes. *Nature Reviews Microbiology*, *16*(2), 67–79. https://doi.org/10.1038/nrmicro.2017.137

InfoOGM. (2024, May 15). *Novonesis, a new Danish industrial giant promoting "biosolutions"?* https://infogm.org/en/novonesis-a-new-danish-industrial-giant-promoting-biosolutions/

Innovative Genomics Institute. (2023, April 17). *IGI's 'Audacious' New Frontier for CRISPR: Editing Microbiomes for Climate and Health*. https://innovativegenomics.org/news/audacious-project-crispr-microbiome/

ISAAA. (2018). *Global StatuS of CommerCialized biotech/Gm Crops in 2018: Biotech Crops Continue to help meet the Challenges of increased population and Climate Change*. ISAAA. https://www.isaaa.org/resources/publications/briefs/54/download/isaaa-brief-54-2018.pdf

Jiao, N., Zhu, L., & Zhu, R. (2024). The search for authentic microbiome–disease relationships. *Nature Medicine*, *30*(5), 1243–1244. https://doi.org/10.1038/s41591-024-02920-z

Kawall, K. (2019). New Possibilities on the Horizon: Genome Editing Makes the Whole Genome Accessible for Changes. *Frontiers in Plant Science*, *10*, 525. https://doi.org/10.3389/fpls.2019.00525

Ke, J., Wang, B., & Yoshikuni, Y. (2021). Microbiome Engineering: Synthetic Biology of Plant-Associated Microbiomes in Sustainable Agriculture. *Trends in Biotechnology*, *39*(3), 244–261. https://doi.org/10.1016/j.tibtech.2020.07.008

Kidd, J., Manning, P., Simkin, J., Peacock, S., & Stockdale, E. (2017). Impacts of 120 years of fertilizer addition on a temperate grassland ecosystem. *PLOS ONE*, *12*(3), e0174632. https://doi.org/10.1371/journal.pone.0174632

Koller, F., & Cieslak, M. (2023). A perspective from the EU: Unintended genetic changes in plants caused by NGT—their relevance for a comprehensive molecular characterisation and risk assessment. *Frontiers in Bioengineering and Biotechnology*, *11*, 1276226. https://doi.org/10.3389/fbioe.2023.1276226

Kosicki, M., Tomberg, K., & Bradley, A. (2018). Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. *Nature Biotechnology*, *36*(8), 765–771. https://doi.org/10.1038/nbt.4192

Ku, Y.-S., Wang, Z., Duan, S., & Lam, H.-M. (2021). Rhizospheric Communication through Mobile Genetic Element Transfers for the Regulation of Microbe-Plant Interactions. *Biology*, *10*(6), 477. https://doi.org/10.3390/biology10060477

Ledford, H. (2020). CRISPR gene editing in human embryos wreaks chromosomal mayhem. *Nature*, *583*(7814), 17–18. https://doi.org/10.1038/d41586-020-01906-4

Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. *Nature*, *528*(7580), 60–68. https://doi.org/10.1038/nature16069

Lensch, A., Lindfors, H. A., Duwenig, E., Fleischmann, T., Hjort, C., Kärenlampi, S. O., McMurtry, L., Melton, E.-D., Andersen, M. R., Skinner, R., Wyss, M., & Van Kranenburg, R. (2024). Safety aspects of microorganisms deliberately released into the environment. *EFB Bioeconomy Journal*, *4*, 100061. https://doi.org/10.1016/j.bioeco.2023.100061

Lentzos, F., Rybicki, E. P., Engelhard, M., Paterson, P., Sandholtz, W. A., & Reeves, R. G. (2022a). Eroding norms over release of self-spreading viruses. *Science*, *375*(6576), 31–33. https://doi.org/10.1126/science.abj5593

Lentzos, F., Rybicki, E. P., Engelhard, M., Paterson, P., Sandholtz, W. A., & Reeves, R. G. (2022b). Self-spreading vaccines: Base policy on evidence—Response. *Science*, *375*(6587), 1363–1363. https://doi.org/10.1126/science.abo1980

Li, Y., Liu, Z., Liu, C., Shi, Z., Pang, L., Chen, C., Chen, Y., Pan, R., Zhou, W., Chen, X.-X., Rokas, A., Huang, J., & Shen, X.-X. (2022). HGT is widespread in insects and contributes to male courtship in lepidopterans. *Cell*, *185*(16), 2975-2987.e10. https://doi.org/10.1016/j.cell.2022.06.014

