OMEGA YEAST

4720 W Pensacola Ave
Chicago IL 60641

info@omegayeast.com
773.657.3438

Christopher Kampmeyer
Regulatory Review Scientist
Office of Food Additive Safety, HFS-200
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Dear Christopher Kampmeyer:

We received your letter on February 18th, 2022 regarding our submission dated August 9th, 2021, received on August 13th 2021 for the intended use of *Saccharomyces cerevisiae* strain OYR-185 in food that we view to be generally recognized as safe (GRAS).

We are hereby submitting a revision of our Generally Recognized as Safe (GRAS) Notification in accordance with Title 21 C.F.R. §170.30, for Omega Yeast Labs *Saccharomyces cerevisiae* strain OYR-185. This revision has additional and expanded sections in response to your February 18th email and letter questions/concerns including the exposure assessment, ingredient specifications, batch analyses, manufacturing section and GRAS conclusions.

Please do not hesitate to contact me at any time by email at lance@omegayeast.com to discuss details or to request supplemental information as needed.

Thank you for your consideration.

Sincerely,

[redacted]

Lance Shaner

Owner, Omega Yeast

GRAS notice for *Saccharomyces cerevisiae* strain OYR-185

PREPARED FOR:
Office of Food Additive Safety (HFS-200)
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

DATE: March 1st, 2022

§ 170.225 Part 1, GRAS Notice: Signed Statements and Certification

(1) GRAS Notice Submission

Omega Yeast Labs, LLC., in accordance with 21 CFR § 170.225(c)(1), has determined that its *Saccharomyces cerevisiae* strain OYR-185, that has been genetically edited using the CRISPR/Cas9 system to incorporate a naturally-occurring inactivating mutation in *FDC1* gene, is Generally Recognized as Safe ("GRAS") for the intended food application in accordance with Subpart E of 21 CFR § 170.

(2) Name and Address

Omega Yeast Labs, LLC
4720 W Pensacola Ave
Chicago, IL 60641
USA

(3) Name of Notified Substance

The name of the notified substance is a brewing yeast obtained from a *Saccharomyces cerevisiae* strain that has been genetically edited using the CRISPR-Cas9 system to incorporate a naturally-occurring inactivating mutation in *FDC1* gene. This novel yeast strain is called OYR-185.

(4) Intended Use in Food

The OYR-185 strain was developed by Omega Yeast to provide a novel flavor profile to beer by eliminating the phenolic off flavor (POF) phenotype in a Belgian brewing strain. *Saccharomyces cerevisiae* produces many aroma-active metabolites that contribute to the complexity of a beer's flavor including phenolics, esters, carbonyl compounds, higher alcohols, aldehydes and sulfur compounds. Some of these metabolites are considered undesirable off flavors. The phenolic compound, 4-vinyl guaiacol (4-VG), is produced by POF+ strains including wild yeast and wine yeast, along with some Belgian and German brewing strains. 4-VG imparts a clove-like or medicinal flavor in beer and overshadows desirable flavor compounds from yeast and hops. For centuries, brewing POF- yeast strains have been selected for and used in the production of "clean" lagers and ales. These POF- brewing strains harbor inactivating mutations in the *PAD1* and *FDC1* genes that are required for 4-VG production. One of these naturally-occurring mutations in *FDC1* was introduced into the host strain to produce the POF- OYR-185.

By eliminating the POF+ phenotype in the host strain, the fermentation with the resulting OYR-185 strain produces a combination of aroma-active metabolites that is unique to this strain and highly desirable. The flavor characteristics of OYR-185 provide a complement to hops and malt, that would be costly to obtain with other combinations of raw materials.

The OYR-185 yeast strain is intended for use as a commercial liquid yeast culture for the production of fermented beverages in accordance with Good Manufacturing Practices (GMPs). This liquid yeast strain performs alcoholic fermentation in the same manner as its host strain and other traditional brewing strains. The elimination of POF+ phenotype in OYR-185 provides a better-suited brewing yeast strain for American craft beers, hoppy beers and other "clean" beer styles.

(5) Statutory Basis for GRAS Determination

Omega Yeast Labs, LLC has determined that the OYR-185 yeast strain is "Generally Regarded as Safe" (GRAS) for the use as a starter culture in alcoholic beverage fermentation. This determination is based on scientific procedures and conforms to the regulations in accordance with 21 CFR § 170.30(a) and (b). Based on the design of OYR-185, it is substantially equivalent to the host strain, with the sole difference being one single base pair substitution that is a naturally occuring single nucleotide polymorphism common to other commercial brewing strains. The precise change of the *FDC1* allele to the *fdc1-C460T* allele means there is a negligible chance of unintended effects in the OYR-185 strain.

(6) Premarket Approval Statement

Omega Yeast Labs further asserts that the use of the OYR-185 yeast strain ingredient, as described herein, is exempt from the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act, based on a conclusion that the substance is GRAS under the conditions of its intended use.

(7) Availability of Information

The data and information that serve as the basis for this GRAS determination, as well any information that has become available since the GRAS determination, will be sent on request, or are available for the FDA's review and copying during customary business hours at the following address:

Omega Yeast Labs, LLC
Attention: Lance Shaner
4720 W Pensacola Ave
Chicago, IL 60641
USA

(8) Data and Information Confidentiality Statement

None of the data and information in the GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

(9) GRAS Certification

To the best of our knowledge, this GRAS notice for *Saccharomyces cerevisiae* OYR-185 and its use in fermentation of alcoholic beverages in accordance with Good Manufacturing practices (cGMP) is a complete, representative and balanced submission that includes both favorable and unfavorable information known to Omega Yeast Labs, LLC and pertinent to the evaluation of the safety and GRAS status of the use of the strain. Recent reviews of the scientific literature revealed no potential adverse health concerns.

(10) Name/Position of Notifier

[redacted] 3/2/2022

Lance Shaner, Ph.D.
Co-owner and Founder
Omega Yeast Labs, LLC

Date

§ 170.230 Part 2, Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1. IDENTITY OF THE NOTIFIED SUBSTANCE

The subject of this notification is an industrial brewing *S. cerevisiae* strain called OYR-185. This strain was modified with CRISPR-Cas9 for a single nucleotide substitution from wildtype *FDC1* to *fdc1-C460T*. This modification is a naturally occurring inactivating single nucleotide polymorphism found in many industrial brewing strains. Technical details regarding the modification are detailed in sections below. The use of OYR-185 in brewing eliminates phenolic off flavor in beer. The resulting beer will have little to no phenolic character similar to beers fermented with commonly used American and English brewing strains.

2.2 HOST MICROORGANISM

The host yeast strain, OYL-024, is an industrial brewing strain of *S. cerevisiae* that is widely used in commercial beer production of Belgian-style ales. The strain is commonly referred to as the Ardennes strain, referring to the Ardennes region of Belgium, and is sold by multiple commercial yeast suppliers under the names WY3522, WLP550 and OYL-024.

2.2.1. History of use

S. cerevisiae is widely used in industrial fermentation of many food products. It is in the air that we breathe and it grows naturally on many foods that we eat regularly. It is likely that most humans are exposed daily to foods containing millions of live *S. cerevisiae* through consumption of wine, beer, fruits, salads, and cheese and other dairy foods, as well as other traditional and ethnic fermented foods. This yeast species has been used in food production for over five thousand years. Common industrial applications include; baked goods, beer, wine, cider, sake, spirits, coffee and chocolate (Boekhout and Robert, 2014).

S. cerevisiae serves as a model organism for scientific research. Over 30 years of yeast research has contributed to our fundamental understanding of cell and molecular biology. The *S. cerevisiae* genome size is small, is highly homologous with the human genome, genetics are very tractable, and DNA repair pathways are robust allowing for ease of accurate genetic manipulation (Botstein and Fink, 2011). A long history of research and industrial use has indicated that *S. cerevisiae* is safe for use in food and beverage manufacturing (Boekhout and Robert, 2014).

There are over 150 *S. cerevisiae* strains that are commercially sold as brewing yeast. These strains have been isolated from the brewery and farmhouse origins, where the re-pitching or backslopping practices allowed for the propagation and selection of unique brewing characteristics (Gallone *et al.*, 2016). The origin of the host strain, OYL-024, traces back to Brasserie d'Achouffe in Belgium which is used to produce over 300,000 bbls sold in 72 countries. OYL-024 is a popular strain used by many US craft breweries for Belgian-style beers.

The FDA considers *S. cerevisiae* and several derived products safe for consumption. Indeed, FDA has approved dried yeast as an ingredient for food (21 C.F.R. §172.896), and Baker's yeast extract has been

affirmed by the FDA as a GRAS flavoring agent and adjuvant (21 C.F.R. §184.1983). The FDA has also approved various yeast-derived products for their use in food. These include Baker's yeast protein (21 C.F.R. §172.325), Yeast-malt sprout extract (21 C.F.R. §172.590) and Baker's yeast glycan (21C.F.R. §172.898). *S. cerevisiae* is also considered Generally Recognized as Safe as noted in several GRAS Notices; GRNs 120, 175, 239, 260, 284, 350, 353.

2.2.2. Taxonomy

The history of the use of *S. cerevisiae* is reviewed in Section 2.2.1 above. The OYL-024 host strain is a *S. cerevisiae* ale strain that is closely related to other Belgian/Germany "Beer 1" industrial strains. The phylogeny and taxonomy of OYL-024 has been determined from whole genome sequencing of industrial brewing yeast (Gallone *et al.*, 2016).

2.2.3. Characteristics

The host strain exhibits standard industrial brewing characteristics. It is maltose-fermenting, is easily propagated in wort produced from malted barley and exhibits high alcohol tolerance. Fermentations with this strain produce a desirable Belgian-flavor profile attributed to the production of ester and phenolic metabolites. This strain sporulates, but exhibits low spore viability and thus is not well-suited for traditional breeding strategies.

2.3. DONOR ORGANISM

2.3.1. Taxonomy

The *fdc1-C460T* allele is found in many industrial yeast strains. With over 102 industrial beer strains analyzed, >60% had inactivating mutations in *PAD1* and/or *FDC1*. An example of a common industrial brewing strain with the inactivating *fdc1-C460T* mutation is WLP001 (California Ale strain). This C to T mutation at position 460 of the *FDC1* coding sequence replaces a glutamine residue (CAA) by a stop codon (UAA) and is a loss of function mutation.

2.3.2. Genetic material from donor organisms

The source of donor DNA for the modified strain was a synthesized duplex oligonucleotide containing the single *fdc1-C460T* modification and was not derived from a donor *S. cerevisiae* strain. This strategy was validated by a prior research study and publicly available whole genome sequencing data of industrial brewing strains (Gallone *et al.*, 2016).

