

Davis, July 22, 2025

Bernadette Juarez
APHIS Deputy Administrator
Biotechnology Regulatory Services
4700 River Rd, Unit 98
Riverdale, MD 20737

Re: Request for confirmation of regulatory status – Am I Regulated – strains of *Paenibacillus polymyxa* gene-edited with intragenomic insertions

Dear Ms. Juarez,

BioConsortia, Inc. hereby respectfully requests that USDA/APHIS/BRS confirms the regulatory status for the transport, field testing, and commercialization of BioConsortia's gene-edited strains of the diazotrophic *Paenibacillus polymyxa* with several combinations of modifications, including coding and non-coding insertions derived from other *Paenibacillus* species, such as *Paenibacillus durus*, *Paenibacillus odorifer*, *Paenibacillus typhae*, *Paenibacillus graminis*, *Paenibacillus borealis*, and *Paenibacillus donghaensis*.

We include in this Am I Regulated inquiry strains that have already been developed, named BEC265, BEC267, BEC268, BEC269, BEC273, BEC284, and BEC295, but we also extend our claim of BRS no jurisdiction for future *Paenibacillus polymyxa* strains to be developed by BioConsortia with similar purpose and quality control. This request is based on BioConsortia's assertion that:

- The parent strains are not plant pests
- The gene edits are specifically to improve nitrogen fixation activities
- []
- The strains as edited are not a plant pest
- USDA has confirmed that another gene-edited *Paenibacillus polymyxa* strain with similar modifications developed by BioConsortia should not be regulated pursuant to 7 CFR part 340 (25-007-02air)

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Background

BioConsortia is a private US corporation formed in 2014 for the purpose of discovering and developing microbial solutions for increased plant growth and health, pest control and plant yield enhancement. Utilizing its proprietary Advanced Microbial Selection™ (AMS) methods (US Patent 9,777,267), microbes are selected by plants in iterative rounds of plant cultivation in randomly collected soil samples. AMS selected microbes are the basis for our research and development activities because

the microbes in these experiments interact closely with the plants, have evolved along with the plants, and do not exhibit any harmful effects on the plants. BioConsortia has developed several of these plant-associated microbes as fungicides, nematicides, insecticides, biostimulants and nitrogen fixing agents.

It is no secret that one of the greatest contributors to yield in modern agriculture practice is the implementation of effective fertilizer programs. It is reported that by 2010, average US corn yields were nearly 160 bushels per acre, which is more than a six-fold increase in yield in a period of 70 years, much of this credited to the availability of low cost fertilizers created by converting Nitrogen (N) in the air and Hydrogen in water into ammonia, a form of nitrogen readily taken up by plants, using Haber-Bosch process. Nitrogen fertilizer is, in fact, a critical enabler of modern food systems which endeavor to feed the nearly 9 billion people on Earth.

However, synthesis and application of nitrogen fertilizers is not without environmental impact. The production process is energy intensive, requiring about 0.65 tons of natural gas to make 1 ton of ammonia. Much of this nitrogen fertilizer is applied on our best agricultural land in an environment where nitrogen may be lost by nitrate leaching, some of which may reach the marine environment and cause eutrophication of our waterways. Ammonia volatilization from fertilizers and animal manures may also cause eutrophication and soil acidification. Finally, some nitrogen may be lost as nitrous oxide by denitrification and nitrification; and nitrous oxide is a strong greenhouse gas.

Since 2018, BioConsortia has developed and grown its gene editing expertise and capabilities, concentrating its efforts on gram positive spore-forming diazotrophs, trickier to edit, but with greater commercial applicability due to their robust nature. Focusing primarily on *Paenibacillus* and like genera of microbes, BioConsortia successfully edited several potential N-fixing leads.

BioConsortia purposefully selected and advanced its research and development using *Paenibacillus* because of their fit for commercial use due to their long shelf life and applicability as seed treatments, spore-forming capabilities, general safety profile, and abundance in the environment.

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Extensive literature and experimental data provide comprehensive and reliable evidence that BioConsortia's wild-type and gene-edited *Paenibacillus polymyxa* strains are not plant pests and, therefore, they should not be subject to regulation under 7 CFR 340. We hereby respectfully request that BRS confirm our position of BRS' no jurisdiction for the strains outlined in this inquiry and other future *Paenibacillus polymyxa* strains with similar modifications to be developed by BioConsortia, including coding and non-coding DNA deletions and/or intrageneric modifications and proven absence of genetic material from organisms that meet the definition of "plant pest" in 7 CFR 340.

1. Developer (Responsible Party) name and contact information, including email address.

Christina Huben
 Senior Vice President Operations & Administration
chuben@bioconsortia.com

2. Taxonomic description of organism (genus, species, and subspecies, if relevant).

The gene-edited strains outlined in this inquiry are variants of the naturally occurring *Paenibacillus polymyxa* strains BEC150, BEC172 and BEC258.

The BEC150 strain was isolated from the [

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] These strains include *Paenibacillus odorifer*, *Paenibacillus typhae*, *Paenibacillus graminis*, *Paenibacillus borealis*, and *Paenibacillus donghaensis*.

The taxonomical species identification of all strains is based on whole genome sequence analyses.

