Marco Marcucci
Taixing Dongsheng Bio-Tech Co., Ltd.
No.1 Tonglian Rd., Huangqiao Town
Taixing City, Jiangsu Province
People's Republic of China
225411

Re: GRAS Notice No. GRN 001021

Dear Mr. Marcucci,

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001021. We received Taixing Dongsheng Bio-Tech Co., Ltd. (TDS Biotech)'s GRAS notice on June 24, 2021 and filed it on October 22, 2021. TDS Biotech submitted amendments to the notice on April 25, July 13, August 11, and October 7, 2022, providing additional information about the technical effect, intended use, and manufacturing method.

The subject of the notice is transglutaminase enzyme preparation produced by *Streptomyces mobaraensis*[1,2] strain M2020197 (transglutaminase enzyme preparation) for use as an enzyme at a maximum level of 97.6 mg Total Organic Solids (TOS) per kg food in fish products (including restructured fish and shellfish, excluding Siluriformes fish products), dairy products, meat analogs, baked goods (including pastries and breads), pasta and noodles, grain mixtures, and ready-to-eat cereals, and for use as a binder at a maximum level of 65 mg TOS per kg in meat and poultry products. The notice informs us of TDS Biotech's view that this use of transglutaminase 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. TDS Biotech's notice provides information about the components in the transglutaminase enzyme preparation.

[1] TDS Biotech states that *Streptoverticillium mobaraense* was reclassified as *Streptomyces mobaraensis.*
[2] Witt, D., & Stackebrandt, E. (1990). Unification of the genera Streptoverticillum and Streptomyces, and amendation of Streptomyces Waksman and Henrici 1943, 339AL. *Systematic and applied microbiology*, 13(4), 361-371. doi: 10.1016/S0723-2020(11)80234-1

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, transglutaminase is identified by the Enzyme Commission Number 2.3.2.13.[3] The CAS Registry Number for transglutaminase enzyme is 80146-85-6. TDS Biotech states that the primary sequence of transglutaminase is 331 amino acids with a calculated molecular weight of 37.8 kDa.

TDS Biotech states that the *S. mobaraensis* M2020197 production organism is non-pathogenic and non-toxigenic. TDS Biotech confirmed the identity of the production strain with 16S rDNA sequencing. TDS Biotech verified the absence of functional antibiotic resistance genes in the final production strain genome by whole genome sequencing and bioinformatic analyses.

TDS Biotech states that the transglutaminase enzyme preparation is manufactured by submerged fermentation of a pure culture of the *S. mobaraensis* M2020197 production strain under controlled conditions. The transglutaminase is secreted into the fermentation medium and then recovered by plate-pressure filtration, centrifugation, and ultrafiltration. The enzyme is then precipitated with ethanol, recovered with a final centrifugation step, and lyophilized. TDS Biotech states that the entire process is performed using food grade raw materials and in accordance with good manufacturing practices. TDS Biotech states that the fermentation medium contains fish protein concentrate but that this protein is absent in the final formulation as confirmed by PCR.

TDS Biotech has established food grade specifications and states that the transglutaminase enzyme preparation conforms to specifications established in the Food Chemicals Codex (FCC, 11th edition, 2018[4]), 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). TDS Biotech provides data from analyses of three batches of transglutaminase enzyme preparation to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production organism and antibiotic activity.

TDS Biotech intends to use transglutaminase enzyme preparation at a maximum use level of 97.56 mg TOS/kg food in fish products, dairy products, meat analogs (e.g., vegetable protein and soybean products), baked goods (including pastries and bread products), pasta and noodles, grain mixtures (e.g., burritos, tacos, tortillas), and ready-to-eat cereals, and at a maximum use level of 65 mg TOS/kg food in meat and poultry products. TDS Biotech states that the transglutaminase catalyzes acyl transfer reactions between γ-carboxamide groups of protein-bound glutamine and primary amines, resulting in cross-linking of proteins. TDS Biotech notes that the transglutaminase enzyme preparation is added to raw or partially-processed food, where it functions as a texturizer or binder, and will be inactivated or denatured by heat during food processing (e.g., baking, pasteurization) or cooking at the consumer level. TDS Biotech, however, estimates a maximum dietary exposure to transglutaminase enzyme preparation to be 1.22 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses, and

