Joanne Donoghue
Director, EHS & Operations Mascoma LLC
67 Etna Road, Suite 200
Lebanon, NH 03766

Re: GRAS Notice No. GRN 000841

Dear Ms. Donoghue:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000841. We received Mascoma LLC's (Mascoma) GRAS notice on January 29, 2019, and filed it on March 29, 2019. Mascoma provided amendments on January 9 and January 20, 2020, modifying their manufacturing protocol and discussing yeast sensitivities in literature.

The subject of the notice is a genetically engineered *Saccharomyces cerevisiae* expressing a synthetic gene encoding lactate dehydrogenase from *Rhizopus oryzae* (*S. cerevisiae* producing lactate dehydrogenase). The *S. cerevisiae* producing lactate dehydrogenase will be added at up to 1 gram per liter to ferment beer and to enhance its flavor. The notice informs us of Mascoma's view that this use of *S. cerevisiae* producing lactate dehydrogenase is GRAS through scientific procedures.

Mascoma states that the *S. cerevisiae* producing lactate dehydrogenase is non-pathogenic and non-toxigenic and has been modified by the addition of two copies of a synthetic (codon-optimized for *S. cerevisiae*) lactate dehydrogenase genes from wild-type *R. oryzae*.

Mascoma describes the construction of the production strain as targeted integration of an expression cassette carrying a *R. oryzae* LDH gene encoding lactate dehydrogenase, under the regulation of the native *S. cerevisiae* ADH1 promoter and PDC1 terminator. Mascoma confirms the integration,[1] the stability of the insertion for over 100 generations,[2] and that the final production strain does not contain any functional or transferable antibiotic resistance genes.[3]

Mascoma states that *S. cerevisiae* producing lactate dehydrogenase is obtained by fermentation of a pure culture of the production strain under controlled conditions

[1] *Via* whole genome sequencing, and PCR analyses.
[2] *Via* PCR.
[3] *Via* PCR genotyping and dilution plating.

including a source of carbon (typically molasses) and other nutrients. After fermentation is complete, the yeast cells containing the lactate dehydrogenase enzyme are centrifuged and washed to remove non-yeast soluble solids. The resulting yeast slurry contains 20-30% solids (200-300 g dry weight/kg) and packaged. Mascoma states that a dry product is also generated by processing the slurry through a filter press and dewatered under pressure to generate yeast-cake with 35-40% solids. The yeast-cake is then extruded and formulated with a food-grade emulsifier.

Mascoma states that the entire process is performed using food-grade raw materials and in accordance with current good manufacturing practices. Mascoma also states that the *S. cerevisiae* producing lactate dehydrogenase does not contain any major food allergens from the fermentation media. Mascoma states that the liquid product is stable for six months while the dry formulation is stable for two years.

Mascoma has established food grade specifications and states that the *S. cerevisiae* producing lactate dehydrogenase meets the criteria for total bacteria and wild yeast, total viable cells and solids (dry matter). Mascoma provides analytical data for three batches of *S. cerevisiae* producing lactate dehydrogenase to demonstrate that *S. cerevisiae* producing lactate dehydrogenase can be produced in accordance with the provided specifications.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, lactate dehydrogenase is identified by the Enzyme Commission Number 1.1.1.27.[4] Mascoma provides the amino acid sequence of the codon-optimized lactate dehydrogenase from *R. oryzae* present in *S. cerevisiae* producing lactate dehydrogenase.

Mascoma intends to use *S. cerevisiae* producing lactate dehydrogenase at up to 1 g/L to ferment beer and to enhance flavor. Mascoma notes that the *S. cerevisiae* producing lactate dehydrogenase will be non-viable or removed by pasteurization and/or filtration after processing; however, some *S. cerevisiae* producing lactate dehydrogenase may remain in the final food. Therefore, in order to estimate dietary exposure, Mascoma assumes that all the *S. cerevisiae* producing lactate dehydrogenase will remain in the final food. Mascoma estimates dietary exposure to *S. cerevisiae* producing lactate dehydrogenase to be 4.7 x 10^6 yeast cells/kg bw/d.[5,6]

Mascoma relies on published information that discusses the safety of *S. cerevisiae* producing lactate dehydrogenase, the safety of the host strain *S. cerevisiae* as a brewer's yeast (including the current authorizations for *S. cerevisiae* and *S. cerevisiae* derived ingredients), evolutionarily conserved biological roles of lactate dehydrogenase across other microorganisms commonly found in the food supply, and the safety of microbial

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[4] https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC1/1/1/27.html

[5] Mascoma assumes a maximum consumption of 418 mL of beer/person/day based on published literature and NHANES (2003-6), and a maximum 1x10^6 yeast cells/mL of beer.

[6] Mascoma also provided estimated dietary exposure to lactate dehydrogenase enzyme based on units of lactate dehydrogenase activity. FDA calculated the estimated dietary exposure to lactate dehydrogenase to be in 1 μg/kg bw/d based on the use level information provided in the notice.

enzyme preparations used in food processing. Additionally, Mascoma summarizes published toxicological studies of food-grade dried fermentate of *S. cerevisiae* to corroborate safety of the intended uses of *S. cerevisiae* producing lactate dehydrogenase. These studies include several *in vitro* genotoxicity and mutagenicity assays, as well as an acute, a 14-day, a 90-day, and a 1-year oral toxicity studies in rats. Mascoma states that no treatment-related adverse effects were observed in any of the *in vitro* or *in vivo* studies.

To address the potential allergenicity of lactate dehydrogenase, Mascoma discusses publicly available literature, as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes. Further, based on bioinformatic analyses, Mascoma reports that the lactate dehydrogenase does not share any biologically meaningful sequence homology or sequence identity to potential oral allergens. Based on the totality of the information available, Mascoma concludes that it is unlikely that oral consumption of lactate dehydrogenase enzyme will result in allergenic responses.

Based on the data and information summarized above, Mascoma concludes that *S. cerevisiae* producing lactate dehydrogenase 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 Mascoma' notice concluding that *S. cerevisiae* producing lactate dehydrogenase 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 *S. cerevisiae* producing lactate dehydrogenase. Accordingly, our response should not be construed to be a statement that foods containing *S. cerevisiae* producing lactate dehydrogenase, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Mascoma provided, as well as other information available to FDA, we have no questions at this time regarding Mascoma' conclusion that genetically engineered *Saccharomyces cerevisiae* expressing a synthetic gene encoding lactate dehydrogenase from *Rhizopus oryzae* is GRAS under its intended conditions of use. This letter is not an affirmation that genetically engineered *Saccharomyces cerevisiae* expressing a synthetic gene encoding lactate dehydrogenase from *Rhizopus oryzae* 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 000841 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2020.03.10 10:58:27 -04'00'

Susan Carlson, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition