



GRAS Notice (GRN) No. 1094 with amendments  
<https://www.fda.gov/food/generally-recognized-safe-gras/gras-notice-inventory>

2451 Peralta St.  
Oakland, CA 94607

May 27, 2022

GRAS Notification Program  
Office of Food Additive Safety, HFS-200  
5001 Campus Drive  
College Park, MD 20740-3835

**GRAS Notice for *Saccharomyces cerevisiae* strain BY-989**

Dear Sir or Madam,

In accordance with 21 FR §170 Subpart E, Berkeley Fermentation Science Inc. (DBA Berkeley Yeast) hereby informs the United States Food and Drug Administration of the conclusion that *Saccharomyces cerevisiae* strain Y-989 is GRAS on the basis of scientific procedures, for use in beer fermentation to impart tropical fruit flavors/aromas during fermentation, and therefore, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. Information supporting the GRAS status of Y-989, under the intended conditions of use, is enclosed for review by the Agency.

I certify that the enclosed electronic files are virus-free.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process by email at [charles@berkeleyyeast.com](mailto:charles@berkeleyyeast.com). Thank you for your time and your consideration.

Sincerely,

**Berkeley Fermentation Science Inc.**

y: \_\_\_\_\_

Name: Charles Denby, Ph.D.

Title: Chief Executive Officer

# **GRAS notice for *Saccharomyces cerevisiae* strain BY-989**

**PREPARED FOR:**

Office of Food Additive Safety (FHS-200)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5001 Campus Drive  
College Park, MD 20740-3835

**DATE:**

May 27, 2022

## TABLE OF CONTENTS

PART 1: § 170.225 SIGNED STATEMENTS AND CERTIFICATION	4
1.1 Exemption from premarket approval	4
1.2 Basis for GRAS determination	4
1.3 Name and address of the notifier	4
1.4 Common name of the notified substance	4
1.5 Intended conditions of use	4
1.6 Availability of information for FDA review	5
1.7 Disclosure and certification	5
PART 2: §170.230 IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT	6
2.1 Identity of the notified substance	6
2.2 Host microorganism	7
2.3 Donor organisms	8
2.4 The modified microorganism	10
2.5 Intended use of BY-989	13
2.6 Method of manufacture of BY-989	14
PART 3: §170.235 DIETARY EXPOSURE	15
3.1 Intended use of BY-989	15
3.2 Levels of use of the substance	15
3.3 Dietary exposure	15
PART 4: §170.240 SELF-LIMITING LEVELS OF USE	17
PART 5: §170.245 EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958	18
PART 6: §170.250 NARRATIVE	19
6.1 Overview of safety assessment	19
6.2 Safety assessment of the host strain	19
6.3 Safety assessment of the donor strains	19
6.4 Safety assessment of the CSL enzyme	20
6.5 Safety assessment of the promoter and terminator sequences	21
6.6 Safety assessment of BY-989	21
6.7 Precedence for safety of engineered <i>S. cerevisiae</i> strains	25
6.8 Conclusions for GRAS determination	25
PART 7: § 170.255 LIST OF SUPPORTING DATA AND INFORMATION	27
7.1 Supporting information	27
7.2 References	27

## LIST OF FIGURES

Figure 1: CSL is an enzyme that converts conjugated thiols to free thiols	7
Figure 2: Diagram of genomic integration	11
Figure 3: Interdelta analysis of LAY and BY-989	12
Figure 4: Fermentation profiles of LAY and BY-989	22
Figure 5: Identification of 3MH and 3MHA using liquid chromatography mass spectrometry	25

# **PART 1: SIGNED STATEMENTS AND CERTIFICATION**

## **1.1. Exemption from premarket approval**

In accordance with Title 21 C.F.R. §170.30, Berkeley Fermentation Science Inc. hereby informs the United States (U.S.) Food and Drug Administration (FDA) of the view that its *Saccharomyces cerevisiae* strain BY-989 is Generally Recognized as Safe (GRAS) under the conditions of its intended use and is, therefore, exempt from the requirement for premarket approval from the Federal Food, Drug, and Cosmetic Act.

## **1.2. Basis for GRAS determination**

The determination of the GRAS status is based on scientific procedures and conforms to the regulations in accordance with Title 21 C.F.R. §170.30.

## **1.3. Name and address of the notifier**

Berkeley Fermentation Science Inc.  
2451 Peralta Street  
Oakland, CA 94607

## **1.4. Common name of the notified substance**

*Saccharomyces cerevisiae* expressing a gene encoding a carbon-sulfur lyase derived from *Citrobacter freundii*. This novel yeast strain is called BY-989.

## **1.5. Intended conditions of use**

Yeast strain BY-989 is intended for use in beer fermentation to impart tropical fruit flavors/aromas. BY-989 is used as any other commercial yeast would be used in the beer manufacturing process. During fermentation, BY-989 releases the volatile thiol 3-mercapto-1-hexanol (3MH), a desirable aroma molecule in both beer and wine with a high aroma impact, which imparts notes of guava, passion fruit, grapefruit, and gooseberry. BY-989 also produces elevated levels of 3-mercaptohexyl acetate (3MHA), through the action of endogenous alcohol acyltransferase (AAT) enzymes. 3MHA imparts similar sensory notes, most commonly described as passion fruit. Brewers seek out tropical fruit flavors in specialty hops cultivars, and as such, the hop preparations that contain aromas indicating elevated levels of 3MH and 3MHA are prized throughout the industry. However, even the most aromatic hops contain only modest quantities of volatile thiols, making it difficult to produce beers that have high concentrations of volatile thiols and strong tropical flavor notes. In addition, these cultivars are vulnerable to stresses from climate change, having been bred for aroma quality and not drought resistance (or resistance to other abiotic stresses).



## **PART 2: IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT**

### **2.1. Identity of the notified substance**

#### **2.1.1 Identity of the production strain**

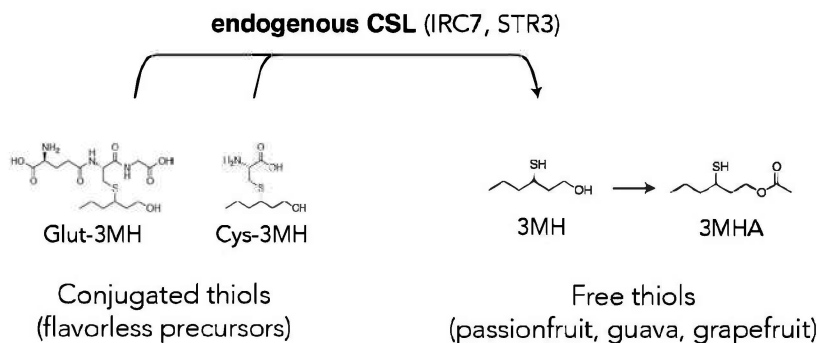
The subject of this notification is an industrial brewing *Saccharomyces cerevisiae* strain called BY-989. This strain contains one heterologous gene derived from *Citrobacter freundii*, a gram-negative bacterium found in the human microbiome, and produces a carbon-sulfur lyase enzyme (CSL). The gene was codon optimized for *S. cerevisiae* and synthesized by oligonucleotide assembly, based on the *C. freundii* amino acid sequence.

#### **2.1.2 Identity of the enzyme**

CSL is an enzyme that converts the conjugated thiols glutathione-3MH and cysteine-3MH to free 3MH (3-mercapto-1-hexanol). Conjugated thiols are flavorless molecules that exist in barley, hops, and grapes and serve as precursors to free thiols. When CSL cleaves the carbon-sulfur bond in the precursor molecules glutathione-3MH and cysteine-3MH, it releases free 3MH, which imparts a tropical fruit flavor and aroma (Figure 1). 3MH has a high aroma impact and is a desirable aroma molecule in both beer and wine. The phenomenon whereby flavorless precursor compounds are converted to flavorful aroma compounds can be referred to as “biotransformation”. In this case, glutathione-3MH and cysteine-3MH are biotransformed into 3MH.

The academic literature has reported that two different *S. cerevisiae* genes, IRC7 and STR3, encode CSL enzyme variants<sup>1,2</sup>. Previous studies that characterized endogenous carbon-sulfur lyase activity, across a panel of different industrial yeast strains, reported limited carbon-sulfur lyase activity, and showed that only 0.2-3.2% of available precursor was converted to 3MH<sup>3,4</sup>.

*S. cerevisiae* also endogenously encodes multiple alcohol acyltransferase (AAT) enzymes, some of which have been reported to catalyze the formation of 3MHA from 3MH (Figure 1)<sup>5</sup>. 3MHA imparts sensory notes similar to 3MH, most commonly described as passion fruit.



**Figure 1. CSL is an enzyme that converts conjugated thiols to free thiols.** Two different *S. cerevisiae* genes, IRC7 and STR3, encode CSL enzyme variants. 3MHA is formed from 3MH through the action of endogenous alcohol acyltransferase enzymes.

BY-989 expresses a more active CSL enzyme that efficiently releases 3MH from both glutathione-3MH and cysteine-3MH precursors. Expression of this CSL increases 3MH concentrations in finished beer compared to beer made with the parent strain. The CSL enzyme expressed in BY-989 is a variant containing five amino acid changes compared with the wild-type *C. freundii* enzyme. These amino acid changes to the *C. freundii* CSL increase the specificity of the enzyme to the conjugated thiols glutathione-3MH and cysteine-3MH, whereas the wild-type enzyme acts on the additional substrate tryptophan (see Section 2.3.1).

In addition, we have found that because BY-989 produces more 3MH, it also produces increased 3MHA concentrations.

## 2.2 Host microorganism

The parent yeast strain from which BY-989 was derived is an industrial brewing strain of *Saccharomyces cerevisiae* called London Ale Yeast (LAY). LAY is an isolate from a London, UK brewery and belongs to the group of brewing strains known as ale strains, which are classified as belonging to the *Saccharomyces cerevisiae* species. LAY has been commonly used in commercial beer production for decades.

### 2.2.1. History of use

*Saccharomyces cerevisiae* has been used in food and beverage production for thousands of years. The first evidence of beer production comes from ancient Iran, Iraq, and Egypt, although spontaneous fermentations resulting in beer were likely happening far earlier than that, as wild yeasts in the air will ferment most grain left unattended<sup>6</sup>. Ancient peoples utilized yeast in the production of other fermented foods and baked goods as well<sup>7</sup>. Today yeast is an integral component in many industries, and millions of tons are produced commercially each year. *S.*



*cerevisiae* has also served as a model organism in scientific research and was the first eukaryote to have its genome completely sequenced.

### **2.2.2. Taxonomy**

The parent strain, LAY, is an isolate from London, UK and belongs to the group of brewing strains known as ale strains. Ale strains are classified as belonging to the *Saccharomyces cerevisiae* species. A considerable amount of genetic diversity exists within the species *S. cerevisiae*, and genome sequencing efforts have covered thousands of individuals<sup>8</sup>. The strains used for various industrial food and beverage manufacturing processes have evolved considerably from their wild ancestors, which likely inhabited primeval forests<sup>9</sup>. These industrial strains have been categorized into distinct clades; LAY falls within the "Beer 1" clade, which includes brewing strains from Britain, the US, Belgium, and Germany<sup>8</sup>.

### **2.2.3. Characteristics**

LAY is commonly used in the brewing industry, and is generally supplied to breweries in the form of a liquid slurry. It is recommended for use in the production of IPAs and classical British beer styles and has become one of the most popular strains used to make hazy IPAs. LAY is popular for making this style because it is known to be a high producer of ester compounds, and is noted for its fruity character and slightly sweet finish.

A common misconception is that the haze associated with hazy IPAs is due to yeast remaining in solution. In actual fact, the haze seen in this style of beer is derived from complexes between proline-rich proteins and hop-derived polyphenols, and is not due to the yeast itself<sup>10-12</sup>.

LAY is a polyploid strain and as such, contains multiple sets of homologous chromosomes. Similar to most industrial beer strains, LAY does not reproduce sexually through sporulation; rather, its reproduction occurs asexually through budding. In addition, brewing strains like LAY do not survive well in conditions outside of the brewery environment.

## **2.3. Donor organisms**

The following organisms contributed DNA to the engineered strain:

### ***Citrobacter freundii***

BY-989 contains a gene that encodes a CSL derived from *Citrobacter freundii*, a facultative anaerobic gram-negative bacterium, and a common component of the healthy human microbiome. It is found in the microbiomes of >84% of healthy individuals<sup>13</sup>. In addition, it is commonly found in soil and water throughout the world, where it plays an important role in nitrogen fixation<sup>14</sup>.

The CSL in the engineered strain is a variant containing five amino acid changes compared with the wild-type *C. freundii* enzyme. These amino acid changes make the enzyme more specific to conjugated thiols, whereas the wild-type enzyme acts on tryptophan as well.

### ***Saccharomyces cerevisiae***

BY-989 contains promoter and terminator DNA sequences derived from the *S. cerevisiae* strain S288C. The history of use of this species is given in Section 2.2.1. The S288C strain, a widely used lab strain, was isolated through genetic crosses for biochemical studies by Robert Mortimer<sup>15</sup> and serves as the reference genome sequence in the Saccharomyces Genome Database. S288C is mainly derived from a wild yeast strain that was isolated from figs; minor portions of its genetic material are derived from baking yeasts and other yeasts isolated from fruit. In addition, the sequences isolated from S288C are 99% identical to the sequences from the host strain and brewing organism LAY.

