Innovative Genomics Institute

Re: Docket No. APHIS-2024-0002 – Exploring Pathways to Commercialization for Modified Microbes

September 3, 2024

Attn: United States Department of Agriculture, Animal and Plant Health Inspection Service

Re: Docket No. APHIS-2024-0002

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The Innovative Genomics Institute (IGI), a public, academic research institute formed in partnership between multiple University of California campuses, below submits comments on the USDA-APHIS request for information on *Exploring Pathways to Commercialization for Modified Microbes*. We thank the Agency for prioritizing this emerging area of research and development and providing an opportunity to share our thoughts on the regulation of genetically modified microbes.

This request for information is particularly relevant to the IGI, as our researchers have recently embarked on a novel project to advance precision microbiome editing, which entails genome editing of target microbial communities in their natural environment. Part of our work focuses on precisely editing methane-producing microbes in the cow rumen in an effort to curb agricultural methane emissions.

We applaud USDA-APHIS for its focus on emerging technologies, such as microbial gene editing, and hope our comments are helpful in the development of regulatory frameworks for research and commercialization. This response reflects the perspectives of a range of scientists across various disciplines who stand ready to address any questions or provide further information as needed.

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Question 1: Describe new or emerging categories of biotechnology products that are relevant to the development and use of modified microorganisms. To assess new and emerging technologies with modified microbes, what expertise and resources are needed in the government to evaluate the overall plant pest risk of modified microbes?

Some emerging areas of research and biotechnological innovation are:

- **Genetic engineering of phages** for various purposes, such as: biocontrol agents selectively infecting harmful bacteria to promote crop health; disease surveillance and management by detecting specific pathogenic bacterial strains; development of diagnostic tools and biosensors owing to their protein or antibody phage display property; advanced wastewater treatment by targeting bacterial contaminants; vaccine delivery vehicles; deploying targeted CRISPR edits (e.g., in the context of precision microbiome editing).
- **Community editing of microbiomes**, which involves targeted genome editing of microbes within their native community and environments. Although community editing of microbes is still in the early stages of research, scientific advances such as genome-resolved metagenomics - an approach to reconstruct all the individual microbial genomes in a complex community - and CRISPR genome editing, have opened avenues for researchers to make precise edits in native microbial communities, such as gut microbiome, soil microbiome, plant microbiomes, etc. Such edits could remove or introduce one or more microbial functions, alter abundance of a particular strain or species or change interactions within the community. Unlike previously where microbes were first isolated and genetically engineered, researchers are now able to simultaneously introduce multiple edits into multiple microbes *in situ,* both the culturable and unculturable microbes. Such precise interventions stand in contrast with broad-spectrum approaches, such as the use of antibiotics or antifungals. The ability to precisely edit one or a subset of microbes within a community could introduce beneficial functions or remove harmful features without significant disruptions to the broader community of microorganisms. Reports by Sheth et al. (2016) and Rubin et al. (2022) provide more in-depth discussions into microbial community editing.

Considering the substantial pace at which the field of genetically engineered microbes is evolving, we recommend that USDA-APHIS maintain adequate resources for the evaluation of these products and train existing staff and/or bring on additional experts, as needed, including: microbial ecologists, phage biologists, computational biologists, general microbiologists, plant pathologists, plant microbiologists, soil microbial specialists, bioinformaticians, and synthetic biologists.

Question 2: Describe areas where the clarity and/or efficiency of regulations governing modified microorganisms could be improved (*e.g.,* definitions that need to be provided or revised, barriers to obtaining the data necessary to achieve commercialization).

Genetically Engineered Microbes (GEMs) that modify other microorganisms: Some GEMs are developed purposely as tools to modify other microorganisms, such as in the gut, and are afterwards eliminated, such that they do not constitute the final modified microbe or will not be present in the final

product (e.g., genetically modified phages). Will such intermediary microorganisms require separate USDA-APHIS regulatory approval, or would the Agency regulate the tool and the end product as one application in such cases?

