JHeimbach LLC

July 28, 2021

Susan J. Carlson, Ph.D., Director
Office of Food Additive Safety (HFS-200),
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Dr., College Park, MD 20740

Dear Dr. Carlson:

Pursuant to 21 CFR Part 170, Subpart E, Danstar Ferment AG (operating as Lallemand), through me as its agent, hereby provides notice of a claim that the use of *Metschnikowia pulcherrima* strain DANMET-A and *Metschnikowia fructicola* strain DANMET B, individually and in combination, as secondary direct additives during the post-harvest processing of coffee, is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Danstar Ferment AG has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

A CD is enclosed containing Form 3667, the GRAS monograph, and the signatures of members of the GRAS panel in a zip directory produced through COSM.

If you have any questions regarding this notification, please feel free to contact me at 202-320-3063 or jh@jheimbach.com.

[redacted]

James Ph.D., F.A.C.N.
President

Encl.

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GRAS DETERMINATION FOR THE USE OF

Metschnikowia pulcherrima Strain DANMET-A and *Metschnikowia fructicola* Strain DANMET B, Individually and in Combination, as Secondary Direct Additives in the Post Harvesting Processing of Coffee

July 2021

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Danstar Ferment AG
Zug, Switzerland

Prepared by
JHeimbach LLC

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Part 1. Signed Statements and Certification

1.1. GRAS Notice Submission

Danstar Ferment AG, Zug, Switzerland, through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration that the use of the two yeast strains *Metschnikowia pulcherrima* strain DANMET-A and *Metschnikowia fructicola* strain DANMET B, individually and in combination, as described below, is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because Danstar Ferment AG, operating as Lallemand, has determined through scientific procedures that this use is generally recognized as safe (GRAS).

[redacted] July 28, 2021

James T. Heimbach, Ph.D., F.A.C.N. Date
President, JHEIMBACH LLC

1.2. Name and Address of the Notifier

Notifier contact :
Danstar Ferment AG (operating as Lallemand)
Francine Vidal – Project Leader Lalcafé
fvidal@lallemand.com
+33 6 14110908

Agent contact
James T. Heimbach, Ph. D.
JHeimbach LLC,
923 Water Street #66
Port Royal VA 22535,
jh@jheimbach.com
+1-804-742-5543

1.3. Names of Notified Organisms

The subjects of this Generally Recognized as Safe (GRAS) notice are the two yeast strains *Metschnikowia pulcherrima* strain DANMET-A and *Metschnikowia fructicola* strain DANMET-B, individually and as a combination of the two strains (designated as NESY2).

The two yeast strains are registered in the National Collection of Yeast Cultures (NCYC) in Norwich, UK. *M. pulcherrima* strain DANMET-A is registered as NCYC CODE R801 and *M. fructicola* strain DANMET-B is NCYC CODE R802. The NCYC acknowledges that long-term storage stocks of both strains have been made and successfully passed quality control.

1.4. Intended Use and Consumer Exposure

Metschnikowia pucherrima DANMET-A and *Metschnikowia fructicola* DANMET-B are intended to be added individually or together as a combination as secondary direct additives to

better control the post-harvest processing of coffee. The intended addition level of the strains individually or the combination of the two strains is up to $2.5x10^7$ cfu/g of freshly harvested coffee fruits.

The two strains of yeast are not intended for use in infant formula or other products intended for consumption by infants and toddlers, or in any product regulated by the U.S. Department of Agriculture.

1.5. Statutory Basis for GRAS Status

Lallemand's GRAS determination for the intended use of *Metschnikowia pulcherrima* DANMET-A and *Metschnikowia fructicola* DANMET-B, individually and as a combination of the two strains is based on scientific procedures as described under 21 CFR §170.30(b). A comprehensive search of the literature through February 2021 was conducted by Lallemand and reviewed and extended through May 2021 by JHeimbach LLC; the information was critically evaluated and summarized in this GRAS monograph. The complete literature review summarizes the totality of the generally available information germane to determining the safety of the intended use of the two yeast strains as described in this monograph.

Determination of the safety and GRAS status of the intended use of *Metschnikowia pulcherrima* DANMET-A and *Metschnikowia fructicola* DANMET-B was made through the deliberations of a panel of experts (GRAS Panel) consisting of Joseph F. Borzelleca, Ph.D., James T. Heimbach, Ph.D., and Michael W. Pariza, Ph.D., who reviewed information in this monograph and other generally available information they deemed appropriate. The GRAS Panel critically reviewed the available information, including the potential intake of the two yeast strains, and unanimously concluded that the generally available information on the strains contains no evidence that demonstrates or suggests reasonable grounds to suspect a hazard to the public health under their intended conditions of use.

It is the unanimous opinion of the GRAS Panel that other qualified scientists reviewing the same generally available information would reach a similar conclusion. Therefore, the intended use of *Metschnikowia pulcherrima* DANMET-A and *Metschnikowia fructicola* DANMET-B as secondary direct additives for the processing of post-harvesting coffee is GRAS by scientific procedures.

1.6. Premarket Exempt Status

Lallemand's GRAS intended use of *Metschnikowia pulcherrima* DANMET-A and *Metschnikowia fructicola* DANMET-B is not subject to the premarket approval requirements of the Federal Food Drug and Cosmetic Act based on Lallemand's conclusion that such use is GRAS.

1.7. Availability of Information

The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times

at the office of James T. Heimbach, Ph.D., President, JHeimbach LLC, 923 Water Street #66, Port Royal, Virginia 22535, telephone 804-742-5543 and e-mail jh@jheimbach.com.

1.8. Freedom of Information Act Statement

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

1.9. Certification

To the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information as well as favorable information known to me and pertinent to the evaluation of the safety and GRAS status of the intended use of *Metschnikowia pulcherrima* and *Metschnikowia fructicola*, individually or in a combination of the two strains.

1.10. FSIS Statement

Not applicable.

1.11. Name, Position, and Signature of Notifier

[redacted]

James T. Heimbach, Ph.D., F.A.C.N.
President
JHeimbach LLC,
Agent to Danstar Ferment AG

Part 2. Identity, Method of Manufacture, Specifications, and Technical Effect

2.1. Names of the Notified Organisms

The subjects of this GRAS notification are:

-*Metschnikowia pulcherrima* strain DANMET-A

-*Metschnikowia fructicola* strain DANMET-B

-The combination of the two strains, trade-named Lalcafé™ NESY2 (designated as NESY2)

2.2. Sources of the Notified Organisms

Metschnikowia pulcherrima DANMET-A was isolated from a Chilean vineyard in Santiago in 2004, initially isolated by a team from the university of Santiago de Chile (USACH).

Metschnikowia fructicola DANMET-B was isolated from Pinot Noir grapes in a French vineyard in Burgundy in 2009 by a team from IFV Beaunes.

Both strains are registered in the National Collection of Yeast Cultures (NCYC) in Norwich, UK. The taxonomy of the species is as follows:

- Kingdom: Fungi
- Phylum: Ascomycota
- Subphylum: Saccharomycotina
- Class: Saccharomycetes
- Order: Saccharomycetales
- Family: Metschnikowiaceae
- Genus: *Metschnikowia*
- Species: *fructicola* and *pulcherrima*

2.3. Descriptions of the Notified Organisms

Strains of *Metschnikowia* are acknowledged for their biocontrol capabilities (Agate and Bhat 1966; Janisiewica et al. 2001; Turkel et al. 2014; Oro et al. 2018; Hranilovic et al. 2020; Binati et al. 2020). They are found on numerous plants and fruits species across the world and have been described as such since 2000.

Their benefits are exploited in different applications such as winemaking or plant-care to control various pests. Numerous technological innovations involving antagonistic *Metschnikowia* strains have been patented (e.g., JPH01117778A, 1989; US6991930B1, 2006; NZ528225A, 2008; P0800775, 2008; ITTO20070655A1, 2009; WO2010149370, 2010; WO2010149369, 2010; CN101946805A 2011; CN103642705A, 2014; EP3266305A1, 2018; CN107904180A, 2018; CN110684678A, 2020;) and several *Metschnikowia*-based products have been commercialized around the world [Excellence Bio-Nature (Lamothe-Abiet); Flavia, Gaïa, Guardia, and Initia (Lallemand); Shemer (Bayer, Koppert Biological Systems); Zymaflore Egide

(Laffort)] as active dry yeast (ADY) for inoculated fermentation agents or as biocontrol agents for inhibiting plant pathogens and post-harvest plant diseases.

The organisms that are the subject of this GRAS notice are two thoroughly characterized strains belonging to this genus. Because of the intrinsic nature of yeasts, the strains are not resistant to antibiotics and do not produce biogenic amines.

Metschnikowia is a large ascomycetous genus currently comprising 79 species (Mycobank 2020); however, the genus is continually being expanded as new species are discovered. The *M. pulcherrima* clade of the genus contains seven validly described species that share the ability to produce pulcherrimin, a maroon-red pigment that has the ability to control bacterial growth (Turkel et al. 2014; Arnaouteli et al. 2019; Sipiczki 2020). These species and their strains have broad biotechnological potential for application in various industrial processes, given their ability to produce this biocontrol agent. In wine fermentation, these yeasts can modulate the population dynamics of the fermenting yeast communities and produce enzymes and a broad range of compounds that can improve the aromatic complexity of the wine (Sipicki 2020).

Over the past two decades, large numbers of strains isolated from various substrates have been assigned to one of these species in the clade (preferentially to *M. pulcherrima*) based on genetic identity, preferentially using the D1/D2 domains of the LSU rRNA genes and the ITS1-5.8S-ITS2 segments of the rDNA repeats (Figure 1). Recently, two additional species, *M. persimmonesis* and *M. citriensis* were proposed to accommodate pulcherrimin-producing strains (Mycobank 2020). The taxonomic name *M. persimmonesis* was proposed for a single Korean isolate but without providing a complete taxonomic description. The phylogenetic position of the strain is uncertain because its different rDNA barcode sequences (D1/D2, ITS, and 18S) show the highest similarities to sequences of type strains belonging to different species. Many pulcherrimin-producing isolates were not identified at the species level or could not be assigned to any species and were therefore only classified as *Metschnikowia* sp. *M.* aff. *pulcherrima or M.* aff. *fructicola*. Since pigmentation is an irrelevant property in most biotechnological processes, the strains isolated for industrial purposes are normally not tested for pulcherrimin production. Therefore, and because of the sensitivity of pulcherrimin synthesis to the culturing conditions, it is unknown whether pigmentation is a general ability of all strains of the clade (Sipicki 2020).

2.3.1. *Metschnikowia pulcherrima*

Due to the large fatty globules in their chlamydospores (“pulcherrima cells”), strains in this species are outstanding candidates for low-cost lipid production. However, their most intensively studied property is the strong antimicrobial activity.

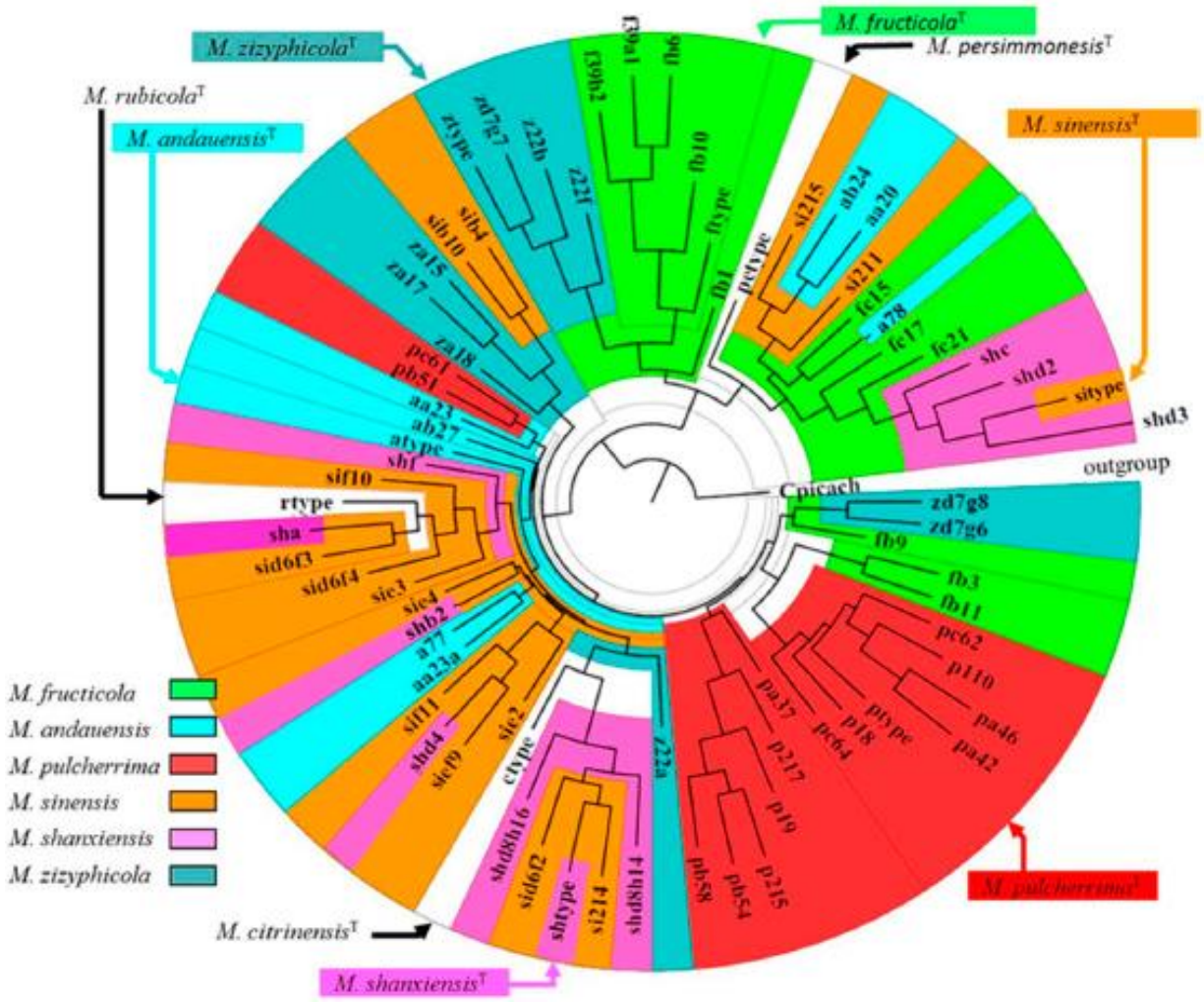

Figure 1. *Metschnikowia* Phylogenetic Tree.

