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

June 1, 2020

Reference: *Lactobacillus plantarum* DSM 33452

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 *Lactobacillus plantarum* (*L. plantarum*) DSM 33452 is generally recognized as safe for use in malolactic fermentation of wine and musts and is not subject to the pre-market approval requirements. The recommendation is to inoculate the pure starter culture of *L. plantarum* DSM 33452 into wine or must at an inoculation level of 1.0E+07 CFU/g at the time of crushing grapes or as early as possible in the fermentation tanks. *L. plantarum DSM 33452* is sensitive to alcohol concentration, so the concentration of the organism will decrease as the concentration of alcohol in the wine or must increases. Though *L. plantarum* DSM 33452 is safe to consume, it would be present at negligible levels, if at all, in the finished product.

It should also be noted that due to recent taxonomic changes to the genus *Lactobacillus*, *Lactobacillus plantarum* will be known as *Lactiplantibacillus plantarum* moving forward (Zheng, et al., 2020).

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

Yours sincerely,

Arie Carpenter

Senior Regulatory Affairs Specialist
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CHR. HANSEN, INC.

Generally Recognized As Safe (GRAS) Determination for *Lactobacillus plantarum* DSM 33452

Prepared by Chr. Hansen, Inc.

May, 2020

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Abbreviations

ABV	Alcohol By Volume
ADI	Arginine Deiminase
BA	Biogenic Amine
BE	Bioengineering
BLASTN	Nucleotide Basic Local Alignment Search Tool
CCP	Critical Control Points
CFR	Code of Federal Regulations
CFU	Colony Forming Units
cGMP	Current Good Manufacturing Practice
DNA	Deoxyribonucleic Acid
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EC	Ethyl Carbamate
EFFCA	European Food and Feed Cultures Association
EFSA	European Food Safety Authority
EU	European Union
FCC	Food Chemical Codex
FDA	Food and Drug Administration
FSIS	Food Safety and Inspection Service
FSSC	Food Safety System Certification
GM	Genetic Modification
GMP	Good Manufacturing Practice
GRAS	Generally Recognized As Safe
HACCP	Hazard Analysis and Critical Control Point
IDF	International Dairy Federation
ISO	International Standardization Organization
LAB	Lactic Acid Bacteria
MIC	Minimum Inhibitory Concentration
MLF	Malolactic Fermentation
MRS	de Man, Rogosa, and Sharpe
NBFDS	National Bioengineered Food Disclosure Standard
OPRP	Operational Prerequisite Program
PRP	Prerequisite Program
QPS	Qualified Presumption of Safety
USDA	United States Department of Agriculture

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. has concluded, through scientific procedures, that *Lactobacillus plantarum* DSM 33452 is GRAS and is not subject to the premarket approval requirements for use in malolactic fermentation of wine and musts.

Name and Address of Organization

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Milwaukee, WI 53214
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Fax: (414) 607-5959

Contact Person:
Arie Carpenter
Sr. Regulatory Affairs Specialist
Food Cultures and Enzymes

1.2 Name of GRAS organism

Food culture / culture / Malolactic bacteria / Lactic acid bacteria (LAB) / *L. plantarum* / *Lactobacillus plantarum* DSM 33452 / *Lactiplantibacillus plantarum*

1.3 Conditions of use

Lactobacillus plantarum is intended for use in the production of wine and musts. Prior to the primary fermentation, *L. plantarum* is added to increase the rate of malolactic fermentation (turning malic acid into lactic acid) and to prevent the growth of microorganisms that could cause off flavors in the finished product. *L. plantarum* is sensitive to alcohol so as alcohol levels rise, the *L. plantarum* dies off.

1.4 Basis for GRAS determination

Pursuant to the GRAS rule [81 Fed. Reg. 159 (17 August 2016)], Chr. Hansen has concluded that *Lactobacillus plantarum* DSM 33452 is GRAS through scientific procedures, in accordance with 21 CFR 170.30 (a) and (b).

1.5 Premarket approval status

It is the opinion of Chr. Hansen that *L. plantarum* DSM 33452 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.

1.6 Availability of information

The data and information that are the basis for Chr. Hansen's conclusion that *L. plantarum* DSM 33452 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.
Arie Carpenter
Regulatory Affairs Specialist
9015 W Maple St., Milwaukee, WI 53214
usarbr@chr-hansen.com

1.7 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.

1.8 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 *L. plantarum* DSM 33452.

1.9 FSIS statement

Not Applicable

1.10 Name, position and signature of responsible person who signs dossier and signature

May 28, 2020

Arie Carpenter, Senior Regulatory Affairs Specialist — Date

May 28, 2020

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

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

In preparing this dossier, Chr. Hansen has consulted and applied the Pariza *et al*. "Decision Tree for Determining the Safety of Microbial Cultures to be Consumed by Humans or Animals" (2015). The decision tree is composed of thirteen questions which, when applied, provide a "comprehensive approach for determining the safety of microbial cultures that lack an established history of safe use for their intended new applications". These questions include criteria related to characterization, antimicrobial substances, genetic engineering, and other relevant topics. The sections in Part 2 satisfy those criteria. The criteria will be further discussed and met in sections 3 and 6.

2.1 Name of the GRAS organism

The subject of this GRAS determination is a strain of the bacterial species *Lactobacillus plantarum* designated as DSM 33452.

Recent taxonomic changes to the genus *Lactobacillus* published by Zheng *et al.* in April of 2020 will effectively change the nomenclature of this organism from *Lactobacillus plantarum* to *Lactiplantibacillus plantarum* moving forward (Zheng, et al., 2020).

2.2 Source of the GRAS organism

The strain was isolated from South African red wine.

2.3 Description of the GRAS organism

Lactobacillus plantarum was originally described in literature by (Orla-Jensen) Bergey *et al.* in 1923 as non-motile, Gram-positive rods that tend to become longer as the acidity becomes greater. It was noted that the natural habitat of *Lactobacillus plantarum* was widely distributed especially in fermenting plant and animal products, the organism could also be isolated in fermented dairy products, bread, and other fermented food products such as pickles or sauerkraut. (Bergey & Pederson, 1935)

The taxonomic identification of *Lactobacillus plantarum* DSM 33452 was confirmed in-house at Chr. Hansen and was deposited in the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) under the accession number DSM 33452 on February 20, 2020. The taxonomic lineage of *Lactobacillus plantarum* DSM 33452 is provided in Table 2-1.

Table 2-1: Taxonomic lineage of Lactobacillus plantarum DSM 33452

Taxonomy	**Taxonomic Assignment**
Kingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Lactobacillales
Family	Lactobacillaceae
Genus	*Lactobacillus*
Species	*Lactobacillus plantarum*
Strain	*Lactobacillus plantarum* DSM 33452

The identity of the organism was confirmed through whole genome sequencing using 16S rDNA and *rpoA* genes as identifiers. Sequencing of 16S gene is standard for the identification of microorganisms, but 16S is not able to resolve all species of *Lactobacillus.* To further speciate, a second housekeeping gene, *rpoA,* was analyzed. In the *Lactobacillus plantarum* group, the suitability of *rpoA* sequence for reliable species identification has been demonstrated (Naser, et al., 2007). The strain has therefore been species identified by use of 16S rDNA sequencing and sequencing of *rpoA*.

2.3.1 Phenotypic analysis of *L. plantarum* DSM 33452

Lactobacilli are Gram positive, catalase negative, facultative anaerobic, non-spore-forming, rod shaped bacteria producing lactic acid (Bergey & Pederson, 1935). *Lactobacillus plantarum* is a heterofermentative *Lactobacillus* producing gas when metabolizing gluconate (Stiles & Holzapfel, 1997). They have complex nutritional requirements (De Angelis & Gobbetti, 2016) and able to ferment several carbohydrates such as glucose (dextrose), fructose, maltose, and saccharose (sucrose), and others as determined by use of Api50 CHL method (Table 2-2).

Table 2-2 Carbohydrate fermentation (api50 CHL) of the DSM 33452 strain

Control	-	Esculine	+
Glycerol	-	Salicine	+
Erythritol	-	Cellobiose	+
D-Arabinose	-	Maltose	+
L-Arabinose	+	Lactose	+
Ribose	+	Melibiose	+
D-Xylose	-	Saccharose	+
L-Xylose	-	Trehalose	+
Adonitol	+	Inuline	-
β-Methyl-xyloside	-	Melezitose	+
Galactose	+	D-Raffinose	+
D-Glucose	+	Amidon	-
D-Fructose	+	Glycogen	-
D-Mannose	+	Xylitol	-
L-Sorbose	-	β-Gentiobiose	+
Rhamnose	-	D-Turanose	+
Dulcitol	-	D-Lyxose	-
Inositol	-	D-Tagatose	-
Mannitol	+	D-Fucose	-
Sorbitol		L-Fucose	-
α-Methyl-D-mannoside	+	D-Arabitol	-
α-Methyl-D-glucoside	-	L-Arabitol	-
N-acetyl glucosamine	+	Gluconate	+
Amygdaline	+	2-keto-gluconate	-
Arbutine	+	5-keto-gluconate	-

Lactobacilli are native to many different habitats such as plants, silage, meat, milk, and soil. They can also be found in abundance in the human oral cavity, intestinal tract, and vagina (Stiles & Holzapfel, 1997). Lactobacillus plantarum is also found in many different ecological niches such as vegetables, meat, fish, and dairy products including many fermented vegetable and dairy food product. In addition, it is found in the gastro-intestinal tract and the oral cavity and is also used as a probiotic strain (Holt & et al., 1994).

Lactobacillus plantarum has long history of use as a starter culture in fermented meat, milk, cereal, and vegetable products. It can also be found as a spoilage organism in wine, citrus juice, and some cheese (Stiles & Holzapfel, 1997).

2.3.2 Safety assessment of *L. plantarum* DSM 33452

2.3.2.1 Biogenic amines

No standardized method exists to test for the production of biogenic amines, but several methods have been published in the scientific literature (Cid, Miguelez-Arrizado, Becker, Holzapfel, & Vidal-carou, 2008). The occurrence of biogenic amines is attributed to the decarboxylase activity in certain bacteria (Fernandez, Hudson, Korpela, & de los Reyes-Gavilan, 2015). Histamine and tyramine, along with cadaverine and putrescine, have been identified in literature and by EFSA to be the biogenic amines most relevant to food safety (Pariza, Gillies, Kraak-Ripple, & Leyer, 2015) (EFSA Panel on Biological Hazards, 2011).

The ability of L. plantarum DSM 33452 to produce histamine, tyramine, cadaverine, and putrescine were tested by use of an in-house validated GS-MS method modified from (Smart, Aggio, Van Houtte, & Villas-Boas, 2010) e.g. (Garcia-Moruno, Carrascosa, & Munoz, 2005) (Costantini, Cersosimo, Del Prete, & Garcia-Moruno, 2006) (Cid, Miguelez-Arrizado, Becker, Holzapfel, & Vidal-carou, 2008). The induction step was performed by growing the strain in MRS broth anaerobically at 30 °C in the presence of the corresponding amino acids. A negative control and two positive controls were included.

The results showed that *L. plantarum* DSM 33452 does not produce the four biogenic amines of concern.

2.3.2.2 Antibiotic resistance

The genome sequence of the *L. plantarum* DSM 33452 strain was analyzed *in silico* for the presence of known antibiotic resistance genes by BlastN analysis against the ResFinder database (version downloaded 10.01.2019) (Zankari, et al., 2012). Absence of antibiotic resistance genes shows that any phenotypic resistance observed is intrinsic and not a result of acquired antibiotic resistance genes.