Community editing of Microbes: As research into community editing of microbes gains momentum and products that can alter microbiomes *in situ* become a reality, we offer the following suggestions:

a. We encourage USDA-APHIS to take community microbial engineering into account in regulatory guidances. For instance, the USDA-APHIS could engage with stakeholders and experts to develop a clear regulatory definition of community editing of microbes and to have a common understanding of what this may include:
    i. How is a microbial community defined?
    ii. How narrowly is a community genome edit defined on the taxonomic level? Should it be confined at species, genus or a higher level? What constitutes an off-target event?
    iii. How are bacteriocins (bacterially-derived antimicrobial peptides) with the ability to impact microbial community composition defined? What types of products should be included/excluded from a regulatory definition of community editing?

b. How do engineered probiotics that alter the genome of target species in the microbiome (i.e., GEMs that modify other microbes) differ from those that introduce a new function into the microbiome, or edit community makeup, without editing any native species' genome (e.g., by promoting or suppressing specific microbial functions or by selectively killing specific microbial species)? How does regulatory oversight differ between engineered probiotics intended to be transiently present (such as those that modify target microbes) and those that persist in the microbial community (such as those that confer a desired function or induce an intended change in the microbial community)?

c. Single or multiple edits can be introduced into multiple microbial strains and/or species simultaneously using community editing techniques. This may be necessary when modulating a gene present in multiple strains/species; how will these be regulated? Establishing an exemption framework similar to that for modified plants (i.e., single edit in one species) is not suitable for community editing technologies.

APHIS Plant Pest List: We propose that APHIS continually update the plant pest list to reflect current scientific evidence and relate new genomic findings to phytopathogenicity. We ask that such updates and additional evidence be communicated clearly to stakeholders.

Classification in the APHIS BRS Draft Microbial Plant Pest List: How do 'high', 'medium', 'low' and 'not yet determined' evidence categories translate in practice? Does editing a 'low evidence' or "not yet determined" pest translate to exemptions? There is need for clear experimental data on what is a plant pest risk and we recommend that APHIS BRS should not label as pathogenic what is not known to be or only loosely related to a pathogen.
Additionally, community editing enables researchers to make edits in microbes that are unculturable. We recognize that some unculturable microbes are already included in the 'high evidence' category of the draft microbial plant pest list. However, it is unclear what evidence is used to develop and maintain the list of plant pests or what evidence developers should seek to collect when intending to modify an

unculturable microbe not already identified by USDA. We ask that USDA further elucidate what constitutes a 'significant' body of evidence to establish a causal link?

Pathogenic sequences database: How is BRS helping researchers identify pathogenic genetic sequences, especially for high throughput research? We recommend provision of a database that catalogs the nucleic acid sequences of pathogenic microbes or of virulent factors so that users can profile whether the microbe/DNA sequence they are working with or intend to modify is pathogenic. These databases will be especially useful and cost effective for academic or small/medium scale biotech developers.

Inter-Agency coordination: As it currently stands, a microbial product developer needs to consult USDA, EPA, and FDA, their different offices, and statutes to know exactly what regulations apply to them - often creating confusion. There is overlap and redundancy of the information required. While we acknowledge that efforts are being made such as the exemption of EPA regulated microbial pesticides that do not pose a plant pest risk from the APHIS biotechnology regulation 7 CFR part 340, more can be done. We recommend that the agencies categorically indicate what is regulated by other agencies and what they **do not** regulate for clarity purposes and ease of compliance.

Question 3: Describe key elements of a regulatory framework that would enable a scientifically sound assessment of a modified microorganism's plant pest risk, in order to inform regulatory decision-making by APHIS.

a. **Describe any biological features of microorganisms that APHIS should consider when determining whether a modification changes the plant pest risk, and thus the regulatory status of a modified microorganism (e.g., the potential for horizontal gene transfer, the production of airborne spores, its ecological role, or the ability to remain dormant for long periods of time).**

Given the complexity of microbial communities, and the range of emerging technologies that enable their modification, we propose that USDA-APHIS develop a tiered regulatory framework that considers the sum of factors that increase or attenuate plant pest risk of a modified microbe. Below we outline several factors that could change plant pest risk. We encourage the Agency to establish such a tiered framework in consultation with a range of relevant experts. We also refer to these lists below in our discussion of risk-based exemptions.

