BERKELEY YEAST

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 CFR §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 BY-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 BY-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. Thank you for your time and your consideration.

Sincerely,

Berkeley Fermentation Science Inc.

By: _____
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

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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).

To address these challenges, we propose the use of engineered strain BY-989. Both barley and hops contain high concentrations of conjugated thiols, flavorless precursor molecules that can be enzymatically converted into flavor-active volatile thiols through the activity of carbon-sulfur lyase (CSL) enzymes. BY-989 expresses a highly active CSL that efficiently releases 3MH from these precursors. In a representative brewing process, expression of this CSL in a London Ale yeast strain increases thiol concentrations in finished beer compared to beer made with the parent strain. As the brewing industry is forced to rely on agricultural inputs generated through more sustainable farming practices, generating difficult-to-achieve flavors with engineered yeast strains - flavors that are traditionally derived from stress-sensitive and low-yield hop cultivars - will allow for adoption of hop cultivars that are bred for resilience and yield, rather than flavor and aroma.

1.6. Availability of information for FDA review

Berkeley Fermentation Science Inc. undertakes that all data and information used in this GRAS determination for use of BY-989 yeast in brewing will be made available for the FDA to review and copy at the following address:

Berkeley Fermentation Science Inc.
Attention: Charles Denby
2451 Peralta Street
Oakland, CA 94607
USA

Data will be sent to the FDA upon request.

No information used in this part of this notification is trade secret or confidential commercial information.

1.7. Disclosure and certification

No data or information contained in parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

To the best of our knowledge, this GRAS notice for *Saccharomyces cerevisiae* strain BY-989 and its use in brewing is a complete, representative, and balanced submission that includes both favorable and unfavorable information, known to Berkeley Fermentation Science Inc. and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

[redacted] _____

Charles Denby, Ph.D. Date
Chief Executive Officer
Berkeley Fermentation Science Inc.

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[1,2]. 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[3,4].

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)[5]. 3MHA imparts sensory notes similar to 3MH, most commonly described as passion fruit.

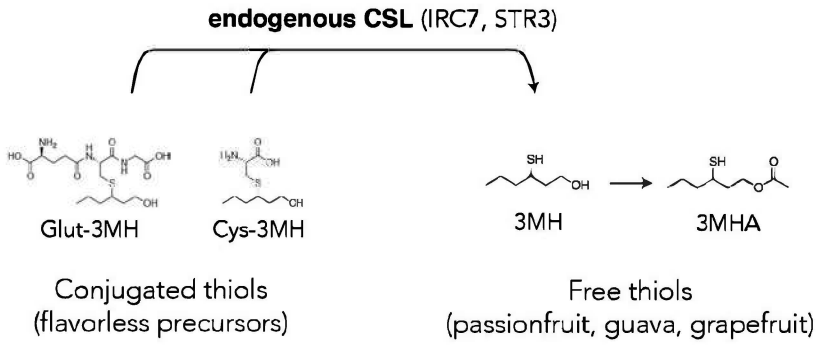

Figure 1. CSL is an enzyme that converts conjugated thiols to free thiols. Two different *S. cerevisiae* genes, IRC7 and STR3, encode CSL enzyme variants. 3MHA is formed from 3MH through the action of endogenous alcohol acyltransferase enzymes.

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[6]. Ancient peoples utilized yeast in the production of other fermented foods and baked goods as well[7]. 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[8]. The strains used for various industrial food and beverage manufacturing processes have evolved considerably from their wild ancestors, which likely inhabited primeval forests[9]. 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[8].

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[10–12].

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[13]. In addition, it is commonly found in soil and water throughout the world, where it plays an important role in nitrogen fixation[14].

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[15] 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)[16,17], 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[18]. 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.

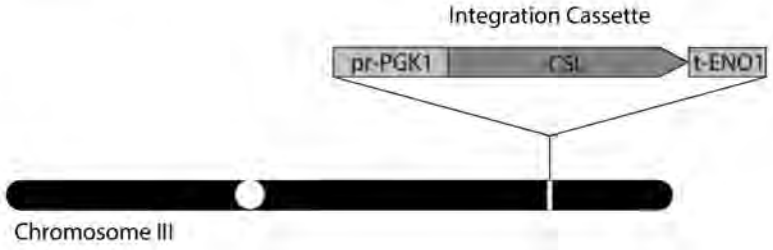

Figure 2. Diagram of genomic integration. The integration cassette, containing the PGK1 promoter, CSL gene sequence, and the ENO1 terminator, was directly integrated into the chromosome of the parent strain at a specific and known site.

