PROZURE

A Technology Licensing Company

June 11th, 2020

Dear Sir or Madam,

Please find enclosed a copy of the GRAS notification for the use of the bacterial strain *Lactobacillus johnsonii* 456 as an ingredient in food and beverage products. This submission conforms with the requirements of 21 CFR § 170, and is in accordance with the FDA issued GRAS final rule 81 FR 54960.

Please do not hesitate to contact me directly if you have any further questions.

Michael Davoren Rodriguez
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22224 Collington Drive
Boca Raton, FL 33428

Notice of Generally Recognized as Safe (**GRAS**)
Status for the Use of

***Lactobacillus Johnsonii* Strain 456**

In Food and Beverage Products

Submitted by
Prozure, Inc.

To

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Park Drive.
College Park, MD 20740

June 2020

Table of Contents

Part 1: Signed Statements and Certification

1.1 Submission of the GRAS Notice

Prozure, Inc. hereby submits this notification of GRAS (Generally Recognized as Safe) status in accordance with 21 CFR §170.225.

1.2 Submitting Organization

Prozure, Inc.
22224 Collington Drive
Boca Raton, FL 33428

1.3 Name of the Notified Substance

The substance that has been determined as GRAS in this notice is the bacterial strain *Lactobacillus johnsonii* 456. Patent rights to the use of this strain are held by Prozure, Inc, and the intellectual property rights for this strain are the property of the University of California, where it was originally isolated (Yamamoto et al., 2013). Worldwide rights to said patents, and thus the use and manufacture of the strain, are exclusively licensed to Prozure, Inc.

1.4 Intended Conditions of Use

Lactobacillus johnsonii 456 is intended to be added as an ingredient to food and beverage in a manner consistent with cGMP. This GRAS substance qualifies as a *Nutrient Supplement* as defined by 21 CFR §170.3. No specific medical or therapeutic claims are made with regards to this substance.

Intended applications of the substance as an ingredient include, but are not limited to, the following exemplary food and beverage uses: products such as yogurt, yogurt drinks, juice drinks, cereals, chocolate, alcoholic beverages and meal replacement drinks. The substance may be present in a lyophilized (freeze-dried), microencapsulated, or active form. Such products may contain up to 10^{11} (100 billion) CFU (colony-forming units) per serving.

1.5 Statutory Basis for GRAS Determination

Lactobacillus johnsonii 456 has been determined to be GRAS through the use of scientific procedures, in accordance with 21 CFR §170.30 parts (a) and (b).

1.6 Exemption from Premarket Approval Requirements

Lactobacillus johnsonii 456 is exempt from the requirements for premarket approval as detailed in the Federal Food, Drug, and Cosmetic Act based on the conclusion that the notified substance is GRAS under the conditions of intended use described above.

1.7 Data Availability

The data, information, and individual documents used to determine the GRAS status of *Lactobacillus johnsonii* 456 are available to the FDA upon request. Such documentation may be sent to the FDA in paper or electronic format by the designated contact person listed below:

Michael Davoren Rodriguez
mdavoren@prozure.net
1 (415) 847-1238

or viewed during standard business hours at Prozure, Inc.'s business address.

1.8 Freedom of Information Act

None of the information as listed in Parts 2 through 7 of this notice is exempt from disclosure under the Freedom of Information Act, 5 USC §552.

1.9 Certification and Signature

To the best of our knowledge, this GRAS notice 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 safety and GRAS status of the use of this substance.

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Michael Davoren Rodriguez
Chief Scientific Officer
Prozure, Inc.

Part 2: Identification, Manufacture, Specifications, and Physical or Technical Effect

2.1 Identification of the Strain

2.1.1 Identity

Lactobacillus johnsonii 456 is a strain of lactic acid bacteria (LAB). It is an anaerobic, gram-positive, catalase-negative, rod-shaped bacterium that does not undergo spore formation. An image of *L. johnsonii* 456 bacilli is included as **Figure 1**. The strain has a particularly high tolerance to low pH, even as compared to other *Lactobacilli*, and is capable of surviving in gastric acid for a period of time (Davoren et al., 2018). On LAB-selective de Mann, Rogosa, and Sharpe (MRS) agar plates, it can be identified as smooth, round, off-white colonies (**Figure 2**).

