BioPolicy Solutions, LLC

May 30, 2023

Kristi Muldoon-Jacobs Ph.D, Acting Director
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Re: GRAS Notice for the Use of Phoenix Oyster Mushroom (*Pleurotus pulmonarius*) Mycelium as an Ingredient in Food

Dear Dr. Muldoon-Jacobs,

In accordance with 21 CFR 170 Subpart E (170.203 through 170.285), BioPolicy Solutions LLC (Houston, Texas, USA) is submitting this GRAS Notice as an agent on behalf of Mushlabs GmbH (Humboldtst. 59, 22083 Hamburg, Germany). We are submitting one hard copy and one electronic copy (on a CD also containing Form 3667) of summaries of data and information that supports our conclusions that Mushlabs' Phoenix Oyster Mushroom Mycelium (*Pleurotus pulmonarius*) as an ingredient in food is GRAS on the basis of scientific procedures. Attached to this letter for review and evaluation by FDA is all the information and data upon which Mushlabs' GRAS conclusion has been reached.

Please direct any questions or concerns regarding this GRAS Notice that may arise during the review process to BioPolicy Solutions LLC to ensure a timely response. BioPolicy Solutions can be reached by e-mail at lmp@bpsllc.us (Dr. Laura Plunkett) or lxr@bpsllc.us Dr. Larisa Rudenko).

Sincerely,

Laura M. Plunkett, Ph.D., DABT
Co-Founder BioPolicy Solutions LLC

GRAS NOTICE FOR THE USE OF PHOENIX OYSTER MUSHROOM (*Pleurotus pulmonarius*) MYCELIUM AS AN INGREDIENT IN FOOD

PREPARED BY

BioPolicy Solutions LLC
Houston, Texas

PREPARED FOR

Mushlabs GmbH
Humboldtstr. 59
22083 Hamburg
Germany

May 30, 2023

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Part 1. Signed Statements and Certification

Name of GRAS Substance

Mycelia of *Pleurotus pulmonarius* [Phoenix Oyster Mushroom Mycelium][1]; the fruiting bodies of this mushroom are known as Phoenix Oyster Mushroom, Oyster Mushroom, Summer Oyster Mushroom, or Indian Oyster Mushroom.

Intended Conditions of Use

An ingredient in various processed products including meat, poultry, and fish analogs,[2] dairy analogs, and baked goods at a maximum level of 25% (dry mycelium weight/100 g product). Mycelia are not intended for use in USDA-regulated products or infant formula. Table 1.1 lists the intended food uses and the maximum levels at which mycelia could be added to those foods.

TABLE 1.1
Intended Food Uses for Mushlabs Mycelium Product

Food Category	Maximum Use Level of Mushlabs *P. pulmonarius* mycelia (g/100g)
Milk analogs	10
Ice cream analogs	15
Cream analogs	10
Processed cheese	25
Yogurt analogs	15
Meat analogs	25
Fish analogs	25
Seafood analogs	25
Baked goods	25

Basis for Conclusion of GRAS Status

Mushlabs GmbH (hereafter referred to as Mushlabs) concludes that the intended uses of *P. pulmonarius* mycelia are Generally Recognized As Safe (GRAS) through Scientific Procedures. Mushlabs understands that although there is a history of common use of the fruiting body in food prior to 1958, there is no documentation of mycelia produced via submerged fermentation being used as food before 1958.

[1] *P. pulmonarius* has been deemed to be identical to *P. sajor-caju* (Shnyreva and Shnyreva, 2015; Zmitrovich and Wasser, 2016), and will be referred to a *P. pulmonarius* throughout this Notice.

[2] For clarity, none of these products will contain animal-derived components that would cause them to be considered as meat under the Federal Meat Inspection Act or Poultry Products Inspection Act.

Pre-market Approval Exclusion Claim

Mushlabs concludes that the uses of *P. pulmonarius* mycelia are not subject to the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act (FD&C) Act based on its conclusion that the uses and intended conditions of use of *P. pulmonarius* mycelia are GRAS.

Certification

To the best of my knowledge, this GRAS Notice is a complete, representative, and balanced submission that includes unfavorable and favorable information known to Mushlabs and pertinent to the evaluation of the safety and GRAS status of the intended uses of *P. pulmonarius* mycelia.

Name, Position, and Signature of Authorized Individual and Date of Signature

Chief Science Officer	26 May 2023
Name (Thibault Godard)	**Position**	**Date Signed**

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

2.1 Identity

The subject of this notice can be described as mycelium of *Pleurotus pulmonarius,* strain PXM1906, Phoenix Oyster Mushroom Mycelium, the fruiting bodies of which are commonly known as Phoenix oyster mushroom or oyster mushroom. Taxonomically, *P. pulmonarius* is categorized as *Pleurotus pulmonarius* (Fr.) Quél. The fruiting body of *P. pulmonarius* is commonly found in European, North American, and Asian diets.

Taxonomy
Kingdom: Fungi
Phylum: Basidiomycota
Class: Agaricomyces
Order: Agaricales
Family: Pleurotacaea
Genus: Pleurotus
Species: pulmonarius

Phoenix Oyster Mushroom Mycelium was confirmed as an isolate of *P. pulmonarius* by genetic comparison of the isolate with the MycoBank database. Appendix A to this document contains the details on the testing performed.[3]

2.1.1 Synonyms

A synonym for *P. pulmonarius* is *P. sajor-caju*, while *P. sapidus* is a subspecies of *P. pulmonarius* (Bao *et al.* 2004; Shnyreva and Shnyreva, 2015). *P. pulmonarius* has been shown to be 100% comparable to *P. sajor-caju* (Shnyreva and Shnyreva, 2015). Zmitrovich and Wasser (2016) reported ITS homology of 97 to 100% between *P. pulmonarius* and *P. sajor-caju*. Therefore, published data on *P. sajor-caju* are directly relevant for the evaluation of the safety of Phoenix Oyster Mushroom Mycelium; such data are included in this GRAS Notice.

2.2 Method of Manufacture

Mushlabs initiates its production cultures from *P. pulmonarius* PXM1906 mycelia grown on agar plates containing appropriate commercially available culture media[4] and then transfers the mycelia to liquid culture medium with a sterile loop. A pure culture of Phoenix Oyster Mushroom Mycelium is used to inoculate sterile liquid culture medium.[5] The media (see Table 2.1) contain food grade materials including

[3] Mushlabs engaged a contract testing laboratory (Charles River) to employ the "genetic barcode" of the internal transcribed spacer (ITS) region from the nuclear ribosomal RNA cistron (concluded to be a sequence that identifies a wide range of fungal species by Schoch *et al.* 2012) to confirm the identity of its *P. pulmonarius* strain, including specifically the *Pleurotus* species (Shnyreva and Shnyreva, 2015; Rugolo *et al.,* 2019).
[4] Some examples are potato dextrose agar, malt extract agar, and malt extract peptone agar.
[5] As noted above, the medium composition can be adjusted to modulate relative levels of proximates.

amino acids, mineral salts, acids or bases for pH adjustment, sugars as carbon sources, and sources of nitrogen. This culture is then transferred to sterile flasks and the culture is submerged in medium. Once the requisite mass of mycelia has been reached, the culture is transferred to sterile growth medium in a fully automated fermenter (bioreactor). This apparatus can be thoroughly cleaned, sterilized, and reused. Biomass is removed from the bioreactor, separated from the fermentation broth by standard separation techniques, washed with slightly acidic water (pH between 4 and 7), concentrated to achieve 3-30% dry mass, and stored at 4°C until use, which is generally after a few days and not weeks. Products are then formulated with either wet or dry mycelia[6] to fractional compositions of up to 25% (w/w) on a dry weight basis. Mushlabs conducts all processing in accordance with current Good Manufacturing Practice (cGMP), 21 CFR § 117, Subpart B.

A list of raw materials and processing aids that may be used in the production of Phoenix Oyster Mushroom Mycelium is provided in Table 2.1. A review of the Certificates of Analysis for the raw materials used in Mushlabs' production confirmed that specifications for raw materials and processing aids are consistent with food-grade quality.

TABLE 2.1
List of Substances Used in Various Formulations of Mushlabs' Growth Media

Category	Substances Used
Amino acids	20 common amino acids
Mineral salts	Calcium carbonate and calcium chloride Copper chloride and copper sulfate Diammonium hydrogen phosphate Disodium hydrogen phosphate Iron chloride and iron sulfate Magnesium chloride and magnesium sulfate Manganese chloride and manganese sulfate Potassium dihydrogen phosphate Sodium chloride Sodium phosphate monobasic hydrate Sodium selenate Zinc sulfate
Vitamins	Biotin Choline chloride Folic acid[7] Myo-inositol Niacinamide Pantothenic acid Pyridoxal Riboflavin Thiamine Cobalamin Ascorbic acid
pH adjustment agents (Acids and bases)	Sodium hydroxide

[6] The use of dry versus wet material is dependent on the application of the product. This difference does not pose a safety concern.

[7] Folic acid is added to a level needed for growth of the mycelia; it is not added to provide fortification of the food, as evident from the relatively low levels present in the mycelia (data provided in Table 6.3).

TABLE 2.1 List of Substances Used in Various Formulations of Mushlabs' Growth Media	
Category	**Substances Used**
	Potassium hydroxide Sulfuric acid Phosphoric acid Hydrochloric acid Citric acid Acetic acid Ammonium sulfate, ammonium chloride and ammonium carbonate
Nitrogen sources	Corn steep liquor Yeast extract Peptone Ammonia Urea
Carbon sources	Sucrose Glycerol Glucose Fructose Lactose Galactose Maltose Xylose Arabinose Starch Cellulose Malt extract Beet molasses Corn molasses
Solid medium for isolation	Potato dextrose agar Malt extract agar Malt extract peptone agar
Additional reagents	Agar agar
Processing Aids (safe and suitable for food use)	Tween 20 Tween 80 Food grade antifoam agent

2.3 Specifications[8]

Demonstration that Mushlabs can make Phoenix Oyster Mushroom Mycelium consistently over time is key to ensuring identity and quality. Once acceptance criteria and the safety of the qualifying batches have been established, demonstrating the consistency of batches helps ensure the food safety of all batches meeting those qualifications. Specifically, data on the levels of proximates in the Phoenix Oyster Mushroom Mycelium, as well as levels of certain heavy metals and limitations on levels of microbes are important to the demonstration of food safety. As a result, Mushlabs has established acceptance criteria

[8] Although ICH considers "specifications" to refer to tests and/or references to analytical procedures, as well as appropriate acceptance criteria (i.e., numerical limit ranges, or other criteria for tests described), in this document the terms are used interchangeably with the assumption that the numerical limits have been set by an accepted and validated test method. https://www.ich.org/page/quality-guidelines

and specifications for Phoenix Oyster Mushroom Mycelium taking each of these three quality parameters into account.

Eight batches of Phoenix Oyster Mushroom Mycelium were tested to determine a compositional profile; the same eight batches were analyzed for the presence of heavy metals and microbes. The data were used to set acceptance criteria for commercial batches. Batch samples were freeze-dried prior to analysis to generate more accurate compositional analyses as high-water content can interfere with measurements. Freeze-drying ensures that nutritional components were conserved, and sensitive compounds were not damaged during sample preparation. Table 2.2 summarizes the acceptance criteria for release of Phoenix Oyster Mushroom Mycelium.

TABLE 2.2
Acceptance Criteria and Specifications for Phoenix Oyster Mushroom Mycelium

Parameter	Specification	Method of Analysis
Protein	10 to 65	[1]ASU L 06.00-7 mod.[#]
Fiber	25 to 75	ASU L 6.00-18, mod.
Fat	0 to 10	ASU L 6.00-6
Ash	1 to 15	ASU L 06.00-4
Lead	<0.05	[2]DIN [3]EN 15763 mod.
Cadmium[4]	<0.1	DIN EN 15763 mod.
Arsenic[4]	<0.2	DIN EN 15763 mod.
Mercury[4]	<0.1	DIN EN 15763 mod.
Aerobic plate count	<5000	DIN EN ISO 4833-1
Yeast and molds	< 1000	ISO 21527-2
Enterobacteriaceae	<1000	DIN EN ISO 21528-2
E. coli	<10	DIN ISO 16649-2
Salmonella sp.	negative	DIN 10135 - PCR

[1]ASU = Official collection of analysis methods according to § 64 of the German Food and Feed Code;
[2]DIN = Deutsches Institut für Normung
[3]EN = Europaische Norm
[4] These three metals will not be part of a routine specification for Phoenix Oyster Mushroom Mycelium. This decision was made based on the results where values were all below the limit of detection (LOD) for the assays and the fact that raw materials used in production of Phoenix Oyster Mushroom Mycelium will be verified to have levels of these three metals that are below the LOD values set forth here.
[#]mod. = modification
*Tests for other organisms such as *B. cereus* will be performed should an outbreak be detected.

The Phoenix Oyster Mushroom Mycelium batch data also are important in the food safety assessment to demonstrate that the batches produced over time are consistent under the conditions of production, and that safety has not been affected by virtue of changes in the composition of different batches. Proximate analysis is a well-established method for describing the key physical characteristics of a food or food ingredient. Protein, fiber, fat, and ash are quantified in proximate analyses; carbohydrate levels are generally estimated by calculation. Mushlabs notes the ranges listed in Table 2.2 for protein, fiber, fat, and ash were set considering that the composition of mycelia can be modulated through changes in the

substances used for growth and other growth conditions (Table 2.1 and Table 6.1). Published data supports that different carbon sources can affect the composition of polysaccharides and that lipid composition can be modulated by various culture conditions (reviewed by Bakratsas *et al.* 2021).

Table 2.3 provides the results of the proximate analyses for the Phoenix Oyster Mushroom Mycelium adjusted for dry weight. Little variability was observed across all compositional endpoints, indicating that the Mushlabs process is reproducible in terms of the basic composition of the product. The results of all these batch analyses demonstrate that Mushlabs' production process can yield a consistent Phoenix Oyster Mushroom Mycelium product that conforms to product quality acceptance criteria as set forth in Table 2.2.

TABLE 2.3
Results of Proximate Analyses of Eight Batches of Phoenix Oyster Mushroom Mycelium

Batch No. (g /100 g)	1	2	3	4	5	6	7	8	Mean (SD)
Protein	35.6	29.3	33.4	33.4	32.4	30.4	30.7	30.9	32.0 (1.9)
Fiber	60.4	63.1	66.9	63.8	64.9	68.1	65.0	65.2	64.7 (2.2)
Fat	1.1	1.1	1.0	0.9	1.1	1.1	0.9	1.1	10.0 (0.1)
Ash	3.3	2.9	2.9	2.6	2.7	3.0	2.6	2.7	2.8 (0.05)
Carbohydrates	-[+]	3.3	-	-	-	-	0.7	0.1	1.7 (1.7)

[*]All values are based on dry weight.
[+] -- = not detected

Table 2.4 provides the results of the heavy metals analyses for Phoenix Oyster Mushroom Mycelium. Levels of heavy metals including arsenic, cadmium, mercury, and lead were below the established specification limits of 0.1 mg/kg for arsenic, cadmium, and mercury, and 0.2 mg/kg for lead. The results of these batch analyses demonstrate that Mushlabs production process can yield a consistent product that conforms to product quality acceptance criteria as set forth in Table 2.2, and that levels of heavy metals do not pose food consumption risks.

TABLE 2.4
Results of Heavy Metals Analyses of Eight Batches of Phoenix Oyster Mushroom Mycelium

Batch No.	1	2	3	4	5	6	7	8	Method of Analysis
Arsenic (mg/kg)	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	DIN EN 15763 mod.
Cadmium (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	DIN EN 15763 mod.
Lead (mg/kg)	0.044	0.045	0.039	0.037	0.041	0.042	0.034	0.036	DIN EN 15763 mod.
Mercury (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	DIN EN 15763 mod.

Eight batches of Phoenix Oyster Mushroom Mycelium were analyzed for microbial contamination as part of stability testing; analyses were at Weeks 4 and 8 following Phoenix Oyster Mushroom Mycelium production. The results of Week 4 and 8 testing are provided below in Table 2.5. Although in some cases

total aerobic plate count did not meet specifications, the introduction of an acidic wash (pH between 4 and 7) for Batches 3 through 8 was effective in limiting bacterial growth generally to below the specification of <5000 CFU/g (met in 5 of 6 batches at week 4 and 6 of 6 batches at week 8 after the acidic wash was introduced).

For Batch 7, a total aerobic plate count of 18,000 CFU/g was observed at week 4; the specification value of < 5000 CFU/g was met for the 8-week Batch 7 sample. Batch 7 samples were prepared manually and handled independently, which may have led to the observed difference in total aerobic plate count at the two time points. Aerobic plate count is generally easier to control in highly automated and controlled commercial facilities compared to pilot scale plants. As a result, it is expected that any microbial levels detected would be lower in batches of commercial Phoenix Oyster Mushroom Mycelium compared to those produced in Mushlabs' pilot facility. This is demonstrated by the total aerobic plate count data for Batch 8. Batch 8 was produced using an upscaled 96 m^3 process and the microbial data met the specifications at all time points of analysis (Table 2.2). Based on the Mushlabs' specifications, a finding similar to the one observed in Batch 7 (week 4) for any commercial Phoenix Oyster Mushroom batch would result in the batch not being released into commerce. Moreover, Phoenix Oyster Mushroom Mycelium is not intended to be stored for weeks before being processed to end products or consumed raw. It will be used as an ingredient in food with further processing steps that would reduce further the risk of any microbial contamination in food products (*e.g.,* heating, pH adjustments).

TABLE 2.5
Results of Microbial Analyses After Four and Eight Weeks of Eight Batches of Phoenix Oyster Mushroom Mycelium

Batch No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Method of Analysis**
Week 4	120,000	32,000	2,000	<1000	<1000	<1000	18,000	<1000	DIN EN ISO 4833-1
Week 8	22,000	<1000	<1000	<1000	<1000	<1000	1000	1000	DIN EN ISO 4833-1
Week 4	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	ISO 21527-2
Week 8	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	ISO 21527-2
Week 4	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	DIN EN ISO 21528-2
Week 8	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	DIN EN ISO 21528-2
		=							
Week 4	<10	<10	<10	<10	<10	<10	<10	<10	DIN ISO 16649-2
Week 8	<10	<10	<10	<10	<10	<10	<10	<10	DIN ISO 16649-2
			=						
Week 4	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	DIN 10135-PCR
Week 8	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	DIN 10135-PCR

2.4 Physical or Technical Effect

The intended effect of *P. pulmonarius* mycelia is as a source of protein, fiber, and other nutrients in various processed food products including meat and dairy analogues and baked goods.

Part 3. Dietary Exposure

3.1 History of Use of Mushroom Mycelium as a Food Ingredient

Unlike the fruiting bodies of *P. pulmonarius*, there is little documented history of the use of isolated *P. pulmonarius* mycelia produced through submerged fermentation as a food source in the US, yet mycelia from other species of fungi are commonly consumed. For example, tempeh is composed of the mycelium of *Rhizopus oligosporus*, and in the US, a bacon-alternative product derived from mycelia (MyForest Foods[9]) recently entered the marketplace. Further, the US Food and Drug Administration (FDA) issued a Compliance Policy Guide in 1988 regarding labelling of food containing mycelia stating that mushroom mycelium is suitable for use in food when grown in acceptable media provided that food products are labelled accordingly (see CPG Sec. 585.525; https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-585525-mushroom-mycelium-fitness-food-labeling).

In 2002, the FDA issued a "no questions" letter related to GRAS Notice GRN91, directed to the use of fungal protein preparations from *Fusarium* spp. in food; it is marketed by Marlow foods under the trade name of "Quorn". Quorn™ has been marketed and consumed for decades without apparent adverse effects (Berger *et al.,* 2022). Other GRAS Notices evaluated by FDA relate to products of mycelia from various fungal organisms and have received "no questions" letters in the recent past (*i.e.,* GRN848; GRN904; GRN945).

In addition to the protein component of Phoenix Oyster Mushroom Mycelium, the mycelia contain significant levels of fiber. GRN997, the subject of which was fiber extracted from white button mushrooms (*Agaricus bisporus*), was filed in 2021 and received a "no questions" letter from FDA. As described in GRN997, the fiber was for use as an antimicrobial ingredient at levels of from 0.01 to 0.150 g/100g (equivalent to 100 to 1,500 ppm or 100 to 1,500 mg/kg). This same fiber received GRAS status from the Flavor and Extract Manufacturers Association (FEMA) for use as a flavor-modifying ingredient (FEMA No. 4946) at levels up to 2,000 ppm.

As the intended uses of the substances discussed in the various GRAS Notices are similar to those proposed for Phoenix Oyster Mushroom Mycelium, they provide a basis for a dietary exposure assessment.

3.2 Intended Use and Characteristics of the Dietary Exposure Assessment

For the purposes of this safety assessment, dietary exposure assessment for the US includes all age groups (both children and adults) that typically could consume products that may incorporate mycelia. Mushlabs initially will be incorporating Phoenix Oyster Mushroom Mycelium into foods at a maximum level of 25% of the final product (g dry mycelia/100 g food).

Phoenix Oyster Mushroom Mycelium has been developed as an ingredient to be incorporated into food as an alternative protein source. Anticipated product categories are alternative milks, yogurts, cheeses, shrimp, meat, fish, seafood products, and bakery products.

[9] https://myforestfoods.com/

Available US dietary surveys, such as the National Health and Nutrition Examination Survey (NHANES), have not collected data on intakes of mycoprotein or even specific types of alternative protein products incorporated into foods, *e.g.,* plant-based dairy and meat products. GRAS notices GRN91, GRN904, and GRN945 have therefore been used as data sources for estimating dietary exposure for Phoenix Oyster Mushroom Mycelium *in toto*, as well as to estimate dietary exposures to the protein (only) component of Phoenix Oyster Mushroom Mycelium. As Phoenix Oyster Mushroom Mycelium contains significant levels of fiber, an estimate of dietary fiber exposure resulting from consumption of the mycelium based on estimates of daily intake of mushroom fiber found in GRN997 has been used in this Notice.

3.3 Estimated Dietary Exposure to Phoenix Oyster Mushroom Mycelium

GRAS notices served as the primary sources of information in developing the dietary exposure assessment for Phoenix Oyster Mushroom Mycelium (Table 3.1). Estimates of intake of both "mycoprotein"[10] and other alternative protein sources (*e.g.,* plant-based protein) were considered relevant to Mushlabs' dietary intake assessment. GRN91 and GRN945 provided estimates of daily intake of mycoprotein in the US. Estimates of daily intake of protein and/or plant-based protein in US adults also were identified (GRN848; Fulgoni, 2008; Gropper *et al.* 2019; Pikosky *et al.* 2022). Additionally, Fulgoni (2008) and Pilosky *et al.* (2022) provide analyses of dietary data collected as part of the National Health and Nutrition Survey (NHANES), a U.S. government resource for dietary data often used for food safety assessments. Gropper *et al.* (2019) describe analysis of data collected from 2012 to 2014 as part of the Healthy Aging Research Initiative (HARI) study, a long-term investigation performed by Florida Atlantic University.[11]

Table 3.1
Summary of Available US Dietary Intake of Protein and Fiber (Children and Adults) Relevant for Phoenix Oyster Mushroom Mycelium Dietary Assessment

Reference	Data Source	Type of Ingredient	Average Daily Intake (g/person/day)[a]	Maximum Level of Daily Intake (g/person/day)[a]	Comments
GRN91 "Mycoprotein (Quorn™)"	Published studies available in 2001 when the GRAS notice was filed	Mycoprotein intake/day	0.6-10.8 [general US population] 14.4-27.6 [vegetarians]	28.8-55.2 [calculated based on FDA guidance for a "pseudo-90th percentile"][b]	Mycoprotein-specific ingestion estimates are relevant to this exposure assessment No distinction as to age-associated intake values Intake values calculated by FDA based on the agency's data evaluation[12]
GRN945	NHANES data 2003-2016	Mycoprotein in meat,	37.7 (2-5 years) 51.6 (6-11 years) 68.8 (12-19 years)	75 (2-5 years) 103 (6-11 years) 138 (12-19 years)	Mycoprotein-specific ingestion estimates are

[10] Mycoprotein is the term that refers to the type of protein that comes from a fungal organism.
[11] http://med.fau.edu/research/labs/ouslander.php#collapseTwo

Table 3.1
Summary of Available US Dietary Intake of Protein and Fiber (Children and Adults) Relevant for Phoenix Oyster Mushroom Mycelium Dietary Assessment

Reference	Data Source	Type of Ingredient	Average Daily Intake (g/person/day)[a]	Maximum Level of Daily Intake (g/person/day)[a]	Comments
"Mycoprotein (Quorn) as a Food Ingredient"		poultry and seafood only	79 (20+ years) 73.3 (all ages) 18 (taken from GRN91)	158 (20+ years) 147 (all ages) 36 (taken from GRN91) [calculated based on FDA guidance for a "pseudo-90th percentile"]	relevant to this exposure assessment Age-associated intake rates provided Intakes based on replacement of 58% of meat protein with mycoprotein. That level is more than twice Mushlabs' maximum incorporation of 25%
GRN904 "Fermented microbial protein of *Fusarium novum Yellowstonensis*"	NHANES data 2011-2012	Mycoprotein	24.4 (all ages)	48.8 (all ages) [calculated based on FDA guidance for a "pseudo-90th percentile"]	Ingestion estimates relevant to this exposure assessment
GRN848 "Pea and Rice Protein Fermented by Shiitake Mycelia"	NHANES data 2011-2012	Pea and rice protein	10.3 (all ages)	17.3 (all ages) [90th percentile]	Although not only mycoprotein intake, these data provide a context for other US alternative protein intakes
Pikosky *et al.* 2022. *Front. Nutr.* 9:873512	NHANES data 2011-2014 [adults 19+]	Plant-based protein intake/day (all sources)	27.4 (adults)	43.7 (adults) [4th quartile]	Likely over-estimates intake for this exposure assessment
GRN997 "Fiber Extracted from White Button Mushrooms"	NHANES data 2015-2016	Fiber extracted from mushrooms (*A. bisporus*)	1.35 ("consumers only"[c])	2.7 [90th percentile]	Relevant mushroom fiber data for the dietary assessment
USDA What We Eat in America dataset (accessed September 2022)	NHANES data 2017 – March 2020 Pre-pandemic	Dietary fiber (total)	16.6	33.2 [calculated based on FDA guidance for a "pseudo-90th percentile"]	Overestimate of fiber intake for the dietary assessment as it considers all food sources of fiber (daily)

[a] Intake of ingredient in grams dry weight/person/day.

Table 3.1 Summary of Available US Dietary Intake of Protein and Fiber (Children and Adults) Relevant for Phoenix Oyster Mushroom Mycelium Dietary Assessment					
Reference	**Data Source**	**Type of Ingredient**	**Average Daily Intake (g/person/day)[a]**	**Maximum Level of Daily Intake (g/person/day)[a]**	**Comments**
[b] FDA (2006) *Guidance for Industry: Estimating Dietary Intake of Substances in Food*, August (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-estimating-dietary-intake-substances-food). [c] "Consumers only" was defined in the notice as intake by individuals who reported consuming at least one food product in which the use of the ingredient was under consideration.					

As seen in Table 3.1, daily protein intakes (grams of dry weight/day) are available for non-animal-based proteins as a group, *i.e.,* pea protein, rice protein, and mycoprotein. Mycoprotein intake was selected as the most appropriate surrogate for mycelia ingestion as Phoenix Oyster Mushroom Mycelium is likely to be used as a protein replacement in various types of foods. To provide a conservative, health-protective assessment, the highest reported 90th percentile values for intake, 28.8-55.2 g/person/day[13] calculated by FDA and used in the GRN91 notice were employed in this exposure assessment. Although higher mycoprotein intake values were discussed and listed in GRN945 (see Table 3.1 above), the actual dietary exposure assessment for that notice relied on the values estimated (by calculation) by FDA in GRN91.

Of importance, these values are estimates of daily intake by vegetarians, who would be expected to seek alternative protein products such as Phoenix Oyster Mushroom Mycelium products, and thus are appropriate upper-bound estimates of intake for individuals likely to consume alternative protein-source products. In the safety assessment provided in GRN 91, an average 90th percentile daily intake of mycoprotein was estimated to be 42 g/person/day.[14] For dietary fiber intakes, the highest 90th percentile estimate reported in USDA's most recent dietary intake nutrient survey was 33.2 g/person/day. This value is lower than the mycoprotein daily intake value previously indicated; as Phoenix Oyster Mushroom Mycelium consists of both protein and fiber, this safety assessment focuses on daily intakes related to the higher nutrient value, 42 g mycoprotein/person/day as a conservative assessment of exposure. This value is representative of the estimated exposure of, or intake by, vegetarians (see GRN91).

Even though there are no individual values found for daily US intake of plant-based milks, cheeses, creamers, yogurts, meat, baked goods, and shrimp or fish as individual products, data allowing for estimating US daily intakes of these food categories are available. When those values are considered in light of the fraction composition intended for incorporation into various foods by Mushlabs (Table 3.2), it is clear that estimates of intake for individual food category estimates are lower than the values estimated for daily intakes of mycoprotein in vegetarians (up to 55 g/person/day as a 90th percentile value: average daily intake 90th percentile value of 42 g/person/day).

[13] GRN 91

[14] Calculated by taking the mean of the 90th percentile values calculated by FDA (dividing 28.8 + 55.2 by 2).

TABLE 3.2 Daily US Intakes (g/person/day) in Adults and Children for Different Foods Where Phoenix Oyster Mushroom Mycelium May Be Included as a Food Ingredient				
Food Type	**US Mean Intake (g/person/day)**	**Fractional (%) Composition of Mycelium In Food**	**Estimated Daily Mycelium Intake (g/person/day)**	**Comments**
Yogurt	150 (children) 182 (adults)	15	22.5 (children) 27.3 (adults)	Yogurt daily values from analysis of NHANES data (Cifelli *et al.* 2020[a])
Milk	170 (2+years)	10	17	Milk (fluid) daily intake value from a 2010 USDA source based on NHANES data (https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/DBrief/3_milk_consumption_0506.pdf)
Processed Cheese	170 (all ages)	25	43	Processed cheese daily value from a 2019 USDA source (https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=103984)
Ice Cream	14 (all ages)	15	2.1	Estimate of *per capita* US ice cream daily consumption (https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=101589)
Baked Goods (all categories)	130 (2+ years)	25	32.5	Daily value from 2006 FDA guidance *"Guidance for Industry: Estimating Dietary Intake of Substances in Food"* (FDA, 2006)
Crustaceans (Shrimp)	1.2 (all ages)	25	0.3	Estimated per capita US daily intake for shrimp (https://www.seafoodsource.com/news/foodservice-retail/nfi-releases-new-top-10-list-detailing-the-seafood-species-americans-consume-most)
Fish (Canned Tuna)	17 (all ages)	25	4.25	Canned tuna daily intake estimated based on per capita numbers (https://fsi.colostate.edu/canned-tuna/)
Meat (all types of animal protein)	82 (19+ yrs)	25	20.5	Animal protein daily intake from analysis of NHANES 2007-2010 data (Pasiakos *et al.* 2015)[b]

[a] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7696083/pdf/nutrients-12-03435.pdf
[b] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4555161/pdf/nutrients-07-05322.pdf

Part 4. Self-Limiting Levels of Use

In general, no self-limiting levels of use for the product have been identified. One possible exception is the incorporation of mycelium product into fluid milk, as amounts in excess of 15% may affect its texture. Mushlabs limits incorporation as a food ingredient in various food products to no more than 25% (w/w on a dry basis).

Part 5. Experience Based on Common Use in Food Before 1958

Although there is a history of common use of the fruiting body in food prior to 1958, there is no documentation of *P. pulmonarius* mycelia produced via submerged fermentation being used as food before 1958.

Part 6. Safety Narrative

Mushrooms of the *Pleurotus* genus are common constituents of the US and world-wide diets. They are commonly referred to as "oyster mushrooms" without any further indication of species at the retail level. In 2020-2021, over 72 million pounds of "oyster" mushrooms (including the *Pleurotus* spp.) were produced in the US.[15] *Pleurotus* mushrooms are among the most cultivated fungi in the world (Lee *et al.* 2021) and are second in global mushroom consumption (Cohen *et al.* 2002; Sanchez, 2010). In addition to their use in food, many traditional medicines employ mushrooms for therapeutic uses, possibly because of their antioxidant properties[16] (Jose, 2002). There is extensive literature studying the beneficial properties of mushrooms, including *Pleurotus* spp. (see Section 6.3).

Although often referred to generally as oyster mushrooms, there is, in fact, genetic diversity within the *Pleurotus* genus of fungi. Genetic relationships among *Pleurotus* spp. have been discussed in depth by Shnyreva and Shnyreva (2015), who cited differences in the population of distinct ecological niches: temperate forests for *P. pulmonarius* and humid, subtropical environments for *P. sajor-caju* as being sufficient to ascribe different species names. Subsequently, however, Zmitrovich and Wasser (2016) confirmed that these two organisms are in fact genetically the same organism growing in different environments and parts of the world.

Due to their commercial importance, several studies have investigated the genetic relationships among various *Pleurotus* species (Pawlik *et al.* 2012; Zhao *et al.* 2016; Khan *et al.* 2017; Panek *et al.* 2019; Steenkamp *et al.* 2018; Li *et al.* 2019; Lin *et al.* 2022). Despite similar morphologies, there are often significant differences among the different species when genomic analyses are performed as demonstrated by Panek *et al.* (2019). A sequence-based analysis of population differentiation to measure evolutionary divergence, as well as a more traditional cardinal temperature analysis (which differentiates among different isolates based on rates of growth at different temperatures) clearly indicates, for example, that *P. pulmonarius* and *P. ostreatus* are relatively far apart phylogenetically and are unlikely to interbreed (*i.e.,* the classical distinction among species). Even though *P. pulmonarius* maintains genetic identity through different ecological niches, *P. ostreatus* comprises two significantly genetically separated groups (OA and OB) with limited mutual gene flow.

Several reports note that fungal hyphae in mycelia and fruiting bodies behave as totipotent stem cells and can be assembled into mycelia, stems, or fruiting bodies (*i.e.,* a mycelial culture can be started using cells from a fragment of a fruiting body or a stem; Money, 2002; Moore *et al*. 2008). As shown in Table 6.1, cells comprising fruiting bodies and mycelia are quite similar in composition at the level of proximates. Demonstration of compositional similarity between fruiting bodies and mycelia of *P. pulmonarius* would be important to support the food safety of Phoenix Oyster Mushroom Mycelium, based on extensive consumption of the fruiting body (generally referred to as "mushroom"), and the lack of significant reported toxicity after consumption of the fruiting body.

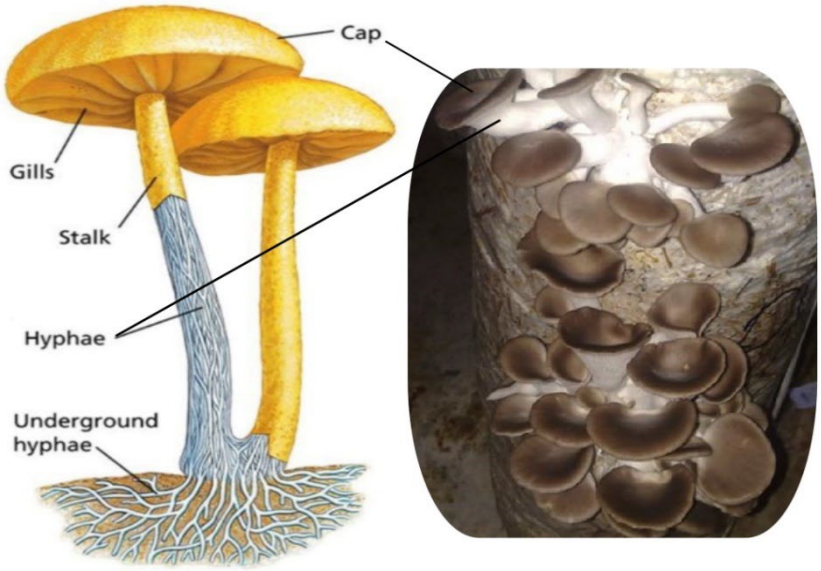
6.1. Developmental Concordance Between Mushroom Mycelia and Fruiting Bodies

Compared to higher plants and animals, fungi can be considered as relatively simple eukaryotic organisms. The basic structural element of most mushrooms, including *P. pulmonarius,* is the hypha (hyphae, pl) as

[15] https://www.nass.usda.gov/Publications/Todays_Reports/reports/mush0821.pdf
[16] https://www.mskcc.org/cancer-care/integrative-medicine/herbs/oyster-mushroom

shown in Figure 1. As shown in the left panel of Figure 1, hyphae are the building blocks of mushrooms. As described in the literature[17]: *"The main body of the fungus is made up of fine threads (hyphae) that group together to make a mycelium. Most of the time the mycelium is hidden from view because it is growing through the soil or under fallen logs or decaying plant and animal remains…When conditions are just right (which may be once or twice a year or sometimes more!) the mycelium can gather together to form a fruit body. So the fine threads that make up the mycelium that lies hidden from view can also form the fruit body of the fungus that we see."* The fruiting bodies of oyster mushrooms in the wild are generally found on rotting trees or stumps, where the mycelia extend into the wood substrate and extract nutrients to feed the reproductive structure (*i.e.,* the fruiting body). Generally, fruiting bodies are the forms found at the market level. More recently, however, cultivated mycelia have been introduced into the food supply as meat substitutes (see Section 3.1).

FIGURE 1: Depiction of the Structures of Oyster Mushroom Structure (Left Panel Adapted from: https://plantfacts.osu.edu/wiki/index.php/Hyphae; Right Panel Adapted from: Myronycheva, O., Bandura, I., Bisko, N., Gryganskyi, A. P., and Karlsson, O. (2017). "Assessment of the growth and fruiting of 19 oyster mushroom strains for indoor cultivation on lignocellulosic wastes," BioRes. 12(3), 4606-4626.



[17] https://www.britmycolsoc.org.uk/mycokids/mycokids-how-are-fruit-bodies-made

6.2. Composition

Analyses of Phoenix Oyster Mushroom Mycelium show that they are composed of proteins, carbohydrates that include fiber, and appreciable levels of some vitamins and minerals. Approximately 90% of the dry weight of the Phoenix Oyster Mushroom Mycelium is fiber (a type of carbohydrate) and protein, with other proximates present in lower amounts. Smiderle *et al.* (2012) have provided data showing that the composition of mycelia can be modulated through changes in the growth media; use of alternative carbon sources may come at the expense of yield.

6.2.1 Proximates in Phoenix Oyster Mushroom Mycelium

Mushlabs surveyed the literature for data and information on the composition of *P. pulmonarius* mycelia and fruiting bodies, including published accounts of what was previously referred to as *P. sajor-caju*. Table 6.1 provides a summary of the proximates analyses for eight batches of substantially dried Phoenix Oyster Mushroom Mycelium and compares those values with published values for proximates in mycelia and fruiting bodies of *Pleurotus* spp. This comparison shows that the proximate levels measured in Phoenix Oyster Mushroom Mycelium are within the expected normal biological variability that is reported in the published literature for *P. pulmonarius* mycelia. Additionally, Phoenix Oyster Mushroom Mycelium compositional data are consistent with data found in the literature on *P. pulmonarius* fruiting bodies. Figures 2 and 3 are depictions of the data in Table 6.1 and allow for ease of comparison.

Table 6.1
Proximates Data Comparison of Phoenix Oyster Mushroom Mycelium (n=8 Batches) and Published Values for *P. pulmonarius* Mycelia and Fruiting Bodies

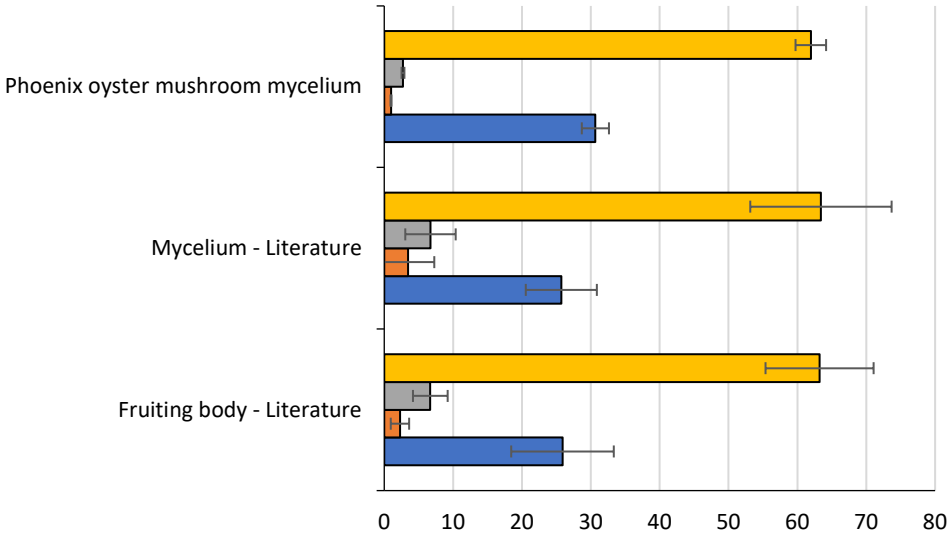
Phoenix Oyster Mushroom Mycelium	% Moisture [ASU L 0.600-3][a]	% Protein [ASU L 06.00-7 mod.]	% Fat [ASU L 06.00-6]	% Ash [ASU L 06.00-4]	% Carbohydrates [calculated]	% Fiber[b] [ASU L 00.00-18 mod.]	% Total Carbohydrates [calculated]
Mean	4.28	30.66	0.98	2.72	1.30	61.95	61.95
S.D.	0.43	1.97	0.07	0.21	1.59	2.22	2.22
Minimum	3.70	27.90	0.86	2.47	0.10	57.70	57.8
Maximum	4.80	34.00	1.03	3.13	3.10	64.90	68.0
Published Values for Mycelia of *P. pulmonarius*[18]							
Mean	--[c]	25.7	3.48	6.71	41.13[b]	22.29	63.42
S.D.	--	5.16	3.79	3.66	27.36	18.11	10.26
Minimum	--	23.09	1.69	3.91	4.10	2.53	50.55
Maximum	--	32.10	10.20	9.09	68.77	46.45	72.07
Values for *P. pulmonarius* Mycelia (Combined Literature Values and Phoenix Oyster Mushroom Mycelium Values)							
Mean	--	28.19	2.23	4.72	20.56	22.29	62.69
S.D.	--	3.5	1.77	2.82	29.08	28.04	1.04
Minimum	--	23.09	0.80	2.47	0.10	2.53	50.55

[18] Ojo *et al.* 2017; Yogachitra, 2019; Confortin *et al.* (2016); Kausar *et al.* (2006); Smiderle *et al.* (2012)

Table 6.1 Proximates Data Comparison of Phoenix Oyster Mushroom Mycelium (n=8 Batches) and Published Values for *P. pulmonarius* Mycelia and Fruiting Bodies							
Phoenix Oyster Mushroom Mycelium	**% Moisture [ASU L 0.600-3][a]**	**% Protein [ASU L 06.00-7 mod.]**	**% Fat [ASU L 06.00-6]**	**% Ash [ASU L 06.00-4]**	**% Carbohydrates [calculated]**	**% Fiber[b] [ASU L 00.00-18 mod.]**	**% Total Carbohydrates [calculated]**
Maximum	--	34.00	10.20	9.09	68.77	64.90	72.07
Published Values for Fruiting Bodies of *P. pulmonarius*[19]							
Mean	--	25.89	2.28	6.69	35.85[b]	26.73	63.22
S.D.	--	7.45	1.33	2.52	17.60	19.11	7.85
Minimum	--	14.73	0.13	1.06	3.73	2.36	46.86
Maximum	--	42.90	6.62	10.35	65.41	63.61	83.29

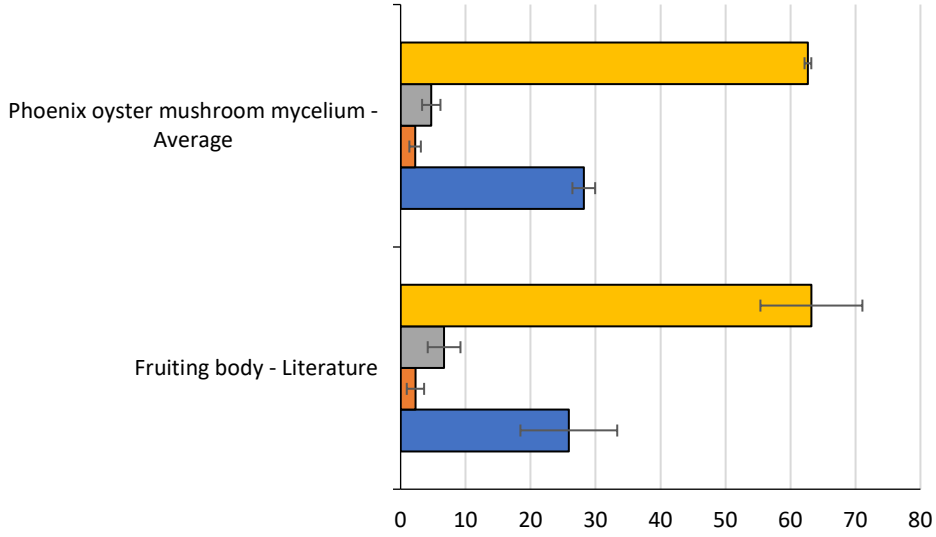
[a] Information in [ ]'s indicates the method of analysis used by Mushlabs.
[b] There was a wide range of levels allocated between fiber and carbohydrates among the different publications, likely due to different assays used by various laboratories and noting that carbohydrates are usually calculated by difference.
[c] NA = not available or not reported

FIGURE 2: Proximates Data Comparison for Eight Batches of Phoenix Oyster Mushroom Mycelium with Published Data on *P. pulmonarius* Mycelia and Fruiting Bodies



[19] Adewoyin and Ayandele, 2018; Akyuz *et al.* 2022; Alam *et al.* 2008; Bonatti *et al*. 2004; Chirinang and Intarapichet. 2009; Cogorni *et al*. 2014; Eke-Ejofor and Pollyn, 2020; Familoni *et al.* 2018; Finimundy *et al.* 2018; Goswami *et al.* 2020; Goyal *et al.* 2006; Gupta *et al.* 2013; Hassan, 2017; Irshad *et al.* 2023; Islam *et al.* 2017; Jonathan *et al.* 2011; Nwoko *et al.* 2017; Patil, 2013; Raman *et al.* 2020; Rana *et al.* 2015

FIGURE 3: Proximates Data Comparison Depicting the Combined Mycelia Values (Eight Batches of Phoenix Oyster Mushroom Mycelium and Values from Published Data on *P. pulmonarius* Mycelia) and Literature Values[20] for Fruiting Bodies



6.2.2 Amino Acids in Phoenix Oyster Mushroom Mycelium

Table 6.2 provides further information about protein composition data shown in Table 6.1 by comparing the amino acid composition of Phoenix Oyster Mushroom Mycelium (the same eight batches) with the values from a series of publications[21] on *P. pulmonarius* fruiting bodies. The amino acid composition of the proteins is quite similar, again emphasizing the similarity of mycelium composition to that of fruiting bodies that have a long history of consumption. This is shown graphically as well in Figure 4.

Table 6.2
Amino Acid Composition of Proteins: Phoenix Oyster Mushroom Mycelium Batch Data Compared with Aggregated Literature Values[22] (Mean ± Standard Deviation) for Fruiting Bodies of *P. pulmonarius*

Amino Acid	Phoenix Oyster Mushroom Mycelium	Aggregated Literature Values for Fruiting Bodies [Min/Max Values]
Alanine	6.88 ± 0.69	6.80 ± 2.21 [2.86/ 11.43]
Arginine	5.64 ± 0.96	7.52 ± 2.05 [3.64/ 12.54]

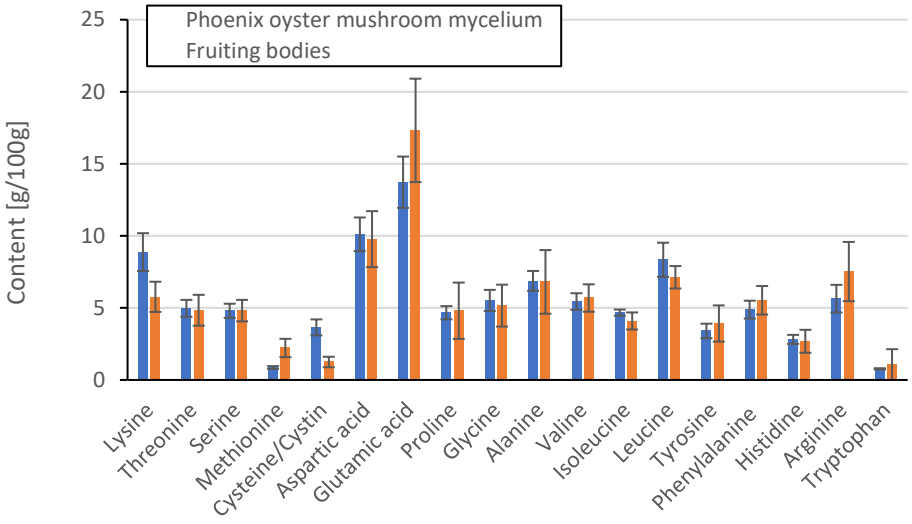
[20] Adewoyin and Ayandele, 2018; Akyuz *et al.* 2022; Alam *et al.* 2008; Bonatti *et al*. 2004; Chirinang and Intarapichet. 2009; Cogorni *et al*. 2014; Eke-Ejofor and Pollyn, 2020; Familoni *et al.* 2018; Finimundy *et al.* 2018; Goswami *et al.* 2020; Goyal *et al.* 2006; Gupta *et al.* 2013; Hassan, 2017; Irshad *et al.* 2023; Islam *et al.* 2017; Jonathan *et al.* 2011; Nwoko *et al.* 2017; Patil, 2013; Raman *et al.* 2020; Rana *et al.* 2015

[21] Chirinang and Intaqrapichet, 2009; Gupta *et al.,* 2013; Kayode *et al.* 2015; Adebayo and Oloke, 2017; Fasoranti *et al.,* 2019; Wang *et al.,* 2022

[22] Chirinang and Intaqrapichet, 2009; Gupta *et al.,* 2013; Kayode *et al.* 2015; Fasoranti *et al.,* 2019; Wang *et al.,* 2022; Mukhopadhyay and Guha, 2015

Table 6.2 Amino Acid Composition of Proteins: Phoenix Oyster Mushroom Mycelium Batch Data Compared with Aggregated Literature Values[22] (Mean ± Standard Deviation) for Fruiting Bodies of *P. pulmonarius*		
Amino Acid	**Phoenix Oyster Mushroom Mycelium**	**Aggregated Literature Values for Fruiting Bodies [Min/Max Values]**
Aspartic acid	10.11 ± 1.17	9.77 ± 1.94 [6.32/ 12.52]
Cysteine/Cystine	3.65 ± 0.56	1.25 ± 0.36 [0.74/ 2.09]
Glutamic acid	13.72 ± 1.78	17.32 ± 3.59 [8.43/ 21.00]
Glycine	5.52 ± 0.73	5.16 ± 1.45 [2.02/ 7.29]
Histidine	2.81 ± 0.31	2.68 ± 0.80 [2.13/ 4.76]
Isoleucine	4.67 ± 0.21	4.09 ± 0.59 [3.34/ 5.43]
Leucine	8.34 ± 1.18	7.13 ± 0.78 [5.78/ 8.13]
Lysine	8.87 ± 1.31	5.77 ± 1.05 [3.88/ 6.91]
Methionine	0.87 ± 0.09	2.22 ± 0.64 [1.27/ 3.08]
Phenylalanine	4.88 ± 0.62	5.53 ± 0.99 [4.28/ 7.88]
Proline	4.67 ± 0.46	4.80 ± 1.95 [1.64/ 10.03]
Serine	4.80 ± 0.49	4.81 ± 0.75 [3.08/ 6.15]
Threonine	4.97 ± 0.59	4.84 ± 1.07 [1.82/ 6.58]
Tryptophan	0.77 ± 0.04	1.06 ± 1.08 [--/2.42]
Tyrosine	3.40 ± 0.50	3.92 ± 1.26 [2.83/ 6.69]
Valine	5.44 0.58	5.69 ± 0.95 [4.88/ 8.56]

FIGURE 4: Levels of Amino Acids (per 100 g Protein) in Phoenix Oyster Mushroom Mycelium (Blue Bars) Compared with Levels Reported for Fruiting Bodies (Orange Bars) in Published Literature.



6.2.3 Polysaccharides in Phoenix Oyster Mushroom Mycelium

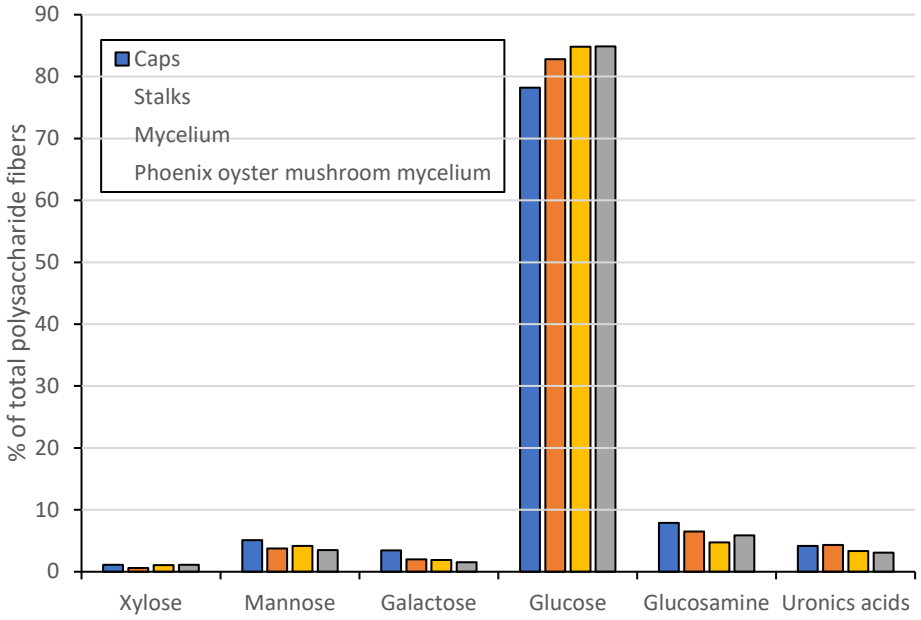
In addition to the data on amino acid levels, Mushlabs has performed more detailed compositional comparisons of fiber polysaccharide content in Phoenix Oyster Mushroom Mycelium (Table 6.3 and Figure 5). Again, the comparisons demonstrate that the compositional data are within the range of biological variability.

TABLE 6.3
Comparison of Fiber Structure (per 100 g protein) in Phoenix Oyster Mushroom Mycelium Batches and Published Literature Values

	Biological Source	Xylose	Mannose	Galactose	Glucose	Glucosamine	Uronic acid
Published Values for P. pulmonarius							
Cheung, 1996	**Cap**	1.14	5.12	3.47	78.2	7.9	4.17
	Stalk	0.62	3.77	2.01	82.8	6.5	4.34
	Fruiting Body	0.88	4.445	2.74	80.5	7.2	4.255
	Mycelia	1.06	4.15	1.88	84.8	4.73	3.34
Guo *et al.* 2022	**Fruiting body**	2	5	3	90.0	Not measured	Not measured
Smiderle *et al.* 2011	**Mycelia**	2	9.8	3.2	84.1	Not measured	Not measured

TABLE 6.3 Comparison of Fiber Structure (per 100 g protein) in Phoenix Oyster Mushroom Mycelium Batches and Published Literature Values							
	Biological Source	**Xylose**	**Mannose**	**Galactose**	**Glucose**	**Glucosamine**	**Uronic acid**
Phoenix Oyster Mushroom Mycelium							
Batch Data	**Mycelium**	1.14	3.50	1.55	84.87	5.86	3.09

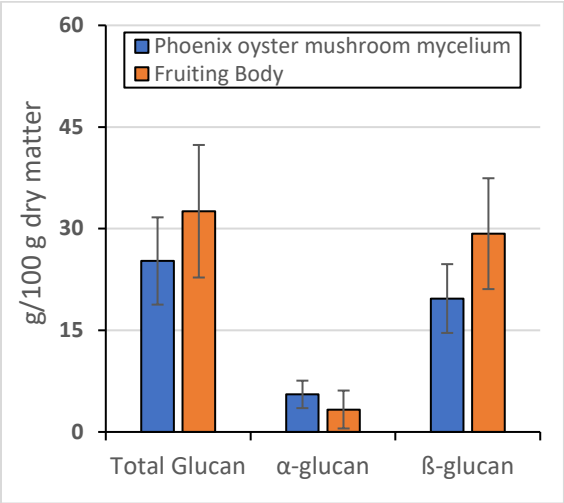
FIGURE 5: Comparison of Total Polysaccharide Content (per 100 g fiber) in Phoenix Oyster Mushroom Mycelium Batches and Published Literature Values for Phoenix Oyster Mushroom Structures (Source Cheung, 1996, published as *P. sajor-caju*)



In addition to the analysis of fiber polysaccharides shown in Figure 5, Table 6.4 show that levels of the soluble fibers α-glucan and β-glucan levels are similar as well when analyses of Phoenix Oyster Mushroom Mycelium are compared with data found in the published literature on *P. pulmonarius* mycelia (*i.e.,* values found in Avni *et al.* 2017; Stastny *et al.* 2022; Sari *et al.* 2016). Figure 6 provides the comparison of Phoenix Oyster Mushroom Mycelium glucan composition levels with levels reported in the published literature for *P. pulmonarius* fruiting bodies as well.

TABLE 6.4 Comparison of Levels of Soluble Fiber Found in Phoenix Oyster Mushroom Mycelium (8 Batches) with Values Reported in the Published Literature[23] for *P. pulmonarias* Mycelia			
Source Material	**Total Glucan Content**	**α-Glucan Content**	**β-Glucan Content**
Values in grams / 100 grams			
Phoenix Oyster Mushroom Mycelium	25.2 ± 6.4	5.5 ± 2.0	19.7 ± 5.1
Literature Average value	32.57 ± 9.78	3.31 ± 2.8	29.26 ± 8.18
Glucan composition - Values Expressed as % of Total Glucan			
Phoenix Oyster Mushroom Mycelium	--	21.8 ± 5.6	78.2 ±5.6
Literature Average Value	--	9.75 ± 6.0	91.1 ±6.0

FIGURE 6: Comparison of Glucan Composition (per 100 g dry weight) in Phoenix Oyster Mushroom Mycelium Batches and Published Literature Values[23] for *P. pulmonarius* Fruiting Bodies (Source Avni *et al.,* 2017, Stastny *et al.,* 2022, Sari *et al.* 2022)



More careful consideration of available data on total carbohydrate levels (including fiber) was also performed. The composition of carbohydrates reported in literature for both fruiting bodies and mycelium of *Pleurotus* spp. Shows some variability. Fractional composition of simple carbohydrates ranges from 3-7% (Hassan, 2017; Salehi, 2019; Akyüz *et al.* 2022) to 50-60% (Yogachitra, 2019; Ojo *et al.,* 2017; Bonatti *et al.,* 2004; Patil, 2013). Likewise, fractional fiber content ranges from 6-7% (Patil, 2013; Nwoko *et al.,* 2017; Eke-Ejiofior and Pollyn, 2020) to 55-60% (Hassan, 2017; Chilanti *et al.,* 2022; Irshad *et al.,* 2023). The wide range in values can be explained by the methods used to assess the level of carbohydrates;

[23] Avni *et al.* (2017); Stastny *et al.* (2022); Sari *et al.* (2022)

carbohydrate levels are usually determined by calculation (*i.e.,* the remaining component when crude protein, crude fat, ash, and moisture have been summed and subtracted from 100%) and thus often includes fiber (Chang *et al.* 1981; Laforteza *et al.,* 2020). Others prefer subtracting the level of fiber to differentiate between simple and complex carbohydrates.

Different assays to determine total fiber content are listed in the Codex Alimentarius and have been interchangeably used. The methods differ in their abilities to accurately quantify fiber, however, either because they do not solubilize resistant fiber or because other compounds are precipitating during the analysis and are quantified as fiber.[24] This can result in underestimation or overestimation of fiber content and, result in apparently different levels of simple carbohydrates after subtraction of the other components. With respect to Phoenix Oyster Mushroom Mycelium, single carbohydrates were detected at low levels only (up to 3.1 g/100g) while complex carbohydrate levels (fiber) accounted for a major part of the macro composition (57.7 to 64.9g/ 100g) of Phoenix Oyster Mushroom Mycelium. Overall, these data are consistent with observations of others for *P. pulmonarius* fruiting bodies (Hassan*,* 2017; Salehi, 2019; Akyüz *et al,* 2022; Irshad *et al.,* 2023).

6.2.4 Fatty Acids in Phoenix Oyster Mushroom Mycelium

Data on the fatty acid profile of Phoenix Oyster Mushroom Mycelium was also collected. After extraction and formation of methyl esters of fatty acids, eight batches were analyzed using gas chromatography with flame ionization detection (ISO 12966); results are provided Table 6.5, with all values reported as g/100 g dry weight. For comparison purposes, the fatty acid profile for *P. pulmonarius* fruiting bodies and mycelium reported in the published literature are included in Table 6.5 as well (also g/100 g dry weight). Overall, values for fatty acids were low because total fat is present at low levels in fungi (around 1%). In terms of specific types of fat, the data on Phoenix Oyster Mushroom Mycelium are consistent with literature data showing that fatty acid composition in both *P. pulmonarius* mycelium and fruiting bodies is a balance of saturated and unsaturated fatty acids with C18:2 (linoleic acid), C18:1 (oleic acid) and C16:0 (palmitic acid) fatty acids being the predominant components (Finimundy *et al.* 2018; Dimou *et al.* 2002; Irshad *et al.* 2023; Goyal *et al.* 2015; Nair *et al.* 1989). Figure 7 depicts the comparison as well.

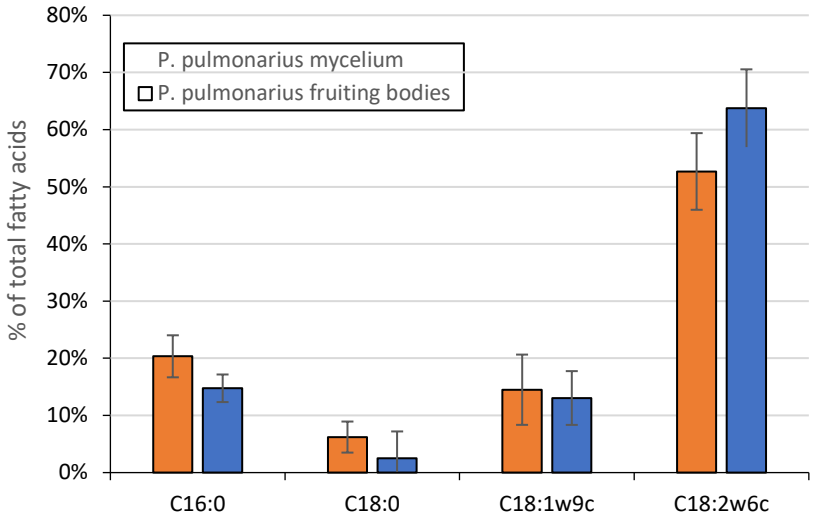
TABLE 6.5
Fatty Acid Profile of Phoenix Oyster Mushroom Mycelium (8 Batches) and Literature Values Reported for *P. pulmonarius* Mycelia and Fruiting Bodies

Sample Type	Saturated Fat	Monosaturated Fat	Polyunsaturated Fat	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid
Mushlabs' Phoenix Oyster Mushroom Mycelium							
Batch 1	34.6%	27.9%	37.5%	20.2%	6.3%	21.2%	36.5%
Batch 2	26.7%	26.7%	46.5%	14.9%	5.7%	23.8%	43.6%
Batch 3	34.4%	22.9%	42.7%	20.8%	6,6%	19.8%	41.7%
Batch 4	35.7%	16.7%	47.6%	21.4%	6.2%	14.3%	47.6%
Batch 5	47.1%	21.6%	31.4%	24.5%	12.7%	18.6%	31.4%
Batch 6	51.5%	27.7%	20.8%	25.7%	18.8%	25.7%	19.8%
Batch 7	64.8%	18.2%	17.0%	34.1%	15.9%	14.8%	17.0%
Batch 8	50.0%	27.5%	22.5%	25.5%	17.6%	24.5%	22.5%

[24] See discussion at: https://www.megazyme.com/focus-areas/dietary-fiber-portal/measurement-of-dietary-fiber

TABLE 6.5 Fatty Acid Profile of Phoenix Oyster Mushroom Mycelium (8 Batches) and Literature Values Reported for *P. pulmonarius* Mycelia and Fruiting Bodies							
Sample Type	**Saturated Fat**	**Monosaturated Fat**	**Polyunsaturated Fat**	**Palmitic Acid**	**Stearic Acid**	**Oleic Acid**	**Linoleic Acid**
Average Value (S.D.)	42.9% (11.8)	23.9% (5.9)	33.2% (11.7)	27.8% (5.1)	11.3% (5.9)	20.6% (5.4)	32.4% (11.1)
P. pulmonarius Mycelium Literature Values[25]							
Average Value (S.D.)	28.5% (4.9)	16.8% (6.2)	52.7% (6.7)	20.3% (3.7)	6.2% (2.7)	14.5% (6.2)	52.7% (6.7)
P. pulmonarius Fruiting Bodies Literature Values[26]							
Average Value (S.D.)	22.0% (5.1)	13.8% (5.5)	64.5% (6.6)	14.8% (2.4)	2.5% (1.9)	13.1% (4.7)	63.7% (6.8)

FIGURE 7: Comparison of Fatty Acid Composition (per 100 g dry weight) in Phoenix Oyster Mushroom Mycelium[25] and Published Literature Values[26] for *P. pulmonarius* Fruiting Bodies



The Table 6.5 data related to fatty acid profiles in lipids of *P. pulmonarius* and Phoenix Oyster Mushroom Mycelium are further supported by discussion int the published literature around similarities in lipids in mycelium and fruiting bodies generally (Diamantis *et al.* 2022): *"Several studies have suggested that the cell wall composition of mycelium can be similar to that of mushroom fruiting body. Smiderle et al. reported total lipids of 0.8% w/w in P. pulmonarius mycelium synthesized when glucose was used as the*

[25] Values reported in Nair *et al.* (1989), Maheshwari *et al.* (2020), Mukhopadhyay *et al.* (2015) and Dimou *et al.* (2002). Expressed as average of mean values (g/100 g dry weight) with standard deviation (S.D.).

[26] Values reported in Nair *et al.* (1989), Maheshwari *et al.* (2020), Mukhopadhyay *et al*. (2015, Dimou *et al.,* (2002), Goyal *et al.* (2015), Finimundy *et al.* (2018), and Irshad *et a*l. (2023). Expressed as average of mean values (g/100 g dry weight) with standard deviation (S.D.).

main carbon source. Kavishree et al.[27] reported fat content of P. djamor and P. sajor-caju at 0.5 % w/w and 0.8% w/w, respectively."

6.2.5 Vitamins and Minerals in Phoenix Oyster Mushroom Mycelium

Levels of certain vitamins and minerals in Phoenix Oyster Mushroom Mycelium are also relevant for food safety assessment. The eight batches of Phoenix Oyster Mushroom Mycelium were also tested for levels of vitamins and minerals that are essential for normal growth and development in humans. In some cases, experts in nutrition have set levels needed in the diet of humans to maintain health, referred to collectively as dietary reference values. These values are referred to as "Recommended Daily Intakes" or "RDIs" and "Recommended Daily Allowances" or RDAs.

An RDI is a population-adjusted value derived from an RDA and is defined as the average daily dietary intake level sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a group. Because certain vitamins and minerals can have toxic effects when levels of exposure greatly exceed recommended daily intakes, particularly if exceeded on a repeated basis, scientists have established "upper limits" on some dietary reference values for vitamins and minerals, referred to a "tolerable upper intake level" or "UL". These values represent the highest level of daily nutrient intake that is likely to pose no significant risk of adverse health effects to almost all individuals in the general population.

Levels of vitamins and minerals from Phoenix Oyster Mushroom Mycelium batches were used to inform the safety of the ingredient for use in food (Table 6.6). Measurable amounts of the following vitamins were detected in the eight lots: vitamin B1, vitamin B2, vitamin B6 (as pyridoxamine), niacin, pantothenic acid (B5), biotin (B8), folic acid (B9), vitamin B12, vitamin C, vitamin E, and α-tocotrienol (Table 6.6). There were also measurable amounts of sodium, calcium, potassium, phosphorus, magnesium, iron, copper, zinc, and manganese (Table 6.6). These data show a high degree of consistency across Mushlabs' batches in the detected levels of vitamins or minerals.

Other vitamins and minerals were assayed but were not detected at levels above the LOD for the methods used. These included vitamin A, pyridoxal, pyridoxine, vitamin D2, β-tocopherol, Δ-tocopherol, γ-tocopherol, α-carotene, β-carotene, vitamin K1, selenium, and cobalt. Cobalt is not allowed in human food in the US, making the result of particular importance to the food safety assessment.

The following methods of analysis were used: vitamin B1 (DIN EN 14122, HPLC/Fl); vitamin B2 (DIN EN 14152, HPLC/Fl); pyridoxamine (DIN EN 14663, HPLC/FI); niacin (AOAC 944.13); pantothenic Acid (AOAC 945.74); biotin (SOP M 3552, LC-MS-MS); vitamin C (SOP M 2885, LCMS-MS); vitamin B12 (AOAC 952.20/986.23); vitamin D3 (SOP M2885, LC/MS-MS); vitamin E (DIN EN 12822, HPLC/Fl); sodium, calcium, potassium, phosphorus, magnesium, and iron, (DIN EN 15621, mod.); copper (DIN EN 15763, mod.); zinc (DIN EN 15621, mod); selenium, manganese, and cobalt (DIN EN 15763, mod).

[27] Kavishree, S. *et al.* 2008. Fat and fatty acids of Indian edible mushrooms. *Food Chem.* 106:597-602.

TABLE 6.6 Vitamin and Mineral Levels Detected in Eight Batches of Phoenix Oyster Mushroom Mycelium									
Parameter[a]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean Value**
Vitamins									
Vitamin B1 (mg/kg)	0.403	0.374	0.469	0.417	0.450	0.444	0.393	0.459	0.426
Vitamin B2 (mg/kg)	0.860	0.819	0.994	0.885	0.934	0.888	0.817	0.890	0.886
Pyridoxamine	0.20	0.182	0.203	0.196	0.215	0.157	0.167	0.177	0.187
Vitamin B6 (total) (mg/kg)	0.201	0.183	0.204	0.197	0.216	0.158	0.168	0.178	0.188
Niacin (mg/kg)	8.75	8.18	9.14	8.82	9.56	8.72	8.28	9.07	8.82
Pantothenic acid (mg/kg)	1.81	1.63	1.71	1.80	1.67	1.57	1.74	1.69	1.70
Biotin (µg/kg)	4.72	4.30	4.43	4.12	4.56	4.61	4.03	4.82	4.45
Folic acid (µg/kg)	120	134	162	143	133	131	133	141	137
Vitamin B12 (µg/kg)	1.61	1.63	1.13	0.833	0.905	0.902	0.878	1.13	1.13
Vitamin C (mg/kg)	2.29	2.48	3.03	1.43	2.84	1.42	1.59	1.52	2.08
Vitamin D3(µg/kg)	<0.05[b]	<0.05[b]	<0.05[b]	18.7	1.48	13.9	<0.05[b]	<0.05[b]	4.29
Vitamin E (mg/kg)	0.257	0.242	0.191	0.274	0.335	0.320	0.346	0.287	0.282
A-Tocopherol (mg/kg)	0.041	0.041	0.030	0.037	0.107	0.087	0.124	0.077	0.068
Minerals									
Sodium (mg/kg)	3,330	2,620	2,640	2,310	2,140	2,240	2,180	2,370	2,479
Calcium (mg/kg)	3,910	3,770	3,910	3,520	3,930	4,400	3,120	3,370	3,741
Potassium (mg/kg)	1,600	1,400	1,670	1,460	1,470	1,610	1,490	1,540	1,530
Phosphorus(mg/kg)	5,350	4,850	5,520	4,910	5,000	5,330	4,900	5,270	5,141
Magnesium (mg/kg)	778	772	785	705	727	762	703	784	746
Iron (mg/kg)	83.7	86.2	86.8	89.2	83.6	100	71.7	75.2	84.6
Copper (mg/kg)	11.2	9.68	11.7	9.87	10.7	10.1	10.1	10.9	10.5
Zinc (mg/kg)	34.6	29.9	35.6	30.1	32.4	31.1	32.2	32.2	32.3
Manganese (mg/kg)	7.60	6.45	7.35	6.57	6.87	6.95	5.95	6.96	6.84

[a] Values listed for each vitamin or mineral are based on dry weight.
[b] Values were below the limit of quantification.

Because some vitamins and minerals have UL values set, *i.e.,* maximum intake levels expected to be free of any adverse health effects, Mushlabs estimated margins of exposure (MOEs) between UL for those substances with levels detected in Phoenix Oyster Mushroom Mycelium batches (Table 6.7). MOEs are commonly used in food safety assessment to determine whether a level of exposure would raise a food safety concern compared to some already established physiological or regulatory level. MOEs were calculated only for those vitamins and minerals with established ULs and that also were detected at quantifiable levels in the eight batches of Phoenix Oyster Mushroom Mycelium. Inspection of the data in Table 6.7 indicates that the MOEs for components in Phoenix Oyster Mushroom Mycelium are at appropriate levels for safe consumption in food under the intended conditions of use.

TABLE 6.7 Margin of Exposure (MOE) Between Dietary Reference Values for Individual Vitamins and Minerals with Established UL Values (UL of a RDA[a] or RDI[b]) and Levels of Measured Vitamins and Minerals in Phoenix Oyster Mushroom Mycelium				
Vitamin	**Mean Level Detected[c] (mg/kg)**	**Level in a Daily Serving of Mycelia[d] 19 g [or 42 g]**	**Adult UL of RDA [RDI] Level**	**MOE US UL Value: Daily Serving Amount**
Vitamins				
Folic acid	0.137	2.6 µg [5.8 µg]	1000 µg/d	172 to 385
Pantothenic acid	1.70	32.3 µg [71.4 µg]	[5 mg/d]	70 to 155
Vitamin B6	0.188	3.6 µg [7.9 µg]	100 mg/d	12,658 to 27,778
Vitamin C	2.08	39.5 µg [87.4 µg]	2000 mg/d	22,883 to 50,633
Vitamin E	0.282	5.4 µg [11.8 µg]	1000 mg/d	84,746 to 185,185
Vitamin B12	0.001	1.9E-5 mg [4.2E-5 mg]	[2.4 µg/d]	57 to 126
Minerals				
Sodium	2479	47 mg [104 mg]	2.3 g/d	22 to 49
Calcium	3741	71.1 mg [157 mg]	2.5 g/d	16 to 35
Phosphorus	5141	98 mg [216 mg]	4 g/d	19 to 41
Iron	84.6	1.61 mg [3.55 mg]	40 mg/d	11 to 25
Copper	10.5	0.20 mg [0.44 mg]	10 mg/d[e]	23 to 50
Zinc	32.3	0.614 mg [1.36 mg]	40 mg/d	29 to 65
Manganese	6.84	0. 13 mg [0.287 mg]	11 mg/d	38 to 85

[a] RDA = Recommended Daily Allowance
[b] RDI = Recommended Daily Intake
[c] Levels listed in this column reflect the average (mean) levels detected in the eight batches of Mushlabs' mycelia product.
[d] The estimated US maximum intake per day of mycoprotein of from 19 to 42 g/person/day is assumed for the purposes of comparison.
[e] The UL for copper intake in children is 1 mg/day and is based on a single acute intake of copper (one time exposure).

6.2.6 Other Compositional Data Relevant for Safety Assessment

Other relevant compositional data for food safety assessment would include the heavy metals and microbial contamination data that were described previously in Section 2.3. Specifications for Phoenix Oyster Mushroom Mycelium have been set to ensure that neither heavy metals nor microbial burden present a food safety concern.

It is important to note that unlike commercially grown mushroom fruiting bodies, mycelial preparations are grown under controlled fermentation conditions and are thus unlikely to carry significant microbial loads. Data shown in Table 2.5 supports the conclusion that Phoenix Oyster Mushroom Mycelium is safe for consumption from a microbiological perspective.

6.3 Allergenicity

Two types of allergenicity are relevant to the discussion of Phoenix Oyster Mushroom Mycelium: food allergy associated with ingestion of allergenic mushroom species, and respiratory allergy associated with inhalation of allergenic spores of mushrooms.

No reports of food allergy related to ingestion of either fruiting bodies or mycelia of this species have been identified. There is one listed report of an adverse reaction to ingestion of *Pleurotus* (species not listed) in an individual in which alcohol was implicated in the adverse reactions reported of gastrointestinal distress, flushing, hypotension, muscle spasm, and tachycardia (Beug *et al.* 2006). The same review (Beug *et al.* 2006) identified nine cases of gastrointestinal symptoms in individuals ingesting *P. ostreatus*. None of these reactions to *P. ostreatus* or undefined *Pleurotus* spp. were identified specifically as food allergy. Considered together, the literature does not indicate that food allergy is a food safety concern for Phoenix Oyster Mushroom Mycelium.

As with other cultivated mushroom species, inhalation-based allergies have been documented especially among workers who process mushrooms. It is well known that some antigens present on spores are allergenic, resulting in allergic reactions to inhaled antigens. For example, Branicka *et al.* (2021) recently published a case report of an allergic asthmatic reaction in a woman processing and packaging *P. ostreatus*, a related species of edible mushrooms. In an earlier work, Fischer *et al.* (2002) described delayed hypersensitivity-type reactions in some individuals upon intra-dermal challenge of hypersensitive subjects with *P. pulmonarius* spore extract. Characteristic symptoms of *Pleurotus* spp. allergy have included allergic rhino-conjunctivitis, asthma, and hypersensitivity pneumonitis (Lehrer *et al.* 1994; Saikai *et al.* 2002; Hebling *et al.* 1998; Mori *et al.* 1998). In addition, allergic contact dermatitis after exposure to *Pleurotus ostreatus* has been described (Rosina *et al.* 1995).

Because food products consisting of mycelia produced with submerged fermentation do not contain spores, allergenicity would not pose a food safety concern for use of Phoenix Oyster Mushroom Mycelium as a food ingredient.

6.4 Toxins

Oyster mushrooms are considered edible, with the implication that like other edible mushrooms, they are not toxic *i.e.,* do not contain toxins that could pose a food safety concern. Nonetheless, fungi (including edible mushrooms) have evolved chemical means to protect themselves. These take the form of various peptides or proteins that can harm predators. For example, the most consumed mushrooms in the US, *Agarius bisporus*, a species that contains the white button, cremini, and portobello mushrooms contains agaritine, a toxic compound implicated in mutagenesis. Despite the wide use of these mushrooms in the US diet and their long history of safe use as a food, LaGrange and Vemoux (2022) urge that as a "matter of prudence" that *A. bisporus* not become an "everyday" food, particularly not one that is consumed raw.

There are reports that members of the *Pleurotus* genus also have evolved chemoprotective mechanisms. *P. ostreatus* has been reported to contain a cytolytic protein, ostreolysin, primarily as a defense against nematodes (Zuzek *et al.* 2006; Frangez *et al.* 2017). This pore-forming protein is heat-labile and sensitive to pH, losing its activity at pH below 5 and temperatures above 25°C (Berne *et al.* 2005). The genus also contains the closely related pleurotolysins A and B.

In order to determine whether Phoenix Oyster Mushroom Mycelium contained any similar toxins, Mushlabs had a Blastx search conducted with all known annotated fungal toxin genes, including a literature search for the known *Pleurotus* toxin genes, ostreolysin A6 and pleurotolysins A and B. The results indicated some closely related nucleotide sequences were found in the genome. Further analysis of all fungal peptides did not result in identification of coding sequences with a high degree of identity. (See Appendix B).

Despite the apparent concordance of the DNA sequences in the data collected by Mushlabs, the genomic concordance does not translate to a food consumption risk associated with Phoenix Oyster Mushroom Mycelium. Vidic *et al.* (2005) confirmed the findings of Berne *et al*. (2002) that ostreolysin is not found in the mycelia of basidiomycetes (of which *Pleurotus spp.* is one). Ostreolysin expression is turned on during the stage of early primordia, and increases during the maturation of the fruiting bodies, resulting in a relatively high concentration in young fruiting bodies. Ostreolysin was measured both by immunofluorescence and hemolytic activity (the toxin forms pores in lipid-rich vesicles such as erythrocytes). No immunofluorescence or hemolytic activity was detected in the mycelia, leading the investigators to hypothesize that the lysin participates in hyphal differentiation and the formation of basidia and basidiospores during the early differentiation of the fruiting body.

These observations indicate that although genetic sequences encoding ostreolysin are present in the Phoenix Oyster Mushroom Mycelium genome, these genes are not expressed in mycelia and no ostreolysin protein has been identified in mycelia. Therefore, this hazard is not present in Phoenix Oyster Mushroom Mycelium.

Apart from toxins that may be produced by the mushrooms themselves, as previously discussed, food safety concerns may arise due to the potential for the mushrooms to contain chemical contaminants from environmental contamination. These are only present as the result of the environment in which mushrooms grow (*e.g.,* pesticides or other environmental toxins). Regardless, and despite Mushlabs' controlled conditions of growth, the company contracted a third-party laboratory to assay for the presence of a series of chemical contaminants often encountered in agricultural practice. All testing was negative.[28] The production of Phoenix Oyster Mushroom Mycelium under controlled conditions precludes the need for further analyses of potential chemical contaminants.

6.5 Overall Summary of Published Literature

An extensive literature search was performed to identify data or information relevant to assessing the safety of *P. pulmonarius* as a food ingredient. Much of the identified literature provides reviews of the use of *P. pulmonarius* for its medicinal properties (*e.g.,* Dalonso *et al.* 2015; Golak-Siwulska *et al.* 2018; Krakowska *et al.* 2020; Tung *et al.* 2020). Although such studies were not designed as traditional toxicological evaluations, some studies focusing on pharmacological or biological activity of either the *P.*

[28] Data are provided in summary form in Appendix C.

pulmonarius mushroom itself (fruiting bodies) or extracts of the mushroom (both fruiting bodies and mycelia), do provide data relevant to a food safety assessment. The key observations from the published literature related to safety of *P. pulmonarius* as a food ingredient are presented in Appendix D.

Appendix D provides a tabular overview of 18 studies on the bioactivity of different *P. pulmonarius* preparations (*e.g.,* dried mushroom; aqueous and/or organic extracts of fruiting bodies of *P. pulmonarius*; aqueous and/or organic extracts of mycelia of *P. pulmonarius*). The studies overall indicate that extracts of *P. pulmonarius* appear to have beneficial effects on endpoints of human health studied. Although most of these studies were not designed as safety or toxicology evaluations, the data can serve as indicators of the lack of toxicity (or its inverse, safety) of *P. pulmonarius* after oral exposure (*e.g.,* no change in body weight; no histopathological changes in organs after oral dosing; *etc.*).

The *in vivo* studies included were selected because they met one or more of the following criteria:

- Published in a peer-reviewed journal (relevant to study quality)
- Used a common laboratory animal species (relevant to study quality and interpretation of any findings/ observations)
- Involved systemic exposure of animals to preparations of *P. pulmonarius*, either after oral dosing or intraperitoneal injection (relevant to food safety assessment)
- Included control groups in the study design (aids in interpretation of any reported effects/ observations)
- Included more than one exposure or dose level in the study design (dose-response evaluation)
- Investigated duration of exposure (relevant to food safety assessment)
- Provided details on the nature of the *P. pulmonarius* substance being administered (relevant to food safety assessment)

A review of the key features and outcomes of these studies (Appendix D) shows that regardless of the type of *P. pulmonarius* preparation/extract studied, there were no reports that the mushroom or its extracts were toxic at "bioactive" doses. In this context, "bioactive" doses refer to levels of exposure affecting basic cellular physiology in disease models, *e.g.,* animal models of diabetes or cancer in the case of *P. pulmonarius* and its extracts. The studies consistently indicated that bioactivity was not accompanied by early indications or frank signs of toxicity. Appendix D includes studies (*e.g.,* Xu *et al.* 2014, Balaji, P. *et al.* 2020), in which data were collected from control groups *i.e.,* those in which the only intervention was administration of a *P. pulmonarius* preparation to non-disease induced or otherwise untreated animals. No toxicity was reported in the outcomes of such studies.

6.6 Safety of Phoenix Oyster Mushroom Mycelium Based on Compositional Analyses and Consideration of Dietary Exposure Reference Values

The data and information provided in this document demonstrate that Phoenix Oyster Mushroom Mycelium is fully defined with no unidentified compounds present at levels above the limit of detection. Toxins associated with the fruiting body of *Pleurotus spp.* are not present in the mycelia. Compositional data on eight batches of Phoenix Oyster Mushroom Mycelium have demonstrated the ingredient to be primarily protein and carbohydrate (including fiber). No microbial contamination and no heavy metals

were detected at levels that would raise a food safety concern. Other toxins and chemical contaminants also were not detected in the eight batches.

Once ingested in the diet, Phoenix Oyster Mushroom Mycelium, like any food including *P. pulmonarius* fruiting bodies, will undergo digestion. Bachmann *et al.* (2021) reported on an *in vitro* digestion of *Aspergillus niger* mycelium high in fiber and protein content (similar to Phoenix Oyster Mushroom Mycelium). The substrates were analyzed to determine *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) coefficients. The study authors reported *A. niger* mycelium had 28% IVDMD and 31% IVOMD, which correlated to nutrient density. Other substrates also were tested by the authors, and the IVDMD for *A. niger* mycelium was comparable to soybean shells and wheat bran, while the IVOMD for *A. niger* mycelium was comparable to powdered cellulose, soybean shells, and wheat bran. The IVDMD and IVOMD values for the mycelium were higher than those of sugar beet pulp and were less than those of lignocellulose. Phoenix Oyster Mushroom Mycelium would be expected to have a similar degree of digestibility to that of *A. niger* mycelium.

Considering potential exposure under the proposed conditions of use, levels of intake of certain vitamins and minerals for the use of Phoenix Oyster Mushroom Mycelium as a food ingredient were estimated by calculation and have been presented in Table 6.5. These comparisons are relevant to the food safety assessment because some vitamins and minerals can be toxic at levels of exposure that exceed the ULs established for them. Inspection of the data in Table 6.5 confirms that levels of exposure to various vitamins and minerals detected in the eight batches of Phoenix Oyster Mushroom Mycelium produced were within acceptable daily dietary intake ranges, confirming that the mycelia would be safe to eat. The calculated MOEs were almost always at least an order of magnitude above the estimated intake levels of exposure, and in many cases, exceeded several orders of magnitude. Given that the intake estimates are based on the worst-case scenario (*i.e.,* intake levels for vegetarians in which Mushlabs' Phoenix Oyster Mushroom Mycelium comprised 100% of the mycoprotein-equivalent intake each day), consumption of vitamins and minerals from Phoenix Oyster Mushroom Mycelium is not expected to be nutritionally disadvantageous or present a safety concern to children and adults ingesting foods containing the mycelia on a daily basis.

6.7 Safety Conclusions

Phoenix Oyster Mushroom Mycelium is safe to eat as an ingredient in food based on the following observations and weight of evidence evaluation:

- The mushroom mycelia will be limited to a factional composition of no more than 25% (dry basis) as an alternative source of protein in commonly consumed foods such as alternative milks, cheeses, creams, yogurts, meat, and shrimp and fish products.
- The compositional data obtained from Phoenix Oyster Mushroom Mycelium do not indicate the presence of any toxicants, including heavy metals or microbial toxins.
- The compositional data are consistent, within expected biological variability, with compositional information from publicly available literature on *P. pulmonarius* mycelia and fruiting bodies, the latter of which is currently present in the US diet.
- The company's manufacturing methods and operations are conducted under food Good Manufacturing Practice, and do not introduce microbial contamination or other toxic substances.

- A body of publicly available information on the safety of various *Pleurotus* spp. preparations, including concentrated extracts of *P. pulmonarius*.

Part 7. List of Supporting Data and Information

7.1 Cited Publications and References

Abidin, M.H.Z. *et al.* 2018. Antiatherogenic potential of extracts from the gray oyster medicinal mushroom, *Pleurotus pulmonarius* (Agaricomycetes), *in vitro*. *Int. J. Medic. Mushrooms* 20(3):283–290.

Abidin, M.H.Z. *et al.* 2016. Protective effect of antioxidant extracts from grey oyster mushroom, *Pleurotus pulmonarius* (Agaricomycetes), against human low-density lipoprotein oxidation and aortic endothelial cell damage. *Int. J. Medic. Mushrooms* 18(2):109-121.

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7.2 APPENDIX A

Data Demonstrating Identity of Phoenix Oyster Mushroom Mycelium Source Material

Processing Lab:
Detlev-Karsten-Rohwedder-Straße 7
41564 Kaarst Germany
www.criver.com/accugenix
Accugenix.labEU@crl.com

Accugenix®
AccuGENX-ID® Report

Customer:	Mushlabs GmbH	Account:	605530 (MUH1)
Address:	Rosenthaler Str. 13, Berlin, berlin, 10119, Germany		
Accugenix®C# / Run Date:	**C5206557-20220225127 / 2022-02-24 16:40:28**	ID Request Form#:	536070
Customer Sample ID:	**ML_ITS_22**	Due Date:	2022-02-25

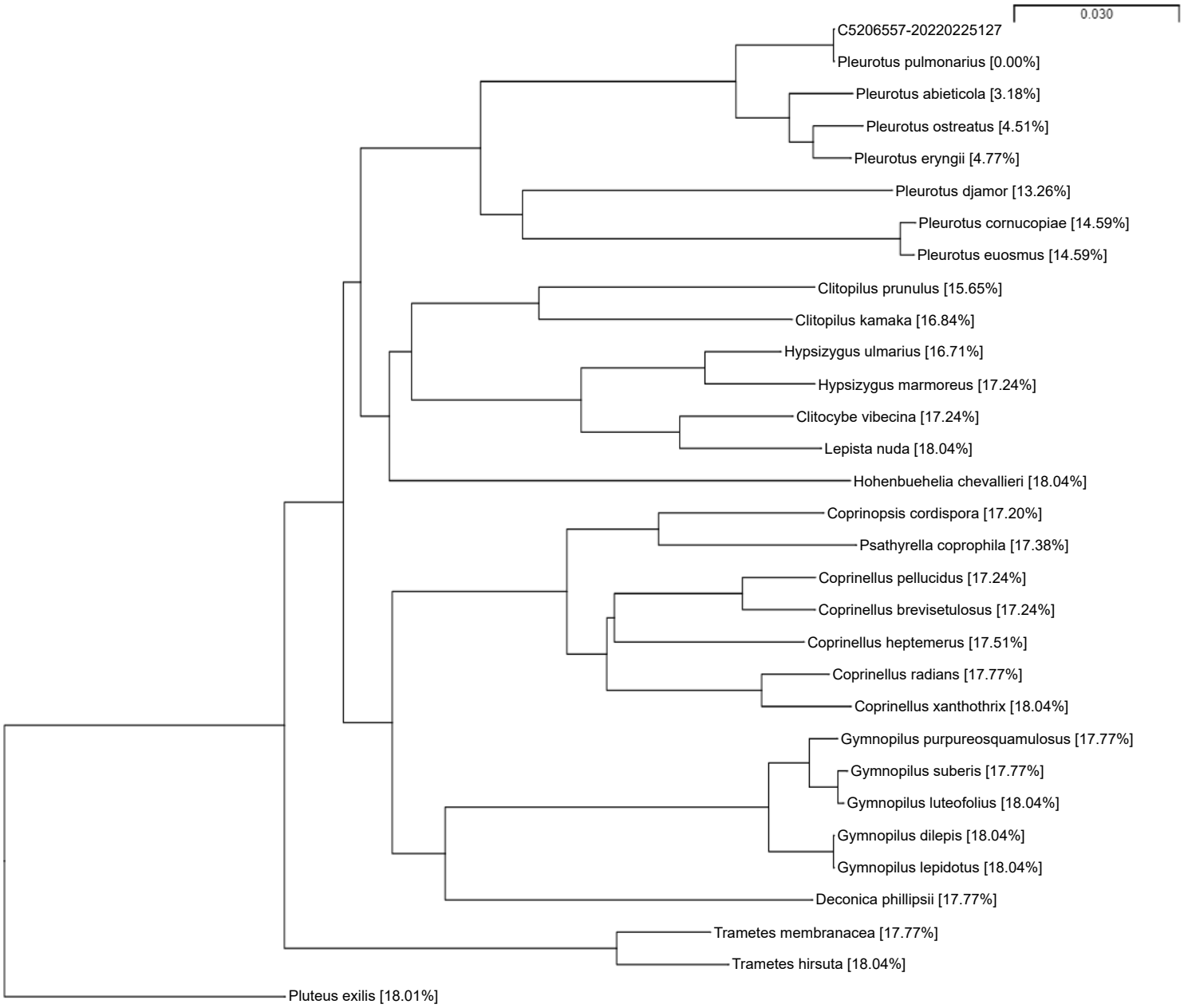
AccuGENX-ID® Database Search Result - Fungal ITS Library

Identification: ***Pleurotus pulmonarius***

Confidence Level: **Species**

●Based on published literature, the above identified species has been described as a filamentous fungi. Click HERE for more information on this organism.

Neighbor Joining Tree



The value shown in the bracket represents the percent difference in the sequence alignment between the unknown and each individual library entry.

Not intended for *in vitro* diagnostic use. The results apply to the sample as received.

Prepared By Michael D. Testa at Newark,DE, United States on 2022-02-25 10:31:08
Reviewed By Robert Stevens at Newark, DE, United States on 2022-02-25 10:32:06
QA Approved By Alyssa K Ohara at Newark, DE, United States on 2022-02-25 16:14:07

This document has been signed electronically by the individual(s) shown above, in compliance with FDA regulation CFR 21 Part 11.

7.3 APPENDIX B

Study Report on Coding Sequences for Toxins Produced by *Pleurotus* spp.

charles river

Submitting Laboratory: Mushlabs
Special Project Number: MUH1_1
Customer Sample Identification: ML_CR_WGS_01
Accugenix C Number: 5303367
Accugenix IRF Number: 543400

NEXT GENERATION SEQUENCING

1. Sample: Organism streaked on agar plate
2. DNA Extraction: Master Pure Yeast DNA Purification kit
3. Library Preparation: Illumina DNA Prep Sequencing Library Preparation protocol
4. Next Generation Sequencing: 1 Gbp of short reads using 150 x pair end reads, Illumina SBS chemistry on Illumina NextSeq 2000 and 900 Mbp of long reads using Oxford Nanopore for a ~39.87 Mb *Pleurotus pulmonarius* genome.
5. Bioinformatics: Genome assembly and annotation. Blastx search of genome assembly with all known, annotated fungal toxin genes, data derived from PubMed. Literature search for known *Pleurotus* toxin genes.

ASSEMBLY & ANNOTATION RESULTS

Quality

Table 1: Quality parameter metrics

Sequencing	DNA extraction	Total reads	Total bases	Coverage	Quality (%>Q30)*
Illumina short reads	65.1 ng/µl	15,984,900	2,408,250,036	60X	90.758%

Sequencing	DNA extraction	Total Trimmed reads	Total trimmed bases	Coverage	Quality (%>Q30)
Oxford Nanopore long reads (ONT)	65.1 ng/µl	897,232	1,120,518,160	28X	Not applicable

*Base calling accuracy, measured by the Phred quality score (Q score), is the most common metric used to assess the accuracy of a sequencing platform. It indicates the probability that a given base is called incorrectly by the sequencer. If Phred assigns a Q score of 30 (Q30) to a base, this is equivalent to the probability of an incorrect base call 1 in 1000 times. This means that the base call accuracy (i.e., the probability of a correct base call) is 99.9%.