Derived from the neighbor-joining analysis of the cloned D1/D2 sequences of the type strains of six pulcherrimin-producing Metschnikowia species and the D1/D2 sequences of the type strains available in databases. Outgroup: Candida (M)

2.3.1.1. Phenotypic Identification of *Metschnikowia pulcherrima*

Metschnikowia pulcherrima was first identified by Pitt and Miller (1968). The species grows as a pink strain due to its production of pulcherrimin. On selective media, Sabouraud, *M. pulcherrima* forms small pink and smooth colonies (Figure 2). It is budding and non-motile, 1 to 1.5 µm in width by 4 µm in length (Figure 3).

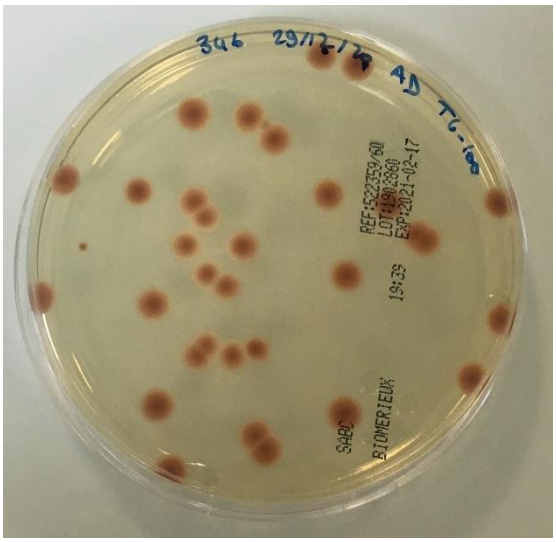

Figure 2. *M. pulcherrima* Strain DANMET-A on Sabouraud Screening Medium after 5-Day Growth at Ambient Temperature Followed by 3-Day Growth at 24°C.

Figure 3. Electron Microscopic View of *M. pulcherrima* Strain DANMET-A.

2.3.1.2. Genotypic Identification of *Metschnikowia pulcherrima* Strain DANMET A

The genetic profile (Figure 4) was obtained by Delta-PCR with primers targeting inter-delta sequences. For the PCR migrations, the Promega DNA Ladder was used (Promega #PR-G6951).

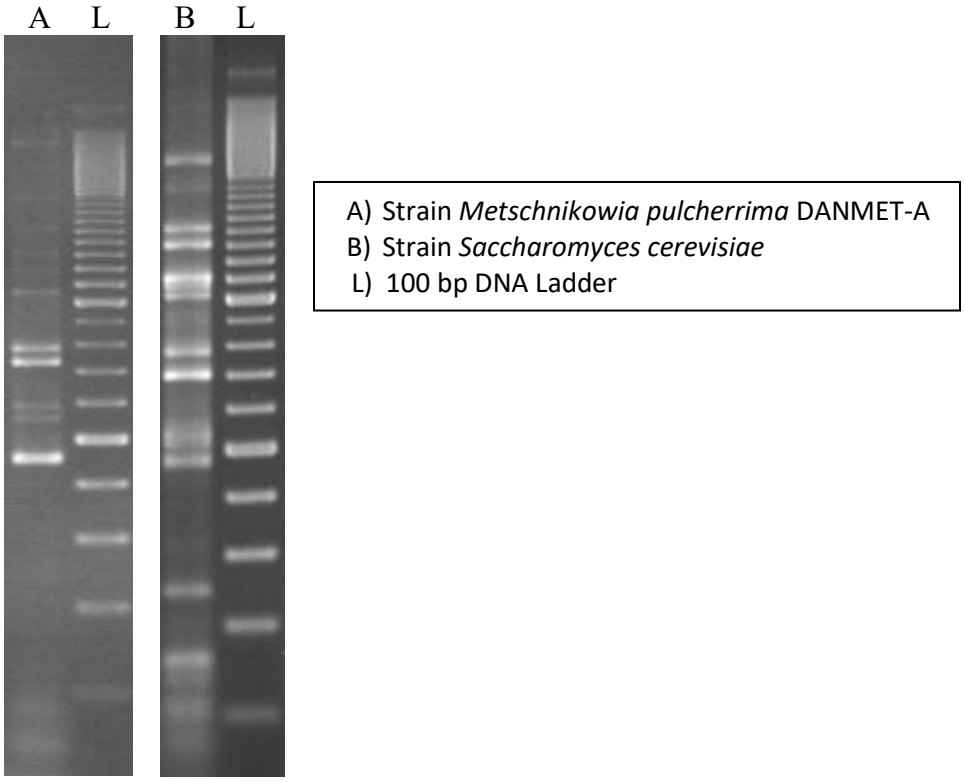

Figure 4. Genetic Profile of *M. pulcherrima* in Comparison with *Saccharomyces cerevisiae*.

2.3.2. *Metschnikowia fructicola*

2.3.2.1. Phenotypic Identification of *Metschnikowia Fructicola*

Metschnikowia fructicola is phenotypically similar to *M. pulcherrima* but with a less intense pinkish color (Figure 5).

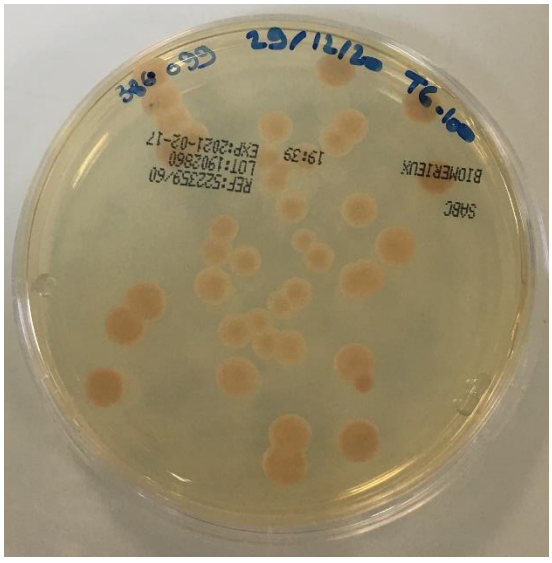

Figure 5. *M. fructicola* Strain DANMET-B on Sabouraud Screening Medium after 5-Day Growth at Ambient Temperature Followed by 3-Day Growth at 24°C.

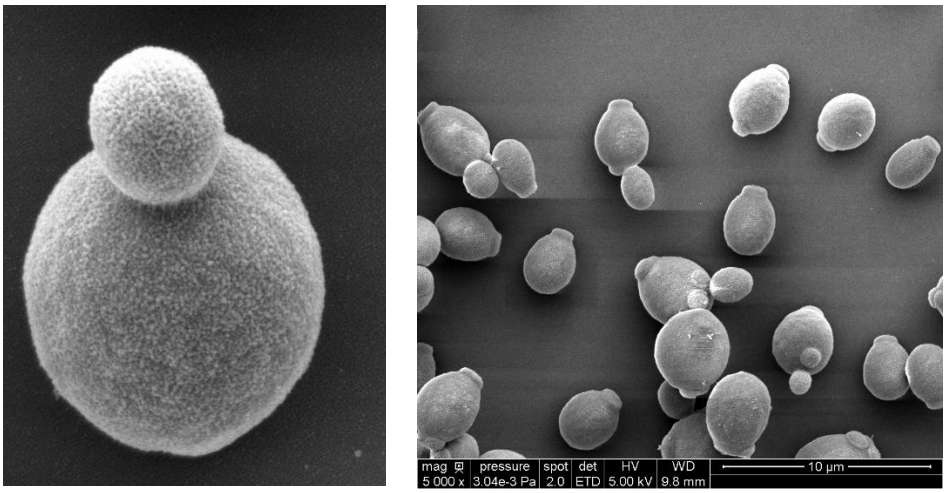

Figure 6. Electron Microscopic View of *M. fructicola* Strain DANMET-B.

2.3.2.2. Genotypic Identification of *Metschnikowia fructicola* Strain DANMET-B

The genetic profile is obtained by Delta-PCR with primers targeting inter-delta sequences. For the PCR migrations, the Promega DNA Ladder was used (Promega #PR-G6951).

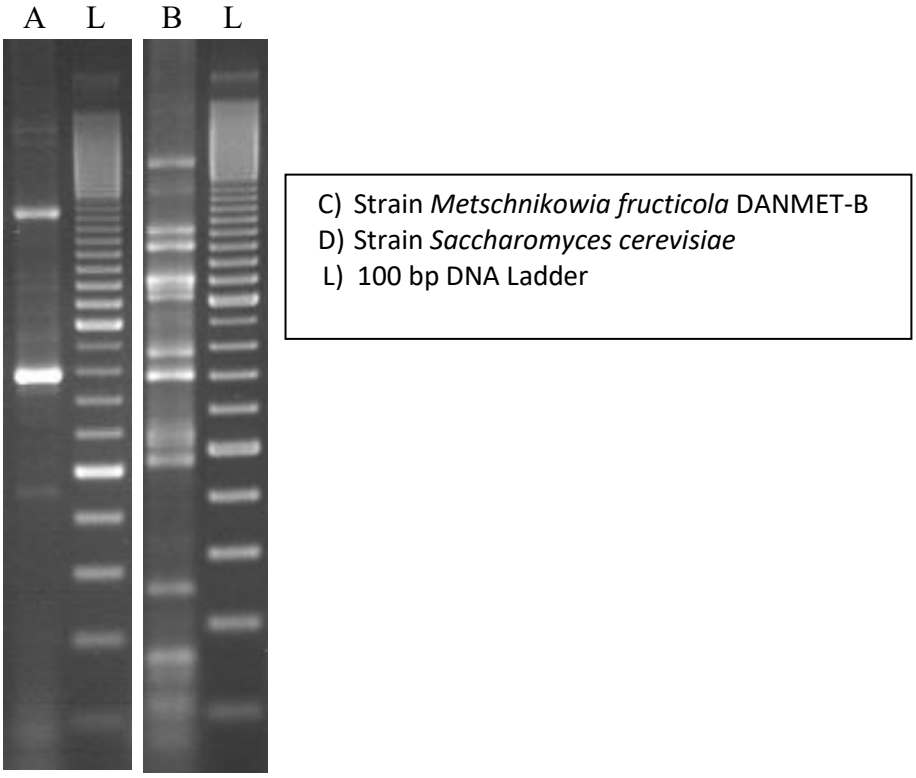

Figure 7. Genetic Profile of *M. fructicola* in Comparison with *Saccharomyces cerevisiae*.

2.4 Genomic Analysis

The genomes of both yeast strains have been sequenced, assembled, and annotated for the following purposes:

- To verify the taxonomic assignment of each strain via a comparison of its D1/D2 sequences with the D1/D2 sequence of type strains for each species;
- To confirm that the strains do not encode genes known to be involved in the production of mycotoxins;
- To screen the strains for genes that enable them to produce process-favorable enzymes, pulcherrimic acid, and volatile aromatic esters.

Genome assemblies for both strains (including annotations) are made available in GenBank format in support of this regulatory filing.

2.4.1. Taxonomic verification via D1/D2 sequence comparison

M. pulcherrima and *M. fructicola* are sister species within *Metschnikowia*, and the taxonomic affiliation of individual strains in this lineage is not always clear. To verify the assignment of DANMET-A to *M. pulcherrima* and of DANMET-B to *M. fructicola*, the 26S rRNA D1/D2 sequence region in DANMET-A was extracted from each annotated genome assembly and aligned against the D1/D2 sequence of the *M. pulcherrima* type strain U45736 (https://www.ncbi.nlm.nih.gov/nuccore/U45736) and that from DANMET-B to the *M. fructicola* type strain AF360542 (https://www.ncbi.nlm.nih.gov/nuccore/AF360542) using default pairwise alignment parameters in the Geneious Prime genome browser (https://www.geneious.com/prime/). Both DANMET-A and B exhibited high sequence similarity to their type strains, particularly at key diagnostic variant sites (Figures 8 and 9 [line 1 is DANMET-A, line 2 is the *M. pulcherrima* type strain, line 3 is the *M. fructicola type* strain, and line 4 is DANMET-B]).

