UDC 582.284:577.151.02:547.458 *review*
ISSN 1330-9862

(FTB-1244)

Current Techniques for the Cultivation of *Ganoderma lucidum* for the Production of Biomass, Ganoderic Acid and Polysaccharides

Ricardo Wagner[1], *David Alexander Mitchell*[1]*, *Guilherme Lanzi Sassaki*[1], *Maria Angela Lopes de Almeida Amazonas*[2] *and Marin Berovič*[3]

[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Cx. P. 19046, Centro Politécnico, Curitiba 81531-990, Paraná, Brasil

[2]Centro Nacional de Pesquisa de Florestas, Embrapa Florestas, Cx. P. 319 Colombo, 83411-000 Paraná, Brasil

[3]Department of Biotechnology, National Institute of Chemistry, Hajdrihova 19, SI-1115 Ljubljana, Slovenia

Received: June 18, 2003
Accepted: November 10, 2003

Summary

Ganoderma lucidum is a medicinal mushroom that has been used in the Orient for more than 2000 years. Due to the long time required for basidiocarp formation, attention has recently been given to the use of submerged fermentation for the production of mycelial biomass and its bioactive components, such as polysaccharides and ganoderic acids. We review the current state of knowledge about the cultivation of *G. lucidum* by modern fermentation techniques, focussing on the effects of fermentation conditions and how knowledge of these effects has been used to develop strategies for improving the production of biomass, polysaccharides and ganoderic acid. We also outline the methods that have been used for biomass and product recovery and point out potential problems involved in these steps. Studies to date have been almost entirely limited to laboratory scale and much more understanding of the physiology of *G. lucidum* and its relationship with growth morphology will be necessary before it will be possible to develop economical large scale processes.

Key words: Ganoderma lucidum, submerged fermentation, polysaccharide, ganoderic acid

Introduction

Ganoderma lucidum (Leyss.: Fr.) Karst, is a basidiomycete belonging to the Polyporaceae, also known as »Ling zhi« in China and »Reishi« in Japan. It has been widely used as a traditional medicine in the Orient for more than 2000 years. The first mention of this mushroom dates from the period of the first emperor of China, Shih-huang of the Ch'in Dynasty (221–227 B.C.) (*1*).

Even today the basidiocarp (fruiting body) of *G. lucidum* is a popular remedy to treat conditions like hepatopathy, chronic hepatitis, nephritis, hypertension, hyperlipemia, arthritis, neurasthenia, insomnia, bronchitis, asthma, gastric ulcer, arteriosclerosis, leukopenia, diabetes, anorexia and cancer (*1–3*). *G. lucidum* produces several metabolites with biological activity, such as polysaccharides and terpenoids, which might be respon-

* Corresponding author; Phone: ++55 41 3611 658; Fax: ++55 41 2662 042; E-mail: davidmitchell@ufpr.br

sible for some of these medicinal properties. Polysaccharide fractions containing (1→3)-*β*-D-glucans, branched mainly at the C-6 position, demonstrate high antitumor activity (*4*). Terpenoids are cytotoxic to hepatoma cells (*3*), they inhibit the response of platelets to various aggregating agonists (*5*) and inhibit eukaryotic DNA polymerase and HIV type 1 reverse transcriptase (*6*).

Although (1→3)-*β*-D-glucans and a group of terpenoids known as ganoderic acids are found both in basidiocarp and cultured mycelium (*2,7*), most of the commercial *G. lucidum* products derive from its basidiocarp. There has been recent interest in producing these biologically active substances by fermentation techniques, prompted by the fact that *G. lucidum* is rarely found in nature and traditional basidiocarp production in logs or sawdust takes months (*3,8*). In addition, these traditional techniques do not guarantee a standardized product since the composition of the substrate, which affects basidiocarp composition, varies from batch to batch.

This review focuses on how fermentation techniques have been used to obtain ganoderic acids (GA) and intracellular and extracellular polysaccharides (IPS and EPS, respectively), which are composed mainly of (1→3)-*β*-D-glucans. Both submerged fermentation and solid-state fermentation techniques have been used, but most attention has been given to submerged fermentation. We not only review various aspects of the fermentation strategies that have been used but also analyze critically the methods that have been used to monitor growth and product formation.

Submerged Fermentation of *Ganoderma lucidum*

The advantage of submerged fermentation over traditional basidiocarp cultivation is the reduction in the time spent to obtain the product of interest. The production of basidiocarp takes at least 3 to 5 months, while reasonable amounts of ganoderic acid and polysaccharides can be obtained by submerged fermentation after only 2 to 3 weeks (*9*).

Table 1 summarizes the published data on submerged fermentations with *Ganoderma*. There is only one report of a large scale fermentation, in which *G. tsugae* was cultivated in tanks with a volume of 20 m^3. All reported studies of fermentation with *G. lucidum* were done in volumes of 10 L or less. Roughly half the studies were undertaken in Erlenmeyer flasks and half in bioreactors. The advantage of using bioreactors is that it is easier to control environmental conditions such as temperature, dissolved oxygen and pH.

These fermentations were done with different objectives. Some aimed simply to produce biomass, with no concern for its composition. Others aimed to maximize the production of either ganoderic acids or polysaccharides, and to understand how different variables affect their production. The best yields reported to date are 22.1 g/L for biomass (*10*), 1.71 g/L for EPS (*11*), 2.49 g/L for IPS (*9*) and 582 mg/L for GA (*12*). The growth and product formation kinetics are discussed later.

Culture maintenance

Potato dextrose agar (PDA) has been favored for culture maintenance. Special care should be taken when preparing PDA with potatoes of unknown origin because pesticide residues, if present, will interfere with fungal development. Therefore, commercial brands of PDA are recommended. An alternative medium for culture maintenance is: malt extract 1 %, yeast extract 4 %, D-glucose 0.4 % and agar 2 % (*4*). The incubation time used is normally 7 days at 24–30 °C. The cultures are then usually stored at 4 °C, although studies of culture viability at this temperature are not available.

Inoculum

The production of the inoculum is a crucial step that often receives only minor attention. The size, viability and homogeneity of the inoculum can affect the performance of the subsequent fermentation significantly, and therefore must be standardized in order to obtain a reproducible process. In this respect, *Ganoderma* presents some interesting challenges, because, unlike filamentous fungi, with which it is quite easy to produce spores and use them as inoculum, it is not practical to use spore inocula with macrofungi such as *Ganoderma*, since spores are only produced in the basidiocarp. Therefore mycelium-based inocula must be used. There are two main strategies for preparation of mycelial inocula for submerged fermentation, either preparation of the seed culture on agar or preparation in submerged fermentation, although both are potentially problematic, as described below.

When the volume of the growth medium to be inoculated is low, small pieces of mycelium, together with some of the agar medium on which they are growing, can be cut from slants or Petri dishes and put directly into the fermentation broth (*13,14*). However, a long lag phase would be expected since the mycelium is transferred from a solid to a liquid environment and typically the inoculum densities achieved by this method are quite low. Unfortunately, no information is available about how *Ganoderma* adapts during the early stages of the fermentation when this inoculum strategy is used. Further, if the inoculum is to be used in comparative studies involving fermentations undertaken simultaneously in several bioreactors or flasks, the size of the pieces of mycelium must be standardized and they must be removed from the same radial distance from the colony center, in order to guarantee that different inocula contain the same amount of mycelium at the same stage of development.

