Candice Cryne
AB Enzymes GmbH
Feldbergstr. 78
D-64293 Darmstadt
GERMANY

Re: GRAS Notice No. GRN 000746

Dear Ms. Cryne:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000746. We received AB Enzymes GmbH (AB Enzymes)'s GRAS notice on November 13, 2017, and filed it on December 21, 2017. We received amendments containing additional safety information on April 17, 2018 and May 28, 2018.

The subject of the notice is maltogenic amylase enzyme preparation produced by *Bacillus subtilis* expressing a synthetic gene encoding maltogenic amylase from *Geobacillus stearothermophilus* (maltogenic amylase enzyme preparation) for use as an enzyme in the production of baked goods at a maximum level of 20 mg Total Organic Solids (TOS)/kg flour. The notice informs us of AB Enzymes' view that this use of maltogenic 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 amylase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, maltogenic amylase is identified by the Enzyme Commission Number 3.2.1.133. The accepted name for this enzyme is glucan 1,4-alpha-maltohydrolase, and the systematic name is 4-alpha-D-glucan alpha-maltohydrolase. The enzyme is also known as maltogenic alpha-amylase, 1,4-alpha-D-glucan alpha-maltohydrolase, and glucan-1,4-alpha-maltohydrolase. Maltogenic amylase catalyzes the hydrolysis of (1-4)- alpha-D glucosidic linkages in starch and related polysaccharides and oligosaccharide substrates to remove successive alpha-maltose residues from the non-reducing ends of the chains. The CAS No. for maltogenic amylase is 160611-47-2. AB Enzymes states that the primary amino acid sequence of the mature maltogenic amylase enzyme has been determined and it consists of 686 amino

acids. AB Enzymes states that the calculated molecular weight of maltogenic amylase is 75 kDa.

AB Enzymes states that the *B. subtilis* production strain RF12029[1] was derived from the *B. subtilis* recipient strain RH11634.[2] AB Enzymes states that this recipient strain was modified at several chromosomal loci to delete genes necessary for sporulation. AB Enzymes describes *B. subtilis* as a non-pathogenic, non-toxigenic, 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 RF12029 by transformation of the recipient strain with a plasmid containing an expression cassette carrying a synthetic gene encoding a wild-type maltogenic amylase from *G. stearothermophilus* linked to a *B. amyloliquefaciens* signal sequence, under the control of a *B. amyloliquefaciens* promoter, and a *Thermoactinomyces vulgaris* transcriptional terminator. AB Enzymes states that the maltogenic amylase amino acid sequence is 100% identical to the published sequence of the wild-type maltogenic amylase from *G. stearothermophilus*. AB Enzymes states that the plasmid also carries a gene encoding a *B. subtilis* orotidine 5-phosphate decarboxylase, which is used for selection of transformants. AB Enzymes states that the structural and segregational stability of the introduced plasmid DNA has been demonstrated for approximately 200 generations. AB Enzymes also states that the final production strain does not contain any functional or transferable antibiotic resistance genes.

AB Enzymes states that maltogenic amylase enzyme is produced by submerged fed-batch fermentation of a pure culture of the production strain. AB Enzymes states that fermentation is carried out under controlled conditions and that the enzyme is secreted into the fermentation broth. After fermentation, flocculants or filter aids are added to the broth containing the desired enzyme, at controlled pH and temperature to initiate enzyme separation. The enzyme is then recovered from the fermentation media by filtration or centrifugation, and concentrated by filtration. AB Enzymes provides analytical data from analyses of two batches of maltogenic amylase enzyme concentrate to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production strain. The liquid enzyme is further concentrated and used for toxicological studies. The liquid concentrate is filtered, and formulated to the maltogenic amylase enzyme preparation with food-grade sunflower oil, maltodextrin derived from maize, and sodium chloride. AB Enzymes states that the entire process is performed in accordance with current good manufacturing practices. AB Enzymes states that the maltogenic amylase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 10th edition, 2016), 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

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[1] AB Enzymes states that *B. subtilis* strain RF12029 is deposited in the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands as CBS141004.

