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In Vitro Studies of Secondary Metabolite–Related Responses in Some Species of Genus *Grifola* (Agaricomycetes) from Argentina

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ABSTRACT: *Grifola gargal* Singer and *Grifola sordulenta* (Mont.) Singer mushrooms are related to *Grifola frondosa* (Dicks.) Gray, which is well known for its medicinal properties. *In vitro* studies were performed to find a useful guide for optimizing the environmental parameters through biotransformation of lignocellulosic materials and basidiome development, also considering secondary metabolism–related responses (SMRRs) associated with these processes and the variability among species and strains; this optimization is necessary to make the mushroom's industrial cultivation profitable. Morphological features of mycelial cultures revealed that intraspecific variability was of taxonomic relevance. A low ligninolytic capacity in studied *Grifola* species was observed when compared with 2 control species: *G. frondosa* and *Ganoderma lucidum*. Experiments with nutrient media containing different carbohydrate sources indicated that *G. gargal* mycelia grew better in xylulose and *G. sordulenta*, in xylulose or cellulose; in addition, the latter species presented cellobiose dehydrogenase activity. An additional study of SMRRs under different light conditions (aroma, pigmentation, and morphogenic manifestations) showed that white light was better than blue, green, or red-filtered light at inducing advanced SMRRs. The results of SMRR stimulation are proposed as useful guidance for optimizing the environmental parameters for bioprocesses aimed at metabolite production.

KEY WORDS: agar cultures, Andino-Patagonian forests, *Grifola*, growth differentiation, medicinal and edible mushrooms

ABBREVIATIONS: CDH, cellobiose dehydrogenase; **DCPIP,** dichlorophenolindophenol; **LiP,** lignin peroxidase; **MYPA,** malt yeast peptone agar media; **SMRR,** secondary metabolism-related response

I. INTRODUCTION

Grifola gargal Singer and *Grifola sordulenta* (Mont.) Singer (Meripilaceae, Agaricomycetes) mushrooms have recently been receiving much attention because of their nutritional content[1] and their medicinal properties, including a hypotensive response[1]; antioxidant activity[2]; antioxidant activity in biotransformed wheat grains, mycelia, and basidiomes[3,4]; anti-inflammatory activity[5]; amelioration of atherosclerosis[6]; and antigenotoxic properties in biotransformed wheat grains, mycelia, and basidiomes.[7] As expected, this new evidence of their medicinal potential was followed by research on different forms of cultivation.[8–11] The aim of this study was to describe the ligninolytic properties, the carbohydrate preferences, and the effect of medium composition and light quality on the appearance of secondary metabolism–related responses in the available strains of *G. gargal* and *G. sordulenta*.

II. MATERIALS AND METHODS

A. Mushroom Sources

G. gargal strain CIEFAP #191 (hereafter referred to as strain A) and *G. sordulenta* strain CIEFAP #154 were obtained from CIEFAP-CONICET. Two new strains (B and G9) of *G. gargal* were isolated during field trips undertaken at Lake Lacar (Lanín National Park, Argentina). Two polypore white-rot fungi

were used for comparison purposes: *Grifola frondosa* (of Taiwanese origin, donated by Mushworld, South Korea) and *Ganoderma lucidum* strains E47 (Guelph University, Guelph, Ontario, Canada) and S (Fungi Perfecti, Olympia, WA). The latter species were considered as positive controls for lignocellulolytic activity and for an appropriate response to environmental stimulation.[10,12] Unless otherwise specified, the malt yeast peptone agar (MYPA) medium was used as the growth culture, according to Postemsky et al.[9]

B. *In Vitro* Evaluation of Ligninolytic Activity

Petri dishes containing MYPA medium in the presence or absence of gallic acid (5 g/L; Sigma), tannic acid (5 g/L; Fluka), Poly R-478 (polyvinylamine sulfonate anthrapyridone, 0.2 g/L; Sigma-Aldrich), and Azure B (trimethyl thionin, 15.3 g/L; Sigma) were used for studying the ligninolytic activity, as described by Levin et al.[13] Mycelia colony diameters and reactions producing changes in their halos, together with the secondary metabolism–related responses (SMRRs) in the mycelia, were recorded weekly. "Initial SMRRs" include exudates, aroma, pigmentation, and morphogenic manifestations in the surface appearance (e.g., pores), whereas "advanced SMRRs" are aggregated mycelia, primordia, and lamellae.

