



Memorandum

Date April 21, 2004

From Division of Petition Review (HFS-265)

Subject FAP 4A4754 – Mannitol produced by a fermentation method using *Lactobacillus intermedius (fermentum)*

To Division of Petition Review (HFS-265)
Attention: Celeste Johnston
Through: Robert I. Merker, Ph.D., Division of Biotechnology and GRAS Notice Review (HFS-255) Robert I. Merker

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REF

INTRODUCTION

In a petition to the Food and Drug Administration, zuChem, Inc. proposed to amend the food additive regulations under 21 CFR 180.25 to allow for the safe use of mannitol produced by a fermentation method using *Lactobacillus intermedius (fermentum)*. This memorandum presents the conclusions drawn from the data submitted by the petitioner in support of the microbiological safety of mannitol produced in such a manner. All page numbers referenced indicate the location of the information in the original petition.

STRAIN IDENTITY

The petitioner indicated that their microorganism was determined to be most similar to *Lactobacillus intermedius (fermentum)* NRRL B-3693, originally deposited in the USDA ARS Culture Collection at the National Center for Agricultural Utilization Research (formerly the Northern Regional Research Laboratory; <http://nrrl.ncaur.usda.gov/>) in Peoria, Illinois, and redeposited as *L. intermedius* B-30560. The petitioner had culture samples compared for similarity to other microorganisms at independent laboratories by 1500 bp analysis and 500 bp analysis; in both cases it was identified as being most similar to *L. fermentum* NRRL 3693. However, it is not clear whether the petitioner obtained their organism from the USDA ARS Collection, or if they have isolated their own strain that just happens to be similar. The petitioner should clarify this point.

The USDA ARS Culture Collection was contacted to determine the status and availability of the *L. intermedius (fermentum)* strains identified. The curator of the Bacterial Collection (Alejandro Rooney) indicated that *L. intermedius* B-3693 is available to the public by request. In addition, the same organism was redeposited into their Patent Collection reclassified as *Lactobacillus fermentum*. The Curator of the Patent Collection (James Swezey) indicated that no U.S. Patent has issued that cites this strain and so it is restricted from any public distribution from that collection.

The selection of *L. intermedius* B-3693 for its production of mannitol is described in a paper by Saha and Nakamura (page 436). This strain of *L. intermedius* had a yield of 0.70 for mannitol following fermentation using fructose. The organism has not been genetically modified, and the organism does not produce antibiotics.

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Given *L. intermedius (fermentum)* B-3693 is freely available from the USDA ARS Culture Collection and it has a very high yield for mannitol, it is probably reasonable to assume that, in the short term, mannitol produced using *L. intermedius (fermentum)* would be produced by utilizing that specific strain.

MANNITOL PRODUCTION

Mannitol will be produced by pure culture fermentation of fructose using *Lactobacillus intermedius (fermentum)*. The petitioner did not provide information regarding the procedures they use to maintain the cultural purity and genetic stability of the microorganism. Additionally, they did not provide information regarding the procedures they would use to guarantee cultural purity during the fermentation of mannitol, quality control procedures, criteria for purity or steps to be taken if contamination is observed.

While the criteria above are evaluated as part of the safety assessment, there is little enforcement capability for other producers of the subject food additive if the specifications are not written into the final regulation. The final regulation for mannitol produced using *L. intermedius (fermentum)* should include language such as that in 21 CFR 184.1924. For example, the regulatory language might include the following: "Mannitol is produced by a pure culture fermentation of sugars using the nonpathogenic, nontoxicogenic bacterium *Lactobacillus intermedius (fermentum)*." By specifying a pure culture fermentation and the nonpathogenic, nontoxicogenic bacterium, cultural purity and genetic stability of the starter culture and cultural purity during fermentation are regulatory requirements of the final product. Additionally, there are GMP and Food Chemical Codex requirements on the final product, and fermentation processes have a long history of reliable production of a variety of compounds on an industrial scale.

MANNITOL PROCESSING

Viable cells of *L. fermentum* must not be present in the final product. The petitioner has stated that _____ are included in the purification of their product.

The _____ step will remove bacteria and other particles greater than _____ in size. While this step would not remove all cells, it would greatly reduce their number. The _____ step will remove entirely *Lactobacillus* cells, and other particles and molecules greater than _____ in size. Additionally, _____ will be used to remove other impurities and contaminants from the broth. Subsequently, there will be no *Lactobacillus* present in the final product and no exposure to *Lactobacillus* by the final consumers of the mannitol produced.

