Susan Cho, Ph.D.
NutraSource, Inc.
6309 Morning Dew Court
Clarksville, MD 21029

Re: GRAS Notice No. GRN 000861

Dear Dr. Cho:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000861 submitted on behalf of GeneScript and Bestzyme Biotech, Inc. (GeneScript/Bestzyme). We received GeneScript/Bestzyme's GRAS notice on May 14, 2019, and filed it on July 30, 2019. We received an amendment clarifying information about the enzyme production, its use, and administrative language on May 18, 2020.

The subject of the notice is pullulanase enzyme preparation produced by *Bacillus subtilis* carrying a synthetic pullulanase gene from *Bacillus deramificans* (pullulanase enzyme preparation) for use as an enzyme at a maximum level of 186 mg Total Organic Solids (TOS)/kg raw material during processing of starch, beer, and cereal-based beverages. The notice informs us of GeneScript/Bestzyme's view that these uses of pullulanase enzyme preparation are 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. GeneScript/Bestzyme's notice provides information about the components in the pullulanase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, pullulanase enzyme is identified by the Enzyme Commission Number 3.2.1.41[1] and CAS Reg. No. 9075-68-7. GeneScript/Bestzyme provides the amino acid sequence of pullulanase and states that its molecular weight is approximately 92 kDa.

GeneScript/Bestzyme states that *B. subtilis* is non-pathogenic and non-toxigenic with a history of safe use in food. GeneScript/Bestzyme states that the *B. subtilis* host strain

[1] https://enzyme.expasy.org/EC/3.2.1.41

was derived by genetic modification of the parental *B. subtilis* BS-YF strain (CICC 20632).[2] GeneScript/Bestzyme describes the construction of the *B. subtilis* production strain by targeted integration of an expression cassette carrying a synthetic pullulanase gene [3] into the AmyE locus of the *B. subtilis* recipient strain by single cross-over recombination.

GeneScript/Bestzyme indicates that no other components of the pullulanase expression plasmid were incorporated into the production strain. GeneScript/Bestzyme confirms that the production organism was stable for five generations. GeneScript/Bestzyme confirms the insertion of the pullulanase gene by halo formation on red-pullulan plates and PCR.

GeneScript/Bestzyme states that pullulanase enzyme preparation is manufactured by submerged fermentation of the production strain under controlled conditions; the enzyme is secreted into the fermentation medium. After the fermentation is complete, the enzyme is pretreated with flocculant, filtered and concentrated. The enzyme concentrate is used for the safety studies prior to standardization as a preparation with glucose, sodium benzoate and potassium sorbate. GeneScript/Bestzyme states that pullulanase enzyme preparation is manufactured under current good manufacturing process and that all raw materials used in the manufacturing process are food grade. GeneScript/Bestzyme also states that the pullulanase enzyme preparation does not contain any major food allergens from the fermentation medium.

GeneScript/Bestzyme lists methods used to assess specifications and provides data from analyses of three nonconsecutive lots of pullulanase enzyme concentrate. GeneScript/Bestzyme states that the pullulanase enzyme preparation complies with specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 11th edition, 2018) and 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). GeneScript/Bestzyme states that the production strain is absent in the commercial enzyme preparation.

GeneScript/Bestzyme states the intended use of the pullulanase enzyme preparation is as an enzyme at a maximum use level of 186 mg TOS/kg raw material to hydrolyze α-1,6-glucosidic bonds in starch during the processing of starch, beer and cereal-based beverages. GeneScript/Bestzyme notes that the pullulanase enzyme preparation will be deactivated during the processing and likely absent in the final food. However, in estimating dietary exposure, GeneScript/Bestzyme assumes that all the pullulanase enzyme preparation will remain in the final food and will be active. GeneScript/Bestzyme estimates a maximum dietary exposure to pullulanase enzyme preparation from all the intended uses to be 0.5 mg TOS/kg body weight per day (mg

[2] GeneScript/Bestzyme notes that the host *B. subtilis* strain was produced by deleting six genes and a gene cluster from the parental strain *B. subtilis* BS-YF (CICC 20632); these modifications improve the expression and secretion of pullulanase and confer resistance of the strain to phage lysis.
[3] The expression cassette consists of a synthetic promoter, a synthetic ribosome binding site, a synthetic truncated form of the pullulanase from *B. deramificans*, and a synthetic terminator.

TOS/kg bw/d).[4]

GeneScript/Bestzyme relies on published information for the safety of microbial enzyme preparations used in food processing, including the safety of *B. subtilis* as a production organism. Additionally, GeneScript/Bestzyme summarizes unpublished toxicological studies using pullulanase enzyme concentrate to corroborate safety of the intended uses of the enzyme preparation. These include bacterial reverse mutation assay and a rat acute oral toxicity study. As supportive evidence, GeneScript/Bestzyme also discusses published and unpublished toxicity studies conducted with pullulanase preparations produced using pullulanase genes from the same or related *Bacillus* strains.

GeneScript/Bestzyme discusses publicly available literature as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes to address potential allergenicity due to pullulanase enzyme preparation. Further, based on bioinformatic analyses, GeneScript/Bestzyme reports that pullulanase enzyme does not share any biologically meaningful sequence homology or sequence identity to potential oral allergens. Based on the totality of the information available, GeneScript/Bestzyme concludes that it is unlikely that oral consumption of pullulanase enzyme will result in allergic responses.

Based on the data and information summarized above, GeneScript/Bestzyme concludes that pullulanase enzyme preparation is GRAS for its intended use.

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 GeneScript/Bestzyme's notice concluding pullulanase 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 pullulanase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing pullulanase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

[4] GeneScript/Bestzyme uses the pullulanase enzyme preparation at the use levels described in GRN 000645. We evaluated this GRAS notice and responded in a letter dated November 1, 2016 stating that we had no questions at that time regarding the notifier's GRAS conclusion. GeneScript/Bestzyme uses the Budget method to estimate dietary exposure to pullulanase enzyme preparation based on a maximum use level of 186 mg TOS/ kg raw material, a consumption of a maximum of 12.5 g of solid foods and 25 g of beverages per kg body weight per day and, all solid processed food to contain 25% starch dry matter, and processed beverages to contain 13% hydrolyzed starch dry matter.

Conclusions

Based on the information that GeneScript/Bestzyme provided, as well as other information available to FDA, we have no questions at this time regarding GeneScript/Bestzyme's conclusion that pullulanase enzyme preparation produced by *B subtilis* carrying a synthetic pullulanase gene from *B. deramificans* is GRAS for its intended conditions of use. This letter is not an affirmation that pullulanase enzyme preparation produced by *B. subtilis* carrying a synthetic pullulanase gene from *B. deramificans* 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 000861 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlso
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Digitally signed by Susan J. Carlson -S
Date: 2020.07.21 13:04:34 -04'00'

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