C. *In Vitro* Evaluation of Carbohydrate Utilization

Basal medium contained in Petri dishes was prepared with 1.7% agar, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.2 g/L $MnSO_4 \cdot 5H_2O$, and 4 g/L asparagine; a mineral micronutrients complement described by Murashige and Skoog[14] was added separately with 1 g/L of carboxymethylcellulose, soluble starch, pectin, or xylulose. Cellobiose dehydrogenase (CDH) activity was tested by bleaching dichlorophenolindophenol (DCPIP; 0.015 g/L; Sigma), using sucrose as the carbon source (10 g/L). Incubation was carried out in darkness (18 ± 1°C). At the end of the trial, the cellulose utilization was revealed by spraying an aqueous solution of 0.3% Congo red; after washing with 1 N sodium chloride, the halo was measured. Xylulose, pectin, and starch utilization were demonstrated using the same protocol but ended with an aspersion of 1% sodium triiodide solution. Data recorded were the same as those determined for ligninolytic activity (see Section II.B).

D. Biological Responses to Light

G. gargal (strains A and B) and *G. sordulenta* were cultivated *in vitro* in a growth chamber under controlled temperature, humidity (90%), and photoperiod in an attempt to mimic natural conditions for the production of basidiomes. Temperature cycles of 17°C (17 hours) and 19°C (7 hours) in darkness were used to promote vegetative mycelial growth. After full colonization (22 days), the temperature was reduced to 5°C for 36 days. Afterward, the experimental units (n = 6–7) were placed under different light treatments (white, blue, green, and red light) and a control (dark). Lights of the colors of the main wavelengths were used to discriminate the presence of blue, green, or red receptors; positive responses under dark treatment indicated that there was no strict receptor requirement for producing the advanced SMRRs. To do this, either light irradiation from fluorescent tubes (Philips TLD 36W/54) was used directly (white-light treatment), or white light was filtered using 4-mm-thick colored acrylic sheets (Plexiglas). To find the color band emitted by these materials, an absorption spectrum was obtained using a spectrophotometer (Pharo 300; spectral data are available upon request). The photosynthetically active radiation was determined using LICOR equipment and resulted in 4 $\mu mol\ m^{-2}\ s^{-1}$ in the white, 2 $\mu mol\ m^{-2}\ s^{-1}$ in the blue, 3 $\mu mol\ m^{-2}\ s^{-1}$ in the green, and 2 $\mu mol\ m^{-2}\ s^{-1}$ in the red bands. We used a thermo-photoperiod comprising 12°C for 10 hours during the day and 2°C at night. Mycelia growing in agarized media contained in Petri dishes were grown under these conditions for 34 days, after which colonies under the same conditions were opened for ventilation once a week during an additional 3-week period.

Data were recorded weekly as the number of colonies presenting either initial or advanced

TABLE 1: Mycelia Growth in Cultures of *Grifola gargal* and *Grifola sordulenta* in Different Phenolic and Dye-Containing Media

Species and Strain	Growth Days (n) to Reach 5-cm Colony Diameter*					Maximum Mycelial Growth Rate (mm/Day)					Age of the Crop (Days) at Maximum Growth Rate				
	M	G	T	A	P	M	G	T	A	P	M	G	T	A	P
Grifola gargal															
Strain A	13 B a	20 B ab	38 C c	21 C b	17 A ab	4.7	2.8	1.4	3.2	3.6	18	15	58	25	25
Strain B	15 B a	28 C c	62 E d	25 D bc	19 B a	4.3	1.9	0.7	2.4	3.0	18	30	58	37	30
Strain G9	14 B a	24 BC b	—	18 BC ab	17 B a	4.6	2.2	—	3.4	3.5	18	25	—	25	11
Grifola sordulenta	14 B a	26 BC c	49 D d	21 C b	17 B a	4.1	2.5	1.5	3.0	3.2	18	11	58	25	25
Grifola frondosa	14 B a	30 C c	24 B b	17 B a	19 B ab	3.6	1.8	2.1	2.9	2.8	18	7	30	11	15
Ganoderma lucidum															
Strain E47	5 A a	9 A b	12 A c	6 A a	6 A a	8.9	5.6	4.7	7.9	7.8	7	15	48	25	25
Strain S	6 A a	9 A ab	12 A b	7 A a	7 A a	8.6	4.8	4.4	8.0	7.6	7	15	58	25	25

Standard errors (SEs) were calculated with the formula $SE_{ANOVA} = (MS_{error}/ni)^{½}$; SEs for rows (in descending order for each species and strain) were 2.3, 1.8, 1.7, 1.2, 1.4, 0.6, and 0.9; SEs for columns (for M, G, T, A, and P, respectively) were 1.7, 2.1, 1.7, 1.1, and 1.0. *Grifola frondosa* and *Ganoderma lucidum* species (E47 and S strains) were included for a growth-performance comparison. *Capital letters in columns represent the least squares difference (LSD) of the Fisher exact test between species for the same media culture. Lowercase letters in the rows represent the LSD of the Fisher exact test between media cultures of the same strain. Statistical significance is α = 0.05. A, Azure B; G, gallic acid; M, malt yeast peptone agar (control); P, Poly R-478; T, tannic acid.

