CHR. HANSEN
Improving food & health

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Division of Biotechnology and GRAS Notice Review
Center for Food Safety & Applied Nutrition (HFS-255)
U.S. Food & Drug Administration

Reference: *Pichia kluyveri* DSM 33235

April 29, 2020

Dear Sir or Madam,

In accordance with the Federal Register [81 Fed. Reg. 159 (17 August 2016)] issuance on Generally Recognized as Safe (GRAS) notifications (21 CFR Part 170), Chr. Hansen is pleased to submit a notice that we have concluded, through scientific procedures that *Pichia kluyveri* (*P. kluyveri*) DSM 33235 is generally recognized as safe and is not subject to the pre-market approval requirements used to enhance flavor in fermentation of beverages including but not limited to brewing of alcohol free beer and low alcohol beer. The recommendation is to inoculate the pure culture at a level of 0.1 g/L in fermentation. Prior to bottling, alcohol free and low alcohol beers undergo pasteurization or filtration prior to bottling, which removes most if not all *P. kluyveri*.. Though *P. kluyveri* is safe to consume, *P. kluyveri* would only be present in negligible levels, if at all, in the finished food product due to pasteurization or filtration.

If there are any questions or concerns, please contact us.

Yours sincerely,

Arie Carpenter

Senior Regulatory Affairs Specialist
usarbr@chr-hansen.com
414-777-7526

CHR. HANSEN, INC.

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Abbreviations

DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EFSA: European Food Safety Authority
FDA: Food and Drug Administration
CFR: Code of Federal Regulations
NCBI: National Center for Bioinformatics
GRAS: Generally Recognized As Safe
ISO: International Standardization Organization
LAB: Lactic Acid Bacteria
P. kluyveri: Pichia kluyveri
MIC: Minimum Inhibitory Concentration
BA: Biogenic Amine
AA: amino acid
Spp: species
GMP: Good manufacturing practice
PRP: Prerequisite program
OPRP: Operational Prerequisite Program

CCP: Critical control point
FALCPA: US Food Allergen Labeling and Consumer Protection Act of 2004
NHANES: National Health and Nutrition Examination Survey
OIV: International Organization of Vine and Wine
VFDB: Virulence Factor Database
EUCAST: European Committee on Antimicrobial Susceptibility Testing
ECOFF: Epidemiological Cutoff
CLSI: Clinical and Laboratory Standards Institute
FALCPA: Food Allergen Labeling And Consumer Protection Act
CFU: Colony Forming Units

1 Signed statements and certification

1.1 Statement of intent

In accordance with the 21 CFR 170 Subpart E, regulations for Generally Recognized as Safe (GRAS) notifications, Chr. Hansen, Inc. is pleased to submit a notice that we have concluded, through scientific procedures, that *Pichia kluyveri* DSM 33235 is GRAS and is not subject to the premarket approval requirements for use in fermentation of beverages including but not limited to brewing of alcohol free beer and low alcohol beer.

1.2 Name and address of notifier

Chr. Hansen, Inc.
9015 W Maple St.
Milwaukee, WI 53214
Tel: (414) 607-5700
Fax: (414) 607-5959

1.3 Common or usual name

Yeast / Fermentation Yeast / *Pichia kluyveri* / *P. kluyveri* / DSM 33235 / Food culture

1.4 Conditions of use

Pichia kluyveri DSM 33235 is intended to be used at an inoculation level of 0.1 g/L (1kg for 10,000 L) as a pure starter culture in fermentation of alcohol-free beer and low alcohol beer characterized by a similar flavor profile of regular beers.

Due to selective fermentation of glucose, low alcohol production and high volatile esters productivity, *P. kluyveri* DSM 33235 exhibits optimal characteristics for the purpose of providing superior flavor in these applications. At the end of beer fermentation, this yeast is removed by filtration or centrifugation and killed by pasteurization, therefore it is not present in viable form in the finished product.

1.5 Basis for GRAS determination

Pursuant to the GRAS rule [81 Fed. Reg. 159 (17 August 2016)], Chr. Hansen has concluded that *P. kluyveri* DSM 33235 is GRAS through scientific procedures, in accordance with 21 CFR 170.30 (a) and (b).

1.6 Premarket approval status

It is the opinion of Chr. Hansen that *P. kluyveri* DSM 33235 pure culture for brewing is not subject to premarket approval requirements of the Federal Food, Drug and Cosmetics Act, based on our conclusion that the notified substance is GRAS under the intended use conditions.

1.7 Availability of information

The data and information that is the basis for Chr. Hansen's conclusion that *P. kluyveri* DSM 33235 is GRAS are available for review and copying by FDA during customary business hours, at the location below, or will be sent to FDA upon request, made to:

Chr. Hansen, Inc.
c/o Arie Carpenter
Senior Regulatory Affairs Specialist
9015 W Maple St., Milwaukee, WI 53214
usarbr@chr-hansen.com

1.8 Freedom of Information Act

It is our opinion that the information contained in this notification is not exempt from disclosure under the Freedom of Information Act.

1.9 Certification

To the best of our knowledge, this GRAS notification is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of *Pichia kluyveri* DSM 33235.

1.10 Signature

Arie Carpenter, Senior Regulatory Affairs Specialist — April 29, 2020 Date

Katharine Urbain, Head of Regulatory Affairs – North America — April 29, 2020 Date

2 Identity, method of manufacture, specifications, and physical or technical effect

2.1 Name of the GRAS organism

The subject of this GRAS determination is the yeast, *Pichia kluyveri,* designated as DSM 33235.

2.1.1 Source and description of the GRAS organism

Pichia kluyveri DSM 33235 was isolated from spontaneous ferment of mature Chardonnay grapes and published by (Goddard, 2008). The strain has been deposited into the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) under the accession number DSM 33235. *P. kluyveri* is placed in the *Pichia* genus (Kurtzman, Robnett, & Basehoar-Powers, 2008), which belongs to the *Pichiaceae* family and the order of *Saccharomycetales* along with other yeast species relevant for food and biotechnology such as *Saccharomyces cerevisiae, Debaryomyces hansenii, Yarrowia lipolytica* and *Komagataella pastoris* (reclassified from *Pichia pastoris*).

Yeasts of the *Pichia* genus can be found in diverse natural habitats ranging from soil and water to a variety of plants and mature or rotten fruits. *Pichia* spp. are frequently isolated from food (Villa-Carvajal, Querol, & Belloch, 2006) (Loureiro & Malfeito-Ferreira, 2003). In the microbiome of some agronomically important fruits, such as grape, *Pichia spp*. are even considered predominant (Sabate, Cano, Esteve-Zarzoso, & Guilllamon, 2002).

As a species, *P. kluyveri* is a wild non-pathogenic yeast. True to the genus, its natural habitat is diverse and varies from the fleshy part of plants, fruit insects such as *Drosophila* sp., fruits and vegetables. Literature has shown a diversity of fruits and vegetables in which *Pichia kluyveri* can be found. *Pichia kluyveri* naturally occurs in produce such as grapes, persimmon, apple, cocoa beans, coffee beans, soy, olives, tomato and cactus necrotic tissues (Vaudano, et al., 2019) (Bozoudi & Tsaltas, 2016) (Kim, Lee, Jeon, & Park, 2019) (Wei, Zhang, Yuan, Dai, & Yue, 2019) (Holt, Mukherjee, Lievens, Verstrepen, & Thevelein, 2018) (Gross, Kunz, Muller, Santos Kron, & Freimoser, 2018) (Broissin-Vargas, Snell-Castro, Godon, Gonzalez-Rios, & Suarez-Quiroz, 2018) (Vadkertiova, Molnarova, Vranova, & Slavikova, 2012) (Hamby, Hernandez, Boundy-Mills, & Zalom, 2012)

Pichia is characterized by multilateral budding on a narrow base, presence or absence of true hyphae but pseudohyphae may occur, ascospores may be hat-shaped, hemispheroidal, or spherical with or without a ledge, sugars may be fermented and nitrate is not utilized as a source of nitrogen (Kurtzman C. P., 2011) (Kurtzman, Fell, & Boekhout, The Yeasts : a taxininuc study, 2011) (Villa-Carvajal, Querol, & Belloch, 2006). *Pichia kluyveri* Bedford ex Kudryavtsev (1960) – having the synonyms *Pichia kluyveri* Bedford (1942), *Hansenula kluyveri* Bedford ex Kudryavtsev (1960), *Pichia belgica* Dekker (1941) and *Zygosaccharomyces bisporus* Anderson (1917) - is an aerobic teleomorph yeast producing two to four hat-shaped spores in each ascus, able to ferment only glucose and produce pectin degrading enzymes (Kurtzman, Fell, & Boekhout, The Yeasts : a taxininuc study, 2011) (Pichia kluyveri, 2019).

Classification of the organism *Pichia kluyveri* is as follows:

- Kingdom: Fungi
- Phylum: Ascomycota
- Subphylum: Saccharomycotina
- Class: Saccharomycetes
- Order: Saccharomycetales
- Family: Pichiaceae
- Genus: *Pichia*
- Species: *kluyveri*

2.1.2 GM status

Pichia kluyveri DSM 33235 is not genetically modified by use of recombinant DNA techniques (APPENDIX 1: PRODUCT INFORMATION SHEET 715390).

2.1.3 Species identification

Pichia kluyveri DSM 33235 was species identified at an external laboratory according to the current standard for yeast identification using ITS5/4 and D1/D2 primers as described by Vu *et al*. (2016). The species *Pichia kluyveri* was first described by Bedford (1942) but it has been modified since, thus the correct reference for *Pichia kluyveri* is Kudryavtsev (1960). The *P. kluyveri* type strain is the CBS 188 strain.

2.1.4 Genome sequencing and functional gene annotation

To obtain a high-quality genome sequence of *P. kluyveri* DSM 33235, the strain was genome sequenced using the Illumine MiSeq Technology and Oxford Nanopore Technology. Output from the MiSeq sequencing (4,909,396 raw reads and coverage 78x) was assessed and trimmed as previously described Agersø *et al.* (2018) and combined with the ONT reads (26,619 raw reads and coverage 37x) to obtain high quality hybrid assembly. The hybrid assembly from both sequencing technologies resulted in 92 contigs with a total assembly size of 13.6 Mbp with a GC content of 28.4%.

The genome sequence of the *P. kluyveri* DSM 33235 was subjected to gene finding and functional annotation using the state-of-the-art program GeneMark-ES in 'fungi'-mode and predicted proteins were annotated the using a Swiss-Prot database. GeneMark-ES predicted 6,474 proteins of which 93% were functional annotated.

The genome size, GC content and number of proteins in the *P. kluyveri* DSM 33235 were comparable to the genome sequence of *Pichia kluyveri* CBA6002 (QEFR01.1) published by Kim *et al.* (2019) as well as the genome sequences of other *Pichia* spp. in the NCBI genome database.