When the volume of the growth medium to be inoculated is large, the inoculum typically must be the whole volume of a submerged seed culture or an aliquot of it (*11,15*). In this case it is possible to standardize the inoculum size based on the dry weight of mycelium $m(X)$ per liter of broth. Table 1 shows that workers commonly use a pre-culture of a given percentage of the final working volume and do not report the mycelium concentration in the seed culture at the time of inoculation.

When aliquots are removed from a single preculture to inoculate several bioreactors or flasks, homogeneity

Table 1. Fermentation experiments undertaken with *Ganoderma* and key results obtained

Ref.	Media, with components in g/L unless otherwise specified	Working volume, bioreactor and conditions. Inoculum (density of the inoculum culture)	Maximum concentration obtained (day)				Linear production rate [R^2] (interval used, day)			
			γ(X) g/L	γ(EPS) g/L	γ(IPS) g/L	γ(GA) mg/L	ρ(X) g/(L.d)	ρ(EPS) g/(L.d)	ρ(IPS) g/(L.d)	ρ(GA) mg/(L.d)
(13)	Medium A	100 mL in 500-mL flask; initial pH=4.0; 100 rpm on rotary shaker; 30 °C. Disk inoculum.	5.5 (14)	–	–	–	–	–	–	–
(11)	Medium A	100 mL in 500-mL flask; initial pH=4.0; 100 rpm on rotary shaker; 30 °C. Inoculum*	–	1.71 (7)	–	–	–	–	–	–
(11)	Medium A	1 L in 2-L bioreactor; 6-blade impeller; 200 rpm; initial pH=4.0; 30 °C; 1 vvm; Inoculum 10 %	–	1.26 (7)	–	–	–	0.17 [0.85] (2–7)	–	–
(11)	Medium A	As previous entry but agitation 400 rpm. Inoculum 10 %	–	1.48 (3)	–	–	–	–	–	–
(11)	Medium A	3 L in 5-L bioreactor; pH=4.0; 30 °C; 100 rpm; 1 vvm; 7 days. Inoculum*	–	–	–	–	–	–	–	–
(14)	Medium A	100 mL in 250-mL flask; initial pH=4.0; 100 rpm on rotary shaker; 30 °C. Disk inoculum.	2.3 (8)	0.13 (8)	–	–	0.3 [0.84] (2–8)	0.03 [1.00] (4–8)	–	–
(14)	Medium A + olive oil 1 %	As previous entry. Disk inoculum.	3.28 (7)	0.16 (7)	–	–	–	–	–	–
(14)	Medium A + safflower oil 1 %	As previous entry. Disk inoculum.	2.78 (7)	0.18 (7)	–	–	–	–	–	–
(14)	Medium A + oleic acid 1.5	As previous entry. Disk inoculum.	4.6 (7)	0.16 (7)	–	–	–	–	–	–
(14)	Medium A + palmitic acid 1	As previous entry. Disk inoculum.	4.4 (8)	0.16 (8)	–	–	0.6 [0.94] (2–8)	0.03 [0.97] (2–8)	–	–
(14)	Medium A + palmitic acid 2.5	As previous entry. Disk inoculum.	3.4 (7)	0.21 (7)	–	–	–	–	–	–
(14)	Medium A + oleic acid 1.5	100 mL in 250-mL flask; initial pH=4.0; rotary shaker 100 rpm; 30 °C. Polyurethane foam sheet as inert support. Disk inoculum.	8.7 (7)	0.27 (7)	–	–	–	–	–	–
(14)	Medium A + palmitic acid 1	As previous entry. Disk inoculum.	6.1 (7)	0.32 (7)	–	–	–	–	–	–
(16)	Medium B + Glc 35; YE 10; P 5	50 mL in 250-mL flask; initial pH=5.5; rotary shaker 120 rpm; 30 °C. Inoculum 10 % (ρ(X)=325 mg/L)	15.8 (8)	0.62 (8)	0.86 (8)	150 (8)	–	–	–	–
(16)	Medium B + Glc 35; YE 5; P 5	As previous entry. Inoculum 10 % (ρ(X)=325 mg/L)	15.4 (8)	0.81 (8)	0.81 (8)	171 (8)	–	–	–	–
(16)	Medium B + Glc 65; YE 5; P 5	As previous entry. Inoculum 10 % (ρ(X)=325 mg/L)	12.6 (10)	1.08 (10)	0.95 (10)	159 (10)	–	–	–	–
(16)	Medium B + Glc 50; YE 5; P 5	As previous entry. Inoculum 10 % (ρ(X)=325 mg/L)	16.7 (10)	0.85 (10)	1.19 (10)	212 (10)	–	–	–	–
(15)	Medium B + Glc 35; YE 5; P 5	As previous entry but initial pH=3.5 Inoculum 10 % (ρ(X)=325 mg/L)	13.8 (8)	1.02 (14)	0.94 (8)	155 (8)	–	0.08 [0.97] (1–12)	–	–

Ref.	Media, with components in g/L unless otherwise specified	Working volume, bioreactor and conditions. Inoculum (density of the inoculum culture)	Maximum concentration obtained (day)				Linear production rate [R^2] (interval used, day)			
			γ(X)	γ(EPS)	γ(IPS)	γ(GA)	ρ(X)	ρ(EPS)	ρ(IPS)	ρ(GA)
			g/L	g/L	g/L	mg/L	g/(L.d)	g/(L.d)	g/(L.d)	mg/(L.d)
(*15*)	As previous entry	As previous entry but initial pH=5.5. Inoculum 10 % (ρ(X)=325 mg/L)	15.5 (8)	0.63 (8)	1.06 (8)	193 (8)	–	–	0.15 [0.99] (1–8)	–
(*15*)	As previous entry	As previous entry but initial pH=6.5. Inoculum 10 % (ρ(X)=325 mg/L)	17.3 (8)	0.59 (8)	1.05 (8)	208 (8)	2.3 [0.97] (1–8)	–	–	27 [0.98] (2–8)
(*15*)	As previous entry	50 mL in 250-mL flask; 120 rpm on rotary shaker; 30 °C; 10 g/L of Glc added on day 8. Inoculum 10 % (ρ(X)=325 mg/L)	–	1.41 (14)	–	–	–	0.19 [0.98] (8–12)	–	–
(*12*)	*	50 mL in 250-mL flask; rotary shaker 120 rpm; 30 °C; static after 4 days. Inoculum 10 % (ρ(X)=325 mg/L)	20.9 (16)	–	–	582 (12)	0.9 [0.99] (4–16)	–	–	56 [0.97] (4–12)
(*17*)	*	Bioreactor*. Inoculum ρ(X)=170 mg/L	15.3 (8)	0.71 (8)	0.79 (8)	267 (8)	–	–	–	36 [1.00] (2–8)
(*17*)	*	Bioreactor*. Inoculum ρ(X)=330 mg/L	15.7 (8)	0.79 (8)	1.08 (8)	214 (8)	2.8 [0.99] (1–6)	–	–	–
(*17*)	*	Bioreactor*. Inoculum ρ(X)=670 mg/L	14.2 (8)	0.88 (8)	1.22 (8)	180 (8)	–	0.11 [0.87] (1–8)	0.16 [0.97] (1–8)	–
(*9*)	Medium B + sucrose 35; YE 5; P 5.	50 mL in 250-mL flask; initial pH=5.5; rotary shaker 120 rpm; 30 °C. Inoculum 10 % (ρ(X)=625 mg/L)	3.5 (2)	0.63 (day*)	0.25 (day*)	40 (day*)	–	–	–	–
(*9*)	Medium B + Glc 35; YE 5; P 5.	As previous entry. Inoculum 10 % (ρ(X)=625 mg/L)	10.1 (10)	0.47 (day*)	0.97 (day*)	144 (day*)	–	–	–	–
(*9*)	Medium B + maltose 35; YE 5; P 5.	As previous entry. Inoculum 10 % (ρ(X)=625 mg/L)	12.2 (10)	0.57 (day*)	1.16 (day*)	119 (day*)	–	–	–	–
(*9*)	Medium B + lactose 35; YE 5; P 5.	As previous entry. Inoculum 10 % (ρ(X)=625 mg/L)	12.3 (12)	0.53 (day*)	1.43 (day*)	180 (day*)	–	–	–	–
(*9*)	Medium B + lactose 50; YE 5; P 5.	As previous entry. Inoculum 10 % (ρ(X)=625 mg/L)	13.8 (14)	0.79 (14)	1.65 (12)	110 (14)	–	–	0.11 [0.97] (2–12)	–
(*9*)	Medium B + lactose 65; YE 5; P 5.	As previous entry. Inoculum 10 % (ρ(X)=625 mg/L)	14.4 (14)	0.94 (14)	1.41 (14)	92 (14)	0.81 [0.95] (2–14)	0.06 [0.98] (2–14)	–	–
(*9*)	Medium B + lactose 35; YE 5; P 5.	50 mL in 250-mL flask; rotary shaker 120 rpm; 30 °C; lactose added on day 8. Inoculum 10 % (ρ(X)=625 mg/L)	~20 (12)	0.96 (20)	1.97 (16)	258 (20)	–	0.49 [0.98] (8–20)	–	–
(*9*)	As previous entry	As previous entry but lactose added on day 10. Inoculum 10 % (ρ(X)=625 mg/L)	~20 (14)	0.80 (22)	2.01 (14)	334 (20)	–	–	0.24 [0.96] (10–14)	20 [1.00] (10–20)
(*9*)	As previous entry	As previous entry but lactose added on day 12. Inoculum 10 % (ρ(X)=625 mg/L)	~20 (14)	0.76 (18)	1.78 (18)	270 (20)	–	–	–	–
(*9*)	As previous entry	2 L in agitated bioreactor, 2 x 6-blade impellers; 100-180 rpm; 0.25-0.5 vvm. Inoculum %* (ρ(X)=625 mg/L)	16.7 (10)	0.61 (12)	1.68 (20)	178 (10)	–	0.03 [0.96] (4–12)	0.10 [0.95] (4–12)	–

Ref.	Media, with components in g/L unless otherwise specified	Working volume, bioreactor and conditions. Inoculum (density of the inoculum culture)	Maximum concentration obtained (day)				Linear production rate [R^2] (interval used, day)			
			γ(X)	γ(EPS)	γ(IPS)	γ(GA)	ρ(X)	ρ(EPS)	ρ(IPS)	ρ(GA)
			g/L	g/L	g/L	mg/L	g/(L.d)	g/(L.d)	g/(L.d)	mg/(L.d)
(*9*)	As previous entry	Bioreactor and operation as previous entry. [lactose] increased 15 g/L on day 10. Inoculum %* (ρ(X)=625 mg/L)	21.9 (12)	0.87 (20)	2.49 (22)	367 (12)	–	0.02 [1.00] (12–20)	0.06 [0.86] (12–22)	–
(*10*)	As previous entry	3.5 L in stirred bioreactor; 100–250 rpm; 30 °C; 0.25–1.0 vvm; initial pH ~5.5. Inoculum 10 %	18.4 (10)	0.81 (14)	–	–	1.7 [0.98] (4–10)	0.05 [0.99] (4–14)	–	–
(*10*)	As previous entry	Bioreactor and operation as previous entry but lactose fed on day 10. Inoculum 10 %	22.1 (12)	1.25 (16)	–	–	–	0.12 [0.99] (10–16)	–	–
(*19*)	Medium B + lactose 35; YE 2.5; P 5	3.5 L in bioreactor with 2 × 6-bladed turbines (6.5 cm i.d.). 30 °C. DOT maintained at 10 %. Inoculum*	4.1 (12)	0.70 (12)	0.60 (12)	147 (9)	–	0.05 [1.00] (4–12)	0.05 [0.99] (4–12)	–
(*19*)	As previous entry	As previous entry but DOT maintained at 25 %. Inoculum*	14.7 (10)	0.60 (12)	1.56 (10)	340 (10)	1.4 [1.00] (4–10)	0.05 [0.99] (4–12)	0.20 [0.98] (4–10)	34 [0.87] (4–10)
(*19*)	As previous entry	As previous entry but agitation 200 rpm, $Q_{(air)}$=0.22 L/min (k_La = 16.4 h^{-1}). Inoculum*	12.1 (8)	0.97 (13)	1.91 (10)	245 (10)	–	0.06 [0.99] (2–12)	–	–
(*19*)	As previous entry	As previous entry but agitation 200 rpm, $Q_{(air)}$=1.75 L/min (k_La = 78.2 h^{-1}). Inoculum*	15.6 (10)	0.92 (13)	2.19 (10)	338 (10)	2.1 [1.00] (2–8)	–	0.25 [0.98] (2–10)	–
(*19*)	As previous entry	As previous entry but agitation 200 rpm, $Q_{(air)}$=3.5 L/min (k_La = 96.0 h^{-1}). Inoculum*	13.9 (14)	0.92 (13)	2.09 (10)	450 (10)	–	–	–	53 [0.99] (2–10)
(*18*)	Glc 60; KH_2PO_4 0.5; YE 6; $(NH_4)_2HPO_4$ 5; Sigma antifoam 289 0.5 %	2 L in 3-L air-lift fermenter; 25 °C; 2.5 vvm.; initial pH at 6.0 (not controlled). Inoculum 5 %	7.9 (8)	4.1 (8)	–	–	1.0 [0.96] (1–7)	0.6 [1.00] (1–7)	–	–
(*18*)	As previous entry	As previous entry but pH controlled at 3.0. Inoculum 5 %	12.9 (6)	6.4** (8);	–	–	2.4 [1.00] (1–6)	0.7 [0.96] (1–8)	–	–
(*18*)	As previous entry	As previous entry but pH controlled at 6.0. Inoculum 5 %	5.7 (4)	13.6** (7);	–	–	1.7 [0.99] (1–4)	2.2 [0.99] (1–8)	–	–
(*18*)	As previous entry	As previous entry but bistage control of pH=3.0 initially and 6.0 later. Inoculum 5 %	8.2 (5)	20.0** (6)	–	–	1.7 [0.99] (1–5)	4.2 [1.00] (2–6)	–	–
(*22*)	Glc 20; YE 2; P 2; K_2HPO_4 1; KH_2PO_4 0.46; $MgSO_4 \cdot 7H_2O$ 0.5	3 L in 5-L Jar fermenter; initial pH=5.0; 25 °C; 150 rpm; 2 vvm. Inoculum 4 %	6.9 (15)	1.16** (15)	–	–	–	–	–	–
(*22*)	Glc 10; ME 3; P 5; YE 3	As previous entry. Inoculum 4 %	5.7 (15)	0.66** (10)	–	–	–	–	–	–
(*22*)	PDB 24; ME 10; P 1	As previous entry. Inoculum 4 %	16.3 (11)	6.5** (12)	–	–	1.52 [0.95] (1–11)	0.60 [0.98] (1–12)	–	–
(*4*)	Glc 50; ME 40; YE 1; KH_2PO_4 0.5; K_2HPO_4 0.5; $MgSO_4 \cdot 7H_2O$ 0.5	Working volume*. Shaking culture at 28 °C. Inoculum*	–	0.85** (7)	–	–	–	–	–	–

Ref.	Media, with components in g/L unless otherwise specified	Working volume, bioreactor and conditions. Inoculum (density of the inoculum culture)	Maximum concentration obtained (day)				Linear production rate [R^2] (interval used, day)			
			γ(X) g/L	γ(EPS) g/L	γ(IPS) g/L	γ(GA) mg/L	ρ(X) g/(L.d)	ρ(EPS) g/(L.d)	ρ(IPS) g/(L.d)	ρ(GA) mg/(L.d)
(*20*)	3 kg of peeled potatoes autoclaved and made up to 10 L with water; Glc 20 g/L; olive oil 2 %	Working volume*. 10-L reactor, 3 Rushton turbines; 300 rpm; initial pH=5.8; 30 °C; $p_{(O_2)}$=70–80 %; redox potential *E*=100–450 mV; $Q_{(air)}$=30 L/min. Inoculum 17 % wet weight of 6-day shake flask culture.	5.4 (8)	–	–	–	0.9 [0.99] (1–5)	–	–	–
(*20*)	As previous entry	As previous entry but after 8 days 7 L of broth substituted with fresh sterile medium. Inoculum as previous entry	9.6 (15)	–	–	–	1.1 [0.95] (10–14)	–	–	–
(*26*) ***	Glc 30; P 3; K_2HPO_4 1.5; $MgSO_4$ 0.7; vit. B_1 0.01	Working volume*, 20 t tank; *t*=(26±1) °C; 7–10 days. Inoculum*	25 kg total	–	–	–	–	–	–	–

*not possible to determine from the article; **determined by the gravimetric method; ***the fungus used was *Ganoderma tsugae.*

Medium A = Glc 50; K_2HPO_4 0.5; KH_2PO_4 0.5; $MgSO_4{\cdot}7H_2O$ 0.5; YE 1; NH_4Cl 4; Medium B = $KH_2PO_4{\cdot}H_2O$ 1; $MgSO_4{\cdot}7H_2O$ 0.5; vitamin B_1 0.05.

Abbreviations: EPS = exopolysaccharide; GA = ganoderic acid; IPS = intracellular polysaccharide; Glc = glucose; vvm = volumes of air per working volume per minute; DOT = dissolved oxygen tension; P = peptone; ME = malt extract; PDB = potato dextrose broth; YE = yeast extract; Disk inoculum = several 5 x 5 mm disks of agar with mycelium

of the inoculum can be improved by first treating the seed culture with a blender (*13*). However, no evaluation has been made of the mycelial damage that this may cause or the consequences for growth in the subsequent fermentation.

In general, the nutritional and environmental conditions used to prepare the seed culture are the same as those used in the fermentation, resulting in a shorter lag phase. Fang and Zhong used sucrose as the carbon source during the pre-cultures and glucose in the fermentation, but did not explain why they did this (*15*). Possibly the fact that the mycelia cultivated on sucrose were shorter (*9*) meant that it was easier to take homogeneous and standardized aliquots of inoculum.

Media for the production step

All the media listed in Table 1 contain a complex carbon and nitrogen source such as yeast extract, peptone or malt extract. Some workers have compared media containing these complex carbon and nitrogen sources with completely defined media, *i.e.* with only a single carbon source, a single nitrogen source and salts. In none of these comparisons were quantitative data presented; the authors simply claimed that growth was better when a complex source was added (*16*). It is common to add yeast extract and peptone, either singly or together, each at concentrations from 1 to 5 g/L. Note that peptone at concentrations above 10 g/L inhibits cell growth (*16*). Interestingly, vitamin B1 at a concentration of 0.05 g/L is also commonly added, even if both peptone and yeast extract are already present (Table 1).

In addition to the complex nutrient source, all workers add sugar as the major carbon source. Systematic studies to determine the best sugar have not been done. Most workers have used glucose, although some have used lactose (Table 1). The only study that compared different sugars was that of Tang and Zhong, who compared sucrose, glucose, maltose and lactose (*9*). The authors claimed that lactose was the best sugar both for cell growth and for production of components such as IPS and ganoderic acid. However, a statistical analysis of the results, using Student's *t* test with p = 0.05, suggests that biomass productivity was higher on maltose, that IPS productivity on lactose was not significantly better than on maltose and that ganoderic acid productivity was not significantly better on lactose than on glucose.

Effects of Process Variables on Growth and Product Formation in Submerged Culture

The optimum fermentation conditions depend on whether the desired product is biomass, ganoderic acid, IPS or EPS. Various factors interact to influence the relative productivity of these products. The effects of fungal morphology, initial sugar concentration, agitation rate, aeration rate, temperature, pH, inoculum density and fatty acids on *Ganoderma* are discussed below.

Morphology

The metabolism of *Ganoderma* is related to its morphology. Growth occurs in pellet form in shaking cul-

tures, with the relative productivity of the various metabolites depending on pellet size (*12*,*16*,*17*). The yield of ganoderic acid, for example, is higher in pellets that are larger than the critical size for oxygen diffusion because limitation of oxygen in the pellet center stimulates the production of ganoderic acid by the cells in this region. On the other hand, IPS concentrations are lower in larger pellets. Pellet size is itself influenced by variables such as the sugar concentration in the medium, the agitation regime and the inoculum density. Unfortunately, many studies devoted to exploring the relationship between growth morphology and metabolite production simply describe the effect of changing a variable and do not elucidate the underlying mechanism that causes the effect.

Initial sugar concentration in the medium

Fang and Zhong noted a decrease in the average growth rate of *G. lucidum* as the initial glucose concentration was increased above 35 g/L and attributed this to the increased osmotic pressure of the medium (*16*). The highest sugar concentrations used resulted in the highest values for the specific oxygen uptake rate, while the yield coefficient decreased linearly from w(X, sugar) =0.58 at 20 g/L of glucose to w(X, sugar)=0.35 at 60 g/L of glucose. This suggests that the growth is less efficient at higher sugar concentrations, possibly due to increased maintenance requirements.