L. plantarum DSM 33452 did not contain any antibiotic resistance genes in the genome and was susceptible to most antimicrobial agents with the exception of vancomycin. Resistance to vancomycin is intrinsic to many *Lactobacillus* spp. including *L. plantarum* (Deghorain, et al., 2007) (Goffin, et al., 2005) and is subsequently not a safety concern in food.

To measure antimicrobial susceptibility of *L. plantarum* DSM 33452, the minimal inhibitory concentration (MIC) was determined using the standard methods recommended by ISO 10932 | IDF 223. The strain was tested for resistance to nine antibiotics (ampicillin, vancomycin, gentamycin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, and chloramphenicol) as recommended by EFSA, and the results were interpreted using epidemiological cut-off values also recommended by EFSA (EFSA, 2018).

Table 2-3 MIC values for DSM 33452

Antibiotic type	Antibiotic	MIC in µg/ml	EFSA cut-off values in µg/ml[a]
Aminoglycoside	Gentamicin	2-4	16
	Kanamycin	64	64
	Streptomycin	32-64	n.r.
Tetracycline	Tetracycline	32	32
Macrolide	Erythromycin	0.5	1
Lincosamide	Clindamycin	1	4
Chloramphenicol	Chloramphenicol	8	8
β-lactam	Ampicillin	0.25-0.5	2
Glycopeptide	Vancomycin	>128	n.r.

n.r.: not required to be tested by EFSA

a: EFSA cut-off values for *Lactobacillus plantarum/pentosus* as listed in 'Guidance on the characterisation of microorganisms used as feed additives or as production organisms', EFSA Journal 2018,16:5206

2.3.2.3 Citrulline formation from arginine degradation

Ethyl carbamate (EC) is a food processing contaminant in fermented foods and beverages and has been classified as probably carcinogenic to humans. Many factors influence the occurrence of EC in wine, including the presence of precursors, such as hydrocyanic acid or urea. To a lesser extent can citrulline serve as an EC precursor. Certain wine lactic acid bacteria are capable of forming small amounts of citrulline during arginine biosynthesis through the arginine deiminase (ADI) pathway.

The genome sequence of *L. plantarum* DSM 33452 was analyzed for genes encoding the enzyme arginine deiminase, the first enzyme in the ADI pathway that catalyzes the degradation of arginine to citrulline and ammonia. It was found that the strain does not harbor genes encoding arginine deiminase.

Furthermore, the concentrations of arginine and citrulline were determined in culture supernatants after growth of the *L. plantarum* DSM 33452 in wine must supplemented with arginine. There was no difference in the concentration of arginine and citrulline after 24 and 48 hours of growth showing that citrulline is not produced by *L. plantarum* DSM 33452 under these conditions.

The results from these experiments show that *L. plantarum* DSM33452 does not encode arginine deiminase and does not produce citrulline.

2.3.3 Conclusion of the safety assessment of *L. plantarum* DSM 33452

To determine if a bacterial strain is safe for consumption, the Pariza decision tree ask if a strain has been characterized and its genome has been analyzed. It asks if the strain produces any toxins, any antimicrobial substances, or if it can transfer antibiotic resistance to other organisms.

In section 2 up to this point, we have shown that *L. plantarum* DSM 33452 has been characterized and its genome has been analyzed.

We can conclude that resistance to antibiotics is not a safety concern for *L. plantarum* DSM 33452. Results from the genotypic analysis show that *L. plantarum* DSM 33452 was intrinsically resistant to vancomycin as expected for any organism in the *Lactobacillus* genus. No other antibiotic resistance genes were found. Furthermore, MIC testing showed that *L. plantarum* DSM 33452 fell at or below EFSA epidemiological cut off values for *L. plantarum* (Table 2-3) for most relevant antibiotics with the exception of vancomycin, to which resistance is intrinsic in the genus *Lactobacilli* (EFSA, 2018).

Studies examining the ability of *L. plantarum* DSM 33452 to produce the biogenic amines histamine, tyramine, cadaverine and putrescine, or the EC precursor citrulline, all potentially harmful substances, have been performed and it can be concluded that *L. plantarum* DSM 33452 does not produce any of these compounds.

These conclusions indicate that *L. plantarum* DSM 33452 is free from undesirable attributes and metabolites and are safe to consume.

2.4 Genetic Modification Status

L. plantarum DSM 33452 is not genetically modified by use of recombinant DNA techniques. In accordance with the European Union (EU) regulations 1829/2003 and 1830/2003 the use of Chr. Hansen cultures including *L. plantarum* DSM 33452 does not trigger Genetic Modification (GM) labeling of the final food product.

According to US regulations, Chr. Hansen cultures and enzyme products are not subject to bioengineered (BE) labeling under NBFDS, codified in 7 CFR Part 66.

2.5 Method of Manufacture

L. plantarum DSM 33452 is being manufactured at two facilities following Chr. Hansen's global protocol for the production of wine bacteria (APPENDIX 3: HACCP FLOW SHEET – WINE BACTERIAL CULTURES). The culture is grown, concentrated, frozen, and packed in France. Some of the product is sold into market as frozen culture and some of it is transported to our facility in Denmark where it is freeze dried.

Both of these plants have fully implemented HACCP plans, standard operating procedures and quality control programs to ensure quality of the product being produced. As part of the HACCP plan, each 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 publicly available from our website: www.chr-hansen.com. In addition, each plant has an appointed local OPRP (Operational Pre-Requisite Program) that includes PRP issues and CCPs (Critical Control Points). The OPRP, PRP, and CCP's are documented and classified as specifically critical for the safety of food ingredients produced in the plant. Both plants maintain FSSC 22000 certification as seen in APPENDICES 7 AND 8 FSSC22000. The two current locations of manufacture are in France (Route d'Aulnay, F-91292 Arpajon, France), and in Denmark (Jernholmen 1-27, DK-2650 Hvidovre).

L. plantarum DSM 33452 is produced by industrial fermentation. Pure strains of the microorganism are inoculated into sterilized growth medium specifically designed to meet the nutritional needs of *L. plantarum*. Strict conditions throughout the fermentation process ensure optimal growth. These conditions include maintaining an anaerobic environment and strictly controlling the temperature and pH. Once the fermentation enters stationary growth, it is cooled to stop the growth process. The fermentation is centrifuged to concentrate the microorganisms and eliminate the growth medium. The

concentrated microorganisms are then frozen into pellets. Finally, the pellets are packaged into plastic film bags and labeled with the product name, item number, batch number, amount, and storage temperature. Some of the frozen pellets are sold into market and some of them get shipped to Denmark for freeze-drying.

Frozen pellets destined for freeze drying are lyophilized resulting in a very low water activity and ensuring stability of the culture. The freeze dried powder is then packed into bags and labeled with the relevant information and sold.

The process flow, including critical control points, can be seen in (APPENDIX 3: HACCP FLOW SHEET GLOBAL – WINE BACTERIAL CULTURES).

2.5.1 Raw Materials and Processing Aids

L. plantarum DSM 33452 is produced 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.

2.5.2 Quality Program

Chr. Hansen's extensive quality program includes a FSSC 22000 standard and hygienic monitoring program. This program serves to verify the process control of the production facility. It includes testing surfaces of process equipment and air quality to document the cleanliness of the production environment.

2.5.3 Allergen Control

Chr. Hansen controls all allergens listed in EU Labeling Regulation 1169/2011 and the US Food Allergen Labeling and Consumer Protection Act of 2004. Chr. Hansen also communicates the allergen status of our products in accordance with these two regulations. Allergen control is managed via our GMP and HACCP programs that are FSSC 22000 certified at all of our production sites. Allergen communication is managed via our Quality Management and HACCP programs that are ISO 22000 certified. Further information can be found in APPENDIX 5: ALLERGENS IN PLANT.

2.6 Product Specifications and product stability

2.6.4 Specifications

L. plantarum DSM 33452 pellets are frozen concentrated pure culture. The pellets have an off-white to slightly brown appearance and have a cell concentration at or above 13.3 log CFU/g.

Enumeration of *L. plantarum* DSM 33452 is performed following ISO 15214 standard and use of de Man, Rogosa, and Sharpe (MRS) agar and incubation under anaerobic conditions for 72 hours at 30 ^{o}C.

Purity is controlled as described in Table 2-4 and additionally in APPENDIX 2: PRODUCT SPECIFICATION SHEET. All product testing is compliant with guidelines set by the International Oenological Codex of the OIV and the regulation EC 606/2009. This statement can be found in APPENDIX 9: OENOLOGICAL CODEX OF OIV – VINIFLORA. Additionally, heavy metals including Arsenic, Lead, Cadmium, and Mercury are controlled per the Commission Regulation (EC) No. 1881/2006 and International Oenological Codex (OIV-Oeno 328-2009) as seen in Table 2-5 It should be noted that cultures are generally considered foodstuffs

in the EU, but wine cultures are not specifically mentioned. Subsequently, Chr. Hansen sets our chemical contaminant standards as the lowest limits that are mentioned for heavy metals.

Table 2-4: Purity Specifications for Wine Bacterial Cultures

Microorganism	Criteria (CFU/g unless otherwise stated)	Frequency of Testing
Acetobacter	<100	Every Batch
Enterobacteriaceae	<100	Every Batch
Moulds	<10	Every Batch
Non lactic acid bacteria	<500	Every Batch
Yeasts	<10	Every Batch
Listeria monocytogenes	Absent in 25g	Per Monitoring Program
Salmonella spp	Absent in 25g	Per Monitoring Program

Table 2-5: Chemical Contamination Limits for Wine Bacterial Cultures

Contaminant	EU Limits (Except Arsenic) as Stated in the Commission Regulation (EC) No. 1881/2006 for final food products (values in mg/kg)	Limits According to the International Oenological Codex on Lactic Acid Bacteria use in Onelogy (OIV-Oeno 328-2009) (values in mg/kg)
Arsenic (As)	<3*	<3
Lead (Pb)	<0.20	<2
Cadmium (Cd)	<0.05**	<1
Mercury (Hg)	<0.1**	<1

* Chr. Hansen uses the limit valid for enzymes, regulated by French legislation: Arreté du 19 octobre 2006 relatif á l'emploi d'auxiliaries technologiques dans la fabrication de certains denrées alimentaires Article Annexe II
**No limit applies to wine cultures or wine products, so the lowest limit for food products is the standard

2.6.5 Product stability

L. plantarum DSM 33452 frozen culture has a shelf life of 12 months if stored according to recommendations (≤ -45^{o}C) and frozen pellets are transported in dry ice. The freeze-dried product has a shelf-life of 36 months from the date of manufacture when stored at <-18^{o}C or a shelf life of 6 months if stored at 5^{o}C in the original or tightly closed foil pouch.

The genetic stability of *L. plantarum* DSM 33452 is demonstrated by DNA fingerprinting and plasmid profiling comparing the reference material for *L. plantarum* DSM 33452 with inoculation material produced in 2013. Chr. Hansen's reference stock material and inoculation material produced in 2013 show identical fingerprints and plasmid profiles as shown in figure 2-1. The genetic stability of *L. plantarum* DSM 33452 shows that the strain safety analysis will hold true over time.

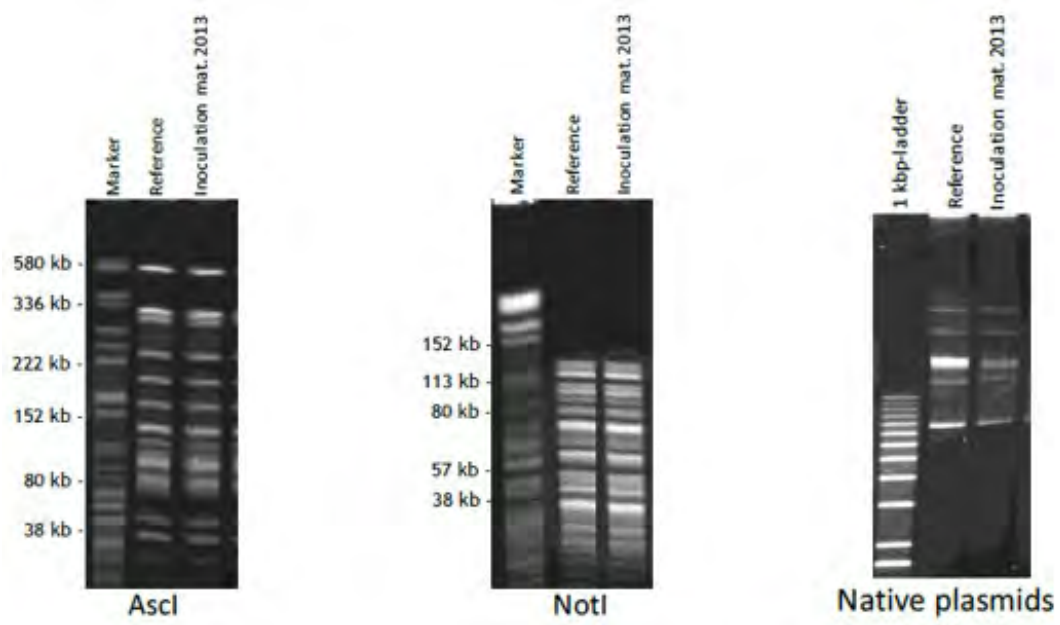

Figure 2-1: Asc1 and Not1 fingerprints and plasmid profiles for the DSM 33542 reference and inoculation material produced in 2013.

2.7 Intended Technical Effect & Amount Required

Traditionally, the production of red wine requires two fermentation steps. During the first fermentation, yeasts ferment sugars to produce ethanol and some flavors. The second fermentation step is performed by lactic acid bacteria and turn malic acid into lactic acid providing a fuller flavor profile.

There are several benefits to the wine maker when malolactic fermentation occurs prior to alcohol fermentation. Malolactic fermentation takes far less time if it occurs prior to alcohol fermentation which allows faster tank turnover and minimizes the risk of microbial contaminants such as errant lactic acid bacteria, acetic acid bacteria, molds, or yeasts. Since *Lactobacillus plantarum* DSM 33452 prefers to metabolize malic acid over sugars, the addition of this culture also enhances fruit complexity and overall wine aroma.

Lactobacillus plantarum DSM 33452 is intended to be used for malolactic fermentation in grape juice with low to moderate malic acid levels (<2.5 g/L). The grape juice also should be produced with the thermovinification procedure. *L. plantarum* DSM 33452 is intended to be inoculated into must at an inoculation level of 1.0E+07 CFU/g at the time of crushing grapes (as long as there is no acid addition) or as early as possible in the fermentation tanks. It should be noted that *L. plantarum* DSM 33452 is sensitive to total sulfites (<15ppm), pH (<3.4), and ethanol concentration (4-5%). Since *L. plantarum DSM 33452* is sensitive to alcohol concentration, the organism will die as the concentration of alcohol increases.

Because of its sensitivity, it is recommended to inoculate grape juice with the following conditions (also see Appendix: Product Information sheet)

Table 2-6: Physiological Parameters at Inoculation

Inoculation Temperature Range	20-25°C (68-77°F)
pH minimum*	3.4
Total SO2 maximum at inoculation*	5 ppm
Alcohol maximum*	5% volume

*note that these inhibitory factors are antagonistic towards each other. The individual tolerances are valid only if other conditions are favorable. The level of sulfite produced by the yeast used for primary fermentation as well as free sulfite should be checked.

2.7.4 Application and Plant Safety

Lactobacillus plantarum DSM 33452 is intended to be added into the product at the time of crushing or into the vat early in fermentation. Good industrial hygiene practices should be followed when handling and storing the product. This includes wearing safety glasses when handling frozen culture (see APPENDIX: SAFETY DATA SHEET). There are no known hazards towards workers.

There are no labeling requirements for this strain, however please consult with local legislation if in doubt.

3 Dietary Exposure

L. plantarum DSM 33452 is intended to be used to assist in malolactic fermentation contributing to flavor development in the production of wine and must. It is intended to be inoculated at 1.0E+07 CFU/g prior to alcohol fermentation. This strain is sensitive to alcohol, so as the alcohol content rises *L. plantarum* dies. Though ABV can vary based on a variety of factors, red wine typically has above a 6.5% alcohol by volume. *L. plantarum* cannot survive alcohol contents above 5%. *L. plantarum* is not viable in the finished product and does not contribute to lactic acid bacteria in dietary exposure.

If by chance, *L. plantarum* were to survive alcoholic fermentation, it is known that the adult microbiome is very stable and only shifts with significant dietary changes or extreme weight loss (Faith, et al., 2013). *L. plantarum* are commonly found in the human oral cavity and intestinal tract of healthy individuals (Stiles & Holzapfel, 1997) (Holt & et al., 1994) and lactic acid bacteria, including *Lactobacillus plantarum,* are found in high numbers in most of the fermented foods we consume daily from yogurt to sauerkraut to sausage. The addition of *L. plantarum* DSM 33452 to wine would not cause a significant increase in the gut or in the diet and therefore, need not be calculated.

4 Self-Limiting Levels of Use

The proposed use of *L. plantarum* DSM 33452 is as a food ingredient to be inoculated directly into grape juice or must with less than 1% ethanol v/v to perform a fast and safe malolactic fermentation in most red and white musts. The self-limiting levels of use are:

- cGMP – Following the use level prescribed by Chr. Hansen, *L. plantarum* DSM 33452 will only be added to the product at levels required to achieve the technical effect in wine and musts. There would be no benefit to the customer to add the product at a higher level.
- Alcohol maximum – *L. plantarum* DSM 33452 is sensitive to the level of alcohol and will not be able to survive alcohol content above 5.0 percent by volume. As fermentation progresses, *L. plantarum* will be limited by the building alcohol content in the finished product.

5 Experience Based on Common Use in Food

The conclusion of this GRAS notification regarding *L. plantarum* DSM 33452 is based on scientific data and procedures and not common use in food before 1958.

6 Narrative

In the following sections, the data and information providing the basis for our conclusion that *L. plantarum* DSM 33452 is GRAS, through scientific procedures, under the conditions of its intended use is presented. The information provided below, and elsewhere in this document that is generally available has been properly cited. Chr. Hansen has rigorously applied the decision tree recommended by Pariza *et al.* (Pariza, Gillies, Kraak-Ripple, & Leyer, 2015) and has taken into consideration the risk assessment conducted by EFSA per the Qualified Presumption of Safety (QPS) approach for the determination of the safety of *L. plantarum* DSM 33452.

6.1 History of consumption of *L. plantarum* DSM 33452

Consumption of lactic acid bacteria including *L. plantarum* has occurred for longer than recorded history. *L. plantarum* can be found in high numbers in most foods that have been fermented with lactic acid bacteria, especially when the fermented food is plant based such as brined olives, sauerkraut, salted gherkins, sourdough, Nigerian ogi (made from maize or sorghum), Ethiopian Kocho, Ethiopian sourdough made from teff, and cassava. People who are eating food that has been fermented with lactic acid bacteria are subsequently consuming large numbers of *L. plantarum.* (Molin, 2003)

The fermentation of grapes into wine has incidentally been with us since the dawn of civilization. Grapes would get damaged while being picked, sit in a container and rudimentary wine would be produced from endogenous flora present on the grape skins (Chambers & Pretorius, 2010). Through cultivation, it was found that if malolactic fermentation occurred resultant wine would be less acidic. Malolactic fermentation (MLF) is a secondary fermentation turning malic acid into lactic acid and carbon dioxide and results in less acidic wine and also positive sensory changes (Lonvaud-Funel, 2010). In 1891, Hermann Muller-Thurgau attributed bacteria to less-acidic wine (malolactic fermentation) and in 1900, Robert Koch intentionally inoculated wine with bacteria to show that he could reduce the wine's acidity (Chambers & Pretorius, 2010).

Though *Oenococcus oeni* has been reported as a key player in malolactic fermentation, *L. plantarum* has also been used as a starter culture for MLF and has shown to have some advantages over *O. oeni* including survival in harsher conditions and providing additional aromatic compounds to be produced (Brizuela, et al., 2018).

In conclusion, *L. plantarum* DSM 33452 is safe for consumption and for this intended use of malolactic fermentation in wine and musts.

6.2 Safety of *L. plantarum* DSM 33452

The genus *Lactobacillus* has been the subject of several safety assessments and has in every case been found to be a safe genus with limited concern regarding adverse effects (Salminen & Tuomola, 1998)

(Borriello, et al., 2003) (Bernardeau, Vernoux, Henri-Dubernet, & Gueguen, 2008) (Bernardeau, Guguen, & Vernoux, 2006).

Lactobacillus including *L. plantarum* are widely used as probiotics in the dairy industry where they have a long history of safe use (Bourdichon, et al., 2012). There have been extremely rare cases of morbidity linked to the consumption of *Lactobacillus rhamnosus* as a probiotic; two cases of sepsis in preterm infants (Dani, et al., 2015) and two cases of *Lactobacillus spp.* abscesses in immunocompromised elderly patients (Saarela, Matto, & Mattila-Sandholm, 2002). After extensive literature searches, we were unable to find any cases in which the consumption of *L. plantarum* was linked to infection.

Opportunistic infections by *Lactobacillus* spp. are described but they are extremely rare and restricted to severely immuno-compromised individuals (Saarela, Matto, & Mattila-Sandholm, 2002) (Sullivan & Nord, 2006) (Bernardeau, Vernoux, Henri-Dubernet, & Gueguen, 2008) (Salminen, et al., 2004) (Dani, et al., 2015) (Doron & D.R., 2015). Infection or pathology linked to *L. plantarum* species is even more rare. In a review of 89 cases of patients with *Lactobacillus* bacteremia, *L.* plantarum was only found as the infecting organism one time in a case of endocarditis stemming from poor oral hygeine. In the same study, it was noted that 82% of patients had severe or fatal comorbidities. (Salminen, et al., 2004). A second review followed 45 cases of *Lactobacillus* bacteremia over 15 years. The conclusions mirrored the results of the Salminen study. The researcher concluded that *Lactobacilli* are relateively avirulent pathogens that produce bacteremia in patients with serious underlying illnesses and have received antibiotic therapy that may select out for the organism (Husni, Gordon, Washington, & Longworth, 1997). In both of these reviews, *L. plantarum* infection was only found as an opportunistic infection and was not linked to its consumption in food or as a food ingredient.

6.3 Recognition of Safety by an Authoritative Group of Qualified Experts

EFSA assesses the safety of microorganisms used in food and feed, before they are authorized for use in the European market. The Authority introduced the concept of qualified presumption of safety (QPS) to harmonize the safety evaluation of microorganisms used as food or feed additives, food enzymes, novel foods or pesticides. The list of QPS recommended biological agents is updated annually, with the latest version being released in May 2020 (EFSA, 2020). To be granted QPS status, a microorganism must have a well-defined taxonomic identity, the available body of knowledge must be sufficient to establish safety, the lack of pathogenic properties must be established and substantiated, and its intended use must be clearly described (EFSA, 2020). Essentially, QPS status is given if the taxonomic group does not raise safety concerns. Absence of specific characteristics such as transmissible antibiotic resistance, food poisoning toxins, surfactant activity and enterotoxin activity are required for QPS status. Additionally, EFSA carries out an extensive literature search every six months to ensure that the list is up to date. In addition, *L. plantarum* is included on the International Dairy Federation (IDF) list of microorganisms with technological beneficial use when it was published in 2012 (Bourdichon, et al., 2012).

The species *L. plantarum* has been evaluated by the EFSA Panel on Biological Hazards (BIOHAZ) and found to be suited for the QPS (Qualified Presumption of Safety) status since the start in 2007 (EFSA, 2007; EFSA BIOHAZ Panel, 2017). The inclusion of the species on this list provides a strong foundation of support that any strains of *L. plantarum* are safe for human consumption.

L. plantarum has been assessed for food safety domestically as well as internationally. There are four relevant notified GRAS dossiers for various strains of *L. plantarum* per (U.S. Food and Drug Administration, 2020) as listed in Table 6-1 below. Though these are different strains of *L. plantarum*, it illustrates that the species *L. plantarum* is safe for human consumption in many kinds of food applications.

Table 6-1 Lactobacillus plantarum has received no objection letters for multiple GRAS notices illustrating the safety of the species

GRN No.	Intended Use	Date of Closure
847	For use as an ingredient in foods generally, excluding infant formula and foods that would require additional regulatory review by USDA, at levels up to 1 x 10^{10} CFU per serving.	Sep 30, 2019
722	For use as an ingredient in yogurt and other dairy products; soy products; beverages; chewing gum, confectionary snacks and other foods at levels not more than 5 x 10^{11} CFU/serving.	Feb 16, 2018
685	Intended for use as a probiotic microorganism in conventional foods at a use level up to 10^{11} CFU per serving	Oct 31, 2017
378	As antimicrobial agents in a variety of food categories typically at levels of 0.1 to 4.5%, including meat and poultry, but excepting infant formula and infant foods	Mar 26, 2012

As is mentioned in the Pariza *et al.* publication (2015), experts have asserted that "microorganisms listed on the IDF and EFFCA/QPS inventories meet the criteria for GRAS for their traditional uses". As *L. plantarum* traditionally is used in many kinds of fermented food products, it is not novel to think of it as an ingredient added to wine and must fermentations. Using the decision tree proposed by Pariza *et al*., it can be concluded that *L. plantarum* DSM 33452 is safe for use in the applications presented in this GRAS notice.

6.4 *L. plantarum* DSM 33452 is safe

6.4.1 *L. plantarum* does not produce biogenic amines

Biogenic amines in foods are known to be toxic and can cause an allergic reaction, especially in susceptible individuals. The aromatic amines histamine and tyramine are considered the most toxic and relevant to food safety. Fermented foods are of particular concern for biogenic amines due to associated intensive microbial activity and biogenic amine formation (EFSA Panel on Biological Hazards, 2011). The biogenic amines cadaverine, and putrescine are not as toxic, but may enhance the toxicity of the aromatic amines by blocking their metabolism or increasing their absorption (Cid, Miguelez-Arrizado, Becker, Holzapfel, & Vidal-carou, 2008). The Pariza decision tree demands that biogenic amine production must be taken into consideration in the selection and implementation of food cultures to reduce toxicological risks (Pariza, Gillies, Kraak-Ripple, & Leyer, 2015).

It should also be noted that during malolactic fermentation, indigenous bacteria often produce biogenic amines from amino acids. *L. plantarum* DSM 33452 has been selected for malolactic fermentation because it is unable to produce biogenic amines. Furthermore, since *L. plantarum* speeds up malolactic fermentation, other indigenous organisms that could produce biogenic amines are less likely to grow.

L. plantarum DSM 33452 was tested for histamine, tyramine, cadaverine, and putrescine. The results showed that *L. plantarum* DSM 33452 does not produce these four biogenic amines of concern.

6.4.2 *L. plantarum* is susceptible to antimicrobial agents tested and does not encode transmissible antibiotic resistance genes

It is important to verify that culture strains used as food cultures do not present transferable antimicrobial resistance. This is also criterion number 4 of the Pariza decision tree (Pariza, Gillies, Kraak-Ripple, & Leyer, 2015). Minimum inhibitory concentrations (MIC) of nine antibiotics were determined for *L. plantarum* DSM 33452 according to the ISO 10932/IDF 223 international standards. The results showed that *L. plantarum* DSM 33452 was susceptible to most antimicrobial agents tested with the exception of vancomycin for which *L. plantarum* species is intrinsically resistant. *L. plantarum* cannot transfer this vancomycin resistant characteristic to other microorganisms; including pathogenic species (Danielsen & Wind, 2003)

The genome sequence of the *L. plantarum* DSM 33452 strain was analyzed *in silico* for the presence of known antibiotic resistance genes and it was found that *L. plantarum* DSM 33452 does not contain any antibiotic resistance genes.

In conclusion, resistance to antibiotics is not considered a safety concern for *L. plantarum* DSM 33452.

6.4.3 *L. plantarum does not produce citrulline, an ethyl carbamate pre-cursor*

Ethyl carbamate (EC), also referred to as urethane, is classified as a probable human carcinogen (Group 2A by the Int. Agency for Research on Cancer (IARC) in 2007. EC is formed in alcoholic beverages and fermented foods during fermentation process and/or during storage. The presence of EC primarily results from the reaction of ethanol and EC precursors (compounds containing carbamyl groups) which include urea, citrulline, carbamyl-phosphate, and allantoin (Ough, Crowell, & Mooney, 1988).

The key reaction for EC formation is between urea and ethanol, but lactic acid bacteria can also be a source of citrulline under winemaking conditions. Lactic acid bacteria can metabolize arginine to ornithine during malolactic fermentation with citrulline as an intermediary product. In wine LAB, the degradation of arginine occurs through the arginine deiminase pathway, catalyzed by inducible arginine deiminase, ornithine transcarbamylase and carbamate kinase. The first step is the conversion of arginine to citrulline by arginine deiminase. (Liu & Pilone, 1998) concluded that the ADI pathway is the exclusive route of arginine catabolism in wine. In general, lactobacilli from the wine environment do not seem to harbor this pathway for arginine degradation. Citrulline may also promote the growth of undesirable lactobacilli and is therefore unwanted.

Spontaneous malolactic fermentation by undefined strains can increase levels of EC precursors or EC in wine. When malolactic fermentation is desired and to avoid EC or it's precursors, winemakers may use a commercial strain that does not produce high levels of citrulline.

L. plantarum DSM 33452 strain has been examined for its ability to produce citrulline. It was found that the strain does not harbor the arginine deiminase gene and thus cannot degrade arginine to citrulline (ADI negative). In addition, the concentration of arginine and citrulline is not changed during incubation of DSM33452 in wine must at winemaking conditions showing that arginine is not degraded to citrulline.

It is concluded that *L. plantarum* DSM33452 does not produce citrulline and does not contribute to EC in wine.

6.5 Conclusion of GRAS status

Chr. Hansen has applied the framework of the Pariza *et al.* decision tree to determine safety of *L. plantarum* DSM 33452 for consumption in human food. The data presented in this document fully supports the conclusion that *L. curvatus* DSM 33452 is GRAS for the intended use as described. The basis for this conclusion can be summarized in the following five main points.

- The publicly available scientific literature documents the history of safe use of this microorganism in fermented foods as well as being found as part of the endogenous flora of the food matrix in which Chr. Hansen intends for it to be used.
- *L. plantarum* DSM 33452 is not genetically modified, is not able to produce the four most prevalent biogenic amines, does not carry any transferrable genes coding for antibiotic resistance and do not produce the EC precursor citrulline.
- Chr. Hansen's manufacturing and quality control programs ensure the safety and quality of the final *L. plantarum* DSM 33452 product.
- The estimated daily intake of *L. plantarum* DSM 33452 does not increase the overall intake of lactic acid bacteria in the diet.
- *L. plantarum* has been evaluated and deemed safe and nonpathogenic by qualified individuals including the FDA, EFSA per the QPS approach, and has been included in the IDF list since 2012.

6.6 Pariza Decision Tree Analysis

As indicated above, in assessing the safety of *Lactobacillus plantarum* DSM 33452, Chr. Hansen has consulted and applied the Pariza et al. "Decision Tree for Determining the Safety of Microbial Cultures to be Consumed by Humans or Animals" (2015). The decision tree is composed of thirteen questions which, when applied, provide a "comprehensive approach for determining the safety of microbial cultures that lack an established history of safe use for their intended new application." This approach is described below:

Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology?

YES (go to 2)

Has the strain genome been sequenced?

YES (go to 3)

Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity?

YES (go to 4)

Is the strain genome free of functional and transferable antibiotic resistance gene DNA?

YES (go to 5)

Does the strain produce antimicrobial substances?

NO (go to 6)

Has the strain been genetically modified using rDNA techniques?

NO (go to 8a)

Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component?

YES (go to 9a)

Has the species, to which the strain belongs, undergone a comprehensive peer-reviewed safety evaluation and been affirmed to be safe for food by an authoritative group of qualified scientific experts?

YES (go to 10a)

Do scientific findings published since completion of the comprehensive peer-reviewed safety evaluation cited in question 9a continue to support the conclusion that the species, to which the strain belongs, is safe for use in food?

YES (go to 11a)

Will the intended use of the strain expand exposure to the species beyond the group(s) that typically consume the species in "traditional" food(s) in which it is typically found?

NO (go to 12a)

Will the intended use of the strain expand intake of the species?

NO (go to 14a)

The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.

Chr. Hansen concludes that the intended use of *Lactobacillus plantarum* DSM 33452 is GRAS based on the information presented.

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Viniflora® NoVA™

Product Information

Version: 4 PI GLOB EN 05-26-2017

Description

Viniflora® NoVA™ is a frozen concentrated pure culture of Lactobacillus plantarum. It is a homofermentative malolactic bacteria in must and wine conditions which has been selected to ensure a fast and safe malolactic fermentation in must. It has therefore a very low tolerance to alcohol.

The Viniflora® NoVA™ culture is ready for inoculation directly into grape juice or musts without previous reactivation.

The culture can be used both in red and white wines.

Culture composition:
Lactobacillus plantarum.

Material No:	712535	Color:	Off-white to slightly brown
Size	6X5000 L	Format:	F-DVS
Type	Bag(s) in box	Form:	Frozen pellets

Storage

< -45 °C / < -49 °F

Transport condition

The frozen cultures should be transported using dry ice, with a maximum transit time of 72 hours.

Shelf life

When stored according to recommendation the product has a shelf life of 12 months.

Application

This culture has been selected for its overall outstanding performance and capability to perform a fast and safe malolactic fermentation in most red and white musts. Among the features are:

o Direct inoculation into grape juice or must with less than 1% ethanol v/v
o High numbers of active cells which ensure a quick start of fermentation
o High level of microbiological purity
o Low production of volatile acidity
o Does not produce biogenic amines*

*During malolactic fermentation, indigenous bacteria often produce biogenic amines from amino acids. This strain of malolactic bacteria has been selected using state-of-the-art techniques in screening, analyses or production to deliver malolactic cultures unable to produce the following biogenic amines: histamine, tyramine, putrescine, phenylethylamine, isoamylamine, cadaverine.