Results

Assembly:
Quality control and adapter trimming was performed with bcl-convert [1] and porechop [2] for Illumina and ONT sequencing respectively. Long read assembly with ONT reads was performed with flye [3]. The long-read assembly was polished with pilon [4]. To reduce erroneous assembly artifacts caused by low quality nanopore reads, long read contigs with an average short read coverage of 15x or less were removed from the assembly. Assembly statistics were recorded with QUAST [5].

Table 3: Summary of assembly statistics

Total Length	Number of contigs	Longest contig	N50	GC (%)
48,904,992	1,017	1,017,662	148,311	49.92

Annotation:
Assembly annotation was performed with funannotate [6]. 15,996 genes were predicted.

The assembly and annotation files are "5303367-MUH1.fasta" and "5303367-MUH1.gbk" respectively.

SUMMARY OF TOXIN SEARCH

Pleurotolysins

Pleurotolysins are well known toxins in *Pleurotus* sp. [7] Three genes, closely related to known pleurotolysins A and B, are found in the assembly.

1) Locus which is Ostreolysin A6 [Pleurotus pulmonarius] on contig 161

Assembly_contig	GenBank_best_match	percent_ identity	match_ length	contig_ start	contig end	query_start	query_ end	e_value
contig_161	KAF4577128.1	94.495	109	12693	13019	32	140	1.73E-75
contig_161	KAF4577128.1	92.105	38	12545	12658	1	38	1.73E-75

2) Locus which is Pleurotolysin B on contig_686

Assembly_contig	GenBank_best_match	percent_ identity	match_ length	contig_ start	contig_ end	query_start	query_ end	e_value
contig_686	KAF4577132.1	82.941	340	6256	7275	67	351	0.0
contig_686	KAF4577132.1	85.616	146	7317	7754	347	473	0.0
contig_686	KAF4577132.1	92.593	54	7798	7959	468	521	0.0
contig_686	KAF4577132.1	77.108	83	5957	6205	1	67	0.0

3) Locus which is Pleurotolysin A/Ostreolysin A6 on contig_686 and collocated with Pleurotolysin B

Assembly_contig	GenBank_best_match	percent_ identity	match_ length	contig_ start	contig_ end	query_start	query_ end	e_value
contig_686	BAD66666.1	84.713	157	5239	4769	1	138	4.17E-82

Other toxins

Additionally, all fungal peptides which contain the keywords "toxin" OR "defensin" were collected from GenBank on May 19th, 2022. Defensins are toxins which are frequently short, often not well conserved, and are used to protect from bacterial or eukaryote attack. Defensins are often overlooked with standard annotation pipelines because they are typically only 18-45 amino acids in length. There were 529,645 peptides found using the above keyword search. These peptides were downloaded.

Diamond blastx was then used to map the assembly to the fungal toxin peptide dataset collected with a cut-off e-value of $1e^{-10}$, which minimizes false positives for blast (note: a higher cut-off would mean exponentially more false positives). The blast identified 4,516 genomic loci which map, at least somewhat, to any fungal peptide annotated as a toxin or toxin related.

The blastx output was then converted into bed format and the bedfile flattened using bedtools merge. This merges overlapping loci into single spanning loci. The bed file was converted to nucleotide fasta using bedtools command getfasta resulting in 971 coding loci of interest.

These loci were annotated using best Diamond blastx match to non-redundant peptides (downloaded 20th March, 2022) and the fungal toxins identified in the first step.

Each of these loci were individually examined and the original fungal toxin entry in GenBank peptides considered. In some cases NCBI web blast was used to identify conserved domains and to match peptides with their function. This reduced the dataset down to 26 loci (Table 4), only 8 of these coding regions overlapped the gene annotation (Table 5).

Table 4. blastx of the coding loci with the toxin related fungal genes

Coding_locus	GenBank_fungal_toxin_peptide	% identity	match_length	e_value
contig_1053:108946-110566	VWO98972.1	54.7	353	2.10E-105
contig_1103:2136-3758	VWO98972.1	52.0	542	1.60E-153
contig_1218:31115-32734	VWO98972.1	49.0	537	3.40E-140
contig_140:17799-18982	VWO98972.1	49.6	240	4.80E-59
contig_182:56559-59763	QIM40768.1	33.4	960	8.20E-122
contig_200:2-436	VWO98972.1	52.8	144	1.30E-33
contig_213:29188-30534	VWO97593.1	55.7	449	5.70E-125
contig_232:154291-155793	VWO98972.1	52.9	499	2.20E-146
contig_243:3950-4378	UFQ31923.1	39.2	143	6.60E-19
contig_310:6415-7962	VWO98972.1	53.0	515	1.20E-155
contig_322:13186-14655	VWO98972.1	52.7	488	1.90E-142
contig_341:59619-61576	VWO96292.1	70.7	532	3.60E-213
contig_407:18432-20079	VWO98972.1	46.2	333	1.10E-77
contig_414:188592-190202	VWO98972.1	47.5	547	1.10E-130
contig_430:551-2229	VWO98972.1	52.2	446	8.90E-128
contig_432:2120-3934	VWO98972.1	51.6	560	9.60E-160
contig_44:7-810	VWO98972.1	56.9	267	4.00E-86
contig_507:1524-3173	VWO98972.1	52.5	551	4.30E-159
contig_548:564-2213	VWO98972.1	54.3	547	5.50E-162
contig_555:32610-34258	VWO98972.1	54.5	473	1.40E-141
contig_559:9694-10983	VWO98972.1	48.3	429	1.10E-109
contig_595:971-2524	VWO98972.1	50.4	516	1.00E-141
contig_627:2565-4978	VWO95044.1	27.6	644	6.30E-42
contig_779:1715-2545	VWO97386.1	29.8	289	3.90E-15
contig_93:202855-204507	A0A384XHA3.1|STR11_STRTC	60.1	551	5.60E-175
contig_93:204546-205207	A0A384XHA3.1|STR11_STRTC	57.4	136	2.10E-32

Table 5. Annotation of fungal toxin peptides.

Peptide_ID	**Descriptor**
VWO98972.1	Dual O-methyltransferase/FAD-dependent monooxygenase CTB3 (Cercosporin toxin biosynthesis cluster protein 3) [Ganoderma boninense]
VWO97593.1	O-methyltransferase CTB2 (EC (Cercosporin toxin biosynthesis cluster protein 2) [Ganoderma boninense]
VWO97386.1	Cercosporin MFS transporter CTB4 (Cercosporin toxin biosynthesis cluster protein 4), partial [Ganoderma boninense]
VWO96292.1	Ketoreductase CTB6 (EC (Cercosporin toxin biosynthesis cluster protein 6) [Ganoderma boninense]
VWO95044.1	Cercosporin MFS transporter CTB4 (Cercosporin toxin biosynthesis cluster protein 4) [Ganoderma boninense]
sp|A0A384XHA3.1|STR11_STRTC	Full=Phenylalanine ammonia-lyase str11; Full=Strobilurin biosynthesis cluster protein r11
UFQ31923.1	ergot alkaloid biosynthetic protein A, partial [Claviceps africana]
QIM40768.1	prolyl oligopeptidase [Amanita pallidorosea]

The list provided in Table 5 are potential toxin genes or genes associated with toxin production. These peptides are distantly related species to the *Pleurotus* genus so it is probable that many of the above genes aren't involved in toxin synthesis. Further functional studies on these genes may be required to confirm toxin production.

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Appendix: Bioinformatic tools used.

Tool	Version
bcl-convert	3.9.3
porechop	0.2.3_seqan2.1.1
flye	2.8
pilon	1.23
quast	5.0.2
funannotate	1.7.4
guppy	5.0.16
Diamond blastx	v0.9.24.125
bedtools	v2.27.1

Database	Type	Version	Date	Num_Records
pfam	hmmer3	33.1	2020-04	18259
gene2product	text	1.64	8/25/20	33640
interpro	xml	81	8/13/20	37821
dbCAN	hmmer3	9	8/4/20	641
busco_outgroups	outgroups	1	9/16/20	8
merops	diamond	12	10/4/17	5009
mibig	diamond	1.4	9/16/20	31023
uniprot	diamond	2020_04	8/12/20	563082
go	text	8/11/20	8/11/20	47224
repeats	diamond	1	9/16/20	11950
blastx	diamond	v0.9.24.125	3/20/2022	529,645

References

[1] bcl-convert: A proprietary Illumina software for the conversion of bcl files to basecalls. https://support-docs.illumina.com/SW/BCL_Convert/Content/SW/FrontPages/BCL_Convert.htm

[2] An open source software for the QC and adapter trimming of ONT technologies. https://github.com/rrwick/Porechop

[3] Yu Lin, Jeffrey Yuan, Mikhail Kolmogorov, Max W Shen, Mark Chaisson and Pavel Pevzner, "Assembly of Long Error-Prone Reads Using de Bruijn Graphs", PNAS, 2016 doi:10.1073/pnas.1604560113

[4] Walker, Bruce J et al. “Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement.” PloS one vol. 9,11 e112963. 19 Nov. 2014, doi:10.1371/journal.pone.0112963

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[6] Jon Love, Jon Palmer, Jason Stajich, Tyler Esser, Erik Kastman, Daniel Bogema, & David Winter. (2019, November 10). nextgenusfs/funannotate: funannotate v1.7.0 (Version 1.7.0). Zenodo. http://doi.org/10.5281/zenodo.3534297

[7] References to Pleurotolyins:

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https://pubmed.ncbi.nlm.nih.gov/28379176/
https://pubmed.ncbi.nlm.nih.gov/16860832/
https://pubmed.ncbi.nlm.nih.gov/32971130/
https://pubmed.ncbi.nlm.nih.gov/29145176/
https://pubmed.ncbi.nlm.nih.gov/30911026/
https://pubmed.ncbi.nlm.nih.gov/15912956/
https://pubmed.ncbi.nlm.nih.gov/19524606/

7.4 APPENDIX C

Data Collected by Mushlabs Showing a Lack of Detectable Environmental Contaminants Attributable to Agricultural Practices in Phoenix Oyster Mushroom Mycelium

As a measure of extra caution, Mushlabs screened for the presence of mushroom toxins and toxins produced by potential fungal contaminants (collectively known here as mycotoxins) using liquid chromatography with tandem mass spectrometry or LC-MS/MS; SOP M 3650. The mycotoxins included in the screening are listed in Table 2.6. The levels of these toxins were all below the limit of detection (LOD). Further, as some of these mycotoxins are only produced during certain growth phases by *Aspergillus* and *Fusarium* species, they would only be present if Mushlabs' mycelia were contaminated by fungal microbes. Mushlabs believes the yeast and mold specifications for Mushlabs' mycelia are sufficient to preclude any food safety concerns related to the presence of toxins.

Analyzed Mycotoxins with Associated Limits of Detection	
Toxin	**Limit of Detection (µg/kg)**
Aflatoxin B1	< 0.2
Aflatoxin B2	< 0.2
Aflatoxin G1	< 0.2
Aflatoxin G2	< 0.2
Ochratoxin A	< 0.5
Deoxynivalenol (DON)	< 10
Zearalenone	< 5.0
Deoxynivalenol (DON)3-Acetyl-Deoxynivalenol	< 10
15-Acetyl-Deoxynivalenol	< 20
Nivalenol	< 10
T-2 Toxin	< 2.0
HT-2 Toxin	< 2.0
4,15-Diacetoxyscirpenol	< 10
Fusarenon-X	< 10
Fumonisin B1	< 50
Fumonisin B2	< 50

7.5 APPENDIX D

Tabular Summary of Published Studies with Data or Information Relevant to the Food Safety of *P. pulmonarius*

Summary of Published Studies with Data or Information Relevant to the Food Safety of *P. pulmonarious*				
Study Title (reference)	**Study Design**	**Type of Test Article**	**Outcomes/ Endpoints Measured**	**Reported Results**
In Vitro Studies				
Antiatherogenic Potential of Extracts from the Gray Oyster Medicinal Mushroom, *Pleurotus pulmonarius* (Agaricomycetes), *In Vitro* (Abidin, M.H.Z. *et al.* 2018*. Int. J. Medic. Mushrooms.* 20(3):283–290)	*In vitro* study of potential for affecting atherogenic mechanisms (same lab as the 2016 study below) Different mushroom extracts were tested versus a control (water) Test concentrations=10 mg extract/ml	Extracts of *P. pulmonarius* fruiting bodies • crude aqueous (CA) • crude hot water (CHW) • hexane (H) • methanol/ dichloromethane (MDC)	Responses (oxidative stress; nitric oxide bioavailability and endocan expression) in human aortic endothelial cells (HAECs) or in enzyme assays (ACE inhibition and HMG-CoA reductase inhibition)	Authors cite to wide medicinal use of edible mushrooms including *P. pulmonarius* species *In vitro* data supports medicinal use of this species
Protective effect of antioxidant extracts from grey oyster mushroom, *Pleurotus pulmonarius* (Agaricomycetes), against human low-density lipoprotein oxidation and aortic endothelial cell damage (Abidin, M.H.Z. *et al.* 2016. *Int. J. Medic. Mushrooms* 18(2):109-121)	*In vitro* study of antioxidant activity and potential ability to affect coronary disease Different mushroom extracts were tested versus a control (water)	Extracts of *P. pulmonarius* fruiting bodies • crude aqueous (CA) and protein fractions (protein fractions PF30, PF60, PF90) • crude hot water (CHW) • partially purified polysaccharide fraction (PP) • hexane (HF) • methanol/ dichloromethane (MD) • water fraction (WF)	*In vitro* antioxidant assays included: Folin-Ciocalteu, 1,1-diphenyl-2-picrylhydrazyl free radical scavenging, metal chelating, cupric ion reducing antioxidant capacity, and lipid peroxidation inhibition Effects on human aortic endothelial cell (HAEC) viability and hydrogen peroxide-induced endothelial cell damage	Toxicity endpoint of cell viability: "*Based on the results (Fig. 5), cell viability was not less than 80% when treated with up to 200 µg/mL concentration of the extracts. The extracts possessed IC50 values higher than 200 µg/mL. It is proven that HF, WF, CA, and CHW extracts were not toxic to the HAEC."* Studies support antioxidant activity of various types of *P. pulmonarius* extracts
Antioxidant, antifungal, and anticancer activities of Se enriched *Pleurotus spp.* mycelium extracts	*In vitro* studies of anti-fungal and anti-cancer potential, with and without selenium enrichment	Extracts of *P. pulmonarius* strain HAI 573 mycelia	Cytotoxicity to cells using extracts of the mushroom mycelia (12.5, 25, 50, 100 and 200 µg/ml)	The authors report: *"Cytotoxic activity against both HeLa and LS174 cell lines was very low…"*

Summary of Published Studies with Data or Information Relevant to the Food Safety of *P. pulmonarious*				
Study Title (reference)	**Study Design**	**Type of Test Article**	**Outcomes/ Endpoints Measured**	**Reported Results**
(Milovanovic, I. *et al.* 2014. *Arch. Biol. Sci.* 66(4):1379-1388)		(aqueous extraction and filtration)	Minimal inhibitory concentrations (MICs) against fungal organisms Radical-scavenging activity in relation to phenol and flavonoid content of the extract	Selenium enrichment influenced the anti-fungal activity of *P. pulmonarius* extracts (reductions) Radical scavenging capacity of extracts directly correlated with phenol/ flavonoid content.
Antioxidant and immunomodulating activities of exo- and endopolysaccharide fractions from submerged mycelia cultures of culinary-medicinal mushrooms (Jeong, S.C. *et al.* 2013. *Int. J. Med. Mushrooms* 15(3):251–266)	*In vitro* assays to evaluate and compare antioxidant and immunomodulatory effects of exopolysaccharides (EXPs) and endopolysaccharides (ENPs) of submerged cultures of mushroom mycelia	Extracts of *P. pulmonarius* mycelia (aqueous extract)	Antioxidant activity assays: biological assay using *Saccharomyces cerevisiae;* DPPH radical scavenging activity; chelating ability for ferrous ions; and ferric reducing antioxidant power. Immunomodulation assessed by production of IFN-γ, IL-2, IL-4 and IL-5, and macrophage enzyme activity	Authors cite to wide medicinal use of edible mushrooms including *P. pulmonarius* species. Mycelia extract of *P. pulmonarius* exhibited both antioxidant and immunomodulatory activity.
In Vivo Animal Studies				
Evaluation of antidiabetic activity of *P. pulmonarius* against streptozotocin-nicotinamide induced diabetic Wistar albino rats (Balaji, P. *et al.* 2020. *Saudi J. Biol. Sci.* 27:913–924)	*In vivo* rat study of anti-diabetic activity of mushroom extracts in a rat model of diabetes LD50 values determined and then extracts administered orally for 4 weeks (200 and 400 mg extract/kg bw; both extracts tested separately)	*P. pulmonarius* mycelia extracts (aqueous and organic)	Normalization of blood glucose and insulin levels Levels of HDL and cholesterol in blood Pathology in pancreas. Liver and kidney	Only the aqueous extract exhibited activity to normalize glucose and insulin levels and reduced blood lipids Reversal of pathological changes in pancreas, liver, and kidney in untreated animals No reported adverse outcomes in the rats treated with single doses of either *P. pulmonarius* extract: *"The results of the acute toxicity study of HWE [hot water extract] and AE [acetone extract] showed no mortality even*