SMRRs at each stage of growth: vegetative growth, cold-shock induction, and light treatments.

E. Data Analysis

The mycelial growth rate data were subjected to 1-way analysis of variance, and the differences were analyzed with the Fisher least significant difference test (α = 0.05) using Infostat software.[15] The maximum mycelium growth rate corresponded to the inflection point of the growth curve. The χ^2 test and the Fisher exact test (α = 0.05), according to Lowry,[16] were used to compare the number of experimental units between treatments presenting initial and advanced SMRRs.

III. RESULTS AND DISCUSSION

A. *In Vitro* Evaluation of Ligninolytic Activity

Table 1 shows the growth characteristics of mycelia in media containing phenolic and dye compounds. Figure 1 shows representative images of the mycelial colonies. The time needed for the colonies to reach a 5-cm diameter in MYPA medium (control) did not differ between species and strains of *Grifola* spp., with maximum mycelia growth rates of 3.6 and 4.7 mm/day. Under the conditions used, a single dose of gallic acid reduced the mycelial growth rate, except in *G. gargal* strain A and *G. lucidum* strain E47; whereas a single dose of tannic acid reduced the growth rate in all strains. Mycelia of both *G. gargal* (strain B) and *G. sordulenta* began to grow exponentially 40 and 35 days after inoculation, respectively. Moreover, in both the *G. gargal* and the *G. sordulenta* colonies, in the presence of Poly R-478 and Azure B there was a lag in the time required to reach a 5-cm diameter, suggesting that these strains and species had to adapt their metabolism to thrive when these chemicals were used as recalcitrant compounds.

Thus, the time needed to obtain the maximum mycelium growth rate reveals the period of adaptation of the mycelium to new substrates. Indeed,