For further information about biogenic amines in wines and how to avoid them please consult the OIV code of good vitivinicultural practices to minimize biogenic amines: http://www.oiv.int/oiv/info/enguidesoiv#biogenic

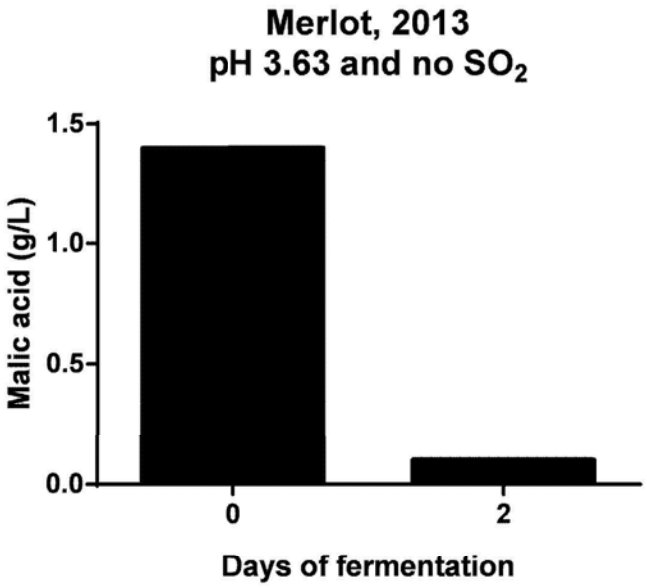
Directions for use

This frozen culture should always be inoculated directly into the wine. No rehydration, reactivation or acclimatization is required.

Remove cultures from the freezer just prior to use to ensure optimal performance. Prolonged exposure to temperatures abov -
pellets directly into the wine. Make sure the culture is added to the liquid phase of the wine, and that the culture is completely dissolved in the wine. For more information on inoculation, please contact your local Chr. Hansen Representative to acquire the appropriate inoculation protocol.

Technical Data

Viniflora® NoVA™ degraded the malic acid of the grape juice in 2 days, from 1,4 g/L to 0,1 g/L.



Physiological data

Inoculation temperature range	20-25°C (68-77°F)
pH minimum*	3.4
Total SO_2, max. at inoculation*	5 ppm
Alcohol maximum *	5 % vol

** note that these inhibitory factors are antagonistic towards each other.*
The individual tolerances are valid only if other conditions are favourable.
Check level of SO_2 produced by the yeast used for primary fermentation and be aware of level of free SO_2.

Legislation
The product is intended for food use as an œnological product and complies with the current International Oenological Codex. 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.

The product is intended for food use.

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. Good Manufacturing Practise (GMP) is implemented in all plants manufacturing Chr. Hansen cultures. Chr. Hansen has made a risk assesment of microbiological, physical and chemical risks in our manufacturing and distribution plants for dairy, wine and meat cultures. Control points (CP´s) and Critical Control Points (CCP´s) are based on the risk assesment. A HACCP team as well as HACCP plans are established for each plant.

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 used under license. Trademarks appearing in this document may not be registered in your country, even if they are marked with an ®.

Additional Information
Check the latest news on www.chr-hansen.com/food-cultures-and-enzymes/wine

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* Viniflora® NoVA™ 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 Viniflora® NoVA™ 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.

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*

Viniflora® NoVA™

Product Specification

Form:	Frozen DVS
Material No:	712535
Culture Composition:	Lactobacillus plantarum

Performance	**Specification**
Total cell count/pouch cfu/pouch	>=2E+13

Purity	**Specification**
Acetobacter cfu/g	<100
Enterobacteriaceae cfu/g	<100
Moulds cfu/g	<10
Non lactic acid bacteria cfu/g	<500
Yeasts cfu/g	<10
Listeria monocytogenes *	Absent in 25 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.

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

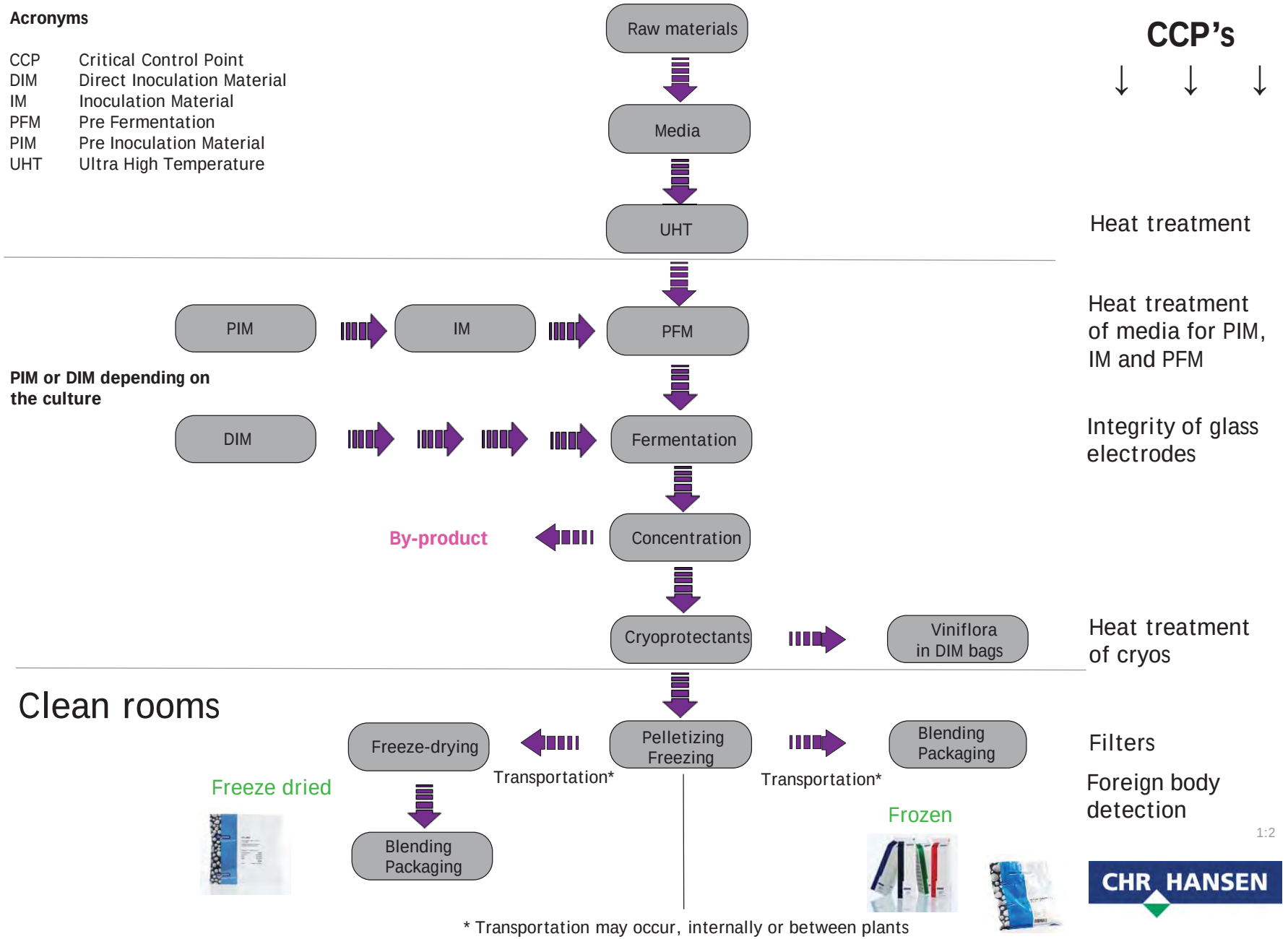
Electronically generated - signature not required

Global production process flow – bacterial wine cultures

December 3, 2018
Valid three years from date of issue

Acronyms

CCP	Critical Control Point
DIM	Direct Inoculation Material
IM	Inoculation Material
PFM	Pre Fermentation
PIM	Pre Inoculation Material
UHT	Ultra High Temperature



1:2

CHR. HANSEN

Global culture production - bacterial wine cultures
Critical Control Points (CCP's)

FSSC 22000 standard

- Each plant comply 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 is available from our website: www.chr-hansen.com
- On top of that, each plant has an appointed local OPRP (Operational Pre-Requisite Program), that includes PRP issues, which need to be documented, and are classified as specifically critical for the food safety.
- The following CCP's (Critical Control Points) are global, and per site adopted to the local equipment and processes:
  - Glass electrodes in fermentors
  - Heat treatments (UHT, Autoclaving, Heat-in-place)
  - Filters
  - Foreign body detection (metal detection or X-ray)

Chr. Hansen A/S -10-12 Bøge Allé - DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 www.chr-hansen.com

CHR. HANSEN

Statement

March 27, 2020
Valid two years from date of issue

To whom it may concern

Shipment of freeze dried bacterial wine cultures at ambient temperatures

Thank you for your inquiry into Chr. Hansen's products.

In general freeze dried products are microbiologically stable, due to the low water content, and therefore Chr. Hansen's freeze-dried bacterial wine cultures are shipped at ambient temperatures. However shipment at 15-30ºC (59-86°F) should not exceed 10 days, as this may compromise product quality.

If the cultures are stored as recommended, at -18º C (0º F) or below, Chr. Hansen guarantees a shelf life according to the information given in the Product Information sheets. Furthermore, we guarantee a shelf life of 6 weeks at 5º C (41º F). Once a pouch has been opened, Chr. Hansen recommends that the product is used at once, to guarantee shelf life.

If you have further questions, please contact us.

Yours sincerely
Chr. Hansen A/S – Food Cultures & Enzymes
Global Business Support

Electronically generated, therefore not signed

Chr. Hansen A/S -10-12 Bøge Allé - DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 www.chr-hansen.com

Viniflora® NoVA™

Allergen Information

Material No: 712535

Version: 4 AL EN 02-11-2016

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

F-DVS Viniflora® NoVA™

Version: 2 GHS / EN
Revision Date: 08-04-2015

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

1.1 Product identifier

Product name:	F-DVS Viniflora® NoVA™
Material No:	712535

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

Application:	For wine 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: bacteria.

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.

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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.

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9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance:	Frozen pellets
Color:	Off-white to slightly brown
Odor:	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.

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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.

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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: None known, unless listed below.
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)

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16. OTHER INFORMATION

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

Wording of Hazard Statements
-

BUREAU VERITAS
Certification

Chr. Hansen France SAS

Le Moulin d'Aulnay, BP 64, 91 292 ARPAJON Cedex, France

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 (multiplication, extraction, pelletization) and filling in plastic bags of frozen dairy cultures.

Product category: CI

Certification cycle start date:	22-09-2017
Subject to the continued satisfactory operation of the organization's Management System, this certificate expires on:	21-09-2020
Original certification date:	22-09-2011
Certificate No./Version:	DK009976-2
Contract No.	10278474
Issue date:	26-11-2018

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

MIX Paper from responsible sources FSC® C074440

BUREAU VERITAS
Certification

Chr. Hansen A/S Avedøre Culture

Jernholmen 1-27, 2650 Hvidovre, Denmark

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 food, dietary supplements and infant products.

Product category: K

Certification cycle start date: Subject to the continued satisfactory operation of the organization's Management System, this certificate expires on:	23-11-2018 22-11-2021
Original certification date:	23-11-2012
Certificate No./Version:	DK010327
Contract No.	10543656
Issue date:	14-11-2018

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

MIX Paper from responsible sources FSC® C074440

Statement

November 5, 2018
Valid three years from date of issue

To whom it may concern

Viniflora® range - compliance with the International Oenological Codex and EU regulation

Thank you for your inquiry into Chr. Hansen's products.

The Viniflora® range of malolactic bacterial cultures based on strains of the species *Oenococcus oeni* and *Lactobacillus plantarum* are meant for direct human consumption, and comply with the standards and guidelines laid down in the International Oenological Codex of the OIV, as well as the Regulation EC 606/2009.

If you have further questions, please contact us.