2.4. THE MODIFIED MICROORGANISM

2.4.1. Final construct used in the CRISPR-Cas9 strategy

2.4.1.1. Construction strategy

To generate OYR-185, wildtype *FDC1* loci in OYL-024 were edited to the naturally occurring *fdc1-C460T* allele utilizing CRISPR-Cas9 technology. This C to T mutation at position 460 of the *FDC1* coding sequence, replaces a glutamine residue (CAA) with a stop codon (UAA) and is a loss of

function mutation. CRISPR-Cas9 methodology involves the recruitment of Cas9 to the *FDC1* loci with a small guide RNA (sgRNA) that is homologous to the sequence surrounding the position 460 in *FDC1*. In addition, a PAM sequence (NGG) is in close proximity, which is crucial for the correct guidance of Cas9 to its target. Once recruited, Cas9 generates a double strand break (DSB) adjacent to the C at position 460 of *FDC1*. A repair template with homology to each end of the double strand break is supplied in excess and is utilized by the yeast cells endogenous homologous recombination pathway for repair. This repair template contains the C to T substitution at position 460. Once the repair occurs, the *FDC1* sgRNA homology is disrupted and thus Cas9 is unable to efficiently target the *fdc1-C460T* locus. Successful CRISPR-Cas9 targeting results in the editing of each *FDC1* loci to *fdc1-C460T*. Transformation events were screened through an absorbance-based POF measurement to confirm the POF- phenotype. The POF- isolates were further sequenced to confirm the *fdc1-C460T* substitution. The resulting *fdc1-C460T* substitution is a naturally occurring loss of function mutation resulting from a premature stop codon and a truncated protein and thus there is no concern for the introduction of toxic or allergenic material.

2.4.1.2. Generating the FDC1 CRISPR plasmid and fdc1-C460T repair template

The final DNA used for transformation of the host organism are the *FDC1* CRISPR plasmid and *fdc1-C460T* repair template. To generate the *FDC1* CRISPR plasmid, the *FDC1* targeting sgRNA was cloned into a parent yeast shuttling vector containing Cas9 regulated by *S. cerevisiae PGK1* promoter and terminator. The parent yeast shuttling vector was digested with BsmBI. An *FDC1* sgRNA expression cassette containing the tRNA phe promoter, tRNA phe, *FDC1* sgRNA, and the tSRN52 sequences with homology to the linearized ends of the parent yeast shuttling vector was synthesized. The gap repair yeast cloning method was used where two linear fragments with overlapping homology are transformed into *S. cerevisiae* and repaired through homology-directed repair pathways. The parent yeast shuttling vector also contains the *KanMX* gene, and thus successful gap repair results in G418-resistant colonies. The resulting *FDC1* CRISPR plasmid was isolated from the *S. cerevisiae* G418 transformants and confirmed through restriction digestion and sequencing. The *FDC1* CRISPR plasmid allows for co-expression of Cas9 and the *FDC1* sgRNA. The plasmid map and FASTA sequence is provided in Figure 1 and Appendix 1.

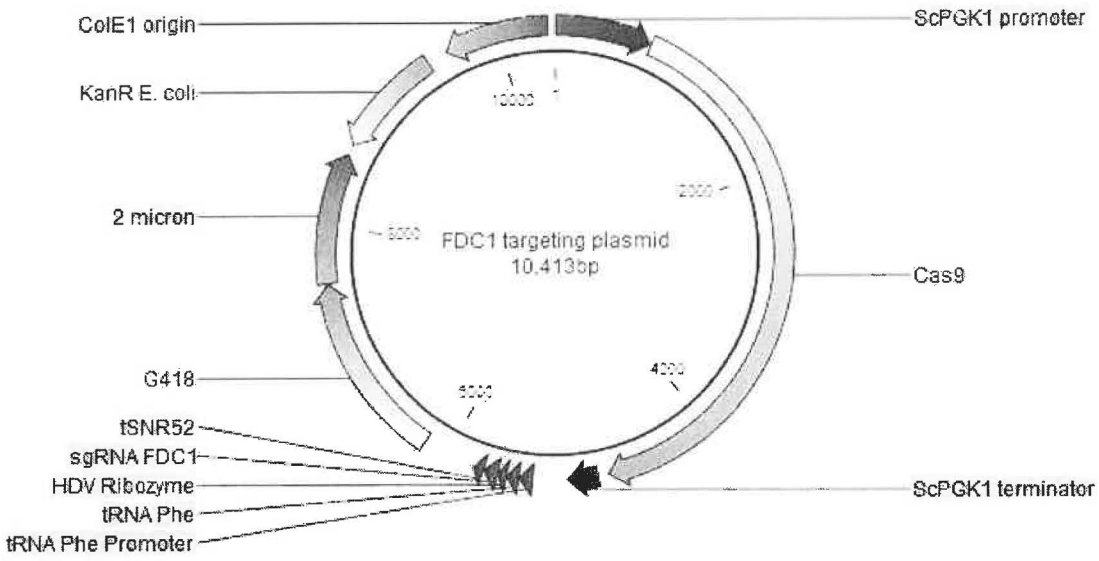

Figure 1. Schematic of the *FDC1* targeting CRISPR/Cas9 plasmid. The FDC1 targeting plasmid contains the Cas9 gene (with PGK1 promoter and terminator regulatory sequences), the sgRNA targeting FDC1 (with tRNA promoter, HDV ribozyme, SNR52 terminator regulatory sequences). For propagation and selection in yeast, the plasmid contains the 2 micron origin of replication and the G418 drug resistance marker, respectively. For propagation and selection in E. coli, the plasmid contains the ColE1 origin of replication and KanR drug resistance marker, respectively.

A double stranded (ds) DNA oligonucleotide was synthesized as the repair template for homology-directed repair of the induced DSB. The sequence of the repair template also contains the *fdc1-C460T* base substitution and is provided in Figure 2 and Appendix 2.

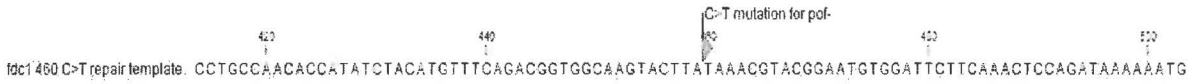

Figure 2. Schematic of the *fdc1-C460T* repair template. The *fdc1-C460T* repair template contains the nucleotide sequence of FDC1 spanning 414-503, and the C460T single base pair substitution.

2.4.1.3. Detailed description of the final *FDC1* CRISPR plasmid and *fdc1-C460T* repair template

The *FDC1* CRISPR plasmid is 10,413 bp long. The sequence contains the Cas9 ORF flanked by the ScPGK1 promoter and terminator (2-5,111 bp), the *FDC1* sgRNA expression cassette containing the tRNA phe promoter, tRNA phe, *FDC1* sgRNA, and the tSRN52 (5,362-5,799 bp), the *KanMX* gene for G418-selection in *S. cerevisiae* (8,631-9,446 bp), the yeast 2 micron multi-copy selfish DNA

element (7,591-8,553 bp), the *KanR* gene for Kanamycin-selection in E. coli (8,631-9,446 bp), and the ColE E. coli origin of replication (9,595-10,358 bp). The plasmid map and FASTA sequence is provided in Figure 1 above. The sequence of the repair template containing the *fdc1-C460T* base substitution is provided in Figure 2 above.

2.4.2. The transformation event

2.4.2.1. Genetic material used for the transformation method

The *FDC1* CRISPR plasmid and *fdc1-C460T* repair template were the only DNA sequences used for the transformation of the host OYL-024 and generation of the modified OYR-185.

2.4.2.2. Screening method for transformants

Logarithmically growing cultures of OYL-024 were transformed with 500 ng of the *FDC1* CRISPR plasmid and 25 µg of the *fdc1-C460T* repair template using standard LiOAc transformation protocol. The transformation reaction was outgrown in YPD overnight and plated onto YPD-G418 agar plates to select for successful transformants. Colonies were restruck to YPD-G418 agar plates to confirm resistance and then individual transformants were screened through an absorbanced-based POF assay. Briefly, yeasts were inoculated into 2ml of YPD growth medium, supplemented with 100 mg/L ferulic acid. The OYL-024 parental was included as a positive control for POF+ and OYL-088 was included as a negative control for POF-. After 3 days of incubation at 30° C, 220 rpm, cultures were centrifuged and the absorbance of the supernatant was measured at 325 nm. Results are reported in Figure 3.

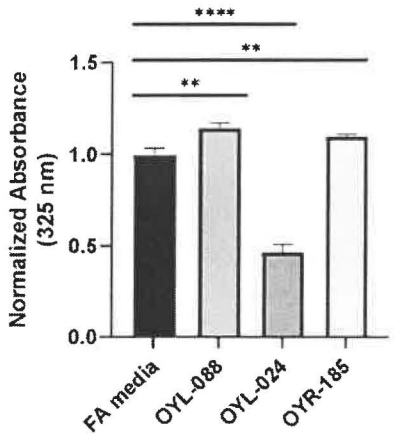

Figure 3. Ferulic Acid Depletion in CRISPR/Cas9 Isolates. The OYR-185 modified strain does not deplete ferulic acid and exhibits the POF- phenotype. Strains were grown in YPD+ferulic acid media for 3 days and the remaining ferulic acid was measured using a UV-vis absorbance-based assay. Ferulic acid exhibits an absorbance peak at 325 nm. Depicted is the average relative absorbance at 325 nm of the tested strains relative to a YPD+ferulic acid media control. OYL-088 california ale strain was included as a POF- control, which like OYR-185 does not result in FA depletion, whereas

the OYL-024 host strain shows clear ferulic acid depletion and the POF+ phenotype.

Colony PCR of the resulting POF- isolates was performed to amplify the region surrounding position 460 in *FDC1* and further sequenced by Sanger sequencing. Sequencing results are reported in Figure 4.

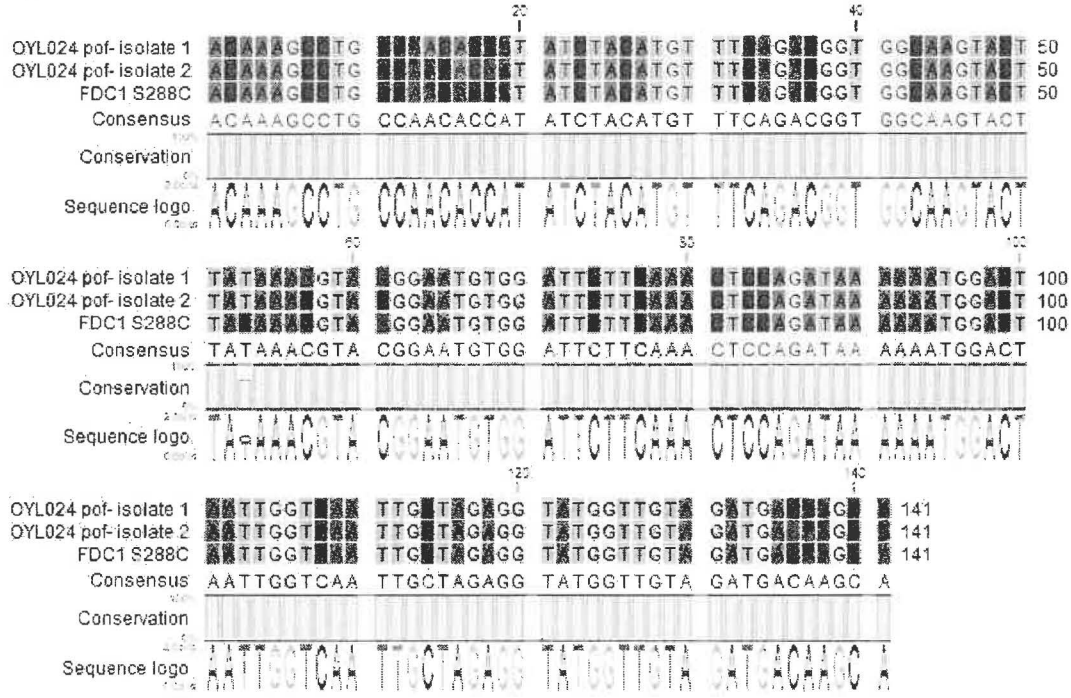

Figure 4. Sequencing results confirm CRISPR/cas9 modified *fdc1-C460T* nucleotide. Sanger sequencing results of the *FDC1* gene confirms the *fdc1-C460T* mutation. Two isolates of OYL-024 after CRISPR editing confirmed a POF- phenotype were sequenced. The *fdc1-C460T* was the only nucleotide alteration observed.