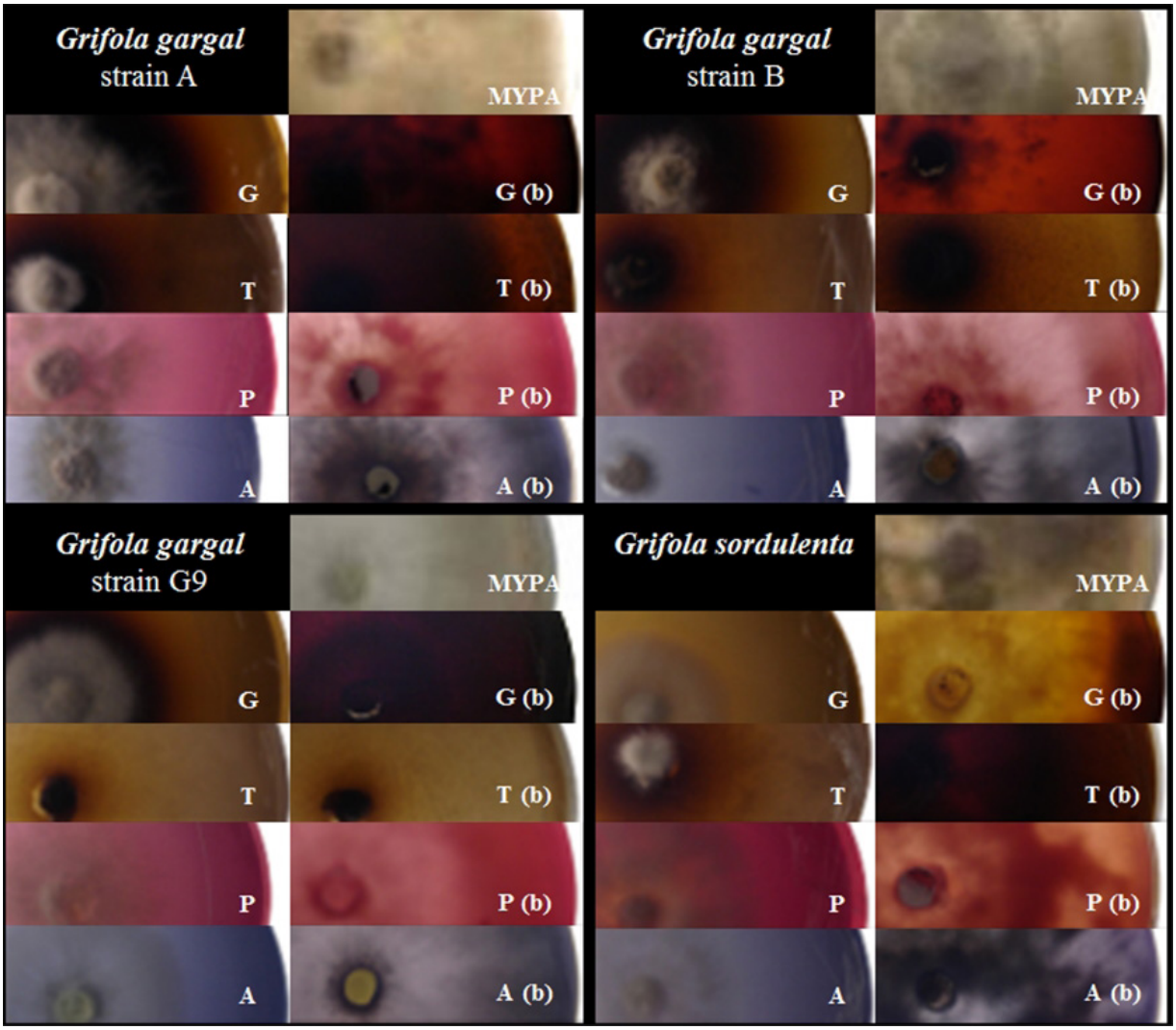

FIG. 1: Representative images of *Grifola gargal* and *Grifola sordulenta* growing in different phenolic and dye-containing media. Media are malt yeast peptone agar (MYPA), gallic acid medium (G), tannic acid medium (T), poly-R478 (P), and Azure B (A). Incubation times were either 28 days (MYPA medium) or 18 days (phenolic media, Poly-R478, and Azure B media). A better view of media discoloration is shown in photographs taken from the bottom side of cultures at 46 days, marked with "(b)."

under the same culture conditions, the mycelia growth rates were considerably faster in *G. frondosa* and *G. lucidum*, which showed their higher ligninolytic capacity; this was in good agreement with the reported performance of the biodegradation of lignocellulosic materials.[10,12,17,18] On the other hand, *G. gargal* and *G. sordulenta* were able to grow at lower temperatures despite less ligninolytic activity, which is an interesting trait to know about during the cultivation of these medicinal mushrooms in cold climates.[10,11] Table 2 presents traits of the biotransformation abilities and the presentation of initial SMRRs. *G. gargal* strains presented a lower biotransformation rate than the 2 strains of *G. lucidum*; this was revealed by the halos of discoloration in the gallic acid–enriched media (*G. frondosa* and *G. sordulenta* did not produce any halos).

The subsequent fading process took place between 30 and 46 days in different ways: centrifugally (*G. gargal* strains A and B, *G. sordulenta*, and

TABLE 2: Biotransformation Ability and Secondary Metabolism–Related Responses Evaluated in Cultures of *Grifola gargal* and *Grifola sordulenta*

Species and Strain	Biotransformation of Phenolic and Dye-Containing Media												Initial SMRR									
	Maximum Rate of Halo Diffusion (mm/Day)		Age of Crop (Days) at Maximum Halo Diffusion		Age at Fading (Days)				Discoloring Direction				Exudates (Days)					Aggregates (Days)				
	G	T	G	T	G	T	A	P	G	T	A	P	M	G	T	A	P	M	G	T	A	P
Grifola gargal																						
Strain A	5.3	5.5	11	7	46	58	30	37	CF	NP	CF	CF	—	—	—	—	60	60	—	—	60	—
Strain B	4.8	4.5	11	7	30	—	37	37	CF	—	CF	NP	—	60	—	46	46	58	37	—	51	37
Strain G9	5.6	4.0	15	7	—	—	37	37	—	—	CF	CF	—	60	—	—	—	—	—	—	60	—
Grifola sordulenta	—	—	—	—	37	—	37	30	CF	—	CF	NP	—	58	—	—	—	—	—	—	—	—
Grifola frondosa	—	6.7	—	7	46	37	37	37	CF	NP	CF	NP	60	25	58	—	—	—	—	—	—	—
Ganoderma lucidum																						
Strain E47	8.9	5.9	7	7	37	37	37	37	NP	NP	CP	CP	—	—	—	58	—	—	37	—	—	—
Strain S	7.4	6.6	7	7	30	30	37	30	CP	NP	CP	NP	58	—	58	46	—	60	—	—	58	—

A, Azure B; CF, centrifugal; CP, centripetal; G, gallic acid; M, malt yeast peptone agar (control); NP, no appreciable pattern; P, Poly R-478; SMRR, secondary metabolism–related response; T, tannic acid.