Yours sincerely
Chr. Hansen A/S – Food Cultures & Enzymes

Trine Hansen
Business Support Specialist

Electronically generated, therefore not signed

Chr. Hansen A/S -10-12 Bøge Allé - DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 www.chr-hansen.com

From:	Arie Carpenter
To:	Hice, Stephanie
Cc:	Kate Urbain
Subject:	RE: GRN 000946 - Questions for Notifier
Date:	Friday, November 20, 2020 3:10:23 PM
Attachments:	image001.png 2020-11-20 GRN 946 FDA Answers.pdf CoA_Viniflora_NoVA__712535_3355396_EN.pdf CoA_Viniflora_NoVA__712535_3412837_EN.pdf CoA_Viniflora_NoVA__712535_3439982_EN.pdf CoA_Viniflora_NoVA__712535_3474497_EN.pdf CoA_Viniflora_NoVA__712535_3537681_EN.pdf

Hi Dr. Hice,

Please find attached answers to the questions that you presented November 6th regarding GRAS Notice No. 000946 as well as 5 non-consecutive COA's for L. plantarum.

Please let me know if you have any further questions.

Thanks so much,

Arie Carpenter
Sr. Regulatory Affairs Specialist, Food Cultures and Enzymes
Cell: 414-544-2317 Desk: 414-777-7526
usarbr@chr-hansen.com | www.chr-hansen.com

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Friday, November 6, 2020 2:36 PM
To: Arie Carpenter <USARBR@chr-hansen.com>
Subject: GRN 000946 - Questions for Notifier

Dear Ms. Carpenter,

During our review of GRAS Notice No. 000946, we noted further questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

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

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

Division of Biotechnology and GRAS Notice Review
Center for Food Safety & Applied Nutrition (HFS-255)
U.S. Food & Drug Administration
Dr. Stephanie Hice
5100 Campus Drive, College Park, MD 20740

Chr. Hansen, Inc.
9015 West Maple Street
Milwaukee, WI 53214 - 4298

Telephone: +1 (414) 607 5700
www.chr-hansen.com
info@chr-hansen.com

November 20, 2020
USARBR

Supplement to GRN 946

Dear Dr. Hice,

In response to your email dated November 6, 2020 please accept this supplementary information regarding *Lactobacillus plantarum* DSM strain DSM 33452 for use in the production of wine and musts to increase the rate of malolactic fermentation and to prevent growth of microorganisms that could cause off flavors in the finished product.

We appreciate the opportunity to clarify our position on the safety of *L. plantarum* DSM 33452.

Yours sincerely,

Arie Carpenter
Sr. Regulatory Affairs Specialist
Food Cultures and Enzymes North America
usarbr@chr-hansen.com
Mobile: 414-544-2317

(1) Please note that the term "probiotic" is neither a regulatory term, nor a scientific term, and its use in the notice appears to have context as a marketing term denoting or connotating beneficial effects. We note that the Agency's evaluation of GRAS notices focuses exclusively on the safety of the ingredient in food and not about purported beneficial effects of the substance.

We appreciate your feedback and note that the FDA would not evaluate probiotics or dietary ingredients in a GRAS notice. We emphasize that *L. plantarum* is not intended to be used as a dietary ingredient or probiotic in this notice for use as a food ingredient. The term "probiotic" is simply used in this dossier to mirror the terminology used in the literature cited in this dossier and as the common and usual name for a product category. Further, we note that we refer to probiotics and discuss the probiotic use of the *L. plantarum* species only in the context of demonstrating one of a number of safe uses for human consumption of the species in accordance with FDA guidance on the content of dietary intake assessments of GRAS notified substances wherein the Agency notes that all uses should be taken into account.

(2) In Table 6-1 (page 18), the notifier lists the intended use(s) for GRNs 000722 and 000685 as "For use as an ingredient in yogurt and other dairy products; soy products; beverages; chewing gum, confectionary snacks and other foods at levels not more than 5 x 1011 CFU/serving" and "Intended for use as a probiotic microorganism in conventional foods at a use level up to 1011 CFU per serving", respectively. We note that the intended uses listed in our response letters for GRNs 000722 (February 16, 2018) and 000685 (October 31, 2017) are "… for use as an ingredient in conventional foods, including yogurt and other dairy products, soy products, beverages, chewing gum, and confectionary snacks at 1 x 1010 colony forming units (CFU)/serving" and "… for use as an ingredient in conventional foods at up to 1 x 1010 CFU/serving1", respectively. For the administrative record, please make a statement that corrects this reference.

Please correct Table 6-1 on page 18 of GRN 946 with the following information. The changes have been bolded below for clarity.

GRN No.	Intended Use	Date of Closure
847	For use as an ingredient in foods generally, excluding infant formula and foods that would require additional regulatory review by USDA, at levels up to 1 x 10^{10} CFU per serving.	Sep 30, 2019
722	For use as an ingredient in yogurt and other dairy products; soy products; beverages; chewing gum, confectionary snacks and other foods at levels not more than 1 x $\mathbf{10^{10}}$ CFU/serving.	Feb 16, 2018
685	Intended for use as a probiotic microorganism in conventional foods at a use level up to $\mathbf{10^{10}}$ CFU per serving	Oct 31, 2017
378	As antimicrobial agents in a variety of food categories typically at levels of 0.1 to 4.5%, including meat and poultry, but excepting infant formula and infant foods	Mar 26, 2012

(3) For the administrative record, please provide a description of the phenotypic (e.g., pathogenicity, toxigenicity) characteristics of *Lactiplantibacillus plantarum* strain DSM 33452.

For the administrative record, *L. plantarum* DSM 33452 is a Gram-positive, non-spore forming, homofermentative and non-motile rod able to ferment a wide range of carbohydrates. (Zheng, 2020) *L. plantarum* is non-pathogenic and non-toxigenic as established by its presence on the qualified presumption of safety (QPS) list. (EFSA BIOHAZ Panel, 2020) Chr. Hansen takes the approach for food cultures that once a microorganism belongs to the QPS list we rely in part on QPS evaluation since safety has already been established by a group of qualified experts and is reviewed every 6 months.

The European Food Safety Authority (EFSA) established a systematic approach to assess the safety of microorganisms used in food at a species level. To make it onto the QPS list, a species must meet four criteria:

1. Its taxonomic identity must be well defined
2. The available body of knowledge must be sufficient to establish its safety
3. the lack of pathogenic properties must be established and substantiated
4. Its intended use must be clearly described.

If any safety concerns arise, the species is not granted QPS status. Furthermore, literature reviews are conducted on each species on the QPS list every 6 months; the list is modified if any safety concerns come up. (EFSA, 2020)

(4) For the administrative record, please describe whether *L. plantarum* strain DSM 33452 produces antibiotics.

For the administrative record, while *L. plantarum* DSM 33452 is not known to produce any antibiotics of interest in human or veterinary medicine, the members of the species can produce antimicrobial substances that have been used to inhibit other microorganisms capable of causing foodborne illness or food spoilage (Behera, Ray, & Zdolec, 2018).

(5) Please specify how the purity of the initial inoculum is ensured.

The inoculum material is pure *L. plantarum* DSM 33452. This is ensured by its preparation in a HACCP controlled sterile environment. The seed preparation is tested for purity prior to freezing. During this first purity check, the inoculum is tested for microbial contaminants of safety concern (*Staphylococcus,* yeast and mold, *Salmonella*, etc) but are also tested for cross-contaminants (other lactic acid bacteria that may produce off flavors or textures). A DNA analysis is also run to ensure stability of the strain over time as shown in Figure 2-1 of the dossier. More importantly though, because the inoculum is the starter material for larger fermentations, any microbial contaminant would be amplified and grown along with the intended culture. Each batch of culture is put through rigorous microbiological quality testing before it is sold into the market. As shown in GRN 946 in Table 2-4 (page 13), each lot is tested for contaminants. If any contaminating microorganisms were present in the inoculum, they would be amplified through fermentation and found in the finished product. As part of our quality control program, an investigation is conducted on any lot that does not meet the criteria listed in Table 2-4; an investigation would identify contaminated inoculum material.

(6) Please provide the results from a minimum of three, but preferably five, non-consecutive batch analyses to demonstrate that *L. plantarum* strain DSM 33452 meets the established specifications. In addition, please confirm that all analytical methods, including internally-developed methods, used to test for each specification parameter are validated for that purpose. If using standard methods, please provide complete and appropriate citations.

Please see attached certificates of analysis for 5 non-consecutive batches of *L. plantarum*.

The following table shows references to the test methods used. Some of our testing methods have slight modifications on internationally recognized standard methods. Modifications typically surround dilution or incubation time and have been made based on internal verification work to optimize recovery of contaminants from our extremely concentrated cultures.

It should be noted that this culture has been commercially available since 2014 and Chr. Hansen has never received a customer complaint on the microbial purity of the strain.

Salmonella and Listeria testing is performed using a VIDAS method which is widely known and accepted in the food industry and has been validated according to ISO 16140.

Parameter	Reference Test Method
Acetobacter	OIV-MA-AS4-01
Enterobacteriaceae	ISO 21528-1
Moulds	ISO 6611 | IDF 94
Non-lactic acid bacteria	ISO 13559 | IDF 153
Yeasts	ISO 6611 | IDF 94
Listeria monocytogenes	ISO 11290-1 (ISO 16140)*
Salmonella	ISO 6579 (6) (ISO 16140)**

*VIDAS Listeria is an alternative method to ISO 11290-1 and has been validated according to ISO 16140.
**VIDAS Salmonella is an alternative method to ISO 6579 (6) and has been validated according to ISO 16140.

(7) In Table 2-4 (page 13), the notifier lists the frequency of testing for *Listeria monocytogenes* and *Salmonella* serovars as "Per Monitoring Program". For the administrative record, please clarify what this means.

Based on a risk assessment of our production process, *Salmonella* and *Listeria* are monitored by testing ten percent of culture batches produced along with a robust environmental monitoring program. We ensure that every piece of production equipment is tested on a monthly basis.

(8) Please state whether any of the raw materials used in the fermentation are major allergens or derived from major allergens. If any of the raw materials used are major allergens or derived from major allergens, please discuss why these materials do not pose a safety concern.
In regard to allergens present in raw materials used in fermentation, there are none. The fermentation media essentially only consists of yeasts, sugars, salts, and acids. None of these components contain major allergens identified in FALCPA (Food Allergen Labeling and Consumer Protection Act, 2004).

(9) Please state whether the fermentation process is conducted in a contained, sterile environment.

The fermentation process is conducted in an entirely closed and sterile environment. As stated, production facilities have fully implemented HACCP plans and are FSSC22000 certified.

(10) In Section 2.5.1, the notifier indicates that *L. plantarum* strain DSM 33452 is produced using standard fermentation techniques and includes the use of fermentation and standardizing ingredients that are safe and suitable for use in human food. Please confirm that all processing aids are approved for their respective use via a regulation in Part 21 of the U.S. Code of Federal Regulations, are the subject of an effective food contact notification, or are GRAS for that use in the U.S.

We confirm that when processing aids are present, they are GRAS or approved food additives for their respective uses via regulations found in Part 21 of the U.S. Code of Federal Regulations.

(11) On page 14, the notifier indicates that the grape juice in which *L. plantarum* strain DSM 33452 is intended to be used should be produced using a thermovinification procedure. Please clarify if this procedure is part of the intended use.

The thermovinification procedure is not part of the intended use; it is simply one method by which wine can be prepared in some parts of the world.

(12) In Section 3 (page 15), the notifier indicates that *L. plantarum* cannot survive alcohol contents above 5% and is therefore, not viable in the finished product. Please provide a published reference(s) to support this statement.

Please correct this statement to say that *L. plantarum* DSM 33452 cannot tolerate alcohol contents above 5%. We have found through the scientific literature and in house experimentation that alcohol tolerance is strain specific.

