



Dicentra, LLC
Attention: Mr. Dylan Fronda, B.Sc.
Regulatory and Scientific Specialist
2 Bloor Street W #700
Toronto, Ontario M4W 3E2
CANADA

Re: GRAS Notice Submission – *Ligilactobacillus salivarius* L28

Dear Mr. Fronda:

The Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM) refers to a generally recognized as safe (GRAS) notice, dated July 2025, received November 12, 2025, submitted on behalf of your client, NexGen Innovations, LLC (the notifier). The subject of the notice is *Ligilactobacillus salivarius* L28 (hereafter known as *Ligilactobacillus salivarius* L28 or the notified substance) for use as a viable microorganism in beef and dairy cattle at 1×10^6 CFU of *Ligilactobacillus salivarius* L28/head/day. The submission informs us of the notifier's conclusion that the subject of the submission is GRAS through scientific procedures. You were notified in a letter, dated January 9, 2026, that the GRAS notice was acceptable for filing, and the notice was designated as animal GRAS notice number (AGRN) 90. CVM received an amendment on May 12, 2026 from the notifier containing additional information.

We have completed our evaluation of the GRAS notice and amendment as well as other data and information that are available to the Agency. As discussed more fully below, the notice does not provide a sufficient basis for a conclusion that the notified substance is GRAS under conditions of its intended use as a viable microorganism in beef and dairy cattle.

To address the identity, method of manufacture, and specifications of the notified substance, the notifier provided information for manufacture and process controls, specifications of the market formulations, analytical methods to enumerate viable cell count, and analytical methods for potential microbial contaminants and heavy metals. The notified substance is produced through batch fermentation. After the fermentation, the biomass is harvested by centrifugation, mixed with cryoprotectants, and freeze-dried. The freeze-dried cell concentrate is further mixed with excipients to produce market formulations. The notifier provides specifications, packaging, and some stability information for the market formulations.

Based on the information contained in the notice CVM has questions at this time regarding the information provided to address the method of manufacture and specifications for the notified substance for its intended use for the following reasons: 1) the notice lacks the specifications and batch analyses information of the notified substance, which is the freeze-dried powder of *Ligilactobacillus salivarius* L28 with cryoprotectants before mixing with the excipients to produce the market formulations, and 2) the notice does not provide sufficient data and information to support the stability of the notified substance and the market formulations.

To address safety of the notified substance in the target animal through molecular biology, identity, and microbial safety the notifier provides a description of the genome and microbial safety analysis conducted for the notified substance. The notifier used Illumina Miseq (short

reads sequencing) to sequence the whole genome of *Ligilactobacillus salivarius* L28. Subsequently the sequenced genome was processed for downstream analyses. The taxonomic identification was carried out using whole genome Average Nucleotide Identity analysis. Subsequently, a series of bioinformatics tools and online databases were used to analyze the coding sequence of the *Ligilactobacillus salivarius* L28 genome for the presence of potential plasmids, antimicrobial resistance genes, and pathogenicity and virulence related genes. The notifier conducted a literature search to address the microbial safety of the notified substance.

The notifier cites publicly available and unpublished data and information including the microbial and genome safety information, historical natural occurrence of *Ligilactobacillus salivarius* in humans and animals, and several feeding trials in which the notified substance was included in diets of beef and dairy cattle.

Based on the data and information contained in the notice, and other information available to the agency, CVM has questions regarding the information provided to support safety to the target animal through molecular biology, identity, and microbial safety analysis for the notified substance. Genomic analysis confirmed the presence of the tet(M) antimicrobial resistance gene in the notified substance, yet the notifier's assertion that mobile genetic elements are absent is contradicted by the NCBI whole-genome sequencing assembly, indicating horizontal gene transfer potential.

To address the human food safety of the intended use of the notified substance to humans consuming edible products from beef and dairy cattle, the notifier provides information to support that there is no expectation that the notified substance will be transferred from the rumen to edible tissues of target animals and refers to the microbial and genome safety assessments and the natural occurrence of the species *Ligilactobacillus salivarius* in gastrointestinal tracts of humans and animals.

Based on the information contained in the notice CVM has questions at this time regarding the notifier's conclusion on the safety to humans consuming edible products from beef and dairy cattle that have consumed the notified substance. CVM questions the conclusion from data provided to demonstrate lack of transmissibility of the antimicrobial resistance gene tet(M) in the notified substance.

Section 403(a) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Under section 403(a) of the FD&C Act, a food is misbranded if its labeling is false or misleading in any particular. NexGen Innovations, LLC did not provide any information to demonstrate that the notified substance functions as intended because the notifier concluded that the intended use would not be expected to impact safety. Therefore, we did not evaluate whether the notified substance would achieve the effect claimed for it. However, please note that if products containing the notified substance bear any claims on the label or in labeling regarding the function of the notified substance, these claims should be supported by appropriate data and information. FDA may take enforcement action if any claims on labels or labeling are found to be false or misleading.

Conclusion

Based on the information that you provided, as well as other information available to FDA, the notice

does not provide a sufficient basis for a conclusion of GRAS status for the intended use of *Ligilactobacillus salivarius* L28 for use as a viable microorganism in beef and dairy cattle at 1×10^6 CFU of *Ligilactobacillus salivarius* L28/head/day.

In accordance with 21 CFR 570.275(b)(2), the text of this letter responding to AGRN 90 is accessible to the public on our website for the Current Animal Food GRAS Notices Inventory at <https://www.fda.gov/animal-veterinary/generally-recognized-safe-gras-notification-program/current-animal-food-gras-notices-inventory>.

If you have any questions concerning this letter, please contact Ms. Wasima Wahid at animalfood-premarket@fda.hhs.gov.

Sincerely,

/s/

David Edwards, Ph.D.
Director, Division of Animal Food Ingredients
Center for Veterinary Medicine