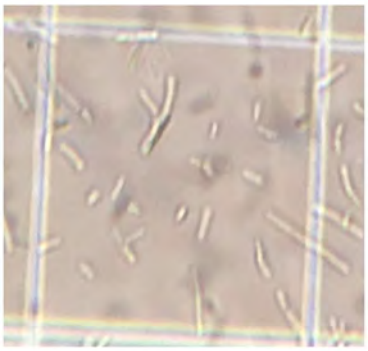

Figure 1 – *Lactobacillus johnsonii* 456 under light microscope (visible square is 0.5 x 0.5 mm)

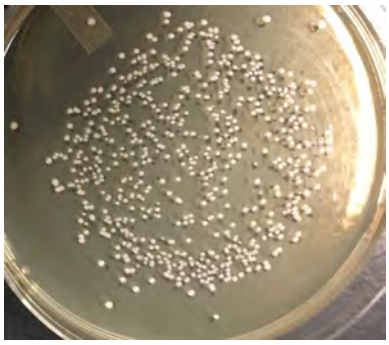

Figure 2 - *Lactobacillus johnsonii* 456 colonies grown under anaerobic conditions on MRS agar

L. johnsonii is a heterofermentative strain, capable of generating lactic acid, ethanol, and carbon dioxide from sugars. The strain's fermentation profile by the API 50 CH panel is:

Fermentation Strong Positive: 11-Glucose ; 12-Fructose ; 22-N-acetylGlucosamine ; 25-Esculine ; 28-Maltose ; 31-Saccharose ; 32-Trehalose
Fermentation Incomplete/ Partial Positive: 13-Mannose ; 27-Cellobiose ; 36-Starch ; 39-Gentobiose.

2.1.2 Source of Derivation

Lactobacillus johnsonii 456 was originally isolated from the intestinal microflora of *ATM*-deficient C57BL/6J mice in the specific pathogen free (SPF) vivarium facility at the University of California, Los Angeles. This strain was found to be of elevated abundance in mice with restricted microflora; this was originally investigated because these mice had suppressed rates of genotoxicity and cancer. It was later found that oral gavage with this strain alone into other mice could drastically cut their rates of genotoxicity and inflammation (Yamamoto et al., 2013).

2.1.3 Sequencing and Genotyping

16S rRNA gene sequences of the fecal microbiome of the mice in the abovementioned study were analyzed, and this strain was identified as a *Lactobacillus johnsonii* strain via BLAST (Yamamoto et al., 2013). Strain specific primers, detailed in the table below, were later designed using primerBLAST for discrimination between this strain and other species of *L. johnsonii* using qPCR (Davoren et al., 2018). The strain carries no plasmids.

Table 1: *Lactobacillus johnsonii* 456 specific primer sequences

Primer Name	Description	
LJ4F	LBJ 456 specific forward primer	AGA CCC AAA GGC GCT TAT AGA
LJ4R	LBJ 456 specific reverse primer	TGT AAG TTC AGA AAA ATG TAT CCC G

The whole genome of the strain was sequenced using PacBio Sequel to an average coverage of 100x. This genome was uploaded to the NCBI database under accession code QGQW00000000.

2.1.4 Culture Collection Deposit

A strain deposit of *Lactobacillus johnsonii* 456 was made with the American Type Culture Collection (ATCC) in accordance with the Budapest Treaty (Budapest Treaty Regulations, 1977). The strain is listed under the accession number PTA-124205.

2.2 Method of Manufacture

The manufacture and production of *Lactobacillus johnsonii* 456 for commercial use will be carried out specifically by Prozure or by partnering suppliers under contract and direct supervision of Prozure. All ingredients and growth media components are food grade in their own right, and all equipment is approved for food contact and permitted for use in this application. Any and all use of soy, milk, gluten or other potentially allergenic products as part of growth media will be clearly disclosed in product labelling.

2.2.1 Strain Maintenance and Storage

In addition to copies of the strain maintained in Culture Collections, such as the ATCC, Prozure, Inc. maintains master copies of *Lactobacillus johnsonii* 456 for long term storage. The master strain copies are maintained at -80° C.

When new working cultures of the strain need to be prepared, an aliquot of the master strain will be inoculated into autoclave-sterilized liquid MRS media to select for lactobacillus growth. The strain will be allowed to grow for 18-24 hours at 37° C under anaerobic conditions, yielding a new working stock. This working stock is then tested to re-confirm strain identity by phenotypic analysis and strain-specific qPCR. After testing, it is suitable for inoculation of primary production ampoules.

2.2.2 Manufacturing Flow Chart

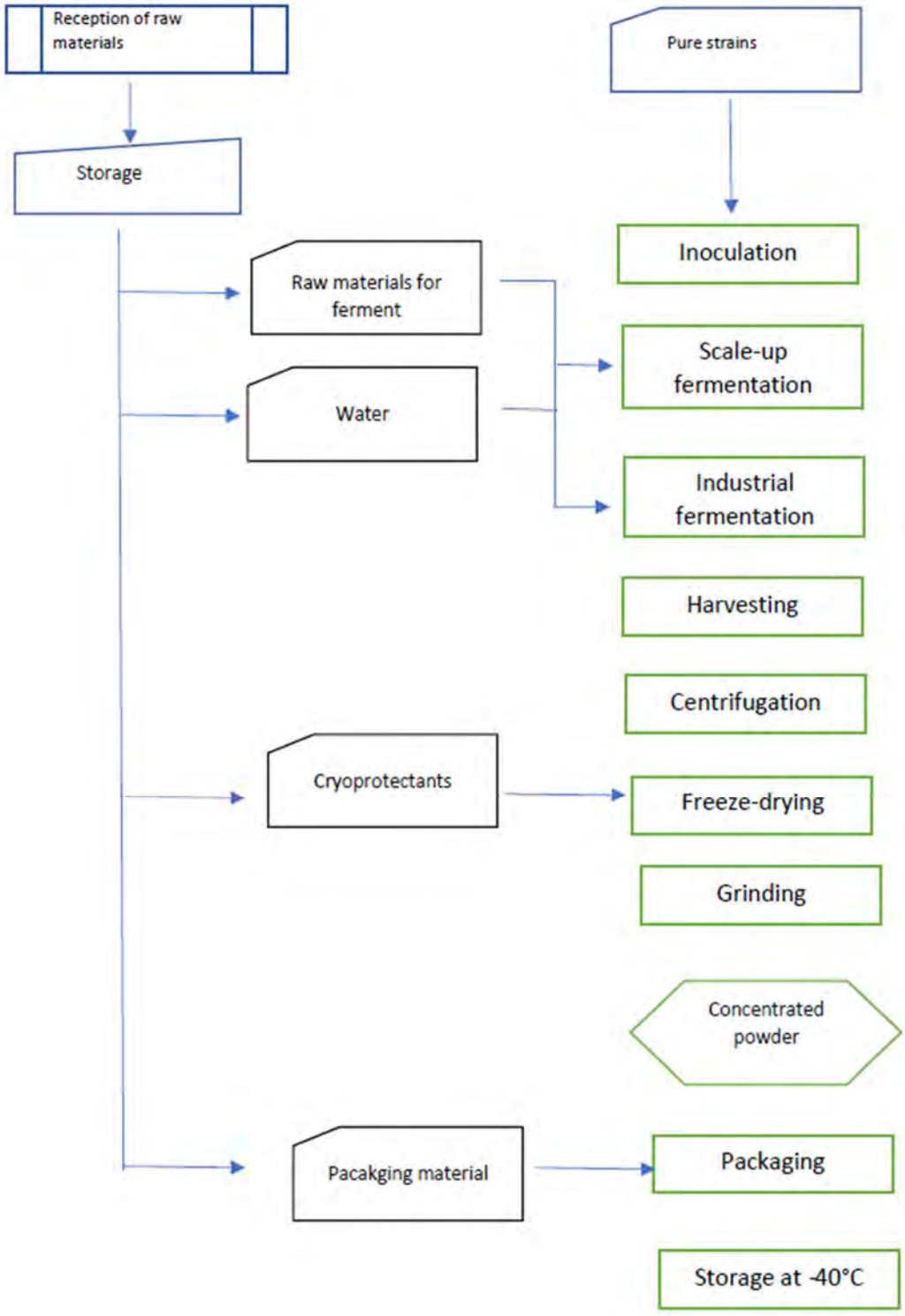

Figure: Representation of the manufacturing process resulting in food grade *Lactobacillus johnsonii* 456.

2.2.3 Fermentation

Lactobacillus johnsonii 456 will be grown using industry standard fermentation techniques, using conditions and ingredients suitable for human consumption. Culture media for the strain will consist of sugars, complex nitrogen sources, vitamins, and minerals necessary for growth. Each individual ingredient will be generally recognized as safe or otherwise compliant with all regulations in its own right.

2.2.4 Concentration and Lyophilization

After completion of fermentation, cells are cooled and concentrated into a slurry via centrifugation. If the end product format demands slurry as the ingredient form, this substance is then prepared with cryoprotectant, frozen, and shipped in containers such as cans. For lyophilized powder, these cells are mixed with prepared cryoprotectant solution and freeze-dried using industrial equipment to generate bricks of freeze-dried product. These bricks are then ground into powder of a uniform particle size.