Initial sugar concentration also influences pellet size. Fang and Zhong determined the effect of initial glucose concentration on the pellet size distribution in 8-day-old cultures. Pellets of diameters smaller than 1.2 mm predominated at initial glucose concentrations of 50 and 65 g/L while pellets of diameters larger than 1.6 mm predominated at initial concentrations of 20 g/L (*16*).

Since larger pellets are correlated with higher ganoderic acid and lower IPS content, as mentioned above, and pellet size is affected by initial sugar concentration, the fermentation conditions can be manipulated in order to favor one product over another as a proportion of the biomass, as desired. However, the overall yield of the desired product depends not only on its content in the biomass, but also on the biomass yield obtained. For example, the ganoderic acid content of the mycelium was higher with an initial glucose concentration of 20 g/L than with an initial concentration of 50 g/L. However, the biomass concentration obtained at 50 g/L was sufficiently high to give a higher yield of ganoderic acid per volume of fermentation broth (*16*).

Agitation and aeration

The optimum agitation rate represents a balance between oxygen transfer into the medium and shear stress, both of which increase with increasing agitation rate. Yang and Liau, testing a range of shaking speeds from 50 to 250 rpm with Erlenmeyer flasks in an orbital shaker, obtained the highest biomass density at 100 rpm, but the highest EPS yield occurred at 150 rpm (*11*). They suggested that higher shaking speeds favor EPS production because they decrease the adsorption of the secreted extracellular polysaccharides on the cell wall, providing stimulus for further EPS synthesis. However, a mechanism by which this stimulus would occur was not proposed.

Although Yang and Liau used a constant rotation rate throughout the fermentation (*11*,*13*), the increased viscosity resulting from higher biomass concentrations and EPS levels may require an increase of the agitation speed. For example, in a stirred bioreactor fitted with two six-bladed turbine impellers, Tang and Zhong started at 100 rpm but increased the impeller speed to 250 rpm in one fermentation and to 180 rpm in another (*9*,*10*).

Agitation in bioreactors is mainly provided by turbines, although some workers have used air-lift fermenters (Table 1). In this case agitation is provided by the rising of bubbles, which should cause less shear stress on mycelium than a turbine does (*18*). A disadvantage of the air-lift fermenter is that oxygen transfer is not as efficient.

There are only two studies that aimed specifically to determine the effect of aeration on biomass and product formation by *G. lucidum*. Yang and Liau compared different sizes of Erlenmeyer flasks, with and without baffles, and used different aeration rates in a bioreactor (*11*). The dissolved oxygen concentration was not measured in these studies, so it was not possible to interpret the results quantitatively. In fact, in studies in which dissolved oxygen levels are not measured, it is not simple to evaluate the effects of aeration independently of those of agitation, since the efficiency of oxygen transfer depends not only on the design and operation of the aeration system itself, but also on the agitation method and agitation speed. Furthermore, the properties of the broth, which can change during the fermentation, also affect the efficiency of oxygen transfer. There may even be agitation effects in studies in which the dissolved oxygen level is controlled in a bioreactor, since this control is usually achieved, at least partially, through control of the agitation rate. Tang and Zhong did measure dissolved oxygen concentrations and reported that the production of EPS was higher at a dissolved oxygen tension (DOT) of 10 % than when the DOT was 25 % (*19*). The contents of IPS and GA in the biomass were also higher at the DOT of 10 % although the higher biomass concentration achieved at the DOT of 25 % meant that in absolute terms IPS and GA production were higher at this DOT. The result for IPS does not agree with the observation reported above that IPS contents are lower in larger pellets due to the relatively poor oxygenation (*12*,*16*,*17*).

Temperature

Fermentations with *G. lucidum* have been done at 25 to 35 °C, with most done at 30 °C (Table 1). The effect of temperature on growth and product formation has not been systematically studied. Both polysaccharide production and mycelial growth rate are favored at temperatures between 30 and 35 °C, being drastically reduced outside this range (*11*,*13*). No information is available regarding the effect of temperature on the production of ganoderic acid.

pH

Little information can be obtained about pH effects from studies done in Erlenmeyer flasks since the pH is not controlled. In this system it is only possible to study the influence of the initial pH on growth and metabolite production. In one such study, Fang and Zhong obtained similar pH profiles after the fourth day even though the initial value varied from 3.5 to 7.0: during the first four days the pH decreased to 3.2 and then remained constant for one week (*15*). After that, around days 10 to 14, when the glucose was almost exhausted, the pH increased rapidly to 7.0. When glucose was fed on day 8 in a quantity sufficient to increase its concentration by 5 to 10 g/L, the pH remained the same until day 14. The authors suggest that the relatively high glucose consumption may result in production of organic acids, which keep the pH low, although they did not do any analyses to support this (*15*). The same pattern of pH change has been observed in bioreactors without pH control (*10,20*).

Although in the studies of Fang and Zhong the pH profile was almost the same after day 4 irrespective of the initial pH, growth and metabolite production did depend on the initial value. Highest yields were obtained with an initial pH of 6.5 in the case of biomass, 5.5–6.5 in the case of ganoderic acid, 5.5–7.0 in the case of IPS and 3.5–4.5 in the case of EPS (*15*). Fang and Zhong used a medium composed of glucose plus peptone and yeast extract. In other studies, the optimum initial pH for biomass formation depended on the major nitrogen source used, being 4.0 in the case of ammonium chloride and 5.0 in the case of malt extract (*13*).

Quite different results were obtained when the pH was controlled in a bioreactor. Whereas the highest biomass yield was obtained by Fang and Zhong with an initial pH of 6.5, in the case of a fermentation in which the pH was controlled, pH=3.0 gave higher biomass densities than pH=6.0 (*18*). With respect to EPS production, control at pH=6.0 gave a higher yield than control at pH=3.0 (*18*) whereas in the initial pH studies of Fang and Zhong the lowest initial pH tested of 3.5 gave a higher yield than did initial pH values of 5.5 and above (*15*). Lee *et al.* suggested that the better EPS production when the pH was controlled at 6.0 was because pellet growth was maintained (*18*). In a cultivation initiated at pH=6.0 but without pH control, the pH fell to 2.6 and the morphology changed from pellet to filamentous form in the latter stages of the cultivation (*18*), leading the authors to suggest that pH influences the morphology, which in turn affects EPS production. However, it is not necessarily the case that the domination of the filamentous form is responsible for the lower EPS production. Both effects could be independent consequences of the decrease in pH from 6.0 to 2.6. In any case, the results of Lee *et al.* for EPS production may be questionable since the method of polysaccharide quantification adopted is doubtful. This matter will be further discussed below.