2.4.3. Genetic characterization of the modified microorganism

2.4.3.1. The loss of the Cas9 plasmid containing the antibiotic resistance gene

Successful POF-, *fdc1-C460T* transformants were inoculated into liquid YPD, grown to saturation and subsequently diluted and plated onto YPD agar plates. Once individual colonies were observed, YPD plates were replica-plated to YPD-G418. Colonies that lost the *FDC1* CRISPR plasmid were G418- were re-struck to YPD and YPD-G418 to confirm the loss of the antibiotic resistance gene (Figure 5).

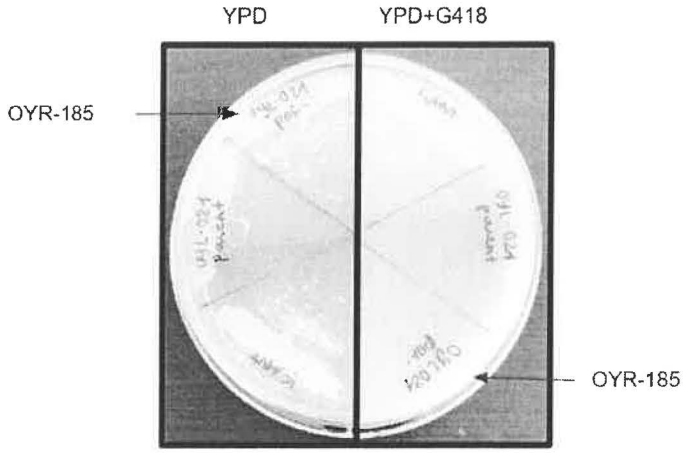

Figure 5. Confirmation of the loss of the FDC1 CRISPR/Cas9 targeting vector and G418 resistance. The FDC1 CRISPR/Cas9 targeting vector contains the G418 resistance marker for selection of transformation events. The OYL-024 POF- isolate was passaged in YPD liquid, plated to single colonies on YPD and replica plated to YPD-G418 selection plates to confirm loss of the plasmid and G418 resistance. Sensitivity of OYL-138 (labeled OYL-024 POF-) to G418 was confirmed by streaking onto YPD and YPD-G418 as shown.

2.4.3.2. DNA sequencing of the *fdc1-C460T* substitution

Colony PCR of the resulting POF- isolates was performed to amplify the region surrounding position 460 in *FDC1* and further sequenced by sanger sequencing. Sequencing results are reported in Figure 4 above.

2.4.4. Absence of difference between genetic profiles of the transformed and the host strain

The genome of *S. cerevisiae* contains long terminal repeat sequences known as δ elements. These δ elements are the remnants of Ty1 transposon integration events. The number and location of these δ elements are specific to a strain and have been used to fingerprint and differentiate between strains of *S. cerevisiae*. Using PCR and primers δ2 (5'- GTGGATTTTTATTCCAAC-3'), δ12 (5'-TCAACAATGGAATCCCAAC-3') and δ21 (5'-CATCTTAACACCGTATATGA-3') to amplify these δ sequences, we have verified the genetic relationship between the host strain OYL-024 and OYR-185 (Figure 6). This strategy is widely used to differentiate between industrial strains of *S. cerevisiae* (Legras and Karst, 2003). The interdelta PCR experiments were performed after serial passages YPD agar plates confirming the genome is stable. Furthermore, the expected genotype and phenotype of OYR-185 remains unchanged when propagated through the liquid yeast manufacturing process (Figure 7) representing greater than 30 cell divisions. The OYR-185 strain is not expected to be any more likely to undergo genomic rearrangement events within the normal environmental conditions of fermenting beer then the parent OYL-024 strain.

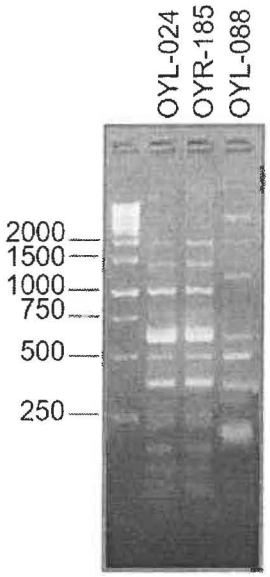

Figure 5: Interdelta PCR patterns of the OYL-024 and OYR-185 yeast strains. Interdelta PCR banding pattern shows that OYL-024 and OYR-185 are genetically identical. PCR was performed using delta12 (5'-TCAACAATGGAATCCCAAC-3') and delta21 (5'-CATCTTAACACCGTATATGA-3') primers on genomic DNA isolated from OYL-024, OYR-185 and OYL-088 (negative control) yeast strains. This technique is further described by Legras et al. 2003 and Schuller et al. 2004.

2.5. METHOD OF MANUFACTURE OF THE MODIFIED MICROORGANISM

2.5.1. Manufacturing of OYR-185

Propagation of the OYR-185 does not require any additional selection or alterations to the propagation methods of the host strain OYL-024 or any other industrial brewing strains. The manufacturing of OYR-185 is identical to that of all liquid yeast used in brewing and is in accordance with cGMP. Packaged product is >98% viable on ship date, and free from detectable levels of contaminants (zero colony forming units (CFUs) of bacteria or yeast detected per 20 million yeast cells). We do not anticipate dangerous levels of heavy metals. A summary of the methods used in manufacturing of OYR-185 can be found in Figure 6.

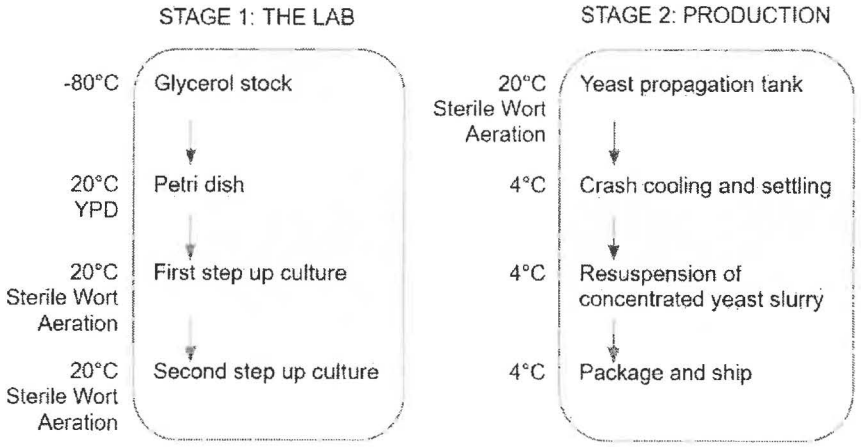

Figure 6. Flow diagram of liquid yeast manufacturing. Strains are started from glycerol stocks by streaking directly to YPD growth media plates. The YPD colonies are inoculated into the first step up culture. After reaching saturation, this culture is inoculated into the second step up culture. The second step up culture is grown to saturation and brought to the production facility to inoculate the propagation tank. This large-scale propagation is the final stage. When the culture has depleted all fermentable sugar, the temperature is lowered to 4°C and the cells are separated through natural processes of flocculation and settling. The propagation media is decanted and the remaining yeast cell mass is resuspended as a concentrated liquid slurry. This concentrated yeast slurry is packaged and shipped to consumers.

2.5.2. Raw Materials

All raw materials are standard food grade ingredients used in the manufacturing of liquid brewing yeast. These ingredients are free of the 8 major food allergens specified under the Food Allergen Labeling and Consumer Protection Act.

2.5.3. Lab Stage

OYL-185 is inoculated onto nutrient agar plates from frozen stock cultures maintained at -80°C in glycerol. The yeast colonies are transferred with a sterile loop from the nutrient agar plate into sterilized flasks of propagation medium and grown with aeration at 20°C to saturation. These starter cultures are inoculated into larger sterilized flasks of propagation medium and grown to cell numbers that are sufficient for the inoculation of a yeast propagation tank.

2.5.4. Fermentation and Recovery

The yeast from the lab is inoculated into propagation tanks in production. The propagation is aerated and grown at 20°C. Once fermentation is completed, the temperature is lowered to 4°C for settling. The yeast slurry is resuspended to a slurry concentrate of 30% yeast solids (percent pellet weight to total volume). The slurry concentrate is transferred to sterile packaging and stored at 4°C until used to inoculate a beer fermentation.

To prevent contamination of foreign microorganisms, the propagation medium is sterilized and all equipment is carefully cleaned and sanitized. The propagation tanks are cleaned in place with acid and base and rinsed to neutral pH. The cleaning is validated with microbiological testing and ATP swabs. The tanks are then sanitized with food grade sanitizer prior to filling with sterile propagation medium. The propagations are sampled throughout for microbiological controls and the yeast percent solids is determined at the end of propagation to insure proper and consistent growth.

2.6. APPLICATION AND USE LEVELS

We recommend that brewers use approximately 1 million cells of OYR-185 per milliliter of wort per degree Plato for brewing wort fermentation, as is standard industrial practice. At this inoculation level, alcoholic fermentation will be efficiently conducted and phenolic compounds will be absent similar to beers produced with brewing strains containing the natural inactivating *fdc1-C460T* mutation (i.e., POF- ale and lager brewing strains).

2.7. PRODUCT SPECIFICATIONS

The specifications for liquid yeast are measured on every batch and subsequently approved for release by Quality Control. The liquid yeast slurry is plated on microbiological growth mediums to detect contaminating bacteria and wild yeast. Any observation of growth results in the product being discarded. At the end of fermentation, cell growth is monitored by percent yeast solids and anything under 3% is discarded. The growth medium and liquid yeast product does not contain any of the 8 major food allergens specified under the Food Allergen Labeling and Consumer Protection Act. Product specifications are listed in the Table 1.

Table 1. Liquid Yeast Specifications

Parameter	Specification Range
Percent Yeast Solids	>3%
Total Viable Cells	>98%
Total Bacteria	<1 per 2 x 10^6 yeast cells
Total Wild Yeast	<1 per 2 x 10^6 yeast cells

If a liquid yeast propagation does not meet the Quality Control specifications, then the batch is rejected. Table 2 contains data for three batches of the OYR-185 liquid yeast.

Table 2. Data for three batches of OYR-185 liquid yeast

Parameter	Specification Range	4/21/2021	5/22/2021	10/20/2021
Percent Yeast Solids	>3%	4.39	4.12	4.94
Total Viable Cells	>98%	100%	100%	100%
Total Bacteria	<1 per 2 x 10^6 yeast cells	n.d.	n.d.	n.d.
Total Wild Yeast	<1 per 2 x 10^6 yeast cells	n.d.	n.d.	n.d.

n.d. not detected

§ 170.235 Part 3, Dietary Exposure

3.1. INTENDED USE OF OYR-185

Omega Yeast Labs, LLC has proposed the use of OYR-185 in brewing to remove the phenolic character of OYL-024 and highlight the fruity esters, among other potential desirable flavor metabolites. Belgian character is often desired for these fruity flavors, but the phenolic character is off-putting for certain beer styles. OYR-185 will expand the use of this traditional Belgian strain to beer styles such as juicy IPAs, fruited sours and will provide versatility to strain choices in the development of future brewing trends. OYR-185 is intended to be added at approximately 1 million cells per milliliter of wort per degree plato for brewing wort fermentation, as is standard industrial practice.

