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Process for the Production and Purification of Cytochalasin B from *Phoma exigua* var. *heteromorpha*†

ANTONIO BOTTALICO,*[,1] RENATO CAPASSO,[2] ANTONIO EVIDENTE,[2] AND MAURIZIO VURRO[3]

[1]*Istituto di Patologia vegetale, Università degli Studi, Sassari;* [2]*Dipartimento di Scienze chimico-agrarie, Università di Napoli "Federico II," Portici; and* [3]*Istituto Tossine e Micotossine da Parassiti Vegetali, CNR, Bari, Italy*

ABSTRACT

A patented process to produce and purify large amounts of cytochalasin B is described. The fungal microorganism, *Phoma exigua* var. *heteromorpha*, is grown on a solid medium, constituted by autoclaved wheat kernels. The fungal culture, obtained growing the fungus in optimized environmental conditions, is extracted by organic solvent, to obtain crystalline cytochalasin B, in more than 3 g/kg of dried wheat culture.

The advantages of this process are: production higher than that obtained with other known processes; lower production expenses; lower quantities of solvents; exclusion of chromatographic purifications.

Moreover, the production of large amounts of cytochalasin B allow to prepare a wide number of derivatives, with different biological activities.

Index Entries: Cytochalasin B; cytochalasins; toxins; biological activity; patented process.

*Author to whom all correspondence and reprint requests should be addressed.

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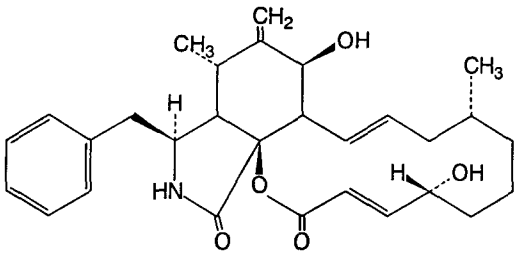

Fig. 1. Chemical structure of cytochalasin B.

INTRODUCTION

Cytochalasins are a large group of fungal metabolites produced by several species of fungi, having primarily cytotoxic effects on mammalian cells (*1*), as well as other biological activities. Up to now, more than 50 different cytochalasins have been purified and identified. Investigations on the biological activity of cytochalasins, mainly referred to cytochalasin B (*see* Fig. 1), indicated that they showed toxicity both in vitro and in vivo toward animal cells and animals, as well as on microorganisms such as bacteria, algae, fungi, and protozoa (*2,3*). Moreover, some cytochalasins were also observed to have phytotoxic activities (*4–6*).

Investigations on the cytochalasins produced by *Ascochyta heteromorpha* (later reclassified as *Phoma exigua* var. *heteromorpha*), a foliar pathogen of Oleander, led to the purification of cytochalasins A and B from culture filtrates (*7*). In considering the chemical and biological interest of these compounds, further studies were carried out, using more suitable environmental and nutritional conditions, to identify other metabolites and to obtain higher production of toxins (*8–11*). This article describe a patented method to obtain pure and crystalline cytochalasin B in more than 3 g/kg of dried culture.

EXPERIMENTAL

Fungus

Phoma exigua var. *heteromorpha* (Schulzer *et* Sacc.) Noordeloos *et* Boerema, first classified as *Ascochyta heteromorpha* (Schulzer *et* Sacc.) Curzi, was isolated from necrotic spots of Oleander (*Nerium oleander* L). leaves, and deposited in the fungal collection of Centraalbureau voor Schimmelcultures, Baarn, The Netherlands (CBS 548.90). The fungus was grown on autoclaved wheat kernels at 25°C, for 24 d.

Extraction and Isolation of Cytochalasin B

Wheat cultures were dried and finely minced. The dried material was extracted by stirring at room temperature with CH_2Cl_2 (three times × 24 h each). The organic extracts were combined and evaporated under reduced pressure, affording a brown-red oil residue. The extract was resuspended in a methanol-H_2O (1% NaCl) mixture (55:45, v:v), defatted by *n*-hexane and then extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined and dried under reduced pressure, yielding a solid suspended in abundant brown oil. The oil was washed with several small amounts of CH_2Cl_2 (5 × 10 mL) yielding a solid material, that was crystallized by ethyl acetate-*n*-hexane (2:8, v:v) yielding white needles of cytochalasin B (2.02 g/kg of dried culture). The residue obtained by evaporation of the mother liquors was crystallized as previously described, yielding a further amount of cytochalasin B (1.13 g/kg of dried culture). The total amount of cytochalasin B obtained with this process is 3.15 g/kg of dried culture.

Identification of Cytochalasin B

The melting point (218–220°C) of the crystalline compound, its elementar analysis, and its UV, IR, MS, $^1H-$, and ^{13}C-NMR spectra were identical to those of an authentic sample of cytochalasin B, and were consistent with the data reported in the literature (*12–14*).

RESULTS AND DISCUSSION

This process allows to obtain a large amount (more than 3 g/kg of culture) of cytochalasin B, which is considerably higher than that obtained by other known processes. For example, Aldridge et al. (*12*) obtained 50 mg of cytochalasin B per liter of medium, growing *Helminthosporium dematioideum* in liquid culture. One more advantage of this process is that wheat kernels as medium are very easy to prepare and not expensive. Moreover, it is possible to purify and crystallize cytochalasin B using small amounts of solvents, and without the use of chromatographic methods. These make the purification easier, quicker, and cheaper compared to the other known processes. Preliminary studies (*15,16*) showed that essentially the size and the conformational freedom of the macrocyclic ring of the cytochalasin molecule, as well as the kind of groups bonded to the ring, can greatly influence the toxic properties of these compounds. The availability of large amounts of cytochalasin B at low costs will also allow to prepare several derivatives, with a wide range of biological activities.

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