



Brinda Mahadevan, Ph.D.  
Brincor Associates, LLC  
6056 Haybury Drive  
New Albany, OH 43054

Re: GRAS Notice No. GRN 001159

Dear Dr. Mahadevan:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001159. We received the notice that you submitted on behalf of Pellucid Lifesciences Pvt., Limited (Pellucid) on August 10, 2023, and filed it on December 5, 2023. Pellucid submitted amendments to the notice on February 15, 2024, March 4, 2024, and March 21, 2024, providing clarifying information on the intended use, manufacturing process, specifications, and safety.

The subject of the notice is *Heyndrickxia coagulans* strain MTCC 25460<sup>1</sup> spore preparation for use as an ingredient at a maximum level of  $2 \times 10^9$  colony forming units (CFU)/serving in baked goods and baking mixes; breakfast cereals; non-alcoholic beverages and beverages bases; coffee and tea; milk and milk products; dairy product alternatives; fruit juices; condiments and relishes; confections and frostings; frozen dairy desserts and mixes; fruit and water ices; jams and jellies; gelatins, puddings and fillings; grain products and pastas; hard candy; soft candy; chewing gum; extracts, flavorings, herbs, seeds, spices, seasonings, and blends; nuts and nut products; plant protein products; processed fruits; processed vegetables and vegetable juices; snack foods; soups and soup mixes; sugar; and sweet sauces, toppings, and syrups.<sup>2</sup> The notice informs us of Pellucid's view that these uses of *H. coagulans* MTCC 25460 spore preparation are GRAS through scientific procedures.

Pellucid describes *H. coagulans* MTCC 25460 spore preparation as a free flowing, white to pale brownish powder. Pellucid states that *H. coagulans* MTCC 25460 was isolated from soil and is a Gram-positive, spore forming, rod-shaped, slightly acidophilic, thermotolerant, aerobic to microaerophilic bacterium. Pellucid discusses the results of phenotypic and genotypic characterization used to confirm the strain's identity. Pellucid states that *H. coagulans* MTCC 25460 is deposited in the Indian Microbial Type Culture Collection and Gene Bank (MTCC).

---

<sup>1</sup> Pellucid states that *Bacillus coagulans* was reclassified as *Weizmannia coagulans* as reported in Gupta, et al. (Ref. 1) We note that *Weizmannia coagulans* was reclassified as *Heyndrickxia coagulans* as reported in Narsing Rao, et al. (Ref. 2)

<sup>2</sup> Pellucid states that *H. coagulans* MTCC 25460 is not intended for use in infant formula, foods formulated for infants, in products under the jurisdiction of the United States Department of Agriculture, or in foods where standards of identity preclude its use.

Pellucid describes the manufacturing process for *H. coagulans* MTCC 25460 spore preparation, stating that it is produced by fermentation of a pure culture of *H. coagulans* MTCC 25460 in a contained, sterile environment. The fermentation process is monitored for temperature, dissolved oxygen, and pH. Pellucid states that microscopy checks are performed to confirm the morphological parameters of *H. coagulans* MTCC 25460 and ensure the production of spores. After fermentation is complete, the bacterial culture is centrifuged to separate the spore biomass from the fermentation media. The biomass undergoes diafiltration using phosphate buffered saline and is then subjected to spray drying and then optionally blended with formulating agents such as maltodextrin. Pellucid states that *H. coagulans* MTCC 25460 spore preparation is manufactured according to current good manufacturing practices and that all raw materials used in the manufacturing process are food grade. Pellucid states that *H. coagulans* MTCC 25460 spore preparation does not contain any major allergens.

Pellucid provides specifications for *H. coagulans* MTCC 25460 spore preparation, including spore count ( $\geq 1.5 \times 10^{11}$  CFU/g); loss on drying ( $\leq 7\%$  w/w at 105 °C for 1 hour); lactic acid producing capacity ( $\geq 10$  ml of 0.05 N NaOH consumed); heavy metals, including lead ( $\leq 0.2$  mg/kg); and limits for other microorganisms, including yeast and mold ( $\leq 100$  CFU/g), *Escherichia coli* (negative in 10 g), *Salmonella* (negative in 10 g), *Pseudomonas aeruginosa* (negative in 1 g), *Staphylococcus aureus* (negative in 1 g), and *Bacillus cereus* (not more than 10 CFU/g). Pellucid provides the results from the analyses of three non-consecutive batches to demonstrate that the *H. coagulans* MTCC 25460 spore preparation can be manufactured to meet these specifications. Based on the results of stability testing, Pellucid concludes that the shelf life of *H. coagulans* MTCC 25460 spore preparation is 36 months at 25° C and 60% relative humidity.