3.2. ESTIMATED DIETARY EXPOSURE

3.2.1. History of consumption

S. cerevisiae has been used in food production for thousands of years. Bread and alcoholic beverages fermented by *S. cerevisiae* have been at the heart of many ancient civilizations. The use of yeast in fermentation can be dated as far back as ancient Egypt. Today the industrial food and beverage applications of *S. cerevisiae* range from bread, alcoholic beverages, coffee, and chocolate to name a few. The manufacturing of *S. cerevisiae* is a global industry with millions of tons of industrial *S. cerevisiae* manufactured each year.

Through the process of selection, brewers and industrial yeast manufacturers have obtained natural POF- isolates harboring this same *fdc1-C460T* mutation. The use of POF- brewing strains is widespread and accounts for >90% of commercial beer produced worldwide (Gallone *et al.* 2016).

3.2.2. Estimated consumption

Beer produced with OYR-185 will contain comparable levels of alcohol and flavor metabolites as the host strain, with a reduction in phenolic off flavors to levels comparable to historical examples shown in 3.2.1.

Exposure to the engineered OYR-185 will not differ from the exposure to industrial yeast used in other commercial beer applications. This yeast is flocculant and rapidly declines in viability at the end of fermentation. The settled, non-viable yeast is discarded at the completion of fermentation. The resulting beer contains trace levels of yeast (<1 million cells/ml) and any yeast remaining will have a limited viability and metabolic activity in the packaged beer (Boulton *et al.* 2001).

The following maximum potential exposure estimates based on average potential beer intake calculations for the modified OYR-185 yeast. It is assumed that only men and women over the age of 21 will be exposed to OYR-185 due to the legal drinking age in the United States.

Assumptions for Calculations:

- Craft Brewery maximum of 1 million cells/ml in the beer product.
- Consumption (Guenther et al. 2013, based on NHANES studies, 2009-2010) of beer for people over the age of 21; Weight (McDowell et al. 2008, National Health Statistics Report).

Population	Daily Beer Consumption (ml)	Weight (kg)	Exposure to Yeast (cells/kg bw/day)
Men	426	88.3	4.8×10^6
Women	142	74.7	1.9×10^6

- Using an average weight estimate of 60 kg, the estimated daily exposure to OYR-185 for men and women would be 7.1×10^6 cells and 2.4×10^6 cells, respectively.

This estimated maximum exposure to OYR-185 does not differ to the exposure to *S. cerevisiae* strains commonly used to ferment beer. The single difference between OYL-185 and the OYL-024 host strain is the targeted single base pair substitution, *fdc1-C460T,* which is present in many commercial brewing strains. In addition, commercial brewers will remove yeast by cold conditioning, centrifuging and/or filtering, thereby reducing or eliminating the human exposure to OYR-185.

§ 170.240 Part 4, Self-Limiting Levels of Use

The use of the proposed yeast in brewing is considered to be self-limiting for technological reasons, such as product flavor profile, which could affect consumer acceptance.

§ 170.245 Part 5, Experience Based on Common Use in Food

While there exists an extensive history of the safe consumption of *S. cerevisiae* yeast and yeast products by both humans and animals, and *S. cerevisiae* products have FDA GRAS status, the statutory basis for our conclusion of its GRAS status in the notice is based on scientific procedures and not common use in food.

§ 170.250 Part 6, GRAS Narrative

6.1. SAFETY ASSESSMENT OF THE HOST STRAIN

The host strain, OYL-024 is an industrial brewing strain of *S. cerevisiae*. The origin of OYL-024 traces back to Brasserie d'Achouffe in Belgium where this strain is used to produce over 300,000 barrels sold in 72 countries. OYL-024 is a popular strain used by many US craft breweries for Belgian-style beers.

S. cerevisiae is considered GRAS for use in brewing, baking and winemaking industries (21 CFR §172.896). The genome of *S. cerevisiae* has been extensively studied and it has been determined that the yeast is free of known pathogenicity traits. In addition, several genetically modified S. cerevisiae strains for use in fermented beverages have been granted GRAS status, with no question, by the US FDA (GRN 120, GRN 175, GRN 350, GRN 798 and GRN 841).

In the 27th report of The Scientific Committee for Human Food of the European Community the authors state that *S. cerevisiae* has a safe history of use in food and belongs to a species that is known not to produce toxins. In addition, the Environment Protection Agency (EPA) has included *S. cerevisiae* as a recipient microorganism for exemptions from EPA review and expedited EPA review (40 CFR 725.420). This exemption was made because this species is found to have little adverse effects. They also determined that the introduction of genetic material would not increase the potential for adverse effects, provided that the genetic material is limited in size, well-characterized, free of certain sequences and poorly mobilizable.

According to the European Food Safety Agency (EFSA), yeasts used in food production, particularly baker's/brewer's yeast, are considered among the safest of microorganisms (EFSA, 2007, 2013). *S. cerevisiae* has been designated Qualified Presumption as Safe (QPS) status in Europe, which indicates that no additional safety assessment is needed according to the established guidelines (EFSA, 2007, 2008).

The OYL-024 host strain belongs to the *Saccharomyces cerevisiae* species which has been used for more than 7,000 years by humans in fermented beverages. It can therefore be concluded that species will remain GRAS, even after genetic transformation, as long as the transformation does not introduce new or changed capabilities for harmful effects.

6.2. SAFETY ASSESSMENT OF THE GENETIC MATERIAL USED TO CONSTRUCT THE MODIFIED ORGANISM

6.2.1. Foreign genetic material source and product

No foreign (non-*Saccharomyces*) genetic material is in the OYR-185 yeast strain. The same basis of assessment applied in Section 6.1 would apply to this section.

6.2.2. Native genetic material source and product

Native genetic material source and product: *FDC1* sequences

6.2.2.1 The *fdc1-C460T* repair template

Source: *Saccharomyces cerevisiae*

A naturally occurring inactivating mutation *fdc1-C460T*, is a common variant found in industrial brewing strains. Duplex oligos that contain this single base substitution were synthesized as described in Section 2.2.2.

Product: The C to T mutation at position 460 of the *FDC1* coding sequence replaces a glutamine residue (CAA) by a stop codon (UAA). This truncated fdc1 protein disrupts the dimerization domain and the catalytic pocket of the protein is not formed anymore.

Therefore, to the best of our knowledge, the naturally occurring variant *fdc1-C460T* introduced into OYR-185 strain encodes a non-functional fdc1 protein.

6.2.3. Construction of the modified organism

6.2.3.1. Vector and repair template

The *FDC1* CRISPR plasmid (Figure 1) was constructed using the gap repair subcloning procedure in *S. cerevisiae*. A parent yeast shuttling vector containing Cas9 regulated by *S. cerevisiae PGK1* promoter and terminator was digested with BsmBI. An *FDC1* sgRNA expression cassette containing the tRNA phe promoter, tRNA phe, *FDC1* sgRNA, and the tSRN52 sequences with homology to the linearized ends of the parent yeast shuttling vector was synthesized. The two linear fragments with overlapping homology were transformed into *S. cerevisiae* and repaired through homology-directed repair pathways.

A double stranded (ds) DNA oligonucleotide was synthesized as the repair template for homology-directed repair of the induced DSB. The sequence of the repair template contains the *fdc1-C460T* base substitution (provided in Figure 2).

6.2.3.2. Transformation of the host strain

The FDC1 CRISPR plasmid and *fdc1-C460T* repair template were the only DNA sequences used in the transformation method.

OYL-024 was transformed with *FDC1* CRISPR plasmid and *fdc1-C460T* repair template and plated onto YPD-G418 media to select for transformed cells. Single colonies were chosen and re-streaked to YPD-G418 media. These transformants were screened with an absorbance based POF assay for POF- isolates. The POF- isolates were sequenced to confirm successful *fdc1-C460T* editing. The resulting OYR-185 was passaged in liquid YPD media, plated to single colonies and replica plated on YPD-G418 plates. Colonies that no longer displayed G418-resistance were re-streaked to YPD and YPD-G418 medias. The lack of G418-resistance confirmed the loss of the FDC1 CRISPR plasmid (Figure 4).

The results show that the *FDC1* CRISPR plasmid containing the G418 marker is not present in OYR-185 and that the CRISPR-Cas9 editing event was specifically targeted to the *FDC1* locus.

6.3. SAFETY ASSESSMENT OF THE MODIFIED ORGANISM

The safety assessment evaluates the exposure to OYR-185, though the presence of OYR-185 in the final beer product is minimal and is further reviewed in section 6.4.2.3.

6.3.1. Characterization of the transformation event

The genome of *S. cerevisiae* contains long terminal repeat sequences known as δ elements. These δ elements are the remnants of Ty1 transposon integration events. The number and location of these δ elements are specific to a strain and have been used to fingerprint and differentiate between strains of *S. cerevisiae*. Using PCR and primers δ2 (5'- GTGGATTTTTATTCCAAC-3'), δ12 (5'-TCAACAATGGAATCCCAAC-3') and δ21 (5'-CATCTTAACACCGTATATGA-3') to amplify these δ sequences, we have verified the genetic relationship between the host strain OYL-024 and OYR-185 (Figure 5).

Loss of the G418 antibiotic resistance marker was confirmed by streaking OYR-185 onto YPD-G418 media and confirming sensitivity. Figure 4 clearly shows that OYR-185 is sensitive to this antibiotic like the parental strain OYL-024.

Colony PCR of the resulting POF- isolates was performed to amplify the region surrounding position 460 in *FDC1* and further sequenced by sanger sequencing. Sequencing results reported in Figure 3 confirm the single C to T substitution at position 460.

6.3.2. Consequence of the genetic modification on the physiology of the OYR-185

6.3.2.1. Overview of the function of the *FDC1* gene

FDC1 is a non-essential gene in *Saccharomyces cerevisiae*, encoding a phenyl acrylic acid decarboxylase. Together, *FDC1* and *PAD1* decarboxylate ferulic acid, p-coumaric acid, and cinnamic acid to generate phenolic off-flavors (POF) in alcoholic beverages such as beer and wine. In beer, *FDC1* most notably contributes to the formation of 4-vinyl guaiacol (4-VG) which imposes a very distinct spicy, clove-like flavor. Fdc1 is 503 amino acids long and 56 kDa in weight.

6.3.2.2. Growth and fermentation rates of OYL-024 and OYR-185 during laboratory-scale fermentations

Brewing trials with OYL-024 and OYR-185 showed similar fermentation rates, terminal platos and terminal pH. Propagation trials with OYL-024 and OYR-185 resulted in similar peak cell densities. No growth advantages were observed in OYR-185 relative to the OYL-024 host strain. All data indicates that the only difference between OYL-024 and OYR-185 is the depletion of ferulic acid and production of the phenolic-off flavor (POF).

Therefore it can be concluded that the OYR-185 strain is substantially equivalent to the host strain OYL-024 except for the absence of the phenolic-off flavor (POF) phenotype.