Figure 8. 26S rRNA D1/D2 sequence similarity between DANMET-A LYCC 7475 and ...

Figure 8. D1/D2 Sequences of Strains DANMET-A and DANMET-B and Their Type Strains.

Figure 9. Close-Up of Diagnostic Variant Sites from Figure 8.

2.4.2. *Metschnikowia pulcherrima* Strain DANMET-A

2.4.2.1. Sequencing

A cell pellet of *Metschnikowia pulcherrima* strain DANMET-A was destocked and grown from the Lallemand Yeast Culture Collection on 2020-01-20 (culture collection ID LYCC 7475) and was shipped on dry ice to the sequencing provider SNPsaurus (http://www.snpsaurus.com) for DNA extraction and PacBio sequencing on a dedicated Sequel II SMRT cell (internal sequencing project code MSEQ99). SNPsaurus sheared extracted DNA to ~15 kb and performed a size selection for fragments above 7 kb. SNPsaurus assembled PacBio sequence reads with Flye 2.6 (Kolmogorov et al. 2019). Whole genome sequencing and assembly statistics are displayed in Table 1. Quality assessment of Pacbio reads (read count and read length distribution) was performed with FASTQC (Andrews 2010) and compiled with MULTIQC (Ewels et al. 2016).

Table 1. DANMET-A Whole Genome Sequencing and Assembly Statistics.

Element	Quantity
Reads	1,444,928
Mean read length (bp)	14,602
Genome coverage	1,330-fold
Final assembly contigs	60
Genome size (nt)	15,857,410
GC content (%)	45.8

2.4.2.2. Annotation of the Genome

The genome sequence of DANMET-A was annotated with AUGUSTUS v3.3.3 (Stanke et al. 2008) for ORF finding and BLASTP for functional annotation: 5,556 ORFs were annotated on the assembled sequence.

2.4.3. *Metschnikowia fructicola* Strain DANMET-B

2.4.3.1. Sequencing

A cell pellet of *Metschnikowia fructicola* strain DANMET-B was destocked and grown from the Lallemand Yeast Culture Collection on 2020-01-20 (culture collection ID LYCC 7705), and was shipped on dry ice to the sequencing provider SNPsaurus (http://www.snpsaurus.com) for DNA extraction and PacBio sequencing on a dedicated Sequel II SMRT cell (internal sequencing project code MSEQ99). SNPsaurus sheared extracted DNA to ~15 kb and performed a size selection for fragments above 7 kb. SNPsaurus assembled PacBio sequence reads with Flye 2.6 (Kolmogorov et al. 2019). Quality assessment of Pacbio reads (read count and read length distribution) was performed with FASTQC (Andrews 2010) and compiled with MULTIQC (Ewels et al. 2016).

Table 2. DANMET-B Whole Genome Sequencing and Assembly Statistics.

Element	Quantity
Reads	719,131
Mean read length (bp)	15,948
Genome coverage	705-fold
Final assembly contigs	112
Assembled genome size (bp)	16,262,156
GC content (%)	45.8

2.4.3.2. Annotation of the Genome

The genome sequence of DANMET-B was annotated with AUGUSTUS v3.3.3 (Stanke et al. 2008) for ORF finding and BLASTP for functional annotation: 5,664 ORFs were annotated on the assembled sequence.

2.4.4. Gene Screening and Strain Comparison

2.4.4.1. Screening for Genes Involved in the Production of Mycotoxins

ToxFinder, a BLAST+ analysis tool developed by the Center for Genomic Epidemiology at the National Food Institute of the University of Denmark (https://www.genomicepidemiology.org/), was used to screen the assembled genome sequences of DANMET-A and -B for the presence of genes involved in the production of 7 mycotoxins: aflatoxin, citrinin, patulin, ergot, fumonisin, ochratoxin, and trichothecene. The genome sequences, in FASTA format, were each analyzed online at https://cge.cbs.dtu.dk/services/ToxFinder/ with ToxFinder 1.0, using database version 2021-01-29, setting a detection threshold of 70% sequence identity and 60% minimum coverage. The analysis detected no hits for genes involved in the production of aflatoxin, citrinin, patulin, ergot, fumonisin, ochratoxin, or trichothecene in DANMET-A or DANMET-B.

2.4.4.2. Screening for Genes Involved in the Production of Process-Favorable Enzymes

DANMET-A encodes 3 copies of endo-1,3(4)-beta-glucanase and DANMET-B encodes 4 copies (gene ID=g1099 in genome assembly; blastp ID=NP_014465.1). DANMET-A also encodes an allantoate permease that is absent in DANMET-B (gene ID=g4586 in genome assembly, blastp ID=NP_012686.3).

2.4.4.3. Screening for Genes Involved in the Production of Volatile Aromatics (Esters)

The production of volatile compounds may bring favorable aromas to coffee (esters). In yeast, esters are primarily produced when an acyl-CoA and an alcohol are coupled by alcohol acyl transferases (AATs; Kruis et al. 2018). AAT paralogs ethanol hexanoyl transferase (EHT1) and ethyl ester biosynthesis (EEB1) are acyl-coenzymeA:ethanol O-acyltransferases that produce medium chain fatty acid (MCFA) ethyl esters by coupling MCFA-CoA with ethanol (Saerens et al. 2006). MCFA esters confer a range of flavors to yeast-fermented products, including ethyl butanoate (pineapple), ethyl hexanoate (apple, anise), ethyl octanoate (sour apple), and ethyl decanoate (waxy, apple, cognac; Knight et al. 2014). Paralogs alcohol acetyltransferase 1 and 2 (ATF1 and ATF2) produce acetate esters (Lilly et al. 2006), including isoamyl acetate (banana), phenylethyl acetate (rose), isobutyl acetate (floral, tropical, pineapple), and ethyl acetate (sweet,

solvent, off-flavor; Dzialo et al. 2017). Ethanol acetyltransferase genes (e.g., EAT1) also make acetate esters (Kruis et al. 2018).

The blastp-generated genome annotations of DANMET-A and B were screened for EHT1, EEB1, ATF1, ATF2, and EAT1 via a text-based search of gene descriptions and gene IDs, and no annotations for those genes were detected in either strain. Reference sequences for all five ester genes were also downloaded from the NCBI Gene database (https://www.ncbi.nlm.nih.gov/gene/, accession numbers NC_001134 EHT1, NC_001139 ATF2 and EAT1, NC_001147 ATF1, NC_001148 EEB1), and a blastn search was conducted for hits to each sequence in the DANMET-A and -B genome assemblies. Default settings were: match mismatch scoring = 2-3, gap cost = 5 2, word size = 11, maximum E-value = 0.05. Hits with query coverage <10% of the gene sequence were discarded. This analysis revealed no hits for any of the 5 ester production genes in DANMET-A and -B.

2.5. Production Process

The manufacturing process presented in the following sections comprises the production of each dried yeast strain as a powder (Section 2.5.1), followed by combining the two dried yeast strains *Metschnikowia pulcherrima* DANMET-A and *Metschnikowia fructicola* DANMET-B.

Information regarding the facilities involved in the manufacture and testing of each dried yeast strain and their combination, including the responsibilities of each, is provided in Table 3.

Table 3. Production Facilities.

Name and Address	Activity
Producing Plant De Sanske Gærfabrikker Bredstrupvej 33 8500 Grenaa Denmark	**Production of Yeast:** Culture Collection, Fermentation, Concentration, Drying, Quality Control, Storage
Producing Plant Lallemand GmbH Ottakringerstrasse 89 Einfahrt Festgasse Vienna, Vienna A-1160 Austria	**Production of Yeast:** Culture Collection, Fermentation, Concentration, Drying, Quality Control, Storage
Mixing and Packing Plant Lallemand Denmark A/S Vejlevej 10 7000 Fredericia Denmark	**Production of Combination:** Combining, Packing, Quality Control, Storage

The facility in Grenaa is compliant with the International Food Standard (IFS Food) in the product scope of dry products, other ingredients, and supplements. (See Appendix 1: Bureau Veritas Certification for Grenaa's plant for International Food Standard (IFS) for yeast production.)

The production plant in Vienna has undergone an audit by the FDA on its compliance to produce according to the FSMA requirements. (See Appendix 2: Statement FSMA.)

The plant in Fredericia complies with ISO 9001:2015 and meets the requirements set out for the BRC Global Standard for Food Safety. (See Appendices 3 and 4: Certification for Federicia's plant for International Food Standard (IFS) for Mixing and Packaging in flow pack aluminum foil vacuum pouches of dry yeast, and Certification for Federicia's plant for ISO 9001.)

2.5.1. Manufacturing Process of the Yeast Strains

The process is similar to any other food application yeast production, and the same process is followed with both strains. A flowchart of the production process is presented in Figure 10. A pre-culture phase is started with a small number of yeast cells from the yeast cell bank. More yeasts grow over several laboratory stages, until there is enough yeast to start a seed fermentation in a large production fermenter. At this step, filtered and sterilized food-grade sugar-cane or beet molasses is the main nutrient, supplemented with vitamins and minerals.

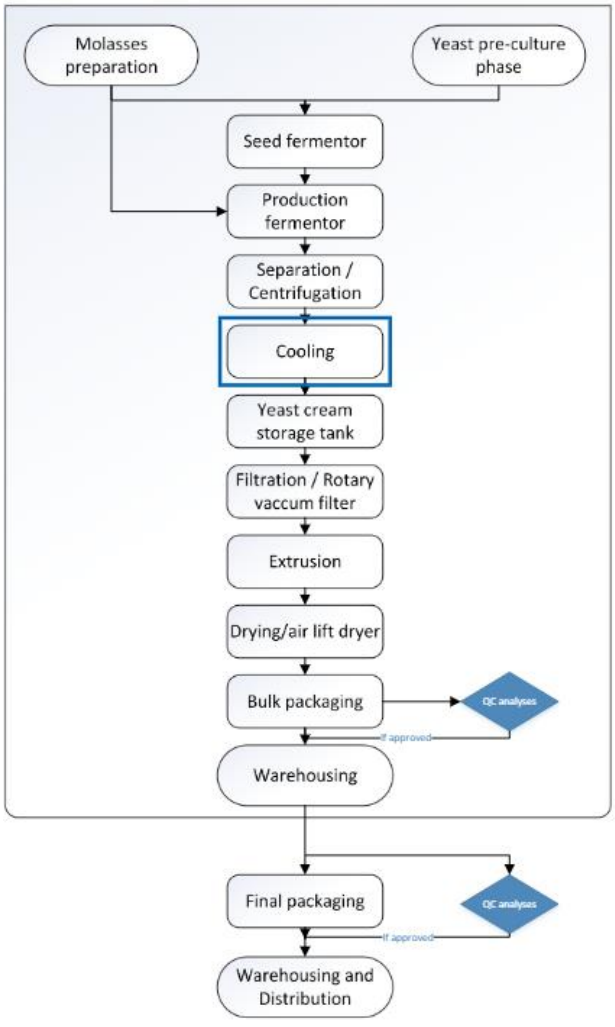

Figure 10. Manufacturing Flowchart.

At the end of the fermentation process, the yeast is separated from the surplus of water and residues from the media. The obtained cream yeast (approximately 20% dry matter) is cooled and stored in tanks.

The first step of the drying process is filtration via a rotary vacuum filter to increase the dry matter from ~20% to ~35%. This crumbly yeast is fed to an extruder, where the typical "noodle" shape of the dry yeast is forged. The extruded yeast is fed to a fluid bed dryer, where the yeast is dried with warm and dehumidified air to reach a moisture level not exceeding 8%.

As soon as the optimum dry matter is reached, the dryer is emptied, and the dried yeast is stored and packed in 20-kg bulk packaging at 4°C. At this point samples are taken and extensive QC testing is performed as shown in Table 4.

If all QC analyses are successfully passed, the final packaging in 500-g or 10-kg lots is done and stored at 4°C before being distributed in a cool transportation system. In addition to QC testing, other physicochemical analyses are performed to provide information of interest to customers, including moisture level, dispersion, rehydration, and reactivation test of glucose consumption. (See Appendix 5.)

Table 4. Quality Control Testing.

Test	Passing Level
Viable yeasts	Estimation of CFU/g
Mold	$<10^3$ CFU/g
Lactic acid bacteria	$<10^5$ CFU/g
Acetic acid bacteria	$<10^4$ CFU/g
Salmonella spp.	Absent in a 25-g sample
Escherichia coli	Absent in a 1-g sample
Staphylococcus spp.	Absent in a 1-g sample
Coliforms	$<10^2$ CFU/g
Arsenic	<1 mg/kg
Lead	<1 mg/kg
Mercury	<1 mg/kg
Cadmium	<1 mg/kg

2.5.2. Combining the Two Yeast Strains

Both strains are produced as described in the section above. Once they are dried and ready to be mixed, they are combined and packed as shown in Figure 11.