[3] https://iubmb.qmul.ac.uk/enzyme/EC2/3/2/13.html

[4] Specifications for enzymes remain the same in the most recent edition of the Food Chemicals Codex (FCC, 13th edition, 2022)

the assumption that the transglutaminase enzyme preparation will be active and remain in the final food.[5]

TDS Biotech relies on published information that discusses the safety of the *S. mobaraensis* M2020197 production organism and the safety of transglutaminase enzyme preparations used in food processing. TDS Biotech discusses published and unpublished toxicological studies with transglutaminases from other sources. In addition, TDS Biotech discusses unpublished toxicological studies using their transglutaminase produced by *S. mobaraensis* M2020197 to provide corroborative evidence for safety of their enzyme preparation.[6]

TDS Biotech discusses publicly available literature to address potential allergenicity and immunogenicity due to transglutaminase. Based on bioinformatic analyses, TDS Biotech reports no matches between the amino acid sequences of the transglutaminase and the primary sequences of known allergens based on the guidelines developed by the FAO/WHO in 2001 (Food and Agriculture Organization of the United Nations January 2001) and the Codex Alimentarius Commission in 2009 (Codex, 2009). TDS Biotech discusses publications that implicated transglutaminases being associated with celiac disease. However, TDS Biotech concludes that dietary exposure to transglutaminase under the conditions of its intended use is limited; any potential clinically relevant immunogenicity is anticipated to be negligible because the enzyme has been demonstrated to be inactivated during food processing. Based on the totality of the information available, TDS Biotech concludes that it is unlikely that oral consumption of transglutaminase enzyme from the intended use will result in allergenic or adverse immune responses.

Based on the data and information summarized above, TDS Biotech concludes that transglutaminase enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, TDS Biotech states its intention to use transglutaminase 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.

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,

[5] TDS Biotech uses the Budget Method to estimate dietary exposure to transglutaminase enzyme preparation based on consumption of a maximum of 0.0125 kg of solid foods per kg bw/d containing transglutaminase.

[6] TDS Biotech estimated a margin of safety based on their unpublished 90-day rat repeat-dose oral toxicity study and estimated TOS. FDA notes that because the margin of safety is based on an unpublished study, this evidence is considered corroborative.

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. Transglutaminase enzyme standardized with lactose may require labeling under the FD&C Act because it may contain protein derived from milk. 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.

Use in Products under USDA Jurisdiction

As provided under 21 CFR 170.270, during our evaluation of GRN 001021, we coordinated with the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture. Under the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act, FSIS determines the efficacy and suitability of ingredients used in meat, poultry, and egg products, and prescribes safe conditions of use. Suitability relates to the ingredient's effectiveness in performing its intended technical effect and the assurance that the ingredient's use will not result in products that are adulterated or misleading for consumers.

FSIS has completed its review and has no objection to the use of transglutaminase enzyme preparation described in GRN 001021. Regarding labeling, meat or poultry products (excluding Siluriformes fish products) containing transglutaminase enzyme are required to be labeled in the ingredients statement of the products in which it is used.

FSIS requested that we advise you to seek regulatory guidance from its Risk Management and Innovations Staff (RMIS) about the use of transglutaminase enzyme preparation in meat, poultry, and egg products. You should direct such an inquiry to Dr. Melvin Carter, Director, RMIS, Office of Policy and Program Development, FSIS by email at Melvin.Carter@fsis.usda.gov.

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

Conclusions

Based on the information that TDS Biotech provided, as well as other information available to FDA, we have no questions at this time regarding TDS Biotech's conclusion that transglutaminase enzyme preparation produced by *S. mobaraensis* M2020197 is GRAS under its intended conditions of use. This letter is not an affirmation that transglutaminase enzyme preparation produced by *S. mobaraensis* M2020197 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 001021 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2022.11.03 16:32:45 -04'00'

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

cc: Melvin Carter, Ph.D.
Director
USDA/FSIS/OPPD/RMIS
Stop Code 3782, Patriots Plaza III
1400 Independence Ave. SW
Washington, DC 20250-3700