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[19]. 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 δ elements. These δ elements are the remnants of Ty1 and Ty2 transposon integration events[20]. The number and location of these δ elements are strain-specific and serve as an effective means of distinguishing between strains of *S. cerevisiae*[21], and detecting large chromosomal rearrangements. We verified the genetic relationship between the host strain and the engineered strain by amplifying these δ 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.

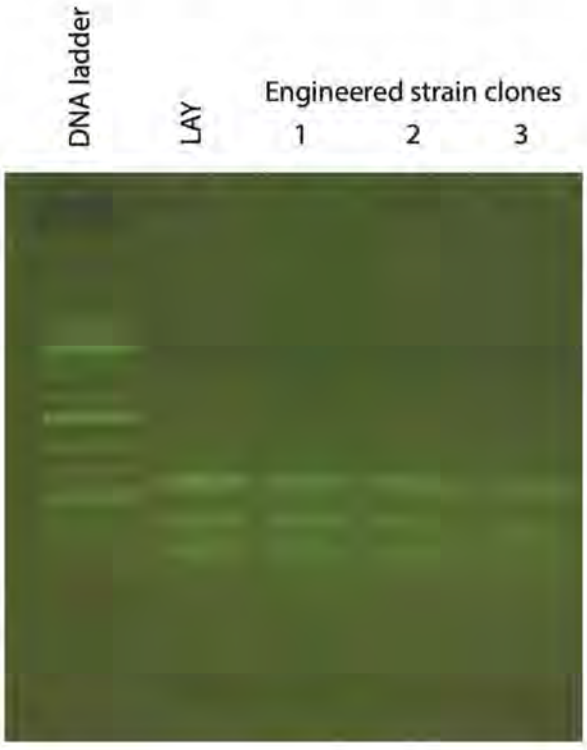

Figure 3. Interdelta analysis of LAY and BY-989. Interdelta analysis verifies the genetic relationship between the host strain and the engineered strain. The identical banding pattern shows that the engineered strain does not contain any large chromosomal changes. PCR was performed using the delta2/delta12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

described in detail by Legras *et al.* 2003)[21] and Schuller *et al.* 2004)[22]. 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[23] and barley[24] 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[4,25]. 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[26–28]. 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[29,30].

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[31] and the FAO/WHO[32] 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[29].

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[10–12]. 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[33–35]. 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[36]. 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[13]. In addition, it is found in soil and water, where it plays an important role in nitrogen fixation[14].

Intestinal microorganisms can, in rare cases, act as opportunistic pathogens. Similarly, *C. freundii* can act as an opportunistic pathogen[37]. 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[38], 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[39]. 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[40], and SSD1[41] 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[42,43]. 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/Toxigenic 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[44, 45] and modified by Codex Alimentarius Commission, 2009[46], 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[47]. 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[48]. 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.

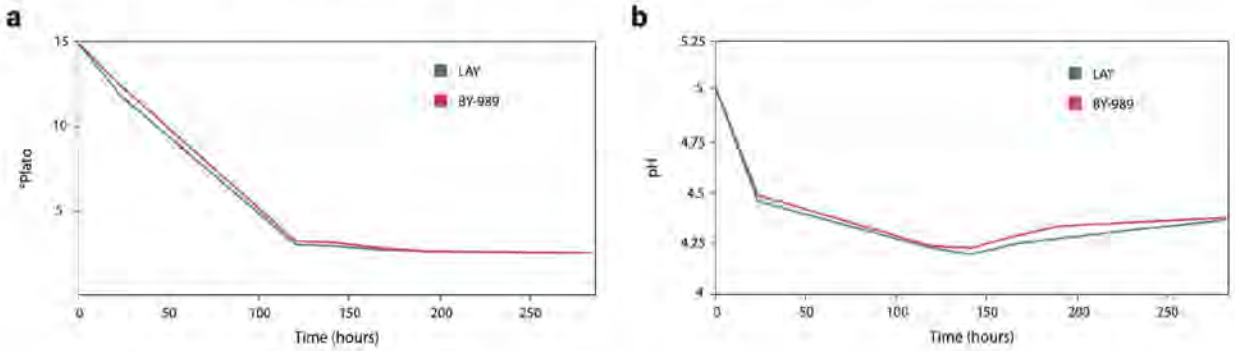

Figure 4. Fermentation profiles of LAY and BY-989. a) This fermentation was performed with 15 °P wort as a starting substrate. Gravity (°P), which corresponds to sugar concentration, was measured throughout brewing trials as a means of monitoring sugar consumption. These values were measured using an 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[49–51]. 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_{18} 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 µM 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[52].