2.1.5 Analysis of the *P. kluyveri* DSM 33235 genome

Databases of virulence genes and antimicrobial resistance genes does not exist for yeast strains in similar fashion as several curated databases for virulence and antibiotic resistance genes are published for bacterial strains. In addition to this, acquired resistance is not a concern in yeast as it is in bacteria (Arendrup & Patterson, 2017) (EFSA Panel on Biological (BIOHAZ), 2020).

2.1.5.1 Search of functional gene annotations for words of potential safety concern.

The gene annotations of *P. kluyveri* DSM 33235 were searched to identify terms that could be linked to antifungal resistance. A total of 39 proteins included one or more of these words in its annotation. Many proved to be housekeeping genes or transporters, five genes had functional annotations that could be linked with resistance to fluconazole i.e. 'Negative regulator of PDR1-mediated fluconazole resistance JJJ1', 'Fluconazole resistance protein 1', 'Fluconazole resistance protein 3' or 'Lanosterol 14-alpha demethylase' (CYP51/ERG11). As acquired antifungal resistance is not an issue in yeast strains (Arendrup and Patterson, 2017; EFSA Panel on Biological Harzards (BIOHAZ), 2020) and as homologous proteins were observed in other *Pichia* spp. and other budding yeast, these genes are conserved and therefore not considered a concern. Several of these genes are assumed to play a role in the intrinsic fluconazole resistance in *P. kudriavzevii* (anamorph form *C. krusei*). Both Lamping *et al*. (2009) and Whaley *et al*. (2017) stated that the main resistance mechanism is assumed to be efflux pump activity i.e. reduced drug accumulation in combination with reduced azole affinity at the target protein, Erg11p. Whereas Feng *et al.* (2016) find that it is point mutations and increased expression of Erg11 that lead to azole resistance, however they also state that the mechanism is not fully investigated in *P. kudriavzevii* (Feng, Yang, Wang, Chen, & Xi, 2016). The remaining functional annotations that were linked to 'resistance' were associated with drug, multidrug, oligomycin, vanadate, ethionine, alkylphosphocholines, nitrosoguanidine and homologs were observed in other *Pichia* spp.. These conserved genes are putatively involved in the intrinsic fluconazole resistance observed in the DSM 33235 strain and they were dismissed as safety concerns.

In a similar fashion the gene annotations were searched for terms that could be linked to virulence. This search identified 19 genes of which all were found to be of no safety concern.

Finally, because biogenic amines are produced by decarboxylation of amino acids, the gene annotations were searched for the word 'decarboxylase'. Only one such gene was found, labeled 'ornithine decarboxylase' putatively involved in decarboxylation of ornithine to putrescine. (Rocha & Wilson, 2019) describes that polyamines, such as putrescine, are essential metabolites found in yeasts and the biosynthesis of putrescine to spermidine to spermine are involved in critical roles in the cell. A homolog of ornithine decarboxylase is observed in *Pichia* spp. strains and many different *Saccharomycetales* strains including *S. cerevisiae* supporting the presence in other yeast strains including strains important for food productions.

2.1.5.2 Search for the Candida albicans toxin candidalysin

Candidalysin is regarded as the first and only 'true' virulence factor identified in *C. albicans.* It is a cytolytic peptide toxin secreted by the invasive form of the number one human pathogenic yeast and is critical for mucosal and systemic infections and triggers protective immune responses (Moyes, et al., 2016) (Naglik, Gaffen, & Hube, 2019). Candidalysin is a 31 amino acid peptide produced by cleavage of the Ece1p protein (Moyes, et al., 2016) (Naglik, Gaffen, & Hube, 2019). Blast search of the 31 aa peptide against *P. kluyveri* DSM 33235 strain did not detect any homologous peptides in the DSM 33235 strain.

2.1.5.3 Genome analysis for mycosins in the DSM 33235 genome sequence

A variety of eukaryotic and prokaryotic microorganisms produce antimicrobial substances such as bacteriocins that enable the ability to dominate in a certain environment by antimicrobial activity to closely related microorganisms (Schaffrath, Meinhardt, & Klassen, 2018). Antimicrobial activity or

inhibitory activity of yeast against other yeast is often described to be caused by secondary metabolites known as 'killer toxins' or 'mycocins' (Hatoum, Labrie, & Fliss, 2012). *Pichia* spp. have great potential for antimicrobial activity and screening studies have identified *P. kluyveri* as the most frequently isolated yeast with killer activity (Starmer, Ganter, & Aberdeen, 1992) (Abranches, Morais, Rosa, Mondonca-Hagler, & Hagler, 1997).

Search for killer toxins in the genome of the *P. kluyveri* DSM 33235 strain showed that the strain encodes a putative killer toxin, which has homology to the protein sequence of the killer toxin KT395 and preproteins of other killer toxins. Furthermore, in the DSM 33235 strain is also identified proteins related to β-1,3-glucanase activity, which in some publication referred to killer activity. It is however debated in the literature if lytic enzymes should be referred to as antagonistic toxins (Pretscher, et al., 2018). Pretscher *et al*. (2018) did detect extracellular amylase, cellulase, β-glucosidase and protease activity in two different *P. kluyveri* strains including the 395 strain and the above finding support that DSM 33235 strain might have similar antimicrobial activity.

There are no indications that 'killer toxins' or antimicrobial substances produced by yeast strains including yeasts important for food industry are real toxins and are therefore not harmful to human beings. Thus, they are therefore not of any safety concerns.

2.1.5.4 Search for secondary metabolite biosynthesis pathways in the DSM 33235 strain

To analyze for biosynthesis genes for secondary metabolites, the genome of DSM 33235 and genomes of yeast species relevant for the food industry were analyzed using published methods. The analysis showed that the DSM 33235 strain does not encode any secondary metabolites. The results of the analysis are in line with the general impression that yeasts do not produce secondary metabolites and are of low biosafety concern (Freimoser, Rueda-Mejia, Tilocca, & Migheli, 2019).

2.2 Phenotypic characteristics

2.2.1 Antifungal susceptibility

Minimum inhibitory concentrations (MICs) of 8 antifungals were determined for *P. kluyveri* DSM 33235 according to the EUCAST standards (EUCAST definitive document E.Def 7.3.1) (Table 2-1). In line with the recommendation by EUCAST, the MICs were compared with the cut-off values established for the breakpoint values for *P. kudriavzevii (*the teleomorph version of *C. krusei)* by EUCAST and CLSI as no breakpoints are published for *P. kluyveri.* The range of antifungals tested are in line with the recommendation given by EFSA 'Scientific Opinion on the maintenance of the list of QPS microorganisms intentionally added to food or feed (2013 update)' to test clinically relevant antifungals (EFSA, 2020).

Table 2-1: MIC values for *Pichia kluyveri* DSM 33235.

Antifungal group	Antifungal	MIC value (mg/l)	EUCAST breakpoint *Candida krusei*[a]	EUCAST ECOFF *Candida krusei*[b]	CLSI Breakpoint *Candida krusei*[c]
Azoles	Fluconazole	>64	-[d]	128	-
	Itraconazole	0.5	-	1	-
	Posaconazole	0.125	-	ND	-
	Voriconazole	1	-	1	≥2
Echinocandins	Anidulafungin	≤0.008	0.064	0.064	≥1
	Micafungin	≤0.008	-	0.25	≥1
Polyenes	Amphotericin	0.06	1	1	-
Pyrimidine analogue	Flucytosine	2	-	-	-

a: EUCAST Antifungal Clinical Breakpoint for *Candida krusei (P. kudriavzevii)* in as listed Table v. 9.0 valid from 2018-02-12.
B: EUCAST ECOFF for *Candida krusei (P. kudriavzevii)* given at https://mic.eucast.org/Eucast2/ (Reference date: February 11th, 2020)
c: CLSI breakpoints for resistant *C. krusei (P. kudriavzevii)* as listed in Table 1 'Minimal Inhibitory Concentrations Breakpoints for *In Vitro* Broth Dilution Susceptibility Testing of *Candida* spp. And Selected Antifungal Agents After 24-Hours Incubation' in the CLSI standard M60 'Performance Standards for Antifungal Susceptibility Testing of Yeasts' (2017).
D: no breakpoint or ECOFF provided in M60 or EUCAST.

Pichia kluyveri DSM 33235 is sensitive to most of the antifungal tested with MIC values that are below the CLSI breakpoints and the EUCAST ECOFF values for the *P. kudriavzevii (C. krusei)* (Table 2-1). The strain has low susceptibility to fluconazole and this is considered to be intrinsic resistance as a similar resistance pattern is observed for other *P. kluyveri* strains (Data generated internally at Chr. Hansen and (Xiao, *et al.*, 2018). *Pichia* spp. are reported to be intrinsically resistant to fluconazole in literature as well (Cuenca-Estrella, 2013) (Arendrup & Patterson, 2017).

Regarding resistance in other *Pichia* species, *P. kudriavzevii (C. krusei)* is described as either intrinsically resistant or insensible to fluconazole in several reviews (Cuenca-Estrella, 2013) (Arendrup & Patterson, 2017) (Wiederhold, 2017) and similar is shown for small population of clinical isolates of *Pichia norvegensis* (the teleomorph version of *Candida norvegensis*) isolated nearly 60 years apart (Sandven, Nilsen, Digranes, Tjade, & Lassen, 1997). The observed fluconazole intrinsic resistance in *Pichia* spp. is supported by larger studies such as a study of 6,082 blood sepsis isolates (Pfaller, *et al.,* 2004) and the ARTEMIS DISK study of 256,882 isolates (Pfaller M. D., 2010). Accordingly, the ECOFF set by EUCAST and the absence of breakpoint set by CLSI for *P. kudriavzevii (C. krusei)* are well supported.

2.2.2 Biogenic amine production

The DSM 33235 strain was tested for the production of histamine, tyramine, cadaverine and putrescine using an in-house procedure based on published methods. The strain did not produce any of the four biogenic amine compounds tested when grown in presence of specific amino acid precursors known to induce production of the biogenic amines.

2.3 Method of manufacture

Pichia kluyveri DSM 33235 can be manufactured by Chr. Hansen GmbH Giessener Str. 94, Pohlheim, in accordance with current Good Manufacturing Practices (cGMP) consistent with 21 CFR Parts 110 and 117 and following Chr. Hansen's global protocol for the production of yeasts to be used in fermented

beverages. The production plant complies with a set of basic GMP-rules, also called Pre-Requisite Program (PRP) according to Chr. Hansen's Quality, GMPs and Food Safety Principles, which are available from our website: www.chr-hansen.com. In addition, the production plant has an appointed local OPRP (Operational Pre-Requisite Program) that includes PRP issues and CCPs (Critical Control Points), which are documented and are classified as specifically critical for the safety of food ingredients produced in the plant. The Pohlheim production plant maintains the following certifications: FSSC 22000 and ISO 22000 (APPENDIX 7: FSSC 22000 CERTIFICATE POHLHEIM and APPENDIX 8: ISO 22000 CERTIFICATE POHLHEIM).