[2] The parental strain was isolated in year 1974 from soil, and was characterized as *B. subtilis* by the Deutsche Sammlung von Mikroorganismen (DSMZ) in Germany. This strain was further modified by AB Enzymes via conventional mutagenesis.

(JECFA, 2006). AB Enzymes also states that the final maltogenic amylase enzyme preparation does not contain any major food allergens from the fermentation media.

AB Enzymes intends to use maltogenic amylase enzyme preparation in the production of baked goods at a maximum level corresponding to 20 mg TOS/kg flour. AB Enzymes notes that the maltogenic amylase enzyme preparation will be deactivated or removed during the production of the baked goods. To estimate dietary exposure to maltogenic amylase enzyme preparation, AB Enzymes assumes that the enzyme preparation will be used at the maximum intended levels, and that all of the enzyme preparation will remain in the final food. AB Enzymes estimated dietary exposure from all uses of maltogenic amylase enzyme preparation to be 0.18 mg TOS/kg body weight per day (mg TOS/kg bw/d).[3]

AB Enzymes relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the production organism. Additionally, AB Enzymes summarizes unpublished toxicological studies using the maltogenic amylase enzyme liquid concentrate to corroborate safety of the intended uses. AB Enzymes states that the maltogenic amylase enzyme is not mutagenic based on results from a bacterial reverse mutation assay, and on results from an *in vitro* micronucleus assay in cultured human lymphocytes. A 13-week oral toxicity study in rats using the maltogenic amylase enzyme concentrate did not cause any treatment-related adverse effects up to the highest dose tested (equivalent to 1,000 mg TOS/kg bw/d). Based on the highest dose tested in the 13-week study and the estimated dietary exposure from the intended uses of the maltogenic amylase enzyme preparation, AB Enzymes calculates a margin of exposure to be approximately 5000. FDA notes the margin of exposure is based on unpublished safety studies, and is corroborative of the published information regarding enzyme preparations used in food processing.

AB Enzymes discusses potential food allergenicity of maltogenic amylase enzyme. AB Enzymes states that naturally occurring food enzymes, if present in the final food, are unlikely to have allergenic potential because they are present in low concentrations and are susceptible to digestion in the gastrointestinal system. AB Enzymes further cites the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes due to their low use levels and the extensive processing of enzyme-containing foods during manufacturing. Additionally, AB Enzymes conducted a sequence homology search using the peptide sequence of the maltogenic amylase against known allergens stored in the FARRP allergen protein database. Eighty-mer sliding window search analyses returned homology hits above 35% threshold to four allergens; 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 allergens. Based on the totality of the information available, AB Enzymes concludes that it is unlikely that oral consumption of

[3] AB Enzymes uses the Budget Method to calculate estimated dietary exposure to maltogenic amylase enzyme preparation based on consumption of a maximum of 25 g of solid foods per person per day. AB Enzymes assumed that 50% of all solid foods (i.e., 12.5 g) will be baked goods and will contain the maltogenic amylase enzyme preparation.

maltogenic amylase enzyme will result in any allergenic responses. AB Enzymes also assessed the sequence homology of maltogenic amylase to known toxins using the BLAST-P database and did not identify any significant homology to any protein sequence identified or known to be a toxin.

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

Standards of Identity

In the notice, AB Enzymes states its intention to use maltogenic 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.

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 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 amylase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing maltogenic 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 amylase enzyme preparation produced by *B. subtilis* expressing a synthetic gene encoding maltogenic amylase from *G. stearothermophilus* is GRAS under its intended conditions of use. This letter is not an affirmation that maltogenic amylase enzyme preparation produced by *B. subtilis* expressing a synthetic gene encoding maltogenic 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 000746 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Michael A. Adams -S

Digitally signed by Michael A. Adams -S
Date: 2018.06.13 16:29:01 -04'00'

Dennis M. Keefe, Ph.D.
Director
Office of Food Additive Safety
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