Susan Cho, Ph.D.
AceOne RS
5903 Hampton Forest Way
Fairfax, VA 22030

Re: GRAS Notice No. GRN 001109

Dear Dr. Cho:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001109. We received the GRAS notice you submitted on behalf of Dyne Bio Inc. (Dyne Bio) on September 25, 2022, and filed it on May 5, 2023. Dyne Bio submitted an amendment to the notice on March 18, 2024, containing additional information on enzyme and preparation identity, specifications, analytical methods, and allergenicity.

The subject of the notice is β-agarase DagA enzyme preparation produced by *Streptomyces coelicolor* (β-agarase enzyme preparation) for use as an enzyme at up to 20 mg Total Organic Solids (TOS)/g agar-agar in the production of neoagaro-oligosaccharides (NAO) intended for use in food. The notice informs us of Dyne Bio's view that this use of β-agarase 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. Dyne Bio's notice provides information about the components in the β-agarase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, β-agarase is identified by the Enzyme Commission Number 3.2.1.81,[1] and the Chemical Abstracts Service Number is 37288-57-6. Dyne Bio states that the primary amino acid sequence of the β-agarase consists of 309 amino acids with a molecular weight of 32 kDa.

Dyne Bio states that the *S. coelicolor* production organism is a non-pathogenic and non-toxigenic bacterium. Dyne Bio states that the *S. coelicolor* production strain A3(2) M22-2C43 was produced by UV irradiation of strain A3(2) M22-2C43-WT. Dyne Bio states

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/81.html

that the sequence integrity of β-agarase is confirmed by PCR and DNA sequencing and that the final production strain does not contain any transferable antibiotic resistance genes.

Dyne Bio states that β-agarase enzyme preparation is manufactured by controlled fermentation of a pure culture of the *S. coelicolor* production strain. The enzyme is secreted into the fermentation medium. After fermentation, the medium containing the enzyme is separated from the biomass, recovered, and concentrated by centrifugation and a series of filtration and ultrafiltration steps. The resulting β-agarase enzyme preparation is a light to dark brown colored liquid and is formulated with water. Dyne Bio states that the entire process is performed in accordance with current good manufacturing practices and with food-grade raw materials. Dyne Bio also states that the β-agarase enzyme preparation does not contain any major food allergens.

Dyne Bio has established food-grade specifications and states that the β-agarase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 13th edition, 2022), 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). Dyne Bio provides results from analyses of three non-consecutive batches of β-agarase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism in the final product.

Dyne Bio states β-agarase enzyme preparation is intended for use at a maximum level of 20 mg TOS/g agar-agar to produce NAO. β-agarase hydrolyzes the 1,6 β-glucosidic bonds of agarose in the agar-agar. Dyne Bio notes that the β-agarase enzyme is removed during purification of NAO and states that the maximum residual enzyme content in NAO is 0.04 mg TOS/g NAO. Dyne Bio estimates a maximum dietary exposure to the β-agarase enzyme preparation to be 0.01 mg TOS/kg body weight (bw)/day from the use of NAO in food and drinks with the assumption that NAO is the only source of dietary fiber in the American diet and the added β-agarase enzyme preparation remains present in the final food containing NAO.

Dyne Bio relies on published information that discusses the safety of the *S. coelicolor* β-agarase and the safety of microbial enzyme preparations used in food processing. Dyne Bio summarizes the results of published toxicological studies using the β-agarase enzyme concentrate. Dyne Bio concludes that the β-agarase enzyme is not mutagenic or clastogenic. In the 90-day oral toxicity study using the β-agarase enzyme preparation, the highest dose tested, equivalent to 159 mg TOS/kg bw/d, was determined to be the no observed adverse effect level (NOAEL). Dyne Bio calculates an acceptable daily intake of 1.59 mg TOS/kg bw/day by applying a safety factor of 100 to the NOAEL, and notes that the maximum exposure to the β-agarase enzyme preparation from the intended uses, 0.01 mg TOS/kg bw/day, is well below this acceptable daily intake level.

Dyne Bio discusses publicly available literature, as well as the conclusions of several organizations and working groups, about the low risk of allergenicity posed by enzymes from their intended use, to address potential allergenicity due to β-agarase. Based on

bioinformatic analyses, using criteria recommended by FAO/WHO (FAO/WHO, 2001; Codex Alimentarius, 2009; JECFA, 2016), Dyne Bio reports that no sequence homology of *S. coelicolor* β-agarase to known allergens that would raise allergenicity concerns were identified. Based on the totality of the information available, Dyne Bio concludes that it is unlikely that oral consumption of β-agarase will result in allergenic responses from its intended uses.

Based on the data and information summarized above, Dyne Bio concludes that β-agarase enzyme preparation is GRAS for its intended use.

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

Conclusions

Based on the information that Dyne Bio provided, as well as other information available to FDA, we have no questions at this time regarding Dyne Bio's conclusion that β-agarase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that β-agarase enzyme preparation 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 001109 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2024.04.15 15:51:14 -04'00'

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