#### **2.3.1. Genetic material from donor organisms**

##### **The CSL gene sequence**

The CSL gene sequence encodes an enzyme variant that is highly similar to the enzyme encoded by the gene sequence present in *Citrobacter freundii*. The CSL enzyme is a carbon-sulfur lyase that cleaves carbon-sulfur bonds to release 3MH from glutathione and cysteine conjugates. The CSL enzyme encoded in the engineered strain contains five amino acid changes compared with the wild-type *C. freundii* enzyme. The wild-type gene encodes an enzyme that exhibits both tryptophanase and carbon-sulfur lyase activities. As a tryptophanase, the enzyme catalyzes the degradation of tryptophan to indole; as a CSL, the enzyme catalyzes the cleavage of the carbon-sulfur bond in the precursor molecules glutathione-3MH and cysteine-3MH and releases free 3MH. The amino acid changes make the enzyme highly specific to conjugated thiols, and reduce the activity towards tryptophan, resulting in levels of indole that are sensorially undetectable. Although beer produced with BY-989 contains only trace levels of indole, it should be noted that indole is itself safe for human consumption. Though considered a fault in fermented beverages above a certain threshold (faulty wines can contain as much as 350 ug/L indole)<sup>16,17</sup>, wines typically contain significant levels of indole, on the order of 10 ug/L.

##### **The promoter sequence**

The promoter sequence (i.e. the genetic element immediately 5' of the CSL gene) is derived from *S. cerevisiae* strain S288C. The PGK1 promoter controls CSL expression. This promoter was chosen because it is commonly used for heterologous gene expression in yeast, it is well characterized, and it is thought to drive strong constitutive expression. In an endogenous context, the PGK1 promoter controls expression of 3-phosphoglycerate kinase, a key enzyme in glycolysis and gluconeogenesis. The PGK1 promoter used in this strain consists of a 700 bp fragment ending at the base pair immediately preceding the ATG start codon of the PGK1 gene.

## The terminator sequence

The terminator sequence (i.e. the genetic element immediately 3' of the heterologous gene) is derived from *S. cerevisiae* strain S288C. The ENO1 terminator follows CSL. The terminator sequence provides a signal that triggers the release of mRNA from the transcription complex during gene expression. The ENO1 terminator used in this strain consists of a 225 bp fragment starting immediately after the stop codon of the ENO1 gene.

## 2.4. The modified microorganism

### 2.4.1.1. General construction strategy

The strain was constructed using a single genomic integration. The molecular tools and practices used during the construction of BY-989 are standard to the field of molecular biology and yeast genetics.

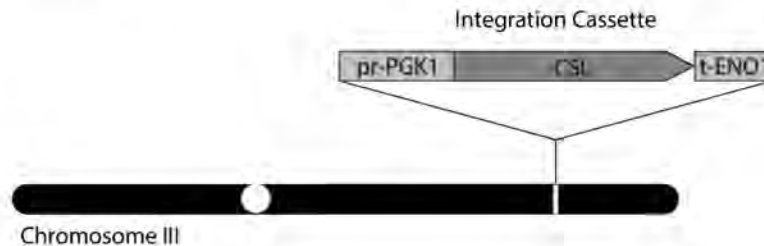
First, we generated the construct containing the genetic material described in section 2.3.1 using standard methods of genetic cloning as follows: The gene coding for the CSL enzyme was codon optimized for *S. cerevisiae*, synthesized by oligonucleotide assembly, and inserted into a cloning vector, which was then sequenced. We chose to synthesize the gene rather than extracting DNA from *C. freundii* and amplifying the coding sequence using PCR to ensure that there was no transfer of DNA from *C. freundii* to *S. cerevisiae* during the construction of BY-989. The promoter and terminator sequences were amplified by PCR from *S. cerevisiae* strain S288C genomic DNA and inserted into a cloning vector, which was then sequenced.

After the three plasmids—containing the the promoter sequence, the CSL gene sequence, and the terminator sequence—were sequenced, further cloning to generate the "integration cassette" was performed by Golden Gate Assembly, a restriction enzyme-based cloning method that avoids mutations introduced by PCR-based methods<sup>18</sup>. The three plasmids, each containing one genetic "part", were assembled into a single plasmid using Golden Gate Assembly. The resulting plasmid was sequenced to confirm that it contained all three parts and then linearized using a restriction enzyme, resulting in an "integration cassette" DNA fragment.

The linear integration cassette fragment, containing the gene encoding the CSL enzyme under the regulation of a native *S. cerevisiae* promoter and terminator, was directly integrated into the genome of the parent strain LAY at a specific and known site (Figure 2). This direct integration approach results in a strain that is highly stable. Based on PCR methods, we confirmed that the cassette was successfully integrated at the target locus in the BY-989 strain and confirmed the stability of the engineered site.

No genes encoding for virulence factors, protein toxins, or enzymes involved in the synthesis of toxins, allergens, or other undesirable substances are expected based on our knowledge of the strain, the gene sequence, and the promoter and terminator. During construction of BY-989, a single plasmid was used, which contained the geneticin (G418) resistance gene. However, this plasmid was only used as a co-transformation aid and none of its genetic material was

integrated into the cell. The plasmid was cured during subsequent plating and passaging of the transformant. Confirmation of plasmid loss was confirmed by plating on selective medium containing G418. Therefore, any antibiotic resistance genes were removed from the final strain and no antibiotic resistance is conferred to BY-989.



**Figure 2. Diagram of genomic integration.** The integration cassette, containing the PGK1 promoter, CSL gene sequence, and the ENO1 terminator, was directly integrated into the chromosome of the parent strain at a specific and known site.

#### 2.4.2. Screening method for transformants

The transformed strain was plated onto G418-containing media in order to select transformed cells. The integration event at all homologous copies of the locus was confirmed by diagnostic PCR. The presence of the cassette was confirmed using two sets of primer pairs, targeting the 5' end and the 3' end of the cassette. Each primer pair contains one primer that binds inside the cassette and one primer that binds outside the cassette. The intended integration results in PCR bands with both primer pairs, whereas the parental control does not produce bands. Homozygosity was confirmed using an additional pair of primers that flank the integration cassette at the integration locus. Homozygous integration results in a larger band, whereas the parental control produces a smaller band, and heterozygous integration results in both bands. Following confirmation, the strain was passaged on YPD media for plasmid loss, which was confirmed by testing for growth on G418. A colony that was sensitive to G418 was selected for further study. Functionality of the pathway was then assessed by fermentation and thiol analysis using liquid chromatography mass spectrometry (Figure 5).

#### 2.4.3. Genetic characterization of the modified microorganism

##### 2.4.3.1. The loss of the plasmid containing the antibiotic resistance gene

Loss of the plasmid containing the antibiotic resistance gene was confirmed by testing for growth on media containing G418 and confirming sensitivity. BY-989 is sensitive to this antibiotic, like the parental strain LAY.

##### 2.4.3.2. Genetic stability of the introduced DNA sequence

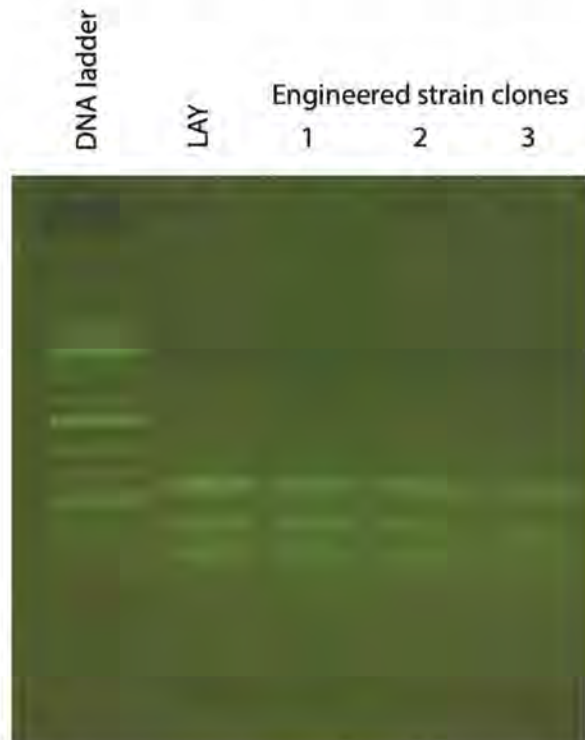
The inserted DNA is integrated into the *S. cerevisiae* chromosome at all copies of the targeted locus, such that the newly inserted DNA is homozygous. In previous research, we have demonstrated that homozygosity in polyploid yeast strains is critically important for integration

stability<sup>19</sup>. If integration results in heterozygosity, the insert is highly unstable, often reverting to the parental allele within only a few generations. On the other hand, homozygous integration is stable over hundreds of generations.

We confirmed the stability of the integration over 100 generations by serially passaging BY-989 on YPD medium. After passaging, the presence of the inserted DNA was re-confirmed by diagnostic PCR (see Section 2.4.2), using the parental strain as a control.

#### 2.4.4. Similarity between genetic profiles of BY-989 and the host strain

The genome of all *Saccharomyces cerevisiae* strains contain long terminal repeat sequences known as  $\delta$  elements. These  $\delta$  elements are the remnants of Ty1 and Ty2 transposon integration events<sup>20</sup>. The number and location of these  $\delta$  elements are strain-specific and serve as an effective means of distinguishing between strains of *S. cerevisiae*<sup>21</sup>, and detecting large chromosomal rearrangements. We verified the genetic relationship between the host strain and the engineered strain by amplifying these  $\delta$  sequences by PCR with primers 5'-GTGGATTTTTATTCCAAC-3' and 5'-TCAACAATGGAATCCCAAC-3' (delta2/delta12 primer pair), a technique called interdelta analysis (Figure 3). This analysis did not detect any large chromosomal changes between the parental and engineered strain.



**Figure 3. Interdelta analysis of LAY and BY-989.** Interdelta analysis verifies the genetic relationship between the host strain and the engineered strain. The identical banding pattern shows that the engineered strain does not contain any large chromosomal changes. PCR was performed using the delta2/delta12 primer pair on genomic DNA isolated from LAY and BY-989 strains. This technique is

described in detail by Legras *et al.* 2003)<sup>21</sup> and Schuller *et al.* 2004)<sup>22</sup>. Three clones of BY-989 are shown.

We therefore conclude that the engineered strain is substantially equivalent to the LAY parent strain except for the CSL cassette present at the targeted locus.

## 2.5. Intended use of BY-989

Berkeley Fermentation Science Inc. has proposed the use of BY-989 in brewing to impart tropical fruit flavors/aromas during fermentation by releasing specific volatile thiol compounds from precursor compounds present in barley and hops. The main volatile thiol responsible for these desirable aromas is 3-mercapto-1-hexanol (3MH), which imparts an aroma of guava, passion fruit, grapefruit, and gooseberry to beer and wine. 3-Mercaptohexyl acetate (3MHA), an ester formed from 3MH, has a similar flavor profile. Brewers seek out these flavors in hops, and as such, the hop preparations that contain aromas indicating elevated levels of 3MH and 3MHA are prized throughout the industry. However, even the most aromatic hops contain only modest quantities of volatile thiols, making it difficult to produce beers that have high concentrations of volatile thiols and strong tropical flavor notes. In addition, these cultivars are vulnerable to stresses from climate change, having been bred for aroma quality and not drought resistance (or resistance to other abiotic stresses). Conversely, hops bred for stress resistance lack the aromas brewers prize and consumers want. In either case, it is difficult to achieve appreciable levels of volatile thiol compounds, and it will grow increasingly difficult as farmers become increasingly reliant on more resilient but less flavorful cultivars.

An alternative strategy is for yeast to create volatile thiols during the brewing process from flavorless precursor compounds that are already present in both barley and hops. The flavorless precursor molecules can be enzymatically converted into flavor-active volatile thiols through the activity of carbon-sulfur lyase (CSL) enzymes (Figure 1). Conjugated 3MH precursors include glutathione-3MH and cysteine-3MH, which are present in both hops<sup>23</sup> and barley<sup>24</sup> in much higher quantities than free 3MH.

*S. cerevisiae* endogenously encodes multiple CSL enzymes (IRC7 and STR3). These CSLs are inefficient, converting only 0.2-2% of the available precursor to 3MH, resulting in limited 3MH production<sup>4,25</sup>. The engineered strain, however, expresses a CSL that more efficiently releases 3MH from both glutathione-3MH and cysteine-3MH precursors. Expression of this CSL in a London Ale yeast strain increases thiol concentrations in finished beer compared to beer made with the parent strain.

BY-989 is used as any other commercial yeast would be used in the beer manufacturing process. We recommend that BY-989 be inoculated between 9.00E+06 and 1.50E+07 cells per mL of wort for beer fermentation, as is standard industrial practice.

