



Ankit Rathi  
Specialty Enzymes and Probiotics  
13591 Yorba Avenue  
Chino, CA 91710

Re: GRAS Notice No. GRN 000971

Dear Mr. Rathi:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000971. We received the notice that you submitted on behalf of Advanced Enzyme Technologies Ltd.'s (Advanced Enzymes) notice on September 24, 2020 and filed it on February 24, 2021. Advanced Enzymes submitted amendments to the notice on May 20, 2021, June 14, 2021, August 11, 2021, and November 2, 2021, providing clarifying information about the microorganism, additional manufacturing specifications, and results from the analysis of three non-consecutive batches.

The subject of this notice is *Alkalihalobacillus clausii* strain MCC 0538<sup>1</sup> (*A. clausii* MCC 0538) spore preparation at a use level up to  $2 \times 10^9$  colony forming units (CFU)/serving as an ingredient<sup>2</sup> in baked goods and baking mixes; breakfast cereals; cheeses; non-alcoholic beverages and beverage bases; coffee and tea; milk and milk products; dairy product analogs; fats and oils; fruit juices; condiments and relishes; confections and frostings; frozen dairy desserts and mixes; fruit and water ices; gelatins, puddings, and fillings; jams and jellies; grain products and pastas; hard candy and cough drops; soft candy; chewing gum; herbs, seeds, spices, seasonings, blends, extracts, and flavorings; 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. The notice informs us of Advanced Enzymes' view that these uses of *A. clausii* MCC 0538 spore preparation are GRAS through scientific procedures.

Advanced Enzymes describes *A. clausii* MCC 0538 spore preparation as a light brown to brown-colored powder. Advanced Enzymes states that *A. clausii* MCC 0538 is a non-pathogenic, non-toxic Gram-positive, motile, spore-forming, rod-shaped bacterium. The strain is deposited in the strain collection of the National Centre for Microbial Resource (NCMR) in Pune, India. Advanced Enzymes discusses the results of the phenotypic and genotypic characterization used to confirm the strain's identity. Advanced Enzymes describes the manufacture of *A. clausii* MCC 0538 spore

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<sup>1</sup> We note that *Bacillus clausii* was reclassified as *Alkalihalobacillus clausii* as reported in Patel and Gupta (Ref. 1).

<sup>2</sup> Advanced Enzyme states that *A. clausii* MCC 0538 spore preparation is not intended for use in foods under the jurisdiction of the United States Department of Agriculture.

preparation by batch and fed-batch fermentation of a pure culture under controlled conditions. When the desired spore count is reached, the fermentation is complete. The bacterial culture is then heat treated at 60 °C<sup>3</sup> for 30 minutes, centrifuged to separate the spores from the soluble media components, and the spores are then washed, and spray dried in the presence of approved food-grade stabilizers, such as maltodextrin. Advanced Enzymes states that no components of the fermentation media are allergens or are derived from allergenic sources. Advanced Enzymes states that the manufacturing process is monitored for contamination, and that *A. clausii* MCC 0538 spore preparation is manufactured in accordance with current good manufacturing practice using food-grade materials.

Advanced Enzymes provides specifications for *A. clausii* MCC 0538 spore preparation that include total viable spore count (not less than  $1.5 \times 10^{11}$  CFU/g); moisture ( $\leq 7.0\%$ ); limits for heavy metals, including lead ( $\leq 0.3$  mg/kg); and limits for other microorganisms, including yeast and mold ( $\leq 100$  CFU/g), coliforms ( $\leq 100$  CFU/g), *Escherichia coli* (absent in 10 g), *Salmonella* serovars (absent in 10 g), *Pseudomonas aeruginosa* (absent in 1 g), *Staphylococcus* spp. (absent in 1 g), and *Listeria monocytogenes* (absent in 25 g). Advanced Enzymes provides the results of three non-consecutive batch analyses to demonstrate that the *A. clausii* MCC 0538 spore preparation can be manufactured to conform with the provided specifications. Based on the results of stability testing, Advanced Enzymes concludes that the shelf life of *A. clausii* MCC 0538 spore preparation is two years at 25 °C and 60% relative humidity.

Advanced Enzymes estimates dietary exposure to *A. clausii* MCC 0538 spore preparation based on an estimated consumption of 18.2 food servings/d and an assumption that all servings of food contain *A. clausii* MCC 0538 spore preparation at the maximum use level of  $2 \times 10^9$  CFU/serving.