Summary of Published Studies with Data or Information Relevant to the Food Safety of *P. pulmonarious*				
Study Title (reference)	**Study Design**	**Type of Test Article**	**Outcomes/ Endpoints Measured**	**Reported Results**
				after 72 hrs at 2000 mg/kg. The AE showed mild sedation at 2000 mg/kg but however, HWE didn't alter any of the general behavior. No fatality or noxious reactions were found during and after the treatment period with both extracts."
Water extract from *Pleurotus pulmonarius* with antioxidant activity exerts in vivo chemoprophylaxis and chemosensitization for liver cancer (Xu, W.W. *et al.* 2014. *Nutrit. Cancer* 66(6):989-998)	*In vivo* study in mice of the effects of mushroom extracts on experimental liver cancer prevention and progression Prevention group received oral *P. pulmonarius* extract (200 mg/kg bw) daily for 2 weeks before cancer induction (control received no extract) Sensitization group had cancer induction before oral daily administration of the extract (50 mg/kg bw) for 28 days (control received no extract)	Extracts of *P. pulmonarius* fruiting bodies (aqueous)	Tumor induction and tumor progression were monitored as was body weight and histopathology of "critical organs"	Toxicity evaluation: *"…critical organs of mice were collected and subjected to H&E staining for checking their histological features. No obvious difference in these organs was observed in the Pp-treated [P. pulmonarius only animals] and control groups… There was also no significant change in the body weight of the animals between treated groups and control group from the beginning until the end of the experiments (Fig. 3B and Fig. 4B)."*
Extract of *Pleurotus pulmonarius* suppresses liver cancer development and progression through inhibition of VEGF-Induced PI3K/AKT signaling pathway (Xu, W. *et al.* 2012. *PLoS ONE* 7(3): e34406)	*In vivo* study in mouse xenograft model of anti-tumor potential of extract of *P. pulmonarius* Mushroom extract administered orally (200 mg/kg bw) or i.p. (50 mg/kg bw) three times a week for 27 days (total of nine doses) [*In vitro* mechanistic data also provided but not discussed here]	*P. pulmonarius* fruiting body extracts (aqueous)	The tumor volumes and body weight were measured every three days in all mice Histopathology of organs at sacrifice	Authors report the extract was non-toxic: *"Furthermore, no obvious side effects or changes were observed by comparing the histological features of internal organs including the liver, lung and kidney (Fig. 7A) and body weight of the animals (Fig. 7B) in the treatment and control group suggesting the safety and clinical implication of PP [P. pulmonarius] as an anticancer agent."*
Glucans from the edible mushroom *Pleurotus pulmonarius*	*In vivo* study of anti-cancer activity of mushroom extracts in	Extracts of *P. pulmonarius* fruiting bodies and mycelia	Monitored: • formation of aberrant crypt foci	Both extracts reported to inhibit events linked to colon carcinogenesis: "*We conclude that P. pulmonarius FBE and ME inhibit colitis-*

Summary of Published Studies with Data or Information Relevant to the Food Safety of *P. pulmonarious*				
Study Title (reference)	**Study Design**	**Type of Test Article**	**Outcomes/ Endpoints Measured**	**Reported Results**
inhibit colitis-associated colon carcinogenesis in mice (Lavi, I. *et al*. 2012. *J. Gastroenterol.* 47:504–518)	a mouse model of colorectal cancer Mice fed a daily diet (80 days) containing 2 or 20 mg of FBE (fruiting body extract) or ME (mycelia extract) per mouse	(aqueous extracts of both)	• expression of proliferating cell nuclear antigen • number of apoptotic cells in colon • expression of TNF-a in colon tissue	*associated colon carcinogenesis induced in mice through the modulation of cell proliferation, induction of apoptosis, and inhibition of inflammation."* No toxicity of the extracts is mentioned in the narrative of the paper.
Anti-leukemic and Immunomodulatory Effects of Fungal Metabolites of *Pleurotus pulmonarius* and *Pleurotus ostreatus* on Benzene-induced Leukemia in Wister Rats (Olefumi, A.E. et al. 2012. *Korean J. Hematol.* 47:67-73)	*In vivo* study in a rat model of leukemia (intravenous injection of benzene) An aqueous solution of mycelial fungal "metabolites" (20 mg/mL) orally administered (0.2 mL) before, during, and after leukemia induction One treatment group (n=16 rats) served as the control group for adverse reactions and toxicity that may be linked to intake of mycelia (Group D; only treated with the mycelia and given a control diet)	Extracts of *P. pulmonarius* mycelia (aqueous)	Hematological parameters at baseline and after leukemia induction Immunomodulatory potential assessed by a phagocytic assay	Results in the mycelia only group (control group) reported included: *"Results of various hematological parameters in group D revealed that there was no adverse reaction or toxicity experienced by the rats as a result of administration of the 2 metabolites."; "The healthy animals treated with P. ostreatus and P. pulmonarius metabolite presented in group D showed no significant alterations in erythrocyte counts, leukocyte counts, and hematological indices, when compared with the healthy animals in group F who received commercial feed and water only."*
Antimicrobial and anti-inflammatory potential of polysaccharide from *Pleurotus pulmonarius* LAU 09 (Adebayo E.A. *et al.* 2012. *Afr. J. Microbiol. Res.* 6(13):3315-3323)	*In vivo* study rat studies to investigate the potential anti-inflammatory actions of polysaccharides from *P. pulmonarius* Polysaccharides prepared by submerged fermentation culture of mycelia mat; harvested after 10 days, filtered, centrifuged and precipitated with acetone. Polysaccharide activity (6 mg/kg oral administration for 15 days)	Extract of *Pleurotus pulmonarius* mycelia (strain known as LAU 09; GenBank accession number JF736658) (acetone/organic extract)	Paw edema and sperm motility were monitored after 15 days	No polysaccharide toxicity reported for any dose level.

Summary of Published Studies with Data or Information Relevant to the Food Safety of *P. pulmonarious*				
Study Title (reference)	**Study Design**	**Type of Test Article**	**Outcomes/ Endpoints Measured**	**Reported Results**
	tested in a rat model of inflammation (paw edema induced by formalin or carrageenan).			
Antinociception of β-d-glucan from *Pleurotus pulmonarius* is possibly related to protein kinase C inhibition (Baggio, C.H. *et al.* 2012. *Int. J. Biol. Macromol.* 50:872– 877)	Follow-on study to Baggio et al. 2010. See next listing. Mice injected i.p. with mushroom extract (doses from 0.1 to 100 mg/kg bw) and acute effects monitored	Extracted B-glucan from milled *P. pulmonarius* (aqueous)	Anti-nociceptive activity assessed 30 minutes after intraplantar (paw) injections of capsaicin, cinnamaldehyde, menthol, acidified saline and phorbol myristate acetate (PMA) Also monitored PKC activation in skin and connective tissue	Anti-nociceptive activity *in vivo* reported at doses as low as 1 mg/kg B-glucan extract No polysaccharide toxicity reported for any dose level.
Antinociceptive effects of (1/3),(1/6)-linked b-glucan isolated from *Pleurotus pulmonarius* in models of acute and neuropathic pain in mice: evidence for a role for glutamatergic receptors and cytokine pathways (Baggio, C.H. *et al.* 2010. *J. Pain* 11(10): 965-971)	*In vivo* studies of mushroom polysaccharides in mouse pain models Mice injected i.p. (single or repeated doses) with B-glucan (30 mg/kg)	Extracted B-glucan from milled *P. pulmonarius* (aqueous)	ACUTE PAIN: intra-plantar injection of glutamate; intrathecal injection of glutamatergic receptor agonists, substance P, IL-1b, TNF-a CHRONIC NEUROAPTHIC PAIN: partial sciatic nerve ligation	Extract inhibited acute and neuropathic pain in mice via inhibition of glutamate receptors and IL-1b pathways The IC50 in the acute pain model = 0.34 mg/kg Chronic treatment with extract (2X/day for 7 days) reversed mechanical allodynia caused by partial sciatic nerve ligation (chronic pain treatment). Doses effective to treat pain did not affect locomotor activity of mice (30 mg/kg; measure of toxicity) No extract toxicity reported
Orally administered glucans from the edible mushroom *Pleurotus pulmonarius* reduce acute inflammation in dextran sulfate sodium-induced experimental	*In vivo* study in mouse colitis model (induced by 3.5% dextran sulfate sodium); doses of 2 and 20 mg/mouse extracts tested in mice with induced colitis	Extracts of *P. pulmonarius* fruiting bodies (aqueous; HWS group) and	Endpoints monitored: • Colon damage (macroscopic and histological)	Dietary HWS and ME reversed histological and biochemical alterations in experimentally induced colitis

Summary of Published Studies with Data or Information Relevant to the Food Safety of *P. pulmonarious*				
Study Title (reference)	**Study Design**	**Type of Test Article**	**Outcomes/ Endpoints Measured**	**Reported Results**
Colitis (Lavi, I. *et al.* 2010. *Br. J. Nutrit.* 103:393–402)	Extract doses approximated dietary fiber intakes in humans Control groups: 20 days administration of extract in diet, no colitis induction, and (a) hot water extract (HWS) at 20 mg/mouse/day, mycelial extract (ME) at 20 mg/mouse/day	mycelia (aqueous; ME group)	• Changes in colon length • Levels in colon of MPO activity and mRNA levels of IL-1b IL-10	No adverse histopathological changes in colons of mice receiving only extracts for 29 days in diet; no adverse effects on inflammatory modulators in these same groups
Clastogenicity potential screening of *Pleurotus pulmonarius* and *Pleurotus ostreatus* metabolites as potential anticancer and antileukaemic agents using micronucleus assay (Akanni, E.O. *et al.* 2010. *Br. J. Pharmacol. Toxicol.* 1(2):56-61)	*In vivo* genotoxicity study (micronucleus assay) Wister rats injected i.p. (16 and 64 mg/kg; 3.2 and 12.8% of the LD50) and then sacrificed (24, 48 and 72 h post treatment); both positive and negative controls were included	Unspecified extracts of *P. pulmonarius*	Rate of micronucleus formation over three time points compared to positive and negative controls	Extracts of *P. pulmonarius* were negative Only the positive control was associated with clastogenicity Provides evidence for a lack of mutagenic potential *in vivo*
Mushroom polysaccharide extracts delay progression of carcinogenesis in mice (Wasonga, C.G.O. *et al.* 2008. *J. Exp. Therapeut. Oncol.* 7:147-152)	*In vivo* study in a mouse model of liver cancer (DRNA injection followed by DENA in drinking water from week 3 to week 12) Mushroom extract mixed in the diet (mixed in a ratio of 1:4 with pellets; 25% of the diet); controls included no treatment and positive controls	Extracts of dried *P. pulmonarius* (aqueous)	Development/progression of liver cancer assessed by histological analysis and tumor markers (LDH and sialic acid)	Extracts of *P. pulmonarius* delayed progression of liver cancer Mice treated with mushroom extract alone (no carcinogen pretreatment) did not exhibit liver histopathology (same outcome as untreated controls) No other toxicity endpoints reported
Effect of usuhiratake (*P. pulmonarius*) on sneezing and nasal rubbing in BALB/c mice (Yatsuzuka, R. *et al.* 2007. *Biol. Pharm. Bull.* 30(8):1557-1560)	*In vivo* study in BALB/c mice (repeated oral doses of *P. pulmonarius* powder in solution; 200 or 500 mg/kg/day) for 4 weeks	Powder of *P. pulmonarius* (whole mushroom)	Monitored sneezing and nasal rubbing behavior after antigen stimulation of histamine release as well as total IgE levels	No effects on IgE, indicating direct effects on histamine release, consistent with *in vitro* study data (rat mast cell)

Summary of Published Studies with Data or Information Relevant to the Food Safety of *P. pulmonarious*				
Study Title (reference)	**Study Design**	**Type of Test Article**	**Outcomes/ Endpoints Measured**	**Reported Results**
			Monitored release of histamine from rat mast cells (*in vitro* method)	
Hypoglycemic activity of aqueous extract of *Pleurotus pulmonarius* in alloxan-induced diabetic mice (Badole, S.L. *et al.* 2006. *Pharmaceut. Biol.* 44(6):421–425)	*In vivo* study of mushroom extract in diabetic and non-diabetic (untreated) mice Oral doses of 250, 500, and 1000 mg/kg bw (untreated and alloxan-treated mice) up to 28 days Acute oral toxicity also determined in untreated mice (doses up to 5000 mg/kg bw)	Dried *P. pulmonarius* powder extract (aqueous)	Serum glucose and body weight measured One group subjected to an oral glucose tolerance test (OGTT) Mortality after acute doses up to 5000 mg/kg	No mortality and no toxic effects reported at doses up to 5000 mg/kg (single dose) Repeated doses for 28 days reversed body weight loss, improved serum glucose levels, and reduced mortality compared to diabetic control mice No reports of other toxic effects attributed to the extract
Antioxidant, anti-inflammatory and antitumor activities of culinary-medicinal mushroom *Pleurotus pulmonarius* (Fr.) Quel. (Agaricomycetideae) (Jose, N. *et al.* 2002. *Int. J. Med. Mushrooms* 4:329-335)	*In vivo* studies in mice tumor models (ascites and solid tumor models) Mice injected i.p. with mushroom extract (250, 500 and 1000 mg/kg bw) 24 hours after tumor inoculation (5 doses in the ascites model; for 10 days in the solid tumor model)	Extract of *P. pulmonarius* fruiting bodies (methanol; organic)	Tumor regression was monitored	Significant tumor regression at all doses of the *P. pulmonarius* extract in both tumor models No extract toxicity reported for any dose level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

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

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001152	May 31, 2023
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
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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

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Telephone Number	Fax Number	E-Mail Address
281-493-5702	281-493-5781	lplunkett@biopolicysolutions.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Phoenix Oyster Mushroom Mycelium

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 1

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 91

☐ 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)* GRN945

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

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

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8) and 170.250(d) and (e))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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

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

☐ No

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

☐ Yes, a redacted copy of the complete submission

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

☐ No

SECTION D – INTENDED USE

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

An ingredient in various processed products including meat, poultry, and fish analogs, For clarity, none of these products will contain animal-derived components that would cause them to be considered as meat under the Federal Meat Inspection Act or Poultry Products Inspection Act. dairy analogs, and baked goods at a maximum level of 25% (dry mycelium weight/100 g product). Mycelia are not intended for use in USDA-regulated products or infant formula.

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 Mushlabs GmbH

(name of notifier)

has concluded that the intended use*(s)* of Phoenix Oyster Mushroom Mycelium

(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. Mushlabs GmbH *(name of notifier)* 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.

Humboldtstr. 59, 22083 Hamburg, Germany

(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)
Laura M Plunkett Digitally signed by Laura M Plunkett Date: 2023.05.30 12:19:40 -05'00'	Laura Plunkett	05/30/2023

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)
	GRAS NOTICE FOR THE USE OF PHOENIX OYSTER MUSHROOM MYCELIUM 30 May 2023.pdf	1-64
	GRAS Notice cover letter for submission.pdf	1

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, **PRAStaff@fda.hhs.gov**. (Please do NOT return the form to this address). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.