Chr. Hansen's *P. kluyveri* DSM 33235 product is sold as a frozen block of liquid, and is produced by inoculating the microorganism into sterilized growth substrate. Aerobic conditions are maintained during the fermentation; pH and temperature are controlled. When the microbiological growth peaks, fermentation is stopped by cooling.

Sterile plastic food grade bags are labeled with product name, item number, batch number, amount, and storage temperature. These labeled bags are filled with liquid containing above 1.0E + 09 CFU/g of *P. kluyveri* and frozen for storage. The process flow including critical control points is shown in APPENDIX 3: HACCP FLOW SHEET GLOBAL.

2.3.1 Raw materials and processing aids

Chr. Hansen's *P. kluyveri* DSM 33235 product is manufactured using standard fermentation techniques. This includes the use of fermentation and standardizing ingredients that are safe and suitable for use in human food. These ingredients have no technical function in the finished food product and are all permitted for this application in addition to meeting the specifications of the Food Chemical Codex.

Chr. Hansen's *P. kluyveri* DSM 33235 product does not contain any allergens in accordance with the list of common allergens in accordance with the US Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA), and by the EU Regulation 1169/2001/EC Annex II, as shown in APPENDIX 5, ALLERGENS IN PLANT. Though there are allergens present in the production facility, there is no risk that those allergens could contaminate *P. kluyveri* production line (APPENDIX 3: HACCP FLOW SHEET).

2.4 Specifications

Purity is controlled as described in Table 2-2 and additionally in APPENDIX 2: PRODUCT SPECIFICATION SHEET 715390. Moreover, absence of chemical contamination is assessed per a global monitoring program along with a vendor management program.

Enumeration of *P. kluyveri* DSM 33235 strain is carried out based on ISO 7954:1987, ISO 6611 | IDF 94, along with OIV-OENO 576B-2017 recommendations. Yeast extract Glucose Chloramphenicol Agar (YGC) plates are inoculated with *P. kluyveri* culture and are incubated for 72 hours at 25°C in aerobic conditions.

The testing for contaminating microorganisms is carried out following standard test methods outlined in OIV monograph of non-Saccharomyces yeasts (Aurand, 2017) and published in the International Oenological Codex. While this international standard is focused on wine, it provides purity standards for non-Saccharomyces yeasts in fermented beverages where such an internationally recognized standard does not exist for beer to date.

Table 2-2: Quality control testing Schedule for *P. kluyveri* DSM 33235.

Microorganism	Specification	Frequency
Coliforms	<100 cfu/g	Every batch
E. coli	Absent in 1 g	Every batch
Lactic Acid Bacteria	<1000 cfu/g	Every batch
Mould	<1000 cfu/g	Every batch
Staphylococci	Absent in 1 g	Every batch
Salmonella	Absent in 25g	Per monitoring program

2.4.1 Genetic stability

Genetic stability of *P. kluyveri* DSM 33235 has been demonstrated by DNA fingerprinting comparing the stock culture in the cell bank and various batch of inoculation material produced since 2014 (Figure 2-1).

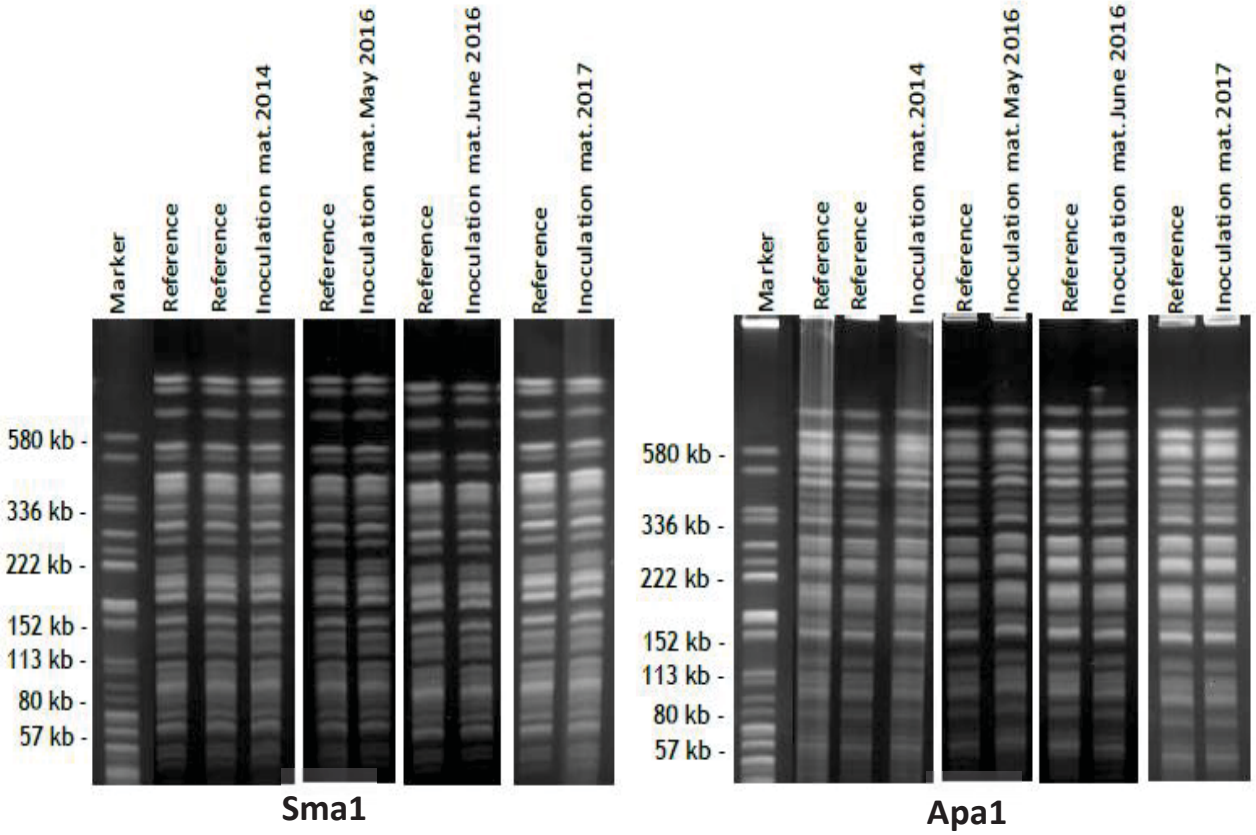

Figure 2-1: Fingerprints Profiles of *P. kluyveri* DSM 33235 Reference Stock and Inoculation Materials.

2.5 Intended technical effect & amount required

In nature, *Pichia kluyveri* can be found primarily in fruits, plants, vegetative fermentation and decay. As an example, *P. kluyveri* is one of the organisms that helps to impart organoleptic characteristics such as fruitiness, sweetness, and cocoa aroma in the sensory profile of chocolate (Barista, Ramos, Ribeiro, Pinheiro, & Schwan, 2015) (Crafack, Mikkelsen, Saerens, Knudsen, Blennow, Lowor, Takrama, Swiegers, Petersen, Heimdal, Nielsen, 2013).

Currently, *P. kluyveri* is a commercialized oenological yeast, and it is widely used in winemaking as non-*Saccharomyces* starter culture known as Frootzen™ (FROOTZEN - first ever Pichia kluyveri yeast, 2019). With this dossier, we would like to demonstrate the safety and versatility of *P. kluyveri* in the production of non-alcoholic and low alcoholic beer applications in order to improve fruity flavors developed during the natural fermentation process.

When used in beer brewing alcohol-free and low alcohol beer, *P. kluyveri* DSM 33235 strain is added at the start of fermentation where it consumes glucose in the wort and produces ester compounds (flavor) together with carbon dioxide. After fermentation the beer is left to mature. Once maturation is complete, alcohol free and low alcohol beer must be filtered or centrifugated prior to pasteurization and bottling. The process of filtration or pasteurization eliminates *P. kluyveri* DSM 33235 culture from the product.

At the time of use, the frozen *P. kluyveri* DSM 33235 culture should be defrosted in lukewarm water (30°C) for 5 – 10 minutes before being directly inoculated into the fermentation tank. The culture containing *P. kluyveri* DSM 33235 has a total cell count above 1.0E + 09 cfu/g and is intended to be inoculated at a concentration of 0.1 g/L (approximately 1.0E + 05 cfu per ml / 1 KG per 10,000L). Frozen *P. kluyveri* DSM 33235 product has a shelf life of 18 months when stored at -50°C.

3 Dietary exposure

P. kluyveri DSM 33235 is either pasteurized or filtered out of the finished low or non-alcoholic beer. Because of this, only a negligible amount of non-viable microorganisms, if any, would be present in the bottled beverages. The dietary intake of *P. kluyveri* yeast cells, therefore, are not expected to increase due to consumption of the final product.

If by chance *P. kluyveri* DSM 33235 is ingested, literature and the conducted strain specific safety assessment on the DSM 33235 shows that it would be safe to consume (Aponte & *et al*., 2010) (Banjara, Suhr, & Hallen-Adams, 2015) (DEAK & BEUCHAT, 1993) (Liang, Zhang, Wu, Liu, & Zhang, 2016) (Ogunremi, Sanni, & Agrawal, 2015). Furthermore, it is well established that the microbiome of an adult is very stable and would only shift in microbial composition due to significant dietary changes or extreme weight loss (Faith, et al., 2013). Therefore, if *P. kluyveri* DSM 33235 strain, as viable yeast, would reach the digestive system of consumers, due to sporadic consumption of fermented beverages, it's presence should be considered transient and is not expected to change the gastrointestinal flora.

4 Self-limiting levels of use

The proposed use of the *P. kluyveri* DSM 33235 strain is as a food ingredient added for fermentation of low alcohol beer and alcohol-free beer with the intent of enhancing flavor and aromatic profiles. The self-limiting levels of use are:

- Current GMP – Following the use level prescribed by Chr. Hansen, the *P. kluyveri* DSM 33235 strain will only be added for fermenting beverages at levels required to achieve the technical effect in the final product. There would be no benefit to the customer to add *P. kluyveri* product at higher levels due to the following:
    - Increased cost to the customer
    - Negative impact on flavor or aromatic profiles

5 Experience based on common use in food

The basis for the GRAS conclusion for the *P. kluyveri* DSM 33235 is based on scientific procedures and not common use in food before 1958.

6 Narrative

6.1 *Pichia kluyveri* is endogenous in fermented plants and as a yeast isolated from food

Yeasts of the *Pichia* genus are widely distributed in diverse natural habitats such as soil, freshwater, tree exudates, plants, mature or rotten fruits, and frequently they are reported as yeasts isolated from food (Villa-Carvajal, Querol, & Belloch, 2006) (Loureiro & Malfeito-Ferreira, 2003). *Pichia* species are considered predominant in the microbiome of some agronomically important fruits such as in grapes (Sabate, Cano, Esteve-Zarzoso, & Guilllamon, 2002). Carbohydrate utilization is species specific and while the genus is heterogeneous, each species within the genus generally assimilates only a few sugars and other carbon compounds (Kurtzman, Fell, & Boekhout, 2011).