## **2.6. Method of manufacture of BY-989**

The engineered strain is produced in accordance with current good manufacturing practices. Production is conducted at a fermentation facility with established procedures and equipment suitable for large-scale contained production of *S. cerevisiae*. At all steps, appropriate analyses are conducted to ensure that the yeast meets the finished product specifications.

BY-989 is sold as a liquid slurry. No selective media is used for propagation during manufacturing. All starting raw materials are standard food-grade ingredients used in traditional yeast production. The raw materials include malt extract (the carbon source) and other essential nutrients. The only other material used is a food-safe, 100% vegetable oil-based defoamer, which is commonly used in commercial beer production. None of the components of the manufacturing process or finished liquid yeast product include or derive from one of the eight major food allergens. Malt extract is derived from barley only, and does not contain milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, or soybeans.

Each lot of finished, food-grade liquid yeast slurry is tested to be greater than 95% viable on ship date and free from detectable levels of contaminants (zero colony forming units CFUs) of bacteria or yeast detected per 250 million yeast cells). pH is checked to ensure that it is below pH 4.6, and is measured to be at pH 4 or below. Yeast is packaged in PET containers that meet FDA specifications for food contact surfaces, stored at 4 °C, and shipped overnight in an insulated box with ice packs.

## **PART 3: DIETARY EXPOSURE**

### **3.1. Intended use of BY-989**

Berkeley Fermentation Science Inc. has proposed the use of BY-989 in brewing to impart tropical fruit flavors/aromas to beer during fermentation. The yeast can be used as a drop-in replacement for other commercial yeasts in the beer manufacturing process. This strain produces 3MH and 3MHA at concentrations desirable in beer styles such as hazy IPAs. These concentrations are similar to those found in certain white wine styles, with comparable levels found especially in New Zealand Sauvignon Blanc, as well as tropical fruits such as guava.

### **3.2. Levels of use of the substance**

BY-989 is used as a processing aid in the fermentation of beer. We recommend a pitch rate between  $9.00E+06$  and  $1.50E+07$  cells per mL of wort for beer fermentation, as is standard industrial practice.

### **3.3. Dietary exposure**

#### **3.3.1. History of consumption**

*Saccharomyces cerevisiae* has been used in food production for thousands of years, as discussed above in Section 2.2.1.

The volatile thiols 3MH and 3MHA have been identified as primary flavor determinants by several sensory analyses of tropical fruits like guava and passion fruit and certain wine and beer styles<sup>26-28</sup>. Not all beers and wines contain 3MH and 3MHA above the limit of detection, but these compounds are present at concentrations well above flavor threshold in heavily-hopped IPAs and certain styles of wine. For example, volatile thiols give Sauvignon blanc its distinctive aroma profile<sup>29,30</sup>.

#### **3.3.2. Estimated consumption**

The CSL enzyme in BY-989 results in biotransformation of flavorless precursor compounds to 3MH during fermentation, and other yeast endogenous enzymes convert a portion of this 3MH into 3MHA. Beer produced with BY-989 will contain levels of 3MH and 3MHA that are similar to or lower than what is present in other foods and beverages, including beer produced with highly aromatic hop preparations. Independent reviews conducted by both FEMA<sup>31</sup> and the FAO/WHO<sup>32</sup> determined that there is no safety concern regarding the use of 3MH and 3MHA based on current levels of intake. The judgment of the FEMA expert panel was that these substances are GRAS.

In order to quantify 3MH and 3MHA in beer made with BY-989, a number of brewing trials were conducted with BY-989 at several different pilot brewing facilities (Berkeley Yeast, Oakland, CA;



Oregon State University, Corvallis, OR; Alvarado Street Brewery, Monterey, CA), using a variety of different recipes that were representative of the IPA style. Beer made with BY-989 was found to contain 7-9 ug/L 3MH and 0.3-1 ug/L 3MHA. Comparatively, 3MH and 3MHA have been found in wines at concentrations up to 18 ug/L and 2.5 ug/L, respectively<sup>29</sup>.

Exposure to the engineered yeast in finished beer will be limited because yeast is removed from beer as part of the standard brewing process. Removing yeast from finished beer is intrinsic to the brewing process: standard industrial brewer's yeast exhibit a strong propensity to sediment at the bottom of the fermentation vessel as fermentable sugars are exhausted. In a typical brewing process, the yeast is added to the wort and allowed to ferment at 64 - 74 °C until fermentable sugars are fully consumed and standard gravity has stabilized, typically 5 - 10 days. Once stabilized, the fermentation vessel is cooled to 0 °C for 1 - 7 days to promote sedimentation. After sedimentation is complete, the sedimented yeast is removed from the finished beer by racking the yeast from the bottom of the vessel. Using this method, only trace levels of yeast will remain in the finished beer. It is worth noting the common misconception that the haze in the hazy IPA beer style is due to unsedimented yeast; however, the haze is actually derived from high molecular weight complexes between proline-rich proteins and hop-derived polyphenols<sup>10-12</sup>. Indeed, finished beer that contains residual yeast is considered a strong character flaw and a source of off-flavors.

In light of this, it is extremely unlikely that commercial beer made with BY-989 would contain a large amount of yeast in solution. However, it is possible that trace levels of yeast will be present in finished beer for some subset of brewing processes. Given that the parental yeast strain LAY has been used for centuries with no known adverse safety effects, it is highly unlikely that BY-989 would give rise to adverse effects.

Finally, it is worth noting that the only full-length yeast-derived proteins documented in finished beer are those that are cell-wall associated<sup>33-35</sup>. Because CSL protein is contained within the BY-989 cell cytoplasm and because yeast is removed from beer before consumption, it is extremely unlikely that CSL protein will be present in appreciable amounts in finished beer. Therefore, consumption of CSL protein present in beer produced with BY-989 will be negligible. Further, because the enzyme is derived from a microbe that is present in most human microbiomes, the enzyme activity is already abundant in the gastro-intestinal system of most humans.

## **PART 4: SELF-LIMITING LEVELS OF USE**

As described in Section 3.3.2, self-limitation of BY-989 in finished beer is intrinsic to the brewing process. That is, the yeast sediments to the bottom of the fermentation vessel as fermentable sugars are exhausted; the sedimented yeast is then removed from finished beer prior to packaging, leaving only trace levels of yeast in the finished beer.

Pitch rate - the amount of yeast added to wort to start a fermentation - varies depending on strain selection and fermentation conditions. Regardless of pitch rate, however, the yeast sediments to the bottom of the fermentation vessel and is removed from the finished beer. In addition, brewers are unlikely to use more yeast than is necessary because yeast fermentation performance and beer quality may be adversely affected<sup>36</sup>. Elevated levels of yeast are known to: 1, result in an increase in off-flavors; 2, increase cell stress which can result in physiological changes that may affect beer quality; 3, lead to reduced growth during fermentation and thus a higher percentage of aged cells, which can also affect subsequent fermentations. In addition, while there is a time advantage to overpitching, this diminishes with increasing pitch rate. Finally, higher pitch rates are not needed to achieve desired thiol concentrations.

## **PART 5: EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958**

This part does not apply.

## PART 6: NARRATIVE

### 6.1 Overview of safety assessment

In this section, we present evidence that the modified *S. cerevisiae* strain, BY-989, is safe for use in industrial brewing because it is substantially equivalent to the parent strain with the exception of 3MH and 3MHA biotransformation, and there is no safety concern regarding the consumption of 3MH and 3MHA based on current levels of intake in other foods and beverages. Specifically, we detail the safety of the host and donor strains, the CSL enzyme, the gene regulatory regions, and our experimental validation that the strains are otherwise substantially equivalent.

### 6.2. Safety assessment of the host strain

The host is an industrial brewing strain of *Saccharomyces cerevisiae* called London Ale Yeast (LAY), a strain that has been commonly used in commercial beer production in the USA for decades. *S. cerevisiae* is an organism that has an extensive history of safe use. It has been used for more than 7,000 years by humans in fermentation processes, such as bread leavening and beer and wine production. Fermentation products of this yeast are not associated with adverse effects in humans (when consumed in recommended quantities for alcohol intake).

*S. cerevisiae* is considered GRAS through its use in the brewing, baking, and winemaking industries. In the 27th report of The Scientific Committee for Human Food of the European Community, the authors state that *Saccharomyces cerevisiae* has a safe history of use in food and does not produce toxins.

### 6.3 Safety assessment of the donor strains

The donor strain is *Citrobacter freundii*, a facultative anaerobic gram-negative bacterium and a common component of the healthy human microbiome. It is found in the intestinal microbiomes of >84% of healthy individuals<sup>13</sup>. In addition, it is found in soil and water, where it plays an important role in nitrogen fixation<sup>14</sup>.

Intestinal microorganisms can, in rare cases, act as opportunistic pathogens. Similarly, *C. freundii* can act as an opportunistic pathogen<sup>37</sup>. That is, *C. freundii* does not infect healthy individuals, but in neonatal and immunocompromised patients, it can lead to disease. To provide context, it is worth noting that *S. cerevisiae* can also act as an opportunistic pathogen<sup>38</sup>, and *S. cerevisiae* has been used in myriad food processes since the beginning of civilization. Commonly used probiotic microorganisms that have proven health benefits, such as *Saccharomyces boulardii*, can also act as opportunistic pathogens in immunocompromised patients<sup>39</sup>. In the context of the CSL gene in BY-989, the important aspect is not whether the source organism can act as an opportunistic pathogen, but whether the CSL gene is likely to increase the pathogenicity of the modified brewer's yeast, BY-989. There is a large body of

literature on the genetic determinants of opportunistic pathogenicity for both *Saccharomyces* and *Citrobacter* species. In *Saccharomyces* species, genes such as TCB2, BIO5, PDR5, CUP1<sup>40</sup>, and SSD1<sup>41</sup> have been found to be associated with pathogenicity. The CSL gene is unrelated to these genes. In *Citrobacter* species, pathogenicity is associated with genes involved in flagellar apparatus biosynthesis/function, iron uptake, secretion system function, and resistance to antimicrobial agents<sup>42,43</sup>. The CSL gene is not involved in any of these processes. In addition, because the gene was cloned from an artificially synthesized gene, rather than from genomic DNA extracted from *C. freundii*, there was no possibility of transfer of DNA from *C. freundii* to *S. cerevisiae* during the construction of BY-989.

The safety assessment of donor strain *S. cerevisiae* has been previously established.

#### **6.4. Safety assessment of the CSL enzyme**

Source: *Citrobacter freundii*

Product: Carbon-sulfur lyase

The CSL gene codes for a carbon-sulfur lyase enzyme which cleaves the carbon-sulfur bond in glutathione-3MH and cysteine-3MH to release free 3MH (also see Section 2.1.2; Figure 1).

##### **6.4.1. Allergenic/Toxicogenic potential of the CSL enzyme and formation of unwanted substances in beer**

It is extremely unlikely that intracellular CSL could cause a food allergy. This is based on the following considerations:

1. The vast majority of enzymes present in the natural world are not allergens, and a wide variety of enzyme classes and structures are naturally present in plant and animal based foods.
2. In this use case, the concentration of CSL in finished beer is anticipated to be nominal (see Section 3.3.2). The total amount of CSL protein is a small fraction of the total yeast protein and because the CSL is expressed inside the cell, and because the yeast is removed from beer before consumption, only extremely low levels of CSL protein or peptide fragments will be found in finished beer.
3. This enzyme is derived from a microbe that is present in most human microbiomes; therefore, the enzyme (and proteolysis products) are abundant in the gastro-intestinal system of most humans.

In order to further evaluate the possibility that the enzyme expressed from the integration cassette will induce a reaction in an already sensitized individual, amino acid sequence similarity to known allergens was assessed. Following the guidelines developed by FAO/WHO, 2001<sup>44, 45</sup> and modified by Codex Alimentarius Commission, 2009<sup>46</sup>, the CSL amino acid

sequence was compared to allergens from the Food Allergy Research and Resource Program (FARRP allergen protein database <http://allergenonline.org>) as follows:

We conducted an alignment of the expressed protein to each of the allergens in the FARRP. No significant similarity was found between the CSL amino acid sequence and any of the allergens from the FARRP database; no matches with more than 50% identity were found. This was repeated using a sliding window of 80 amino acid segments of the CSL amino acid sequence; no matches with more than 35% identity were found. In addition, a search for 100% identity over 8 contiguous amino acids was completed; no matches were found.

In order to further evaluate the possibility that the peptide expressed from the integration cassette has toxigenic potential, we next set out to determine whether the expressed enzyme is similar to known toxins. We extracted all peptide sequences in the UniProt database annotated as “toxin”, then queried these sequences with the expressed CSL protein using the BLASTP algorithm and default parameter settings<sup>47</sup>. In total, this queried more than 89,000 sequences in the UniProt database. All hits obtained during this search had “Expect Values” (E-values) > 0.5, indicating that the alignments were due to short random sequence identities. We therefore conclude that the expressed protein is not similar to any of the annotated toxins in the UniProt database.

On the basis of the available evidence, it is concluded that oral intake of the protein expressed from the integration cassette in BY-989 does not pose any food allergenic or toxigenic concerns. The CSL gene present in BY-989 is not substantially similar to either toxic or allergenic proteins. In addition, the enzyme is typically removed or denatured during beer production.