2.2.5 Packaging, Storage, and Transport

Lyophilized product is packaged into sterile bags or other food-grade containers. Storage temperature of product is maintained at -20 to -40° C. Samples from each batch are taken to ascertain identity, compliance with minimum standards, and to prevent contamination with foreign microorganisms or substances. Containers are shipped at storage temperature until delivery using boxes with temperature control elements.

2.3 Specifications for Food Grade Material

Prozure and its manufacturing partners use industry standard microbiological specifications to demonstrate that any batch of *Lactobacillus johnsonii* 456 is of food grade. These specifications are listed below, in an included exemplary Certificate of Analysis document.

Identification of the sample: Lactobacillus johnsonii 456			
Description:	Powder sample containing *Lactobacillus johnsonii 456*		
Batch Number:	NO71901737	***Production date:***	10/2019
		Analysis date:	28/10/2019
Conservation before analysis:	-40 °C	***Expiration date:***	10/2021

Composition:	
Freeze dried microbial powder	100 %
Microbial concentration:	$1,7*10^{11}$ CFU/gram
this includes:	
Lactobacillus johnsonii 456	$1,7*10^{11}$ CFU/gram

Specifications:

Test	Unit	Limits Min	Limits Max	Result
Appearance	-	Thin white powder		Conform
Dry Matter	%	92	-	> 95
Water Activity (Aw)		-	> 0,200	0.06
Aerotolerant microflora[1]	CFU/g	$1.5*10^{11}$		$1.7*10^{11}$
Total Aerobic Microbial Count (24h, 37°C) Petrifilm 3M	CFU/g	-	10000	Conform
Bile Tolerant Gram Negative (48h, 30°C) VRBD agar	CFU/g	-	100	Conform
Yeast's and moulds (5 days, 30°C) SD agar	CFU/g	-	100	Conform
Total coliforms (72h, 30°C) MacConkey agar	CFU/g	-	100	Conform
Salmonella (BIO N° 12/16-09/05)[2]	CFU/g	-	Absent	Absent
Listeria monocytogenes (BIO N° 12/09-07/02)[2]	CFU/g	-	Absent	Absent
Mercury	ppm (mg/kg)	-	0.1	Conform
Lead	ppm (mg/kg)	-	3.0	Conform
Cadmium	ppm (mg/kg)	-	1.0	Conform
Arsenic	ppm (mg/kg)	-	1.0	Conform

1. Method based upon international standards (ISO 6887, ISO 4833, ISO 15214, ISO 29981, ISO/TS 19036:2006)

Part 3: Dietary Exposure

As stated previously, *Lactobacillus johnsonii* 456 is intended to be added to food and beverage products as an ingredient. Intended applications of the substance include, but are not limited to, the following exemplary uses: yogurt, yogurt drinks, juice drinks, cereals, chocolate, alcoholic beverages and meal replacement drinks. The substance may be present in a lyophilized (freeze-dried), microencapsulated, or active form. Such products may contain up to 10^{11} (100 billion) CFU (colony-forming units) per serving.

Therefore, *L. johnsonii* 456 would be present in a number of food and beverage products at concentrations from 10^{9} to 10^{11} CFU/serving. Internal and 3rd party testing has shown that, even in active culture form, live bacteria do not overproliferate and reach excess levels within an active culture product, such as yogurt.

As such, a regular consumer of probiotic product containing *Lactobacillus johnsonii* 456 might be exposed to, in extreme cases, 10^{12} (one trillion) CFU per day. It is important to note that even this relatively heavy dose is well within levels known to be safe. Indeed, one of the most important attributes of *Lactobacillus* as a completely safe genus is that any excess cells are simply passed through the gut with normal defecation. A study of fourfold excess consumption of a *Lactobacillus johnsonii* containing milk drink (360g/day) found no adverse side effects other than increased frequency of defecation (Fukushima et al., 2004). According to recent estimates of total bacterial load, the gut of a healthy adult male would already contain over thirty times the amount introduced in this manner (Sender, Fuchs, & Milo, 2016).

Other substances involved in the growth of *Lactobacillus johnsonii* 456, such as soy or milk proteins, may be present in the end product. Presence of such substances will be screened for and measured. If detected, they will be listed on the ingredients list, and the product will be clearly labelled to disclose their presence. An allergen statement with regards to the final product is available on request.

Part 4: Self-Limiting Levels of Use

Under the fields of use described in this document, *Lactobacillus johnsonii* 456 does not have any self-limiting levels of use.