In order to determine under which tier a novel modified microbe should fall, USDA-APHIS should outline evaluation strategies for plant pest risk in the context of escalating trials. For example, data on the impacts of a genetically engineered microbe or editing tool on plant growth, root development, photosynthetic capacity, and so on could first be tested in a vat and a greenhouse, which should then serve as the basis for determination of the need for trials in a semi-contained field. Where plant pest risk is high, metagenomic and metatranscriptomic methods can help understand how native microbial communities in target and non-target environments may be altered by the introduction of a modified microbe. They can also help understand the impact of various environmental conditions, such as humidity, temperature, soil composition, etc., on the survivability and spread of a modified microbe. We do note that field trials can act as a critical barrier to obtaining the necessary data for further

development due to cost. We therefore propose that a tiered regulatory framework also explore to what extent field trials are needed and outline the essential data requirements for continued development in each tier.

Biological features that may increase plant pest risk:

- Modifications encoded within highly mobile elements, especially plasmids and phages, without additional mitigation sequences (see below)
- Editing systems that can self-perpetuate and/or are mobile, increasing the ability to spread to non-target microorganisms
- Modifications introduced through self-mobilization elements whose dynamics are poorly understood within native environments. If these elements are used in the process of engineering a modified microbe, but inactivated or removed in the end product, this should not be considered an increased plant pest risk.
- Modifications that provide fitness advantages dependent on factors that are highly abundant in the environment.
- DNA sequences that can enhance pathogenicity, such as persistence, virulence, antibiotic resistance, or mechanisms that co-opt plants to support the microbial life cycle. Such sequences should be regulated even if not derived from a known plant pest.
- Modifications that permanently enhance a microbe's capacity to transmit or receive DNA sequences. Examples include suppression of a microorganism's immune response with the goal of increasing its ability to uptake external DNA, or modifications that increase the ability to take up naked DNA from the environment. However, if such changes, which may be useful in engineering a modified organism, are transient, they should not be grounds for inclusion in a higher regulatory tier.

Biological factors that may reduce plant pest risk:

- Engineering of stable kill switches
- Integration of target DNA sequences into or genetic edits of chromosomal regions that are not expected to mobilize
- Regulation of a plasmid (or other mobile element) expressing a desired function could be dependent on an engineered modification stably integrated into chromosomal regions that are not expected to mobilize
- Engineered systems that encode a bacterial toxin onto a mobile genetic element (e.g., plasmid or phage) and an anti-toxin into a chromosomal region not expected to mobilize
- Engineering of orthogonal DNA sequences and macromolecular machinery to translate non-canonical amino acids, minimizing impacts in native microbes including plant pests
- Engineering of sequences that render a microbe susceptible to microbial inhibitory compounds present or being applied to the environment. For example, a cow rumen microbe could be made susceptible to an inhibitory compound abundantly present on farms in the Midwest or that could be introduced onto farms without negative ecological impacts.
- Engineering growth dependencies that are conditional on the presence of externally provided and/or synthetic factors
- Engineering mobilization systems that are unable to move out of a non-target recipient cell to reduce the risks of horizontal gene transfer. This could be particularly impactful if combined with a secondary mitigation strategy, such as toxicity in non-target microbes.

- Engineering oxygen sensitivity to minimize survivability in non-target environments (e.g., anaerobic microbes being exposed to aerobic environments). This could be augmented by DNA sequences encoding non-canonical amino acids to limit use of naked DNA taken up by environmental microorganisms.

Lastly, because novel technologies may enable editing of microbes that are unculturable in the laboratory setting, making studies of their plant pest risk more challenging or potentially unfeasible, we propose that BRS regulate these microbes based on sequence similarity with known plant pests. For example, editing of an unculturable, anaerobic microbe that may be released into the environment through wastewater or manure should be classified as 'low risk', and regulated accordingly, if it shares little sequence similarity/identity with a known plant pest and does not contain mobilizable virulence genes. To implement this, we suggest that USDA-APHIS, in collaboration with experts, develop a searchable database of microbial genomic sequences with annotations of pathogenic sequences. The Agency should then clearly define the sequence similarity/identity thresholds that would result in regulatory action.

b. What criteria, data, and information should be considered when assessing a modified microorganism's plant pest risk?

USDA-APHIS should develop a regulatory framework that considers the impacts of the aforementioned criteria and biological features on plant pest risk. In addition, the Agency could explore the spatial and temporal survivability (and spread, if applicable) of modified microbes in non-target environments, especially where they interact with plants. Soil microcosm studies could help better understand specific risks. USDA-NIFA could direct some BRAG funding to help fund such risk evaluation studies and generate the necessary evidence basis. This is especially critical where microbes with moderate-to-high plant pest risk may be released into environments where related microbial species are abundant. If such a framework is developed, clear thresholds for regulatory consequences should be communicated to stakeholders.

