

Original Article

Effects of Carbon Sources on Growth and Production of Antifungal Agents by *Gymnopilus Spectabilis*

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Abstract

Effects of different carbon sources on growth and production of antifungal agent(s) by *Gymnopilus spectabilis* were investigated. Different carbon sources including; Glucose, fructose, lactose, maltose, manitol and sucrose at the concentration of 10 g/l and slow releasing carbon sources including malt extract and soluble starch at the concentration of 2g/l were used. Inhibition of spore germination of *Aspergillus niger* was used to test antifungal activity. The results showed that the highest activity (90% inhibition of spore germination) and biomass concentration (2.4 g/l) were obtained when glucose was used as carbon source. Other media with different carbon sources showed antifungal activity ranging from 30% to 70% inhibition of spore germination. Combination of glucose with malt extract as a slow releasing carbon source was also increased activity to 100% inhibition.

Keywords: *Gymnopilus spectabilis*; Antifungal; Basidiomycetes and Carbon sources.

Introduction

Mushrooms are considered as popular and effective folk medicines which have been used for diseases, such as hepatitis, hypertension and hypercholesterolemia biochemical potential and their adaptation to extreme life conditions in liquid media have been exploited to produce useful substances such as antibacterial, antifungal and enzymes (1).

With each newly observation of antimicrobial activity or other activity by microorganisms, the problem of concentration arises at once. The problem is, of course, well accepted for biologically active compounds that have been commercial interest, but the problem exists at the early stage of discovery (2).

Therefore carbon source as a component of culture medium plays an important role

in growth and in the production of bioactive compounds by basidiomycetes and other fungi. Various compounds can be used as a source of carbon and energy by fungi. Carbon compounds range from simple small molecules like sugars, organic acids and alcohols to like proteins, polysaccharides and lipids (3). Also a number of waste materials have been considered for this purpose, for example a waste product obtained in Brazil from the distillation of fermented cane juice (4). Basidiomycetes utilize a wide range of carbon sources for growth and production of active compounds. The sources include simple sugars such as glucose (5, 6 and 7) sucrose (8), maltose (7), lactose (9) and complex sugar such as molasses (8).

Gymnopilus spectabilis is a large and colorful wood rotting species that occurs in small groups at the base of dead broad-leaf trees and very occasionally conifers from spring through the early winter. The study on the chemical composition of this fungi showed

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that many species of *Gymnopilus* contain psilosibin, which is the major psychoactive alkaloid. Different biological activities have been reported due to presence of some lead compounds like; tryptamines and psilocibin. These mushrooms represent a growing problem regarding hallucinogenic drug abuse (10 and 11). A cytotoxic fatty acid was also isolated from *Gymnopilus spectabilis* (12).

However, no study was found on the antifungal activity and the effects of carbon sources on growth and for the production of antifungal agents via fermentation process. Hence the present investigation evaluated the effects of different carbon sources for biomass and antifungal agent(s) production by *G. spectabilis*.

Materials and Methods

Organism

G. spectabilis was collected from the north of Iran and identified kindly by Pesticide and Plant Research of Iran (PPDRI) and a voucher specimen (B 428) deposited at the department of Pharmacognosy, Shaheed Beheshti Medical University. Tissue culture was carried out as previously explained by Stamets *et al* (13).

The stock culture was maintained on malt extract agar slants. Slants were incubated at 25°C for 10 days and stored at 4°C. The seed culture was grown in a 250 ml flask containing 0.3% (w/v) yeast extract, 0.3% (w/v) malt extract, 0.5% (w/v) mycological peptone and 1% (w/v) glucose.

The flask culture experiments were performed in a 250 ml flask containing 100 ml of medium inoculated with 5% V/v of the seed culture.

Inoculum preparation

G. spectabilis was initially grown on malt extract agar medium in a Petri dish and then transferred into the seed medium by punching out 5 mm of the agar plate culture with self designed cutter.

Medium preparation

Basal medium containing yeast extract 0.3% (w/v), mycological peptone 0.1% (w/v) KH_2PO_4

0.2% (w/v) and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2% (w/v) was used. Different carbon sources including; 1% (w/v) glucose, fructose, maltose, lactose, sucrose, manitol and xylose and slow releasing carbon sources including soluble starch and malt extract 0.2% (w/v) were also applied.

Extraction

Each culture growth medium was extracted by using ethyl acetate. The extracts were concentrated to dryness under reduced pressure. The obtained products were weighed and stored at 4°C prior to use.