G. frondosa); centripetally (*G. lucidum* strain S), and across the entire surface without any defined pattern (*G. lucidum* strain 47).

With regard to the culture media containing tannic acid, changes in the color of almost all species was considered to be a trait derived from the presence of phenoloxidases.[19,20] *G. gargal* strain G9 did not grow in this medium, but a halo of coloration spread up from the site of inoculation to the margin of the agar-based culture media, possibly as a result of the enzymes released from the initial inoculum—for example, laccase enzymes, which have been described in previous studies.[10]

The discoloration process began between 30 and 37 days in *G. frondosa* and *G. lucidum* culture media; it began close to the end of the trial (day 58) in *G. gargal* strain A—the one with the best response in this media. Fading did not occur in *G. gargal* strain G9, which did not grow; this may indicate that fading activity could be controlled by an enzyme generated *de novo* that was absent in the inoculum. The weak growth responses in tannic media observed in strains A and B are consistent with previous descriptions of other strains of *G. gargal*.[21]

Discoloration of the medium containing Poly R-478 reveals the activity of ligninolytic enzymes, that is, laccases, manganese peroxidase, lignin peroxidase (LiP), and Azure B medium, which is a good indicator of the LiP enzyme.[13] The discoloration process occurred just a week before the observation of initial SMRRs (day 37). All species tested showed LiP activity (revealed by Azure B) with different extents of discoloration. Discoloration occurred centrifugally in *Grifola* spp., whereas in the 2 strains of *G. lucidum* it occurred centripetally. Concerning the initial SMRRs, *G. gargal* strains showed a greater quantity of aggregates, and those of *G. lucidum* showed a higher quantity of exudates (Table 2). Finally, all fungal species and strains grew rapidly in the Poly R-478 medium. Furthermore, the discoloration time in cultures with Poly R-478 and Azure B was consistent, supporting the idea that the same active enzyme occurred in both dye-supplemented cultures.

TABLE 3: Mycelium Growth of *Grifola gargal* and *Grifola sordulenta* Cultures in Different Carbon-Source Media

Species and Strain	Age of the Colony (Days) When 5-cm Diameter Reached*					Carbohydrate Degradation (Present or Absent)					Apparent Density of Growth**				
	Cellulose	Xyulose	Pectin	Starch	DCPIP	Cellulose	Xyulose	Pectin	Starch	DCPIP	Cellulose	Xyulose	Pectin	Starch	DCPIP
Grifola gargal															
Strain A	35 C b	37 E ab	55 F d	31 D a	39 D c	Present	Present	Present	Present	Absent	+	+	++	+	++
Strain B	43 D c	27 D a	29 C a	36 E b	53 E d	Present	Present	Present	Present	Absent	+	+	++	+	++
Grifola sordulenta	20 B a	20 C a	31 D c	25 C b	33 C c	Present	Present	Present	Present	Present	+++	+++	++	++	++
Grifola frondosa	22 B a	22 C a	37 E b	22 B a	38 D b	Present	Present	Present	Present	Present	+	+	+++	+++	+++
Ganoderma lucidum															
Strain E47	11 A b	8 B a	22 A d	11 A b	18 B c	Present	Present	Present	Present	Absent	+++	+++	+	+	+++
Strain S	11 A a	12 A a	14 B a	13 A a	12 A a	Present	Present	Present	Present	Absent	+++	+++	+	++	+++

G. frondosa and *G. lucidum* (strains E47 and S) species were included as controls. Standard errors (SEs) were calculated with the formula $SE_{ANOVA} = (MS_{error}/ni)^{½}$; SEs for rows (in descending order for each species and strain) were 0.6, 1.5, 0.8, 0.5, 0.4, and 0.7; SEs for columns (for cellulose, xyulose, pectin, starch, and dichlorophenolindophenol [DCPIP], respectively) were 1.4, 0.6, 0.8, and 0.9.

*Capital letters in columns represent the least squares difference (LSD) of the Fisher exact test between species for the same media culture. Lowercase letters in the rows represent the LSD of the Fisher exact test between media cultures of the same strain. Statistical significance is $\alpha = 0.05$.