Pichia kluyveri is a yeast that finds its natural habitat in the fleshy part of plants, fruit insects (e.g. *Drosophila* sp.), fruits and vegetables such as grapes, persimmon, apple, cocoa beans, coffee beans, soy, olives, tomato and cactus necrotic tissues (Vaudano *et al*. , 2010) (Vaudano, *et al.*, 2019) (Bozoudi & Tsaltas, 2016) (Kurtzman, Fell, & Boekhout, The Yeasts : a taxininuc study, 2011) (Kim, Lee, Jeon, & Park, 2019) (Wei, Zhang, Yuan, Dai, & Yue, 2019) (Holt, Mukherjee, Lievens, Verstrepen, & Thevelein, 2018) (Gross, Kunz, Muller, Santos Kron, & Freimoser, 2018) (Broissin-Vargas, Snell-Castro, Godon, Gonzalez-Rios, & Suarez-Quiroz, 2018) (Vadkertiova, Molnarova, Vranova, & Slavikova, 2012) (Hamby, Hernandez, Boundy-Mills, & Zalom, 2012) (Gross, Kunz, Muller, Santos Kron, & Freimoser, 2018) (Broissin-Vargas, Snell-Castro, Godon, Gonzalez-Rios, & Suarez-Quiroz, 2018)(Kurtzman and Fell, 1998) (Kurtzman & Fell, 1998) (Vadkertiova, Molnarova, Vranova, & Slavikova, 2012) (Hamby, Hernandez, Boundy-Mills, & Zalom, 2012).

Based on the wide array of food products that *P. kluyveri* are associated with, it can be concluded that *P. kluyveri* has a long history of safe human consumption.

6.2 History of safe use of *Pichia* genus

Yeast strains of the *Pichia* genus can be found in a variety of foods that we eat as well as beverages that are consumed regularly. Several *Pichia* spp. occur naturally in the spontaneous microbiome of foods and beverages. Some selected species have also been intentionally added as starter cultures for imparting characteristic flavors and aromatic profiles.

6.2.1 History of safe use in food of *Pichia kluyveri*

Pichia spp. have been isolated, in viable form, in bakery sourdoughs, partially processed vegetables, cheeses, beers, must and wines among others (Banjara, Suhr, & Hallen-Adams, 2015) (Marquina, et al., 1992) (Nuobariene, Arneborg, & Hansen, 2014) (Hammes, et al., 2005) (KING, Magnuson, Torok, & Goodman, 1991) (Pereira-Dias, Potes, Marinho, Malfeito-Ferreira, & Loureiro, 2000) (Jolly, Augustyn, & Pretorius, 2006) (Drumonde-Neves, Franco-Duarte, Lima, Schuller, & Pais, 2017).

Pichia kluyveri has a well-established and important role in both coffee fermentation and chocolate making. *P. kluyveri* is one of the microorganisms responsible for natural fermentations in coffee beans, essential for pulp removal and sensory quality development (Broissin-Vargas, Snell-Castro, Godon, Gonzalez-Rios, & Suarez-Quiroz, 2018). When inoculated in cocoa as the starting culture in combination with other yeasts, *P. kluyveri* was found to become predominant in the microbiota during cocoa bean fermentation, influencing the chocolate flavor profile with more intense fruitiness, sweetness and cocoa aroma in sensory evaluation (Barista, Ramos, Ribeiro, Pinheiro, & Schwan, 2015) (Crafack *et al*., 2013).

In regard to spontaneously fermented vegetables, *P. kluyveri* has been traditionally reported in the representative yeast flora of olive brines. *P. kluyveri* has also been found in homemade *paocai*, a traditional Chinese preparation containing various fermented ingredients such as cabbage, radish, celery, lettuce, pepper and garlic (Aponte & *et al*., 2010) (Villa-Carvajal, Querol, & Belloch, 2006) (Hurtado, Reguant, Esteve-Zarzoso, Bordons, & Rozes, 2008) (Nisiotou, Chorianopoulos, Nychas, & Panagou, 2010)((Liang, Zhang, Wu, Liu, & Zhang, 2016) (Chang & *et al*, 2008). *P. kluyveri* has also been found to naturally occur in the microbiome of kimchi, contributing to the spontaneous fermentation of this traditional Korean food. Documented cases of *P. kluyveri* overgrowth in kimchi were associated with the presence of aesthetically undesirable white colonies, however no health risk has been associated with the overgrowth of this yeast (Kim J. Y., *et al.,* 2019).

6.2.2 History of safe use of *Pichia kluyveri* in fermented beverages

Due to its high environmental stress tolerance (e.g. towards glucose osmolarity, pH variation and ethanol concentration) and superior sensorial contributions (e.g. significant biosynthesis of ester compounds), *P. kluyveri* is appreciated as alternative to *Saccharomyces* yeasts, or in co-fermentation with them, for improving the flavor bouquets of alcoholic beverages (Kim J. Y., 2019) (Contreras, *et al*., 2015) (Jolly, Augustyn, & Pretorius, 2006) (Padilla, Gil, & Manzanares, 2016) (Kim, Hong, & Park, 2008) (Wei, Zhang, Yuan, Dai, & Yue, 2019). In this respect, *P. kluyveri* has a long and documented history of both intentional and unintentional safe use in traditional production of grape wine, coyol palm (*Acrocomia aculeata*) “taberna” wine, mezcal from agave (*Agave salmiana*), new wine “federweisser”, as well as in brewing of spontaneously fermented beers, likely since ancient times. No safety risks have

been ever reported or associated with fermentations involving *P. kluyveri* in beverages (Santiago-Urbina, Arias-Garcia, & Ruiz-Teran, 2015) (Diaz, Molina, Nahring, & Fischer, 2013).

6.2.2.1 Pichia kluyveri in wine

Vinification or wine making was traditionally based on spontaneous fermentations due to the action of various microorganisms either present on the grape surface or in the winery ecosystem. Alcoholic fermentation is the most important biochemical process that is responsible for converting grape must into wine. Yeasts are the driving force in transforming musts into wine, especially yeasts belonging to the *S. cerevisiae* species.

In traditional winemaking, several non-*Saccharomyces* yeast species intervene in the early phases of the fermentation contributing unique properties to the final flavor. One of the most abundant non-*Saccharomyces* oenological yeasts is *P. kluyveri*, as demonstrated by its ubiquitous and consistent detections in wild fermentations of traditional wines (Pardo, Garcia, Zuniga, & Uruburu, 1989) (Lopandic & *et al*., 2008) (Diaz, Molina, Nahring, & Fischer, 2013). A comprehensive literature review documenting *P. kluyveri* as an indigenous yeast of the natural winery biota is shown in Table 6-1.

In modern winemaking, must is inoculated with selected commercial *Saccharomyces* yeasts to control the microbiological process and prevent undesired spoilage. Recently, however, there has been an increasing interest in the industrial application of non-*Saccharomyces* species that are normally isolated from the wine ecosystem, such as *P. kluyveri*.

In support of this interest, scientific studies investigated the influences on sensorial and organoleptic properties due to the activities of these alternative yeasts isolated from the wine microbiota, and noteworthy positive results were reported (Fleet G. , 2003) (Beckner Whitener *et al*., 2016). When intentionally inoculated, *P. kluyveri* was found to improve the aroma of Sauvignon Blanc and Riesling, by increasing the volatiles thiols content (e.g. 3-mercaptohexyl acetate) and the peach/apricot character, in co-fermentation with *S. cerevisiae* (Anfang, Brajkovich, & Goddard, 2008) (Benito *et al*., 2015).

P. kluyveri nowadays represents a safe and well-established wine microorganism, especially recommended for enhancing tropical fruit aroma in Riesling, Sauvignon Blanc and Chardonnay wines. Additionally, *P. kluyveri* was developed as commercial non-*Saccharomyces* starter culture (Frootzen™), and it can be used as starter pure inoculum, in co-fermentation or in sequential inoculation for vinification (FROOTZEN - first ever Pichia kluyveri yeast, 2019) (Benito *et al*., 2015) (Jolly, Augustyn, & Pretorius, 2006) (Beckner Whitener *et al*., 2016) (New Zealand government-owned Crown Research Institute, 2014).

Table 6-1: Literature review about *Pichia kluyveri* in the wine microbial ecosystem

Sample of origin	Wine cultivar	Country	Publication
Air vineyard, air cellar, tank surface, grape skin, fermenting must	Pinot Noir, Cornalin, Chardonnay, Gutedel, Resi Ermitage	Switzerland	(Diaz, Molina, Nahring, & Fischer, 2013)
Fermenting must	Zweigelt, Grüner Veltliner	Austria	(Lopandic *et al*., 2008)
Grape skin	Žametovka, Modra frankinja	Slovenia	(Raspor, Milek, Polanc, Smole Mozina, & Cadez, 2006)
Fermenting must	Refošk	Slovenia	(Zagorc *et al*., 2001)
Fermenting must	Red and white wines from not specified grapes among the ones in this list: Lista´n Noir, Negramoll, Tintilla, Vijariego, Lista´n Blanc, Malvası´a, Gual, Verdello, Marmajuelo, Moscatel	Spain	(Gonzalez, Barrio, & Querol, 2006)
Fermenting must	Carinyena, Garnacha	Spain	(Sabate, Cano, Esteve-Zarzoso, & Guilllamon, 2002)
Fermenting must	Tempranillo, Macabeo, Bobal	Spain	(Pardo, Garcia, Zuniga, & Uruburu, 1989)
Fermenting must	Tempranillo	Spain	(Ocon *et al.,* 2010)
Fermenting must	Merlot	France	(Fleet, Lafon-Lafourcade, & Ribereau-Gayon, Evolution of yeasts and lactic acid bacteria during fermentation and storage of bordeaux wines, 1984)
Grape skin	Riesling	Canada	(Chamberlain, Husnik, & Subden, 1997)
Grape skins, fermenting must	Grignolino	Italy	(Vaudano, *et al*., 2019)
Grape skins, fermenting must	Pinot blanc Riesling	Italy	(Guzzon *et al*., 2016)
Fermenting must	Catalanesca	Italy	(Di Maro, Ercolini, & Coppola, 2007)
Grape skin	Trincadeira Preta	Portugal	(Barata, Gonzailez, Malfeito-Gerreira, Querol, & Loureiro, 2008)
Fermenting must	Castelão	Portugal	(Baleiras Couto, Reizinho, & Duarte, 2005)