Lyons, K. E., Ryan, C. A., Dempsey, E. M., Ross, R. P., & Stanton, C. (2020). Breast Milk, a Source of Beneficial Microbes and Associated Benefits for Infant Health. *Nutrients*, *12*(4), 1039. https://doi.org/10.3390/nu12041039

Ma, J., Lyu, Y., Liu, X., Jia, X., Cui, F., Wu, X., Deng, S., & Yue, C. (2022). Engineered probiotics. *Microbial Cell Factories*, *21*(1), 72. https://doi.org/10.1186/s12934-022-01799-0

Mao, D., Yu, S., Rysz, M., Luo, Y., Yang, F., Li, F., Hou, J., Mu, Q., & Alvarez, P. J. J. (2015). Prevalence and proliferation of antibiotic resistance genes in two municipal wastewater treatment plants. *Water Research*, *85*, 458–466. https://doi.org/10.1016/j.watres.2015.09.010

Martinez, A., De La Rosa, R., Mujahid, M., & Thakur, N. (2021). Structural racism and its pathways to asthma and atopic dermatitis. *Journal of Allergy and Clinical Immunology*, *148*(5), 1112–1120. https://doi.org/10.1016/j.jaci.2021.09.020

Mechan Llontop, M. E., Tian, L., Sharma, P., Heflin, L., Bernal-Galeano, V., Haak, D. C., Clarke, C. R., & Vinatzer, B. A. (2021). Experimental Evidence Pointing to Rain as a Reservoir of Tomato Phyllosphere Microbiota. *Phytobiomes Journal*, *5*(4), 382–399. https://doi.org/10.1094/PBIOMES-04-21-0025-R

Medicins San Fronriers. (2024, September 23). *Open letter to Novo Nordisk on barriers to accessing diabetes medicines*. https://www.msfaccess.org/open-letter-novo-nordisk-barriers-accessing-diabetes-medicines

Miklau, M., Burn, S.-J., Eckerstorfer, M., Dolezel, M., Greiter, A., Heissenberger, A., Hörtenhuber, S., Zollitsch, W., & Hagen, K. (2024). Horizon scanning of potential environmental applications of terrestrial animals, fish, algae and microorganisms produced by genetic modification, including the use of new genomic techniques. *Frontiers in Genome Editing*, *6*, 1376927. https://doi.org/10.3389/fgeed.2024.1376927

Mitsunaka, S., Yamazaki, K., Pramono, A. K., Ikeuchi, M., Kitao, T., Ohara, N., Kubori, T., Nagai, H., & Ando, H. (2022). Synthetic engineering and biological containment of bacteriophages. *Proceedings of the National Academy of Sciences*, *119*(48), e2206739119. https://doi.org/10.1073/pnas.2206739119

