Production and Purification of Statins from *Pleurotus ostreatus* (Basidiomycetes) Strains

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Pleurotus ostreatus strains were cultured in liquid medium and on wheat straw. The yields of lovastatin were compared.

Key words: Pleurotus ostreatus, Statins, Lovastatin

Introduction

As part of a larger study of chilean mushrooms, we have found in the fungi *Pleurotus ostreatus,* Pleuroteaceae (Basidiomycetes), an important cholesterol reducing molecule. Here we report the presence of statins in fruiting bodies and fermentation processes from *Pleurotus ostreatus* collected from native forest of the VIII Region of Chile. The genus *Pleurotus* is represented in Chile by two species *P. ostreatus* and *P. sutherlandii*, both found growing attached to the cortex of endemic *Nothofagus* trees (France *et al.*, 2000). Lovastatin has been extracted from *Pleurotus pulmonarus* (Basidiomycete) (Gunde-Cimerman and Cimerman, 1995), and Kurashige *et al.* (1997) has proven anticancerigenous properties on the same molecule.

Statins are found to be an inhibitor of the enzyme hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase that catalyzes the reduction of HMG-CoA to mevalonate during synthesis of cholesterol (Endo, 1992; Bobek *et al.*, 1997). All natural statins have a common molecular structure, a hexahydro-naphthalene system and a β-hydroxylactone, but they differ from each other due to side chains and a methyl group around the ring (Fig. 1).

Endo *et al.* (1976) described a process for the production and purification of mevastatin from *Penicillium citrinum.* After this, lovastatin was obtained from cultures of *Monascum ruber* (Manzoni *et al.*, 1999) and in 1980 an industrial process for commercial production was set up using *Aspergillus terreus* which yielded nearly 180 mg lovastatin/l (Buckland *et al.*, 1989).

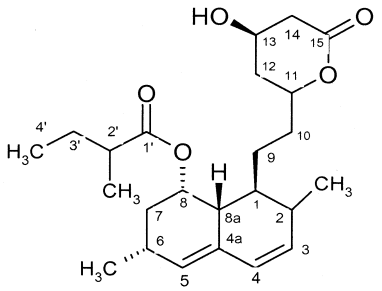

Fig. 1. Lovastatin molecular structure.

Futhermore, the fact that lovastatin is present in high proportion in this edible kind of mushroom, makes this fungi an important food supplement for patients suffering from hipercholesterolemia. Therefore, we report our findings obtained from the screening for statins of several strains of *Pleurotus ostreatus* in their natural environment and also *Pleurotus ostreatus* grown on a variety of natural subtrates inside a greenhouse. Lovastatin and its precursors were isolated and purified from strain cultures that produce higher statins concentrations.

Material and Methods

Organism collection

Fruiting bodies of *Pleurotus ostreatus* were collected at the forest from VIII Región of Chile, growing on *Nothofagus* sp. in autumn and spring of 2001. Mycelial cultures of the strain were derived from the spore print of fruiting body. A voucher specimen of the mushroom was deposited in the herbarium of Instituto de Investigación Agropecuaria, INIA-Quilamapu, Chillán, Chile.

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Fungal strain and culture conditions

Four strains of *Pleurotus ostreatus* were kept on potato dextrose agar (PDA), and incubated for 7–10 days, stored at room temperature. Fermentation was carried out in Hagen medium containing (g/l): $CaCl_2$ 0.05, KH_2PO_4 0.025, $(NH_4)_2HPO4$ 0.25, $MgSO_4$ x $7H_2O$ 0.15, 1.3 ml of $FeCl_3$ 1%, malt extract 3.0 and glucose 10.0; in a 3 liter fermentor with aeration and agitation (150 rpm); 250 ml of well-grown culture (7 days) in the same medium was used as inoculum. The fermentation was stopped after 10 days.

Fruiting bodies production

Mycelia cultures from the greenhouse were developed according to the methodology of France *et al.* (2000) on wheat straw. Fruiting bodies were collected after three months and were processed for extraction using MeOH until exhaution. The methanolic extract was concentrated and further extraction was done using AcOEt. Each of the extracts were analysed looking for lovastatin concentrations (Table I).

Statins isolation

Culture filtrate (3 l) obtained by filtration was acidified to pH 3 with HCl 0.01 M and extracted with ethylacetate (4 × 500 ml). The combined extracts were dried (Na_2SO_4) and concentrated to a final volume of 5 ml.