Grape skin, fermenting must	Blue Frankish, Green Veltliner, Sauvignon blanc	Slovakia	(Nemcova, Breierova, Vadkertiova, & Molnarova, 2015)
Fermenting must	Isabel, Bordeaux, Cabernet Sauvignon	Brazil	(Bezerra-Bussoli, Baffi, Gomes, & Da-Silva, 2013)
Fermenting must	Cabernet Sauvignon	China	(Li, Liu, Xue, & Liu, 2011)
Grape skin, fermenting must	Malbec, Merlot	Argentina	(Lopes, Rodriguez, Sangorrin, Querol, & Caballero, 2007)
Grape skin	Cabernet Sauvignon	Israel	(Zahavi, Droby, Cohen, Weiss, & Ben-Arie, 2002)
Clarified grape juice	Unknown cultivar from the Stellenbosch region	South Africa	(Strauss, Jolly, Lambrechts, & van Rensburg, 2001)
Fermenting must	Veltlin green	Czech Republic	(Suranska, Vranova, Omelkova, & Vadkertiova, 2012)
Grape skin	Unknown from Tokaji region	Hungary	(Sipiczki, 2016)

6.2.2.2 Beer, beer-like and cereal-based beverages

Cereal-based beverages are widespread and include beer, beer-like beverages and several traditional beverages produced in various parts of the world (Chand Bhalla *et al*., 2009). Yeast is a traditional and essential ingredient in the fermentation of these beverages (Center for Food Science and A Nutrion) (BierStDB - Verordnung zur Durchfuhrung des Vorlaufigen Biergesetzes, n.d.) (T. Alcohol and Tobacco Tax and Trade Bureau, n.d.). Spoilage of cereal-based beverages by yeast is not easily defined since the "spoilage" may be perceived as positively contributing to the product aroma and flavor (Loureiro & Querol, 1999). Moreover, after a thorough literature investigation, no food poisoning cases linked with yeast spoilage of cereal based beverages could be found (RASFF portal | Food Safety, 2019) (Boulton & Quain, 2001).

Pichia kluyveri has a documented history of safe use in fermentation of different types of traditional cereal-based beverages and in beer. The presence of this yeast has been documented in certain popular fermented beer-like beverages such as *champús* in Colombia, *chicha* manufactured in Colombia and Ecuador, *kununzaki* and *burukutu* in Nigeria, and *Caxiri* in Brazil (Osorio-Cadavid, Chaves-Lopez, Tofalo, Paparella, & Suzzi, 2008) (Andres Lopez-Arboleda, Ramirez-Castrillon, Adriana Mambuscay-Mena, & Osorio-Cadavid, 2010) (Pilo *et al*., 2018) (Ogunremi, Sanni, & Agrawal, 2015) (Miguel, Collela, de Almeida, Dias, & Schwan, 2015). Likewise, *P. kluyveri* was reported as being present during the fermentation of *tchapalo,* a traditional sorghum beer from Cote d'Ivoire (N'guessan, Brou, Jacques, Casaregola, & Dje, 2011).

Pichia can be found in spontaneously fermented beers that are already present on the United States market. The American coolship ale (ACA) is a type of beer produced by a small number of breweries in the United States and ACA brewing follows practices of the lambic Belgian beer method. In this adaptation of the lambic process by American craft brewers, *Pichia* was found to be part of a yeast community dominated by *Brettanomyces bruxellensis* (Bokulich, Bamforth, & Mills, 2012).

These studies suggest that *P. kluyveri* can be associated with safe fermentations of a varieties of cereals such as corn, sorghum, barley and millet. Moreover, it can be reasonably assumed that *P. kluyveri* can be considered a non-*Saccharomyces* brewing yeast naturally participating in wild cereal-based, beer-like

and beer fermentations that have been safely consumed by humans for a long time in fermented beverages.

6.3 *P. kluyveri* DSM 33235 is a non-pathogenic safe strain

The DSM 33235 strain isolated from spontaneous ferment of mature Chardonnay grapes has been unambiguously species identified to be a *Pichia kluyveri* strain. The Pariza decision tree asks questions concerning an organism's ability to produce harmful substances. Section 6.3 addresses the questions posed in the Pariza decision tree that address pathogenicity.

6.3.1 *P. kluyveri* DSM 33235 does not contain any genes of concern

The genome of *P. kluyveri* DSM 33235 was sequenced and analyzed for genes of concern. The genome did not contain any homologous genes to the one known true virulence factor for yeast, candidalysin, and no functional gene annotations that could be linked with potential safety concerns in regard to 'virulence' were detected.

Furthermore, functional gene annotations were used to search for words connected with potential safety concerns such as "antifungal" or "resistance". As discussed in section 2.2.1, the only hit of potential concern identified might play a role in intrinsic resistance to fluconazole observed in the DSM 33235 strain. When "resistance" was examined, it was associated with drug, oligomycin, vandate, ethionine, aklylphosphochlolines and nitrosoguanidine. Homologues proteins were observed in other *Pichia* species and these were found to be of no safety concern in DSM 33235.

Finally, the genome of DSM 33235 strain was searched genes encoding for antimicrobial substances also referred to as killer toxins in yeast. The *P. kluyveri* DSM 33235 was found to encode genes with putative antimicrobial activity. The genome was also analyzed for genes associated with secondary metabolites and there was no concern of secondary metabolites for the intended uses covered in this dossier.

6.3.2 *P. kluyveri* DSM 33235 is susceptible to most antifungal agents tested

As discussed in Section 2.2.1, *P. kluyveri* DSM 33235 was screened for antifungal resistance against eight common antifungal agents and the strain was sensitive to itraconazole, posaconazole, voriconazole, anidulafungin, micafungin, amphotericin and flucytosine. The *P. kluyveri* DSM 33235 strain was found to be intrinsically resistant to fluconazole, which seem to be a general characteristic of the *Pichia* genus.

6.3.3 *P. kluyveri* DSM 33235 does not produce biogenic amines

As discussed in Section 2.2.2, biogenic amines are produced by decarboxylation of amino acids. Gene annotations were searched for the word "decarboxylase" and only one such gene was found. The gene was annotated "ornithine decarboxylase." A homologous gene is identifiable in many yeast strains widely used in food production such as *S. cerevisiae*. The gene is subsequently not of concern in *P. kluyveri* for use in similar applications.

6.3.4 Literature search on adverse effects of *P. kluyveri*

For thousands of years, yeast strains have been an important and essential ingredient in production of wine, beer, bread and dairy production and, as part of daily life, consumers ingest large amounts of yeast cultures without adverse effects on the health (Fleet G. H., 2007) (Jacques, 2008). Therefore, there is in general a low concern on the biosafety of yeasts based on the facts that yeasts only rarely cause

human infections (Jacques, 2008) (Anoop, Rotaru, Shwed, Tayabali, & Arvanitakis, 2015) (Perez-Torrado & Querol, 2016) (EFSA, 2007) (EFSA , 2009) and are not known as prolific producers of harmful secondary metabolites (Freimoser, Rueda-Mejia, Tilocca, & Migheli, 2019).

As with consumption of any live microorganism there is no such thing as zero risk of infection (Saarela, Matto, & Mattila-Sandholm, 2002) and infections caused by *P. kluyveri* in immuno-compromised patients have been reported in rare occasions (Xiao *et al*., 2018) (Aslani *et al*., 2018). Aslani *et al.* (2018) reported a case of infection with *P. kluyveri* in one patient out of 162 cancer patient included in a study at Mazandaran University Hospital, Sari, Iran, and Xiao *et al.* (2018) one infection caused by a *P. kluyveri* strain among 884 non-candidal infections isolates isolated from invasive fungal infections over a 5-year period from 65 Chinese hospitals. In many other studies or reviews on pathogenic yeasts, *P. kluyveri* strains are not reported to cause human infections or the species is not considered to be pathogenic (Falagas, Roussos, & Vardakas, 2010) (Fernandez-Ruiz & al., 2017) (Guinea, 2014) (Horn & al., 2012) (Seyedmousavi & al., 2018) (Limon, Skalski, & Underhill, 2017) (Yapar, 2014) (Eggimann, Garbino, & Pittet, 2003) (Astvad & al, 2018). The conclusion of the literature study and the performed strain-specific safety assessment of the *P. kluyveri* DSM 33235 strain is that the strain is to be considered safe.

6.4 Conclusion of GRAS status

In summary, the data presented in this document fully supports the conclusion that *Pichia kluyveri* DSM 33235 is GRAS for the production of non-alcoholic beer and low alcoholic beer to add desirable flavors to these products. The basis for this conclusion follows the Pariza *et al.* decision tree.

Pichia kluyveri DSM 33235 has been unambiguously assigned a genus and species, and the genome has been sequenced. The sequenced genome has been analyzed for encoding virulence factors and toxins associated with pathogenicity and was found to be free of such elements. The genome has also been analyzed for genes involved in antimicrobial resistance and found to have conserved genes putatively involved the intrinsic fluconazole resistance observed in the strain. As acquired antifungal resistance is not an issue in yeast strains (Arendrup and Patterson, 2017; EFSA Panel on Biological Harzards (BIOHAZ), 2020), these conserved genes are not considered a safety concern. *P. kluyveri* DSM 33235 was analyzed for the production of antimicrobial substances and found to produce putative genes having antimicrobial activity in line with other *P. kluyveri* strains and yeasts important for the food industry.

Pichia kluyveri DSM 33235 has not been genetically modified and can be found endogenously in many different parts of the plant kingdom. It has a long history of safe use in a variety of foods and has an integral part in the flavor development of those foods and has not been shown to induce any undesirable physiological effects when consumed in food.

Pichia kluyveri DSM 33235 is a nonpathogenic and safe yeast that has been subject to an adequate strain-specific safety assessment mitigating the risk of any negative impact on human health and subsequently, Chr. Hansen has concluded that it is GRAS.

7 List of supporting data and information

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NEER™

Product Information

Version: 3 PI GLOB EN 07-02-2018

Description

NEER™ is a pure culture of *Pichia kluyveri* to be used in non-alcoholic fermented food and beverages: malt or other cereals, fermented based products, fruits, nuts or vegetables juices. The product is delivered as a deeply frozen (-45°C) bag ready for direct inoculation; it does not require re-hydration or acclimatization.

Culture composition:
Pichia kluyveri.

Material No:	715390	Color:	Pale, yellowish brown
Size	1000 U	Format:	F-DVS
Type	Bag(s) in box	Form:	Frozen liquid

Storage

-50 °C / -58 °F

Shelf life

When stored according to recommendation the product has a shelf life of 18 months.

Dosage

It is recommended to use one bag 1000 U in 50000 L / 500 hl / 13200 US gallons

Application

This specific and pure strain of Pichia kluyveri ensures a safe and reliable start to fermentation in wort, fruit or vegetables juices. It gives producers the opportunity to boost fruit flavours, optimising the conversion of soluble derived fruit flavour precursors into volatile flavours, increasing: flavor intensity, spectrum and longevity, sweetness intensity and smoothering the final product.