Part 5: Experience Based on Common Use in Food Prior to 1958

Although *Lactobacillus johnsonii* have been found as part of the fermenting lactic acid bacterial culture of many traditional foods, the specific strain of *Lactobacillus johnsonii* 456 has not been used in historical human food products. *Lactobacillus johnsonii* as a species is used, and has been used, in a number of food and beverage products currently available. For this strain in particular, this GRAS notification is not based on common use in food under 21 CFR §170.30 parts (a) and (c).

Part 6: Narrative

6.1 History of *Lactobacillus* Use and Safety

6.1.1 *Lactobacilli* in Food and the Microbiome

The human gastrointestinal (GI) microbiome is populated by several hundred species of bacteria, although the specific strains vary between individuals (Ciorba, 2012). These microbes have co-evolved towards a symbiotic life with their hosts over the course of 500 million years, and serve a role at least as important as any organ (Cho & Blaser, 2012). *Lactobacilli* represent an important component of the healthy human gut microbiome, although their numbers fluctuate and they rarely colonize permanently in large numbers. Rather, their population is often dynamic and constantly replenished by dietary intake (Walter, 2008). *Lactobacilli* can therefore be found in many parts of the gut at a large range of concentrations, such as from 10^3-10^7 CFU/g in the ileum, and 10^4-10^8 CFU/g in the colon (Bernardeau, Guguen, & Vernoux, 2006).

The use of *Lactobacilli* in the creation of fermented food and drink products dates back to at least 10,000 BC, and was independently developed by multiple human cultures (Gasbarrini, Bonvicini, & Gramenzi, 2016). New strains of *Lactobacilli* continue to be isolated from traditional fermented foods and characterized every year (Pinto, Franz, Schillinger, & Holzapfel, 2006). Natural reservoirs for these bacteria include both the vertebrate microbiome as well as unfermented fruits, vegetables (Vitali et al., 2012).

Lactic acid bacteria, and *Lactobacilli* in particular, are the best studied of all bacteria commonly considered to be probiotic, or thought to induce benefits to human health with their consumption. *Lactobacilli* are associated with the development of immune tolerance and restoration of equilibrium in the intestinal microbiome (Castellazzi et al., 2013). The presence of *Lactobacilli* has been demonstrated to reduce inflammation both in the gut and systemically throughout the body (Yamamoto et al., 2013). Thus, ingestion of *lactobacilli* has stronger associations with benefits to human health than with health risks.

The use of *lactobacilli* in the preparation of food and drink for human consumption has a long and historic record of safety (Holzapfel, 2002). According to most researchers, asking whether a *lactobacillus* species is safe for human consumption is like asking “are apples safe?” (Wallace & MacKay, 2011). In other words, certain measured risks might occur during extraordinary circumstances, but in the vast majority of cases no risk to human health exists. Repeated studies and examination by scientific and regulatory groups have found no evidence of pathogenic or virulence factors across the genus (FAO and WHO Joint Working Group, 2001).

As the presence of *lactobacilli* in the gut is a desired state rather than a site of infection, most of the rare cases of opportunistic *lactobacillus*-derived disease in humans are cases of bacteremia, or presence of bacteria in the bloodstream. Boyle et. al described 7 instances of *lactobacillus*-associated bacteremia, all of which involved the *rhamnosus* species. Importantly, all of these

cases were opportunistic infections associated with other severe disease conditions, such as advanced diabetes and birth defects (Boyle, Robins-Browne, & Tang, 2006). Other potentially vulnerable populations include premature infants and severely immunocompromised individuals. Borriello estimates the risk of *lactobacillus*-associated bacteremia to be less than 1 in 1 million, even in immunocompromised patients (Borriello et al., 2003). However, this risk could be increased in immunocompromised individuals undergoing sustained treatment with broad-spectrum antibiotics (Fruchart et al., 1997) In a comprehensive study of clinically reported *lactobacillus* bacteremia cases, zero out of 85 blood isolates were associated with the *johnsonii* species (M. Salminen et al., 2006).

Epidemiological studies of *lactobacillus* use as a probiotic in humans also provide evidence that the risk of *lactobacillus*-derived infection and bacteremia is vanishingly small. Over a six-year case study period, during which a large increase in probiotic use in general, and *L. rhamnosus* use in particular, was observed in the Swedish population studied, no increase in *lactobacillus* bacteremia was detected (Sullivan & Erik Nord, 2006). A similar study in Finland examined a period of sixfold increased *L. rhamnosus* consumption in the population, also leading to zero increase in bacteremia (M. K. Salminen et al., 2002).

