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Research Paper

Colonization and Optimization of Some Fungal Mycelium through Metal Biosorbent

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Abstract

This study investigated maximum mycelium formation through propagation of a newly isolated *Gliocladium viride* ZIC$_{2063}$. To obtain high mycelium concentrations, the cultural conditions of *Gliocladium viride* ZIC$_{2063}$ were optimized for the first time with the aim of using in further biosorption processes. The fungal mycelium was used as biosorbent. In addition, cultural conditions (pH, temperature, incubation time, inoculum age and size) and culture medium were optimized for the growth of fungal culture as a biosorbent. The optimization led to a doubled amount of mycelium. With a high resistance to chromium, *Gliocladium viride* ZIC$_{2063}$ as a thermostable and acid stable species may be applicable in the leather industry for the treatment of tanning effluent.

Keywords: *Gliocladium Viride*; Biosorbant; Culture

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1. Introduction

Metal binding abilities are well documented for fungal species, for which a considerable tolerance has been reported toward metals and other adverse conditions [1]. The presence of functional groups on their cell walls determines the metal binding capacities of fungi [2]. The surrounding environments of fungi can obstruct or harm these functional groups [3]. Yang and Illman (1999) showed a very sensitiveness of the cell wall chemical composition to the growth milieu. This study, therefore, sought to examine the effects of growth medium composition and other cultural conditions. In order to obtain a high biomass concentration, the optimized cultural conditions achieved herein can be used in the future biosorption trials. Cell surface phenotype is influenced by the growth conditions of microorganisms, which in turn impacts its biosorption potential [5]. A high biosorption was reported for *Aspergillus niger* when the fungus cultured with the addition of potassium hexacyanoferate [2]. Provision of nutrients to Gram-positive bacteria increases biosorption within incubation for 2 h, but such a trend was not observed in Gram-negative bacteria [6]. Addition of sufficient nitrogen source increased the biosorption of *Phormidium laminosum* [7]. The optimizations of pH, temperature, inoculum size, agitation and carbon nitrogen sources were extensively investigated on fungal culture. As the first attempt, the cultural conditions of *Gliocladium viride* ZIC$_{2063}$ were optimized for potato dextrose medium. The present research tried to propagate *Gliocladium viride* ZIC$_{2063}$ as a biosorbent through preparation of an appropriate and cost-effective medium.

2. Material and Methods

2.1 Organism

Tanning unit effluent fungal cultures (> 50) were isolated by the use of potato dextrose agar medium. *Gliocladium viride* ZIC$_{2063}$ was selected for further examination after fungal culture screening, and

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transferred to glycerol at temperatures of −20 °C and 4 °C.

2.2 Biomass analysis

The fungal culture was filtered through a pre-weighed dry Whatman No1 filter paper to evaluate cell biomass gravimetrically. The mycelium subjected to a thorough washing by distilled water followed by weighing.

2.3 Propagation of biomass

Cultural conditions were optimized to obtain maximum quantity of *Gliocladium viride* ZIC$_{2063}$. The fungus was propagated using four culture media, viz. potato dextrose broth (M_1), yeast peptone sucrose medium (M_2), liquid medium (M_3), and Czapek Dox medium (M_4) (Table1). To evaluate their effect on the growth of fungi, the selected mediums were further enriched with different carbon sources (sucrose, dextrose and Sodium acetate) and nitrogen sources (sodium nitrate, urea and yeast extract) at a concentration of 1%**.** The effect of initial pH of growth medium was examined by a variety of pH levels ranging 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 to adjust the acidity using phosphoric acid (1M). Both the incubation temperature and time were optimized in a temperature range of 20 °C to 35 °C at different time periods of 1, 2, 3 and 4 days. Optimizations were also applied to inoculum age (3, 5 and 7 days old) and size (2%, 4%, 6%, 8% and 10%). Additionally, tests were performed to see the role of agitation on mycelium growth.

3. Results and Discussion

3.1 Screening of culture medium

As shown in Table 1, the mycelium formation for the propagation of *Gliocladium viride* ZIC$_{2063}$ was evaluated using four different culture media (M_1, M_2, M_3 and M_4), of which M_1 (potato dextrose broth) yielded the best results (4.9029 g wet weight) and was used in the rest of propagation process. However, M_2, M_3 and M_4 mediums resulted in the growth biomasses of 3.6945 g, 3.0246 g and 2.8301 g wet weight, respectively.