Product fermented with NEER™ will have simultaneously several of the following improved features:

- Increased fruit flavour intensity
- Larger spectrum of fruit flavours adding complexity
- Increased sweetness perception
- Lower volatile acidity
- Rounder mouth-feel

Directions for use

1. Defrosting step: Open the freezer, take one box of product. Open the box with gloves; remove the cap protection placed on top of the bag and place the bag in a bucket of lukewarm water (30°C) for 5 to 10 minutes. This step will help unstick the frozen block from the plastic bag.

2. Activation: Due to the unique production process the *Pichia kluyveri* yeast cells are already activated for inoculation. No further activation is required.

3. Direct inoculation: Cut the top of the bag containing the frozen liquid yeast from end to end with scissors. Pour the content (frozen block of yeast) into your tank. Note: Never place the product in a -18°C freezer, follow carefully the instruction and move the product out of the -45°C freezer right before inoculation. SO_2 will reduce the culture population. Check sulfites before inoculation, limit SO_2 dosage to the minimum possible and always refer to the maximum level indicated for the product.

Technical Data

Fermentation characteristics

Flavors	Acidic balance	Mouth-feel	Other
Enhance fruit flavors (thiols, terpenes, esters) Very low volatile phenols Very low H_2S No diacetyl production	Low volatile acidity Low acetic acid	Medium production of polysaccharides	Low production of SO_2

Timing for inoculation
Application related - consult Chr. Hansen Application specialist

Physiological data

Parameter	Value(s)	Comment
Temperature*		
Tolerance limits	10-28°C (50-82°F)	
Optimum	15-25°C (59-77°F)	
SO_2 tolerance*	45 ppm	
Alcohol tolerance*	6.0%	
Nitrogen requirements	medium	Check YAN or FAN before inoculation
Ethanol production	From 0 to 5% depending applied conditions	Application related - consult Chr. Hansen Application specialist
Glycerol yield	5-8 g/L	Standard

** note that these inhibitory factors are antagonistic towards each other.*
The individual tolerances are valid only if other conditions are favourable.

Legislation
The product is intended for use in food. Chr. Hansen´s cultures comply with the general requirements on food safety laid down in Regulation 178/2002/EC and with Council Regulation (EC) No 606/2009 of 10 July 2009, as amended.

Food Safety
No guarantee of food safety is implied or inferred should this product be used in applications other than those stated above. Should you wish to use this product in another application, please contact your Chr. Hansen representative for assistance.

Labeling
No labeling required, however please consult local legislation if in doubt.

Trademarks
Product names, names of concepts, logos, brands and other trademarks referred to in this document, whether or not appearing in large print, bold or with the ® or TM symbol are the property of Chr. Hansen A/S or an affiliate thereof or used under license. Trademarks appearing in this document may not be registered in your country, even if they are marked with an ®.

Additional Information

Chr. Hansen's worldwide facilities and the personnel of our Application and Technology Center are at your disposal with assistance and instructions.

Technical support

Chr. Hansen's Application and Product Development Laboratories and personnel are available if you need further information.

GMO Information

In accordance with the legislation in the European Union* NEER™ does not contain GMOs and does not contain GM labeled raw materials**. In accordance with European legislation on labeling of final food products** we can inform that the use of NEER™ does not trigger a GM labeling of the final food product. Chr. Hansen's position on GMO can be found on: www.chr-hansen.com

** Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms with later amendments, and repealing Council Directive 90/220/EEC.*
*** Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed with later amendments.*
Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labeling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms amending Directive 2001/18/EC, and with later amendments.

Allergen Information

List of common allergens in accordance with the US Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) and EU Regulation 1169/2011/EC with later amendments	Present as an ingredient in the product
Cereals containing gluten* and products thereof	No
Crustaceans and products thereof	No
Eggs and products thereof	No
Fish and products thereof	No
Peanuts and products thereof	No
Soybeans and products thereof	No
Milk and products thereof (including lactose)	No
Nuts* and products thereof	No
List of allergens in accordance with EU Regulation 1169/2011/EC only	
Celery and products thereof	No
Mustard and products thereof	No
Sesame seeds and products thereof	No
Lupine and products thereof	No
Mollusks and products thereof	No
Sulphur dioxide and sulphites (added) at concentrations of more than 10 mg/kg or 10 mg/litre expressed as SO_2	No

** Please consult the EU Regulation 1169/2011 Annex II for a legal definition of common allergens, see European Union law at: www.eur-lex.europa.eu*

NEER™

Product Specification

Form:	Frozen DVS
Material No:	715390
Culture Composition:	Pichia kluyveri

Performance	**Specification**
Total cell count cfu/g	>1.0E+09

Purity	**Specification**
Coliforms cfu/g	<100
E.coli	Absent in 1 g
Lactic acid bacteria cfu/g	<1000
Mould cfu/g	<1000
Staphylococci	Absent in 1 g
Salmonella *	Absent in 25 g

* Environmental and statistically based product testing is carried out on an ongoing basis, details can be supplied on request.

References and analytical methods are available upon request

The information contained herein is to our knowledge true and correct and presented in good faith. No guarantee against patent infringement is implied or inferred.

Storage and shelf life: See labels and product packaging

Electronically generated - signature not required

HACCP evaluation of Application: Low alcohol beer

Introduction	Generic HACCP risk evaluations of Application of Cultures & Enzymes is part of our ISO 22000 certification

Table of contents

Responsibility

Who	What
Innovation responsible	Fill in template with all information's regarding all informations
HACCP responsible	Confirm results and make overall conclusion
Corporate Quality Partner	Approve conclusion

Procedure	Yeast fermentation in beer including alcoholic fermentation, aromas and protection

Innovation
HACCP evaluation
Application: Low Alcohol Beer, pasteurized

*To be filled in by Innovation Application responsible_*

Project owner:		Hentie Swiegers / Wine Business Group			
Innovation Application responsible		Kristine Bjerre			
Intended Use		**Yeast including both *Saccharomyces* and non-*Saccharomyces* in low alcohol (< 3 vol%), pasteurized beer**			
Application	Product	Human Health:	Animal Health:		
		Dairy:	Wine:		
		Meat:	Other: X		
	Dosage of Culture in Final Application	Cultures included: *S. cerevisiae, T. delbrueckii, and K. thermotolerans:* All fluid bed dried *P. kluyveri*: Frozen product Intended inoculation level in final application is 0.1 g/L for frozen culture (1 kg for 10,000 L), and 0.1-0.2 g/L for dried cultures 500 g package for 25-50 hl. See appendix for more information on inoculation levels. All within the range: 0.01-1.0 g/l or **max 0.1 %**			
	Is the application already covered by a HACCP evaluation?	**Yes:**		**No: X**	
		Reference to HACCP for generic application		*Prepare HACCP for New application* See below	
	Specifications in this intended Application Pathogens Fill in appropriate microorganisms and specification	**Pathogen**	**According to external specifications**		**Reference**
		Salmonella (/25g)	Absent		
		Listeria monocytogenes (/25g)	Not on specifications		
		S. aureus (cfu/g)	<1		
		Enterobacteriaceae (cfu/g)	<1		
		E. coli (/1g) Only on specs for Prelude, Concerto, and Merit	Absent		
		Moulds (cfu/g)	< 1000		
		B. cereus (cfu/g)	Not on specifications		
		* 5x25 g testet (<5/125 g)			
Specifications in this intended	Other Contaminants CH- routine analysis	**Microorganism**			
		Acetic acid bacteria (cfu/g)	< 10000		
		Lactic acid bacteria (cfu/g)	< 1E+05		

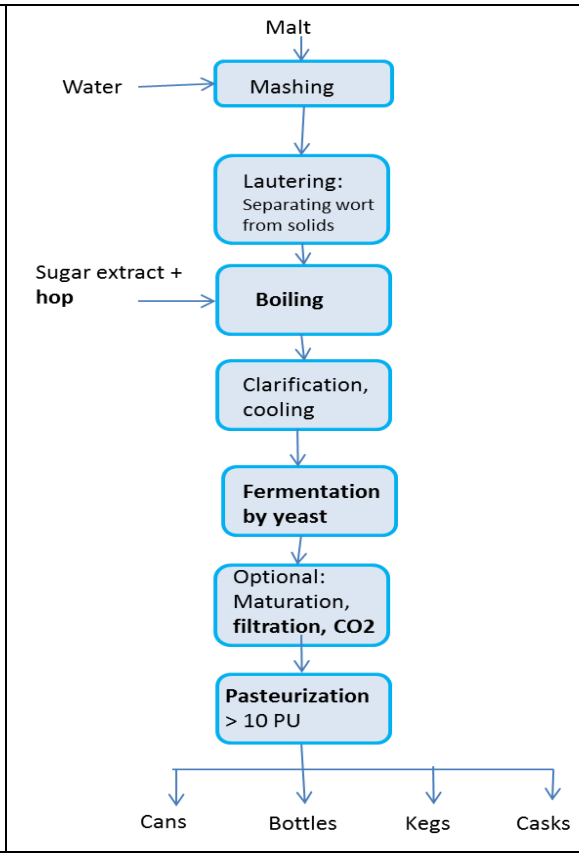
	Fill in appropriate microorganisms and specification			

*To be filled in by Innovation Application responsible_*

Food ready to eat	Hurdle 1: Aw Water activity in Food produced with culture	Aw= 0.98 => No hurdle	**Not c**ritical for Safety**: X**	**Yes c**ritical for Safety**:** *PI –Info*
	Hurdle 2: pH	pH=< 4.5	**Not c**ritical for Safety**:**	**Yes c**ritical for Safety**: X** *PI –Info*
	Hurdle 3. Temperature T ºC	T ºC=25	**Not c**ritical for Safety**: X**	**Yes c**ritical for Safety**:** *PI –Info*
	Hurdle 4: Max Storage time days/weeks/months	days/ weeks/ months	**Not c**ritical for Safety**: X**	**Yes c**ritical for Safety**:** *PI –Info*
	Hurdle 5: please specify	Hop iso-α-acids and Pasteurization	**Not c**ritical for Safety**:**	**Yes c**ritical for Safety**: X** *PI –Info*
	Pathogens relevant in the Food in question, see table encl.	*Cl. botulinum, E coli, Salmonella spp,* and *Yersinia* can be relevant even at pH ≤ 4.5. gram-positive pathogens (*L. monocytogenes* and *St. aureus*) will be limited by the presence of hops (see ref.)		

