



Joab Trujillo
AB Enzymes Inc.
8211 W. Broward Blvd., Suite 375
Plantation, FL 33324

Re: GRAS Notice No. GRN 000974

Dear Mr. Trujillo:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000974. We received AB Enzymes GmbH (AB Enzymes)'s GRAS notice on September 4, 2020 and filed it on February 9, 2021. AB Enzymes submitted amendments to the notice on October 5, 2021 and December 17, 2021, that provided clarification on the genetic modification, lack of allergenicity, identity and composition of maltogenic alpha-amylase enzyme preparation.

The subject of the notice is maltogenic alpha-amylase enzyme preparation produced by *Bacillus subtilis* expressing a modified synthetic gene encoding maltogenic alpha-amylase from *Geobacillus stearothermophilus* (maltogenic alpha-amylase enzyme preparation)¹ for use as an enzyme in the production of baked goods at a level of 22 mg Total Organic Solids (TOS) per kg cereal and baking flours raw materials. The notice informs us of AB Enzymes' view that this use of maltogenic alpha-amylase enzyme preparation is GRAS through scientific procedures.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component that catalyzes the chemical reaction, as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. AB Enzymes' notice provides information about the components in the maltogenic alpha-amylase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, maltogenic alpha-amylase is identified by the Enzyme Commission Number 3.2.1.133.² The Chemical Abstracts Service No. for maltogenic alpha-amylase is 160611-47-2. AB Enzymes states that the primary amino

¹ AB Enzymes states that the maltogenic amylase enzyme preparation also contains a hydrolase enzyme. This is due to the expression of a hydrolase gene present in the genetic construct of the production strain as discussed in Paragraph 5 of this letter.

² <https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/133.html>

acid sequence of the mature maltogenic alpha-amylase enzyme consists of 686 amino acids. AB Enzymes states that the calculated molecular weight of maltogenic alpha-amylase is 75 kDa.

AB Enzymes states that the *B. subtilis* production organism is a non-pathogenic, non-toxicogenic, well-characterized production organism with a history of safe use in the food industry. AB Enzymes describes the construction of the *B. subtilis* production strain RF 13018, which was derived from a *B. subtilis* recipient strain³ by transformation with an expression cassette carrying the modified synthetic gene encoding maltogenic alpha-amylase from *G. stearothermophilus*, under the control of a *B. amyloliquifaciens* promoter and signal sequence and a *Thermoactinomyces vulgaris* terminator. To aid in the fermentation process, a *B. amyloliquifaciens* hydrolase gene,⁴ is inserted upstream of the maltogenic alpha-amylase expression cassette.⁵ AB Enzymes states that it confirmed the sequence integrity and the genetic stability of the production strain by monitoring production of maltogenic alpha-amylase via PCR analysis. AB Enzymes also verified the absence of functional or transferable antibiotic resistance genes in the final production strain genome.

AB Enzymes states that the maltogenic alpha-amylase enzyme preparation is produced by submerged batch-fed fermentation of a pure culture of the *B. subtilis* RF 13018 production strain under controlled conditions. The maltogenic alpha-amylase enzyme is secreted into the fermentation medium and then recovered by pretreatment with flocculants and/or filter aids and a separation step (e.g., centrifugation or filtration). This is followed by concentration, and polish and germ filtration steps. The enzyme concentrate is formulated to a light beige, solid, enzyme preparation with sodium chloride, sunflower oil, and maltodextrin (from corn). AB Enzymes states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices. AB Enzymes states that the fermentation medium used in the manufacturing of maltogenic alpha-amylase enzyme preparation includes a soy-based ingredient that is consumed during fermentation. AB enzymes also states that the equipment used to process the commercial maltogenic alpha-amylase enzyme preparation also processes wheat flour.

AB Enzymes has established food grade specifications and states that the maltogenic alpha-amylase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 12th edition, 2020), and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). AB Enzymes provides data from analyses of three batches of maltogenic alpha-

³ AB Enzymes states that the recipient *B. subtilis* strain was produced by conventional mutagenesis of the parental *B. subtilis* strain, which was isolated from soil and characterized by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). The *B. subtilis* mutant strain has been used since 2010 by AB enzymes to produce maltogenic amylases used in food processing.

⁴ AB Enzymes states that the hydrolase reduces and/or prevents an increase in the thickness of the fermentation broth but has minimal activity in the final maltogenic amylase enzyme preparation.

⁵ The strain is deposited in the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands as CBS145947.

amylase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism and antibiotic activity.

