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Research Article

Optimization of Solid State Fermentation Conditions for the Production of Cellulase by Using *Trichoderma viride* GSG12

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Abstract

Trichoderma viride GSG12 under solid state fermentation technique using cheap and an easily available agricultural waste material, rice bran was used to produce cellulase enzyme.The feasibility of using rice bran for cellulase production by *Trichoderma viride*. under solid state fermentation was evaluated. Our results indicated that optimal pH, initial moisture level of the medium, incubation temperature, inoculums size and incubation time influenced the cellulase production. The optimum pH, initial moisture level, incubation temperature and inoculums size were 5.5, 70%, 32 and 2×10^8 spores/flask, 120h respectively. The supplement of lactose and corn-steep solid to the rice bran favored increased enzyme production.

INTRODUCTION

Cellulases are the hydrolytic enzymes which are responsible for the decomposition of the natural cellulose polymer by acting at 1,4 β-D-glucosidic linkages thus finally converting into glucose monomer (Sternberg *et al*., 2000). The production and applications of cellulases have central importance in bioprocess industries (Headon & Wash, 1994), preparation of medicines, waste treatment, food production, paper industries, perfumes, backing etc,. (Beauchemin *et al*., 2003).

Many fungi produce cellulases to release sugar for cell growth and product formation. More than 14,000 species of fungi have been found to be active in cellulose degradation (Esterbauer *et al*., 1991). *Trichoderma* spp., have been extensively studied due to their strong cellulolytic activity against crystalline celluloses which results into saccharification (Deschamps *et al*., 1985).

Rice bran is the main solid waste generated in Agriculture practice and in an Industrial sector. It accounts for 50% of the dry weight of the processed raw material. It is cheaper carbon and nitrogen source in order to reduce the production costs (Chapple *et al*., 2007). Worldwide, several million metric tonnes of this residue is produced annually (Beatriz *et al*., 2008). Rice bran is a poor animal food because its low protein content. Rice bran does not find any significant commercial application till now and most of this byproduct is generally disposed of in an open area, leading to potentially serious environmental problems. Given this situation, it is necessary to look for processes that allow the controlled elimination of this residue or, even better, its industrial reutilization (Nadagouda *et al*., 2015). Recently much effort has been made to convert this waste in to a variety of value-added products such as cellulose enzyme, biofuels, L-lactic acid, and others (Beatriz *et al*., 2008; Josh *et al*., 2008; Silas *et al*., 2003; Stredansky *et al*., 2000; Berovi_ and Ostroveršnik, 1997; Joshi and Sandhu, 1996; Rahmat *et al*., 1995; Ngadi and Correia, 1992). During fermentation, cultural parameters and nutritional requirements such as ionic concentration, pH, temperature, cultivation time, aeration, and inoculum size have fundamental

role in the growth of microorganisms and subsequent product formation (Mekala *et al*., 2008). The objective of the present study was to optimize various parameters for the enhanced cellulases production by *Trichoderma viride GSG 12* through the use of rice bran.

MATERIALS AND METHODS.

Microorganism

Trichoderma viride. GSG12, a newly isolated cellulase producer, was used in this study. It was identified (Alexopouls and Mims., 2012) and deposited in Dept. of BotanyA.S.M. College for women, Bellary. It was preserved in potato dextrose agar at 4°C.

Substrate: Rice bran was obtained from Agriculture practice. The residue was dried in a hot air oven at 80°C, crushed and sieved to an average size of 300 - 500µm.

Production of cellulase from *Trichoderma viride*. GSG12under solid state fermentation(SSF).

Cellulase production experiments were carried out in 250 mL flasks containing 10 g rice bran moistened with distilled water to a moisture level of 50%. All flasks were sterilized at 121°C for 30 min, inoculated (10^8 spores/flask) and then incubated at 30°C for 144 h. The samples were withdrawn at regular intervals to determine enzyme activities. Various pH values between 4.0 to 7.0 with appropriate buffer at 28°C maintained. To investigate the effect of moisture level on cellulase production, different moisture levels (40, 45, 50, 55, 60, 65, 70, 75 and 80%) were used to compare the enzyme activity. Based on the optimum culture time and moisture level, different culture temperature (25, 28, 30, 32, 35 and 40°C) was compared to investigate the effect of temperature on cellulase production. Different inoculum sizes were also carried out to investigate its effect on cellulase production. Based on the optimum culture time, moisture level, culture temperature and inoculum size, on cellulase production.

Analytical methods.