6.3.2.3. Sensory analysis of beer brewed with OYR-185 and OYL-024

Fermentations were performed for sensory analysis of the OYR-185 and OYL-024 host strain. Wort was prepared to a standard pale ale recipe (14°P extract and 1.5 lb/bbl hopping rate). Fermentations were carried out at 23°C for 10 days. The resulting beer was cooled to 4°C, transferred to a secondary container and carbonated. A tetrad analysis was presented to a panel of 13 trained tasters. 11 were able to discern between the OYR-185 and OYL-024 parent strain, indicating a significant difference. Sensory descriptors for the OYR-185 strain included ripe red fruit, bubblegum, apricot and non-phenolic. The OYR-185 was preferred among 10 tasters. Notably, the

major difference between the OYR-185 and OYL-024 host strain was the targeted disruption of the POF phenotype.

6.3.3. Allergenic/Toxigenic potential

The *fdc1-C460T* allele present in the OYR-185 strain resulting from the genetic modification does not code for either toxic or allergenic proteins, nor proteins implicated in the formation of undesirable compounds. The *fdc1-C460T* is found in many industrial yeast strains with no known associated allergen or toxin risks.

These considerations lead us to conclude that allergenic or toxic risks related to the presence of this *fdc1-C460T* allele within the OYR-185 strain are negligible.

6.3.4. Presence of unintended gene products as a result of the transformation event

The *fdc1-C460T* allele encodes for a truncated fdc1 protein. The C to T mutation at position 460 of the FDC1 coding sequence replaces a glutamine residue (CAA) by a stop codon (UAA). This truncated fdc1 protein disrupts the dimerization domain and the catalytic pocket of the protein is not formed anymore. Industrial brewing strains with this naturally occurring *fdc1-C460T* allele would also express this truncated fdc1 protein, however there are no known adverse effects. Therefore, we conclude that to the best of our knowledge, the presence of the truncated fdc1 protein poses no known-associated risk.

6.4 SAFETY ASSESSMENT OF THE PRODUCT DERIVED FROM THE MODIFIED ORGANISM

6.4.1. Changes in brewing procedures as a result of OYR-185 fermentation

The use of the OYR-185 yeast strain leads to no drastic changes in brewing procedures, as outlined in Section 6.3.2.3

6.4.2. Changes in beer composition as a consequence of OYR-185 fermentation

6.4.2.1. Global Characteristics of beer

The OYR-185 fermentation eliminates the formation of 4-VG and the resulting beers lack spicy, clove-like phenolic-off flavors. Residual extract, pH, and additional sensory descriptors remain consistent to the OYL-024 host strain. Thus, OYR-185 fermentation will have limited impact on the global beer composition (reference the cited source for POF+/- in Part 3/Section 3.2.1).

6.4.2.2. Flavor modification

Laboratory and pilot scale brewing trials showed that the use of OYR-185 beer yeast strain does not lead to any so-called phenolic off-flavors. Descriptive characters of the OYR-185 beer did not significantly vary from the naturally occurring POF- control beers (Figure 2).

6.4.2.3. Yeast cells and release of yeast products during brewing

After alcoholic fermentation, the viable yeast population starts to decrease in beer and yeast autolysis occurs. While many of the solid particles of the beer as well as the yeast have settled to the bottom of the fermented by this point, the majority of beers are also clarified by one or more

of three processes: cooling (which leads to accelerated flocculation), centrifugation, and filtration.

Beer clarification is often performed by letting the solid particles of the beer sediment by gravity, followed by elimination of the sediments. This process is initiated by a "cold crash"--a drop in the temperature of the beer to 33-40 degrees Fahrenheit. Clarification depends on the flocculation ability of the yeast strain: a strong flocculator will result in clear beer, a weak flocculator will result in turbid beer. Both the parent strain OYL-024 and OYR-185 efficiently flocculate, thus the majority of the yeast is purified from the resulting finished beer.

Native and heterologous proteins may be liberated if cells are allowed to lyse. However, clarification processes will remove the bulk of the proteins, as well as larger polypeptide fragments. Hence, only hydrolysis products such as smaller polypeptides and amino acids of these proteins will remain in the beer. The cellular content of an autolyzing yeast is rich in nucleases and phosphatases. As a result of yeast autolysis, nucleic material will normally be found in beer as single nucleic bases or small nucleic base chains.

6.4.2.4. Formation of unwanted substances in beer

S. cerevisiae is considered GRAS for use in brewing, baking and winemaking industries (21 CFR §172.896) and the introduced material has been reviewed for known toxin and allergen sequences. Moreover, to the best of our knowledge, the use of the OYR-185 strain in brewing will not lead to the release or the enhancement of undesirable compounds in beer.

6.5. BASIS FOR THE GRAS DETERMINATION

6.5.1. Introduction

The regulatory framework for determining whether a substance can be considered GRAS in accordance with section 201(s) (21 U.S.C. § 321(s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et. Seq.) ("the Act"), is set forth at 21 CFR 170.30, which states:

> General recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.
>
> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data and information.

These criteria are applied in the analysis below to determine whether the use of the modified *S. cerevisiae* yeast ingredient in brewing that is the subject of this GRAS determination is GRAS based on scientific procedures. All data relied upon in this GRAS determination are publicly available and generally known, and therefore meet the "general recognition" standard under the Federal Food, Drug, and Cosmetics Act.

6.5.2. Safety Determination

The proposed Omega Yeast Labs brewing ingredient (from *S. cerevisiae*) that is the subject of the current GRAS determination is proposed to be added at approximately 1 million cells per milliliter of wort per degree Plato for brewing wort fermentation, as is standard industrial practice. The proposed yeast ingredient is similar to many other yeast products using *S. cerevisiae* and all have a long history of safe consumption by humans as well as animals. Humans have consumed *S. cerevisiae* through the consumption of its fermentative products, which include bread, beer, and wine.

S. cerevisiae is widely used in industrial fermentation of many food products. It is in the air that we breathe, and it grows naturally on many foods that we eat regularly. It is likely that most humans are exposed daily to foods containing millions of live *S. cerevisiae* through consumption of wine, beer, fruits, salads, and cheese and other dairy foods, as well as other traditional and ethnic fermented foods. This yeast species has been used in food production for over five thousand years. Common industrial applications include baked goods, beer, wine, cider, sake, spirits, coffee and chocolate (Boekhout and Robert, 2014).

Brewer's yeast serves as a model organism for scientific research. Over 30 years of yeast research has contributed to our fundamental understanding of cell and molecular biology. The *S. cerevisiae* genome size is small, is highly homologous with the human genome, genetics are very tractable, and DNA repair pathways are robust allowing for ease of accurate genetic manipulation (Botstein and Fink, 2011). A long history of research and industrial use has indicated that *S. cerevisiae* is safe for use in food and beverage manufacturing (Boekhout and Robert, 2014).

There are over 150 *S. cerevisiae* strains that are commercially sold as brewing yeast. These strains have been isolated from the brewery and farmhouse origins, where the re-pitching or backslopping practices allowed for the propagation and selection of unique brewing characteristics (Gallone *et al.*, 2016). The origin of the host strain, OYL-024, traces back to Brasserie d'Achouffe in Belgium which is used to produce over 300,000 bbls sold in 72 countries. OYL-024 is a popular strain used by many US craft breweries for Belgian-style beers.

The FDA considers *S. cerevisiae* and several derived products safe for consumption. Indeed, FDA has approved dried yeast as an ingredient for food (21 C.F.R. §172.896), and Baker's yeast extract has been affirmed by the FDA as a GRAS flavoring agent and adjuvant (21 C.F.R. §184.1983). The FDA has also approved various yeast-derived products for their use in food. These include Baker's yeast protein (21 C.F.R. §172.325), Yeast-malt sprout extract (21 C.F.R. §172.590) and Baker's yeast glycan (21C.F.R. §172.898). *S. cerevisiae* is also considered Generally Recognized as Safe as noted in several GRAS Notices; GRNs 120, 175, 239, 260, 284, 350, 353.

S. cerevisiae has a long history of being considered non-pathogenic. Regulatory authorities have evaluated the safety of *S. cerevisiae* and consider the organism safe for use in food manufacturing. Several GRAS notifications for modified strains *of S. cerevisiae* used in winemaking have been sent to FDA, and the agency has responded that they had no further questions on the GRAS determinations. In summary, regulatory authorities have reviewed the extensive safety database on *S. cerevisiae* and found no issues of concern with respect to its use in human food or human food production.

6.5.3. General recognition of the safety of a modified *S. cerevisiae* for use in brewing

The intended use of the modified *S. cerevisiae* has been determined to be safe through scientific procedures, as set forth in 21 CFR § 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination, and this determination is based on the following:

- The Omega Yeast Labs modified yeast ingredient (OYR-185 strain) that is the subject of the current GRAS determination is derived from *S. cerevisiae*, also (also known as "bakers" or "brewers" yeast). A review of published literature shows a history of safe use of S. cerevisiae in brewing and baking. *S. cerevisiae* products are approved food additives and considered Generally Recognized as Safe.

- The OYR-185 strain was developed by Omega Yeast Labs to eliminate the production of phenolic off flavor, or 4-vinyl guaiacol. *S. cerevisiae* produces many aroma-active metabolites that contribute to the complexity of a beer's flavor including phenolics, esters, carbonyl compounds, higher alcohols, aldehydes and sulfur compounds. Some of these metabolites, such as phenolics, are unpleasant when present in American hop-forward beer styles. This strain was modified with CRISPR-Cas9 introducing a naturally occurring loss of function mutation in *FDC1* to eliminate phenolic character. This modification results in the insertion of only DNA derived from *S. cerevisiae.*

- The OYR-185 yeast strain is intended for use as a commercial liquid yeast culture for the production of fermented beverages in accordance with Current Good Manufacturing Practices (cGMPs). This liquid yeast strain performs alcoholic fermentation in the same manner as its host strain and other traditional brewing strains. The non-phenolic character of OYR-185 provides a well-suited brewing yeast strain for American craft beers, hoppy beers and other "clean" beer styles.

- The proposed Omega Yeast Labs OYR-185 (from *S. cerevisiae*) is proposed to be added at approximately 1 million cells per milliliter of wort per degree Plato for brewing wort fermentation, as is standard industrial practice.

- Beer produced with OYR-185 will contain comparable levels of alcohol and flavor metabolites as the host strain, with the single difference being the elimination of phenolic off flavor (4-vinyl guaiacol). The engineered OYR-185 will not differ from the exposure to industrial yeast used in other commercial beer applications. This yeast is flocculant and rapidly declines in viability at the end of fermentation. The settled, non-viable yeast is discarded at the completion of fermentation. The resulting beer contains trace levels of yeast (<1 million cells/ml) and any yeast remaining will have a limited viability and metabolic activity in the packaged beer.

- The current US daily intake of yeast in the diet for the US population 2 years and older is as follows: the per-capita average daily intake for yeast from all foods was 1.3 g/day (2.9 g/day, 90th percentile), and the per-user average daily intake was 1.5 g/day (3 g/day, 90th percentile) (FDA, 2021).

- Remaining levels of OYR-185 will be limited and will not differ to the exposure to *S. cerevisiae* strains commonly used to ferment beer. Commercial brewing processes remove yeast by cold conditioning, centrifuging and/or filtering, thereby reducing or eliminating the human exposure to OYR-185.

- *S. cerevisiae* has a long history of being considered non-toxigenic and non-pathogenic. It is used in many food manufacturing processes, including bread making, beer brewing, and grape fermentation for wine. The FDA, EPA, and EFSA have evaluated the safety of *S. cerevisiae* and consider the organism safe for use in food manufacturing. Several GRAS notifications for modified strains of *S. cerevisiae* used in winemaking have been sent to FDA, and the agency has responded that they had no further questions on the GRAS determinations.