Effect of inoculum density

The inoculum density influences the fermentation profile. Inoculum densities of ρ(X)=70–670 mg/L gave a lag phase of 1 day whereas the lag phase was 4 days when the inoculum density was only ρ(X)=5 mg/L (*17*). The final biomass density was also affected, although not markedly: it increased from ρ(X)=13.6 g/L at an inoculum density of ρ(X)=70 mg/L to ρ(X)=15.7 g/L at an inoculum density of ρ(X)=330 mg/L, decreasing again to ρ(X)=14.2 g/L at an inoculum density of ρ(X)=670 mg/L. Further, inoculum density affects pellet size distribution. With an inoculum density of ρ(X)=670 mg/L, 68.3 % of the pellets had a diameter smaller than 1.2 mm at day 8, while with an inoculum density of ρ(X)=70 mg/L, 91.0 % of the pellets had a diameter larger than 1.6 mm at this time (*17*).

The effect of the inoculum density on pellet size distribution has consequences for product formation, given that the cell content of IPS is higher in small pellets while the cell content of ganoderic acid is higher in large ones. As expected, Fang *et al.* did obtain higher contents of ganoderic acid in the biomass when the inoculum density was ρ(X)=70 mg/L, because it was this inoculum density that led to a higher proportion of large pellets (*17*). However, despite the higher content of ganoderic acid in the biomass, the overall ganoderic acid yield was greater with an inoculum size of ρ(X) =170 mg/L due to the higher biomass density obtained.

Effect of plant oils and fatty acids

Foam formation is an undesired feature in many submerged fermentation processes. It causes an increase in the working volume, interferes with monitoring and control of fermentation conditions and makes product recovery more difficult. Antifoam agents are sometimes required in the large-scale cultivation of *G. lucidum* (*14*).

Several plant oils, which can be used as antifoam agents, may also be beneficial to the growth of *G. lucidum*. The effects of soy, peanut, safflower, corn, sunflower and olive oils were investigated in submerged fermentation, all at volume fraction of 1 %, presumably. All oils tested stimulated growth, with the highest biomass density being obtained with olive oil (*14*). Yang *et al.* proposed that such stimulation is due to a partial incorporation of lipids in the cell membrane, thereby facilitating the uptake of nutrients from the medium, although they did not suggest a mechanism by which this might occur *(14)*. On the other hand, EPS production was highest with safflower oil, slightly inhibited with soy oil but was not significantly affected by the other oils tested, when compared to a fermentation with the same medium but with no oil added (*14*).

Other than these studies, which were aimed at testing the stimulatory effects of oils on cell growth, there are only two reports about the use of components that act as antifoaming agents. Lee *et al.* used a commercial brand of antifoam agent at volume fraction of 0.5 %, presumably (*18*), while Habijanic *et al.* used olive oil at volume fraction of 2 % (*20*). The advantages of plant oils are their low cost and the possibility of higher growth yields due to the stimulatory properties of the oil. However, the composition of plant oils can differ depending on the plant origin and crop, with small differences in fatty acid composition potentially causing significant

differences in growth of the biomass during the fermentation.

The effects of various fatty acids on biomass and polysaccharide formation in suspension cultures were also evaluated at concentrations ranging from 0.05 to 0.25 % (*14*). No general relationship could be identified between either the length of the carbon chain or the extent of its unsaturation and the level of growth stimulation obtained. Oleic acid resulted in the highest biomass density, followed by palmitic and stearic acids. Linoleic acid had a strong inhibitory effect, which Yang *et al*. found surprising, pointing out that linoleic acid is the main fatty acid in safflower oil, which stimulated both growth and EPS production (*14*). They suggested that other components of the oil might be acting positively, masking the inhibitory effect of linoleic acid. However, two types of safflower oil exist, one that is rich in oleic acid and one that is rich in linoleic acid (*21*) and Yang *et al*. did not specify which type they used.

The production of EPS in suspension cultures was stimulated mostly by the addition of palmitic acid, followed by oleic acid and stearic acid at a concentration below 0.1 % (*14*). Linoleic acid inhibited EPS production, as did stearic acid at concentrations above 0.1 %. The response to fatty acids may differ according to the morphological state of the fungus. When mycelium was grown on a polyurethane foam sheet immersed in the medium, palmitic acid stimulated polysaccharide formation but oleic acid and stearic acid did not cause any significant change at the concentrations tested, which contrasts with the stimulation they had caused in suspension culture (*14*).

Kinetics of Growth and Product Formation

In the majority of the studies in which fermentation profiles are presented, the concentrations of biomass, polysaccharide and ganoderic acid increase approximately linearly for most of the growth or production phase. The linear growth rate and the linear production rates of EPS, IPS and GA were calculated by linear regression of the linear regions of the profiles presented by the authors. It was not possible to analyze the profiles of all the fermentations presented in Table 1 because many either did not present graphs or, if they did, had too few points before the stationary phase. The linear growth rate ranged from $\rho(X)=0.3–2.8$ g/(L.d), whereas the linear production rate varied from $\rho(EPS)=0.02–0.6$ g/(L.d) for EPS, $\rho(IPS)=0.05–0.25$ g/(L.d) for IPS and $\rho(GA)=11–56$ mg/(L.d) for GA. Lee *et al*. achieved an EPS production rate of $\rho(EPS)=4.2$ g/(L.d), however the reliability of this value is questionable due to the rudimentary methodology they used for polysaccharide analysis (*18*).

Fermentation Strategies

Five main strategies have been used for submerged fermentation of *G. lucidum*. The ideas for these strategies arose from the behavior of *Ganoderma* under different environmental conditions. All of the fermentation techniques described below were carried out at laboratory scale and the knowledge generated from each of them will contribute to the scaling-up of the process.

Batch fermentation

Batch fermentation is the simplest technique reported. Most of the studies have been done in Erlenmeyer flasks (*9–17*) although some have been done in bioreactors (*9,11,18–20,22*).

Fed-batch fermentation

The strategy of fed-batch fermentation is to add one or more of the nutrients during the fermentation, based on the possibility that the high concentrations required for high final growth and product yields might inhibit growth if added in total at the start of the fermentation. Potentially, growth and product formation can be extended for long periods compared to a normal batch fermentation.

Tang and Zhong investigated fed-batch fermentation with *G. lucidum*, motivated by the observation that lactose concentrations above 35 g/L had diminished the production of ganoderic acid (*9*). The fermentation was started at 35 g/L. The lactose level was monitored off-line, and when it fell to between 5 and 10 g/L, sufficient lactose was added to increase its concentration by 15 g/L, in a volume equal to 10 % of the working volume of the bioreactor (*9,10*). The production of biomass, polysaccharides and ganoderic acid were improved. The final yields of GA, EPS and IPS were 11, 43 and 48 % higher, respectively, in fed-batch than in batch fermentation (*9*). Therefore lactose feeding stimulates the production of polysaccharides more than it stimulates the production of ganoderic acid.

In Erlenmeyer flasks and with glucose as the carbon source, the production of EPS was 124 % higher for a fermentation in which glucose was added after 8 days of fermentation. At this time, the original glucose had fallen to below 2 g/L and the addition increased its concentration by 10 g/L (*15*). No data was provided concerning ganoderic acid and IPS production in this case.