## **6.5. Safety assessment of the promoter and terminator sequences**

The promoter and terminator sequences were isolated from *S. cerevisiae*. The safety assessment of *S. cerevisiae* has already been established and since the regulatory sequences do not code for proteins, the source of this material does not raise a safety concern.

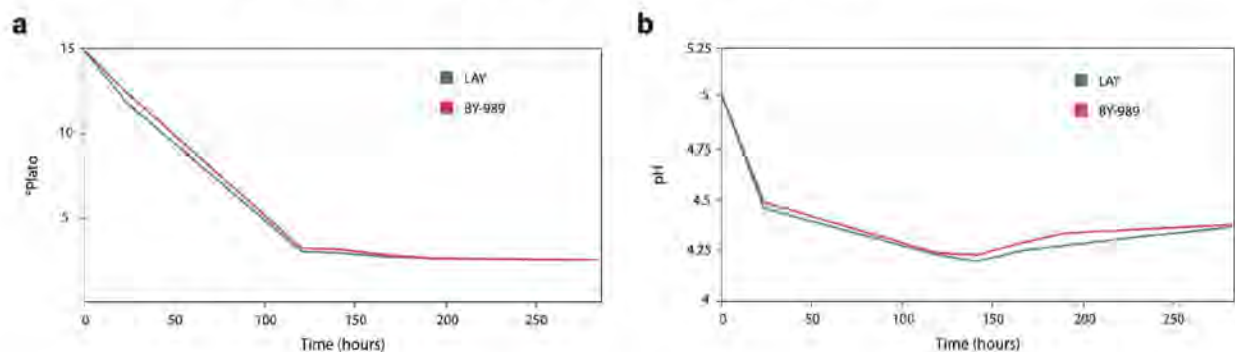
As an additional measure, we tested whether the integration cassette contained open reading frames (ORFs) beyond the one that was intentionally introduced. We searched for ORFs of more than 100 codons using the NCBI ORF Finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). No ORFs were identified.

## **6.6. Safety assessment of BY-989**

### **6.6.1. Fermentation profiles of BY-989 and LAY**

Brewing trials have shown that BY-989 and the LAY parent strain have highly similar fermentation profiles, exhibiting nearly identical rates of sugar consumption and pH progression (Figure 4). Initial brewing trials were conducted in our pilot facility where side-by-side fermentations can be performed in identical conditions at 20 L scale. We have determined that fermentations performed in our pilot facility are generally representative of the industrial brewing

process. Similar results were also obtained at 300 L scale fermentation trials performed at Oregon State University in collaboration with Dr. Thomas Shellhammer<sup>48</sup>. No difference in fermentation rate was found between the parent strain LAY and BY-989 (Figure 4a). In addition, the engineered strain shows the same pH profile throughout fermentation as its parent (Figure 4b). Taken together, these data indicate that the presence of the CSL cassette does not affect fermentation performance, and BY-989 can be regarded as substantially equivalent to the parent strain, with an additional capacity for 3MH and 3MHA production.



**Figure 4. Fermentation profiles of LAY and BY-989.** a) This fermentation was performed with 15 °P wort as a starting substrate. Gravity (°P), which corresponds to sugar concentration, was measured throughout brewing trials as a means of monitoring sugar consumption. These values were measured using an Anton Paar DMA-4500 M-EC density meter (Anton Paar GmbH, Graz, Austria). b) pH was measured throughout fermentation using a Mettler Toledo SevenEasy S20 pH meter (Mettler Toledo Headquarters, Columbus).

### 6.6.2. Changes in brewing procedures or beer composition as a consequence of BY-989 fermentation

The use of BY-989 does not require changes to brewing procedures. BY-989 functions as a drop-in replacement for beer created with LAY, in that a fermentation performed with BY-989 will be substantially equivalent to a fermentation performed with LAY, except for the production of 3MH and 3MHA.

### 6.6.3. Sensory analysis of beer brewed with BY-989

In order to compare the flavors conferred by BY-989 with the flavors made by traditional brewing strains, side-by-side fermentations were performed with BY-989 and the parent strain at 300 L scale. A panel of 18 trained tasters were asked to select aroma and flavor attributes for each beer. The beer fermented with BY-989 was more frequently associated with attributes such as tropical, fruity, guava, and passion fruit compared with the beer fermented with LAY. Notably, initial screening by the tasters identified no off-flavors. These tasting notes are consistent with our findings that the only alteration to yeast metabolism is the production of the CSL enzyme and increased 3MH and 3MHA concentrations.

#### 6.6.4. Metabolomic comparison of BY-989 and LAY

The introduction of the CSL gene resulted, as expected, in the production of 3MH and 3MHA. However, unexpected effects are also possible. In order to evaluate whether the genetic modification introduced unexpected metabolic alterations, un-targeted metabolomics was performed on beer produced with BY-989 and its parent strain, LAY. Untargeted methods agnostically capture metabolite levels in the fermented beer, rather than focusing on ones expected to be present. This provides an unbiased approach to comparing engineered strains to their parent strains.

To assess differences in the metabolite composition of a beer fermentation, quadruplicate cultures of BY-989 and LAY were inoculated into 1 L of standard beer wort (12 °P and fermentation was allowed to proceed for 5 days at 25 °C. The resulting fermentations were processed for both gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS) as described below. Mass spectrometry-based methods can detect thousands of molecules made by yeast during fermentation at concentrations as low as parts per trillion. Comparing the levels of these molecules allowed us to verify that no unexpected changes occurred.

In total, we were able to compare 1,469 distinct metabolites in the BY-989 and LAY fermentations: 1,235 using LC-MS and 234 using GC-MS. None of the metabolites assayed were unique to BY-989, meaning that the genetic modification did not result in the production of new, unexpected compounds. In addition, none of the metabolites exhibited >5-fold increase in BY-989 fermentations compared with parent strain LAY. To provide context, in a number of studies where metabolomics was performed on beers made with different yeast strain isolates, substantial differences in metabolites were observed<sup>49-51</sup>. Overall, these results indicate that the metabolism of BY-989 and LAY are substantially equivalent except for the expected difference in production of 3MH and 3MHA.

Further details of the experimental procedures are as follows: for GC-MS analysis, 1 mL of sample was extracted with 500 µL ethyl acetate. The ethyl acetate was treated with an excess of sodium sulfate to remove water prior to injection into a 6890 Agilent gas chromatograph equipped with a HP-5MS column (30 m x 0.25 mm x 0.25 µM and a 5973N single-quadrupole mass spectrometer. Helium was used as the carrier gas with a flow rate of 1 mL/min. The GC oven temperature was programmed for 50 °C for 5 min followed by a linear ramp to 325 °C over 45 min.

For LC-MS analysis, C<sub>18</sub> Sep-Paks (Waters, PN: WAT020515) were used to extract analytes: each Sep-Pak was first equilibrated with 3 mL methanol, followed by 3 mL water. 25 mL of sample was injected into the Sep-Pak via syringe, after which the Sep-Pak was washed with 6 mL water. The Sep-Pak was then dried, and the sample was eluted with 500 µL methanol. The entire sample volume was next transferred to a 1.5 mL microcentrifuge tube and clarified by centrifugation at 20,000 rcf for 10 minutes. The supernatant was transferred to glass sample vials for analysis via LC-MS. 500 µL cell culture equivalent was analyzed by utilizing an Agilent



1290 UHPLC equipped with an Eclipse Plus C18 column (2.1 x 5.0 mm x 1.8  $\mu$ M) and an Agilent 6130 single-quadrupole mass spectrometer. Chromatographic separation was achieved using a linear gradient of 2-98% (v/v) acetonitrile over 53 min in water with 0.1% (v/v) formic acid followed by 2 min at 98% acetonitrile at a flow rate of 0.5 mL/min.

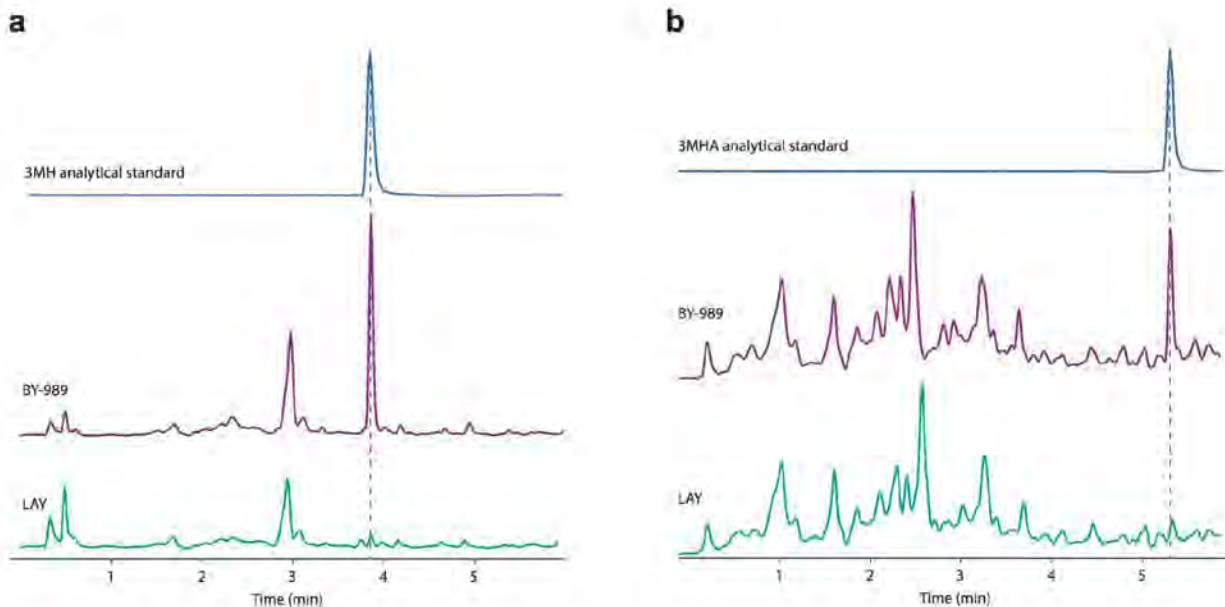
Mass spectral data from both GC-MS and LC-MS analyses were converted to mzdata using MassHunter Qualitative Analysis. Data processing was conducted using XCMS pairwise, group, and meta analyses<sup>52</sup>.

#### **6.6.5. Quantification of 3MH and 3MHA in beers fermented with BY-989**

Following the full characterization of BY-989, the only significant difference between the engineered strain and the parent strain is the production of 3MH and 3MHA (Figure 5). Independent reviews conducted by both FEMA<sup>31</sup> and the FAO/WHO<sup>32</sup> determined that there was no safety concern regarding the use of 3MH and 3MHA as flavorings based on current levels of intake, and beer produced with BY-989 falls within those levels. The judgment of the FEMA expert panel was that these substances are GRAS.

Quantification of 3MH and 3MHA in beer samples was performed following the derivatizing method developed by Capone *et al.*<sup>53</sup>, with some modifications. 20 mL of each clarified beer sample was combined with 4 mL of 10X phosphate buffered saline, pH 7.4, 200  $\mu$ L of 10 mM 4,4'-dithiodipyridine, and 200  $\mu$ L of 100 mg/mL ethylenediaminetetraacetic acid disodium salt solution adjusted to neutral pH. 4,4'-dithiodipyridine acted as the derivatizing agent. The sample was then vortexed for 3 seconds and incubated at room temperature for 1 hour. Thereafter, each sample was extracted with 4 mL of ethyl acetate with vortexing for 20 seconds. Phase separation was assisted by a brief centrifugation step and 1 mL of the resulting organic phase extract was transferred into a glass vial and dried to completion under nitrogen gas. The resulting thin film of off-white solids was dissolved in 200  $\mu$ L methanol, transferred into a 1.5 mL microcentrifuge tube, then clarified by centrifugation at 20,000 rcf for 10 min. The supernatant was transferred into a glass sample vial for analysis by LC-MS.

Samples were chromatographed at 35 °C in a reversed-phase C18 column (2.1 x 5.0 mm x 1.8  $\mu$ m) installed in an Agilent 1290 UHPLC. Derivatized 3MH and 3MHA were detected by mass spectrometry with selected ion monitoring at 244.4 M/Z and 286.4 M/Z, respectively, using an in-line Agilent 6130 single-quadrupole mass spectrometer. Derivatized 3MH and 3MHA were eluted under a linear 5-40% acetonitrile gradient supplemented with 0.1% (v/v) formic acid. Quantification was accomplished by comparing peak areas to standard curves generated by spiking known concentrations of 3MH and 3MHA into beer brewed with LAY, then following the same extraction protocol. Beer made with BY-989 was found to contain 7-9 ug/L 3MH and 0.3-1 ug/L 3MHA. These concentrations are within normal levels of consumption; for example, 3MH and 3MHA have been found in wines at concentrations up to 18 ug/L and 2.5 ug/L, respectively.



**Figure 5. Identification of 3MH and 3MHA using liquid chromatography mass spectrometry.**

Selected ion monitoring chromatograms from LAY (teal) and BY-989 (purple) fermentation samples and analytical standards (blue) are shown. The dotted lines denote the 3MH (a) and 3MHA (b) peaks. The y-axis represents signal intensity in arbitrary units; all three chromatograms are shown on the same scale. Samples were prepared using the method described above. Additional standard curve traces are not shown. The only detectable difference between the engineered strain and the parent strain is the production of 3MH and 3MHA.