In the scientific literature, the reported alcohol sensitivity of *L. plantarum* varies greatly. There is some literature showing that *L. plantarum* has a much higher resistance to alcohol such as G-Alegria *et al* that shows some strains of *L. plantarum* that can survive in the presence of up to 13% ethanol (G-Alegria, et al., 2004). This data are inconsistent though. Volschenk *et al* report that *L. plantarum* is sensitive to alcohol over 5% volume (Volschenk, van Vuuren, & Viljoen-Bloon, 2006) and Guerzoni *et al* show that *L. plantarum* has a high stress tolerance until approximately 6% ethanol (Guerzoni, Sinigaglia, Gardini, Ferruzzi, & Torriani, 1995). This variation indicates that alcohol tolerance is strain specific and cannot be characterized at a species level.

Internal studies of DSM 33452 are consistent with the papers showing that this strain is sensitive to alcohol concentrations above 5%. Since GRAS notification is on strain level, we simply ask to clarify our original statement by saying that *L.plantarum* DSM 33452 cannot survive alcohol concentrations above 5%.

I would also like to reiterate that even if *L. plantarum* DSM 33452 was entirely viable in the finished product and consumed, it is an organism that is commonly found in the human microbiome of healthy individuals. We consume LAB including *L. plantarum* at high numbers in many of the foods we eat daily (both raw and fermented), so the addition of *L. plantarum* DSM 33452 in wine consumption would not cause a significant increase in the diet as explained in Section 3.

(13) On pages 16-17, the notifier cited several publications that concluded the safety of the genus *Lactobacillus,* without providing any discussions or details (Salminen & Tuomola, 1998; Borriello, et al., 2003; Bernardeau, Vernoux, Henri-Dubernet, & Gueguen, 2008; and Bernardeau, Guguen, & Vernoux, 2006). As part of the notifier's GRAS conclusion, please provide a summary of the publicly available literature supporting the safe use of *L. plantarum*.

Lactobacillus spp. and *L. plantarum* are extremely well researched and documented. Using Google Scholar, we can see the sheer number of papers pertaining to food safety and food fermentation of the genus and species:

Category	*Lactobacillus*	*L. plantarum*
Food safety	158,000	47,900
Fermentation	404,000	88,300
Food	657,000	111,000

While it would be too cumbersome to summarize hundreds of thousands of articles on *Lactobacillus* and how it pertains to foods, the papers above are a few among many that discuss the safe consumption of *Lactobacillus.*

Bernardeau *et al* conclude in their paper "Safety assessment of dairy microorganisms: The *Lactobacillus* genus" that eating organisms from the genus *Lactobacillus* is "not at all hazardous since lactobacillemia induced by food… is extremely rare and only occurs in predisposed patients." They go on to say that the primary safety risk associated with *Lactobacillus* is transferrable antibiotic resistance, which has been addressed in our dossier. (Bernardeau, Vernoux, Henri-Dubernet, & Gueguen, 2008) *L. plantarum* DSM 33452 does not have any genes able to transfer antibiotic resistance.

Prior to the paper mentioned above, Bernardeau *et al* presented a paper in 2006 that reviewed the process by which organisms are deemed safe by the QPS (qualified presumption of safety) approach used by EFSA (European Food Safety Authority) and concluded that Lactobacilli present a negligible biological risk (Bernardeau, Guguen, & Vernoux, Beneficial lactobacilli in food and feed: long-term use, biodiversity and proposals for specific and realistic safety assessments, 2006). The EFSA evaluation is especially robust as the agency experts review each species on its list every 6 months to ensure continued and up-to-date safety for use in food applications (EFSA, 2020).

While we are not presenting *L. plantarum* as a probiotic, we note for the purpose of demonstrating the safety of consumption of the organism that many published papers discuss the safety of *L. plantarum* as a microbial ingredient in probiotic products. This is relevant because, when taken as a probiotic, the dietary intake of the organism would be significantly higher than for use in foods; subsequently any safety risk should be more readily apparent in this higher usage. The last two articles talk about the safety of *Lactobacillus* when consumed as a probiotic. Salminen and Tuomola examined how *Lactobacillus spp.* inhibited pathogens (specifically *E. coli*) by adhering to gut endothelium in an in vitro model for both humans and cows. While they showed varying results for adhesion (it was strain dependent), there were no adverse effects reported (Salminen & Tuomola, 1998).

Borriello *et al* again review clinical documentation searching for cases linked to *Lactobacilli*. They conclude that pathogenicity linked to *Lactobacilli* is extremely rare. They too conclude that

antibiotic resistance genes should be monitored as should clinical cases linked to the consumption when used as a probiotic (Borriello, et al., 2003). Again, we are citing reference to probiotic because the level of consumption of the organism would be significantly higher than the intended use for malolactic fermentation of wine so we would expect to see an increase in pathogenicity as a result of such use should any occur. Also of note, continuous monitoring of *Lactobacillus plantarum* for food is carried out by EFSA through QPS.

Here we can see that the genus *Lactobacillus* as a whole is well documented and considered safe for use in food. *L. plantarum* species are isolated from many different fermented foods including fermented vegetables, meats, dairy products, and fermented cereals. (Zheng, 2020)

In their paper entitled "*Lactobacillus* plantarum with Functional Properties: An Approach to Increase Safety and Shelf-Life of Fermented Foods," Behera *et al*. discuss that *L. plantarum* has an extensive and long history of use in traditional fermented foods globally and is now used in both food fermentation applications and as a microbial ingredient because it can increase safety and shelf-life of fermented foods. They included a table illustrating foods associated with the safe use of *L. plantarum* and reference peer reviewed papers for each use. One of the uses that they cite is for malolactic fermentation of wine (Behera, Ray, & Zdolec, 2018). This table is included in Appendix 1.

References

Behera, S., Ray, R., & Zdolec, N. (2018). Lactobacillus plantarum with Functional Properties: An Approach to Increase Safety and Shelf-Life of Fermented Foods. *BioMed Research International*, 1-18.

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Guerzoni, M., Sinigaglia, M., Gardini, F., Ferruzzi, M., & Torriani, S. (1995). Effects of pH, Temperature, Ethanol, and Malate Concentration on Lactobacillus plantarum and Leuconostoc oenos: Modelling of Malolactic Activity. *Am J Enol Vitic* , 368-374.

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Volschenk, H., van Vuuren, H., & Viljoen-Bloon, M. (2006). Malic Acid in Wine: Origin, Function and Metabolism during Vinification. *S. Afr. J. Enol. Vitic.* , 123-136.

Zheng, e. (2020). A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. *Int. J. Syst. Evol. Microbiol.*, 43-44.

Appendix 1 -Table 2 from (Behera, Ray, & Zdolec, 2018)

Lactobacillus plantarum strain mediated fermented food products.

Fermented foods	Fermentable substrate/source	Identified *Lb. plantarum* strain	Special features/application	Reference
Traditional fermented foods				
Chourico [+]	-	*Lb. plantarum* DSMZ 12028	Inducing proinflammatory response	Cammarota et al. [29]
Tofu	Chinese fermented dairy	*Lb. plantarum* C88	Antioxidant activity	Li et al. [30]
Fufu	Cassava (*Manihot esculenta* Crantz) flour	*Lb. plantarum* strain 6710	Protein-fortified product	Rosales-Soto et al. [31]
White (Baek) kimchi	Chinese cabbage without chili	*Lb. plantarum* HAC01	New probiotic development	Park et al. [3]
Fermented table olives	Spanish-style green olives	*Lb. plantarum* B282	Adhesion and antiproliferative effects of colorectal cancer cells	Saxami et al. [32]
Acid beans	*Vigna unguiculata*	*Lb. plantarum* ZDY2013	EPS	Zhang et al. [33]
Kimchi	Chinese cabbage	Enriched with *Lb. plantarum* Ln4	Probiotic effect	Son et al. [34]
Kimchi	Baechu (napa cabbage)	*Lb. plantarum* wikim 18 (KFCC 1188P)	Probiotic effect	Jung et al. [35]
Korean kimchi		*Lb. plantarum* LBP-K10	Antimicrobial activity	Kwak et al. [36]
Cabbage pickle	Korean cabbage	Cell-free supernatant of *Lb. plantarum* NTU 102	Effective against *V. parahaemolyticus* BCRC 12864 and *Cronobacter sakazakii* BCRC 13988	Lin and Pan [9]
Chicken sausage	Minced meat	*Lb. plantarum*	Antioxidant activity	Yadav, [37]
Kunu [ψ]	Millet *(Pennisetum glaucum)*	*Lb. reuteri, Lb. plantarum,* and *Lb. acidophilus*	Enhanced nutrient qualities, shelf-life, and antioxidant potentials	Adedire et al. [38]

Fermented foods	Fermentable substrate/source	Identified *Lb. plantarum* strain	Special features/application	Reference
Pickled cabbage	Cabbage	*Lb. plantarum* ATCCI 4917	-	Turpin et al. [39]
Novel fermented foods				
Fermented oat food	-	*Lb. plantarum* UFG9; *Lb. plantarum* B2	Increased riboflavin (VitB2) concentration	Russo et al. [40]
Fermented soymilk	Soybean	*Lb. plantarum* TWK10	Antimelanogenic property	
Pinot noir wine (Patagonian red wines)	-	*Lb. plantarum* ATCC 14917	Malolactic starter cultures	Brizuela et al. [41]
FRGE	Korean ginseng (*Panax ginseng* Meyer)	*Lb. plantarum* KCCM 11613P	Antioxidant activity	Jung et al. [42]
Litchi juice	Litchi (*Litchi chinensis* Sonn.)	Spray drying of probiotic bacteria (*Lb. plantarum* MTCC 2621) with prebiotics*	Stimulated the digestive system	Kalita et al. [43]
Bread	-	*Lb. plantarum* P8	Improved baking conditions and storage	Zhang et al. [44]

FRGE: fermented red ginseng extract; EPS: exopolysaccharide.•Fructooligosaccharide (FOS), inulin, gum arabic, and pectin. ⁺Portuguese dry fermented sausage. ᵠNonalcoholic beverage of Nigeria.