**Apparent density of growth was classified as +++ (dense),++(soft), or +(faint).[4]

B. *In Vitro* Evaluation of Carbohydrate Utilization

The study of the most beneficial carbohydrate for promoting mycelial growth used low concentrations to obtain a better contrast of the revealed halos of biodegradation. These preliminary evaluations showed that *G. gargal* strain A used starch and xylulose better, whereas pectin caused more growth retardation; pectin was, however, the media in which both strains of *G. gargal* showed a mycelium with an apparently denser growth (Table 3). *G. gargal* strain B showed the highest rate in the culture media with pectin and xylulose. The medium with DCPIP gave a negative result when detecting CDH in both strains of *G. gargal*. Working with other strains of *G. gargal*, Paredes-Leal[21] detected the enzymatic activity of amylase, cellulase, and urease; lipase and protease activity was absent.

Cultivation of *G. sordulenta* in culture media containing cellulose and xylulose showed the best responses in mycelial growth rate and in apparent mycelial density—even more than for *G. frondosa* and *G. gargal*. The formation of an orange halo, a product of the degradation of the substrate by CDH, was observed in the medium with DCPIP. In addition, the presence of initial SMRRs was associated with color change, suggesting that both phenomena are related to the induction of secondary metabolism, which occurs after the amount of easily available nutrients decreases.

C. Biological Responses to Light

Certain ranges of light wavelengths can stimulate or inhibit growth and/or SMRRs of the mycelium.[22] Indeed, Basidiomycota phyla can sense light through phytochromes, opsins, phototropins, cryptochromes, and white-collar photoreceptors.[23] Some SMRRs described in medicinal mushrooms—for example, *Pleurotus ostreatus*, *Lentinus edodes*, *Grifola frondosa*, and *G. lucidum*—include differentiation of vegetative cells, expansion of pileus growth, and pigmentation of basidiomes.[24–27]

Table 4 shows initial and advanced SMRRs to cold shock (temperature decreased from 18°C to 5°C) when the colonization of the available surface area of the nutrient medium was completed.

Before the light treatments were implemented (after 59 days of cultivation), there were no SMRRs in 37% of *G. gargal* experimental units and in 42% of the *G. sordulenta* units. In higher Basidiomycetes, cold shock (among other factors such as nutrient reduction and age of the culture) produces physiological disturbances that modulate the mycelium transition from a vegetative to a reproductive state,[28] and although there were no visible changes in any experimental units here, it is likely that all fungal cells induced by cold shock initiated morphogenic differentiation.

In *G. gargal* cultures, all light treatments generated initial and advanced SMRRs. The SMRRs to light in the 2 strains of *G. gargal* were greater when using white light. Nevertheless, they only differed statistically from those resulting from green light. With regard to *G. sordulenta*, no differences were found between dark or light treatments when comparing the number of experimental units presenting initial SMRRs; however, differences were found when comparing the advanced SMRRs. Primordia were observed only after treatments with white and blue light.

In both species, a lamellar formation emerged from a mature mycelium on the perimeter of the colony adjacent to the edge of the Petri dish, which at this stage is interpreted as a response to the gaseous microclimate being favorable for their induction (i.e., lower CO_2 and higher O_2 concentrations). The most notable effect was produced with red light on *G. gargal* and with white light on *G. sordulenta*.

Both species had biological SMRRs in the absence of light, indicating that light is not an indispensable factor for differentiation. It should also be noted that no phototoxicity symptoms developed under experimental conditions. Conversely, under prolonged exposure to white light, the proportion of SMRRs increased. Even so, there were striking and unexpected responses; for example, in terms of the uniformity of responses to the same treatment, some experimental units had colonies showing a marked response to light, whereas others did not produce that kind of response.

TABLE 4: Secondary Metabolism–Related Responses During the Vegetative Growth Phase and to Cold Shock and Light Treatments in *Grifola gargal* strains A and B and in *Grifola sordulenta*