During the process of enzyme production, 0.5 g sample was withdrawn, extracted with 10 ml distilled water (30 min) and filtered. The supernatant was used for enzyme assay. The activity of cellulase was assayed using 1% Carboxy methyl cellulose, in 0.05 M citrate buffer (pH 4.8) as substrate. The reaction was carried out at 50°C for 30 min. One unit (U) of enzyme activity was defined as the amount of enzyme, which liberates 1 µmol of glucose equivalent from carboxy methyl cellulose per min. Reducing sugars were estimated with 3,5-dinitrosalicylic acid (DNS), using glucose as standard (Miller, 1959). The residue was dried to constant mass at 80°C. The enzyme activity was expressed as U per g dried substrate (U/gds). All values given are means of three determinations.

RESULTS AND DISCUSSION

Time course of cellulase production by *Trichoderma viride GSG* 12. on rice bran.

Figure- 1 shows the effect of incubation time on cellulase production. The production of cellulase increased with increase in incubation time and reached maximum at 120 hr. Further increase in incubation time resulte in decreased cellulase production. Therefore 120hrs incubation time was found to be optimum for cellulase production by *Trichoderma viride GSG* 12.

Time course of 120 h for optimum cellulases production by *T.viride GSG* 12 is in accordance with earlier reports. Cellulase were produced from *Aspergillus niger* KK2 at 120 h incubation (Kang *et al*., 2004). Similarly cellulolytic enzymes were produced by *Aspergillus phoenix* at 120 h incubation (Dedavid *et al*., 2008). Similarly *Aureobasidum pullulans* showed maximum β-glucosidase production at 120 h of cultivation. Likewise, Kirchner et *al*., (2005) produced maximum β-glucosidase activity from *Aspergillus niger* C-6 after 96 to 120 h. Time course required to reach maximum level of cellulase activity may be affected by several factors, including the presence of different ratios of amorphous to crystalline cellulose (Ogel *et al*., 2001). The decrease in the cellulase production with increase in incubation time might be due to depletion of nutrients and accumulation of other byproducts like proteases.

Effect of pH, temperature and incubation time on cellulase production:

The optimum pH for the fungal growth and enzyme production was found to be 5.5 at 32°C for 120h. (Table 1). Cellulase enzyme production gradually increase with increase in pH and become maximum at pH 5.5. Further increase in pH resulted in gradual decrease in the production of cellulase. Therefore pH 5.5 was found to be optimum pH for the production of cellulase by *Trichoderma viride GSG* 12. Cellulase production is greatly influenced by pH of the culture medium.At higher pH cellulase production was greatly decreased due to fact that cellulase is acidic enzyme, neutral pH and alkaline pH values greatly reduce the cellulase production (Chandra *et al*., 2009)

Figure 1. Effect of incubation time on cellulase production.

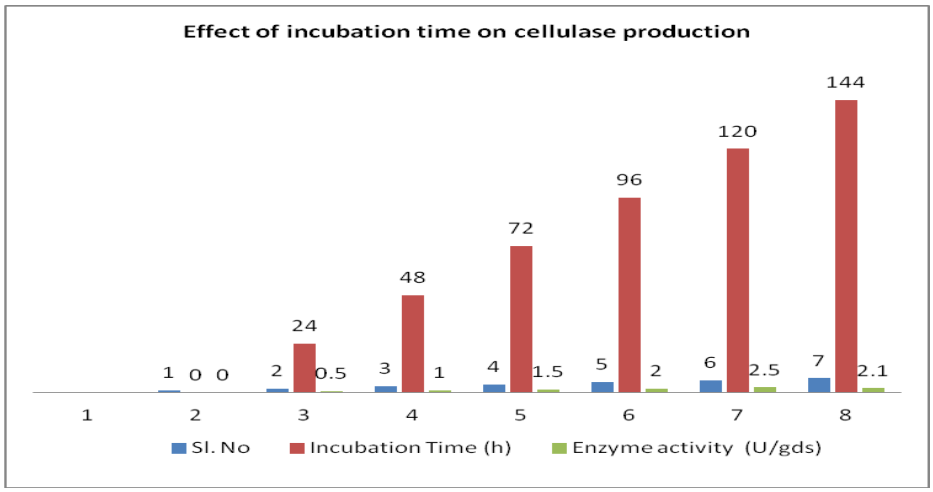
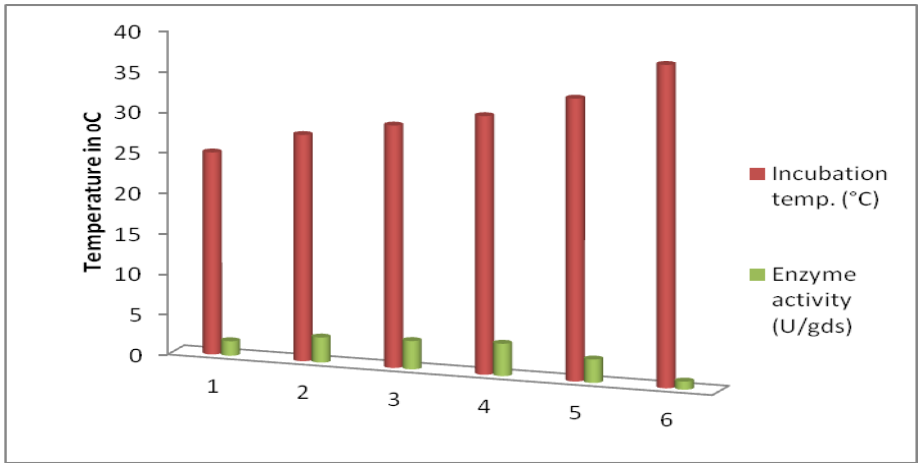