Different sources of carbon and nitrogen were added to potato broth to examine their effects on the fungal growth, which was significantly affected by the type and concentrations of carbon and nitrogen sources. Table 2 indicates that dextrose showed maximum results (4.7813 g wet weight) among other carbon sources. The propagation of *Gliocladium viride* ZIC$_{2063}$ was effectively improved by all nitrogen sources with sodium nitrate being the best nitrogen source (4.9508 g wet weight) for fungal growth (Table 3). Ammonium and nitrate are the kinds of nitrogen sources used by fungi [8]. The most commonly used medium for fungal growth is potato dextrose broth (PDB) [8-10]. Our observations corroborate those reported previously [8].

3.2 Effect of initial pH

It is well known that the charge of fungal cell surface and its attached functional groups can change as a result of pH variations [11]. The initial pH of medium was reported to affect the growth rate with an uppermost biomass (3.8303 g wet weight) during log phase at pH 4.0. Table 4 represents a poor biomass growth at pH above or below 4.0.

3.3 Effect of incubation time

As shown in Table 5, *Gliocladium viride* ZIC$_{2063}$ was incubated for 24, 48, 72 and 96 h. Maximum fungal growth (4.6967 g wet weight) was recorded after incubation for 72 h, which was followed by appearing thick mass of brown to black mycelial pellets. The mycelium concentration did not increase considerably by further rise of the incubation time.

3.4 Effect of incubation temperature

After applying various incubation temperatures of 20, 25, 30 and 35 °C, mycelium growth (4.6847 g wet weight) was maximal at 30 °C (Table 6). The fungal mycelium growth decreased when the temperature dropped below 25 °C. The mycelium concentration also declined by rising temperature beyond 30 °C. As growth is an energy-dependent mechanism, the growth media temperature is of paramount importance [8]. The growth of *Gliocladium viride* ZIC$_{2063}$ is optimized at a temperature of 30 °C. Congeevaram *et al.* (2007), among others, have also reported the same observations.

3.5 Effect of inoculum age & inoculum size (%)

In order to minimize the length of lag phase, the inoculum has to have a healthy and active status. The effect of inoculum age on the propagation of *Gliocladium viride* ZIC$_{2063}$ was examined using different-aged inocula (3, 5 and 7 days). The mycelial pellet (4.8905 g wet weight) was maximized with 5-day-old inoculum (Table 7). Fungal morphology is intensely influenced by inoculum size [12]. In addition, the effect of inoculum size was evaluated by incubating the growth medium (M_1) with 5-day-old inoculum having various sizes (2%, 4%, 6%, 8% and 10%) of mycelium concentration at 2%, 4%, 6%, 8% and 10% inoculum was 3.7794 g, 3.7802 g, 3.7813 g, 4.0023 g and 4.0132 g wet weight, respectively (Table 8). Accordingly, an inoculum size of 8% was used in the next experiments.

Table 1. Effects of different growth media (along with composition) on the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Growth media		Wet weight of mycelial pellet (g)	composition of growth media	Growth rate
1	M_1	PDB Medium [13]	4.9029	Potatoes 300g/500ml Dextrose sugar 1.5g/100ml	++++
2	M_2	YPS Medium [14]	3.6945	Yeast extract 3.0 g/l, Peptone 10g/l Sucrose 20g/l pH 4.5	+++
3	M_3	Liquid Medium [15]	3.0246	Dextrose 20g/l, Peptone 10g/l, Yeast extract 3g/l pH 5	++
4	M_4	Czapek Dox Medium [13]	2.8301	Sucrose 30g/l, $NaNO_3$ 3g/l, $MgSO_4$ $7H_2O$ 0.5g/l, KCl 0.5g/l, K_2HPO_4 1g/l, pH 6.2	+

Note: (+) = very slow growth, (++) = slow growth, (+++) = moderate growth, and (+++) = fast growth.
Cultural conditions: Incubation time 72 h, incubation temperature 30°C, inoculum size 2%, volume of media 50 ml, and shaking velocity 122 rpm.

Table 2. Effects of carbon sources on the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Carbon source	Wet weight of mycelial pellet (g)	Growth rate
1	Sucrose	3.7194	++
2	Dextrose	4.7813	++++
3	Sodium acetate	4.7802	+++
4	Sodium Citrate	4.0123	++

Note: (+) = very slow growth, (++) = slow growth, (+++) = moderate growth, and (+++) = fast growth
Cultural conditions: Incubation time 72 h, pH of media 4, incubation temperature 30 °C, inoculum size 8%, volume of medium 50 ml, and shaking velocity 122 rpm.

Table 3. Effects of nitrogen sources on the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Nitrogen source	Wet weight of mycelial pellet (g)	Growth rate
1	Yeast Extract	4.0014	++
2	Urea	4.1022	+++
3	Sodium Nitrate	4.9508	++++

Note: (+) = very slow growth, (++) = slow growth, (+++) = moderate growth, and (+++) = fast growth
Cultural conditions: Incubation time 72 h, pH of media 4, incubation temperature 30 °C, inoculum size 8%, volume of medium 50 ml, and shaking velocity 122 rpm.