Murphy, A. A., Redwood, A. J., & Jarvis, M. A. (2016). Self-disseminating vaccines for emerging infectious diseases. *Expert Review of Vaccines*, *15*(1), 31–39. https://doi.org/10.1586/14760584.2016.1106942
National Academy of Medicine (U.S.), National Academy of Sciences (U.S.), & Royal Society (Great Britain) (Eds.). (2020). *Heritable human genome editing*. the National Academies Press.
Neckovic, A., Van Oorschot, R. A. H., Szkuta, B., & Durdle, A. (2020). Investigation of direct and indirect transfer of microbiomes between individuals. *Forensic Science International: Genetics*, *45*, 102212. https://doi.org/10.1016/j.fsigen.2019.102212
Norris, A. L., Lee, S. S., Greenlees, K. J., Tadesse, D. A., Miller, M. F., & Lombardi, H. A. (2020). Template plasmid integration in germline genome-edited cattle. *Nature Biotechnology*, *38*(2), 163–164. https://doi.org/10.1038/s41587-019-0394-6
Novo Nordisk Foundation: CO2 as a sustainable raw material in our future food production. (2023, June 13). *PR Newswire*. https://www.prnewswire.com/news-releases/novo-nordisk-foundation-co2-as-a-sustainable-raw-material-in-our-future-food-production-301848326.html
Novonesis. (n.d.-a). *Innova® Drive*. https://www.novozymes.com/en/products/ethanol/fermentation/innova-drive
Novonesis. (n.d.-b). *The era of biosolutions*. https://www.novonesis.com/en/era-of-biosolutions
Nuismer, S. L., Althouse, B. M., May, R., Bull, J. J., Stromberg, S. P., & Antia, R. (2016). Eradicating infectious disease using weakly transmissible vaccines. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1841), 20161903. https://doi.org/10.1098/rspb.2016.1903
Nuismer, S. L., & Bull, J. J. (2020). Self-disseminating vaccines to suppress zoonoses. *Nature Ecology & Evolution*, *4*(9), 1168–1173. https://doi.org/10.1038/s41559-020-1254-y
Ono, R., Ishii, M., Fujihara, Y., Kitazawa, M., Usami, T., Kaneko-Ishino, T., Kanno, J., Ikawa, M., & Ishino, F. (2015). Double strand break repair by capture of retrotransposon sequences and reverse-transcribed spliced mRNA sequences in mouse zygotes. *Scientific Reports*, *5*, 12281. https://doi.org/10.1038/srep12281
Ono, R., Yasuhiko, Y., Aisaki, K., Kitajima, S., Kanno, J., & Hirabayashi, Y. (2019). Exosome-mediated horizontal gene transfer occurs in double-strand break repair during genome editing. *Communications Biology*, *2*(1), 57. https://doi.org/10.1038/s42003-019-0300-2
Papathanasiou, S., Markoulaki, S., Blaine, L. J., Leibowitz, M. L., Zhang, C.-Z., Jaenisch, R., & Pellman, D. (2021). Whole chromosome loss and genomic instability in mouse embryos after CRISPR-Cas9 genome editing. *Nature Communications*, *12*(1), 5855. https://doi.org/10.1038/s41467-021-26097-y
Pearlman, D. N., Zierler, S., Meersman, S., Kim, H. K., Viner-Brown, S. I., & Caron, C. (2006). Race disparities in childhood asthma: Does where you live matter? *Journal of the National Medical Association*, *98*(2), 239–247.
Pfeifer, K., Frieß, J. L., & Giese, B. (2022). Insect allies-Assessment of a viral approach to plant genome editing. *Integrated Environmental Assessment and Management*, *18*(6), 1488–1499. https://doi.org/10.1002/ieam.4577
PHG Foundation. (n.d.). *Viruses for good – the case for phages*. . https://www.phgfoundation.org/blog/viruses-for-good-the-case-for-phages/.
PR Newswire. (2023, March 28). *PacBio and Corteva Agriscience Enable Groundbreaking Plant and Microbial Long-Read Sequencing Workflow on Revio System*. https://www.prnewswire.com/news-releases/pacbio-and-corteva-agriscience-enable-groundbreaking-plant-and-microbial-long-read-sequencing-workflow-on-revio-system-301782411.html
PREEMPT Project. (n.d.). https://ohi.vetmed.ucdavis.edu/programs-projects/preempt
Qiu, S. (2005). A computational study of off-target effects of RNA interference. *Nucleic Acids Research*, *33*(6), 1834–1847. https://doi.org/10.1093/nar/gki324
Ramakrishnan, N. (2023). Bio-surveillance as One Health: A Critique of Recent Definitions and Policy Initiatives. *Development*, *66*(3–4), 215–225. https://doi.org/10.1057/s41301-023-00396-x
Rathbone, C., & Ullah, S. (2023). Ammonia emissions from nitrogen fertilised agricultural soils: Controlling factors and solutions for emission reduction. *Environmental Chemistry*, *21*(1). https://doi.org/10.1071/EN23010
Redmond, C., Akinoso-Imran, A. Q., Heaney, L. G., Sheikh, A., Kee, F., & Busby, J. (2022). Socioeconomic disparities in asthma health care utilization, exacerbations, and mortality: A systematic review and meta-analysis. *Journal of Allergy and Clinical Immunology*, *149*(5), 1617–1627. https://doi.org/10.1016/j.jaci.2021.10.007

Reeves, R. G., Voeneky, S., Caetano-Anollés, D., Beck, F., & Boëte, C. (2018). Agricultural research, or a new bioweapon system? *Science*, *362*(6410), 35–37. https://doi.org/10.1126/science.aat7664

Regalado, A. (2021, August 24). *Is Ginkgo's synthetic-biology story worth $15 billion?* https://www.technologyreview.com/2021/08/24/1032308/is-ginkgos-synthetic-biology-story-worth-15-billion/