Treatment	*Grifola gargal*											*Grifola sordulenta*									
	Strain	N	No SMRR (%)	Initial SMRR (%)				Advanced SMRR (%)				N	No SMRR (%)	Initial SMRR (%)				Advanced SMRR (%)			
				Ex	*Ar*	*Pigm*	*Po*	*Agr*	*Lam P*	*Lam C*	*Prim*			*Ex*	*Ar*	*Pigm*	*Po*	*Agr*	*Lam P*	*Lam C*	*Prim*
Vegetative growth	A	30	57	43	—	—	—	—	—	—	—	33	45	18	—	—	—	55	—	—	—
	B	30	57	43	—	—	—	—	—	—	—										
Cold shock	A	30	37	63	—	—	—	10	—	—	—	33	42	58	—	—	—	24	—	—	—
	B	30	37	63	—	—	—	—	—	—	—										
White light	A	6	0	100	17	100	—	100	100	—	100	6	0	100	—	17	—	67	100	50	83
	B	6	0	33	—	83	33	17	83	—	33										
Blue light	A	6	0	83	—	100	33	50	100	—	—	7	0	71	14	—	14	100	86	—	43
	B	6	0	83	—	83	67	67	83	—	33										
Green light	A	6	0	83	33	33	100	33	100	—	—	7	0	71	29	—	86	100	100	29	—
	B	6	0	100	50	—	83	50	67	—	—										
Red light	A	6	0	—	33	33	50	83	67	67	—	5	0	100	20	—	100	100	100	—	—
	B	6	0	50	50	—	83	33	33	33	17										
Dark (control)	A	6	33	—	50	—	50	33	50	17	17	7	29	14	43	43	43	29	—	—	—
	B	6	100	—	83	—	—	—	—	—	—										

N indicates number of samples. Agr, aggregates; Ar, almond aroma; Ex, exudates; Lam C, central lamellae; Lam P, perimetric lamellae; Pigm, pigmented mycelium; Po, pigmented pores; Prim, primordia; SMRR, secondary metabolism–related response.

The genomic study of fungi in combination with new molecular biology tools will allow deeper insight into the photobiological phenomena in the fungi kingdom; such knowledge may have important practical implications in both mushroom cultivation and in obtaining useful metabolites such as certain nutraceutical, cosmetic, and flavoring compounds.[22]

IV. CONCLUSIONS

The macroscopic and microscopic characteristics of *G. gargal* and *G. sordulenta* cultivated in nutrient agar and the variability found among strains was described and compared. *In vitro* studies using different nutrient media revealed some traits of the ligninolytic enzymatic activity and preferences of the principal carbohydrate sources in *G. gargal* and *G. sordulenta*. In the study of *in vitro* SMRRs, metabolic changes were detected in the physiological phases under different light conditions. The results obtained in this study will help to guide the optimization of bioprocesses for the production of mycelia and/or mushrooms with medicinal properties, as well as secondary metabolites, through submerged or solid-state fermentations.

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REFERENCES

1. Schmeda-Hirschmann G, Razmilic I, Gutierrez M, Loyola J. Proximate composition and biological activity of food plants gathered by Chilean Amerindians. Econ Bot. 1999;53:177–87.