Pellucid states that the intended uses of *H. coagulans* MTCC 25460 are the same as those described in GRN 000949.<sup>3</sup> Pellucid states that because the intended uses of *H. coagulans* MTCC 25460 are substitutional to the current uses of other strains of *H. coagulans*, there will be no increase in the dietary exposure to *H. coagulans* of  $36.4 \times 10^9$  CFU/p/d as stated in GRN 000949.

Pellucid discusses the history of safe use of *H. coagulans* in human food, describing its use in the production of food products and fermented foods. Pellucid discusses published toxicity studies supporting the safety of other strains of *H. coagulans*, with the studies demonstrating no toxicity or significant adverse effects. Pellucid also discusses corroborative unpublished acute and sub-acute studies that assessed the safety of *H. coagulans* MTCC 25460 and these studies showed no reports of significant adverse effects. Pellucid cites published human tolerance studies in which adults ingested other strains of *H. coagulans*, stating that *H. coagulans* was well tolerated with no reports of serious adverse effects or observed safety concerns. Pellucid discusses the results of *in silico* analyses that showed no significant findings for safety related issues associated with *H. coagulans* MTCC 25460, such as antibiotic resistance genes,

---

<sup>3</sup> The subject of GRN 000949 is *Bacillus coagulans* strain DSM 17654 spore preparation. We evaluated the notice and responded in a letter dated January 7, 2021, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

virulence genes, toxin genes, or genes related to the production of biogenic amines. Pellucid states that the species is generally regarded as non-pathogenic and non-toxicogenic and reports of infection associated with *H. coagulans* appear opportunistic in highly immune-compromised populations.

Based on the data and information provided in the submission, Pellucid concludes that *H. coagulans* MTCC 25460 spore preparation is GRAS for its intended use.

### **Standards of Identity**

In the notice, Pellucid states its intention to use *H. coagulans* MTCC 25460 spore preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

### **Potential Labeling Issues**

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), a food is misbranded if its labeling is false or misleading in any way. Section 403(r) of the FD&C Act lays out the statutory framework for labeling claims characterizing a nutrient level in a food or the relationship of a nutrient to a disease or health-related condition (also referred to as nutrient content claims and health claims). If products containing *H. coagulans* MTCC 25460 spore preparation bear any nutrient content or health claims on the label or in labeling, such claims are subject to the applicable requirements and are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition. The Office of Food Additive Safety (OFAS) did not consult with ONFL on this issue or evaluate any information in terms of labeling claims. Questions related to food labeling should be directed to ONFL.

### **Section 301(ll) of the FD&C Act**

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of Pellucid's notice concluding that *H. coagulans* MTCC 25460 spore preparation is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing *H. coagulans* MTCC 25460 spore preparation. Accordingly, our response should not be construed to be a statement that foods containing *H. coagulans* MTCC 25460 spore preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

## Conclusions

Based on the information that Pellucid provided, as well as other information available to FDA, we have no questions at this time regarding Pellucid's conclusion that *H. coagulans* MTCC 25460 spore preparation is GRAS under its intended conditions of use. This letter is not an affirmation that *H. coagulans* MTCC 25460 spore preparation is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 001159 is accessible to the public at [www.fda.gov/grasnoticeinventory](http://www.fda.gov/grasnoticeinventory).

Sincerely,

Susan J.  
Carlson -S

 Digitally signed by Susan J. Carlson -S  
Date: 2024.05.06 17:21:46 -04'00'

Susan J. Carlson, Ph.D.  
Director  
Division of Food Ingredients  
Office of Food Additive Safety  
Center for Food Safety  
and Applied Nutrition

## References

1. Gupta, R., et al. (2020). Robust demarcation of 17 distinct *Bacillus* species clades, proposed as novel *Bacillaceae* genera, by phylogenomics and comparative genomic analyses: description of *Robertmurraya kyonggiensis* sp. nov. and proposal for an emended genus *Bacillus* limiting it only to the members of the Subtilis and Cereus clades of species. *International Journal Systematic and Evolutionary Microbiology*, 70(11):5753-5798. doi: 10.1099/ijsem.0.004475.
2. Narsing Rao, MPN., et al. (2023). Genome-based reclassification of *Bacillus acidicola*, *Bacillus pervagus* and the genera *Heyndrickxia*, *Margalitia*, and *Weizmannia*. *International Journal Systematic and Evolutionary Microbiology*, 73(7):5961-5967. doi: 10.1099/ijsem.0.005961.