Owing to the near impossibility of *lactobacillus*-derived bacteremia in healthy humans, Bernardeau et. al have argued that the sole point of caution with regards to the consumption of *lactobacillus* strains should be the identification of transferable antibiotic resistance genes (Bernardeau, Vernoux, Henri-Dubernet, & Gueguen, 2008). Theoretically, such genes could be transferred laterally to pathogenic species of bacteria in an entirely different genus, rendering them more difficult to treat. While this potential pitfall should be taken into account, such a risk is quite low. Intrinsic vancomycin resistance is a common but entirely nontransferable characteristic in *lactobacilli* (Tynkkynen, Singh, & Varmanen, 1998). Doron et al. note that no clinical evidence of lateral transfer of antibiotic resistance between probiotic organisms and others in the gut has ever been observed (Doron & Snydman, 2015). Beyond that, the fact that many *lactobacillus* strains produce antipathogen factors, inhibiting their growth and adhesion in the gut, would make such a transfer even more difficult.

6.1.2 *Lactobacillus Johnsonii*

Lactobacillus johnsonii is one of the many species of *Lactobacilli* that are specifically adapted to a symbiotic or commensal life associated with vertebrate organisms (Duar et al., 2017). It can be found naturally in the human gastrointestinal tract at locations including the ileum and colon (Aiba, Nakano, Koga, Takahashi, & Komatsu, 2015; Pridmore et al., 2004). In addition to this location, they are also a common component of healthy human vaginal flora (Oliveira et al., 2018; Pramanick, Parab, Mayadeo, Warke, & Aranha, 2018). In humans, *L. johnsonii* strains are associated with positive health benefits and anti-inflammatory outcomes. Specific mechanisms by which these effects are exerted include the inhibition of pathogens and the production of short chain fatty acids (SCFAs) and other important nutrients (Sanders, Benson, Lebeer, Merenstein, & Klaenhammer, 2018). *L. johnsonii* species can also be found naturally in the

gastrointestinal tracts of many other vertebrates, including both birds and mammals (Duar et al., 2017)

Outside of the vertebrate body, *L. johnsonii* species can also be found in food products, such as traditionally fermented vegetables (Zielińska, Rzepkowska, Radawska, & Zieliński, 2015). They can also be isolated from many traditional fermented milk products (G El-Baradei, Delacroix-Buchet, & Ogier, 2008; Gaber El-Baradei, Delacroix-Buchet, & Ogier, 2007; Hassanzadazar, Ehsani, Mardani, & Hesari, 2012). Such products have been consumed by humans for generations, and such consumption has never been associated with outcomes of ill health.

6.2 Strain-Specific Safety Profile

6.2.1 Antibiotic Resistance

In 2002, the FAO/WHO joint working group convened to generate a set of guidelines for the evaluation of bacterial strains sold as probiotics, and included the determination of antibiotic resistance patterns as a potential concern (FAO/WHO, 2002). In addition, the EFSA (European Food Safety Administration) has set a number of cutoff values above which bacteria from a particular group would be considered "resistant" (EFSA, 2012). The European contract research organization Vizera performed an analysis of *Lactobacillus johnsonii* 456's antibiotic resistance profile according to these guidelines, using the cutoffs recommended for the *Lactobacillus acidophilus* group. These data are included in the table below, with values over the recommended cutoffs shown in bold.

	Microbiological cut-off values recommended by EFSA [µg/L]	Antibiotic resistance values for *Lactobacillus johnsonii* 456* [µg/L]
ampicillin	1	0.32
vancomycin	2	**>256**
gentamicin	16	1.25
kanamycin	64	64
streptomycin	16	**20**
erythromycin	1	0.44
clindamycin	1	0.57
tetracycline	4	1.75
chloramphenicol	4	1.25

* A bacterial strain is defined as resistant when it is not inhibited at a concentration of a specific antimicrobial higher than the established cut-off value.

As two antibiotic resistance attributes were above the recommended cutoff range, the *Lactobacillus johnsonii* 456 genome was analyzed for antibiotic resistance genes, and potential for transferability. First, the RGI program was used to comprehensively scan the genome against

the CARD database of many known antibiotic resistance genes (ARGs), as well as mutations in targets of antibiotics known to confer resistance (Jia et al., 2016). No unambiguous, known resistance genes were found. We then performed a manual alignment of *Lactobacillus johnsonii* 456 genes to the ARGs in both the CARD database and the MegaRes database using the sequence analysis tool tblastx, and did not find any strong matches (Lakin et al., 2016; McGinnis & Madden, 2004). Using a threshold of 80% similarity, only two genes had alignments to the card ARG database longer than 100bp, and these were in generally conserved, nontransferable housekeeping genes.