With respect to risks posed by horizontal gene transfer (HGT), this remains a relatively poorly understood process, making it challenging to draw conclusions about plant pest risk. It is highly likely that HGT will be detected if tested; however, it is unclear what an 'acceptable' level of HGT is. Critically, we do not advocate for the Agency to outright dismiss HGT from its assessment of plant pest risk. Rather, it would be helpful if USDA-APHIS, in collaboration with experts, developed experimental methods to clearly interpret data on HGT. For example, the Agency could develop a laboratory test to evaluate the potential of a modified microbe to transfer the engineered DNA sequence to a panel of plant pests, especially those with a greater degree of relatedness or sequence similarity, over a specified period of time. Another test should establish whether such HGT results in increased virulence or persistence of any tested plant pest. These data could be used to help determine under which regulatory tier a modified microbe should fall, as discussed above.

Question 5: Should APHIS consider risk-based exemptions for certain types of microorganisms, or for certain modifications in microorganisms? If so, please provide examples of the types of modified microorganisms that should be exempt from regulation and provide scientific

evidence to support which modifications and types of microorganisms should or should not be exempt.

We propose that USDA-APHIS develop risk-based exemptions as part of the tiered regulatory approach described above. While there may be certain types of commonly used and well-characterized modified microorganisms that could be exempted from regulations already, our focus is on a risk-based exemption pathway for novel GEMs. Generally speaking, highly specific approaches (e.g., by targeting species-specific sequences with guide RNAs and employing delivery methods that minimize entry into non-target microbial species) should be more amenable to deregulation, while broad-spectrum modifications that (could) impact a wide range of species should remain subject to USDA-APHIS regulations, unless they are tuned to only express in specific species. With this in mind, the Agency should develop a framework under which developers are able to obtain risk-based exemptions; categories could include:

a. **If the plant pest risk of the modified microbe is inherently low**, e.g., the modification is unlikely to enhance persistence or virulence of known plant pests, the base microbe is not itself known, or predicted based on sequence analysis, to be a plant pest, and the modification of the microbe does not render it a plant pest. Such an exemption could also be granted to different strains of a microbe that has been previously exempted, as long as the modifications do not encode biological features that increase plant pest risk.

b. **If a microbe is modified without introduction of exogenous DNA and edits do not affect pathogenicity or ecological behavior**, e.g., as determined by sequence comparison with known plant pest pathogenic sequences

c. **If engineered biological features sufficiently attenuate risks**. USDA-APHIS could implement a risk score for various biological features that reduce plant pest risk based on their ability to limit the spread or persistence of engineered microbes or their DNA sequences.

Use of such exemption criteria should be data-driven and could be based on laboratory experiments or trials in greenhouses. USDA-APHIS should make data about exemptions publicly available and continually evolve its regulatory framework with learnings from the field. Importantly, where risk-based exemptions are granted, developers should be able to obtain such a determination in writing. In cases where USDA-APHIS does not consider a modified microbe to be within its jurisdiction, a Letter of No Jurisdiction should be made available to developers. Similarly, USDA-APHIS could clearly state whether a microbe's absence from the plant pest list excludes it from regulation and provide a Letter of No Jurisdiction upon request.

Conclusion

Genetic engineering of microbes is moving at a rapid pace, with novel technologies enabling a future in which precision microbiome editing could address a range of challenges faced in agriculture and by the environment. To ensure both continued innovation and responsible and ethical development and deployment of these technologies, developers need clear regulatory frameworks that remove redundancies and assess risk based on robust scientific evidence. Specifically, we seek clarification on definitions for microbial community editing and some novel targets for engineering; we propose a tiered

regulatory framework that considers how engineered biological features alter plant pest risk; and we propose a dynamic risk-based exemption framework that evolves with current scientific evidence.

We commend USDA-APHIS for prioritizing this area of biotechnological innovation and hope that our comments will be helpful in establishing balanced, evidence-based regulations. We look forward to continued engagement from USDA on this topic and welcome opportunities for IGI experts to further inform the regulatory agencies tasked with implementation of the Coordinated Framework.

References

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