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SAFETY OF *L. INTERMEDIUS (FERMENTUM)*

Lactobacilli form an important component of the normal human intestinal flora of healthy humans (page 447). *Lactobacillus* is a ubiquitous organism of the oropharynx, the gastrointestinal tract and the female genital tract. It is believed to protect the body from pathogenic organisms by competitive inhibition (they often produce bacteriocins or antibiotics). *L. bulgaricus* is specified in FDA's food standard for yogurt (21 CFR 131.200), and prior sanctions were granted for the use of harmless lactic acid producing bacteria, such as *L. acidophilus*, as optional ingredients in specified standardized foods. These bacteria are permitted for use in cultured milk (including buttermilk, §131.112), sour cream (§131.160),

cottage cheese (§133.128), and yogurt (<http://vm.cfsan.fda.gov/~dms/opa-micr.html>). *L. fermentum*, in particular, has a long history of safe consumption in food. It appears to be a normal component of sourdough bread fermentation and has been isolated from fermented maize dough, cheese, and in malt whiskey fermentations (page 103). Strains of *L. fermentum* are used in sourdough bread and pressed curd cheeses, and FDA has affirmed as GRAS a urease preparation from *L. fermentum* for use in wine manufacture.

Lactobacilli are rarely considered pathogens (page 299), and on those occasions they are opportunistic pathogens. Infections involving *Lactobacilli* are rare and, if any, are considered to be of endogenous origin. Additionally, these rare occurrences are usually observed under extraordinary conditions. A more extensive discussion of the safety of *Lactobacilli* is found in GRAS notification #49 (pages 28, 82-84, 179-181).

A single case of endocarditis associated with *Lactobacillus fermentum* has been documented in the literature.^[1] The authors note forty-three previous reports of endocarditis associated with *Lactobacilli*, but contend that this is the first associated with *L. fermentum*. However, one of the reviews noted that 20 of 24 cases of endocarditis occurred in patients with preexisting structural heart disease, and in 18 of the 24 cases, the patients had some form of recent dental infection or manipulation. Additionally, in the case associated with *L. fermentum*, the patient was treated recently for acute appendicitis, had dental work six and ten months prior to admission, and "her dietary history was remarkable for a large daily consumption of milk and milk products." To put this case and other cases associated with *Lactobacilli* into perspective, a recent survey of infective endocarditis covering 26 publications from 1993 to 2003 where 30 or more cases were described and covering 3784 episodes, *Lactobacillus* is not mentioned as a causative agent.^[2] The authors go on to identify four risk factors associated with endocarditis including intravenous drug use, sclerotic valve disease in elderly patients, use of prosthetic valves and nosocomial disease. These reports illustrate that there are circumstances under which a normally benign microorganisms, such as *Lactobacillus*, can breach barriers and become an opportunistic pathogen.

Given the long-standing safe use of *Lactobacilli* in food and the rare occurrence of opportunistic pathogenesis, we have no questions at this time of the petitioner regarding the safety of *Lactobacillus fermentum*.

RECOMMENDATIONS

1. The petitioner should clarify whether they are using the same strain of *Lactobacillus intermedius (fermentum)* in the USDA ARS Culture Collection, or if they have isolated their own strain that just happens to be similar. This request to the petitioner should be included in the comment section of a deficiency letter, or could be clarified by a simple telephone call if there are no other deficiencies noted by the other review disciplines.
2. The final regulation for mannitol produced using *L. intermedius (fermentum)* should include language such as that in 21 CFR 184.1924 specifying fermentation by a pure

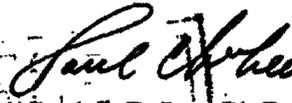
¹ Gallemore, G.H., R.T. Mohon, and D.A. Ferguson. 1995. *Lactobacillus fermentum* endocarditis involving a native mitral valve. *Journal of the Tennessee Medical Association*. 88(8):306-308.

² Moreillon, P. and Y. Que. 2004. Infective endocarditis. *The Lancet*. 363:139-149.

culture and a nonpathogenic, nontoxicogenic strain of *L. intermedius (fermentum)*. For example, the regulatory language might include the following: "Mannitol is produced by a pure culture fermentation of sugars using the nonpathogenic, nontoxicogenic bacterium *Lactobacillus intermedius (fermentum)*."

CONCLUSIONS

ZuChem is petitioning to amend §180.25 to allow for the safe use of mannitol produced by the fermentation of sugars using *Lactobacillus fermentum*. The data provided by the petitioner support the microbiological aspects of the safe use of mannitol produced in such a manner. We have no further questions of the petitioner.


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