Table 4. Effect of initial pH of growth medium on the propagation of *Gliocladium viride* ZIC$_{2063}$

Sr. No.	Initial pH	Wet weight of mycelial pellet (g)	Growth rate
1	2.0	3.0153	+
2	2.5	3.0499	+
3	3.0	3.2360	++
4	3.5	3.6450	++++
5	4.0	3.8303	+++
6	4.5	3.3266	++
7	5.0	3.1704	++
8	5.5	3.0732	+
9	6.0	2.3832	+

Note: (+) = very slow growth, (++) = slow growth, (+++) = moderate growth, and (+++) = fast growth. **Cultural conditions:** Incubation time 5 days, incubation temperature 30 °C, inoculum size 2%, volume of medium 50 ml, and shaking velocity 122 rpm.

3.6 Effect of agitation

The impacts of static and agitated conditions were assessed by incubating the culture at 122 rpm in an agitated orbital shaker for 48 h, and flasks were incubated under static conditions at 30 °C. Interestingly, the agitation process increased the biomass yield almost three times after shaking for 48 h when the cultural conditions were shifted to static conditions. A similar trend was also reported by Kirk et al. (1986) and Yang Illman (1999). A

mycelial pellet of 4.7813 g wet weight was obtained with uniform agitation for 72 h and incubation under static conditions resulted in a very poor biomass growth (2.7194 g wet weight). As shown in Table 9, thick black mycelium reached a maximum (5.2802 g wet weight) when the cultural conditions were shifted to static conditions after shaking for 48 h.

Table 5. Effect of incubation time on the propagation of *Gliocladium viride* ZIC2063

Sr. No.	Incubation time (hr)	Wet weight of mycelial pellet (g)	Growth rate
1	24	3.0245	+
2	48	3.7793	++
3	72	4.7002	++++
4	96	4.6967	++++

Note: (+) = very slow growth, (++) = slow growth, (+++) = moderate growth, and (+++) = fast growth

Cultural conditions: pH of media 4, incubation temperature 30 °C, inoculum size 2%, volume of medium 50 ml, and shaking velocity 122 rpm.

Table 6. Effect of incubation temperature on the propagation of *Gliocladium viride* ZIC2063

Sr. No.	Incubation temperature (°C)	Wet weight of mycelial pellet (g)	Growth rate
1	20	3.0734	+
2	25	3.8979	+++
3	30	4.6847	++++
4	35	3.0245	++

Note: (+) = very slow growth, (++) = slow growth, (+++) = moderate growth, and (+++) = fast growth

Cultural conditions: Incubation time 72 h, pH of media 4, inoculum size 2%, volume of medium 50 ml, and shaking velocity 122 rpm.

Table 7. Effect of inoculum age on the biomass production of *Gliocladium viride* ZIC2063

Sr. No.	Age of inoculum (day)	Wet weight of mycelial pellet (g)	Growth rate
1	3	4.1122	+++
2	5	4.8905	++++
3	7	4.0054	++

Note: (+) = very slow growth, (++) = slow growth, (+++) = moderate growth, and (+++) = fast growth

Cultural conditions: Incubation time 72 h, pH of media 4, incubation temperature 30 °C, inoculum size 8%, volume of medium 50 ml, and shaking velocity 122 rpm.

Table 8. Effect of inoculum size (%) on the biomass production of *Gliocladium viride* ZIC2063

Sr. No.	Inoculum size (%)	Wet weight of mycelial pellet (g)	Growth rate
1	2	3.7794	++
2	4	3.7802	++
3	6	3.7813	++
4	8	4.0023	++++
5	10	4.0132	+++

Note: (+) = very slow growth, (++) = slow growth, (+++) = moderate growth, and (+++) = fast growth.

Cultural conditions: Incubation time 72 h, pH of media 4, incubation temperature 30 °C, volume of medium 50 ml, and shaking velocity 122 rpm.

Table 9. Effect of agitation on the propagation of *Gliocladium viride* ZIC2063

Sr. No.	Agitation rate (rmp)	Wet weight of mycelial pellet (g)	Growth rate
1	Agitat ion for 3 days	4.7813	+++
2	Without agitation for 3 days	2.7194	++
3	2-day agitation followed by 2-day static conditions	5.2802	++++

Note: (+) = very slow growth, (++) = slow growth, (+++) = moderate growth, and (++++) = fast growth.

Cultural conditions: pH of media 4, incubation temperature 30 °C, inoculum size 8%, and volume of medium 50 ml.

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