Of the listed antibiotics, only vancomycin appears to be significantly resisted at levels above EFSA recommended cutoff values. However, as stated previously intrinsic vancomycin resistance is a common but entirely nontransferable characteristic in *lactobacilli* (Tynkkynen et al., 1998). Combined with the dearth of evidence for transferrable ARGs, and the strain's lack of plasmids, there is no meaningful risk associated with this trait.

Streptomycin resistance was also found to be slightly higher (25% higher) than the cutoff value recommended for the *Lactobacillus acidophilus* group. However, this is still not a significant amount of resistance. Indeed, the *lactobacillus acidophilus* group has the lowest cutoff to be considered "resistant" of all *lactobacillus* species in the guidance document – the majority of other groups are considered "normal" under 32 and 64 mg/L (EFSA, 2012). As no transferable ARGs were found to be associated with this small amount of resistance either, the risk associated with this trait is negligible.

6.2.2 Allergic Potential

There is no known instance of human allergy to a *Lactobacillus* strain recorded in the literature (Doron & Snydman, 2015). Indeed, the administration of *Lactobacillus* strains has been demonstrated to be an effective means of reducing IgE-linked allergic responses such as rhinitis and atopic dermatitis, particularly in children (Chen, Lin, Jan, Chen, & Wang, 2010; FAO and WHO Joint Working Group, 2001; Rosenfeldt et al., 2003).

6.3 Research Studies of *Lactobacillus Johnsonii* 456

6.3.1 Animal Studies

Lactobacillus johnsonii 456 was isolated as a microbiome component of C57/BL/6J mice at the University of California, Los Angeles. The strain was noted to be present at higher amounts in mice under RF (restricted flora) conditions that prevented their exposure to many of the other bacterial strains present in a typical mouse colony. RF mice were found to have lower levels of inflammation, resistance to colitis, and healthier gut phenotypes than mice exposed to conventional microflora (Fujiwara et al., 2008; Presley, Wei, Braun, & Borneman, 2010).

The direct administration of *Lactobacillus johnsonii* 456 has been tested in a mouse model (Yamamoto et al., 2013). No adverse effects were observed after heavy oral inoculation with the

strain. In brief, mice had their extant microflora cleared by quadruple antibiotic therapy (ampicillin, neomycin, metronidazole, and vancomycin) delivered in their drinking water for one week. Then, 10^9 CFU of *L. johnsonii* 456 in PBS was administered to each of 8 *ATM*-/- C57BL6/J mice every other day via orogastric gavage for a period of 4 weeks. 10^9 CFU/mL of the strain was also present in their drinking water. After inoculation, viable presence of the strain was confirmed via sequence-selective fecal qPCR. The administration of *L. johnsonii* 456 led to zero adverse health effects in the treated mice over the full observation period.

6.3.2 Human Studies

Lactobacillus johnsonii 456 oral administration was tested in humans over a two month trial period (Davoren et al., 2018). In brief, 13 healthy, mixed gender adults received a 7 day course of yogurt, containing 10^8 CFU/mL of active *L. johnsonii* 456. Participants were instructed to consume a 100mL serving each day for a total of 10 billion bacteria per serving. Of the full group, 11 provided 4 fecal samples, including pre-course day 0, and post-course day 7, day 30, and day 60. These samples were analyzed to confirm the presence of viable *L. johnsonii* 456 after the completion of the course. Strain ID was also confirmed by sequence specific qPCR.

Over the course of the study, no adverse side effects or diarrheal symptoms were observed in any of the subjects, either during the course of administration or during the followup period. Based on the study's estimates, roughly 10^5-10^6 live *L. johnsonii* 456 were present in every gram of feces following the week of inoculation. This amount decreased back to baseline over the course of two months, suggesting temporary, but not permanent, colonization of the human gut after oral consumption. In conclusion, this study demonstrated that the consumption of live *L. johnsonii* 456 is safe and well tolerated in adult humans.

6.3.3 *In Vitro* Studies

Study of *Lactobacillus johnsonii* 456 *in vitro* has elucidated several aspects of the strain that contribute to its probiotic activity. In particular, it has attributes that lend it the capability to survive in the human gut without any adverse side effects. These traits were investigated as part of strain characterization (Davoren et al., 2018).

