



Joab Trujillo  
8211 W. Broward Blvd.  
Suite 420  
Plantation, FL 33324

Re: GRAS Notice No. GRN 001187

Dear Mr. Trujillo:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001187. We received AB Enzymes, Inc.'s (AB Enzymes) notice on April 25, 2024, and filed it on June 24, 2024. AB Enzymes submitted amendments to the notice on October 30, 2024, and January 16, 2025, that provided clarifications on specifications and safety information.

The subject of the notice is polygalacturonase enzyme preparation produced by *Trichoderma reesei* expressing a polygalacturonase gene from *Aspergillus luchuensis* mut *kawachii* (polygalacturonase enzyme preparation) for use as an enzyme at up to 5 mg total organic solids (TOS)/ kg raw material in fruit and vegetable processing, coffee processing, flavoring production, and wine production. The notice informs us of AB Enzymes' view that this use of polygalacturonase 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 polygalacturonase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, polygalacturonase is identified by the Chemical Abstracts Service number 9032-75-1 and the Enzyme Commission Number 3.2.1.15.<sup>1</sup> AB Enzymes states that the primary sequence of polygalacturonase consists of 338 amino acids with a calculated molecular weight of 35.5 kDa.

AB Enzymes states that the *T. reesei* production organism is non-pathogenic and non-toxicogenic and is a well-characterized production organism with history of safe use in the food industry. AB Enzymes states that the production strain, *T. reesei* AR-414, was

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<sup>1</sup> <https://iubmb.qmul.ac.uk/enzyme/EC3/2/1/15.html>

constructed through transformation with an expression cassette carrying an *A. luchuensis* mut *kawachii* polygalacturonase gene. AB Enzymes states that they confirmed sequence integration by whole genome sequencing. AB Enzymes evaluated the stability of the production strain by measurements of enzyme activity and Southern blot analysis. AB Enzymes verified the absence of functional or transferable antibiotic resistance genes in the final production strain genome.

AB Enzymes states that the polygalacturonase enzyme preparation is produced by submerged fermentation of a pure culture of the *T. reesei* production strain under controlled conditions. The polygalacturonase enzyme is secreted into the fermentation medium and then recovered by pre-treatment with flocculants or filter aids, primary separation with centrifugation or filtration, concentration via filtration, and a final polish filtration. The resulting enzyme concentrate is a brown liquid. The polygalacturonase enzyme preparation is then standardized to the desired activity level and preserved with ingredients that include glycerol, potassium chloride, trisodium citrate, and citric acid. AB Enzymes states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices.

AB Enzymes has established food-grade specifications including a limit for lead (<0.5 mg/kg) and states that the polygalacturonase enzyme preparation conforms to the specifications set 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). AB Enzymes provides results from analyses of three non-consecutive batches of polygalacturonase enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism and antibiotic activity.

AB Enzymes intends to use polygalacturonase enzyme preparation at a maximum use level of 5 mg total organic solids (TOS)/ kg raw material as the liquid form in fruit and vegetable processing, coffee processing, flavoring production, and wine production. AB Enzymes states that polygalacturonase is added to the respective raw materials to break down pectin into oligosaccharides (mainly mono-galacturonic acid). AB Enzymes notes that the final enzyme is inactivated, denatured, or removed during food manufacturing. AB Enzymes states that any ingested inactive polygalacturonase in the final food is expected to be digested and not pose any human health risk. AB Enzymes estimates a maximum dietary exposure to polygalacturonase enzyme preparation to be 0.188 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses and assumes that all of the polygalacturonase enzyme preparation remains in the final food.<sup>2</sup>

In support of the safety of the polygalacturonase enzyme preparation, AB Enzymes highlights published information that supports the history of safe use of polygalacturonase in food as well as safety of the donor organism. AB Enzymes states that any active or inactive polygalacturonase remaining in the final food will be digested

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<sup>2</sup> AB Enzymes uses the Budget Method to estimate the dietary exposure to polygalacturonase enzyme preparation based on 12.5 g of solid foods and 25 g of beverages per kg bw/d (worst case scenario) containing the polygalacturonase enzyme preparation at the recommended use level.

and thus will not pose any safety risk. AB Enzymes discusses that enzymatic side activities, common in food enzyme preparations, are present at very low levels and do not pose a safety concern. Additionally, AB Enzymes states that a literature search did not identify any information that would contradict a general recognition of safety of the polygalacturonase enzyme preparation. AB Enzymes states that the amino acid sequence and protein structure of the polygalacturonase enzyme preparation that is the subject of this notice is sufficiently similar to polygalacturonases that were the subject of GRNs 000557 and 000982 such that safety information for those polygalacturonases is relevant to the safety assessment of this polygalacturonase.<sup>3</sup> AB Enzymes relies on published information that discusses the safety of the *T. reesei* production organism.

AB Enzymes discusses publicly available literature to address potential allergenicity due to polygalacturonase. Based on bioinformatic analysis, AB Enzymes reports no significant matches between the amino acid sequences of the polygalacturonase and the primary sequences of known food allergens based on the guidelines developed by the Codex Alimentarius Commission (Codex, 2009). AB Enzymes also discusses bioinformatics analyses of the NCBI Identical Protein Group database containing known toxins and concluded that the polygalacturonase sequence showed no significant homology to known toxins. Based on the totality of information available, AB Enzymes concludes that it is unlikely that oral consumption of polygalacturonase preparation from the intended uses will result in allergic or toxigenic responses.

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

### **Standards of Identity**

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

### **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

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<sup>3</sup> The subject of GRN 000557 is polygalacturonase preparation produced by *Trichoderma reesei* expressing polygalacturonase from *Aspergillus tubingensis*; the subject of GRN 000982 is polygalacturonase enzyme preparation produced by *Aspergillus oryzae* expressing a polygalacturonase from *A. tubingensis* *Mosseray*. We evaluated these notices and responded in letters dated July 23, 2015 and February 2, 2022, respectively, stating that we had no questions at the time regarding the notifier's GRAS conclusions.

section 301(l)(1)-(4) applies. In our evaluation of AB Enzymes' notice concluding that polygalacturonase enzyme preparation is GRAS under its intended conditions of use, we did not consider whether section 301(l) or any of its exemptions apply to foods containing polygalacturonase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing polygalacturonase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(l).

## 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 polygalacturonase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that polygalacturonase 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 001187 is accessible to the public at [www.fda.gov/grasnoticeinventory](http://www.fda.gov/grasnoticeinventory).

Sincerely,  
Susan J.  
Carlson -S

 Digitally signed by Susan J.  
Carlson -S  
Date: 2025.02.28 17:08:39  
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Susan Carlson, Ph.D.  
Director  
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