AB Enzymes intends to use maltogenic alpha-amylase enzyme preparation at a recommended use level of 22 mg TOS/kg raw material to aid in production of baked goods when added to dough before baking.⁶ AB Enzymes notes that the maltogenic alpha-amylase enzyme preparation will be inactivated with high temperatures of baking. AB Enzymes estimates a maximum dietary exposure to maltogenic alpha-amylase enzyme preparation to be 0.195 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses.⁷

AB Enzymes relies on published information that discusses the safety of the *B. subtilis* production organism and the safety of microbial enzyme preparations used in food processing. AB Enzymes discusses the safety of the maltogenic alpha-amylase itself to corroborate safety of the intended uses. AB Enzymes summarizes the results of an unpublished 90-day oral toxicity study in rats showing that consumption of the same maltogenic alpha-amylase enzyme concentrate, produced by a different production strain, does not show any treatment-related adverse effects corresponding to 1000 mg TOS/kg bw/d, the highest dose tested.⁸ Because the new production organism produces ectopically expressed hydrolase gene, AB Enzymes also discusses the safety of the co-expressed hydrolase based on the safety of *B. amylofaciens* from which the hydrolase gene was isolated.

AB Enzymes discusses publicly available literature, as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by oral consumption of enzymes, to address potential allergenicity due to maltogenic alpha-amylase. Based on bioinformatic analyses, AB Enzymes reports that an 80-mer sliding window returned several homology hits above 35% threshold; however, neither the full length FASTA sequence analysis above 50% threshold nor eight contiguous identical amino acids search criteria of maltogenic amylase resulted in identification of known oral allergens. Based on the totality of the information available, AB Enzymes concludes that it is unlikely that oral consumption of maltogenic alpha-amylase enzyme from the intended use will result in allergenic responses.

Based on the data and information summarized above, AB Enzymes concludes that maltogenic alpha-amylase enzyme preparation is GRAS for its intended use.

⁶ AB Enzymes estimates a maximum use of 15.62 mg TOS of maltogenic alpha-amylase/kg food based on the recommended use level of maltogenic alpha-amylase enzyme preparation, and a raw material to final food ratio of 0.71 kg of flour per kg final food.

⁷ AB Enzymes uses the Budget method to estimate dietary exposure to maltogenic alpha-amylase enzyme preparation based on consumption of a maximum of 25 g of solid food per kg bw/d. AB Enzymes assumes that 50% of the solid foods (12.5 g per kg bw/d) will be baked goods and contain the maltogenic alpha-amylase enzyme preparation at the recommended use level.

⁸ GRN 000746 describes uses of maltogenic amylase enzyme preparation produced by *B. subtilis* expressing a synthetic gene encoding maltogenic amylase from *G. stearothermophilus*. We evaluated GRN 000746 and responded in a letter dated June 13, 2018, stating that we had no questions at the time regarding the notifier's GRAS conclusion.

Standards of Identity

In the notice, AB Enzymes states its intention to use maltogenic alpha-amylase enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. 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.

Allergen Labeling

The FD&C Act requires that the label of a food that is or contains an ingredient that contains a “major food allergen” declare the allergen’s presence (section 403(w)). The FD&C Act defines a “major food allergen” as one of nine foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame (effective January 1, 2023)) or a food ingredient that contains protein derived from one of those foods. The maltogenic amylase enzyme preparation may require labeling under the FD&C Act because it may contain an allergen. Questions about petitions or notifications for exemptions from the food allergen labeling requirements should be directed to the Division of Food Ingredients in the Office of Food Additive Safety. Questions related to food labeling in general should be directed to the Office of Nutrition and Food Labeling in the Center for Food Safety and Applied Nutrition.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (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 AB Enzymes’ notice concluding that maltogenic alpha-amylase enzyme 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 maltogenic alpha-amylase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing maltogenic alpha-amylase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions


Based on the information that AB Enzymes provided, as well as other information available to FDA, we have no questions at this time regarding AB Enzymes’ conclusion that maltogenic alpha-amylase enzyme preparation produced by *B. subtilis* expressing a synthetic modified gene encoding maltogenic alpha-amylase from *G. stearothermophilus* is GRAS under its intended conditions of use. This letter is not an affirmation that maltogenic alpha-amylase enzyme preparation produced by *B. subtilis* expressing a synthetic modified gene encoding maltogenic alpha-amylase from *G. stearothermophilus* 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 000974 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

 Digitally signed by Susan J.
Carlson -S
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Susan Carlson, Ph.D.
Director
Division of Food Ingredients
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