*To be filled in by Innovation Application responsible_*

Application Process	Hurdles critical for safety, list values during processing	pH ≤ 4.5, Hops ≥ 15 IBU, Pasteurization ≥ 10 PU Boiling Filtration



To be filled in by HACCP responsible

Predictive model	Has Prediction Models been performed	Yes: X *Name: COMBASE PREDICTOR*		No: *If No, please rationale for omitting tests*
	Inoculation	Salmonella absent in 25 g. => Theoretically 4/100 g Maximum inoculation level 1.0 g/l Salmonella : (4E-2)x(1E-3) = 4E-5 = Roughly 1 pr E-4 g final product Max level 1CFU/25g= 4xE-2 Max dosage 0.1% = 1xE-3 E. coli absent in 1 g. => Theoretically 100/100g. Max dosage = 1xE-3. Max level = 1 E. coli: 1x (1E-3) = 1 pr E-3 g final product Clostridium and yersinia not in specifications.		
	Salmonella with	Aw= 0.997	pH= 4.5	Temp= 20°C
		Conclusion: All 4 species tested will potentially be able to reach 1E+08 CFU/g of final product after storage around 1 week. However clostridium will only grow at pH > 4.7.		**Storage time:** 720 hrs = 1 month

		With all other inhibitory factors, where pateurization >10 PU is the most important taken into consideration, there is no actual risk of these pathogens in the final low alcohol beer product.	
		Results, insert graf 	

To be filled in by HACCP responsible

Predictive model Listeria	Has Prediction Models been performed	Yes:		No: X
		Name: COMBASE PREDICTOR		*If No, please rationale for omitting tests*
	Inoculation			
	Listeria monocytogenes (+acetic acid)	Aw=	pH=	Temp= °C
		Conclusion:		**Storage time:**
		Results, insert graf Not relevant since listeria is inhibited by the presence of hops (and pasteurization).		

To be filled in by Innovation responsible

Challenge tests	Has Challenge tests been performed	**Yes:**	**No: X**
			If No, please rationale for omitting tests
	Results of Challenge test	.	
	Reference Challenge test		

To be filled in by HACCP responsible

References	Science supporting prolongation of shelf life beyond estimation in Predictive Model	Prevalence of contaminant in Production	Salmonella and Listeria not detected in 10.000 samples in plants and products.
		Menz, Aldred, and Vriesekoop. 2011. Growth and survival of foodborne pathogens in beer. Jour. of food protection. **74**(10):1670-1675	Presence of even low levels of hop iso-α-acids prohibit growth and limit survival of gram-positive pathogens (*L. monocytogenes* and *S. aureus*)

To be filled in by HACCP team manager Innovation and Corporate Quality Partner

Conclusion	Production of low alcohol and alcohol free beer will because of the low alc. levels and high presence of nutrients involve a risk for growth of pathogens. Therefore pasteurization > 10 PU must always be employed and monitored for production of low alc. beer. Yeast is approved for Low alcohol beer with ph<4,5 and pasteurized with min. 10PU.	
Date/ signature QA Innovation	*27.11.2014*	*Tine Olesen*
Date/signature	*28.11.2014*	*Mirsad Ajanovic*

Updates	**Version**	**Who**	**What**
	1.	KBr,TiO	New

Low Temperature <10 C

Aw	**pH**							
		St.Au	Listeria	Cl.bot	B.cereus	E.coli	Salmonella	Yersinia
0,83-0,92	<4,0							
	4,0-4,9	x						
	5,0-5,9	x						
	>6,0	x						
0,92-0,93	<4,0							
	4,0-4,9	x	x					
	5,0-5,9	x	x					
	>6,0	x	x					
0,93-0,94	<4,0							
	4,0-4,9	x	x	x				
	5,0-5,9	x	x	x	x			
	>6,0	x	x	x	x			
0,94-0,95	<4,0						x	
	4,0-4,9	x	x	x			x	x
	5,0-5,9	x	x	x	x		x	x
	>6,0	x	x	x	x		x	x
>0,95	<4,0						x	
	4,0-4,9	x	x	x		x	x	x
	5,0-5,9	x	x	x	x	x	x	x
	>6,0	x	x	x	x	x	x	x

Records	R: HACCP-Innovation/ Application or Innovation Quality Team-Site

Flow	NA

Appendix

Beer pasteurization

Kirk-Othmer "Food and Feed Technology"

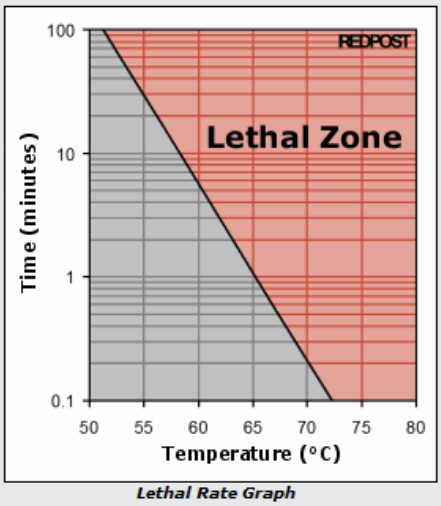
but have made little progress in the United States to the present time.

Most beer in bottles or cans is *pasteurized*. That is, the beer is heated briefly to kill any microorganisms that might be present that could spoil beer flavor. As previously noted, no pathogenic (disease causing) organisms can survive in beer. Because brewers assure that the brewing process is extremely sanitary, few microbes enter the beer, and as a result they use a mild heat treatment. A pasteurizer is a large tunnel through which the beer cans or bottles move on an endless belt. The containers are sprayed with increasingly hot water to raise their temperature to 60–62°C. They are held at this temperature as long as required, and then cooled by water sprays. One pasteurization unit (PU) is 1 min at 60°C (or its heat equivalent) and most beers are pasteurized in the range of 5–15 PUs. There are two alternative techniques to tunnel pasteurization for dealing with the few microbes that might enter beer. The first is "flash" pasteurization in which the beer before packaging flows through a heat exchanger and is rapidly heated up and cooled down. This minimizes heat damage to the beer, but aseptic (sterile or microbe-free) packaging must follow and that is a challenging and expensive technology. Second, bacteria present can be filtered out of the beer by extremely tight membrane filtration. Again, aseptic packaging must follow this, but advantageously the beer can be marketed as "draft" beer in a bottle or can, because the definition of draft beer (in the United States) is that it be unpasteurized. The bottle is now ready for labeling. The packages are loading into six-pack holders, cased, and enter the warehouse from whence the product is distributed to wholesalers and eventually to consumers.

Pasteurisation Units (PUs)

The Pasteurisation Unit (PU) is defined as relating to the sterilising effect observed when the product is held for one minute at a temperature termed the Base value. At this temperature therefore, 1 PU per minute is achieved.

Experiments on various mixtures of the common brewery biological contaminants showed that at temperatures over about 50°C there is an approximately ten-fold increase in sterilising effect for every 7°C increase in temperature. For example, if the time required to kill a population of micro-organisms at 60°C is found to be 5 minutes then if the temperature were to be increased to 67°C the time required would be only 0.5 minutes. The increase in temperature required to produce a ten-fold increase in kill rate is termed the Z value. The Z value in this case is therefore 7°C.



Lethal Rate Graph

The formula for PUs is normally shown like this:

$$PU = t \times 10^{\frac{T-60}{7}}$$

$PU = Pasteurisation\ Units$

$t = Time(minutes)$

$T = Temperature(°C)$

Note this assumes a Base value of 60°C and a Z value of 7°C.

The same definition is often used for soft drinks and other beverages. Where there is any possibility of spore-forming bacterial contamination being present (for example in tomato juice) very much higher temperatures are required for pasteurisation. In these situations a Base value of 80°C and a Z value of 10°C is often used.

External specification for CH yeast:

Pichia (and Concerto) dosage (cfu/ml) is just kept a little low compared to the other dried products in order not to having to apply large volumes to the wine.

	specs CFU/g	batch: CFU/g	Dosage accoring to PI	min CFU/ml
Frootzen	> 1E+09	3008018	1 kg to 100 hl or 0.1 g/l	1,0E+05
Prelude	> 5E+09	1022011: 1.9E+10	500 g to 25-50 hl or 0.1-0.2 g/l	5,0E+05
Concerto	> 1E+09	705079	500 g to 25-50 hl or 0.1-0.2 g/l	1,0E+05
Merit	> 1E+10	1082011	500 g to 25-50 hl or 0.1-0.2 g/l	1,0E+06

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Improving food & health

Viniflora® FrootZen

Product Specification

Form: Liquid
Item no: 703559
Culture Composition: Pichia kluyveri

Performance	Specification
Total cell count cfu/g	>1.0E+09

Purity	Specification
Coliforms cfu/g	<100
Lactic acid bacteria cfu/g	<100000
Mould cfu/g	<1000
Staphylococcus aureus cfu/g	<1
Salmonella spp.	Negative in 25 g

References and analytical methods are available upon request

The information contained herein is to our knowledge true and correct and presented in good faith. No guarantee

Improving food & health

Viniflora® PRELUDE¿

Product Specification

Form:	Fluid Bed Dried Yeast
Item no:	699118
Culture Composition:	Torulaspora delbrueckii

Performance	**Specification**
Total cell count cfu/g	>=5.0E+09

Purity	**Specification**
Acetic acid bacteria cfu/g	<10000
Coliforms cfu/g	<100
Escherichia coli	Negative in 1 g
Lactic acid bacteria cfu/g	<100000
Mould cfu/g	<1000
Staphylococci	Negative in 1 g
Salmonella spp. *	Absent in 25 g

Viniflora® CONCERTO¿

Product Specification

Form:	Fluid Bed Dried Yeast
Item no:	705079
Culture Composition:	Kluyveromyces thermotolerans

Performance	**Specification**
Total cell count cfu/g	>=1.0E+09

Purity	**Specification**
Acetic acid bacteria cfu/g	<10000
Coliforms cfu/g	<100
Escherichia coli	Negative in 1 g
Lactic acid bacteria cfu/g	<100000
Mould cfu/g	<1000
Staphylococci	Negative in 1 g
Salmonella spp. *	Absent in 25 g

* Environmental and statistically based product testing is carried out on an ongoing basis, details can be supplied on request.

Improving food & health

Viniflora® MERIT

Product Specification

Form:	Fluid Bed Dried Yeast
Item no:	673398
Culture Composition:	Saccharomyces cerevisiae

Performance	**Specification**
Total cell count cfu/g	>=1.0E+10

Purity	**Specification**
Acetic acid bacteria cfu/g	<10000
Coliforms cfu/g	<100
Escherichia coli	Negative in 1 g
Lactic acid bacteria cfu/g	<100000
Mould cfu/g	<1000
Staphylococci	Negative in 1 g
Yeasts cfu/g	<100000
Salmonella spp. *	Absent in 25 g

* Environmental and statistically based product testing is carried out on an ongoing basis, details can be supplied on request.

NEER™

GMO statement
Material No: 715390
Version: 1 GMO EN 04-11-2016

GMO statement concerning NEER™

According to the legislation in the European Union genetic modification occurs if certain techniques have been used*. The same legislation also defines techniques, which will not result in genetic modification. In accordance with this legislation we can state that

NEER™ does not contain GMOs and does not contain GM labeled raw materials**.

GM labeling information concerning NEER™

Legislation in the European Union states that a final food product must be labeled if it is a GMO itself, if it contains GMOs, or if it contains ingredients derived from GMOs**. In accordance with European legislation on labeling of final food products we can inform that

The use of NEER™ does not trigger a GM labeling of the final food product.