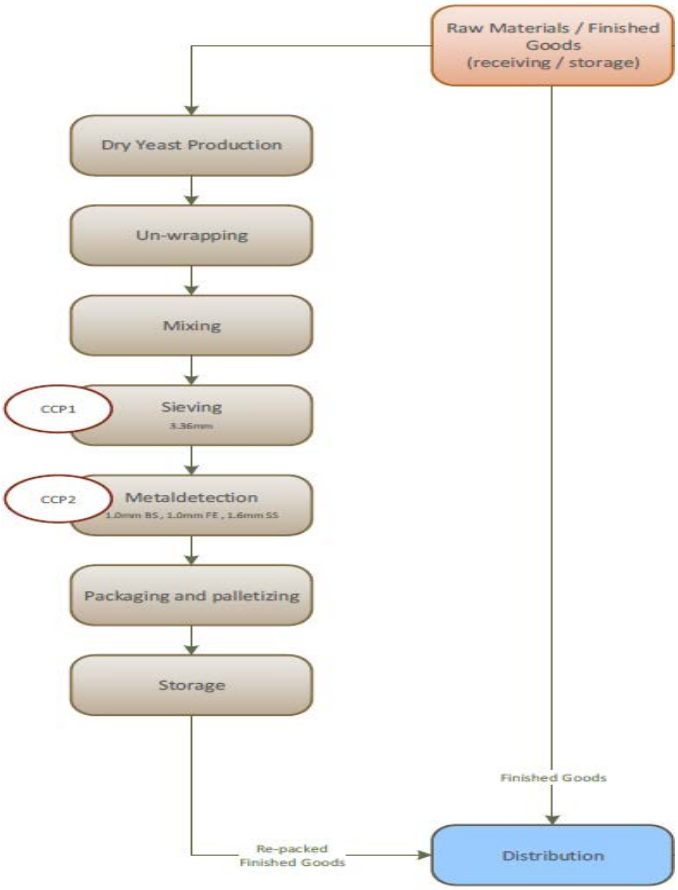

Figure 11. Combining Flowchart.

2.5.3. Production of the Inoculated Coffee

The yeast addition can fit any coffee post-harvest processing, what follows in Figures 12 and 13 presents the most common processes. In any event, however, the coffee is roasted, killing all added yeasts. Neither green coffee nor its cascara (dried pulp) yield living *Metschnikowia* cells after regular post-harvest coffee processing – 12 to 120 hours of tank fermentation, 20 days of sun- and shaded-drying, mechanical hulling, roasting above 200°C, and grinding. The addition of the yeast to coffee and the killing of the yeasts is discussed in more detail in Part 4.

Calculate the volume of potable water for the yeast rehydration. The volume of water is 10 times the weight of the yeast. Fill a clean bucket with an ambient drinking water. .	Slowly suspend the yeast into the potable water. Stir gently to break up any clumps. Wait at least 10 minutes before gently stirring again to break up any remaining clumps and wait 10 to 20 min. before adding to the tank with coffee.	After 20-30 minutes of rehydration, add the yeast suspension to the tank of coffee during filling. In order to ensure the best dispersion of the yeast throughout the coffee, especially for large volumes of coffee, add yeast stepwise as you fill the tank.

Figure 12. Rehydration Steps for Active Dry Yeasts (ADY).

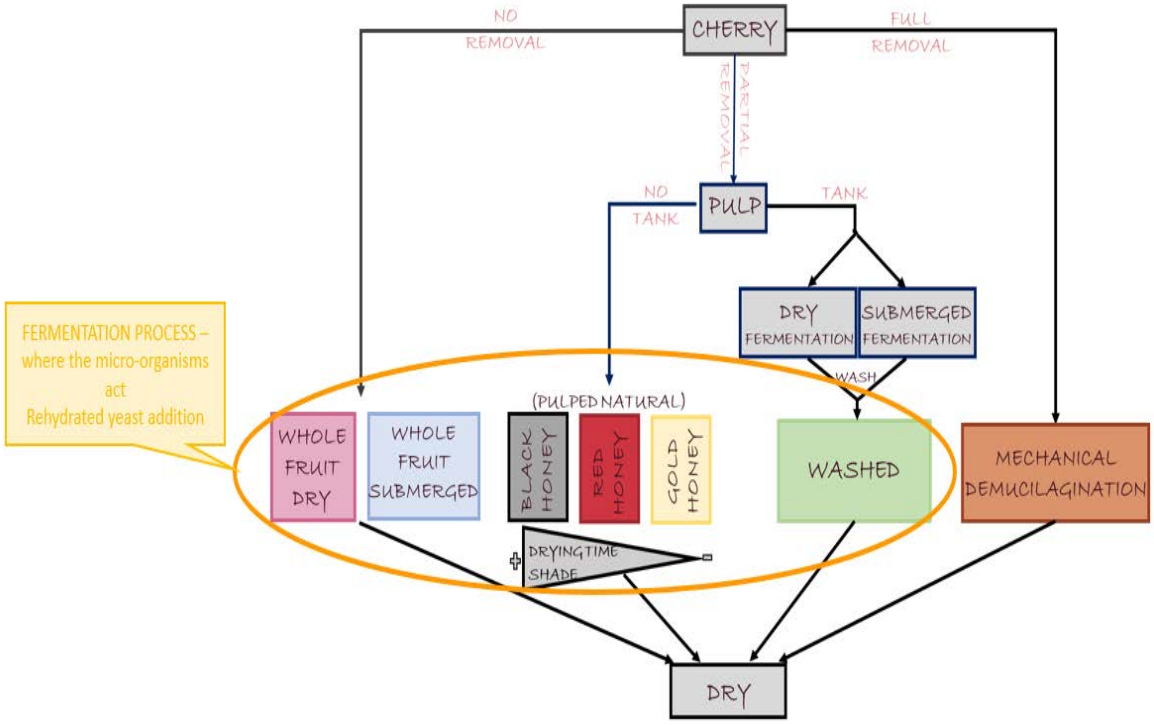

Figure 13. Coffee Post-Harvest Processing Diagram.

2.6. Food-Grade Specifications

2.6.1. Specifications of the Strain DANMET-A

All tested lots of *M. pulcherrima* DANMET-A met the specifications set forth in Table 5.

Table 5. Specifications for *Metschnikowia pulcherrima* DANMET-A.

Parameter	Specification	Methods	Tested Lots		
			346105110	346104110	346068106
Physical aspect	Fine vermicelli	Visual observation	Passes	Passes	Passes
Color	Ivory to beige	Visual observation	Passes	Passes	Passes
Viable *Metschnikowia* spp.	$>10^9$ cfu/g	In-house method	$2.8x10^{10}$	$4.0x10^{10}$	$4.1x10^{10}$
Dry matter	>92%		94.2	94.3	92.2
Heavy metals					
Lead	<1 mg/kg		<1	<1	<1
Mercury	<1 mg/kg		<1	<1	<1
Cadmium	<1 mg/kg		<1	<1	<1
Arsenic	<1 mg/kg		<1	<1	<1
Microbiological Purity					
Coliform	$<10^2$ cfu/g	ISO 4831	<10	<10	<10
E. coli	Absent in 1 g	ISO 7251	Absent	Absent	Absent
S. aureus	Absent in 1 g	ISO 6888-1	Absent	Absent	Absent
Salmonella spp.	Absent in 25 g	ISO 6579	Absent	Absent	Absent

2.6.2. Specifications of the Strain DANMET-B

All tested lots of *M. fructicola* DANMET-B met the specifications set forth in Table 6.

Table 6. Specifications for Metschnikowia fructicola DANMET-B.

Parameter	Specification	Methods	Tested Lots		
			386025203	386102109	386051105
Physical aspect	Fine vermicelli	Visual observation	Passes	Passes	Passes
Color	Ivory to beige	Visual observation	Passes	Passes	Passes
Viable *Metschnikowia* spp.	$>10^9$ cfu/g	In-house method	$1.7x10^{10}$	$1.4x10^{10}$	$1.8x10^{10}$
Dry matter	>92%		92.8	94.3	93.5
Heavy metals					
Lead	<1 mg/kg		<1	<1	<1
Mercury	<1 mg/kg		<1	<1	<1
Cadmium	<1 mg/kg		<1	<1	<1
Arsenic	<1 mg/kg		<1	<1	<1
Microbiological Purity					
Coliform	$<10^2$ cfu/g	ISO 4831	<10	<10	<10
E. coli	Absent in 1 g	ISO 7251	Absent	Absent	Absent
S. aureus	Absent in 1 g	ISO 6888-1	Absent	Absent	Absent
Salmonella spp.	Absent in 25 g	ISO 6579	Absent	Absent	Absent

2.6.3. Specifications of the Combination

All tested lots of combined *M. pulcherrima* DANMET-A and *M. fructicola* DANMET-B met the specifications set forth in Table 7.

Table 7. Specifications for the Combination.

Parameter	Specification	Methods	Tested Lots		
			572001809	572093108	572124112
Physical aspect	Fine vermicelli	Visual observation	Passes	Passes	Passes
Color	Ivory to beige	Visual observation	Passes	Passes	Passes
Viable *Metschnikowia* spp.	$>10^9$ cfu/g	In-house method	$>10^9$	$>10^9$	$>10^9$
Dry matter	>92%		>92	>92	>92
Heavy metals					
Lead	<1 mg/kg		<1	<1	<1
Mercury	<1 mg/kg		<1	<1	<1
Cadmium	<1 mg/kg		<1	<1	<1
Arsenic	<1 mg/kg		<1	<1	<1
Microbiological Purity					
Coliform	$<10^2$ cfu/g	ISO 4831	$<10^2$	$<10^2$	$<10^2$
E. coli	Absent in 1 g	ISO 7251	Absent	Absent	Absent
S. aureus	Absent in 1 g	ISO 6888-1	Absent	Absent	Absent
Salmonella spp.	Absent in 25 g	ISO 6579	Absent	Absent	Absent

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2.7. Stability

For the two strains *Metschnikowia pulcherrima* DANMET-A and *Metschnikowia fructicola* DANMET-B, 12-month stability studies have been completed at 4°C and 25°C, in addition to some accelerated shelf-life studies.

As shown in Tables 8 and 9, *Metschnikowia pulcherrima* continues to meet the specification of $>10^9$ viable cells/g for the 1-year shelf life of the product when stored at either 4 or 25°C. The same stability is shown for *Metschnikowia fructicola* in Tables 10 and 11.

Table 8. Stability Data for *Metschnikowia pulcherrima* at 4°C.

Storage time (months)	0	3	12
Viable Yeasts (cfu/g)	$3.57x10^{10}$	$2.59x10^{10}$	$2.62x10^{10}$

Table 9. Stability Data for *Metschnikowia pulcherrima* at 25°C.

Storage time (months)	0	3	12
Viable Yeasts (cfu/g)	$3.57x10^{10}$	$1.96x10^{10}$	$2.68x10^9$

Table 10. Stability data for *Metschnikowia fructicola* at 4°C.

Storage time (months)	0	1	3	9	12
Viable Yeasts (cfu/g)	$4.94x10^{10}$	$3.87x10^{10}$	$3.85x10^{10}$	$3.79x10^{10}$	$4.01x10^{10}$

Table 11. Stability Data for *Metschnikowia fructicola* at 25°C.

Storage time (months)	0	1	3	9	12
Viable Yeasts (cfu/g)	$4.94x10^{10}$	$2.97x10^{10}$	$1.5x10^{10}$	$1.47x10^{10}$	$9.08x10^{9}$

Part 3. Intended Technical Effect

Bourdichon et al. (2012a) noted that fermentation "has been used by man since the Neolithic period. . . . Fermentation plays different roles in food processing. Major roles considered are:

1. "Preservation of food through formation of inhibitory metabolites . . .
2. "Improving hygiene through inhibition and even elimination of food pathogens . . .
3. "Detoxification of food . . .
4. "Improving wholesomeness through improved digestibility of polymers . . .
5. "Enrichment of food substrates with essential nutrients . . .
6. "Organoleptic properties through effects on flavor, texture, and color . . ."

The intended technical effect of the use of *Metschnikowia pulcherrima* and *Metschnikowia fructicola* on green or ground coffee, individually or together, is for the improvement of hygiene as the inoculated yeast controls fermentation and for organoleptic properties resulting in enhancement of flavor of the coffee.

Part 4. Intended Use and Consumer Exposure

Metschnikowia pulcherrima and *Metschnikowia fructicola*, individually or together (as NESY2) are intended to be added to fresh coffee cherries after harvesting at a current maximum addition level of 1 g yeast per kg coffee fruit cherries. This provides a cell count of about $2x10^6$ and $2x10^7$ cfu/g coffee. The addition of the yeast is during the post-harvest process, where it acts as a processing-aid. As shown below, the yeasts are almost completely removed and totally deactivated during processing of the coffee grounds.

All along the transformation process, the coffee is less and less loaded with yeast. It starts at 1g of active dry yeast added after rehydration in water per kg coffee cherries. As the cherries are dried, they go through a process that removes the dried skin and pulp, removing almost all yeasts from the green coffee beans. In addition to this removal of yeast cells, the coffee undergoes many processes that stress and kill the remaining yeast: during drying, the sun exposure brings the temperature up to about 45°C on the patio while only a little water activity remains. The dried beans then undergo roasting; during this process, the beans are exposed to a temperature higher than 200°C, a temperature far above that needed to kill 100% of the viable yeast cells remaining on the coffee and to destroy the integrity of genetic material. A final step, grinding, submits the coffee to high stress which would also kill the yeast if any were remaining. This ensures that the final consumer, the coffee drinker, will not be in contact with any viable microorganisms.