Bistage control of pH

Based on the observations that while a constant pH of 3.0 improved growth, a constant pH of 6.0 favored exopolysaccharide production, a fermentation with bistage pH control was proposed, with the pH commencing at 3.0 and being changed to 6.0 after 2 days (*18*). The production of EPS was the highest yet reported in the literature (20.04 g/L on day 6). However, given that the polysaccharides were dried by heat and not freeze-dried as in the other works and also that they were quantified by a gravimetric method, the results must be considered preliminary and must be confirmed by more accurate and specific methods.

Two-stage culture processes

The fact that ganoderic acid production was favored at a low oxygen tension led Fang and Zhong to undertake a two-stage process in Erlenmeyer flasks (*12*). The first stage was realized with agitation in a rotary shaker. After 4, 8 or 12 days the agitation was stopped and the culture then remained static until the 24th day. A con-

trol culture was shaken for the whole time. In static culture glucose was consumed at a slower rate and converted to biomass more efficiently. The highest biomass density was obtained with 4 days of agitation followed by 12 days of static culture. Unfortunately, no fermentation was left static from the time of inoculation. Results from such an experiment would contribute to a better understanding of the effects of agitation on the growth of *G. lucidum*.

The highest production of ganoderic acid that has yet been reported (582 mg/L at day 12) was obtained in the culture that was agitated for only the first 4 days. The production of ganoderic acid was almost double that in the continuously-shaken control culture. A thick layer of mycelium was noted in this culture and, although the authors did not say whether it was at the surface of the medium or submerged, its large thickness would have restricted oxygen diffusion into the layer, and low oxygen availability appears to stimulate GA production. No thick mycelial layer was found for the culture agitated during the first 12 days and then left static for further 12 days. In this case the production of GA did not increase significantly compared to a shaking culture without any static stage.

Immobilized culture

Yang *et al*. introduced a polyurethane foam sheet into the medium of a submerged fermentation in an Erlenmeyer flask (*14*). The mycelium adhered to the surface of the foam matrix with almost no mycelia remaining free in the bulk liquid. The biomass density and the amount of EPS obtained were both markedly higher in this culture than in freely suspended cultures, indicating that the mycelial morphology adopted on a solid support was more favorable for both cell growth and polysaccharide formation. The polysaccharide secretion occurred at a slow rate and after 2–3 weeks of fermentation a large portion of polysaccharide was adsorbed on the support. As suggested by the authors, this may enable an alternative strategy for product and biomass recovery in which the support can simply be removed and pressed. There is no report on ganoderic acid production in immobilized cultures.

Solid state fermentation

Very little information about the production of *Ganoderma lucidum* mycelium in solid state fermentation (SSF) is available. Most of the studies have focused on basidiocarp formation with less interest shown in obtaining mycelial biomass and its products. The only report available was carried out in a horizontal stirred tank reactor with a total working volume of 30 L (*23*). The conditions were controlled as follows: a temperature of 30 °C, an airflow of 2 L/min and an agitation rate of 80 rpm for 2 min every second day during the first 7 days, and every day during the latter stages of the cultivation.

The effect of initial moisture content was evaluated in this study. At least $w(H_2O, \text{d.m.})=0.74$ was necessary to give satisfactory rates of cell growth and exopolysaccharide production. This water content corresponded to a water activity of 0.85.

The advantage of SSF over other techniques is that a concentrated product can be obtained from a cheap substrate, such as an agricultural residue with little pretreatment or enrichment. The substrate used in this case was composed of beech sawdust, olive oil, $(NH_4)_2SO_4$, KH_2PO_4, $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, $FeSO_4{\cdot}7H_2O$ and distilled water. On the other hand, the use of an undefined medium, such as sawdust, might make the product purification process more difficult. For this reason this cultivation technique would be most appropriate for the production of immunostimulatory animal feed supplements, because in this case the whole fermented substrate is used as the product.

The content of exopolysaccharides in the solids increased rapidly during the first 7 days, remained relatively constant until 21 days and then decreased, suggesting that the polysaccharide was actually degraded in the latter stages of the process. The period during which the polysaccharide content decreased corresponded with the period in which the water mass fraction was falling rapidly, from the values of 70–80 % that were maintained during the first 21 days, to 20 % at 35 days. However, it is not clear whether there is a direct cause-and-effect relationship between these two observations.

The mechanisms of the processes controlling exopolysaccharide production and consumption are not known, however, the authors suggested that exopolysaccharides serve to fasten the hyphae to the surface of the solid particle, and to protect the hyphae both from mechanical damage during agitation and from desiccation at low moisture contents (*23*).

Techniques of Product Recovery and Analysis

This section presents and discusses the main methodologies used with *Ganoderma lucidum* for the recovery and quantification of products and biomass.

Biomass

In submerged fermentation biomass is generally recovered by filtration under suction or centrifugation. When it is intended to quantify the biomass of a sample, it is generally filtered through a pre-weighed filter paper (GF/C Whatman) (*14*) or a membrane with a standardized pore size (*15,16,22*). Some authors filter the biomass, wash it off the filter with distilled water, recover it from the washings by centrifugation and then dry it until constant weight at temperatures ranging from 50 to 105 °C. No studies have been done about the effect of drying temperature on charring of the biomass or the loss of volatile cell components other than water.

The recovery of the biomass obtained by SSF is a difficult task because the mycelium binds tightly to the solid particles of the substrate. Therefore, it is normally impossible to determine the dry weight directly by the gravimetric method. Alternative methods for accompanying mycelium growth are based on indirect measurements of biomass components, although these are problematic because the level of the component in the biomass may vary during the growth cycle (*24*).

Polysaccharides

The filtered broth obtained from submerged fermentation contains water soluble exopolysaccharides that can be recovered by precipitation with 3–4 volumes of ethanol 95–96 %. The use of 2 volumes of acetone has also been reported (*18*). Some workers dialyze the filtered broth before adding it to the ethanol solution (*4,15,17*) to eliminate smaller molecules, such as oligo- and monosaccharides, which might interfere with the quantification of polysaccharides. Yang and Liau ultrasonicated the mycelium with medium for 2 hours, with the intention to liberate cell-bound polysaccharide (*11*). However, they did not show that this treatment did not disrupt the cell, so it is impossible to affirm that IPS and EPS were quantified individually in this case.

IPS is usually extracted with a solution of 1 M NaOH at 60 °C for 1 h. However, this is not the only fraction of IPS that can be obtained. Other solvents can be used for extraction, such as hot water, ammonium oxalate 1 % and NaOH 5 % (*20*).

In SSF the EPS fraction is not dissolved in a liquid phase. In order to recover it, the fermented substrate, which also contains mycelium, must be extracted with cold water. The extraction of polysaccharides in boiling water for 5 h was reported in SSF (*23*), however, such a procedure is likely to extract not only the IPS and EPS produced by the organism, but also some of the polysaccharides from the solid substrate itself.