Advanced Enzymes states that *A. clausii* MCC 0538 spore preparation does not produce antibiotics. Advanced Enzymes further states that *in vitro* data showed no significant findings for safety-related issues associated with the strain (i.e., antibiotic resistance, bacterial virulence, and biogenic amine production). Advanced Enzymes summarizes and discusses acute, subacute, subchronic, and chronic studies, in which no reports of toxicity or significant adverse effects associated with *A. clausii* MCC 0538 spore preparation were reported. Advanced Enzymes describes the history of safe use of *Bacillus* spp. in human food and explains that *A. clausii* has been isolated from several fermented foods. Advanced Enzymes performed a literature search through June 2021 and summarizes peer-reviewed scientific journals and governmental reviews, concluding that the publications support the safe consumption of *A. clausii*. Advanced Enzymes notes that *A. clausii* bacteremia was reported in some high-risk populations consisting of individuals who are immunocompromised, critically ill, or following surgery; however, aside from these cases in at-risk populations, other studies reported no adverse effects. Advanced Enzymes states that *A. clausii* has been safely consumed by humans for decades and that the reports of infection associated with *A. clausii*

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<sup>3</sup> Advanced Enzymes states that heat-treatment at 60 °C for 30 minutes assures that any vegetative cells are killed, and the only viable organisms in the final product are spores.

appear coincidental and opportunistic in immunocompromised populations. Additionally, Advanced Enzymes describes published human tolerance studies in which children and adults were fed *A. clausii* and states that no significant adverse effects were noted in any of these studies.

Advanced Enzymes includes the report of a panel of individuals (Advanced Enzymes' GRAS panel). Based on its review, Advanced Enzymes' GRAS panel concluded that *A. clausii* MCC 0538 spore preparation is safe under the conditions of its intended use.

Based on the totality of evidence, Advanced Enzymes concludes that *A. clausii* MCC 0538 spore preparation is GRAS for its intended use.

### **Standards of Identity**

In the notice, Advanced Enzymes states its intention to use *A. clausii* MCC 0538 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 & 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 *A. clausii* MCC 0538 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.

### **Potential Requirement for a Color Additive Petition**

There is no GRAS provision for color additives. In the notice, Advanced Enzymes describes *A. clausii* MCC 0538 spore preparation as a light brown to brown-colored powder. As such, the use of *A. clausii* MCC 0538 spore preparation in food products may constitute a color additive use under section 201(t)(1) of the FD&C Act and FDA's implementing regulations in 21 CFR Part 70. Under section 201(t)(1) and 21 CFR 70.3(f), a color additive is a material that is a dye, pigment, or other substance made by a synthetic process or similar artifice, or is extracted, isolated, or otherwise derived from a vegetable, animal, mineral, or other source. Under 21 CFR 70.3(g), a material that otherwise meets the definition of a color additive can be exempt from that definition if it is used (or is intended to be used) solely for a purpose or purposes other than coloring. Our response to GRN 000971 is not an approval for use as a color additive nor is it a

finding of the Secretary of the Department of Health and Human Services within the meaning of section 721(b)(4) of the FD&C Act. Questions about color additives should be directed to the Division of Food Ingredients in the OFAS.

### **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 Advanced Enzymes' notice concluding that *A. clausii* MCC 0538 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 *A. clausii* MCC 0538 spore preparation. Accordingly, our response should not be construed to be a statement that foods containing *A. clausii* MCC 0538 spore preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

### **Conclusions**

Based on the information that Advanced Enzymes provided, as well as other information available to FDA, we have no questions at this time regarding Advanced Enzymes' conclusion that *A. clausii* MCC 0538 spore preparation is GRAS under its intended conditions of use. This letter is not an affirmation that *A. clausii* MCC 0538 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 000971 is accessible to the public at [www.fda.gov/grasnoticeinventory](http://www.fda.gov/grasnoticeinventory).

Sincerely,

Susan J.  
Carlson -S

Susan Carlson, Ph.D.  
Director

Division of Food Ingredients  
Office of Food Additive Safety  
Center for Food Safety  
and Applied Nutrition

Digitally signed by Susan J.  
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## Reference

1. Patel, S., and Gupta, R. S. (2020). A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus *Bacillus*: Proposal for six new genera of *Bacillus* species, *Peribacillus* gen. nov., *Cytobacillus* gen. nov., *Mesobacillus* gen. nov., *Neobacillus* gen. nov., *Metabacillus* gen. nov. and *Alkalihalobacillus* gen. nov. *International Journal of Systematic and Evolutionary Microbiology*, 70(1), 406-438. doi: 10.1099/ijsem.0.003775