Both the manufacturer and the producer performed analyses to assess whether any viable yeast is present on the finished product, i.e., coffee. Both laboratories reported results of plating of the green and roast and ground coffees, which showed no growth. The spread was the result of maceration of the coffee with some peptone water with 10-fold dilution on yeast and mold screening medium (Sabouraud).

-maceration of green coffee which has been inoculated with NESY2
-maceration of roasted coffee which has been inoculated with NESY2
-rehydrated yeast NESY2 (control)

All platings were done following a similar protocol. Both green and roasted coffees were either plated from a direct contact with an inoculation loop or from the macerating peptone water. The coffee was macerated with 10 times its weight of peptone water and then went to a stomacher bag and mixed for 3 minutes. The resulting water was then plated on Sabouraud screening growth medium.

For the plating of the rehydrated yeast, the active dry yeast was rehydrated in tepid water for 20 minutes, then underwent a ten-fold dilution and was plated on a Sabouraud screening growth medium. All three petri dishes were left in an oven at 30°C for 2 days. The results are shown in Figure 14. The first petri dish resulted from green coffee inoculated with NESY2; the second petri dish resulted from roasted coffee inoculated with NESY2; and the third petri dish resulted from pure rehydrated yeast NESY2. The media in the first two petri dishes show no growth of microorganisms, indicating that all coffee beans, green or roasted, were lacking any viable yeast cells. The post-harvest processing and roasting have annihilated the last living microorganisms on the coffee matter. The third petri dish, the control, demonstrates that *Metschnikowia* spp. grow well on Sabouraud medium.

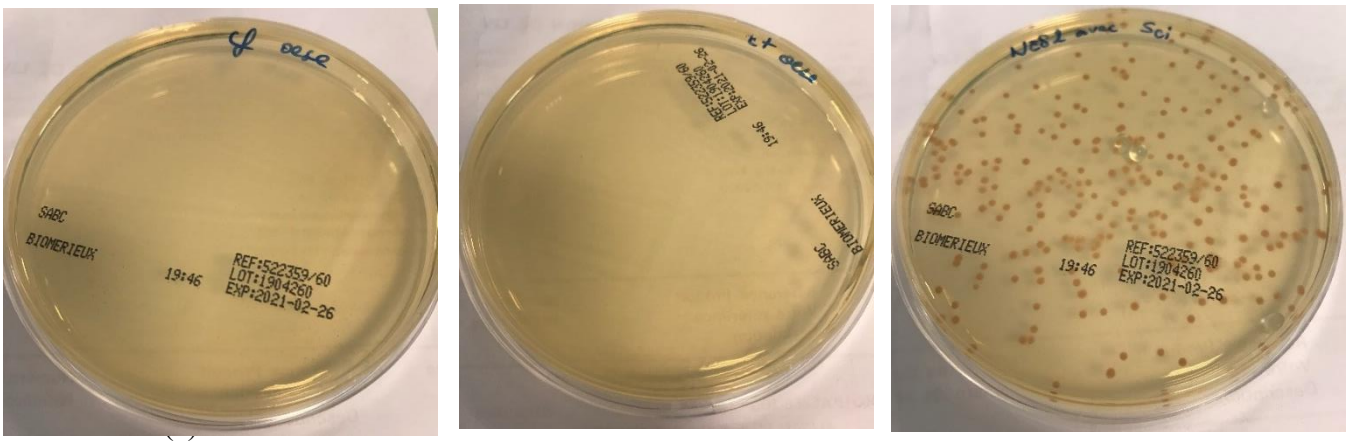


Figure 14. Plating Results.

Part 5. Self-Limiting levels of Use

There is no technological limitation to the concentration of the combination of the two strains *Metschnikowia pulcherrima* and *Metschnikowia fructicola*, or of either of these two notified strains individually, which may be added to post-harvest coffee processing. However, it is not impossible that some organoleptic effects might occur from excess yeast addition.

Part 6. Experience Based on Common Use in Food

The conclusion that the intended use of the combination of the two strains *Metschnikowia Pulcherrima* DANMET-A and *Metschnikowia Fructicola* DANMET-B or of any of these two notified strains individually, is GRAS is based on scientific procedures rather than experience based on common use in food prior to 1958.

Part 7. Narrative

7.1. History of Consumption of Notified Yeast Strains

The microorganism is naturally found on several fruits such as apples and grapes as a bioprotective agent. The *Metschnikowia* spp. are extensively cited in the scientific literature and are positioned as safe for humans and impacting positively on plant health (Oro et al. 2018). It is not always clear in such citations exactly which *Metschnikowia* species are discussed; the indistinct species boundaries make the taxonomic identification of the pulcherrimin-producing strains difficult or even impossible. (Janisiewicz et al. 2001)**.** Both strains show very similar physical characteristics. Kurtzman and Droby (2001) reported that, "Diagnostic separation of species from growth responses on various carbon sources and other tests is difficult because of the considerable similarity of assimilation reactions among species."

The International Dairy Federation (IDF) assembled a list of microorganisms with a documented history of safe use in food (Bourdichon et al. 2012b). The species *Metschnikowia pulcherrima* is listed on this inventory, with documented use in wine production dating to 1940 (Charoenchai et al. 1997).

For grape protection and in winemaking to prevent spoilage, Kurtzman and Droby (2001) indicated that the newly recognized species *Metschnikowia fructicola* (type strain NRRL Y-27328, CBS 8853) has a biocontrol activity against *Botrytis* rot of stored grapes, isolated in Israel. The DNA analysis of *M. fructicola* proved it to be a sister species of *M. pulcherrima* (Janisiewicz et al. 2001). Kurtzman and Droby (2001) reported that "*Metschnikowia fructicola* and *M. pulcherrima* give the same responses on the standard growth tests used in yeast taxonomy. . . . definitive separation of the two species appears to require gene sequence comparisons."

Among the naturally occurring yeasts, a wide range of biodiversity is seen from one origin to another. *Metschnikowia* spp. have been isolated from a wide range of fruits and plants, especially on grapes in France and Chile. Wine is a well-researched area and an extensive literature exists showing the benefits of *Metschnikowia* spp. in fermentation processes (OIV 2019; Hranilovic et al. 2020; Binati et al. 2020).

Metschnikowia spp. have been observed on other foods and fruits, especially as a biocontrol when a fruit is wounded or as a plant care bioactive compound. Many studies mention the positive impact of *Metschnikowia*, helping plants and fruits prevent the growth of pathogenic microbes (Janisiewica et al. 2001; Binati et al. 2020; Plascencia-Jatomea 2014). Of importance regarding the safety of *Metschnikowia* spp. is the conclusion of research reported by Oro et al. (2014) that, "The antimicrobial activity of *M. pulcherrima* does not seem due to proteinaceous compounds such as killer phenomenon, but to the pulcherriminic acid (the precursor of pulcherrimin pigment) that depletes iron present in the medium, making it not available to the other yeasts."

Pawlikowska and Kregiel (2017) investigated the enzymatic profiles and antimicrobial activity of 5 strains of *M. pulcherrima* from the culture collections of Slovakia and the U.K. All strains produced pulcherrimin in the presence of Fe^{3+} and showed α-glucosidase and leucine-arylamidase activities. They all inhibited the growth of tested molds and bacteria. The authors suggested that "strains of the yeast *M. pulcherrima* have a great potential to become a leading natural and biological control agent against a broad spectrum of spoilage microorganisms." In

addition, "*Metschnikowia* spp. with a wide temperature tolerance do not produce either allergic spores or harmful mycotoxins." They also reported that, "the antibacterial activities of *M. pulcherrima* are associated with changes in the extracellular pH values and not with the biosynthesis of killer toxins."

Ianieva and Podgorsky (2020) tested the ability of 36 yeast strains to produce biogenic amines by plating them on YPD agar medium containing a mix of amino acids (tyrosine, histidine, phenylalanine, leucine, tryptophan, arginine, and lysine) at concentrations of 1% or 2%. Six of the 36 strains tested produced biogenic amines, but *Metschnikowia pulcherrima* did not.

Lallemand has produced and sold *Metschnikowia (M. pulcherrima* and *M. fructicola*) yeast species as two separate products to the wine industry since 2012. The two commercial products are sold to 41 different wine producing countries with the key markets being France, Italy, USA, Spain, Russia, Australia, and Switzerland.

In total, Lallemand has sold 40 tons of *Metschnikowia* over the past 8 years to the wine industry as processing aids. Based on an inoculation rate of 7 g/hL for the two products, this accounts for a combined processed wine volume of 10 million hL, approximately 3% of the world's wine production.

No safety issue with such supplies has been reported.

Although winemaking has been the primary application of *Metschnikowia* spp. to date, this is not the only application. These yeast species have also found use in plant-care to prevent contamination by disease-provoking microorganisms and in post-harvest biocontrol of molds in fresh fruit and vegetables. Wang et al. (2018) reported that both *M. fructicola* and *M. pulcherrima* "have been shown to effectively control a variety of postharvest pathogens of citrus." Table 12 lists some applications from the published scientific literature.

Table 12. Some Published Applications of *Metschnikowia pulcherrima* and *fructicola*.

Strain	Origin	Use	Mode of Action	Metabolites	Reference
*M. pulcherrima (*UMY15)	Vineyards (Turkey)	-biocontrol activity against various microorganisms (against *Penicillium roqueforti*, *P. italicum*, *P. expansum*, and *Aspergillus, Fusarium* spp. in *in-vitro* plate tests) -inhibited the germination and mycelia growth of *A. oryzae*, *A. parasiticus*, and *Fusarium* spp. spores on artificial wounds of apples or grape juice	iron immobilizing pigment pulcherrimin = depletion of iron in the growth medium	-secondary metabolite pulcherrimin -lytic enzymes such as chitinase and glucosidase	Türkel et al. 2014
M. fructicola (type strain NRRL Y-27328, CBS 8853)	Grapes (Israel)	-biocontrol activity against *Botrytis* rot of stored grapes		No pulcherrim production	Kurtzman and Droby 2001

M. pulcherrima (T4-A2, T5-A2, ST1-D10, ST2-A10, ST3-E1, ST3-E13, FMB-140H-7A)	Apple Orchards (US)	-Biocontrol against blue mold of apple		None of the strains produced killer toxins against an Indicator strain of *Saccharomyces cerevisiae*	Janisiewicz et al. 2001
M. spp. (local culture collection of the Dept of Biotechnol of the University of Verona)	Vineyard (Italy)	-*Metschnikowia* spp./*S. cerevisiae* positively modulated wine aroma profile.	*Metschnikowia* spp. promoted the formation of higher alcohols and esters, and reduced volatile phenols	-esters -higher alcohols	Binati et al. 2020
M. pulcherrima MP2	From an in-house collection	-production of lower-alcohol wines. (Alcohol decrease in white wines ranged between 0.6 and 1.2% (v/v).)	-divert carbon away from ethanol production	-higher concentrations of fumarate, succinate, and glycerol -lower concentrations of acetic acid. - increased production of acetate esters and higher alcohols	Hranilovic et al. 2020
M. pulcherrima (ICS 1, 46 & 48, DH3, 5, 10, 18 & 21 and commercially available NCYC 2580 & 30470)	Isolated from fruit and flower, UK	-production of edible microbial oils as well as a renewable feedstock for oleochemicals.	-excellent suitability for industrial biotechnology since it produces a range of valuable metabolites, most prominently microbial lipids and 2-phenylethanol. Microbial lipids can be used as a source of food, biofuels, surfactants, or polymers	-microbial lipids -2-phenyl-ethanol	Abeln et al. 2019
M. pulcherrima (MACH1)	Isolated from the carposphere of apple cv Golden Delicious, Italy	Biocontrol agent against *Botrytis cinerea*, *Penicillium expansum,* and *Alternaria alternata* on apples stored for 8 months at 1°C	Competitive ability for iron against postharvest pathogens of apple (pigmented inhibition zone against both pathogens in low iron amendments)	Pulcherrimin	Saravanakumar et al. 2008

M. pulcherrima (Disva 267)	From the collection of the Department of Life and Environmental Sciences (Polytechnic Univ of Marche, Ancona, Italy)	Yeast volatile organic compounds can reduce *in vitro* growth of decay-causing fungi on strawberries.	-Ethyl acetate at 8.97 mg/cm^3 completely inhibits *B. cinerea* growth in the *in vitro* trials. -Ethyl acetate at 0.718 mg/cm^3 can control gray mold on strawberry fruit	Ethyl acetate	Oro et al. 2018

7.2. Use of *Metschnikowia pulcherrima* and *fructicola* in Coffee Processing

As is the case with many fermented food products, coffee producers are starting to use yeast as a processing aid to control its fermentation (Pereira et al. 2014). In 2005, a patent application was filed by Nestec S.A.for "a coffee beverage base comprising a fermented coffee component comprising coffee aroma, which fermented coffee component has a modulated coffee aroma with fruity and/or floral notes due to the fermentation of the coffee aroma." The patent application listed a variety of yeast strains suitable for this purpose, including *Metschnikowia* spp. (WO 2005/048727 A1, 2005).

