Perspectives on Genetically Engineered Microorganisms and Their Regulation in the United States

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***Abstract*—Genetically engineered microorganisms (GEMs) offer a new way to respond to the needs of a growing, changing world. GEMs are being used in agriculture, food production and additives, manufacturing, and commodity and non-commodity products, environmental remediation, etc., and there are further applications in development. Coupled with modern advances in genome manipulating technologies, new manufacturing processes, markets and attitudes are propelling a boom of new products containing or made from GEMs. As a result, researchers and developers are on track to interact with biotechnology regulatory policies that have been in place for decades, and are out of sync with fast-paced scientific advances and knowledge. In the United States, biotechnology regulation is done by multiple agencies with overlapping jurisdictions, which is difficult for both regulators and developers, as they must simultaneously ensure a burgeoning innovation and product market, along with safety/efficacy for the public and environment. This article aims to provide clarity on the factors that interact between regulatory policy and the development of GEMs in the United States, with thoughts from regulators and developers. We will provide summaries of a workshop held at the University of California, Berkeley in 2022, where regulators from U.S. regulatory agencies and industry were convened.**

***Index Terms*—microorganisms, genetic engineering, biotechnology, synthetic biology, regulations, science policy,**

1. INTRODUCTION

Genetic engineering, as the direct manipulation of genomes through either recombinant DNA or molecular biology, has thrust both our understanding and that of biological systems into a new biotechnology era. Breaking through these boundaries are new developments in genome editing, oligonucleotide synthesis, sequencing and bioinformatics, which has pushed us into synthetic biology, or, the de novo synthesis of life. Most of these changes began with the smallest and therefore, most manipulatable life forms, microorganisms. Their small size and rapid generation time allows researchers to implement genetic changes and isolates engineered organisms in a much lower time frame. As a consequence, genetic engineering of microbes has enabled the production of numerous products. Today, microbes are used in several businesses to produce high-value chemicals, such as additives, pharmaceuticals, fragrances, and flavors. Common antibiotics (penicillin, erythromycin, vancomycin, etc.), food additives (vitamins, monosodium glutamate), and pharmaceuticals have been scale produced using microbial biosynthesis. Biomaterials, pesticides, and environmental remediation strategies can be developed from engineered microorganisms, and other types of microbial products can be made using the same methods. Any future that includes genetically engineered microorganisms must also promote responsible design and regulation. The regulation of bioengineered products is a challenge that also must keep pace with the development of bioengineered products, and, at the same time be compatible with products already in the pipeline.

Data Collection

The Future of Microbial Biotechnology: From Research to Regulation In February 2022, the Innovative Genomics Institute, the USDA, and Phytobiomes Alliance organized a workshop that encouraged open discussions among regulators, scientists, and developers about the future of genetically engineered microorganisms (GEMs). This workshop was public entitled: "The Future of

Microbial Biotechnology: from Research to Regulation". The intention of this workshop was to gather those who research, develop, and regulate GEMs and products into a collaborative space to better understand each group's pursuits with GEMs. The participants listened, explained, and emphasized that they use engineering strategies to use the capabilities already provided through natural microbes, and many participants used both natural microbes and engineered microbes to achieve the same desired outcomes. While it was likely that the participants also used several classic wild-type microorganisms, participants articulated that new products resulting from developing wild-type microorganisms appears small and as a result, synthetic biology and genetic engineering have good opportunities. The timing of the workshop is relevant for the ongoing quick transformation in biotechnology, particularly GEMs. Biotechnology, and to a larger extent GEMs, have received attention as a solution to seemingly intractable problems such changes in climate. Climate change now requires that we start using a broad range of solutions quickly, and engaging in a wider array of diverse solutions to help address emergent global crises

GEMs: What are They?

For thousands of years, microorganisms have been controlled and cultivated in the development of food, fuel, and materials. Microbial processes have always been part of the human experience for thousands of years, even before human knowledge of microbial existence in the production of bread, cheese, wine, and yogurt. The notion of microorganisms causing disease and food spoilage was only discovered as recently as the 1800s, which pushed the desire to manipulate microorganisms for all sorts of new possibilities. Today, genetic engineering of microorganisms is becoming more and more common as a new way to make new products and new processes. In this review we use the phrase "genetically engineered microorganism" or GEMs to define a microbial organism that has an altered genome as a result of biotechnology methods.

There is no defined scientific definition of a microbe or microorganism, and microorganisms encompass entities from all three domains of life (Archaea, Bacteria, and Eukarya). It is more functionally defined as "whole organisms which are measured on a microscopic scale." Global definitional boundaries for the word vary based on what is being defined and regulated. The United Nations Food and Agriculture Organization (FAO) defines microorganisms as "a protozoan, fungus, bacterium, virus, or other microscopic self-replicating biotic entity." United States regulatory agencies tend to use the following definition of microorganisms: yeasts, molds, bacteria, viruses, protozoa, and microscopic parasites, [including]

GEMs: What Are They?

