



Lori Gregg  
Novozymes NA  
77 Perry Chapel Church Rd  
Franklinton, NC 27525

Re: GRAS Notice No. GRN 000853

Dear Ms. Gregg:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000853. We received Novozymes NA's (Novozymes) GRAS notice on April 5, 2019 and filed it on May 28, 2019.

The subject of the notice is lysozyme enzyme preparation produced by *Trichoderma reesei* carrying the gene coding for lysozyme from *Acremonium alcalophilum* (lysozyme enzyme preparation) for use as an enzyme during the manufacture of xanthan gum, gellan gum, and yeast extracts at a maximum use level of 544 mg total organic solids (TOS)/kg raw material. The notice informs us of Novozymes' view that this use of lysozyme 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. Novozymes' notice provides information about the components in the lysozyme enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, lysozyme is identified by the Chemical Abstracts Service number 9001-63-2 and Enzyme Commission Number 3.2.1.17.<sup>1</sup> Novozymes states that the molecular weight of the lysozyme is 23 kDa and that it has determined the nucleotide and amino acid sequences.

Novozymes states that the *T. reesei* production organism is non-pathogenic and non-toxicogenic, and that it does not produce antibiotics or mycotoxins under conditions used to produce enzymes. Novozymes describes the *T. reesei* production strain, AGJG-20A2,

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<sup>1</sup> <https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/17.html>

to consist of an expression cassette containing the lysozyme gene<sup>2</sup> from *A. alcalophilum*,<sup>3</sup> a fragment of the *T. reesei cbh1* (cellubiohydrolase 1) promoter, the transcriptional terminator of *cbh1*, and the selective marker, *amdS*. Novozymes states that the production strain was constructed by targeted homologous integration of the expression cassette into three loci-specific sites in the genome of the recipient strain, *T. reesei* RUTC30.<sup>4</sup>

Novozymes states that it confirmed the insertion of the expression cassettes by Southern blot, PCR analyses, and DNA sequencing. Novozymes evaluated the stability of the introduced gene by Southern blot hybridization. Additionally, Novozymes verified that there are no functional antibiotic resistance genes in the final construct by sequence analysis.

Novozymes states that the lysozyme enzyme preparation is manufactured by submerged fermentation of a pure culture of the *T. reesei* production strain under controlled conditions, and that the enzyme is excreted into the fermentation broth. Novozymes states that the enzyme is separated from the microbial biomass by filtration or centrifugation, followed by multiple concentration steps. This is followed by standardization and preservation with sorbitol, potassium sorbate, and sodium benzoate to a liquid enzyme concentrate. Novozymes states that the entire process is performed using food-grade raw materials and in accordance with current good manufacturing practices. Novozymes also states that the final lysozyme enzyme preparation does not contain any major food allergens from the fermentation media.

Novozymes has established food grade specifications and states that the lysozyme enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 11<sup>th</sup> edition, 2018), 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). Novozymes provides analytical data from analyses of three batches of lysozyme enzyme preparation to demonstrate that the manufacturing acceptance criteria have been met, including the absence of the production strain.

Novozymes intends to use lysozyme enzyme preparation to remove cellular debris at a maximum use level of 544 mg TOS/kg raw material in the manufacture of xanthan gum, gellan gum, and yeast extracts. Novozymes notes that the lysozyme will be denatured or removed during the production of xanthan gum, gellan gum, and yeast extracts. However, in estimating dietary exposure, Novozymes assumes that all the lysozyme will remain in the final food. Novozymes estimated a maximum dietary exposure to lysozyme to be 1 mg TOS/kg body weight per day (mg TOS/kg bw/d) from all the

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<sup>2</sup> Novozymes states that the DNA sequence for the introduced gene is based on the sequence encoding a lysozyme from *A. alcalophilum*.

<sup>3</sup> Novozymes confirms the safety of *A. alcalophilum* and states that its DNA well characterized and that they only used DNA containing the lysozyme coding sequence.

<sup>4</sup> Novozymes states that *T. reesei* RUTC30 (ATCC 56765) is derived from the well characterized *T. reesei* QM6a; the parent of a majority of *T. reesei* production strains. Novozymes states that RUTC30 was modified by classical mutagenesis prior to the integration of the expression cassette carrying the lysozyme gene.

intended uses.<sup>5</sup>

Novozymes relies on published information that discusses the safety of the *T. reesei* production organism and the safety of microbial enzyme preparations used in food processing and the historical uses of lysozyme. Additionally, Novozymes discusses the ubiquity of lysozyme in microorganisms, plants, animals, and humans. Finally, Novozymes performed a literature search on lysozyme in Scopus, reviewed the available abstracts, and found none to be inconsistent with their conclusion of the general recognition of safety of lysozyme.

Novozymes 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 lysozyme. Novozymes further provides unpublished toxicological studies on the article of commerce, including reverse mutation assay, *in vitro* human lymphocyte chromosome aberration assay, and a 13-week oral toxicity study in rats, to corroborate safety of their lysozyme for the intended use. Based on the No Observed Adverse Effect Level from this 13-week oral toxicity study in rats and the estimated exposure, Novozymes estimates a margin of exposure to be 1110. Further, based on bioinformatic analyses, Novozymes reports that the lysozyme does not share any biologically meaningful sequence homology or sequence identity to potential oral allergens. Based on the totality of the information available, Novozymes concludes that it is unlikely that consumption of lysozyme from the intended use will result in allergenic responses.

Based on the data and information summarized above, Novozymes concludes that lysozyme 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 Novozymes' notice concluding that lysozyme 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 lysozyme enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing lysozyme enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

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<sup>5</sup> Novozymes uses the Budget method to estimate dietary exposure to lysozyme enzyme preparation based on a maximum use level of lysozyme enzyme preparation of 544 mg TOS/ kg raw material, a maximum of 5% of xanthan gum, gellan gum, and yeast extracts in processed foods, and consumption of a maximum of 12.5 g of solid foods and 25 g of beverages per kg body weight per day.

## Conclusions

Based on the information that Novozymes provided, as well as other information available to FDA, we have no questions at this time regarding Novozymes' conclusion that lysozyme enzyme preparation produced by *Trichoderma reesei* expressing a gene encoding lysozyme from *Acremonium alcalophilum* is GRAS under its intended conditions of use. This letter is not an affirmation that lysozyme enzyme preparation produced by *Trichoderma reesei* expressing a gene encoding lysozyme from *Acremonium alcalophilum* 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 000853 is accessible to the public at [www.fda.gov/grasnoticeinventory](http://www.fda.gov/grasnoticeinventory).

Sincerely,

Susan J.  
Carlson -S

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

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