### 6.7. Precedence for safety of engineered *S. cerevisiae* strains

Given its history of use, engineered strains of *S. cerevisiae* are GRAS, provided that any and all new changes to the molecular composition of the resulting beer affected by the modification are known to be safe for consumption at the relevant concentrations. The FDA has had no questions on GRAS notifications for a number of *S. cerevisiae* strains where heterologous genes were inserted that altered metabolism so as to eliminate undesirable compounds or to produce compounds known to be safe. Examples include Lesaffre's malolactic yeast strain ML01 (GRN No. 120), Phytterra's urea degrading yeast strain ECMo01 (GRN No. 175), Phytterra's low hydrogen sulfide yeast strain P1Y0 (GRN No. 350), Mascoma's lactic acid-producing yeast strain (GRN No. 841), as well as a monoterpene-producing yeast strain, yBBS002 (GRN No. 798), that we submitted previously.

### 6.8. Conclusions for GRAS determination

Berkeley Fermentation Science Inc. has made the following conclusions regarding the use of yeast strain BY-989 as a processing aid to manufacture beer:

- *S. cerevisiae* has a long history of safe use in food and beverage production. Across a variety of use cases, *S. cerevisiae* and *S. cerevisiae*-derived products have been

approved as food additives, affirmed as GRAS substances, and the subject of previous GRAS Notices.

- The modified strain is derived from an industrial brewing strain of *S. cerevisiae* called London Ale Yeast (LAY) that has been commonly used in commercial beer production for decades. BY-989 is substantially equivalent to the parent strain with respect to overall performance (growth rate, fermentation rate, pH, ethanol production). The strains are substantially equivalent except for the introduction of a single gene, which confers the ability to release 3MH from precursors which are ordinarily present in barley and hops.
- The modified strain is genetically stable. Whereas industrial brewer's yeast are generally polyploid, and DNA insertions that result in heterozygous alleles are unstable, the inserted DNA in BY-989 is integrated into the *S. cerevisiae* chromosome at all copies of the targeted locus, resulting in a strain that is genetically stable. DNA diagnostic methods confirmed the presence of the on-target integration event, as well as the absence of major genetic rearrangements.
- Independent reviews conducted by both FEMA and the FAO/WHO determined that there was no safety concern regarding the use of 3MH and 3MHA as flavorings based on current levels of intake. The judgment of the FEMA expert panel was that these substances are GRAS. BY-989 produces concentrations of 3MH and 3MHA that are within current levels of intake.
- The CSL gene present in BY-989 does not code for either toxic or allergenic proteins, nor is its enzymatic activity implicated in the formation of unanticipated compounds.
- The high degree of similarity between beer composition resulting from LAY and BY-989 fermentations was illustrated by comparative metabolomics.
- BY-989 is produced according to the principles of GMP, using food-grade ingredients. At all steps, appropriate analyses are conducted to ensure that the yeast meets the finished product specifications.
- Yeast is removed from beer as part of the standard brewing process; therefore only trace levels of BY-989 remain in the finished beer.

The modified *S. cerevisiae* strain should be regarded as substantially equivalent to the parent yeast strain in terms of its safety, utility, and functionality, with the exception of its ability to produce the CSL enzyme and increased concentrations of 3MH and 3MHA in beer. Berkeley Fermentation Science Inc. has determined, through the scientific analysis presented in this notice, that yeast strain BY-989 is "Generally Regarded as Safe" (GRAS) for use in brewing and is, therefore, exempt from premarket approval requirements.

# PART 7: LIST OF SUPPORTING DATA AND INFORMATION

## 7.1. Supporting Information

Work underlying the development of BY-989 was funded in part through a USDA SBIR grant: Engineering *Saccharomyces cerevisiae* for increased biotransformation and production of tropical flavors from agricultural products. See link below.

- <https://portal.nifa.usda.gov/web/crisprojectpages/1025791-engineering-saccharomyces-cerevisiae-for-increased-biotransformation-and-production-of-tropical-flavors-from-agricultural-products.html>

Some of the experimentation described herein is in preparation for publication, in collaboration with Dr. Thomas Shellhammer at Oregon State University<sup>48</sup>.

## 7.2. References

1. Cordente, A. G. *et al.* Inactivating Mutations in *Irc7p* Are Common in Wine Yeasts, Attenuating Carbon-Sulfur  $\beta$ -Lyase Activity and Volatile Sulfur Compound Production. *Appl. Environ. Microbiol.* **85**, 2019).
2. Holt, S. *et al.* Engineering *Saccharomyces cerevisiae* to release 3-Mercaptohexan-1-ol during fermentation through overexpression of an *S. cerevisiae* Gene, *STR3*, for improvement of wine aroma. *Appl. Environ. Microbiol.* **77**, 3626–3632 (2011).
3. Swiegers, J. H. Pretorius, I. S. Modulation of volatile sulfur compounds by wine yeast. *Appl. Microbiol. Biotechnol.* **74**, 954–960 (2007).
4. Santiago, M. Gardner, R. C. Yeast genes required for conversion of grape precursors to varietal thiols in wine. *FEMS Yeast Res.* **15**, fov034 (2015).
5. Swiegers, J. H. *et al.* Modulation of volatile thiol and ester aromas by modified wine yeast. in *Flavour Science - Recent Advances and Trends* 113–116 (Elsevier, 2006).
6. Hornsey, I. S. *A History of Beer and Brewing*. (Royal Society of Chemistry, 2003).
7. Legras, J.-L., Merdinoglu, D., Cornuet, J.-M. Karst, F. Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history. *Mol. Ecol.* **16**, 2091–2102 (2007).
8. Gallone, B. *et al.* Domestication and Divergence of *Saccharomyces cerevisiae* Beer Yeasts. *Cell* **166**, 1397–1410.e16 (2016).
9. Duan, S.-F. *et al.* The origin and adaptive evolution of domesticated populations of yeast

- from Far East Asia. *Nat. Commun.* **9**, 2690 (2018).
10. Wang, Y. Ye, L. Haze in Beer: Its Formation and Alleviating Strategies, from a Protein-Polyphenol Complex Angle. *Foods* **10**, (2021).
  11. Bamforth, C. W. Beer Haze. *J. Am. Soc. Brew. Chem.* **57**, 81–90 (1999).
  12. Asano, K., Shinagawa, K. Hashimoto, N. Characterization of haze-forming proteins of beer and their roles in chill haze formation. *J. Am. Soc. Brew. Chem.* **40**, 147–154 (1982).
  13. King, C. H. *et al.* Baseline human gut microbiota profile in healthy people and standard reporting template. *PLoS One* **14**, e0206484 (2019).
  14. Wan, K. H. *et al.* Complete Genome Sequence of the *Citrobacter freundii* Type Strain. *Microbiol Resour Announc* **9**, (2020).
  15. Mortimer, R. K. Johnston, J. R. Genealogy of principal strains of the yeast genetic stock center. *Genetics* **113**, 35–43 (1986).
  16. Capone, D. L. *et al.* Identification and analysis of 2-chloro-6-methylphenol, 2,6-dichlorophenol and indole: causes of taints and off-flavours in wines. *Aust. J. Grape Wine Res.* **16**, 210–217 (2010).
  17. Wine flavours, faults and taints. *The Australian Wine Research Institute* [https://www.awri.com.au/industry\\_support/winemaking\\_resources/sensory\\_assessment/recognition-of-wine-faults-and-taints/wine\\_faults/](https://www.awri.com.au/industry_support/winemaking_resources/sensory_assessment/recognition-of-wine-faults-and-taints/wine_faults/) (2011).
  18. Lee, M. E., DeLoache, W. C., Cervantes, B. Dueber, J. E. A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly. *ACS Synth. Biol.* **4**, 975–986 (2015).
  19. Denby, C. M. *et al.* Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer. *Nat. Commun.* **9**, 965 (2018).
  20. Lesage, P. Todeschini, A. L. Happy together: the life and times of Ty retrotransposons and their hosts. *Cytogenet. Genome Res.* **110**, 70–90 (2005).
  21. Legras, J.-L. Karst, F. Optimisation of interdelta analysis for *Saccharomyces cerevisiae* strain characterisation. *FEMS Microbiol. Lett.* **221**, 249–255 (2003).
  22. Schuller, D., Valero, E., Dequin, S. Casal, M. Survey of molecular methods for the typing of wine yeast strains. *FEMS Microbiol. Lett.* **231**, 19–26 (2004).
  23. Roland, A. *et al.* First identification and quantification of glutathionylated and cysteinylated precursors of 3-mercaptohexan-1-ol and 4-methyl-4-mercaptopentan-2-one in hops (*Humulus lupulus*). *Flavour Fragr. J.* **31**, 455–463 (2016).
  24. Aurélie Roland, Stéphane Delpech, Clément Viel, Florence Reillon, Rémi Schneider and Laurent Dagan. First evidence of cysteinylated and glutathionylated precursors of

- 3-mercaptohexan-1-ol in malts: Toward a better aromatic potential management? Poster presented at: World Brewing Congress; 2016; Denver, CO.
25. Howell, K. S. *et al.* Genetic determinants of volatile-thiol release by *Saccharomyces cerevisiae* during wine fermentation. *Appl. Environ. Microbiol.* **71**, 5420–5426 (2005).
  26. Cannon, R. J. Ho, C.-T. Volatile sulfur compounds in tropical fruits. *J. Food Drug Anal.* **26**, 445–468 (2018).
  27. Roland, A., Schneider, R., Razungles, A. Cavelier, F. Varietal thiols in wine: discovery, analysis and applications. *Chem. Rev.* **111**, 7355–7376 (2011).
  28. Dennenlöhner, J., Thörner, S. Rettberg, N. Analysis of Hop-Derived Thiols in Beer Using On-Fiber Derivatization in Combination with HS-SPME and GC-MS/MS. *J. Agric. Food Chem.* **68**, 15036–15047 (2020).
  29. Coetzee, C. du Toit, W. J. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. *Food Res. Int.* **45**, 287–298 (2012).
  30. Benkwitz, F. *et al.* Identifying the Chemical Composition Related to the Distinct Aroma Characteristics of New Zealand Sauvignon blanc Wines. *American Journal of Enology and Viticulture* vol. 63 62–72 (2012).
  31. GRAS Flavoring Substances 18.  
<https://www.femaflavor.org/sites/default/files/18GRASSubstances3816-3905.pdf>.
  32. WHO FOOD ADDITIVES SERIES: 44. SIMPLE ALIPHATIC AND AROMATIC SULFIDES AND THIOLS. <https://inchem.org/documents/jecfa/jecmono/v44jec09.htm>.
  33. Huismann, M. *et al.* Unfilterable beer haze part II: Identifying suspect cell wall proteins. *J. Am. Soc. Brew. Chem.* **80**, 26–34 (2022).
  34. Berner, T. S., Jacobsen, S. Arneborg, N. The impact of different ale brewer's yeast strains on the proteome of immature beer. *BMC Microbiol.* **13**, 215 (2013).
  35. Konečná, H. *et al.* Exploration of beer proteome using OFFGEL prefractionation in combination with two-dimensional gel electrophoresis with narrow pH range gradients. *J. Agric. Food Chem.* **60**, 2418–2426 (2012).
  36. Verbelen, P. J. *et al.* Impact of pitching rate on yeast fermentation performance and beer flavour. *Appl. Microbiol. Biotechnol.* **82**, 155–167 (2009).
  37. Bai, L. *et al.* Isolation and characterization of cytotoxic, aggregative *Citrobacter freundii*. *PLoS One* **7**, e33054 (2012).
  38. Pérez-Torrado, R. Querol, A. Opportunistic Strains of *Saccharomyces cerevisiae*: A Potential Risk Sold in Food Products. *Front. Microbiol.* **6**, 1522 (2015).