Microorganisms have been manipulated and controlled for thousands of years when it comes to growth of food, fuel, and materials. Almost from the beginning of human civilization, microbial processes were part of the experience of people. Long before humans knew about microbes, we used microbial processes in the production of bread, cheese, wine, and yogurt; it was only in the 1800s that people discovered that microorganisms could also be the causative agents of disease and food spoilage, fueling the desire to manage them for untold new purposes. Today, the genetic engineering of microorganisms for new products and processes is commonplace. In this review we use the term "genetically engineered microorganisms" or GEMs to describe a microbial organism that has a change in its genome due to biotechnological processes.

Advances in Genetic Engineering of Microorganisms

The field of microbial genetic engineering has come a long way since the early recombinant DNA studies of the 1970s, with several recent developments including: CRISPR-Cas Systems: which allow precise and efficient genome editing. Synthetic Biology: which enables the design of completely new biological pathways. Metabolic Engineering: which is the optimization of metabolic processes to increase the yield of desired products.

Scientific Perspectives

- Potential and Promise

GEMs (genetically engineered microorganisms) can be used to produce goods and services at scale and sustainably. There are high levels of precision engineering with GEMs, which opens up opportunities to rely less on traditional (generally polluting) industrial processes.

- Technical Challenges

While immeasurable progress has been made there are still challenges to address:

- the stability of the engineered traits
- the risks of horizontal gene transfer
- containment and control in natural environments.

Ethical and Societal Perspectives

➢ Ethical Issues

- Playing God: Concerns about fundamentally manipulating life.
- Dual Use: Potential for misuse in bioterrorism or manipulations with harmful effects.
- Informed Consent: Potentially for communities impacted by field trials.

➢ Public Perception and Engagement

Public skepticism about any biotech is often seeded in lack of transparency or understanding. Therefore, effective communication with the public about the science is key to developing trust and support.

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Food and Drug Administration

FDA, as the main regulatory body for food safety in the U.S., oversees any GEM product or additive when it is intended for human consumption. There is a variety of ways that FDA regulates GEMs, depending on the product; therefore, there are a variety of terms and definitions that FDA has for these products.

The FDA Center for Food Safety and Applied Nutrition (CFSAN) ensures the safety of foods and food additives through a review process to determine if products are safe or "Generally Recognized As Safe" (GRAS). GRAS is either, scientific data about the substance is publicly available, and there is a consensus among qualified experts that the substance is safe, or the substance has been in common use since 1958 and therefore its safety is determined based on common use.

Any food additive from a GEM or GEM based must be shown to be safe or GRAS prior to market, the data would be provided to CFSAN for approval. If a GEM is present in food, CFSAN too would assess the food for safety, the same as it would any other food In the case of enzymes that are derivatives of GEMs, CFSAN makes recommendations to the affected parties, instead of doing direct review.

Environmental Protection Agency

The EPA also has many offices and statutes regarding the regulation of GEMs in situations where environmental release could become a problem. The EPA Office of Pesticide Programs (OPP) has several functional offices that regulate biopesticides, including GEMs and GM-derived chemicals. The EPA has the authority to set limits on food and food subjected to pesticide residue limitation. Under the Toxic substances Control Act (TSCA), the Office of Pollution Prevention and Toxics (OPPT) regulates different classes of substances, which includes bioremediation agents, biofertilizers, and compounds used to produce biofuels, all of which may utilize GEMs or GM-derived materials. Therefore, EPA has wide authority over any GEMs that could potentially be used to (or are intended to) proliferate in the environment.

The Biopesticides and Pollution Prevention Division (BPPD) contained within OPP regulates biologically-based pesticides, including products based on GEMs or include GEMs (e.g., bacteriophages and fungicides). Like any new formulated pesticide, even if it has previous literature data based on GEMs or GEM-derived products, must provide biological safety data for human exposure, environmental exposure, and any societal and economic impacts to the OPP for permission to use it. OPP also sets a maximum.

GEMs in Food and Agriculture

GEMs used in food and agriculture include food ingredients and processing aids, feed additives, microbes to change plant–microbe interactions or to enhance nutrient availability, and biological pesticides. Although there are many applications in early discovery stages, here we focus on GEM products that are in – or nearing – commercialization.

Food Ingredients and Processing Aids

One of the areas of significant use of microbial biotechnology is food enzymes and additives. Biotechnology has been used for a long time to manufacture chymosin, an enzyme key to rennet production, which is used in most hard cheeses sold commercially. Another dairy product, yogurt, is where CRISPR systems were first discovered as scientists tried to engineer bacteria at Danisco.