The mycelial mass was washed with 0.05 M HCl and stirred at room temperature for 1 h, then filtered and after acidification extracted with methylene chloride (3 × 500 ml) and ethylacetate (2 × 400 ml) for 1 h at 40° under stirring. The extract was dried (Na_2SO_4) and concentrated to a final volume of 5 ml.

The structure of lovastatin was established on the basis of spectroscopic studies, GC-MS and their spectral data was compared to data from literature or from authentic sample (Table II).

Analytical determinations

The identification and quantification of statins were carried out on the culture filtrated and extracts by HPLC, using a Merck LiChrospher 100 RP18 reverse phase column with a diode array detector eluted at a flow rate 2 ml/min and elution with gradient 90:10 water:methanol (v/v).

Results and Discusion

The present study allowed to make a comparison between the content of lovastatin present in the fruiting bodies of *P. ostreatus* cultivated in conservatory on straw of wheat and collected fruiting bodies in the forests of VIII the Region of Chile. The results show that the produced fruiting bodies in conservatories, present a lovastatin level that fluctuates between 0.40 to 2.07% measured in dry fungus. However, the collected fruiting bodies in their natural environment, present levels of lovastatin between 0.7 to 2.8% measured in dry fungus.

The mycelial development in liquid cultures gave a range for lovastatin concentration between 5 and 70 mg/l, depending of the seta type analyzed. It is important to know that the statins are present under two types of structural forms, depending on pH. One of them is the β-hydroxyacid form being in alkaline solution, while the hydroxy acid and β-hydroxylactone form being in equilibrium in acid condition. Our GC-MS and HPLC laboratory analysis showed clearly this equilibrium, which contributes to doublefold the lovastatin concentration.

Table I. Lovastatin production.

Pleurotus ostreatus strain	Lovastatin concentration in fruiting body (%)	Lovastatin concentration in liquid culture [mg/l]
	Wheat straw	
Strain PL-124	0.79	10
Strain PL-127	0.60	5
Strain PL-136	2.07	70
Strain PL-143	0.40	30
	Natural environment	
Strain PLUBB-001	2.80	–
Strain PLUBB-002	0.70	–
Strain PLUBB-003	2.50	–

	NMR		MS
Positions	$^{13}C^a$	$^{1}H^a$	
1	36.7	1.5–1.75 (m, H)	404 [M^+]
2	30.7	2.3–2.4 (m)	302 (2)[$M^+-C_5H_{10}O_2$]
2-Me	13.9	0.91 (3H, d, J = 7)	284 (4) [$M^+-C_5H_{10}O_2-H_2O$]
3	133.1	5.78 (1H, dd, J = 10)	269 (3)[$C_{18}H_{21}O_2$]
4	128.4	6.00 (1H, d, J = 10)	251 (3) [$C_{18}H_{19}O$]
4a	131.6	–	224 (12) [$C_{17}H_{20}$)
5	129.6	5.54 (1H, m)	198 (59) [$C_{15}H_{18}$]
6	27.7	2.45 (1H, m)	159 (100) [$C_{12}H_{15}$]
6-Me	22.9	1.08 (3H, d, J = 8)	
7	32.8	1.8–2.0 (m)	
8	68.0	5.40 (1H, q, J = 3)	
8a	37.4	2,26 (1H, dd, J = 12,3)	
9	24.3	1.35–1.40 (m)	
10	33.0	1.30 (1H,m, H_a)	
		1.8–2.0 (m, H_b)	
11	75.7	4.62 (1H, m)	
12	36.2	1.5–1.75 (m, H_a)	
		1.8–2.0 (m, H_b)	
13	62.6	4.38 (1H, m, J = 4)	
14	38.7	2.62 (1H, m, J = 18,4,2, H_a)	
		2.74 (1H, dd, J = 18, H_b)	
15	170.7	–	
1′	176.9	–	
2′	41.6	2.3–2.4 (m)	
2′-Me	16.3	1.12 (3H, d, J = 8)	
3′	26.8	1.35–1.40 (m, H_a)	
		1.5 – 1.75 (m, H_b)	
4′	11.7	0.89 (3H, t, J = 8)	
OH		2.16 (1H, brs)	

These preliminary results found in poor nutrient culture conditions, suggest to us that it is possible to increase the yield of statins produced by *P. ostreatus* in liquid media. The latter can be done modifying the nutritive quality of the media used.

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