Many reports detailing the benefits of yeast inoculation in coffee have been published in the past 20 years. Either on wet or dry post-harvest processing, several yeast strains have shown a beneficial impact on coffee, either in an organoleptic sense or in the ability to provide biocontrol protection. As early as 1966, Agate and Bhat (1966) published reports of their investigations of the beneficial enzymatic activity of *Saccharomyces (bayanus, cerevisiae, marxianus)* applied to Robusta coffee fruits.

Kwak et al. (2018) measured consumer acceptance of coffee brewed from green coffee beans fermented with three different strains of *Saccharomyces* yeast. They reported that:

> "Yeast fermentation of green coffee beans for 24 h was effective in fortifying the functionality of coffee by inducing a significant increase in antioxidant activity, TPC, and TFC. Yeast fermentation of green coffee beans causes bound phenolic compounds to be released after roasting. The consumer acceptance for the fermented coffee beans was slightly lower than for the controls. Fermentation might negatively influence the aroma and flavor of coffee extracts. However, the consumer segmentation revealed that approximately 39.4% of consumers preferred one of the fermented coffees more than the controls. Therefore, it can be concluded that yeast fermentation did not always generate a negative aroma and flavor for consumers. If fermentation was carried out with properly selected yeasts, fermented coffee can be attractive to coffee consumers, and coffee manufacturers can diversify their products with higher functionality" (Kwak et al. 2018).

A wide range of literature now exists showing the benefits of fermenting coffee cherries with non-saccharomyces yeasts, specifically *Metschnikowia pulcherrima* and *Metschnikowia fructicola*. The following benefits can be attributed to the species (Janisiewica et al. 2001; Kurtzman and Droby 2001; Hranilovic et al. 2020).

1) Biocontrol - avoid the growth of spoilage microorganisms.
2) Better control and management of fermented foods.
3) Expression and revelation of aromatic compounds due to specific enzymatic activities.
4) More consistent quality for final food products.

As for any application of active dry yeast – the format under which the yeast is sold – there is a rehydration step. Once the yeast is active, it is added after the harvest either directly to the coffee fruits or on the pulped coffee and is left to ferment from 12 hours to 5 days. During this fermentation time, the yeasts act in different ways: the most impactful and noticeable is the enzymatic activity through which the cellulose and pectin are degraded into fermentable sugars. Those sugars are then metabolized to volatile organic compounds, higher alcohols, organic acids, etc. Those metabolites shape the sensory signature of each coffee.

All coffees consumed today undergo a fermentation process either by naturally occurring microorganisms, in which case the fermentation is not controlled, or by specially selected yeasts, in which case the fermentation is controlled. Coffee has for centuries been fermented with indigenous flora or microorganisms naturally present in the coffee plantation ecosystem. In the past few years, coffee processors have started inoculating with specifically selected yeasts with the objective of better controlling this crucial step of the process to improve final cup quality. Lallemand commercialized the first yeast for coffee processing in 2017.

Lallemand has developed a range of selected coffee yeasts for post-harvest processing under the trade name Lalcafé™. To date, four commercial products are available worldwide and a distribution network is in place to access more and more producers. This represents more than 2200 tons of coffee produced with addition of Lalcafé™ yeast during fermentation. Examples are shown in Figure 15.

Figure 15. Pictures of Coffees Promoting the Yeast Addition During Fermentation.

7.3. Recognized Safety of Yeast/*Metschnikowia* Spp.

Microbial food cultures have a long, safe history of use in food, and have generally been considered safe and suitable for myriad uses. As long as cultures are used for traditional fermentation and their metabolism and effect on food substrate is well constrained by the substrate and growth conditions, there is a reasonable expectation of safety (Nabors 2009).

The mechanisms by which *Metschnikowia* spp. exert their biocontrol effects are not associated with the production of toxic compounds; thus, these strains can be used safely as bioprotective agents to curb the invasion of pathogenic and saprophytic microorganisms (Abeln et al. 2019). After cytotoxicity testing, Bedir and Kuleasan (2021) reported that *Metschnikowia pulcherrima* "had no toxic effect on L929 mouse fibroblast cells after 24-hour exposure." Muccilli and Restuccia (2015) reported that, "The biocontrol abilities of *S. cerevisiae* and *W. anomalus* strains have been recently proven to be correlated with killer phenotype, while in other yeast species, the antagonistic activity has been mainly attributed to competition for nutrients and space, production of hydrolytic enzymes or volatile organic compounds (VOCs). In particular, the competition for iron was reported to play a significant role in biocontrol interactions of *M. pulcherrima*; yeast strains belonging to this species are effective against postharvest decay of apple, table grape, grapefruit, cherry tomato, sweet cherries and peach." The biocontrol mechanisms of *Metschnikowia* spp. are illustrated in Figure 16.

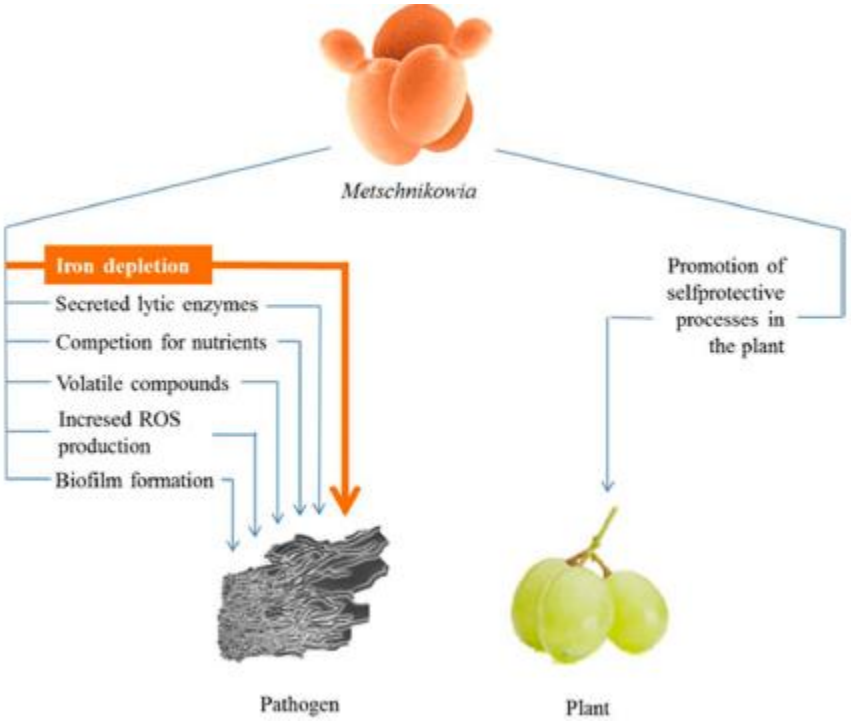


Figure 16. Antimicrobial Antagonism of Strains of the *M. pulcherrima* Clade.

Metabolites production – The two *Metschnikowia* spp. that are the subject of this GRAS notice are reported to be safe. Some metabolites generated are pulcherrimin, which is known to chelate with iron ions to deplete the environment and prevent the growth of spoilage

microorganisms, and -lytic enzymes such as chitinase and glucosidase. Antagonism is not an exclusive property of the *M. pulcherrima* clade; strains of many other yeast species can inhibit other microorganisms. Several of the antimicrobial mechanisms have also been associated with the antimicrobial activity of the pulcherrimin-producing *Metschnikowia* yeasts. The chemical structure of pulcherrimin or pulcherrimic acid and the sites at which iron chelation takes place are illustrated in Figure 17.

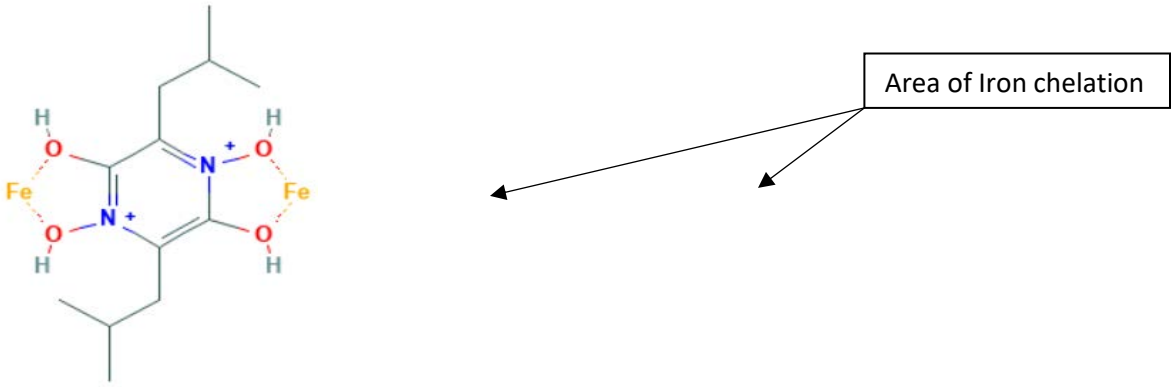


Figure 17. Pulcherrimin - $C_{12}H_{22}Fe_2N_2O_4^{+2}$

Pulcherrimin is an iron chelate in which the four oxygen atoms from pulcherriminic ;

is formed from leucine through the intermediates cyclo-L-leucyl-L-leucyl and pulcherriminic acid. Pigment production is not essential for growth, even under high-iron conditions, so a protection function is unlikely and pigment production must benefit the organism in some other manner (Sipiczki 2006).

Pulcherrimin patents have been registered since 1982. Most of them are related to plant care application, including fruits and vegetables, compost, and fertilizer. Other applications show the benefit of lipids accumulation in *M. pulcherrima* cells. Some patents highlight the interesting production of pulcherrimic acid for food fermentation such as in the (Saravanakumar et al. 2008).

When produced by microorganisms, the pulcherrimic acid depletes the iron ions from the surrounding medium. There has been some research about the self-regulation to balance the production and the richness in Fe to allow the microorganism to keep on growing.

Saravanakumar et al. 2008

7.4. Toxicity Studies

Dan et al. (2020) reported on a series of toxicity studies of *Metschnikowia pulcherrima*. T-2. The published studies included acute and repeated-dose studies of oral toxicity and several

tests of genotoxicity, including bacterial reverse mutation (Ames), mouse sperm aberration, and micronucleus test of mouse bone marrow; it was not reported whether these studies were compliant with guidelines for the testing of chemicals promulgated by the Organization for Economic Co-operation and Development (OECD). In the study of acute oral toxicity, the limit method was used in Kunming mice (number, age, and sex were not reported in the English translation of the Chinese article). Five mice were dosed by gavage sequentially at a limit dose of 10,000 mg/kg bw with no reported indications of toxicity: no mice died, there were no adverse clinical observations, and there were no adverse effects reported in hematological or biochemical measures. The LD50 was determined to be >10,000 mg/kg bw.

In the repeated-dose study, Kunming mice (number, age, and sex were not reported in the English translation of the Chinese article) received feed admixtures of 0, 50, 250, or 500 mg/L of *M. pulcherrima* T-2 for 30 days. There were no significant differences in feed intake or weight gain, no adverse effects on hematological or biochemical parameters, and no pathological changes in examined organs and tissues (heart, liver, spleen, lung, and kidneys). The NOAEL in this study was the highest concentration tested, 500 mg/L. (Assuming consumption of 3 g feed/day, this equates to an exposure of 1.5 mg/day or 50 mg/kg bw/day for 30-g mice.)

The English translation of the Chinese provided little detailed information about the genotoxicity testing. The results of the Ames assay with tester strains TA97, TA98, TA100, and TA109 at doses of 40, 200, 1000, and 5000 µg/plate showed that "the yeast is not mutagenic"; the sperm aberration study reported no teratogenic effect at doses of *M. pulcherrima* ranging from 5 to 500 mg/L; and the mouse bone marrow erythrocyte micronucleus test indicated an absence of induced chromosome damage at doses of 31.25, 125, and 500 mg/L.

Based on the oral toxicity and genotoxicity testing, that authors concluded that "*Metschnikowia pulcherrima* T-2 is safe for humans."

The safety of *Metschnikowia fructicola* NRRL Y-27328 was investigated by the Environmental Protection Agency's Office of Pesticide Programs (EPA/OPP) in response to an application for use of the strain as a microbial pesticide on fruits. The EPA/OPP research included two studies of acute oral toxicity (EPA 2018); compliance with OECD guidelines was not reported. In the first study, 3 female Sprague-Dawley rats (age and bodyweight not reported) were given by gavage a single oral dose of 5,000 mg/kg bw of *Metschnikowia fructicola* preparation containing $1.6x10^{10}$ CFU/g as 40% w/v in distilled water. The study was performed using the up-and-down procedure. Animals were observed for 14 days. Based on the results of this study, *M. fructicola* showed no toxicity to rats after exposure. All animals survived, appeared normal, and gained weight throughout the study. No abnormalities were reported at necropsy.

In the second study, 12 male and 12 female Sprague-Dawley rats (age and bodyweight not reported) were given a single oral dose of $1.4\text{-}2.5x10^{8}$ CFU/animal. The animals were observed for up to 21 days with interim sacrifices (3 animals/sex/day) on Days 4, 8, and 15. Three males and 3 females were treated with heat-inactivated *M. fructicola* as inactive-treated controls, and two additional groups of two males and two females served as untreated "shelf controls" and "non-shelf" controls, respectively. There were no treatment-related deaths, adverse clinical signs, necropsy findings, changes in bodyweight, or changes in bodyweight gain. With a stated limit of detection of 10 CFU/gram, viable test organisms were not reported in the brain, kidneys, spleen, liver, heart, lungs, mesenteric lymph nodes, blood, stomach, small intestine,

cecal contents (all animals), or the feces from treated animals. EPA (2018) concluded that, "A single oral administration of 1.4-2.5x10^8 CFU/rat, constituting a maximum hazard dose, resulted in no signs of infectivity, pathogenicity, or toxicity."