Antifungal test

This assay was used to examine the effect of fungal extract on spore germination and germ tube development of the test organism on liquid culture. *Aspergillus niger* was set – up from the sub master culture. The spore were washed off with sterilized water containing 0.2% tween 80, and the concentration was adjusted to 1×10^6 spore/ml. The assay was carried out using 96 well plates containing growth medium, culture extract and the respective culture broths and spores of the test organisms. 150 μl of fungal extract (at the concentration of 10 mg/ml) were added to 130 μl of fresh culture medium. Each well was inoculated with 20 μl of spore suspension. The total volume of each well was 300 μl . Plates were incubated for 48 h at 25°C. Detection of activity was carried out as previously described by Blocher *et al* (14). The activity was determined by the percentage of germination inhibition. At selected intervals sample was removed aseptically from well. A drop of sample was placed on a clean, cover slip, sample dried. Spore germination was measured using (x400) microscope. A total of 100 spores per samples were counted and classified as refractile (non germinated) or non refractile (germinated). Only fully refractile spores were classified as non germinated.

Mycelial dry weight

The dry weight of mycelia was measured after repeated washing of the mycelial pellets with distilled water and drying overnight at 70°C to a constant weight.

Results and discussion

Many kinds of mushrooms frequently require starch, glucose, sucrose and etc., for their submerged culture. In order to investigate the effects of different carbon sources on growth and antifungal activity of *G. spectabilis*, the fungus was cultivated in the medium containing various carbon sources for 10 days. Initial pH was adjusted in 5.5. Table 1 shows the cell concentration (dry weight, w/v) and the antifungal activity. The maximum mycelial dry weight was obtained from medium containing glucose with a final mycelial biomass concentration of 2.5 g/l. Meanwhile 90% inhibition of spore germination was observed from the medium containing glucose as carbon source. The minimum mycelial biomass level was found in medium containing xylose. The fungus grew very well in the medium containing fructose with a total biomass concentration of 2.3 g/l, being significantly higher than the media containing maltose, lactose and other sugars. Sistorm and Michilis (15) explained that fructose is next best utilized carbon by many fungi. Often adaptation is required before growth begins, but then growth is often as rapid as with glucose. It was unexpected, therefore, the inhibition of spore germination was low when fructose was used (Table 1). It seems that glucose as a carbon source is necessary for production of such bioactive compounds. This was explained that "a fungal species may have the ability to utilize a particular carbon source for vegetative growth but may be unable to use it for production of specialized structure (16).

It is interesting to note that little difference was seen in the inhibition activity of the extracts from the media containing fructose and lactose.

Table 1. Total biomass concentration and antifungal activity of *G. spectabilis* grown in simple sugar

Carbon source	Biomass(g/l)	% inhibition
Glucose	2.5	90
Maltose	1.7	55
Lactose	1.2	47
Sucrose	1.5	26
Fructose	2.3	52
Manitol	1.4	32
Xylose	1.1	35

Table 2. Total biomass concentration and antifungal activity of *G. spectabilis* grown in complex carbon sources

Carbon source	Biomass (g/l)	% inhibition
Malt extract	2	84
Soluble starch	1.6	37
glucose + malt extract	2.3	100
starch+ glucose	2.1	84

The biomass in the growth medium containing maltose was lower than that of fructose; however the bioactivity was found to be similar. Bioactivity per gram was therefore increased when maltose was utilized as the carbon source. It is possible that fructose as a simple sugar interferes with product formation and maltose as a slow releasing carbon sources supported production of bioactive compounds rather than growth of the fungus. Also, with the exception of the medium containing glucose, none of the other cultures totally inhibited spore germination of test organism. Sucrose did not support growth of the fungus and consequently had no effect on production of bioactive compounds. The same result has also been reported that sucrose is a very poor carbon sources for growth of *Coprinus lagopus*, compared to fructose and glucose (17). The results obtained from use of a slow releasing carbon source and the combination of glucose plus malt extract and starch are shown in Table 2. Increased antifungal activity of the fungus in media containing a simple sugar, like glucose plus a slow releasing carbon source, like malt extract can be explained by the high production rate of secondary metabolites when their producing organisms grow in complex media (18). These authors showed that in media containing glucose plus a more slowly utilized carbon source, glucose is usually used first and after glucose depletion, the second carbon source is used for antibiotic biosynthesis.

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