3. Description of intended phenotype(s).

The strains considered in this inquiry are variants of the parent *Paenibacillus polymyxa* strains BEC150, BEC172 and BEC258 and were gene-edited to improve their nitrogen fixation capabilities. [

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P. polymyxa nitrogen fixation gene cluster consists of nine *nif* genes, flanked by genes coding for a hypothetical protein upstream and an ABC transporter downstream (Wang, et al., 2013). The promoter region of the *nif* operon in diazotrophic *Paenibacillus* strains has a *glnR* gene and GlnR/TnrA-binding sites, evidencing the role of GlnR as a regulator of nitrogen fixation (Xie, et al., 2014). In fact, studies have demonstrated that GS stabilizes the binding of GlnR to nitrogen fixation gene operators in *Paenibacillus riograndensis* SBR5 (Fernandes, Hauf, Sant'Anna, Forchhammer, & Passaglia, 2017).

GlnR and TnrA are transcriptional regulators for the nitrogen metabolism in the Gram-positive model organism *Bacillus subtilis* (Kormelink, et al., 2012), forming weak dimers by hydrophobic residues

located on its winged-HTH and residues in its N-terminal helix (Schumacher, Chinnam, Cuthbert, Tonthat, & Whitfill, 2015). Under excess nitrogen, GlnR of *B. subtilis* generally acts as a repressor repressing gene or operons required for ammonium assimilation (Schreier, Brown, Hirschi, Nomellini, & Sonenshein, 1989). In this condition, glutamine (Gln) binds to and feedback inhibits glutamine synthetase (GS, the product of *glnA*) by forming the complex FBI-GS. Formation of the feedback-inhibited GS (FBI-GS) signals the presence of excess nitrogen and transmits that signal by interacting with and affecting the DNA binding and transcription programs of both GlnR and TnrA. FBI-GS functions as a chaperone by a transient interaction with the GlnR autoinhibitory domain and relieves autoinhibition, shifting the equilibrium from the inhibited form to the DNA-binding active form and thus turning on GlnR repression (Wray-Jr. & Fisher, 2008) (Fisher & Wray-Jr., 2007). Under nitrogen limitation, the C-terminal region of GlnR folds back and forms an autoinhibitory helix that prevents dimer formation and thus inhibits DNA binding (Wray-Jr. & Fisher, 2008) (Fisher & Wray-Jr., 2007). In contrast to nitrogen limited conditions, under excess nitrogen, FBI-GS forms a stable complex with TnrA, inhibiting its DNA-binding function. Under nitrogen limitation, TnrA is released from FBI-GS, allowing TnrA dimerization and activation of its transcription program (Brandenburg, et al., 2002) (Belitsky, Wray-Jr., Fisher, Bohannon, & Sonenshein, 2000).

In species of *Paenibacillus* like *P. polymyxa* and *P. odorifer* under nitrogen limitation, GlnR binds to GlnR-binding site I located upstream of -35/-10 regions of *nif* promoter of *nif* operon in a weak and transient association way, activating its transcription. Under conditions of excess nitrogen, glutamine (Gln) binds to and feedback inhibits glutamine synthetase (GS) by forming the complex FBI-GS. FBI-GS interacts with C-terminal domain of GlnR and stabilizes the binding of GlnR to site II located downstream of *nifB* transcription start codon and thus represses *nif* transcription (Wang, et al., 2018).

Given the current understanding of nitrogen fixation by *Paenibacillus* bacteria, one can devise several modifications to the genome that release the stringent control of transcription and translation of the naturally occurring nitrogen fixation genes and ancillary nitrogen fixation regulation genes. The gene edited bacteria described in this document are examples of these efforts.

As a result of the modifications, the strains considered in this application have significantly higher nitrogen fixation activities than their wildtype parents, evidenced in the lab by Acetylene Reduction Assay in the presence of ammonium. [

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4. Description of intended activity (movement or release).

The intended activities for the gene-edited strains of *Paenibacillus polymyxa* are both movement and release.

5. Description of intended genetic change in final product (e.g., insertion, deletion, substitution, other).

The genetic changes in the strains considered in this inquiry are listed below. [

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To create the genetic modifications, we employ the natural process of homologous recombination - exchange of highly similar DNA sequences, from plasmids transformed into the wild-type strain or edited strain, when making combinations of edits. The methods outlined are the same for all modifications, but the specific tools (plasmids) used for each modification are different and will be described next.

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Note that there are no inserted genes or promoters that remain in the final product, and the edits occurred as described above and only in the intended loci, confirmed by genome sequencing.

The names of the specific constructs used to edit the strains considered in this inquiry are detailed in the section below, along with their description.

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9. Description of scientific methodology that you used or intend to use to confirm that the intended genetic changes were achieved.

BioConsortia's gene-editing process includes subjecting all strains to rigorous quality control for the intended edits and complete elimination of the editing plasmid by PCR, Sanger sequencing of the edited region and homology regions, antibiotic sensitivity, and whole genome sequencing. No genes or DNA from plant pests have been inserted into the modified strains considered in this inquiry. Thus, no unintended consequences to the bacteria or metabolic pathways are expected. [

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Given the current understanding of *Paenibacillus polymyxa* species and the targeted nature of our gene editing, we do not perceive any risks associated with potential non-target effects.

We appreciate your consideration and are available to answer any questions you may have regarding this matter.

Sincerely,



Christina E. Huben

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