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[31] and the FAO/WHO[32] 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.*[53], with some modifications. 20 mL of each clarified beer sample was combined with 4 mL of 10X phosphate buffered saline, pH 7.4, 200 µL of 10 mM 4,4′-dithiodipyridine, and 200 µL 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 µL 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 µm 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.

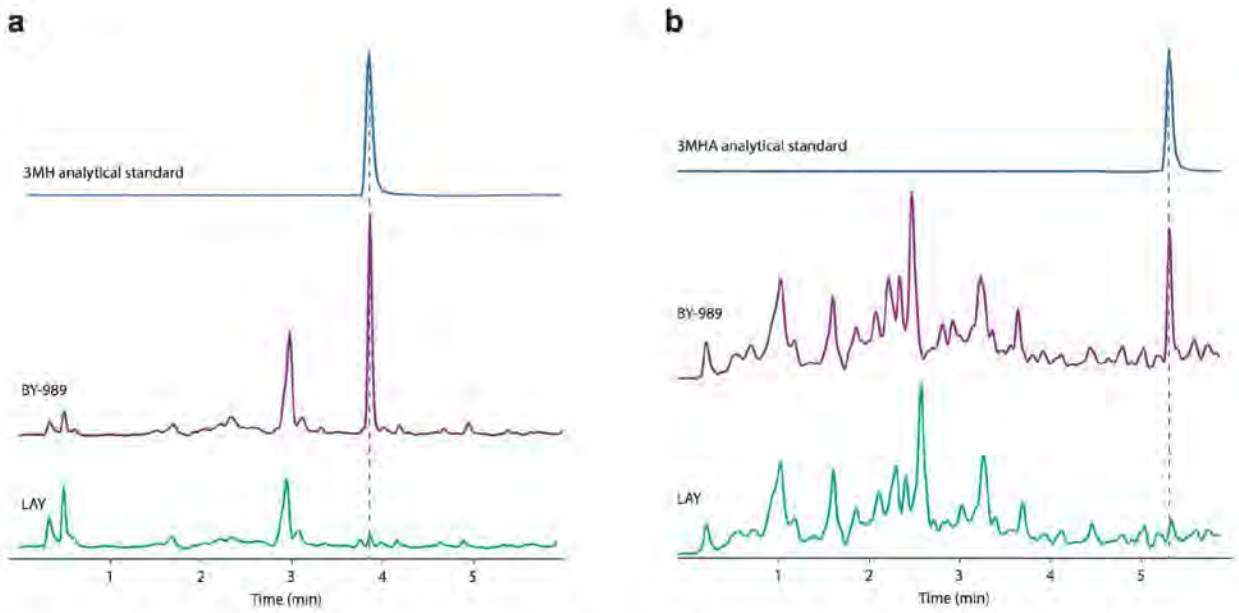

Figure 5. Identification of 3MH and 3MHA using liquid chromatography mass spectrometry. Selected ion monitoring chromatograms from LAY teal) and BY-989 purple) fermentation samples and analytical standards blue) are shown. The dotted lines denote the 3MH a) and 3MHA b) peaks. The y-axis represents signal 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), Phyterra's urea degrading yeast strain ECMo01 (GRN No. 175), Phyterra'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[48].

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Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

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 ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person: Charles Denby		Position or Title: CEO	
Organization *(if applicable)*: Berkeley Fermentation Science Inc.			
Mailing Address *(number and street)*: 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 *(if applicable)*:			
Mailing Address *(number and street)*:			
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. 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.
(name of notifier)

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 or 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	Printed Name and Title	Date (mm/dd/yyyy)
Charles Denby Digitally signed by Charles Denby Date: 2022.06.08 12:19:57 -07'00'	Charles Denby, CEO	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