Chr. Hansen's position on GMO can be found on:
www.chr-hansen.com/About us/Policies and positions/Quality and product safety.

* Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms with later amendments, and repealing Council Directive 90/220/EEC.
** Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed with later amendments.
Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labeling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms amending Directive 2001/18/EC, and with later amendments.

CHR HANSEN
Improving food & health

Allergen Information

Material No: 715390
Version: 1 AL EN 04-11-2016

List of common allergens in accordance with the US Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) and EU Regulation 1169/2011/EC with later amendments.	Present as an ingredient in the product	Ingredient species or type
Cereals containing gluten* and products thereof	No	
Crustaceans and products thereof	No	
Eggs and products thereof	No	Not applicable
Fish and products thereof	No	
Peanuts and products thereof	No	Not applicable
Soybeans and products thereof	No	Not applicable
Milk and products thereof (including lactose)	No	Not applicable
Nuts* and products thereof	No	
List of allergens in accordance with EU Regulation 1169/2011/EC only		
Celery and products thereof	No	Not applicable
Mustard and products thereof	No	Not applicable
Sesame seeds and products thereof	No	Not applicable
Lupine and products thereof	No	Not applicable
Mollusks and products thereof	No	Not applicable
Sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or 10 mg/litre expressed as SO_2	No	

Yes = Allergen labeling required
No = Allergen labeling not required

Please consult the EU Regulation 1169/2011 Annex II for a legal definition of common allergens, see European Union law at: http://eur-lex.europa.eu/

Safety Data Sheet

NEER™

Version: 3 GHS / EN
Revision Date: 04-28-2020

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY/UNDERTAKING

1.1 Product identifier

Product name:	NEER™
Material No:	715390

1.2 Relevant identified uses of the substance or mixture and uses advised against

Application:	For beer or juice application.

1.3 Details of the supplier of the safety data sheet

Supplier:	Chr. Hansen Inc. 9015 West Maple Street 53214-4298 Milwaukee - WI Phone: +1 414 607-5700
Headquarters:	Chr. Hansen A/S Boge Allé 10-12 DK-2970 Horsholm Tel. +45 45 74 74 74

1.4 Emergency telephone number

Emergency telephone:	+45 45 74 74 74

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2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

The product is not classified.

2.2 Label elements

Signal Word None.

Hazard statements None

Precautionary statements None

2.3 Other hazards

Physical and Chemical Hazards:
The hazardous properties of the product are considered to be limited.

Human health:
Risk of local frostbite. Prolonged skin contact may cause redness and irritation.

The product does not contain any carcinogenic substances in amounts to be declared.

Environment:
The harmful effects of the product in the environment are considered to be limited.

---

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.2 Mixtures
The product contains: yeast.

Does not contain substances that must be indicated according to current regulations.

---

4. FIRST-AID MEASURES

4.1 Description of first aid measures

Inhalation: Move into fresh air and keep at rest.

Skin contact: Remove contaminated clothes and rinse skin thoroughly with water.

Eye contact: Do not rub eye. Immediately flush with plenty of water for up to 15 minutes. Remove any contact lenses and open eyelids widely. If irritation persists: Seek medical attention and bring these instructions.

Ingestion: Rinse mouth thoroughly. If uncomfortable: Get medical attention.

4.2 Most important symptoms and effects, both acute and delayed
Risk of local frostbite. Prolonged skin contact may cause redness and irritation.

4.3 Indication of any immediate medical attention and special treatment needed
Symptomatic treatment.

---

5. FIRE-FIGHTING MEASURES

5.1 Extinguishing media
Use fire-extinguishing media appropriate for surrounding materials.

5.2 Special hazards arising from the substance or mixture
No specific precautions.
The explosion limits and the flash point are stated in section 9.

5.3 Advice for firefighters
Selection of respiratory protection for fire fighting: follow the general fire precautions indicated in the workplace.

---

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Avoid contact with skin and eyes. Follow precautions for safe handling described in this safety data sheet.

6.2 Environmental precautions
Avoid discharge into drains, water courses or onto the ground.

6.3 Methods and material for containment and cleaning up
Absorb spillage with suitable absorbent material. Flush contaminated area with plenty of water.

6.4 Reference to other sections
For personal protection, see section 8.
For waste disposal, see section 13.

---

7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Safe handling advice: Avoid contact with skin and eyes. Observe good industrial hygiene practices.

Technical measures: Keep the workplace clean.

Technical precautions: No special precautions.

7.2 Conditions for safe storage, including any incompatibilities
Store in tightly closed original container. Store at super frozen temperature conditions. For detailed information consult the PI sheet.
Store in a dry place.

Technical measures for safe storage: No special precautions.

---

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters
Exposure limits are listed below. No data - no exposure limits noted for ingredient(s).

8.2 Exposure controls
Engineering measures: Provide adequate ventilation.

Respiratory equipment: Not relevant, due to the form of the product.
Risk of inhalation of dust or aerosols use suitable respirator. Use respiratory equipment with particle filter:
EU: FFP3 filter [e.g. 3M 8835 mask]
US: P100 filter [e.g. 3M 8293 mask]
For daily use of more than 3 hours a respirator with a powered air blower should be used.

Hand protection: Wear protective gloves against low temperatures.

Eye protection: Risk of contact: Wear goggles/face shield.

Skin protection: No special precautions.

Hygiene measures: Wash hands after contact.

Environmental Exposure Controls: None.

---

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance:	Frozen liquid
Color:	Pale, yellowish brown
Odor:	Fruity, Peptone-like
pH:	6,00 - 7,00
Melting point:	Not relevant
Boiling point:	Not relevant
Decomposition temperature:	Not relevant
Flash point:	Not relevant
Relative density:	No data available
Solubility:	Water soluble

9.2 Other information
No information available.

---

10. STABILITY AND REACTIVITY

10.1 Reactivity
None known.

10.2 Chemical stability
Stable under normal temperature conditions and recommended use.

10.3 Possibility of hazardous reactions
None known.

10.4 Conditions to avoid
None known.

10.5 Incompatible materials
None known.

10.6 Hazardous decomposition products
None known.

---

11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Inhalation: Not relevant, due to the form of the product.
Inhalation of high concentrations of dust or aerosols may cause toxic alveolitis. Symptoms like fever, coldshivering, coughing, difficulties in breathing, headache, muscle and joint pains etc. may appear 6 to 8 hours after exposure. The symptoms normally disappear completely over night without any treatment.

Skin contact: Risk of local frostbite. Prolonged contact may cause redness and irritation.

Eye contact: Risk of local frostbite.
Ingestion: Risk of local frostbite. May irritate and cause malaise.

Specific effects: Pro-longed exposure to dust/aerosols containing microorganisms may cause allergic alveolitis.
The product does not contain any carcinogenic substances in amounts to be declared.

12. ECOLOGICAL INFORMATION

12.1 Ecotoxicity
The harmful effects of the product in the environment are considered to be limited.

12.2 Persistence and degradability
The product is expected to be biodegradable.

12.3 Bioaccumulative potential
Bioaccumulation: Is not expected to be bio-accumulable.

12.4 Mobility in soil
The product is water soluble and may spread in water systems.

12.6 Other adverse effects
None known.

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods
Dispose of waste and residues in accordance with local authority requirements.

14. TRANSPORT INFORMATION

The product is not covered by international regulations on the transport of dangerous goods (IMDG, IATA, DOT).

14.1 UN number	-

Air (ICAO/IATA):

14.3 Transport hazard class(es)	-
14.4 Packing group	-

Sea (IMDG):

14.3 Transport hazard class(es)	-
14.4 Packing group	-
EmS	-
MFAG	-

Land (DOT):

14.3 Transport hazard class(es)	-
14.4 Packing group	-

14.5 Environmental hazards
Marine pollutant (IMDG): -

14.6 Special precautions for user
None known.

14.7 Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code
Not relevant.

15. REGULATORY INFORMATION

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

NFPA: Health: 1 Fire: 0 Reactivity: 0 Other: -

GHS regulation
Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

---

16. OTHER INFORMATION

The user must be instructed in the proper work procedure and be familiar with the contents of these instructions.

The following sections contain revisions or new statements : 1.

Wording of Hazard Statements
-

BUREAU VERITAS
Certification

Chr. Hansen GmbH

Gießener Straße 94, D35415 Pohlheim, Germany

Bureau Veritas Certification Holding SAS, UK Branch certifies that the food safety management system of the above organization has been assessed and complies with the requirements of:

Standard

FOOD SAFETY SYSTEM CERTIFICATION (FSSC) 22000

Certification scheme for food safety management systems, consisting of the following elements:
ISO 22000:2005, ISO/TS 22002-1:2009 and Additional FSSC 22000 requirements V4.1

This certificate is applicable for the scope of

Production of cultures for the food industry.

Product category: K

Certification cycle start date: 24-04-2018

Subject to the continued satisfactory operation of the organization's Management System, this certificate expires on: 23-04-2021

Original certification date: 23-09-2006

Certificate No./Version: DK009342-2

Contract No. 10435618

Issue date: 20-04-2018

Signed on behalf of BVCH SAS UK Branch

Certification body address: 66 Prescot Street, London E1 8HG, United Kingdom
Local office: Oldenborggade 25-31, DK-7000 Fredericia, Denmark

Further clarifications regarding the scope of this certificate and the applicability of the management system requirements may be obtained by consulting the organization.
To check this certificate validity please contact fooddkmail@dk.bureauveritas.com

FSSC 22000

UKAS MANAGEMENT SYSTEMS

0008

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BUREAU VERITAS
Certification

Chr. Hansen GmbH

Giessener Strasse 94, D-35415 Pohlheim, Germany

Bureau Veritas Certification Holding SAS – UK Branch certifies that the Management System of the above organization has been audited and found to be in accordance with the requirements of the management system standards detailed below.

Standard

ISO 22000:2005

Scope of certification

Research, development, production, applied technology and distribution of biotechnological, functional and natural ingredients and processing aids for the food industry.

Product category: K

Original cycle start date:	**22-09-2006**
Expiry date of previous cycle:	**NA**
Certification/Recertification Audit date:	**NA**
Certification/Recertification cycle start date:	**24-04-2018**

Subject to the continued satisfactory operation of the organization's Management System, this certificate expires on: **23-04-2021**

Certificate No.: DK009343 Version:1 Revision date: **20-04-2018**

[redacted]

MIX Paper from responsible sources FSC® C074440

[redacted]

Certification body address: *5th Floor, 66 Prescot Street, London, E1 8HG, United Kingdom*
Local Office: *Oldenborggade 25-31, 7000 Fredericia, Denmark*

Further clarifications regarding the scope of this certificate and the applicability of the Management System requirements may be obtained by consulting the organization.
To check this certificate validity, please call **(+45) 77 311 000.**

UKAS MANAGEMENT SYSTEMS
0008