- The body of publicly available scientific literature on the consumption and safety of *S. cerevisiae* is sufficient to support the safety and GRAS determination of the proposed yeast ingredient. Because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

Determination of the safety and GRAS status of OYR-185 that is the subject of this evaluation has been made by Omega Yeast Labs. Omega Yeast Labs has critically reviewed and evaluated the publicly available information summarized in this document and has concluded that the yeast ingredient, produced in a manner consistent with cGMP and meeting the specifications described herein, is safe under its intended conditions of use.

Omega Yeast Labs further concludes that use of OYR-185 in brewing described herein is GRAS based on scientific procedures, and that other experts qualified to assess the safety of food and food ingredients for human consumption would concur with these conclusions.

It is also Omega Yeast Labs' opinion that other qualified scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Omega Yeast Labs has concluded that OYR-185 is GRAS under the intended conditions of use on the basis of scientific procedures; and therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

Omega Yeast Labs is not aware of any information that would be inconsistent with a finding that the proposed use of the yeast ingredient in brewing, meeting appropriate specifications, and used according to cGMP, is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

§ 170.250 Part 7, Supporting Data and Information

The following references are all generally available, unless otherwise noted. Appendices 1 and 2 (*fdc1-C460T* CRISPR-Cas9 plasmid sequence and *TDH3-IRC7-CYC1* repair template) are not generally available but are attached for reference.

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APPENDIX 1

FDC1 CRISPR-Cas9 plasmid

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ggtgagtaaggaaagagtgaggaactatcgcatacctgcatttaaagatgccgatttgggcgcgaatcctttattttggcttcaccctcatactattatcagggccagaaa
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APPENDIX 2

fdc1-C460T repair template

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

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

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

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)*

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

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

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person Lance Shaner	Position or Title Owner		
	Organization *(if applicable)* Omega Yeast Labs, LLC			
	Mailing Address *(number and street)* 4720 W Pensacola Ave			
City Chicago	State or Province Illinois	Zip Code/Postal Code 60641	Country United States of America	
Telephone Number (773)657-3438	Fax Number	E-Mail Address lance@omegayeast.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 OYR-185

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

USB flash drive

3. For paper submissions only:

Number of volumes 1

Total number of pages 34

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.

The OYR-185 yeast strain is intended for use as a commercial liquid yeast culture for the production of fermented beverages in accordance with Good Manufacturing Practices (GMPs). This liquid yeast strain performs alcoholic fermentation in the same manner as its host strain and other traditional brewing strains. The elimination of POF+ phenotype in OYR-185 provides a better-suited brewing yeast strain for American craft beers, hoppy beers and other "clean" beer styles. OYR-185 is intended to be added at approximately 1 million cells per milliliter of wort per degree plato for brewing wort 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

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Omega Yeast Labs, LLC
(name of notifier)

has concluded that the intended use(s) of Saccharomyces cerevisiae strain OYR-185
(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. Omega Yeast Labs, LLC 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;
(name of notifier)

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.

4720 W Pensacola Ave., Chicago, IL 60641
(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.

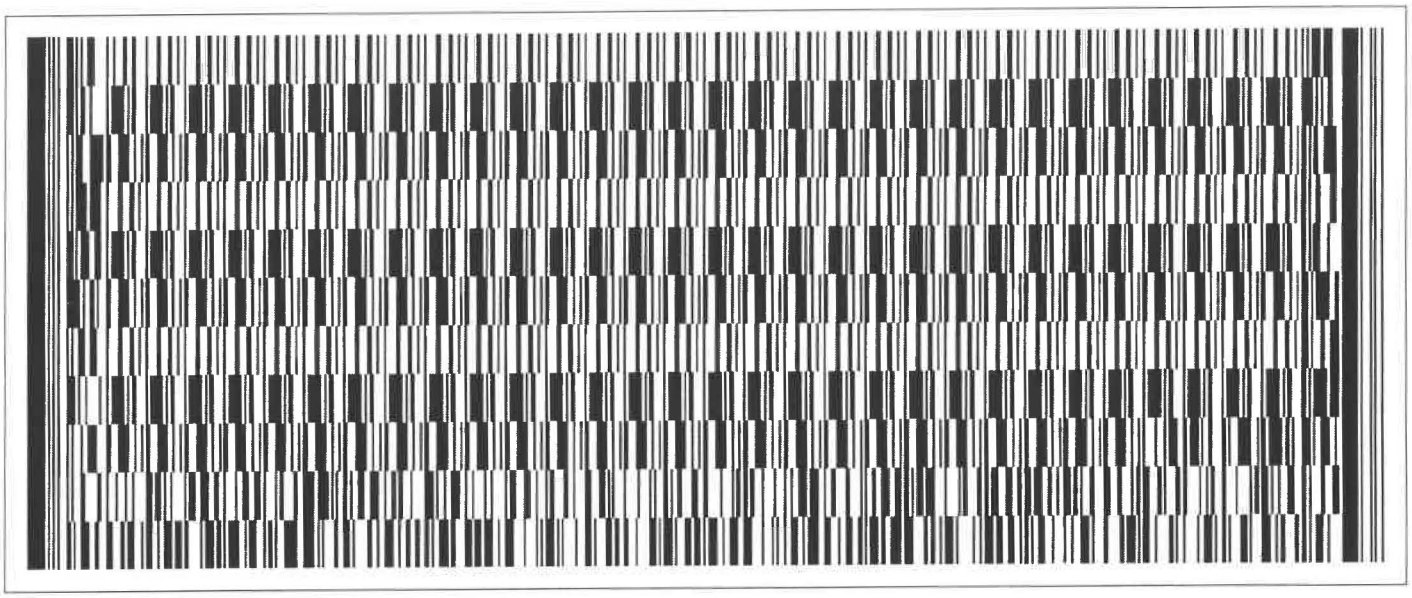
3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Lance Shaner	Lance Shaner, Owner	03/02/2022

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)
1	OYR-185 GRAS Cover Letter.pdf	Submission
2	OYR-185 GRAS.pdf	Submission
3	OYR-185 - FDA 3667 - Signed.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, PRAStaff@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.



Questions/Comments Regarding GRN 001062:

1. In Part 2.6 on page 15, the notifier recommends that brewers use approximately 1 million cells of Saccharomyces cerevisiae "OYR-185" per milliliter (mL) of wort per degree Plato for brewing wort fermentation. Please confirm that "OYR-185" is not intended to be used at levels higher than 1 million cells/mL of wort per degree Plato for brewing wort fermentation.

The recommended use level is set as an intended use for a standard beer fermentation under good manufacturing practices.

The FDA has previously had no questions regarding this recommendation (GRN No 798). Dry yeast substances used in beer and wine fermentations (GRN No 841, GRN No 120) also have recommended use levels that are consistent with the proposed use level. There is a self-limiting use for our product as noted in "Part 4", as brewers would incur significant fermentation performance and quality issues *c*reating a self-limiting use for GMP compliance.

2. In Table 1 on page 16, the notifier provides specifications for "OYR-185". The notifier did not specify the analytical methods employed. Please specify which methods are used to test for the specification parameters and state whether all the analytical methods are validated for their intended uses.

The percent solids, viability, total bacteria and wild yeast protocols are provided by the American Society of Brewing Chemists (ASBC) Methods of Analysis.

Percent Solids by (Yeast 5 ASBC), Viable Count and Yeast Viability by (Yeast 3 ASBC) and (Yeast 4 ASBC) and Total Bacteria and Wild Yeast by (Microbiological Control 2. Detection of Microorganisms ASBC) and (Microbiological Control 5. Differential Culture Media ASBC).

3. In Part 2.5.1 on page 14, the notifier states, "We do not anticipate dangerous levels of heavy metals."

- **Please specify the heavy metals that the statement above refers to.**
- **Please clarify whether the heavy metal levels are monitored, specify the acceptance criteria (i.e., a limit for each heavy metal), the analytical method that is used, and state whether that method is validated for the intended use.**
- **We request that you include a limit for lead in the specifications for "OYR-185" and provide analytical results from a minimum of three non-consecutive batches to demonstrate that the ingredient can be manufactured to meet this specification limit. Please note that the specification limit for lead should reflect the results of the batch analyses and be as low as possible. In addition, please specify the analytical method that is used to test for lead and state whether the method is validated for the intended use.**

Heavy metals are potential risks identified in agriculture ingredients, as well as being present in certain water systems. Omega Yeast Labs controls these risks through cGMPs and other pre-requisite programs, requiring suppliers for agricultural ingredients to be verified by Omega Yeast and each lot of agricultural ingredient is accompanied by a Certificate of Analysis to be within food grade standards for heavy metals, herein defined as < 1.0 ppm lead, < 0.5 ppm arsenic, < 0.03 ppm cadmium, and < 10 ppm (as Pb) total heavy metals. Process water used in the facility is supplied by the municipality of Chicago, and monitored by our facility through collecting annual water quality reports from the city as well as submitting a process water sample to an external laboratory for yearly analysis for total metals (lead with a limit of < 1.1 ppb and mercury with a limit of <0.22 ppb)

Thereby controlling the raw material and water inputs through cGMPs, heavy metals are not introduced during the manufacturing of OYR-185.

Note that the FDA previously accepted this response with no comments within GRN No. 798 (Question for Notifier #5).

Three non-consecutive batches of OYR-185 are scheduled for production and can be sent for heavy metal analysis. These will be available in the coming weeks if necessary.

4. In Part 3.2.2 on page 17, the notifier states that the dietary exposure to "OYR-185" will not differ from the exposure to industrial yeast used in commercial beer applications. Please confirm that the use of "OYR-185" will be substitutional for the use of other S. cerevisiae strains currently used in commercial beer brewing.

Omega Yeast confirms that the use of "OYR-185" will be substitutional for the use of other S. cerevisiae strains currently used in commercial beer brewing.

5. In Part 3.2.2 on page 17, the notifier provides estimates of dietary exposure to "OYR185" for men and women aged 21 years and older, based on the consumption of beer and assuming that 1 mL of finished beer will contain 1 million cells of "OYR-185".

- **Please discuss the relevance of the dietary exposure estimates in the context of the notifier's assertion that "OYR-185" would be removed during the commercial brewing processes and that "OYR-185" would be present in the finished beer at trace levels similar to the levels of other commercial S. cerevisiae strains used in beer brewing.**

  The dietary exposure estimates for 1 million cells/ml represents the trace levels of commercial S. cerevisiae in finished beer. The use levels stated are 7-15 times this amount, and the natural process of settling/flocculation results in levels <1 million cells/ml of S. cerevisiae in the finished beer. This estimate is consistent with the dietary exposure estimate provided in GRN No. 841.

- **For the record, please clarify the population considered in the dietary exposure assessment (eaters only or eaters and non-eaters) and whether the provided estimates represent dietary exposures at the mean or at the 90th percentile**

  The population considered in the dietary exposure assessment are both drinkers and non-drinkers. The dietary exposure estimate is at the mean. The NIAAA definition of heavy drinking is more than 4 drinks on any day or more than 14 drinks per week for men, and 3 drinks on any day or more then 7 drinks per week for women. This is consistent with a 90th percentile estimate of dietary exposure as defined in the referenced study. The dietary estimates of exposure for heavy drinking individuals is outlined below. There is no concern of safety with heavy drinking exposure based on the nature of OYR-185 being cisgenic to other brewing yeast.