7.5. Safety Evaluations by Authoritative Bodies

Non-*Saccharomyces* yeasts are widely used in wine application and winemaking. In Europe, non-*Saccharomyces* species are acknowledged as safe and positive to use for quality improvement and to avoid external spoilage. The European Commission states that "the commercial starters added may be pure cultures or combinations of *Saccharomyces* strains and *non-Saccharomyces* strains. Where active, selected yeasts (*Saccharomyces* and non-*Saccharomyces*) are used, these shall comply with the prescriptions of the International Oenological Codex" (OIV 2019).

EFSA also approved the use of *Metschnikowia fructicola* strain NRRL Y-27328 in plant-care application. Even though some data gaps were identified in terms of toxicity, the evaluation gave a positive opinion on the release of the product on the market. Indeed, no risk has been identified in terms of safety to the soil, to the environment, or to humans (EFSA 2017). In response, on December 6, 2018, *M. fructicola* strain NRRL Y-27328 was approved by the European Commission as an organic pesticide substance (Official Journal of the European Union, 7.122018).

EPA (2018), reviewing the same strain, reported that, "Overall, the supporting information/data provided is sufficient to satisfy the Tier I toxicology data requirements for the human health risk assessment of *Metschnikowia fructicola* strain NRRL Y-27328" and concluded: "EPA concludes that there is a reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of *Metschnikowia fructicola* strain NRRL Y-27328. Therefore, an exemption from the requirement of a tolerance can be established for residues of *Metschnikowia fructicola* strain NRRL Y-27328 in or on the stone fruit group (group 12-12); the small fruit vine climbing subgroup, except fuzzy kiwifruit (subgroup 13-07F); and the low growing berry subgroup (subgroup 13-07G) when used in accordance with label directions and good agricultural practices." This regulation eliminates the need to establish a maximum permissible level for residues of *Metschnikowia fructicola* strain NRRL Y–27328 under FFDCA.

Part 8. Safety Assessment and GRAS Determination

8.1. Introduction

This section presents an assessment that demonstrates that the intended uses of *Metschnikowia pulcherrima* strain DANMET-A, *Metschnikowia fructicola* strain DANMET-B, and their combination are safe, and also GRAS under the Federal Food, Drug, and Cosmetic Act (FFDCA) for their intended use. This safety assessment and GRAS determination entail two steps. In step one, the safety of the intended use of the yeast strains is demonstrated. In the second step, their intended use is determined to be GRAS by demonstrating that their safety is based on generally available information and generally recognized among qualified scientific experts.

The regulatory framework for establishing whether a substance is GRAS in accordance with Section 201(s) of the FFDCA is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under 21 CFR §170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under 21 CFR §170.30(c). This GRAS determination employs scientific procedures established under 21 CFR §170.30(b).

In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components: 1) the data and information relied upon to establish the scientific element of safety must be generally available; and 2) there must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific procedures GRAS determination are applied below in an analysis of whether *M. pulcherrima* strain DANMET-A, *M. fructicola* strain DANMET-B, and their combination are safe and GRAS for the uses and at the use levels intended.

8.2. Safety of the Intended Use of *Metschnikowia pulcherrima* Strain DANMET-A, *Metschnikowia fructicola* Strain DANMET-B, and Their Combination

A scientific procedures GRAS determination requires first that information about the material establish that the intended use of the material is safe. The FDA has defined "safe" or "safety" for food additives under 21 CFR §170.3(i) as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use." This same regulation specifies that three factors must be considered in determining safety. These three factors are:

1) The probable consumption of the substance and of any substance formed in or on food because of its use;

2) The cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet; and

3) Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

The intended use of *M. pulcherrima* strain DANMET-A, *M. fructicola* strain DANMET-B, and their combination has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). A comprehensive search of the literature through May 2021 served as the basis for preparation of a monograph summarizing the information available germane to determining the safety of the intended use of *M. pulcherrima* strain DANMET-A, *M. fructicola* strain DANMET-B, and their combination. Furthermore, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of *M. pulcherrima* strain DANMET-A, *M. fructicola* strain DANMET-B, and their combination has been made through the deliberations of a GRAS Panel comprising Joseph F. Borzelleca, Ph.D., James T. Heimbach, Ph.D., and Michael W. Pariza, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They have independently and collectively critically evaluated the publicly available information summarized in this document and other information deemed appropriate and determined that no evidence exists in the available information on *M. pulcherrima* or *M. fructicola* that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of the strains.

The GRAS Panel applied a decision tree for determination of the safety of microbial cultures (Pariza et al. 2015) to the intended use of *M. pulcherrima* strain DANMET-A and *M. fructicola* strain DANMET-B as follows:

1. Have the strains been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? YES
2. Have the strains' genomes been sequenced? YES
3. Are the strains' genomes free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity? YES
4. Are the strains' genomes free of functional and transferable antibiotic resistance gene DNA? YES
5. Do the strains produce antimicrobial substances? NO
6. Have the strains been genetically modified using rDNA techniques? NO
7. Were the strains isolated from a food that has a history of safe consumption for which the species, to which each strain belongs, is a substantial and characterizing component (not simply an 'incidental isolate')? NO (*Metschnikowia pulcherrima* DANMET-A was isolated from a Chilean vineyard in Santiago and *Metschnikowia fructicola* DANMET-B was isolated from Pinot Noir grapes in a French vineyard in Burgundy)
8. Do the strains induce undesirable physiological effects in appropriately designed safety evaluation studies? NO

The outcome of this decision-tree analysis is a confirmation that "the strains are deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption" (Pariza et al. 2015).

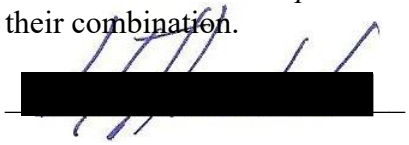
The members of the Expert Panel have independently and collectively critically evaluated the publicly available information summarized in this document and other information deemed appropriate, and have unanimously concluded:

> The safety of the intended use of *M. pulcherrima* strain DANMET-A, *M. fructicola* strain DANMET-B, and their combination has been shown by the extensive history of the safe use of the genus and the two species, their safety as shown in studies of oral toxicity, and the fact that coffee is heated during processing to a level that guarantees that no viable yeast will remain at the time of consumption. The addition of *M. pulcherrima* strain DANMET-A, *M. fructicola* strain DANMET-B, and their combination, complying with the specifications and use described in this GRAS monograph, is safe and GRAS based on scientific procedures.

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach a similar scientific conclusion regarding safety. Therefore, based on scientific procedures, the intended uses of *M. pulcherrima* strain DANMET-A, *M. fructicola* strain DANMET-B, and their combination are safe and GRAS.

8.3. Statement Regarding Information Inconsistent with GRAS

I have reviewed the available data and information and am not aware of any data or information that are, or may appear to be, inconsistent with our conclusion of the GRAS status of the intended use of *M. pulcherrima* strain DANMET-A, *M. fructicola* strain DANMET-B, and their combination.



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Appendix 1

BUREAU VERITAS
Certification

1828

Certificate

Herewith the certification body

Bureau Veritas Certification Denmark

being an accredited certification body for IFS certification and having signed an agreement with the IFS owners, confirms that the processing activities of

De Danske Gærfabrikker (A Subsidiary of Lallemand) A/S

Bredstrupvej 33, 8500 Grenaa, Denmark
COID: **42976**

for the audit scope:

Production of Yeast and Yeast Extract.
Beside own production, company has outsourced processes and/or products.

Product scope: 10. Dry products, other ingredients and supplements

Technology scope: **B, C, D, F**

meet the requirements set out in the

International Food Standard (IFS Food)

Version 6.1, November 2017
And other associated normative documents
at Higher Level
with a score of 96,19 %

Certificate number:	DK010753-1	This certificate is valid until:		05-02-2020
Audit Dates:	03-12-2018 04-12-2018 05-12-2018	Re-audit due date:	From To	17-10-2019 26-12-2019
Auditor:	Ole Knudsen			

Fredericia, 05-02-2019

[redacted]

Lone Nyvang Jensen
Bureau Veritas Certification Denmark,

DANAK

IFS Food

Appendix 2

26.01.2021

Statement FSMA

We hereby confirm that Lallemand GmbH, Ottakringerstrasse 89, 1160 Vienna, Austria, has implemented and is producing according to the FSMA requirements and was successfully audited in 2019 by the FDA.

[redacted]

DI Isabel Schüller
Qualitätssicherung und -management/Quality Assurance and Management
Lallemand GmbH

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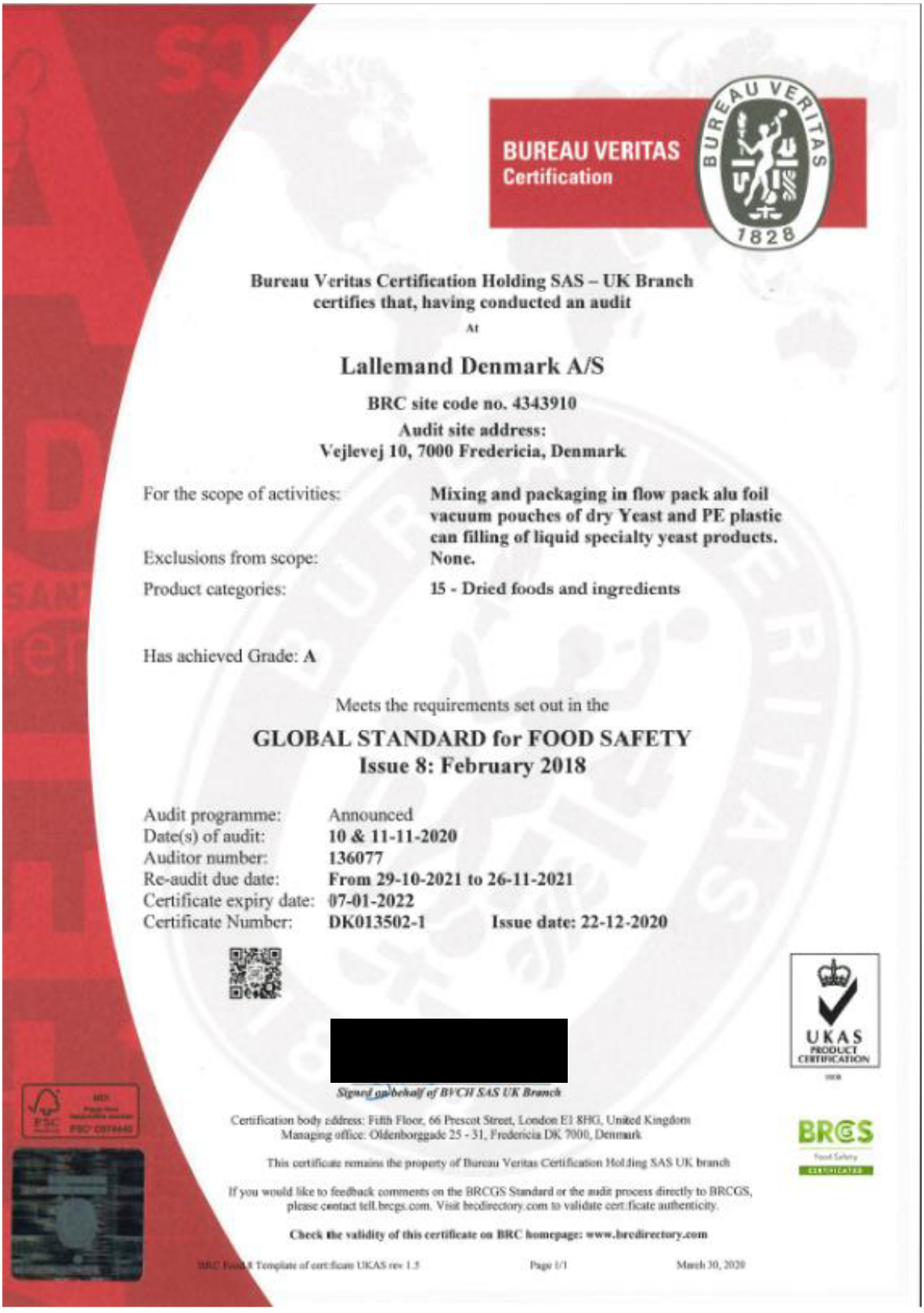
Firmenbuch Gericht: Handelsgericht Wien| FN 173272m
UID.: ATU 45222307 | Geschäftsführer J.G. Steenkamp

Appendix 3



BUREAU VERITAS
Certification

Bureau Veritas Certification Holding SAS – UK Branch certifies that, having conducted an audit

At

Lallemand Denmark A/S

BRC site code no. 4343910

Audit site address:
Vejlevej 10, 7000 Fredericia, Denmark

For the scope of activities:	**Mixing and packaging in flow pack alu foil vacuum pouches of dry Yeast and PE plastic can filling of liquid specialty yeast products.**
Exclusions from scope:	**None.**
Product categories:	**15 - Dried foods and ingredients**