2. de Bruijn J, Loyola C, Aqueveque P, Cañumir J, Cortéz M, France A. Extraction of secondary metabolites from edible Chilean mushrooms. In: Martínez-Carrerra D, Curvetto N, Sobal M, Morales P, Mora VM, editors. Hacia un desarrollo sostenible del sistema de producción-consumo de los hongos comestibles y medicinales en Latinoamérica: avances y perspectivas en el Siglo XXI. Puebla (Mexico): COLPOS-UNS-CONACYT-AMC-UAEM-UPAEP-IMINAP; 2010. pp. 3–18.
3. Postemsky PD, Curvetto NR. Enhancement of wheat grain antioxidant activity by solid-state fermentation with Grifola spp. J Med Food. 2014;17:543–9.
4. Postemsky PD, Curvetto NR. Submerged culture of Grifola gargal and G. sordulenta (higher Basidiomycetes) from Argentina as a source of mycelia with antioxidant activity. Int J Med Mushrooms. 2015;17:65–76.
5. Ito T, Kato M, Tsuchida H, Harada E, Niwa T, Osawa T. Ergothioneine as an anti-oxidative/anti-inflammatory component in several edible mushrooms. Food Sci Tecnol Res. 2011;17:103–10.
6. Harada E, D'Alessandro-Gabazza CN, Toda M, Morizono T, Chelakkot-Govindalayathil A, Roeen Z, Urawa M, Yasuma T, Yano Y, Sumiya T, Gabazza EC. Amelioration of atherosclerosis by the new medicinal mushroom Grifola gargal Singer. J Med Food. 2014;18:872–81.
7. Postemsky PD, Palermo AM, Curvetto NR. Protective effects of new medicinal mushroom, Grifola gargal Singer (higher Basidiomycetes), on induced DNA damage in somatic cells of Drosophila melanogaster. Int J Med Mushrooms. 2011;13:583–94.
8. Shen Q, Royse D. Effects of nutrient supplements on biological efficiency, quality and crop cycle time of maitake (Grifola frondosa). Appl Microbiol Biotechnol. 2001;57:74–8.
9. Postemsky PD, González Matute R, Figlas DN, Curvetto NR. Optimizing Grifola sordulenta and Grifola gargal growth in agar and liquid nutrient media. Micol Aplicada Int. 2006;18:7–12.
10. Postemsky PD, Curvetto NR. Solid-state fermentation of sunflower seed hulls by Grifola gargal and G. sordulenta. Int Biodeterior Biodegradation. 2015;100:52–61.
11. Harada E, Morizono T, Sumiya T, Meguro S. Production of Andean-Patagonic edible mushroom Grifola gargal on wood-based substrates. Mycoscience. 2015;56:616–21.
12. Postemsky PD, Delmastro SE, Curvetto NR. Effect of edible oils and Cu (II) on the biodegradation of rice by-products by Ganoderma lucidum mushroom. Int Biodeterior Biodegradation. 2014;93:25–32.
13. Levin L, Melignani E, Ramos AM. Effect of nitrogen sources and vitamins on ligninolytic enzyme production by some white-rot fungi. Dye decolorization by selected culture filtrates. Bioresour Technol. 2010;101:4554–63.
14. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant.1962;15:473–97.
15. Di Rienzo J, Casanoves F, Balzarini M, Gonzalez L, Tablada M, Robledo CW. InfoStat software. Córdoba (Argentina): Universidad Nacional de Córdoba; 2010. Available from http://www.infostat.com.ar.
16. Lowry R. VassarStats. Arlington (NY): Vassar College; c1998–2016 [cited 2016 Apr 28].. Available from http://faculty.vassar.edu/lowry/tab2x2.html.
17. González-Matute R, Serra A, Figlas D, Curvetto N. Copper and zinc bioaccumulation and bioavailability of Ganoderma lucidum. J Med Food. 2011;14:1273–9.
18. Postemsky PD, Marinangeli PA, Curvetto NR. Recycling of residual substrate from Ganoderma lucidum mushroom cultivation as biodegradable containers for horticultural seedlings. Sci Hort (Amsterdam). 2016;201:329–37.
19. Rajchenberg M. The genus Grifola (Aphyllophorales, Basidiomycota) in Argentina revisited. Bol Soc Argent Bot. 2002;37:19–27.
20. Rajchenberg M, Greslebin A. Cultural characters, compatibility tests and taxonomic remarks of selected polypores of the Patagonian Andes forests of Argentina. Mycotaxon.1995;56:325–46.
21. Paredes-Leal NP. Determinación cualitativa de enzimas relacionadas con la degradación de compuestos carbonados y nitrogenados en cepas de Aphyllophorales [thesis]. Valdivia: Instituto de Microbiología, Universidad Austral de Chile; 2006.
22. Kurtzman RH, Martínez-Carrera D. Light, what it is and what it does for mycology. Micol Aplicada Int. 2013;25:23–33.
23. Idnurm A, Heitman J. Photosensing fungi: phytochrome in the spotlight. Curr Biol. 2005;15:829–32.
24. Sano H, Kaneko S, Sakamoto Y, Sato T, Shishido K. The basidiomycetous mushroom Lentinula edodes white collar-2 homolog PHRB, a partner of putative blue-light photoreceptor PHRA, binds to a specific site in the promoter region of the L. edodes tyrosinase gene. Fungal Genet Biol. 2009;46:333–41.
25. Arjona D, Arago C, Aguilera J, Ramírez L, Pisabarro A. Reproducible and controllable light induction of in vitro fruiting of the white-rot basidiomycete Pleurotus ostreatus. Mycol Res. 2009;113:552–8.
26. Kurahashi A, Shimoda T, Sato M, Fujimori F, Hirama J, Nishibori KA. Putative transcription factor Gf.BMR1 in Grifola frondosa, the homolog of BMR1 in Bipolaris oryzae, was strongly induced by near-ultraviolet light and blue light. Mycoscience. 2015;56:177–82.
27. Poyedinok NL, Mykhailova OB, Shcherba VV, Buchalo AS, Negriyko AM. Light regulation of growth and biosynthetic activity of Ling Zhi or Reishi medicinal mushroom, Ganoderma lucidum (W. Curt.: Fr.) P. Karst. (Aphyllophoromycetidae), in pure culture. Int J Med Mushrooms. 2008;10:369–78.
28. Moore D. Principles of mushroom developmental biology. Int J Med Mushrooms. 2005;7(1–2):79–102.