Heavy Drinking Population	Daily Beer Consumption (ml)	Weight (kg)	Exposure to Yeast (cells/kg bw/day)
Men	1420	88.3	16.1×10^6
Women	1065	74.7	14.3×10^6

Reference:

Guenther PM, Ding EL, Rimm EB. Alcoholic beverage consumption by adults compared to dietary guidelines: results of the National Health and Nutrition Examination Survey, 2009-2010. J Acad Nutr Diet. 2013;113(4):546-550. doi:10.1016/j.jand.2012.12.015

6. In Part 6.5.3 on page 27, the notifier reports dietary exposure estimates to yeast by reference to FDA, 2021. This reference is not listed in Part 7. Please provide the missing reference.

GRAS notice 928, Part 3.

7. For the administrative record, please state whether S. cerevisiae strain "OYR-185" has been deposited in a recognized culture collection and provide the deposit designation. If it has not been deposited, please describe how the strain was taxonomically identified and verified.

It is not customary for brewing strains to be deposited in a recognized culture collection. Culture stocks are maintained in breweries through repitching from batch to batch and at companies that manufacture brewers yeast cultures. The initial source strain (OYL-024) has been confirmed to be S. cerevisiae, and thus the modified OYR-185 is S. cerevisiae. OYL-024 originates from the same source and is supplied by another yeast manufacturer under WLP550.

The whole genome sequence can be found here and confirms the S. cerevisiae taxonomy. https://www.ncbi.nlm.nih.gov/biosample/?term=WLP550

Reference:

Langdon QK, Peris D, Baker EP, et al. Fermentation innovation through complex hybridization of wild and domesticated yeasts. Nat Ecol Evol. 2019;3(11):1576-1586. doi:10.1038/s41559-019-0998-8

Furthermore, we have sent OYR-185 for ITS sequencing to provide confirmation for the S. cerevisiae taxonomy and results will be available by 11/1/2022.

8. For the administrative record, please provide a detailed description (with relevant references, as appropriate) of S. cerevisiae strain "OYR-185" including genotypic (e.g., pathogenicity and toxigenicity) and phenotypic characteristics (e.g., production of antimicrobials, production of secondary metabolites, antimicrobial resistance other than G418 resistance discussed in the notice), and whether these pose a safety concern.

The OYR-185 strain belongs to the S. cerevisiae species which have been used for more than 7,000 years by humans in fermented beverages. It can therefore be concluded that species will remain GRAS, even after genetic transformation, as long as no harmful DNA material is added to it. The modification to the strain is a naturally occurring base pair substitution inactivating the FDC1 gene, and this exact base pair substitution is found in many other S. cerevisiae strains with food and beverage applications. The historic use of S. cerevisiae in food and beverage fulfills criteria for GRAS. In addition, S. cerevisiae (bakers yeast protein/glycan and dried bakers yeast) are approved additives listed in Title 21 of the Code of Federal Regulations Part 172 and 173. The European Food Safety Agency considers bakers/brewer's yeast among the safest of microorganisms. S. cerevisiae also has Qualified Presumption as Safe (QFS) status in Europe.

There is no evidence for genotypic or phenotypic characteristics of OYR-185 that would pose a safety concern.

References:

Gallone B, Steensels J, Prahl T, et al. Domestication and Divergence of Saccharomyces cerevisiae Beer Yeasts. Cell. 2016;166(6):1397-1410.e16. doi:10.1016/j.cell.2016.08.020

Baker' s yeast extract (21 C.F.R. § 184.1983)

Baker' s yeast protein (21 C.F.R. § 172.325)

Dried yeast as an ingredient in food (21 C.F.R. § 172.896);

Baker' s yeast glycan (21 C.F.R. § 172.898)

European Food Safety Authority (EFSA). (2007). Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA - Opinion of the Scientific Committee: Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA - Opinion of the Scientifi. EFSA Journal, 5(12), 587. https://doi.org/10.2903/j .efsa.2007 .587

European Food Safety Authority (EFSA). (2008). The maintenance of the list of QPS microorganisms intentionally added to food or feed - Scientific Opinion of the Panel on Biological Hazards: The maintenance of the list of QPS microorganisms intentionally added to food or feed - Scientific Opinion of the Panel on Biological Hazards. EFSA Journal, 6(12), 923. https://doi.org/10.2903/j .efsa.2008.923.

European Food Safety Agency (EFSA) Panel on Biological Hazards (BIOHAZ). (2013). Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA Journal, 11(1 l). https://doi.org/10.2903/j.efsa.2013.3449

9. On page 10, the notifier discusses the FDC1 plasmid and states, "For propagation and selection in Escherichia coli, the plasmid contains the ColE1 origin of replication and KanR drug resistance marker, respectively." Please state whether the KanR drug resistance marker is present in S. cerevisiae strain "OYR-185". If the marker is not present in S. cerevisiae strain "OYR-185," please describe how it is removed.

The FDC1 plasmid is a shuttling vector with both KanR and G418 drug resistance markers for selection in E. coli and S. cerevisiae. The KanR drug resistance marker was removed during the outgrow. The FDC1 plasmid removal was confirmed with loss of G418 resistance (Figure 5).

10. Please describe how the notifier confirms the insertion point of the FDC1 plasmid and state how many copies are inserted into S. cerevisiae strain "OYR-185."

The FDC1 plasmid is not inserted into the genome of S. cerevisiae strain "OYR-185". The FDC1 plasmid is S. cerevisiae 2 micron plasmid (yeast small circular piece of DNA) that is maintained at a copy number of 20 per cell through G418 selection and is lost through mitotic segregation in the absence of G418 selection. The FDC1 plasmid is no longer in the "OYR-185" strain and was transiently expressed during the strain development and CRISPR/Cas9 editing event.

Reference:

Christianson TW, Sikorski RS, Dante M, Shero JH, Hieter P. Multifunctional yeast high-copy-number shuttle vectors. Gene. 1992;110(1):119-122. doi:10.1016/0378-1119(92)90454-w

11. The specificity of the CRISPR-CAS technique is most likely not 100%. The notifier should confirm that their CRISPR-CAS gene editing system is specific to the intended

target sequence and no inadvertent non-specific cuts in the genome has been made. Any non-specific cuts could potentially have implications for toxicity and allergenicity through the generation of new ORFs.

A bioinformatics tool (https://crispy-pop.glbrc.org/) was used to evaluate the specificity of the sgRNA and exclude any potential off site targets based on 1011 fully sequenced S. cerevisiae genomes. The interdelta PCR method confirmed the absence of any off-target effects that would result in structural alterations to the genome. We chose this method as it has been used in past applications (GRN No. 798) to ensure the specificity of the CRISPR/Cas9 gene editing event in the S. cerevisiae strain yBBS002.

Reference:

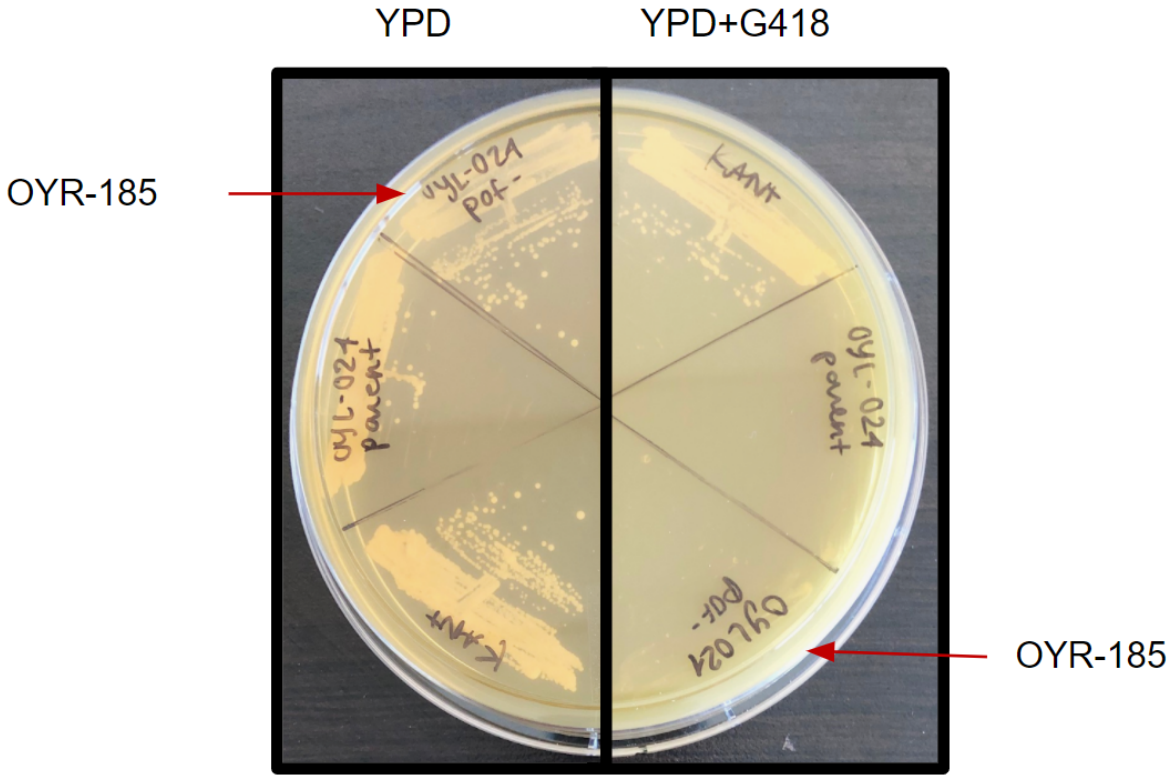
Stoneman HR, Wrobel RL, Place M, et al. CRISpy-Pop: A Web Tool for Designing CRISPR/Cas9-Driven Genetic Modifications in Diverse Populations. G3 (Bethesda). 2020;10(11):4287-4294. Published 2020 Nov 5. doi:10.1534/g3.120.401498

12. On page 12, section 2.4.3.1 discusses the "loss of the Cas9 plasmid." For the administrative record, please describe how the plasmid is lost.

See above response 10.

The FDC1 plasmid (referred here as Cas9 plasmid) is not inserted into the genome of S. cerevisiae strain "OYR-185". The FDC1 plasmid is S. cerevisiae 2 micron plasmid (yeast small circular piece of DNA) that is maintained through G418 selection and is lost in the absence of G418 selection. The FDC1 plasmid is no longer in the "OYR-185" strain and was transiently expressed during the strain development and CRISPR/Cas9 editing event. After the CRISPR/Cas9 editing event, the "OYR-185" strain was grown in non-selective media (outgrow) which results in the spontaneous loss of the plasmid in a percent of the cells. Clonal colonies are then confirmed for loss of G418 resistance to confirm the FDC1 plasmid was lost (Figure 5).

13. For the administrative record, please provide a higher resolution photo of Figure 5 on page 13.



This is the original figure with improved resolution to demonstrate the absence of growth for the OYR-185 strain in the presence of G418 (YPD+G418).

14. For the administrative record, please state whether S. cerevisiae strain "OYR-185" is capable of DNA transfer to other organisms.

The "OYR-185" strain is capable of sexual reproduction and thus the transfer of DNA to other S. cerevisiae strains. The "OYR-185" strain however does not contain any DNA sequence that is foreign to the *S. cerevisiae* gene pool. The fdc1-C460T that resulted from the CRISPR/Cas9 gene editing event is naturally occurring and common among industrial brewing yeast.

