



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

Re: GRAS Notice No. GRN 001011

Dear Mr. Trujillo:

This letter revises our response letter to GRN 001011 signed on July 18, 2022. The purpose of this revised letter is to correct the source of the hydrolase gene in the production organism.

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001011. We received AB Enzymes Inc. (AB Enzymes)'s GRAS notice on May 5, 2021 and filed it on August 19, 2021. AB Enzymes submitted amendments to the notice on February 8, 2022 and March 24, 2022 that provided clarification on the genetic modification, characteristics of the production organism, method of manufacturing and the safety narrative.

The subject of the notice is alpha-amylase enzyme preparation produced by *Bacillus subtilis* strain AR-651 expressing the gene encoding alpha-amylase from *Thermoactinomyces vulgaris* (alpha-amylase enzyme preparation)<sup>1</sup> for use as an enzyme at a maximum level of 100 mg total organic solids (TOS) per kg flour used as raw material in the production of baked goods. The notice informs us of AB Enzyme's view that this use of 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 alpha-amylase enzyme preparation.

According to the classification system of enzymes established by the International

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<sup>1</sup> AB Enzymes states that the alpha-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.

Union of Biochemistry and Molecular Biology, alpha-amylase is identified by the Enzyme Commission Number 3.2.1.1.<sup>2</sup> The Chemical Abstracts Service number for alpha-amylase is 9000-90-2. AB Enzymes states that the primary sequence of alpha-amylase is 453 amino acids with a calculated molecular weight of 52 kDa.

AB Enzymes states that the *B. subtilis* production organism is non-pathogenic, non-toxicogenic, and well characterized with a history of safe use in the food industry. AB Enzymes states that the recipient strain<sup>3,4</sup> used in the construction of the production strain, AR-651, was transformed with an expression cassette carrying the gene encoding alpha-amylase from *T. vulgaris* 94-2A<sup>5</sup> under the control of a *Bacillus spp.* promoter and signal sequence, and a *T. vulgaris* terminator. To aid in the fermentation process, a *Bacillus sp.* hydrolase gene is inserted upstream of the alpha-amylase expression cassette.<sup>6</sup> AB Enzymes confirmed the integration of the expression cassette by PCR and whole genome sequencing. AB Enzymes evaluated the genetic stability of the production strain by monitoring production of alpha-amylase. AB Enzymes also verified the absence of transferable antibiotic resistance genes in the final production strain by whole genome sequencing.

AB Enzymes states that the alpha-amylase enzyme preparation is produced by an aerobic submerged fermentation of a pure culture of the *B. subtilis* AR-651 production strain under controlled conditions. The alpha-amylase enzyme is secreted into the fermentation medium and then recovered by pretreatment with flocculants and/or filter aids to facilitate separation of the biomass. This is followed by concentration and multiple filtration steps. The resulting brown powder is formulated as a liquid concentrate with sunflower oil and wheat flour to obtain the enzyme preparation. AB Enzymes states that the fermentation medium does not contain any allergens or components derived from allergenic sources. AB Enzymes states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices.

AB Enzymes has established food grade specifications and states that the alpha-amylase enzyme preparation conforms to the specifications set in the Food Chemicals Codex (FCC, 12<sup>th</sup> ed., 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 results from analyses of three non-consecutive batches of alpha-amylase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism and antibiotic activity.

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<sup>2</sup> <https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/1.html>

<sup>3</sup> AB Enzymes states that the parental strain was isolated from soil and characterized by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) to be similar to DuPont DUP-12544, as confirmed by multi-locus sequence typing.

<sup>4</sup> AB Enzymes states that the recipient *B. subtilis* strain was obtained by targeted gene deletions in the parental strain to improve production performance and yield.

<sup>5</sup> AB Enzymes states that the production strain is deposited as CBS 147460, in Westerdijk Fungal Biodiversity Institute (formerly the Centraalbureau voor Schimmelcultures (CBS)) in the Netherlands.

<sup>6</sup> AB Enzymes states that the hydrolase has minimal activity in the final alpha-amylase enzyme preparation.

AB Enzymes intends to use alpha-amylase enzyme preparation at a maximum use level of 100 mg TOS/kg of flour in the baking process.<sup>7</sup> AB Enzymes states that alpha-amylase catalyzes the hydrolysis of the alpha-(1,4) glycosidic linkages of starch in cereal-based flours used in baking doughs. AB Enzymes notes that the alpha-amylase enzyme preparation is inactivated or denatured during the baking process. AB Enzymes estimates a maximum dietary exposure to alpha-amylase enzyme preparation to be 0.89 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses, with the assumption that the alpha-amylase will be present and remain active in the final food.<sup>8</sup>

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 also discusses unpublished toxicological studies using an alpha-amylase produced by a related *B. subtilis* production strain.<sup>9</sup>

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 alpha-amylase. Based on bioinformatic analyses, and specific search criteria, AB Enzymes reports no matches between the amino acid sequence of their alpha-amylase and primary sequences of known oral allergens based on the guidelines for evaluation of allergenic potential developed by the Joint FAO/WHO consultation in 2001 (FAO/WHO, 2001) and the Codex Alimentarius Commission in 2003 (Codex, 2003). Based on the totality of the information available, AB Enzymes concludes that it is unlikely that oral consumption of alpha-amylase enzyme from the intended use will result in allergic responses.

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

### **Standards of Identity**

In the notice, AB Enzymes states its intention to use alpha-amylase enzyme 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.

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<sup>7</sup> AB Enzymes estimates a maximum use of 71 mg TOS of alpha-amylase per kg food based on the recommended use level of alpha-amylase enzyme preparation, and a raw material to food ratio of 0.71 kg of flour per kg of final food.

<sup>8</sup> AB Enzymes uses the Budget Method to estimate dietary exposure to alpha-amylase enzyme preparation based on consumption of a maximum of 0.025 kg of solid food per kg bw/d.

<sup>9</sup> AB Enzymes performed their safety studies on a similar *B. subtilis* strain that produces alpha-amylase but does not contain the introduced hydrolase gene from the *Bacillus* spp. AB Enzymes concluded that the introduction of the hydrolase gene does not affect the safety of the production organism or its alpha-amylase enzyme.

## **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 alpha-amylase enzyme preparation requires labeling under the FD&C Act because it contains wheat flour.

## **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 alpha-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 alpha-amylase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing 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 alpha-amylase enzyme preparation produced by *Bacillus subtilis* strain AR-651 expressing a gene encoding alpha-amylase from *Thermoactinomyces vulgaris* is GRAS under its intended conditions of use. This letter is not an affirmation that alpha-amylase enzyme preparation produced by *B. subtilis* expressing a gene encoding alpha-amylase from *T. vulgaris* 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 001011 is accessible to the public at [www.fda.gov/grasnoticeinventory](http://www.fda.gov/grasnoticeinventory).

Sincerely,

Susan J.  
Carlson -S

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J. Carlson -S  
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Susan Carlson, Ph.D.  
Director  
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