39. Imre, A. *et al.* Virulence Factors and in-Host Selection on Phenotypes in Infectious Probiotic Yeast Isolates . *J Fungi (Basel)* **7**, 2021).
40. Strobe, P. K. *et al.* The 100-genomes strains, an *S. cerevisiae* resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen. *Genome Res.* **25**, 762–774 2015).
41. Wheeler, R. T., Kupiec, M., Magnelli, P., Abeijon, C. Fink, G. R. A *Saccharomyces cerevisiae* mutant with increased virulence. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 2766–2770 2003).
42. Yuan, C. *et al.* Comparative Genomic Analysis of and Key Genes Essential for the Pathogenicity of. *Front. Microbiol.* **10**, 2774 2019).
43. Tan, A. *et al.* Evolutionary adaptation of an AraC-like regulatory protein in *Citrobacter rodentium* and *Escherichia* species. *Infect. Immun.* **83**, 1384–1395 2015).
44. Evaluation of Allergenicity of Genetically Modified Foods.  
<http://www.fao.org/docrep/007/y0820e/y0820e00.htm>.
45. The state of food insecurity in the world 2001.  
<http://www.fao.org/docrep/003/y1500e/y1500e00.htm>.
46. Food and Agriculture Organization of the United Nations. *Foods derived from modern biotechnology (2. ed).* 2009).
47. Index of /blast/executables/blast+. <https://ftp.ncbi.nlm.nih.gov/blast/executables/blast/>.
48. Richard W. Molitor, Jeremy I. Roop, Charles M. Denby, Charles J. Depew, Daniel S. Liu, Sara E. Stadulis, Thomas H. Shellhammer. The Sensorial and Chemical Changes in Beer Brewed with Yeast Genetically Modified to Release Polyfunctional Thiols from Malt and Hops. Manuscript in preparation.
49. Seo, S.-H. *et al.* GC/MS-based metabolomics study to investigate differential metabolites between ale and lager beers. *Food Biosci.* **36**, 100671 2020).
50. Pope, G. A. *et al.* Metabolic footprinting as a tool for discriminating between brewing yeasts. *Yeast* **24**, 667–679 2007).
51. GRAS notice 798 for *Saccharomyces cerevisiae* strain yBBS002.  
<https://www.fda.gov/media/133654/download>.
52. Smith, C. A., Want, E. J., O'Maille, G., Abagyan, R. Siuzdak, G. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal. Chem.* **78**, 779–787 2006).
53. Capone, D. L., Ristic, R., Pardon, K. H. Jeffery, D. W. Simple quantitative determination of

potent thiols at ultratrace levels in wine by derivatization and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. *Anal. Chem.* **87**, 1226–1231 (2015).



**FDA USE ONLY**

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Food and Drug Administration  
**GENERALLY RECOGNIZED AS SAFE  
(GRAS) NOTICE** (Subpart E of Part 170)

Transmit completed form and attachments electronically via the Electronic Submission Gateway (*see Instructions*); OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety (*HFS-200*), Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5001 Campus Drive, College Park, MD 20740-3835.

**SECTION A INTRODUCTORY INFORMATION ABOUT THE SUBMISSION**

1. Type of Submission (*Check one*)

New       Amendment to GRN No. \_\_\_\_\_       Supplement to GRN No. \_\_\_\_\_

2.  All electronic files included in this submission have been checked and found to be virus free. (*Check box to verify*)

Most recent presubmission meeting (*if any*) with FDA on the subject substance (*yyyy/mm/dd*): 2021/12/07

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? (*Check one*)  
 Yes If yes, enter the date of communication (*yyyy/mm/dd*): \_\_\_\_\_  
 No

**SECTION B INFORMATION ABOUT THE NOTIFIER**

**1a. Notifier**

Name of Contact Person Charles Denby	Position or Title CEO
Organization ( <i>if applicable</i> ) Berkeley Fermentation Science Inc.	
Mailing Address ( <i>number and street</i> ) 2451 Peralta Street	

City Oakland	State or Province California	Zip Code/Postal Code 94607	Country United States of America
-----------------	---------------------------------	-------------------------------	-------------------------------------

Telephone Number 805-637-1280	Fax Number	E-Mail Address charles@berkeleyyeast.com
----------------------------------	------------	---

**1b. Agent or Attorney  
(if applicable)**

Name of Contact Person	Position or Title
Organization ( <i>if applicable</i> )	
Mailing Address ( <i>number and street</i> )	

City	State or Province	Zip Code/Postal Code	Country
------	-------------------	----------------------	---------

Telephone Number	Fax Number	E-Mail Address
------------------	------------	----------------

## SECTION C GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Saccharomyces cerevisiae strain BY-989

2. Submission Format: *(Check appropriate box(es))*

- Electronic Submission Gateway  Electronic files on physical media  
 Paper  
If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes \_\_\_\_\_

Total number of pages \_\_\_\_\_

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

- Yes *(Proceed to Item 5)*  No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

- a) GRAS Notice No. GRN \_\_\_\_\_  
 b) GRAS Affirmation Petition No. GRP \_\_\_\_\_  
 c) Food Additive Petition No. FAP \_\_\_\_\_  
 d) Food Master File No. FMF \_\_\_\_\_  
 e) Other or Additional *(describe or enter information as above)* \_\_\_\_\_

6. Statutory basis for conclusions of GRAS status *(Check one)*

- Scientific procedures *(21 CFR 170.30(a) and (b))*  Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? *(see 21 CFR 170.225(c)(8) and 170.250(d) and (e))*

- Yes *(Proceed to Item 8)*  
 No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

- Yes, information is designated at the place where it occurs in the submission  
 No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

- Yes, a redacted copy of the complete submission  
 Yes, a redacted copy of part(s) of the submission  
 No

## SECTION D INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Yeast strain BY-989 is intended for use in brewing to impart tropical fruit flavors/aromas during fermentation by releasing specific volatile thiol compounds from precursor compounds present in barley and hops. This strain produces the compounds 3MH and 3MHA, which impart aromas of guava, passion fruit, grapefruit, and gooseberry, at concentrations similar to those found in white wine styles and tropical fruits such as guava. BY-989 is used as any other commercial yeast would be used in the beer manufacturing process. We recommend that BY-989 be inoculated between 9.00E+06 and 1.5E+07 cells per mL of wort for beer fermentation, as is standard industrial practice.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

*(Check one)*

- Yes  No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

*(Check one)*

- Yes  No, you ask us to exclude trade secrets from the information FDA will send to FSIS.

## SECTION E PARTS 2-7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete PART 1 is addressed in other sections of this form)

- PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).
- PART 3 of a GRAS notice: Dietary exposure (170.235).
- PART 4 of a GRAS notice: Self-limiting levels of use (170.240).
- PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).
- PART 6 of a GRAS notice: Narrative (170.250).
- PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

### Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

Yes  No

Did you include this other information in the list of attachments?

Yes  No

## SECTION F SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Berkeley Fermentation Science Inc.

*(name of notifier)*

has concluded that the intended use(s) of Saccharomyces cerevisiae strain BY-989

*(name of notified substance)*

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Berkeley Fermentation Science Inc. *(name of notifier)* agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

2451 Peralta Street Oakland CA 94607

*(address of notifier or other location)*

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance. The notifying party certifies that the information provided herein is accurate and complete to the best of his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official,  
Agent, or Attorney

Charles Denby

Digitally signed by Charles Denby  
Date: 2022.06.08 12:19:57 -07'00'

Printed Name and Title

Charles Denby, CEO

Date (mm/dd/yyyy)

06/08/2022

## SECTION G LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	CoverLetter_BY-989_2022-06-08.pdf	Administrative
	GRASNotice_BY-989_2022-06-08.pdf	Submission

**OMB Statement:** Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, [PRASStaff@fda.hhs.gov](mailto:PRASStaff@fda.hhs.gov). (Please do NOT return the form to this address). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

## Viebrock, Lauren

---

**From:** Rachel Li <rachel@berkeleyyeast.com>  
**Sent:** Friday, June 9, 2023 6:33 PM  
**To:** Charles Denby; Viebrock, Lauren  
**Subject:** [EXTERNAL] Re: GRN 001094 Questions  
**Attachments:** Response to FDA questions regarding GRN 1094.pdf; Certificates of Analysis - Batches 962, 993, 1001.pdf; Lead testing analytical results - Batch 962.pdf; Lead testing analytical results - Batch 993.pdf; Lead testing analytical results - Batch 1001.pdf

**CAUTION:** This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Hi Lauren,

Our response to your questions on GRAS Notice No. 001094 is attached, as well as analytical results from three non-consecutive batches. Please let me know if any additional information is required. Thank you very much and have a good weekend.

Best,  
Rachel

On Tue, May 30, 2023 at 3:36 PM Rachel Li <[rachel@berkeleyyeast.com](mailto:rachel@berkeleyyeast.com)> wrote:  
Hi Lauren,

I wanted to provide a bit more context on my question about using the most recent NHANES data.

In determining dietary exposure, there are 2 recent GRAS notices concerning beer which serve as precedent: one (date of closure March 2020) used NHANES data from 2003-2006 which estimated daily beer consumption (by men) to be slightly under 0.5 L. The other (date of closure December 2019) used National Alcohol Survey data which estimated consumption (by men) to be as high as 1.4 L per day.

We plan to present data on 3MH and 3MHA consumption using both values. For example, based on calculations using JECFA NOELs, even in the extreme - and unlikely - case where all 1.4 L was beer made with BY-989, 3MH consumption would be at 10% of the safe level. Given that the trends in alcohol consumption are declining rather than increasing, we expect these previous values to be, if anything, an overestimate. Given this, and the fact that we have learned that access to the most recent data can take 4 weeks, and be expensive to obtain (requires consultancy), we believe that the most expeditious way to proceed is with this publicly available information. We feel that there is sufficient precedent to justify this course of action. Of course, please let us know if this gives you cause for concern.

Best,  
Rachel

On Tue, May 23, 2023 at 9:29 AM Rachel Li <[rachel@berkeleyyeast.com](mailto:rachel@berkeleyyeast.com)> wrote:  
Hi Lauren,

I hope you had a good weekend. I would like to request additional time for our response to your questions (we are using both approaches to answer question #6 and need more time to make measurements). In addition, would it be acceptable for us to use older NHANES data, or does it need to be the most recent?

Thank you,  
Rachel

On Fri, May 19, 2023 at 3:30 PM Charles Denby <[charles@berkeleyyeast.com](mailto:charles@berkeleyyeast.com)> wrote:

Hi Lauren,

Thanks so much for your response and for the questions. I am working with my team on addressing them. I am out of office next week on work travel, so I'd like to loop in my colleague and co-founder, Rachel Li, who you have met previously during our pre-filing meeting, and is working to finalize our responses. We are hoping to send you answers in the requested 10 day time window. But, in the case that we need more time, how do you suggest we proceed?

Very best regards,

Charles

On Fri, May 12, 2023 at 11:03 AM Viebrock, Lauren <[Lauren.Viebrock@fda.hhs.gov](mailto:Lauren.Viebrock@fda.hhs.gov)> wrote:

Dear Dr. Denby,

During our review of GRAS Notice No. 001094, we noted questions that need to be addressed. Please find the questions attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,

Lauren

**Lauren VieBrock, Ph.D.**

*Regulatory Review Scientist/Microbiology Reviewer*

Center for Food Safety and Applied Nutrition  
Office of Food Additive Safety

U.S. Food and Drug Administration  
Tel: 301-796-7454  
[lauren.viebrock@fda.hhs.gov](mailto:lauren.viebrock@fda.hhs.gov)



--

Rachel Li, PhD  
Co-founder  
Berkeley Yeast  
2451 Peralta St  
Oakland, CA 94607

--

Rachel Li, PhD  
Co-founder  
Berkeley Yeast  
2451 Peralta St  
Oakland, CA 94607

--

Rachel Li, PhD  
Co-founder  
Berkeley Yeast  
2451 Peralta St  
Oakland, CA 94607

June 9, 2023

**GRN 1094 Questions:**

1. Please confirm that none of the components used in the manufacturing process or in the final ingredient include or are derived from sesame.

None of the components used in the manufacturing process or in the final ingredient include or are derived from sesame.

2. Please provide a narrative to describe the manufacturing process, including stating that the fermentation is performed under controlled conditions.

General notes:

- All materials used are approved food contact surfaces.
- Clean-in-place: CIP uses 2% or 3% NaOH solution, depending on the vessel. Fermenters and transfer lines are also heat sanitized. pH of rinse water is measured post CIP to ensure complete evacuation of CIP chemicals. ATP swabs are used to assess completeness of CIP.
- Sanitize-in-place: SIP is held at 88°C (190°F) for 45 minutes for a target of 4.8 x10<sup>5</sup> pasteurization units (PU).
- Preculture and plate media are sterilized at 250°F in a steam sterilizer (autoclave).
- QC using non-differential media is carried out for uninoculated media at many points in the process. "Pass" of a batch assumes zero tolerance and zero CFUs per 500 µL sample.
- QC using differential media is used to assess the presence of a variety of microorganisms at many points in the process.
- Connections between transfer fittings are made in aseptic conditions using HEPA-filtered laminar flow hoods and 70% ethanol solution.
- Transfer of yeast slurry is accomplished using sterile filtered air or pumped using indirect positive displacement (PD) peristaltic pumps.
- For all processes, we adhere to appropriate GMP, HACCP, and safety protocols including all recommendations for PPE.

Our manufacturing process begins with a series of carefully controlled serial steps with cultures being used to inoculate successively larger volumes. Fermentation is performed under controlled conditions. A brief overview of these steps from master stocks through packaging is listed below.

- a. Master stocks of our yeast cultures are stored in redundant cryogenic conditions at -80°C.
- b. Propagation begins by inoculating a plate containing MYPG (Malt, Yeast, Peptone, Glucose) media from this culture with a single-use sterile loop.
- c. Colonies from this plate are transferred to liquid media and grown in increasing volumes. All equipment and food contact surfaces are FDA approved. All media components are food grade and consist of sugars and nutrients that are commonly used in brewing and



yeast manufacturing. Transfer of the inoculum is carried out using peristaltic pumps and autoclaved tubing and fittings.