15. For the administrative record, please briefly specify how the purity of S. cerevisiae strain "OYR-185" is ensured.

OYR-185 was obtained from a single clonal isolate of the CRISPR/Cas9 gene editing event. The purity of the manufactured OYR-185 strain is ensured through GMPs and sterile technique and validated through microbiological testing procedures (details provided in response 16).

16. On page 14, the notifier states, "Packaged product is >98% viable on ship date, and free from detectable levels of contaminants (zero colony forming units (CFUs) of bacteria or yeast detected per 20 million yeast cells)." Please describe how you distinguish between the final ingredient, S. cerevisiae strain "OYR-185", and yeast contaminants in the packaged product.

Omega Yeast tests all product prior to packaging on LCSM and MRS+ to test for Brettanomyces, non-Saccharomyces yeast, and other “wild yeasts” (yeasts not normally used in brewing), and quadrant streaks on WLN to ensure strain purity and that no cross contamination occurred.

References:

Laura T. Burns, Christine D. Sislak, Nathan L. Gibbon, Nicole R. Saylor, Marete R. Seymour, Lance M. Shaner & Patrick A. Gibney (2021) Improved Functional Assays and Risk Assessment for STA1+ Strains of Saccharomyces cerevisiae, Journal of the American Society of Brewing Chemists, 79:2, 167-180, DOI: 10.1080/03610470.2020.1796175

Deng, Y., Liu, J., Li, H., Li, L., Tu, J., Fang, H., Chen, J. and Qian, F. (2014), An improved plate culture procedure for the rapid detection of beer-spoilage lactic acid bacteria. J. Inst. Brew., 120: 127-132. https://doi.org/10.1002/jib.121

17. On page 15, the notifier states, “OYL-185 is inoculated onto nutrient agar plates…” Additionally, on page 18, the notifier states, “The single difference between OYL-185 and the OYL-024 host strain…” For the administrative record, please provide clarification regarding “OYL-185” in reference to the production strain “OYR-185”.

Both of these instances were meant to refer to OYR-185. We apologize for any confusion that resulted from this OYL-185 labeling error.

18. On page 15, the notifier discusses the raw materials used in the manufacturing process and states that the “ingredients are free of the 8 major food allergens specified under the Food Allergen Labeling and Consumer Protection Act. Under the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act, sesame is also considered a major food allergen, effective January 1, 2023 (https://www.fda.gov/food/cfsan-constituentupdates/faster-act-video-food-industry-and-other-stakeholders). For the administrative record, please state whether sesame or substances derived from sesame are used in the notifier’s manufacturing process and whether this poses a safety concern.

All products sold from Omega Yeast have not been produced with sesame or substances derived from sesame.

19. The notifier provides specifications for “Total Bacteria” and “Total Wild Yeast” on page 16.

- **Please provide the analytical methods used to test for these specifications and state whether these methods have been validated for their intended use.**
- **Please clarify what the specification for “Total Bacteria,” refers to. Common microbial tests for ingredients added to conventional food, such as coliforms, Enterobacteriaceae, or foodborne pathogens, such as Salmonella serovars, are not included in the provided list of specifications. The notifier should discuss why testing for “Total Bacteria” and “Total Wild Yeast” is sufficient for their manufacturing process and intended use.**
- **Please clarify the use of the term “Wild Yeast” in the notice.**

The total bacteria and wild yeast protocols are based on American Society of Brewing Chemists (ASBC) methods of analysis Microbiological Control 2. Detection of Microorganisms and Microbiological Control 5. Differential Culture Media.

“Total Bacteria" and “Wild Yeast” refer to common beer spoilage organisms that can cause quality issues and often are more resilient to fermentation hurdles than certain foodborne pathogens. “Total Bacteria” refers to lactic acid bacteria, acetic acid bacteria, and other wort- and beer-spoiling bacteria, while “Wild Yeast” refers to yeasts not normally used in brewing or spoiling organisms such as Brettanomyces and non-Saccharomyces yeast.

References:

Jeon SH, Kim NH, Shim MB, et al. Microbiological diversity and prevalence of spoilage and pathogenic bacteria in commercial fermented alcoholic beverages (beer, fruit wine, refined rice wine, and yakju). J Food Prot. 2015;78(4):812-818. doi:10.4315/0362-028X.JFP-14-431

Menz G, Vriesekoop F, Zarei M, Zhu B, Aldred P. The growth and survival of food-borne pathogens in sweet and fermenting brewers' wort. Int J Food Microbiol. 2010;140(1):19-25. doi:10.1016/j.ijfoodmicro.2010.02.018

Nancy C. L'Anthoën & W. M. Ingledew (1996) Heat Resistance of Bacteria in Alcohol-Free Beer, Journal of the American Society of Brewing Chemists, 54:1, 32-36, DOI: 10.1094/ASBCJ-54-0032

Suzuki K, Asano S, Iijima K, Kuriyama H, Kitagawa Y. Development of detection medium for hard-to-culture beer-spoilage lactic acid bacteria. J Appl Microbiol. 2008;104(5):1458-1470. doi:10.1111/j.1365-2672.2007.03669.x

Lin, Y. (1981), Formulation and testing of cupric sulphate medium for wild yeast detection. Journal of the Institute of Brewing, 87: 151-154. https://doi.org/10.1002/j.2050-0416.1981.tb04005.x

November 29, 2022

Follow-up Questions/Comments Regarding GRN 1062:

1. In response to Question 2 (amendment received on October 27, 2022), you listed the analytical methods used to test for the specification parameters but did not provide the requested information on whether these methods are validated. Please state whether all the analytical methods are validated for their intended uses.

All the analytical methods listed in our October 27, 2022 amendment and this amendment are validated for their intended uses.

2. In Question 3, we requested that you include a limit for lead in the specifications for "OYR-185". In response (amendment received on October 27, 2022), you stated that heavy metals are not introduced during the manufacturing of "OYR-185" and indicated that the results of analyses for heavy metals in "OYR-185" can be made available for our evaluation if necessary. We note that we typically request that a limit for lead be included in the specification for fermentation-derived ingredients. Therefore, we reiterate our request that you include a limit for lead in the specifications for "OYR-185" and provide analytical results from a minimum of three non-consecutive batches to demonstrate that the ingredient can be manufactured to meet this specification limit. Please note that the specification limit for lead should reflect the results of the batch analyses and be as low as possible. In addition, please specify the analytical method used to test for lead and state whether the method is validated for the intended use.

The specification limit for lead in OYR-185 is no greater than 5 ppb. The analytical results from a minimum of three non-consecutive batches of OYR-185 demonstrate that the ingredient can be manufactured to meet this specification limit and is included in the table below.

Data for three non-consecutive batches of OYR-185 liquid yeast

Parameter	Specification Limit	LC208F OYR-185 10/26/2022	LC20AF OYR-185 11/28/2022	LC20B6 OYR-185 12/05/2022
Heavy Metals (Lead, Pb)	5 ppb	<5.00 ppb	<5.00 ppb	<5.00 ppb

The analytical method used to test for lead in OYR-185 has method reference AOAC 2015.01 (modified) and this method is validated for the intended use. This method has a limit of detection (LOD) of 5 ppb and all samples had values below the method LOD and limit of quantification (LOQ).

3. In the October 27, 2022 amendment, you write, "we have sent OYR-185 for ITS sequencing to provide confirmation for the S. cerevisiae taxonomy and results will be available by 11/1/2022." For completeness of the administrative record, please state whether the sequencing results have confirmed that strain "OYR-185" is S. cerevisiae.

The OYR-185 ITS sequencing results confirmed *S. cerevisiae* taxonomy. Briefly, OYR-185 strain was struck to single colonies on YPD-agar plates and was submitted for ITS sequencing with Azenta Life Sciences (https://www.azenta.com/). The ITS sequencing results were queried in NCBI Blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and showed highest percent identity, query coverage and max score to *S. cerevisiae* ITS sequences confirming the OYR-185 *S. cerevisiae* taxonomy.

4. Regarding Question 19 in our October 17, 2022 questions, we asked, "Common microbial tests for ingredients added to conventional food, such as coliforms, Enterobacteriaceae, or foodborne pathogens, such as Salmonella serovars, are not included in the provided list of specifications. The notifier should discuss why testing for "Total Bacteria" and "Total Wild Yeast" is sufficient for their manufacturing process and intended use." In your October 27, 2022 amendment, you did not provide a discussion explaining why testing for "Total Bacteria" and "Total Wild Yeast" is sufficient. For the administrative record, please provide a brief discussion explaining why testing for "Total Bacteria" and "Total Wild Yeast" is sufficient for your manufacturing process and intended use.

Our reported specifications include testing for "Total Bacteria" and "Total Wild Yeast", as defined in our October 27, 2022 amendment, using previously described methods that are validated for their intended uses and represent a culture that is free from bacteria or wild yeast contaminants. Lactic acid bacteria (LAB) have a significantly higher heat-resistance in wort and beer when compared to *E.Coli* O157:H7 and *S. typhimurium*, and gram-negative LAB/acetic acid bacteria (AAB) and wild yeasts are highly adapted for growth in restrictive fermentation environments (pH < 4.2, alcohol content, hop compounds, yeast fermentation metabolites). These quality indicator organisms, "Total Bacteria" and "Total Wild Yeast," represent undesired microbial growth during fermentation-based ingredient manufacturing that is inhibitive to common foodborne pathogens, and are appropriate indicator organisms for microbiological testing for our manufacturing process and intended use.

Additionally, we would like to state a specification limit for Enterobacteriaceae in OYR-185 as no greater than 10 cfu/g. The microbiological results from a minimum of three non-consecutive batches of OYR-185 demonstrate that the ingredient can be manufactured to meet this specification limit and is included in the table below.

Data for three non-consecutive batches of OYR-185 liquid yeast

Parameter	Specification Limit	LC208F OYR-185 10/26/2022	LC20AF OYR-185 11/28/2022	LC20B6 OYR-185 12/05/2022
Enterobacteriaceae	10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g

The analytical method used to test for Enterobacteriaceae in OYR-185 has method reference CMMEF Chapter 9.62 and this method is validated for the intended use.

References

Roberts, T.A. et al. Fermented beverages. Micro-Organisms in Foods 6. Springer, Boston, MA. doi:10.1007/0-387-28801-5_17

Nancy C. L'Anthoën & W. M. Ingledew. Heat Resistance of Bacteria in Alcohol-Free Beer, Journal of the American Society of Brewing Chemists.1996;54(1): 32-36. DOI:10.1094/ASBCJ-54-0032

Jeon SH, Kim NH, Shim MB, et al. Microbiological diversity and prevalence of spoilage and pathogenic bacteria in commercial fermented alcoholic beverages (beer, fruit wine, refined rice wine, and yakju). J Food Prot. 2015;78(4):812-818. doi:10.4315/0362-028X.JFP-14-431

Menz G, Vriesekoop F, Zarei M, Zhu B, Aldred P. The growth and survival of food-borne pathogens in sweet and fermenting brewers' wort. Int J Food Microbiol. 2010;140(1):19-25. doi:10.1016/j.ijfoodmicro.2010.02.018

Suzuki K, Asano S, Iijima K, Kuriyama H, Kitagawa Y. Development of detection medium for hard-to-culture beer-spoilage lactic acid bacteria. J Appl Microbiol. 2008;104(5):1458-1470. doi:10.1111/j.1365-2672.2007.03669.x