Human gastrointestinal tract survival was modelled by exposure to simulated gastric and bile acid containing solutions. The strain is capable of survival in simulated gastric acid at pH under 2 for 2 hours, and is capable of inhibited, but stable, growth in media containing physiological bile acid concentrations. Adhesion to simulated human gut mucosa was also simulated by the use of monolayer-forming human cancer cell lines, including the enterocyte-like Caco-2 line and the goblet cell-like line LS 174T. As Caco-2 cells express solely membrane bound mucus proteins, while LS 174T cells express a more secreted mucin-heavy profile, the two strains provide different substrates for bacteria to adhere to. *L. johnsonii* 456 adhesion was greatest on the LS 174T line, suggesting that the strain's binding capability is more specialized toward a secreted mucin-heavy milieu.

6.4 Qualified Presumption of Safety Status

A wide variety of microorganisms, in particular yeasts and bacteria, have long been used in the production of food and beverages for human consumption. The identification of these food microbes by broad categories has been sufficient to establish certain groups as safe for use. Realizing the need to set priorities within formal risk assessment, the European Food Safety Administration (EFSA) proposed a system to establish the pre-market safety of certain selected groups of microorganisms. This system would grant the status of Qualified Presumption of Safety (QPS) to defined taxonomic groups based on four pillars of analysis: establishing identity, body of knowledge, possible pathogenicity, and end use (European Food Safety Authority, 2007). Only after determination that a group does not raise safety concerns, or if safety concerns can be well defined and excluded, will a group be granted QPS status. After QPS status is granted to a taxon, any strain which can be unequivocally identified as a member is exempt from the need for individual safety assessment other than satisfying any specified qualifications for the QPS group– regardless of the strain's specific application.

As part of the initial recommendation in 2007, the EFSA Scientific Committee submitted a preliminary list of microorganisms suitable to receive QPS status immediately, based on thorough review of available literature (European Food Safety Authority, 2007). As an extraordinarily safe genus of bacteria with a well-documented history of safe incorporation into food products, 33 species of *Lactobacillus* were included in this first set of recommendations, specifically including *Lactobacillus johnsonii*. Since the initial establishment of these guidelines, the EFSA has continued to review emerging information and case reports related to the use of listed QPS microbes. There has never been any need for further review or change to the QPS status of *L. johnsonii*, and all strains of the organism are fully considered safe as of this notification (EFSA Panel on Biological Hazards, 2019; Ricci et al., 2017).

Lactobacillus johnsonii 456 can therefore be considered a QPS microorganism, safe for all human and animal dietary use based on these guidelines.

Part 7: List of Supporting Data and Information

Aiba, Y., Nakano, Y., Koga, Y., Takahashi, K., & Komatsu, Y. (2015). A highly acid-resistant novel strain of Lactobacillus johnsonii No. 1088 has antibacterial activity, including that against Helicobacter pylori, and inhibits gastrin-mediated acid production in mice. *Microbiologyopen, 4*(3), 465-474.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
000957	
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

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

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

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Michael Davoren Rodriguez	Chief Scientific Officer
Organization *(if applicable)*	
Prozure, Inc.	
Mailing Address *(number and street)*	
10741 Moorpark St. Apt. 23	

City	State or Province	Zip Code/Postal Code	Country
North Hollywood	California	91602	United States of America

Telephone Number	Fax Number	E-Mail Address
415 847 1238		mdavoren@prozure.net

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

Name of Contact Person	Position or Title
Organization *(if applicable)*	
Mailing Address *(number and street)*	

City	State or Province	Zip Code/Postal Code	Country

Telephone Number	Fax Number	E-Mail Address

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Lactobacillus johnsonii 456

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

☒ Electronic Submission Gateway

☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

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

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

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

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

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

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

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

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

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Intended conditions for use include both freeze-dried and active lactobacillus johnsonii 456 in fermented and unfermented food and beverage products, including but not limited to milk drinks, yogurt and yogurt drinks, juice drinks, meal replacement beverages, chocolates, and cereals, at concentrations up to 100 billion cfu per serving.

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

(Check one)

☐ Yes ☒ No

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

(Check one)

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

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

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

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

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

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

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

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

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

Other Information

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

☐ Yes ☒ No

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

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Prozure, Inc.
(name of notifier)

has concluded that the intended use*(s)* of Lactobacillus johnsonii 456
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Prozure, Inc. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

22224 Collington Drive, Boca Raton FL 33428
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	**Printed Name and Title**	**Date (mm/dd/yyyy)**
mdavoren@prozure.net Digitally signed by mdavoren@prozure.net Date: 2020.06.15 12:39:13 -07'00'	Michael Davoren Rodriguez, CSO	06/15/2020

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	LBJGRAS620.pdf	Administrative
	LBJGRASCoverLetter620.pdf	Administrative