- d. The final propagation step uses our standard media which is prepared from dry malt extract (DME) and sterilized in a large steam-heated vessel.
- e. Media is transferred to cyindroconical propagation vessels (fermenters) and diluted to target density with sterile water. Sterility of the dilution water is confirmed by plating on non-differential media.
- f. Fermenters are aerated throughout propagation using sterile air, which undergoes multi-stage sterile filtration.
- g. Sugar consumption and pH are monitored throughout the growth cycle. When all available sugar has been consumed, fermenters are cooled. Dense yeast slurry is collected and transferred to a holding vessel. Cell counts, viability, and microbial QC are performed on all vessels.
- h. Cells are counted with an automated cell counter using AOPI fluorescence and confirmed with a hemocytometer using methylene blue staining. After cell enumeration, holding vessels are diluted to target density of  $1.25 \times 10^{12}$  cells/L.
- i. Yeast is packaged in FDA-approved, sterilized HDPE containers. Transfer to these containers occurs in a HEPA-filtered laminar flow hood using peristaltic pumps with autoclaved silicone tubing. Containers are shipped overnight in an insulated box with ice packs.

3. Part 2.6 (page 14) states that each batch of *Saccharomyces cerevisiae* strain BY-989 is tested for several parameters (viable cells, bacteria and yeast, and pH) and provides the corresponding limits.

- a. Please confirm that these parameters and limits are the specifications for your ingredient.

Yes, the specifications listed in Part 2.6 are for our ingredient.

- b. A limit for lead should be included in the specifications for fermentation-derived ingredients. Please include a limit for lead in the specifications for *S. cerevisiae* strain BY-989 that is reflective of the results of the batch analyses and is as low as possible.

The limit for lead in each batch of *S. cerevisiae* strain BY-989 is 10 µg/kg.

- c. Please specify which analytical methods were used to test for each specification parameter and state if those methods have been validated and are fit for purpose.

- **Viable cells:** Viability is determined with an automated cell counter using AOPI fluorescence and with a hemocytometer using methylene blue staining.
- **Bacteria and yeast:** Samples are tested for contaminating microorganisms at several points in the manufacturing process using several different methods. This includes: plating on non-differential media (MYPG), plating on differential media (MYPG plus cycloheximide, MYPG plus copper, WLD, MRS aerobic, and MRS anaerobic), and swabbing to identify the presence of ATP.

- **pH:** Measurements are taken at several points in the manufacturing process using a benchtop pH meter. The value reported on a COA is the endpoint pH.
  - **Lead:** Lead levels are quantified using ICP-MS.
  - All methods have been validated and are fit for purpose
- d. Please provide analytical results from a minimum of three non-consecutive batches to demonstrate that the ingredient can be manufactured to meet the specifications.  
[See attached documentation.](#)
4. Please provide a statement that all processing aids used in the manufacture of *S. cerevisiae* BY-989 are used in accordance with applicable U.S. regulations, were concluded to be GRAS for their respective uses or are subjects of effective food contact notifications.  
[All processing aids used in the manufacture of \*S. cerevisiae\* BY-989 are used in accordance with applicable U.S. regulations.](#)
5. Part 2.5 of the notice (page 13) states that *S. cerevisiae* strain BY-989 “*is used as any other commercial yeast would be used in the beer manufacturing process*” and that the intended use level ( $9.0 \times 10^6 - 1.5 \times 10^7$  cells per mL of wort beer fermentation) is a standard industrial practice. Please confirm that the ingredient is substitutional for other *S. cerevisiae* strains currently used in beer fermentation and its use is not expected to increase dietary exposure to *S. cerevisiae*.  
[BY-989 is substitutional for any other \*S. cerevisiae\* strain currently used in beer fermentation. Its use is not expected to increase dietary exposure to \*S. cerevisiae\*.](#)
6. Please provide a short narrative for the safety of the two flavor molecules (3-mercapto-1-hexanol and 3-mercaptohexyl acetate) that will be present in the beer.  
[See answer below.](#)
- a. You may use the publicly available information (e.g., JECFA, FEMA) for the safety assessment of these flavors using the published ADI values and comparing those numbers with the expected dietary exposure to these flavor molecules from the intended use of *S. cerevisiae* strain BY-989 in beer fermentation. For the safety assessment, we typically request that notifiers provide the 90<sup>th</sup> percentile eaters-only dietary exposure for the U.S. population using recent food consumption data (e.g., the recent National Health and Nutrition Examination Survey (NHANES)). Please state whether the exposure to these flavors will be substitutional for their current intakes related to beer fermentation and therefore not expected to increase the dietary exposure to 3MH and 3MHA.
- b. Alternatively, you may provide information/data showing that the concentrations of 3MH and 3MHA in beer produced by BY-989 will be less than or similar to that which is already present in consumed beer varieties produced using highly aromatic hop preparations. Based on the comparison, please clarify that the exposure to these flavors will be substitutional.

7. A key component of a GRAS conclusion is a complete and balanced review of available scientific literature/data. Please provide the details/parameters of your updated literature search/review.
- Search terms: thiols, volatile thiols, 3MH, 3MHA, beer, wine, beverages, carbon-sulfur lyase
  - Document types: reviews, primary research, toxicology studies, government documents
  - Publication date range for updated search: 2022-current (anything published after submission of GRN 1094)
  - Our review of available scientific literature/data (including the references listed in GRN 1094) is complete and balanced as it covers 4 decades of research up to today, provides a complete understanding of the function of the CSL enzyme, and the presence, origin, and concentration of 3MH and 3MHA in beer and other fermented beverages.

### Question 6:

#### Toxicology data

In the 1999 JECFA evaluation, available at <https://www.inchem.org/documents/jecfa/jecmono/v44jec09.htm>, JECFA did not set a numerical ADI. The report stated that there was no safety concern regarding 3-mercapto-1-hexanol (3MH) and 3-mercaptohexyl acetate (3MHA) consumption at current levels of intake when used as a flavoring agent. JECFA relied on no observed effect levels (NOELs) from 90-day repeated dose studies on related compounds (e.g., 2-mercapto-3-butanol) to assess the margin of safety for these two compounds. An NOEL of 1.9 mg/kg bw/d was reported. To derive a safe level from the NOEL of 1.9 mg/kg bw/d, we applied FDA's typical safety factor of at least 1000, leading to a value of 0.0019 mg/kg bw/d. Using FDA's standard 60 kg body weight for an adult, this safe level would be 0.114 mg/person per day or 114 µg/person/day. In addition, JECFA classified 3MH and 3MHA in Cramer Class I (which covers compounds that have the lowest toxic potential). The intake threshold for compounds in Cramer Class 1 is 1800 µg/person/day or 1.8 mg/person/day.

#### Exposure Model

Based on the use levels described in the notice, we estimated maximum dietary exposure using an exaggerated human intake for beer. This calculation is based on 90<sup>th</sup> percentile consumption of alcoholic beverages in the United States using NHANES Survey<sup>1</sup> 2003-2012 data for a 60 kg person. For these calculations, we used the following assumptions:

- A 90<sup>th</sup> percentile of 15.8 g of beer and beer-like beverages per kg of body weight per day, equal to 948 g per day for a 60 kg person
- All drinks would consist of beer
- The specific gravity, or density, of a standard beer is 1.01<sup>2,3</sup>, meaning 948 g of beer is equivalent to 939 mL

Using these assumptions, we calculated a maximum exposure level if all beer was produced BY-989:

<b>Population</b>	<b>Daily beer consumption</b>	<b>Estimated daily intake of 3MH</b>	<b>Estimated daily intake of 3MHA</b>
90 <sup>th</sup> percentile eaters	939 mL / day	6.57 - 8.45 µg	0.28 - 0.939 µg

We did the same calculations using 2015 National Alcohol Survey<sup>4</sup> data and the NIAAA<sup>5</sup> guidelines for "heavy drinking". For these calculations, we used the following assumptions:

- A 90<sup>th</sup> percentile of 17 drinks/week, as reported for men, and 7 drinks/week for women
- Guidelines for "heavy drinking" as 4 drinks/day for men, and 3 drinks/day for women
- All drinks would consist of beer
- A standard beer consists of 12 fl oz, approximately 355 mL (1 fl oz = 29.5735 mL)
- A body weight of 60 kg

<b>Population</b>	<b>Daily beer consumption</b>	<b>Estimated daily intake of 3MH</b>	<b>Estimated daily intake of 3MHA</b>
Men	6.035 L / week, averaged to 862 mL / day	6.03 - 7.76 µg	0.26 - 0.862 µg
Men	1.42 L / day	9.94 - 12.78 µg	0.43 - 1.42 µg
Women	2.49 L / week, averaged to 355 mL / day	2.49 - 3.2 µg	0.11 - 0.355 µg
Women	1.065 L / day	7.46 - 9.6 µg	0.32 - 1.065 µg

In all cases, the maximum potential exposure for both 3MH and 3MHA is well below the NOEL-derived safe level of 114 µg/person/day and the 1800 µg/person/day intake threshold for Cramer Class I compounds. Potential exposure would be at most 12% of these intake thresholds.

### **Comparison Data**

FDA has asked that we provide data regarding the typical levels of 3-mercapto-1-hexanol (3MH) and 3-mercaptohexyl acetate (3MHA) in beer made with highly aromatic hop preparations. To address this point, we quantified the levels of both 3MH and 3MHA in fourteen commercial beer samples, not made with BY-989. Quantification was performed by the same method as in the main submission with minor modifications to improve sensitivity and ease of handling. Briefly, 10

mL of each duplicated beer sample was combined with 10 mL of phosphate buffered saline, pH 7.4, then treated with 100  $\mu$ M thiol-derivatizing reagent 4,4'-dithiodipyridine for 1 hr at room temperature. The resulting mixture was extracted with 5 mL ethyl acetate by vortexing and 1 mL of the organic layer was dried under a vacuum. The non-volatile solids that remained, including the derivatized thiols, were dissolved with 100  $\mu$ L of methanol followed by 100  $\mu$ L of water. This mixture was analyzed on an LC-MS instrument using the same instrument and protocol as specified before, and the peak areas for 3MH and 3MHA were quantified against external standards prepared via the same protocol. These external standards allow us to directly compare quantified values with data generated by the previous method. We also performed a bridging study to demonstrate that the previous and current methods quantify a beer sample to the same concentration of 3MH and 3MHA within 5% difference. The detailed results and specific identities of these samples are included in the table below.

### 3MH

As reported in our submission, beer made with BY-989 was found to contain 7-9  $\mu$ g/L 3MH. As captured in the table below, eleven of the commercial samples were found to contain detectable levels of 3MH, up to 12.56  $\mu$ g/L in Sample 8. As commercial beers, which are currently consumed, can contain levels of 3MH similar to (or even higher than) those made with BY-989, we expect that any exposure to this flavoring compound from beer made with BY-989 is substitutional.

### 3MHA

As reported in our submission, beer made with BY-989 was found to contain 0.3-1  $\mu$ g/L 3MHA. Seven of the fourteen commercial samples were found to contain detectable levels of 3MHA. The highest 3MHA content in a commercial sample was 0.63  $\mu$ g/L (Sample 8). This level falls within the range of 3MHA content measured in beer made with BY-989. Recognizing that some samples made with BY-989 may contain slightly higher levels of 3MHA, we considered whether the new exposure to 3MHA of an additional 0.37  $\mu$ g/L from beer made with BY-989 for a maximum level of 1  $\mu$ g/L is safe. Based on the NHANES data reported in the exposure model above, it is possible that small new exposures of 0.35  $\mu$ g/person/day to 3MHA will result from use of BY-989 in the production of beer. However, the toxicology data as reported above provides a safe level of 114  $\mu$ g/person/day, which is much higher than the maximum exposure level, even taking the new exposure into account. In addition, other commercial beer samples may have even higher levels; in fact, FEMA<sup>6</sup> reported that the average maximum use level of 3MHA in alcoholic beverages is 100  $\mu$ g/L. If that were the case, the exposure would be 93.9  $\mu$ g/person/day. Based on this, we can conclude that this exposure to 3MHA is unlikely to cause a safety concern.

