July 22, 2022

Kristi O. Smedley, Ph.D.
Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Road
Woodbridge, VA 22192

Re: Animal GRAS Notice No. AGRN 42

Dear Dr. Smedley:

The Food and Drug Administration's (FDA or the agency) Center for Veterinary Medicine (CVM or we) refers to a generally recognized as safe (GRAS) notice, dated December 31, 2020, submitted on behalf of your client Native Microbials, Inc. (Native Microbials or the notifier). The subject of the submission is *Butyrivibrio fibrisolvens* ASCUSDY19, used as a viable microorganism in dairy cattle feed at an intended use rate of $1x10^8$ CFU/cow/day. The submission informs FDA of the notifier's conclusion that the subject of the submission is GRAS through scientific procedures. Following an initial evaluation, you were notified in a letter dated February 12, 2021 that the GRAS notice was acceptable for filing, and the notice was designated as animal GRAS notice number (AGRN) 42. During the evaluation the notifier submitted an amendment on November 2, 2021 to address questions on the identity of the microorganism, molecular biology, utility, microbial safety, and chemistry, manufacturing, and controls. On January 11, 2022 the notifier clarified information in the amendment for utility and chemistry, manufacturing and controls. The notifier provided an amendment to address target animal safety questions on May 24, 2022. We have completed our evaluation of AGRN 42.

Native Microbials provides information about the identity, method of manufacture and specifications of the notified substance. The notified substance, *B. fibrisolvens* ASCUSDY19, is produced through an anaerobic dextrose fed-batch fermentation process. After the fermentation, the biomass is harvested by centrifugation, and mixed with cryoprotectants followed by freeze drying. The freeze-dried cell concentrate is encapsulated with hydrogenated glycerides or other fat to produce the final product, Fat Encapsulated *B. fibrisolvens* ASCUSDY19. The notifier provides specifications for the finished product which include viable cell counts no less than $2x10^7$ CFU/g, coliforms less than 10 CFU/g, *Escherichia coli* less than 10 CFU/g, *Salmonella* negative in 25 g, and *listeria* negative in 25g. The notifier also provides the stability and packaging information for the notified substance and finished product, Fat Encapsulated *B. fibrisolvens* ASCUSDY19.

The notice includes a description of the whole genome sequence analysis conducted for the notified substance *B. fibrisolvens* ASCUSDY19. The notifier used both Illumina Miseq (short reads sequencing) and Oxford Nanopore (long reads sequencing) platforms to sequence whole genome of *B. fibrisolvens* ASCUSDY19. Subsequently the sequenced genome was processed for downstream analyses, including taxonomic identification, plasmid detection, antimicrobial resistance genes, pathogenicity and virulence. The taxonomic identification was performed using 16S rRNA analysis and whole genome Average Nucleotide Identity analysis. The notifier used a bioinformatics tool named Bandage for genome assembly visualization and plasmid detection in the *B. fibrisolvens* ASCUSDY19 genome. The antimicrobial resistance genes were characterized by searching in two databases including Comprehensive Antibiotics Resistance Database (CARD) and National Database of Antibiotic Resistant Organisms (NDARO), as well as on two web servers including ResFinder and AMRFinder Plus using predicted protein sequences. Similarly, the pathogenicity and virulence were analyzed by searching in four databases including Victors, Virulence Factors Database (VFDB), PATRIC_VF, and Pathogen-host interaction database (PHI-base), as well as on two web servers including IslandViewer 4 and PathogenFinder using predicted coding sequences.

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To address identity and microbial safety of *B. fibrisolvens* ASCUSDY19, the notifier provides a narrative based on scientific data and literature that addresses different aspects, including genomic analysis, the presence of the microbial species in animals, pathogenicity, toxin production, and published literature, to support its conclusion that *B. fibrisolvens* ASCUSDY19 is safe for the intended use as a supplemental source of viable microorganisms in dairy cattle feed to "support the digestion of feed in the rumen".

To address the target animal safety of the notified substance, the notifier provides a narrative based on scientific data and literature that addresses different aspects, including genomic analysis, the presence of the microbial species in animals, pathogenicity, toxin production, in silico analyses, published literature, and results from an unpublished feeding trial to support its conclusion that B. fibrisolvens strain ASCUSDY19 is safe for the intended use as a supplemental source of viable microorganisms in dairy cattle feed, at an intended use rate of $1x10^8$ CFU/cow/day. Native Microbials concludes that information on the physical or other technical effect of the notified substance, *B. fibrisolvens* ASCUSDY19, is not necessary because use of the notified substance to support digestion of nutrients in the rumen of dairy cattle does not impact target animal safety.

Native Microbials concludes that *B. fibrisolvens* ASCUSDY19 should not be associated with any human food safety concerns under the intended conditions of use as a viable microbial in the feed of dairy cattle and states that no transfer of viable *B. fibrisolvens* ASCUSDY19 from the rumen to milk or other edible tissues is anticipated under the conditions of intended use. The strain has been characterized as *B. fibrisolvens* and whole genome sequence analysis indicates the absence of any genetic element sequences that code for virulence factors or protein toxins. Native Microbials did not provide data or information to support its conclusion that there is no transfer of viable *B. fibrisolvens* ASCUSDY19 from the rumen to milk or other edible tissues.

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. The notifier did not provide any information to demonstrate that *B. fibrisolvens* ASCUSDY19 functions as intended because Native Microbials concluded that the intended use would not be expected to impact safety. Therefore, we did not evaluate whether *B. fibrisolvens* ASCUSDY19 would achieve the effect claimed for it. However, please note that if products containing *B. fibrisolvens* ASCUSDY19 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.

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 Native Microbials notice, concluding that *B. fibrisolvens* ASCUSDY19 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 *B. fibrisolvens* ASCUSDY19. Accordingly, our response should not be construed to be a statement that foods containing *B. fibrisolvens* ASCUSDY19 if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusion

Based on the information contained in the notice and amendments submitted by Native Microbial, Inc., as well as other information available to the FDA, we have no questions at this time regarding the notifier's conclusion that *Butyrivibrio fibrisolvens* ASCUSDY19 as a supplemental source of viable microorganism in the diets of dairy cattle at an intended use rate of $1x10^8$ CFU/cow/day is GRAS. The agency has not,

however, made its own determination regarding the GRAS status of the intended use of the notified *Butyrivibrio fibrisolvens* ASCUSDY19 in animal food under 21 CFR 570.35. Unless noted above, our evaluation did not address other provisions of the FD&C Act. As always, it is the continuing responsibility of Native Microbials to ensure that animal food ingredients that the notifier markets are safe and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with 21 CFR 570.275(b)(2), the text of this letter responding to AGRN 42 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 about this letter, please contact Ms. Carissa Adams at (240) 402-6283 or carissa.adams@fda.hhs.gov.

Sincerely,

/s/
Timothy Schell, Ph.D.
Director
Office of Surveillance and Compliance
Center for Veterinary Medicine