Reuters. (2023, September 21). *FDA found lapses at Novo Nordisk's main US factory in May 2022, report says*. https://www.reuters.com/business/healthcare-pharmaceuticals/us-fda-found-lapses-novos-main-us-factory-may-2022-report-2023-09-20/

Rubin, B. E., Diamond, S., Cress, B. F., Crits-Christoph, A., Lou, Y. C., Borges, A. L., Shivram, H., He, C., Xu, M., Zhou, Z., Smith, S. J., Rovinsky, R., Smock, D. C. J., Tang, K., Owens, T. K., Krishnappa, N., Sachdeva, R., Barrangou, R., Deutschbauer, A. M., … Doudna, J. A. (2021). Species- and site-specific genome editing in complex bacterial communities. *Nature Microbiology*, *7*(1), 34–47. https://doi.org/10.1038/s41564-021-01014-7

Science. (2024, August 22). *Synthetic biology, once hailed as a moneymaker, meets tough times*. https://www.science.org/content/article/synthetic-biology-once-hailed-moneymaker-meets-tough-times

Sessitsch, A., Wakelin, S., Schloter, M., Maguin, E., Cernava, T., Champomier-Verges, M.-C., Charles, T. C., Cotter, P. D., Ferrocino, I., Kriaa, A., Lebre, P., Cowan, D., Lange, L., Kiran, S., Markiewicz, L., Meisner, A., Olivares, M., Sarand, I., Schelkle, B., … Kostic, T. (2023). Microbiome Interconnectedness throughout Environments with Major Consequences for Healthy People and a Healthy Planet. *Microbiology and Molecular Biology Reviews*, *87*(3), e00212-22. https://doi.org/10.1128/mmbr.00212-22

Simon, S., Otto, M., & Engelhard, M. (2018). Synthetic gene drive: Between continuity and novelty: Crucial differences between gene drive and genetically modified organisms require an adapted risk assessment for their use. *EMBO Reports*, *19*(5). https://doi.org/10.15252/embr.201845760

Sirinathsinghji, E. (2019). *Transferring the laboratory to the wild: An emerging era of environmental genetic engineering*. Third World Network. https://biosafety-info.net/wp-content/uploads/2019/11/Biosafety-briefing_From-lab-to-wild.pdf

Sirinathsinghji, E. (2022). *Bt Crops Past Their Sell-By Date: A Failing Technology Searching for New Markets?* (TWN Biotechnology & Biosafety Series 19). Third World Network and GeneWatch UK. https://www.twn.my/title2/biosafety/bio19.htm

Smits, A. H., Ziebell, F., Joberty, G., Zinn, N., Mueller, W. F., Clauder-Münster, S., Eberhard, D., Fälth Savitski, M., Grandi, P., Jakob, P., Michon, A.-M., Sun, H., Tessmer, K., Bürckstümmer, T., Bantscheff, M., Steinmetz, L. M., Drewes, G., & Huber, W. (2019). Biological plasticity rescues target activity in CRISPR knock outs. *Nature Methods*, *16*(11), 1087–1093. https://doi.org/10.1038/s41592-019-0614-5

Symborg. (n.d.). https://symborg.com/en/what-we-do/

Synlogic. (n.d.). *Synlogic Announces Decision to Discontinue Synpheny-3 Study and Provides Corporate Update*. https://investor.synlogictx.com/news-releases/news-release-details/synlogic-announces-decision-discontinue-synpheny-3-study-and

Tang, S.-S., Biswas, S. K., Tan, W. S., Saha, A. K., & Leo, B.-F. (2019). Efficacy and potential of phage therapy against multidrug resistant *Shigella* spp. *PeerJ*, *7*, e6225. https://doi.org/10.7717/peerj.6225

Tuladhar, R., Yeu, Y., Tyler Piazza, J., Tan, Z., Rene Clemenceau, J., Wu, X., Barrett, Q., Herbert, J., Mathews, D. H., Kim, J., Hyun Hwang, T., & Lum, L. (2019). CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation. *Nature Communications*, *10*(1), 4056. https://doi.org/10.1038/s41467-019-12028-5