	3MH ( $\mu$ g/L)	3MHA ( $\mu$ g/L)
Sample 1	0.00	0.04
Sample 2	0.90	0.00

Sample 3	1.88	0.00
Sample 4	4.92	0.00
Sample 5	7.93	0.21
Sample 6	0.00	0.00
Sample 7	0.00	0.00
Sample 8	12.56	0.63
Sample 9	1.65	0.00
Sample 10	0.88	0.00
Sample 11	6.39	0.14
Sample 12	1.08	0.02
Sample 13	1.18	0.18
Sample 14	7.62	0.10

## References

1. <http://www.cdc.gov/nchs/nhanes.htm>
2. Beer styles – Original Gravity and Final Gravity Chart. *Brewer's Friend*.  
<https://www.brewersfriend.com/2009/02/04/beer-styles-original-gravity-and-final-gravity-chart/> (2009).
3. [https://cdn.brewersassociation.org/wp-content/uploads/2023/05/06143938/2023\\_BA\\_Beer\\_Style\\_Guidelines.pdf](https://cdn.brewersassociation.org/wp-content/uploads/2023/05/06143938/2023_BA_Beer_Style_Guidelines.pdf)
4. As reported in the Alcohol Research Group website based on an analysis by Thomas K. Greenfield, Katherine Karriker-Jaffe, and Yu Ye of the 2015 National Alcohol Survey (NAS) of 7,071 individuals, residing in 50 states and Washington DC. Greenfield, T. K. & Ye, Y. US Drinking Norms Updated. <http://arg.org/news/drinking-norms-in-the-us/> (2019)
5. National Institute on Alcohol Abuse and Alcoholism.  
<https://www.rethinkingdrinking.niaaa.nih.gov/>.
6. <https://www.femaflavor.org/sites/default/files/18GRASSubstances3816-3905.pdf>



## Batch Certificate of Analysis

Strain Name	London Tropics
Strain Number	989
Batch Number	962

Harvest date	4/18/2023
Viability (greater than 95%)	95.10
Attenuation to spec (70-75%)	PASS
pH to spec (below pH 4.6)	PASS
Lead (Less than 10 ug/kg LOQ)	PASS

Glycerol Lot	74
Master Stock Microbial QC	PASS

QC (CFU per 250 uL)	Glycerol lot	Preculture	Uninoculated fermenter	Fermentation endpoint	Harvest	Sales container
MYPG	n/a	n/a	0	n/a	n/a	n/a
MYPG+	0	0	n/a	0	0	0
MYPG++	0	0	n/a	0	0	0
WLD	0	0	n/a	0	0	0
MRS (Aerobic)	0	0	n/a	0	0	0
MRS (Anaerobic)	0	0	n/a	0	0	0





## Batch Certificate of Analysis

Strain Name	London Tropics
Strain Number	989
Batch Number	993

Harvest date	5/12/2023
Viability (greater than 95%)	99.55
Attenuation to spec (70-75%)	PASS
pH to spec (below pH 4.6)	PASS
Lead (Less than 10 ug/kg LOQ)	PASS

Glycerol Lot	73
Master Stock Microbial QC	PASS

QC (CFU per 250 uL)	Glycerol lot	Preculture	Uninoculated fermenter	Fermentation endpoint	Harvest	Sales container
MYPG	n/a	n/a	0	n/a	n/a	n/a
MYPG+	0	0	n/a	0	0	0
MYPG++	0	0	n/a	0	0	0
WLD	0	0	n/a	0	0	0
MRS (Aerobic)	0	0	n/a	0	0	0
MRS (Anaerobic)	0	0	n/a	0	0	0



## Batch Certificate of Analysis

Strain Name	London Tropics
Strain Number	989
Batch Number	1001

Harvest date	5/18/2023
Viability (greater than 95%)	97.88
Attenuation to spec (70-75%)	PASS
pH to spec (below pH 4.6)	PASS
Lead (Less than 10 ug/kg LOQ)	PASS

Glycerol Lot	76
Master Stock Microbial QC	PASS

QC (CFU per 250 uL)	Glycerol lot	Preculture	Uninoculated fermenter	Fermentation endpoint	Harvest	Sales container
MYPG	n/a	n/a	0	n/a	n/a	n/a
MYPG+	0	0	n/a	0	0	0
MYPG++	0	0	n/a	0	0	0
WLD	0	0	n/a	0	0	0
MRS (Aerobic)	0	0	n/a	0	0	0
MRS (Anaerobic)	0	0	n/a	0	0	0

## Viebrock, Lauren

---

**From:** Rachel Li <rachel@berkeleyyeast.com>  
**Sent:** Friday, September 15, 2023 4:07 PM  
**To:** Viebrock, Lauren  
**Subject:** Re: [EXTERNAL] Re: GRN 001094 Questions  
**Attachments:** Certificate of Analysis - Batch 962.pdf; Certificate of Analysis - Batch 993.pdf; Certificate of Analysis - Batch 1001.pdf

**CAUTION:** This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Hi Lauren,

Thank you for clarifying. Please see our batch analysis data in the COAs attached.

Best,  
Rachel

On Thu, Sep 14, 2023 at 12:02 PM Viebrock, Lauren <[Lauren.Viebrock@fda.hhs.gov](mailto:Lauren.Viebrock@fda.hhs.gov)> wrote:

Hi Rachel,

Our question was referring to the batch analysis data for the other specifications in addition to lead; for example, please also provide the results for pH, and microorganisms. Please provide the resultant values for the other specifications for the lots.

Thanks,

Lauren

---

**From:** Rachel Li <[rachel@berkeleyyeast.com](mailto:rachel@berkeleyyeast.com)>  
**Sent:** Thursday, September 14, 2023 2:51 PM  
**To:** Viebrock, Lauren <[Lauren.Viebrock@fda.hhs.gov](mailto:Lauren.Viebrock@fda.hhs.gov)>  
**Subject:** Re: [EXTERNAL] Re: GRN 001094 Questions

**CAUTION:** This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Hi Lauren,

Thank you for the follow-up and apologies if the batch data was unclear. The batch/lot number listed on the analytical reports (962, 993, 1001) are our batch numbers and represent three non-consecutive batches. The values for lead are each < 0.010 mg/kg (please see page 2 of the analytical reports). If I have misunderstood your question, however, could you please clarify?

Best,  
Rachel

On Thu, Sep 14, 2023 at 11:29 AM Viebrock, Lauren <[Lauren.Viebrock@fda.hhs.gov](mailto:Lauren.Viebrock@fda.hhs.gov)> wrote:

Hi Rachel,

Thank you for providing the additional information in response to our questions. We have an additional point to be addressed below.

We note the batch data was provided for the lead specification in the amendment; Please provide the analytical results from a minimum of three non-consecutive batches to demonstrate that the ingredient can be manufactured to meet the all specifications. Please report the resultant value as opposed to “pass” or “conform.” Thank you.

Best,

Lauren

---

**From:** Rachel Li <[rachel@berkeleyyeast.com](mailto:rachel@berkeleyyeast.com)>

**Sent:** Friday, June 9, 2023 6:33 PM

**To:** Charles Denby <[charles@berkeleyyeast.com](mailto:charles@berkeleyyeast.com)>; Viebrock, Lauren <[Lauren.Viebrock@fda.hhs.gov](mailto:Lauren.Viebrock@fda.hhs.gov)>

**Subject:** [EXTERNAL] Re: GRN 001094 Questions

**CAUTION:** This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Hi Lauren,

Our response to your questions on GRAS Notice No. 001094 is attached, as well as analytical results from three non-consecutive batches. Please let me know if any additional information is required. Thank you very much and have a good weekend.

Best,  
Rachel

On Tue, May 30, 2023 at 3:36 PM Rachel Li <[rachel@berkeleyyeast.com](mailto:rachel@berkeleyyeast.com)> wrote:

Hi Lauren,

I wanted to provide a bit more context on my question about using the most recent NHANES data.

In determining dietary exposure, there are 2 recent GRAS notices concerning beer which serve as precedent: one (date of closure March 2020) used NHANES data from 2003-2006 which estimated daily beer consumption (by men) to be slightly under 0.5 L. The other (date of closure December 2019) used National Alcohol Survey data which estimated consumption (by men) to be as high as 1.4 L per day.

We plan to present data on 3MH and 3MHA consumption using both values. For example, based on calculations using JECFA NOELs, even in the extreme - and unlikely - case where all 1.4 L was beer made with BY-989, 3MH consumption would be at 10% of the safe level. Given that the trends in alcohol consumption are declining rather than increasing, we expect these previous values to be, if anything, an overestimate. Given this, and the fact that we have learned that access to the most recent data can take 4 weeks, and be expensive to obtain (requires consultancy), we believe that the most expeditious way to proceed is with this publicly available information. We feel that there is sufficient precedent to justify this course of action. Of course, please let us know if this gives you cause for concern.

Best,  
Rachel

On Tue, May 23, 2023 at 9:29 AM Rachel Li <[rachel@berkeleyyeast.com](mailto:rachel@berkeleyyeast.com)> wrote:

Hi Lauren,

I hope you had a good weekend. I would like to request additional time for our response to your questions (we are using both approaches to answer question #6 and need more time to make measurements). In addition, would it be acceptable for us to use older NHANES data, or does it need to be the most recent?

Thank you,

Rachel

On Fri, May 19, 2023 at 3:30 PM Charles Denby <[charles@berkeleyeast.com](mailto:charles@berkeleyeast.com)> wrote:

Hi Lauren,

Thanks so much for your response and for the questions. I am working with my team on addressing them. I am out of office next week on work travel, so I'd like to loop in my colleague and co-founder, Rachel Li, who you have met previously during our pre-filing meeting, and is working to finalize our responses. We are hoping to send you answers in the requested 10 day time window. But, in the case that we need more time, how do you suggest we proceed?

Very best regards,

Charles

On Fri, May 12, 2023 at 11:03 AM Viebrock, Lauren <[Lauren.Viebrock@fda.hhs.gov](mailto:Lauren.Viebrock@fda.hhs.gov)> wrote:

Dear Dr. Denby,

During our review of GRAS Notice No. 001094, we noted questions that need to be addressed. Please find the questions attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Regards,

Lauren

**Lauren VieBrock, Ph.D.**

*Regulatory Review Scientist/Microbiology Reviewer*

**Center for Food Safety and Applied Nutrition**

**Office of Food Additive Safety**

**U.S. Food and Drug Administration**

Tel: 301-796-7454

[lauren.viebrock@fda.hhs.gov](mailto:lauren.viebrock@fda.hhs.gov)

--

Rachel Li, PhD

Co-founder

Berkeley Yeast

2451 Peralta St

Oakland, CA 94607

--

Rachel Li, PhD

Co-founder

Berkeley Yeast

2451 Peralta St

Oakland, CA 94607

--

Rachel Li, PhD

Co-founder

Berkeley Yeast

2451 Peralta St

Oakland, CA 94607

--

Rachel Li, PhD

Co-founder

Berkeley Yeast

2451 Peralta St

Oakland, CA 94607

--

Rachel Li, PhD

Co-founder

Berkeley Yeast

2451 Peralta St

Oakland, CA 94607





## Batch Certificate of Analysis

<b>Strain Name</b>	<b>London Tropics</b>
<b>Strain Number</b>	<b>989</b>
<b>Batch Number</b>	<b>962</b>

<b>Harvest date</b>	4/18/2023
<b>Viability (&gt;95%)</b>	95.10
<b>Attenuation to spec (&lt;3°P)</b>	1.7
<b>pH to spec (below pH 4.6)</b>	4.19
<b>Lead (Less than 10 ug/kg LOQ)</b>	PASS

<b>Glycerol Lot</b>	<b>74</b>
<b>Master Stock Microbial QC</b>	PASS

QC (CFU per 250 Million Cells)

**MYPG**  
**MYPG+**  
**MYPG++**  
**WLD**  
**MRS (Aerobic)**  
**MRS (Anaerobic)**

Glycerol lot	Preculture	Fermentation endpoint	Harvest	Sales container
n/a	n/a	n/a	n/a	n/a
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0



## Batch Certificate of Analysis

<b>Strain Name</b>	<b>London Tropics</b>
<b>Strain Number</b>	<b>989</b>
<b>Batch Number</b>	<b>993</b>

<b>Harvest date</b>	5/12/2023
<b>Viability (&gt;95%)</b>	99.55
<b>Attenuation to spec (&lt;3°P)</b>	2.4
<b>pH to spec (below pH 4.6)</b>	4.22
<b>Lead (Less than 10 ug/kg LOQ)</b>	PASS

<b>Glycerol Lot</b>	<b>73</b>
<b>Master Stock Microbial QC</b>	PASS

QC (CFU per 250 Million Cells)

	<b>Glycerol lot</b>	<b>Preculture</b>	<b>Fermentation endpoint</b>	<b>Harvest</b>	<b>Sales container</b>
<b>MYPG</b>	n/a	n/a	n/a	n/a	n/a
<b>MYPG+</b>	0	0	0	0	0
<b>MYPG++</b>	0	0	0	0	0
<b>WLD</b>	0	0	0	0	0
<b>MRS (Aerobic)</b>	0	0	0	0	0
<b>MRS (Anaerobic)</b>	0	0	0	0	0



## Batch Certificate of Analysis

<b>Strain Name</b>	<b>London Tropics</b>
<b>Strain Number</b>	<b>989</b>
<b>Batch Number</b>	<b>1001</b>

<b>Harvest date</b>	5/18/2023
<b>Viability (&gt;95%)</b>	97.88
<b>Attenuation to spec (&lt;3°P)</b>	2.6
<b>pH to spec (below pH 4.6)</b>	4.21
<b>Lead (Less than 10 ug/kg LOQ)</b>	PASS

<b>Glycerol Lot</b>	<b>76</b>
<b>Master Stock Microbial QC</b>	PASS

QC (CFU per 250 Million Cells)

	<b>Glycerol lot</b>	<b>Preculture</b>	<b>Fermentation endpoint</b>	<b>Harvest</b>	<b>Sales container</b>
<b>MYPG</b>	n/a	n/a	n/a	n/a	n/a
<b>MYPG+</b>	0	0	0	0	0
<b>MYPG++</b>	0	0	0	0	0
<b>WLD</b>	0	0	0	0	0
<b>MRS (Aerobic)</b>	0	0	0	0	0
<b>MRS (Anaerobic)</b>	0	0	0	0	0