Viniflora® NoVA™

Certificate of Analysis

Form:	Frozen DVS
Material No:	712535
Batch no:	3412837
Date of Manufacture:	06.2018
Best Before Date:	06.2019

Performance	**Result**	**Specification**
Total cell count cfu/g	7.5E+10	-
Total cell count/pouch cfu/pouch	6.4E+13	>=2E+13

Purity	**Result**	**Specification**
Acetobacter cfu/g	<10	<100
Enterobacteriaceae cfu/g	<100	<100
Moulds cfu/g	<10	<10
Non lactic acid bacteria cfu/g	<10	<500
Yeasts cfu/g	<10	<10
Listeria monocytogenes *	* See note below	Absent in 25 g
Salmonella spp. *	* See note below	Absent in 25 g

* Production is systematically tested on an ongoing basis - details can be supplied on request

Electronically generated - signature not required

Viniflora® NoVA™

Certificate of Analysis

Form:	Frozen DVS
Material No:	712535
Batch no:	3474497
Date of Manufacture:	06.2019
Best Before Date:	06.2020

Performance	**Result**	**Specification**
Total cell count cfu/g	1.0E+11	
Total cell count/pouch cfu/pouch	8.5E+13	>=2E+13

Purity	**Result**	**Specification**
Acetobacter cfu/g	<10	<100
Enterobacteriaceae cfu/g	<100	<100
Moulds cfu/g	<10	<10
Non lactic acid bacteria cfu/g	<10	<500
Yeasts cfu/g	<10	<10
Listeria monocytogenes *	* See note below	Absent in 25 g
Salmonella spp. *	* See note below	Absent in 25 g

* Production is systematically tested on an ongoing basis - details can be supplied on request

Electronically generated - signature not required

Viniflora® NoVA™

Certificate of Analysis

Form:	Frozen DVS
Material No:	712535
Batch no:	3355396
Date of Manufacture:	06.2017
Best Before Date:	06.2018

Performance	**Result**	**Specification**
Total cell count cfu/g	7.4E+10	-
Total cell count/pouch cfu/pouch	6.3E+13	>=2E+13

Purity	**Result**	**Specification**
Acetobacter cfu/g	<10	<100
Enterobacteriaceae cfu/g	<100	<100
Moulds cfu/g	<10	<10
Non lactic acid bacteria cfu/g	<10	<500
Yeasts cfu/g	<10	<10
Listeria monocytogenes *	* See note below	Absent in 25g
Salmonella spp. *	* See note below	Absent in 25g

* Production is systematically tested on an ongoing basis - details can be supplied on request

Electronically generated - signature not required

Viniflora® NoVA™

Certificate of Analysis

Form:	Frozen DVS
Material No:	712535
Batch no:	3439982
Date of Manufacture:	11.2018
Best Before Date:	11.2019

Performance	**Result**	**Specification**
Total cell count cfu/g	7.0E+10	
Total cell count/pouch cfu/pouch	6.0E+13	>=2E+13

Purity	**Result**	**Specification**
Acetobacter cfu/g	<10	<100
Enterobacteriaceae cfu/g	<100	<100
Moulds cfu/g	<10	<10
Non lactic acid bacteria cfu/g	<10	<500
Yeasts cfu/g	<10	<10
Listeria monocytogenes *	* See note below	Absent in 25 g
Salmonella spp. *	* See note below	Absent in 25 g

* Production is systematically tested on an ongoing basis - details can be supplied on request

Electronically generated - signature not required

Viniflora® NoVA™

Certificate of Analysis

Form:	Frozen DVS
Material No:	712535
Batch no:	3537681
Date of Manufacture: (DD.MM.YYYY):	23.07.2020
Best Before Date: (DD.MM.YYYY):	22.01.2022

Performance	**Result**	**Specification**
Total cell count cfu/g	8.4E+10	
Total cell count/pouch cfu/pouch	7.1E+13	>=2E+13

Purity	**Result**	**Specification**
Acetobacter cfu/g	<10	<100
Enterobacteriaceae cfu/g	<100	<100
Moulds cfu/g	<10	<10
Non lactic acid bacteria cfu/g	<10	<500
Yeasts cfu/g	<10	<10
Listeria monocytogenes *	* See note below	Absent in 25 g
Salmonella spp. *	* See note below	Absent in 25 g

* Production is systematically tested on an ongoing basis - details can be supplied on request

Electronically generated - signature not required

From:	Arie Carpenter
To:	Hice, Stephanie
Cc:	Kate Urbain
Subject:	RE: GRN 000946 - Questions for Notifier
Date:	Tuesday, December 8, 2020 12:33:36 PM
Attachments:	image001.png 2020-12-01 GRN 946 Response to FDA.pdf

Hi Dr. Hice,

Please see attached and let me know if you need any clarification.

Thanks,

Arie Carpenter
Sr. Regulatory Affairs Specialist, Food Cultures and Enzymes
Cell: 414-544-2317 Desk: 414-777-7526
usarbr@chr-hansen.com | www.chr-hansen.com

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Tuesday, December 1, 2020 2:05 PM
To: Arie Carpenter <USARBR@chr-hansen.com>
Cc: Kate Urbain <USKAUR@chr-hansen.com>
Subject: RE: GRN 000946 - Questions for Notifier

Dear Ms. Carpenter,

During our review of GRAS Notice No. 000946, we noted further questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

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

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients

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Office of Food Additive Safety
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stephanie.hice@fda.hhs.gov

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December 3, 2020
USARBR

Supplement to GRN 946

Dear Dr. Hice,

In response to your email dated December 1, 2020 please accept this supplementary information regarding *Lactobacillus plantarum* DSM strain DSM 33452 for use in the production of wine and musts to increase the rate of malolactic fermentation and to prevent growth of microorganisms that could cause off flavors in the finished product.

We appreciate the opportunity to clarify our position on the safety of *L. plantarum* DSM 33452.

Yours sincerely,

Arie Carpenter
Sr. Regulatory Affairs Specialist
Food Cultures and Enzymes North America
usarbr@chr-hansen.com
Mobile: 414-544-2317

1. For the administrative record, please state whether *L. plantarum* strain DSM 33452 is intended to be used in infant formula and/or foods under the jurisdiction of the U.S. Department of Agriculture (USDA).

As stated in Sections 1.1 and 1.3 of GRAS notice 946, *L. plantarum* DSM 33452 is intended to be used for malolactic fermentation of wine and musts. It is not intended to be used in infant formula. *L. plantarum* DSM 33452 does not fall under the jurisdiction of the USDA.

2. In Table 2-5 (page 13), the notifier provides two different sets of limits for heavy metals. Please clarify which limits are intended to be the specifications for the heavy metals. In addition, please provide complete and appropriate citations for the analytical method(s) used for the analyses of heavy metals and indicate that the method(s) is validated for its intended purpose.

L. plantarum DSM 33452 cultures will comply to the limits set by the International Oenological Codex on Lactic Acid Bacteria used in Oenology (OIV-Oeno 328-2009). Laboratory testing for heavy metals is carried out by an accredited ISO 17025 facility. They utilize two ISO methods to test for heavy metals; ISO 11885 (ICP-OES) or ISO 17294-2 (ICP-MS).

From:	Arie Carpenter
To:	Hice, Stephanie
Cc:	Kate Urbain; Kevin Gillies
Subject:	RE: GRN 000946 - Questions for Notifier
Date:	Monday, December 21, 2020 12:10:01 PM
Attachments:	image001.png 12-09-2020 GRN 946 response to FDA.pdf

Hi Dr. Hice,

Please find a response to the question you sent us December 9th surrounding heavy metal testing in *L. plantarum* in GRN 946.

Please let me know if you have further questions.

Thanks,

Arie

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Monday, December 14, 2020 8:15 AM
To: Arie Carpenter <USARBR@chr-hansen.com>
Cc: Kate Urbain <USKAUR@chr-hansen.com>; Kevin Gillies <kevin.o.gillies@gmail.com>
Subject: RE: GRN 000946 - Questions for Notifier

Dear Ms. Carpenter,

Good morning, and thank you for your email.

We are available for a call from 1:00 to 1:30 PM EST; I have sent you a calendar invitation with the relevant WebEX information.

Thank you again; please do not hesitate to let me know if you have any questions.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

U.S. FOOD & DRUG ADMINISTRATION

From: Arie Carpenter <USARBR@chr-hansen.com>
Sent: Sunday, December 13, 2020 5:17 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Cc: Kate Urbain <USKAUR@chr-hansen.com>; Kevin Gillies <kevin.o.gillies@gmail.com>
Subject: RE: GRN 000946 - Questions for Notifier

Hi Stephanie,

Could I set up some time to talk to you?

Would you by chance, be available on Wednesday 12/16 from 1PM-3PM EST or Friday 12/18 from 11AM-3PM EST?

Thanks so much,

Arie Carpenter
Sr. Regulatory Affairs Specialist, Food Cultures and Enzymes
Cell: 414-544-2317 Desk: 414-777-7526
usarbr@chr-hansen.com | www.chr-hansen.com

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Wednesday, December 9, 2020 3:53 PM
To: Arie Carpenter <USARBR@chr-hansen.com>
Cc: Kate Urbain <USKAUR@chr-hansen.com>
Subject: RE: GRN 000946 - Questions for Notifier

Dear Ms. Carpenter,

During our review of GRAS Notice No. 000946, we noted further questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

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

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
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stephanie.hice@fda.hhs.gov

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December 18, 2020
USARBR

Supplement to GRN 946

Dear Dr. Hice,

In response to your email dated December 9, 2020 please accept this supplementary information regarding *Lactobacillus plantarum* DSM strain DSM 33452 for use in the production of wine and musts to increase the rate of malolactic fermentation and to prevent growth of microorganisms that could cause off flavors in the finished product.

We appreciate the opportunity to clarify our position on the safety of *L. plantarum* DSM 33452.

Yours sincerely,

Arie Carpenter
Sr. Regulatory Affairs Specialist
Food Cultures and Enzymes North America
usarbr@chr-hansen.com
Mobile: 414-544-2317

The exposure to heavy metals should be as low as possible. The proposed specifications based on the limits set by the International Oenological Codex on Lactic Acid Bacteria used in Oenology (OIV-Oeno 328-2009) appear to be high for a fermentation-derived microorganism. Please provide batch analyses from a minimum of three non-consecutive lots to support the proposed specification limits. If the batch data do not support the specification limits, please revise the specification limits to be reflective of the heavy metal content in *L. plantarum* strain DSM 33452. In addition, we note that the stated analytical methods are for the analysis for heavy metals in water. Please indicate if these methods have been validated for the analysis of *L. plantarum* strain DSM 33452 for the respective heavy metals.

Per US regulations, there are not cited limits for heavy metals in food cultures. Furthermore, most international standards and regulations are only set for finished food products and do not apply to food cultures. Since we are producing a culture of lactic acid bacteria to be used solely in wine fermentation, our customers expect us to comply with the limits set by the Oenological Codex (OIV). While Chr. Hansen agrees that the limits listed in the OIV are high, in the absence of regulatory limits, we adapt our specifications to our customer usage and international standards so that our quality management systems are easy to understand and meet the needs of our customers. In practice, we have not recovered heavy metals in testing our food cultures.

It should be understood that heavy metal contamination is not a potential hazard in our process or products. Confirmation that heavy metals are not present in our raw materials occurs for all raw materials through vendor questionnaires and/or vendor testing of raw materials prior to receipt. Potable water is used in our process, which complies with heavy metal limits for drinking water. Seed materials (pre-inoculation material-PIM and direct inoculation material-DIM) are grown with those same standards. PIM and DIM are inoculated into the fermentations at extremely low levels which suggests that their contribution to heavy metal contamination of a production batch is exceedingly small. There are no other sources of potential heavy metal contamination.

Because heavy metals are not a reasonable hazard in food cultures and in the absence of legal limits for heavy metals in food cultures in the US or most other countries globally, heavy metal contaminant testing is not a release criteria and therefore does not appear on certificates of analysis.

As an act of caution, we monitor for heavy metals by selecting products that are representative of the raw materials going into our process at all facilities. Testing is performed annually. Each production site submits representative food cultures to be tested. Testing is performed by one external lab that is ISO 17025 certified and accredited to run heavy metal testing on food matrices. The methods that are being used have been validated for the purpose as it is validated for all food matrices per the testing lab.

The raw materials that go into the production of *L. plantarum* DSM 33452 are represented in the cultures seen in Table 1 along with the results of heavy metal testing. The cultures tested were nonconsecutive. In all cases, no heavy metals were detected.

Table 1: Heavy metal results for 5 nonconsecutive batches of food cultures (reported in parts per million)

Lot ID	Arsenic (As)	Lead (Pb)	Cadmium (Cd)	Mercury (Hg)
718792/3487521	<0.05	<0.02	<0.01	<0.005
704748/3485381	<0.05	<0.02	<0.01	<0.005
666092/3484951	<0.05	<0.02	<0.01	<0.005
704889/3491567	<0.05	<0.02	<0.01	<0.005
709107/3485725	<0.05	<0.02	<0.01	<0.005