There has been a huge uptick in the use of genetic engineering to make beer using engineered strains of brewer's yeast created by companies such as Omega Yeast and Berkeley Yeast. Up to this point there have been many GRAS notices submitted to the FDA by developers of engineered brewer's yeast strains, and most have received a "no questions" response from the FDA.A similarly new area is that of replacement meat using products derived from microbes as a form of replacement.

Nutrient Availability

Microbes are intimately associated with plants and are important for a number of key plant metabolic processes.) Nitrogen (N), phosphorus (P), and potassium (K) are three macronutrients for plant growth and functioning that are traditionally applied to agricultural land in the form of synthetically-derived fertilizers. Microorganisms mediate the bioavailability of all three of these nutrients to the plant. There has been a concentrated effort towards genetically engineering microorganisms to ameliorate the need for synthetic fertilizer or decrease the amount necessary.Nitrogen fixation is a process in which legume plants interact with microorganisms capable of fixing atmospheric nitrogen and ultimately providing nitrogen in the form of ammonia to the plant. Although there has been some advancement in improving this process in legumes that are able to form symbiosis with nitrogen fixing bacteria, to date engineering approaches have had limited value in cereal crops that account for the majority of calories of the world and most of the agricultural synthetic nitrogen fertilizer use.

The distressed state of nitrogen limitation is currently addressed via the industrialized Haber-Bosch process, which combines atmospheric nitrogen and hydrogen gas under high temperature and pressure, to produce ammonia. (There are a number of implications of the energy necessary to use the industrial approach to producing nitrogen fertilizer, but the long-term implications of the release of greenhouse gases (GHG) during the production period, of nitrogen fertilizer is staggering.

Pesticides

Biological pesticides are a key component of advanced agricultural practices. Prevalent in the organic sector, biological pesticides are appealing based on their specificity, low toxicity to nontarget taxa, and biodegradability relative to chemically synthesised, broad-spectrum pesticides. The biopesticide strain Bacillus thuringiensis (Bt) is one of the most commonly used biopesticides which has been marketed as a commercial product to pesticidal lepidopteran pests, because it produces proteins that compromise digestive function. A common practice has been to model the specific genes of B. thuringiensis into the DNA of crop plants to reduce the need for the application of insecticide externally.One example of a microbial biopesticide is a fungicidal amoeba developed by the French company Amoéba. This product has gained approval for field trials in Europe. The engineered amoeba Willaertia magna C2c Maky consumes the fungi causing wheat rust and has similar effectiveness to chemical fungicides, according to the company's press release. Velifer is another commercial product by BASF, which is a strain of the fungus Beauveria bassiana that acts as a biopesticide targeting many insects and plant pathogenic bacteria. Other fungi are being investigated with respect to biocontrol, as a means of reducing toxicity to nontarget organisms, relative to chemical means.

2. CONCLUSIONS

Since the inception of the Coordinated Framework in 1986, biotechnology regulation has occasionally failed to keep pace with the rate of scientific advancement; and this is inevitable (Scientific and technical advancement occurs continuously, exponentially, and often unpredictably, while statutes and regulations evolve steadily over periods of years.) Safeguarding the safety associated with the use of any biotechnology product being made available to the public requires a rigorous assessment which generally means that regulation must lag behind the development of the technology. Advances in biotechnology have come quickly in recent years due to advances in gene-editing, genetic sequencing, nucleic acid synthesis, data science and artificial intelligence, for these reasons developers are capable of creating unparalleled advance in bringing new products to the market. Oversight to approve these products however still involves an arduous and slow review process and consequently requires a harmonization of the oversight of biotechnologies.One takeaway from our workshop was the need for more clarity and synergy between the regulatory approaches called for in the Coordinated Framework. In conjunction with that, approvals require a more flexible and nimble approach to evaluating products so that there is a process for expeditiously approving, or denying, products. In many cases, the approvals processes across multiple agencies and offices has been lengthy and complicated multi-step process. Genetically engineered microorganisms (GEMs) are uniquely positioned at the cutting edge of innovation across biotechnology, environment, and medicine. They are capable of performing highly specific biological functions such as degrading toxic compounds, producing pharmaceuticals, and improving agriculture. GEMs are ideal for improving existing practices, as well as developing new approaches in emerging fields—synthetic biology, precision medicine, and green chemistry.

From an environmental perspective, GEMs have the potential to sustainably address long-standing challenges like heavy metal contamination, oil spills, and greenhouse gas emissions. In medicine, GEMs are rapidly emerging as important tools for targeted drug delivery, probiotic development, and biomanufacturing vaccines. At the same time, advances in CRISPR-Cas systems and synthetic gene circuits are expanding and strengthening the ability to engineer microorganisms with safety and efficacy.

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