Table - 1. Effect of incubation pH on cellulase production.

Sr. No	Incubation pH	Enzyme activity (U/gds)
1	3.5	1.8
2	4.5	3.9
3	5.5	4.0
4	6.5	3.1
5	7.5	2.9
6	8.5	1.0
7	9.5	0.6

Figure 2. Effect of incubation temperature on cellulase production.



Effect of incubation temperature on cellulose production.

In the present study 32°C temperature proved to be the best temperature for the enzyme synthesis. Incubation at lower temperature resulted in longer time to the maximum enzyme activity. Incubation at higher temperature affected the fungus harmfully, which reflected on the enzyme synthesis. Since enzyme is a secondary metabolite produced during exponential growth phase, the incubation at high temperature could lead to poor growth and thus a reduction in enzyme yield (Sabu *et al*., 2002).

Effect of initial moisture level of the medium on cellulase production.

Moisture content is a critical factor for cell growth and enzyme production under SSF, which determines the outcome of the process. As shown in Table 4, the optimum initial moisture level was 70% for cellulase production by *Trichoderma* sp. GSG 12 on a rice bran. Lower or higher than 70% both decreased the cellulase production. Lower moisture level gives a lower degree of swelling and higher water tension and then reduces the solubility of nutrients. Higher moisture level decreases porosity, changes in particle structure, promotes development of stickiness, decreases diffusion, lowers oxygen transfer or increases formation of aerial hyphae.

Table - 2. Effect of initial moisture level of the medium on cellulase production.

Sl. No	Initial moisture level	Enzyme activity (U/gds)
1	40	1.2
2	45	1.8
3	50	2.3
4	55	2.6
5	60	2.9
6	65	3.2
7	70	3.5
8	75	2.9
9	80	2.2

Effect of inoculum size on cellulose production

Lower inoculum size required longer time for the cells to multiply to sufficient number to utilize the substrate rice bran and produce enzyme. An increase in the number of spores in inoculum would ensure a rapid proliferation and biomass synthesis. After a certain limit, enzyme production could decrease because of depletion of nutrients due to the enhanced biomass, which would result in a decrease in metabolic activity (Kashyap *et al.*, 2002). A balance between the proliferating biomass and available nutrient would yield an optimum at which the enzyme synthesis would be maximum (Ramachandran *et al.*, 2004). As shown in Table 5, when the inoculum size was lower than 10^8 spores/flask, the enzyme activity is obviously low. When the inoculum size ranged from 1 x 10^8 to 4 x 10^8 spores/flask, the cellulase activity varied slightly. 2 x 10^8 spores/flask maximized the enzyme production.

Table 3. Effect of particle size on cellulose production.

Sl. No	Inoculum size (10^8 spores/flask)	Enzyme activity (U/gds)
1	0.2	1.8
2	0.5	3.1
3	1	3.5
4	2	4.0
5	3	2.9
6	4	1.0

CONCLUSION.

These studies showed that rice bran could be a good substrate for cellulase synthesis by *Trichoderma* viride. GSG12. Incubation pH, incubation temperature, Initial moisture level of the medium, inoculum size and incubation time greatly influenced the cellulase production. The optimum initial pH, moisture level, incubation temperature, inoculum size and incubation time were 5.5, 70%, 32°C and 2 x 10^8 spores/flask and 120 h respectively.

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