Valles-Colomer, M., Blanco-Míguez, A., Manghi, P., Asnicar, F., Dubois, L., Golzato, D., Armanini, F., Cumbo, F., Huang, K. D., Manara, S., Masetti, G., Pinto, F., Piperni, E., Punčochář, M., Ricci, L., Zolfo, M., Farrant, O., Goncalves, A., Selma-Royo, M., … Segata, N. (2023). The person-to-person transmission landscape of the gut and oral microbiomes. *Nature*, *614*(7946), 125–135. https://doi.org/10.1038/s41586-022-05620-1

Vockley, J., Sondheimer, N., Puurunen, M., Diaz, G. A., Ginevic, I., Grange, D. K., Harding, C., Northrup, H., Phillips, J. A., Searle, S., Thomas, J. A., Zori, R., Denney, W. S., Ernst, S. L., Humphreys, K., McWhorter, N., Kurtz, C., & Brennan, A. M. (2023). Efficacy and safety of a synthetic biotic for treatment of phenylketonuria: A phase 2 clinical trial. *Nature Metabolism*, *5*(10), 1685–1690. https://doi.org/10.1038/s42255-023-00897-6

Wen, A., Havens, K. L., Bloch, S. E., Shah, N., Higgins, D. A., Davis-Richardson, A. G., Sharon, J., Rezaei, F., Mohiti-Asli, M., Johnson, A., Abud, G., Ane, J.-M., Maeda, J., Infante, V., Gottlieb, S. S., Lorigan, J. G., Williams, L., Horton, A., McKellar, M., … Temme, K. (2021). Enabling Biological Nitrogen Fixation for Cereal Crops in Fertilized Fields. *ACS Synthetic Biology*, *10*(12), 3264–3277. https://doi.org/10.1021/acssynbio.1c00049

Wong, C. (2024). Largest genetic database of marine microbes could aid drug discovery. *Nature*, d41586-024-00133–00135. https://doi.org/10.1038/d41586-024-00133-5

Xia, J., Guo, Z., Yang, Z., Han, H., Wang, S., Xu, H., Yang, X., Yang, F., Wu, Q., Xie, W., Zhou, X., Dermauw, W., Turlings, T. C. J., & Zhang, Y. (2021). Whitefly hijacks a plant detoxification gene that neutralizes plant toxins. *Cell*, *184*(7), 1693-1705.e17. https://doi.org/10.1016/j.cell.2021.02.014

Yang, Z., Guo, Z., Gong, C., Xia, J., Hu, Y., Zhong, J., Yang, X., Xie, W., Wang, S., Wu, Q., Ye, W., Liu, B., Zhou, X., Turlings, T. C. J., & Zhang, Y. (2024). Two horizontally acquired bacterial genes steer the exceptionally efficient and flexible nitrogenous waste cycling in whiteflies. *Science Advances*, *10*(5), eadi3105. https://doi.org/10.1126/sciadv.adi3105

Zhang, L., Chen, H., Gao, S., Song, Y., Zhao, Y., Tang, W., & Cui, J. (2024). Antibiotic resistance genes and mobile genetic elements in different rivers: The link with antibiotics, microbial communities, and human activities. *Science of The Total Environment*, *919*, 170788. https://doi.org/10.1016/j.scitotenv.2024.170788

Zhang, T., Li, M., Shi, T., Yan, Y., Niyazbekova, Z., Wang, X., Li, Z., & Jiang, Y. (2022). Transmission of the gut microbiome in cohousing goats and pigs. *Frontiers in Microbiology*, *13*, 948617. https://doi.org/10.3389/fmicb.2022.948617

Zhivkoplias, E., Jouffray, J.-B., Dunshirn, P., Pranindita, A., & Blasiak, R. (2024). Growing prominence of deep-sea life in marine bioprospecting. *Nature Sustainability*, *7*(8), 1027–1037. https://doi.org/10.1038/s41893-024-01392-w

Zhu, C., Bortesi, L., Baysal, C., Twyman, R. M., Fischer, R., Capell, T., Schillberg, S., & Christou, P. (2017). Characteristics of Genome Editing Mutations in Cereal Crops. *Trends in Plant Science*, *22*(1), 38–52. https://doi.org/10.1016/j.tplants.2016.08.009

Zhivkoplias, E., Jouffray, J.-B., Dunshirn, P., Pranindita, A., & Blasiak, R. (2024). Growing prominence of deep-sea life in marine bioprospecting. *Nature Sustainability*, *7*(8), 1027–1037. https://doi.org/10.1038/s41893-024-01392-w