The main method used to quantify polysaccharides is the phenol-sulfuric method (*25*). However, molecules other than polysaccharides, such as monosaccharides, oligosaccharides and proteins containing amino acids with phenolic groups, give false positive responses in this assay. The strategy of dialyzing the filtered broth before precipitating the polysaccharide fraction or washing the precipitate with ethanol eliminates smaller molecules. Proteins, however, will remain together with the polysaccharides and will contribute to the absorbance measured in the assay. Therefore, it is very important to determine the protein content by a specific method in order to assess the accuracy of the phenol-sulfuric assay. Most of the reports do not mention the protein content of the recovered polysaccharide fraction. In fact, many workers do not acknowledge that precipitation with ethanol or acetone does not give a pure polysaccharide fraction. Often, the dried precipitate is weighed and reported as polysaccharide, with the implicit assumption that it is free of contaminants.

Particular care should be taken when an undefined medium is used. Components such as yeast extract and peptone, which are often used in liquid culture, and solid residues used in SSF may add non-polysaccharide components that not only might be extracted together with the polysaccharide produced by the organism but also might be counted as polysaccharide by the analytical method used.

Ganoderic acid

Ganoderic acid extraction and determination is a multistep process. First the dried mycelium is extracted twice with ethanol 50 %, each extraction lasting for one week. The supernatant is then dried and the residue suspended in water. The aqueous solution is then extracted with chloroform and a solution of $NaHCO_3$ 5 % is then added to this organic phase. The aqueous phase is then collected and acidified with HCl to a pH less than 3. The ganoderic acid may be extracted again with fresh chloroform. The chloroform fraction is then evaporated. The resulting residue is suspended in absolute ethanol and the ganoderic acid is measured at 245 nm (*9,12,15–17,19*). The authors do not provide proof that the fraction obtained contains only ganoderic acids. They only say that the GA present in mycelia of *G. lucidum* are a complicated mixture and usually have α,β-unsaturated carbonyl groups, whose absorbance is maximal between λ=230–260 nm (*19*). Other organic acids may be extracted and purified together with the GA, although they may not necessarily absorb significantly in this wavelength range.

Future Perspectives

There is still little information available concerning the use of advanced fermentation techniques for obtaining *Ganoderma lucidum* mycelium and its valuable components. A better understanding of its morphology and physiology is crucial for the development of large scale fermentation processes. All of the work has focused on describing the effects of some alteration in the medium or environmental conditions. No studies have been carried out in a way that might allow an explanation of the cause of the effects observed. This represents a large field for future research which must be addressed if optimal fermentation strategies are to be developed in a rational manner.

Acknowledgements

Ricardo Wagner, Guilherme Sassaki and David Mitchell thank the Brazilian National Council for Scientific and Technological Development (CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico) for research scholarships.

References

1. P. Stamets: *Growing Gourmet & Medicinal Mushrooms*, Ten Speed Press, Berkeley (1993).
2. S. C. John, J. M. Birmingham, *Adv. Appl. Microbiol. 37* (1992) 101–134.
3. T. Mizuno, G. Y. Wang, J. Zhang, H. Kawagishi, T. Nishitoba, J. X. Li, *Food Rev. Int. 11* (1995) 151–166.
4. Y. Sone, R. Okuda, N. Wada, E. Kishida, A. Misaki, *Agric. Biol. Chem. 49* (1985) 2641–2653.
5. C. Y. Su, M. S. Shiao, C. T. Wang, *Thromb. Res. 99* (2000) 135–145.
6. Y. Mizushina, N. Takahashi, L. Hanashima, H. Koshino, Y. Esumi, J. Uzawa, F. Sugawara, K. Sakaguchi, *Bioorg. Med. Chem. 7* (1999) 2047–2052.
7. T. Mizuno, *Int. J. Med. Mushrooms, 1* (1999) 9–29.
8. F. Mayzumi, H. Okamoto, T. Mizuno, *Food Rev. Int. 13* (1997) 365–382.
9. Y. J. Tang, J. J. Zhong, *Enzyme Microb. Technol. 31* (2002) 20–28.
10. Y. J. Tang, J. J. Zhong, *Biotechnol. Lett. 24* (2002) 1023–1026.
11. F. C. Yang, C. B. Liau, *Process Biochem. 33* (1998) 547–553.

12. Q. H. Fang, J. J. Zhong, *Biotechnol. Progr. 18* (2002) 51–54.
13. F. C. Yang, C. B. Liau, *Bioprocess Eng. 19* (1998) 233–236.
14. F. C. Yang, Y. F. Ke, S. S. Kuo, *Enzyme Microb. Tech. 27* (2000) 295–301.
15. Q. H. Fang, J. J. Zhong, *Process Biochem. 37* (2002) 769–774.
16. Q. H. Fang, J. J. Zhong, *Biochem. Eng. J. 10* (2002) 61–65.
17. Q. H. Fang, Y. J. Tang, J. J. Zhong, *Process Biochem. 37* (2002) 1375–1379.
18. K. M. Lee, S. Y. Lee, H. Y. Lee, *J. Biosci. Bioeng. 88* (1999) 646–650.
19. Y. J. Tang, J. J. Zhong, *Enzyme Microb. Technol. 32* (2003) 478–484.
20. J. Habijanic, M. Berovic, B. Wraber, D. Hodzar, B. Boh, *Food Technol. Biotechnol. 39* (2001) 327–331.
21. D. M. Ney, J. B. Lasekan, J. Kim, *J. Nutr. 119* (1989) 857–863.
22. S. W. Kim, H. J. Hwang, J. P. Park, Y. J. Cho, C. H. Song, J. W. Yun, *Lett. Appl. Microbiol. 34* (2002) 56–61.
23. J. Habijanic, M. Berovic, *Food Technol. Biotechnol. 38* (2002) 225–228.
24. D. A. Mitchell: Biomass Determination in Solid State Cultivation. In: *Solid Substrate Cultivation*, H. W. Doelle, D. A. Mitchell, C. E. Rolz (Eds.), Elsevier Applied Science, London (1992) pp. 53–63.
25. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith, *Anal. Chem. 28* (1956) 350–356.
26. J. Zhang, G. Wang, H. Li, C. Zhuang, T. Mizuno, H. Ito, H. Mayuzumi, H. Okamoto, J. Li, *Biosci. Biotechnol. Biochem. 58* (1994) 1202–1205.

Uzgoj *Ganoderma lucidum* za proizvodnju biomase, ganoderske kiseline i polisaharida

Sažetak

Ganoderma lucidum je gljiva koja se u orijentalnoj medicini koristi više od 2000 godina. Zbog dugog vremena potrebnog da bi se oblikovao bazidiokarp, novija su istraživanja usmjerena na submerznu fermentaciju kako bi se proizvela micelijska biomasa i njezine bioaktivne komponente, tj. polisaharidi i ganoderska kiselina. U ovom su radu prikazani najnoviji podaci o uzgoju *G. lucidum* suvremenim fermentacijskim postupcima. Osobito su praćeni uvjeti fermentacije čije poznavanje omogućuje veću proizvodnju biomase, polisaharida i ganoderske kiseline. Opisani su i postupci izdvajanja biomase i produkta te je upozoreno na moguće probleme u procesu rada. Dosadašnja istraživanja bila su obavljana samo u laboratoriju, a za industrijski proces potrebno je više spoznaja o fiziologiji *G. lucidum* te njezinoj povezanosti s morfologijom rasta.