



Charles Denby, Ph.D.
Berkeley Brewing Science
2332 4th Street, Suite E
Berkeley, CA 94710

Re: GRAS Notice No. GRN 000798

Dear Dr. Denby:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000798. We received Berkeley Brewing Science's (BBS) notice on July 3, 2018, and filed it on August 2, 2018. BBS submitted amendments to the notice on March 8 and April 25, 2019. These amendments provide additional information about the identity and method of manufacture of strain yBBS002, as well as additional safety information.

The subject of the notice is *Saccharomyces cerevisiae* strain yBBS002 for use at a level consistent with standard brewing industry practice (1 million cells per mL of wort per degree Plato¹) during wort fermentation to impart flavors associated with dry-hopping to the finished beer.² The notice informs us of BBS's view that this use of strain yBBS002 is GRAS through scientific procedures.

BBS discusses the identity and development of strain yBBS002. BBS states that strain yBBS002 is constructed from *S. cerevisiae* strain California Ale Yeast (CAY), a brewer's yeast strain commonly used in commercial beer production in the United States. BBS describes the construction of strain yBBS002 using genetic engineering techniques to introduce four genes into the strain CAY genome. Expression of the introduced genes results in the production of certain monoterpenoids associated with the flavor of dry-hopped beer.

Specifically, BBS introduced genes that encode the enzymatic domain of a 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and a farnesyl pyrophosphate (FPP) synthase with reduced functionality from yeast (*S. cerevisiae*), a linalool synthase from mint (*Mentha citrata*), and a geraniol synthase from basil (*Ocimum basilicum*). Expression of the four genes is under the control of regulatory elements derived from *S. cerevisiae* strain S288C.

¹ Degrees Plato is used to quantify the concentration of extract as a percentage of weight.

² Dry-hopping is a process whereby brewers add hops to the wort fermentation. The monoterpenoids linalool, geraniol, and citronellol in dry-hopped beer contribute to its characteristic flavor.

BBS discusses the results of DNA sequencing and other strain characterization analyses from which it concludes that strain yBBS002 contains the four genes but does not contain antibiotic resistance genes used for selection during strain development. In its notice, BBS provides additional information about the construction and characterization of strain yBBS002, which is designated as JBEI-16652 in a published study.

BBS explains that the method of manufacture of strain yBBS002 is identical to that used for the current commercial manufacture of liquid brewing yeast, including strain CAY. Briefly, a single colony from the stored strain stock is used to inoculate a small liquid culture, which is then used to inoculate a larger culture grown aerobically in medium containing malt extract. After fermentation, the cells are collected by centrifugation or flocculation and resuspended in a medium with low sugar content. BBS states that none of the components of the manufacturing process include or derive from major allergens and that all materials used in the manufacturing process are food-grade.

BBS provides specifications for yBBS002 strain that include viable cell count (2.75×10^9 yBBS002 colony forming units (CFU)/mL) and limits for other microorganisms.

BBS assesses dietary exposure to strain yBBS002 and to the introduced enzymes and monoterpenoids produced by strain yBBS002 based on the intended use of the strain as a substitute for dry-hopping. BBS describes standard commercial beer production practices and explains that the number of viable yeast cells declines following alcoholic fermentation and that both yeast and proteins are largely removed during beer clarification processes (e.g., cooling, centrifugation, and filtration). BBS reports that strain yBBS002 is comparable to commercial strain CAY in that both efficiently flocculate and will therefore be present, if at all, at negligible levels in finished beer. BBS reports that levels of monoterpenoids present in beer produced using strain yBBS002 (approximately 0.1 mg linalool/L, 0.4 mg geraniol/L, and 0.6 mg citronellol/L) are similar to levels in beer made using dry-hopping. BBS concludes that dietary exposure to strain yBBS002, its proteins, and the monoterpenoids will therefore be comparable to current dietary exposures to yeast, proteins, and monoterpenoids³ from consumption of conventionally dry-hopped beers.

BBS uses a weight-of-evidence approach and publicly available data and information to assess the safety of strain yBBS002, the introduced enzymes, and the resulting monoterpenoids. BBS describes *S. cerevisiae* as a non-pathogenic, non-toxigenic, well-characterized production organism with a history of safe use in the food industry. BBS explains that the genes encoding the four introduced enzymes (the enzymatic domain of the HMG-CoA reductase, FPP synthase with reduced functionality, linalool synthase, and geraniol synthase) are derived from brewer's yeast, mint, and basil, each of which has a history of safe use in food and in food production. BBS states that sequence homology comparisons between the peptide sequences of the four introduced enzymes

³ Assuming consumption of 750 g beer per day, FDA calculates dietary exposures of 0.08 mg/day (d) linalool, 0.3 mg/d geraniol, and 0.4 mg/d citronellol (0.001 mg/kg bodyweight (bw)/d, 0.005 mg/kg bw/d, and 0.007 mg/kg bw/d, respectively) for a cumulative estimated dietary exposure of 0.8 mg/d (0.013 mg/kg bw/d).

and those from databases of known allergens and toxins⁴ support a conclusion that the introduced enzymes lack similarity to food allergens and toxins. BBS explains that the monoterpenoids linalool, geraniol and citronellol are present in foods commonly consumed in the U.S. diet, including mint, basil, and beer.⁵

Based on publicly available scientific data assembled and presented in its GRAS notice, BBS concludes that strain yBBS002 is generally recognized as safe for use as a culture in brewing fermentation to impart flavors associated with dry-hopping.

Section 301(II) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Section 301(II) 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(II)(1)-(4) applies. In our evaluation of BBS's notice concluding that strain yBBS002 is GRAS under its intended conditions of use, we did not consider whether section 301(II) or any of its exemptions apply to foods containing strain yBBS002. Accordingly, our response should not be construed to be a statement that foods containing strain yBBS002, if introduced or delivered for introduction into interstate commerce, would not violate section 301(II).

Conclusions

Based on the information that BBS provided, as well as other information available to FDA, we have no questions at this time regarding BBS's conclusion that strain yBBS002 is GRAS under its intended conditions of use. This letter is not an affirmation that strain yBBS002 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.

⁴ BBS conducted sequence homology comparisons using the AllergenOnline database and the UniProt database (query for peptide annotation "toxin").

⁵ The Joint FAO/WHO Expert Committee on Food Additives group acceptable daily intake (ADI) inclusive of these monoterpenoids, affirmed most recently at the 61st meeting (2003), is 0-0.5 mg/kg bw/d (on a citral basis). FDA notes that the molecular weights of linalool, geraniol, and citronellol are similar to the molecular weight of citral.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 0000798 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

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Director
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