Has achieved Grade: **A**

Meets the requirements set out in the

GLOBAL STANDARD for FOOD SAFETY
Issue 8: February 2018

Audit programme:	Announced
Date(s) of audit:	**10 & 11-11-2020**
Auditor number:	**136077**
Re-audit due date:	**From 29-10-2021 to 26-11-2021**
Certificate expiry date:	**07-01-2022**
Certificate Number:	**DK013502-1** **Issue date: 22-12-2020**

[redacted]

Signed on behalf of BVCH SAS UK Branch

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

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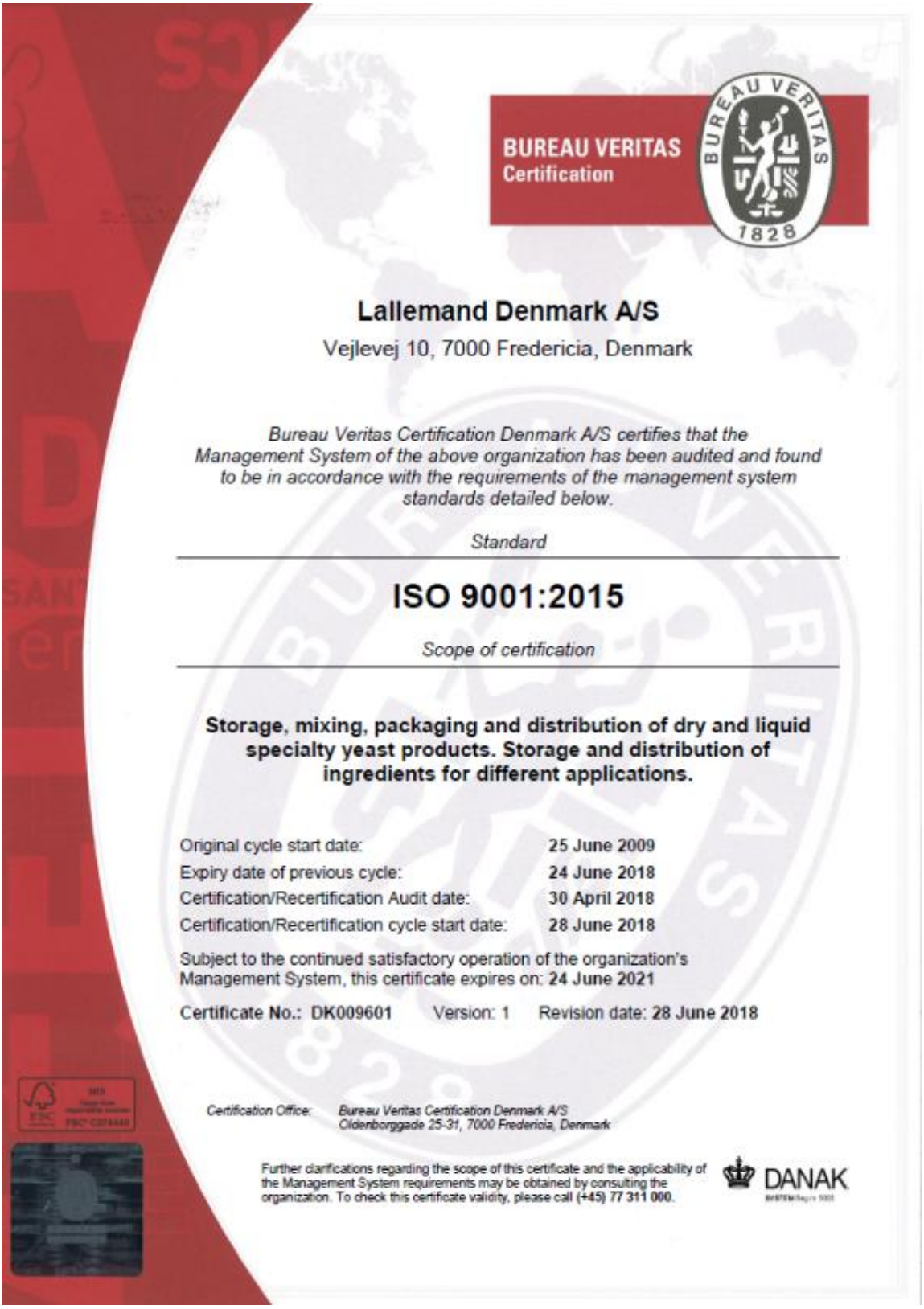
Check the validity of this certificate on BRC homepage: www.brcdirectory.com

BRC Food 8 Template of certificate UKAS rev 1.5 Page 1/1 March 30, 2020

Appendix 4



BUREAU VERITAS
Certification

Lallemand Denmark A/S

Vejlevej 10, 7000 Fredericia, Denmark

Bureau Veritas Certification Denmark A/S 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 9001:2015

Scope of certification

Storage, mixing, packaging and distribution of dry and liquid specialty yeast products. Storage and distribution of ingredients for different applications.

Original cycle start date:	**25 June 2009**
Expiry date of previous cycle:	**24 June 2018**
Certification/Recertification Audit date:	**30 April 2018**
Certification/Recertification cycle start date:	**28 June 2018**

Subject to the continued satisfactory operation of the organization's Management System, this certificate expires on: **24 June 2021**

Certificate No.: DK009601 Version: 1 Revision date: **28 June 2018**

Certification Office: *Bureau Veritas Certification Denmark A/S*
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.**

DANAK

Appendix 5

LALLEMAND	Procedure ADY fermentation activity using Bayer Keto-Diastix®	Ref : CAFE-QC-02 Version: 1 Date: 25/01/2021 Page 1 sur 2

Keeping of the document: Quality Assurance Lallemand

Scope: Applies to ADY to evaluate their fermentation activity.

Distribution of the document:

☐ Product manager

☐ Marketing department

☐ Supply Chain department

☒ R&D department

☒ QC Labs

☐ Production plant :

	Redaction	Verification	Approbation
Name :	Stéphanie Courdesses	Céline Raynal	Stéphanie Courdesses
Function :	QA Manager	R&D project manager	QA manager
Date :	25/01/2021	25/01/2021	25/01/2021
Signature :	[redacted]	[redacted]	[redacted]

Version	Date	Modification
1	25/01/2021	Creation

1- Principle

The method described hereafter will allow to evaluate ADY for fermentation activity. The protocol is also named CLINITEST.

2- Material:

- Bayer's Keto-Diastix strips (Bayer 2882U)
- D-Glucose (ACS grade)
- Defoamer
- Water bath, 35+/-1ºC
- Tap water
- Glassware: glass bottles (250 ml), graduated cylinder, disposable liquid dropper

3- Sample preparation

- In a glass bottle, temper 100 ml tap water at 35ºC in the water bath.
- Weigh 5,0 g of active dry yeast. Transfer in a 250 ml glass bottle.
- Weigh 5,0 g glucose. Transfer in a second 250 ml glass bottle
- Add 25 ml water to the yeast and mix.
- Add 75 ml water to the glucose and mix.
- Allow both bottles to rest in the water bath for 5 minutes mix and allow to rest in the water bath an additional 10 minutes.
- Transfer the glucose solution to the yeast suspension. Mix. This is time 0 min.
- If foaming occurs, add 1-2 drops defoamer.

4- Procedure :

- After 60 minutes, measure the residual sugar;
  - Stir the yeast/sugar suspension
  - Using a disposable dropper, pour a small aliquot of the yeast/sugar suspension over the Keto-Diastix strip (about 3 second).
  - Remove excess liquid from the strip (allow it to drop from the strip)
  - Read after 30 seconds by comparison to the color chart of Keto-Diastix.
- After the initial 60 minutes, measure the residual sugar every 15 minutes until the sugar concentration is less than 0.25%.
- From that time on, test residual sugar every 5 minutes until the sugar concentration is 0%
- Report results as XX minutes.

5- Clinitest stability :

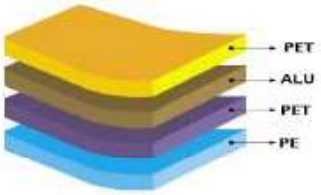
- Place original pack or vacuum sealed foil sachet 7 days at 45C.
- Test as described in the section 3 and 4.
- Report results as Clinitest stability results.

6- Specifications :

The result is variable for each strain. Refer to SharePoint to check the QC specification.

Appendix 7

KOROZO
AMBALAJ SAN. ve TİC. A.Ş.



KOROVAC
A RANGE OF MULTILAYER PE LAMINATES FOR DOYPACK APPLICATIONS

PRODUCT CODE: KV- 1017

Valid For : PET/AL/PET/PE

Metallic bag 500g BAG,12PET/9ALU/12PET/75PE,95X450X63MM

PRODUCT DESCRIPTION:
Biaxially oriented POLYESTER film and ALUMINIUM film for high barrier, laminated to biaxially oriented POLYESTER film and coextruded POLYETHYLENE film.

MAIN USES - APPLICATIONS :
- MAP (Modified Atmosphere Packaging)
- CAP (Controlled Atmosphere Packaging)
- Vacuum Packaging

SPECIAL PROPERTIES :
- Seal performance can be tailor made according to machine speed

FILM CHARACTERISTICS :
- High Oxygen barrier
- High Water Vapor barrier
- Very good heat resistance
- Low temperature sealing
- Good mechanical strength and puncture resistance
- Maximum dimensional stability
- High surface gloss

FOOD SAFETY :
- Specific documents are available on request

SHELF-LIFE & STORAGE CONDITIONS :
- Korovac Film is suitable for use up to 6 months from the date of production maintaining correct storage conditions. Details are available on request.

Properties	Test Method	Target values	Unit
Total Thickness	Micrometer	108 ± 10%	micron
Weigth in Grams	Analytical Balance	134,4 ± 5%	gr/m²
Yield	Analytical Balance	7,44 ± 5%	m²/kg
Solvent Retention	EN 13628	< 15,0	mg/m²
Oxygen Permeability (23°C %0 RH)	ASTM D 3985	< 0,10	cm³/m².day.atm
Water Vapor Permeability (38°C %90 RH)	ASTM F 1249	< 0,05	g/m².day
Width	Meter	± 2	mm
Length	Meter	± 3	mm
Number of Bags in 1 Parcel		According to Requirement	Pieces

The figures and the data provided in this datasheet are consistent with the current state of our knowledge and are intended to provide general information on our products and their applications. They do not constitute a guarantee of any specific applications. We guarantee the properties of these products only within the scope of our Sales Contract. The processor is not released from his obligation to verify the incoming goods or to test and examine the products to ensure that they are suitable for the intended purpose. This material specification is for information only and may be subject to change without further notice.

* COA values will be available upon request.

** Printing control is performed at 5000 Kelvin light.

*** The samples that are taken after slitting/cutting process are kept for two years from production date.

*All TDS values are represented in this document constitute unprinted product specifications. Total thickness and weight are affected by the printing process approx. 2-4(µ-gr).

* Oxygen and water vapour permeability values are "0" for ALU in perfect condition.

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Rev.2018/10

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

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Francine Vidal	Lalcafe Project Leader

Organization *(if applicable)*: Danstar Ferment AG

Mailing Address *(number and street)*: 17975 rue des Gouverneurs

City	State or Province	Zip Code/Postal Code	Country
Mirabel	QC	J7J 2K7	

Telephone Number	Fax Number	E-Mail Address
+33 6 141 10908		fvidal@lallemand.com

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

Name of Contact Person	Position or Title
James T. Heimbach	President

Organization *(if applicable)*: JHeimbach LLC

Mailing Address *(number and street)*: 923 Water Street #66

City	State or Province	Zip Code/Postal Code	Country
Port Royal	VA	22535	

Telephone Number	Fax Number	E-Mail Address
8047425543		JH@JHEIMBACH.COM

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Metschnikowia pulcherrima strain DANMET-A & Metschnikowia fructicola strain DANMET-B

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

☒ Electronic Submission Gateway ☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

3. For paper submissions only

Number of volumes _____

Total number of pages _____

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

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

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

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

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

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

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

☐ 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 two yeast strains are intended to be added individually or together as secondary direct additives to better control the post-harvest processing of coffee. The maximum intended addition level of the strains or combination is 2.5x10E7 cfu/g freshly harvested coffee fruits.

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

(Check one)

☐ Yes ☐ No

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

(Check one)

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

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

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

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

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

☐ Yes ☐ No

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

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Danstar Ferment AG (operating as Lallemand)
(name of notifier)

has concluded that the intended use(s) of Metschnikowia pulcherrima strain DANMET-A and Metschnikowia fructicola strain DANMET-B
(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. Danstar Ferment AG (operating as Lallemand), thro agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

Office of JHeimbach LLC: 923 Water Street #66, Port Royal VA 22535
(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)
	James T. Heimbach, President, JHeimbach LLC	07/27/2021

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)
	Form3667.pdf	Administrative
	GRASforMetschnikowiaStrains.pdf	Administrative
	GRASPanelConclusionRegardingGRASforMetschnikowiaStrains.pdf	Administrative
	GRASPanelSignatures.pdf	Administrative
	MetschnikowiaStrainsCoverLetter20210728.pdf	Administrative