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Full Length Research Paper

Banana peel: A novel substrate for cellulase production under solid-state fermentation

Hai-Yan Sun[1,2], Juanhua Li[1], Pingjuan Zhao[1,2] and Ming Peng[1,2]*

1Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Key Laboratory of Tropical Crop Biotechnology , Ministry of Agriculture, Haikou 571101, China.
2School of Agriculture, Hainan University, Haikou 571101, China.

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The feasibility of using banana peel for the production of cellulase by *Trichoderma viride* GIM 3.0010 in solid-state fermentation was evaluated in this study. The effect of incubation time, incubation temperature, initial moisture content of the medium, inoculum size and supplementation of carbon sources and nitrogen sources on cellulase production was investigated. When banana peel was moistened to the moisture content of 65% with the inoculum size of 1.5 × 10^9 spores / flask and incubated at 30°C for 144 h, the maximum activities of filter paper activity (FPA), carboxy methyl cellulase sodium activity (CMCase) and β-glucosidase (BG) reached 5.56, 10.31 and 3.01 U/gds, respectively. These results indicated that banana peel provided necessary nutrients for cell growth and cellulase synthesis. It can be used as a potential substrate for cellulase production by *T. viride* GIM 3.0010 under solid-state fermentation. To the best of our knowledge, this is the first report on cellulase production using banana peel.

Keywords: Banana peel, cellulase, *Trichoderma viride*, solid-state fermentation.

INTRODUCTION

Cellulose is a fibrous, insoluble and crystalline polysaccharide consisting of D-glucose residues linked by β-1, 4-glucosidic bonds. Cellulose is the most abundant biopolymer in nature and can be degraded to glucose through the synergistical hydrolysis of three classes of cellulase, including endo-β-1, 4-glucanase (EC3.2.1.4), exoglucanase or cellobiohydrolase (EC3.2.1.91) and β-glucosidase (EC3.2.1.21) (Sehnem et al., 2006). Glucose from the hydrolysis of cellulose can be easily fermented into useful products such as ethanol, lactic acid, single cell protein and other value added products (Chandra et al., 2009). Therefore, cellulases are industrially important enzymes having application in diverse industries such as textile, paper and pulp and food industry. Cellulases are relatively costly enzymes and a significant reduction in cost will be important for their commercial use. Production of cellulases using cheaper substrates is an effective strategy to reduce cost. In recent years, much work has been carried out towards efficient utilization of agro-industrial residues such as wheat bran, sugarcane bagasse, coconut coir pith and others (Hao et al., 2006; Muniswaran and Charyulu, 1994; Gutierrez-Correa and Tengerdy, 1997; Krishna 1999). Banana peel is an abundant and low cost agricultural waste residue. It is easily available in large quantities. It accounts for about 30% of the weight of the raw fruit and is rich in carbohydrates, protein and various vitamins and mineral elements (Li et al., 2001). However, banana peel does not find any significant commercial application till now and is generally disposed of in open areas, leading to potentially serious environmental problems. It is necessary to explore its industrial reutilization. This study was carried out to explore the feasibility of using banana peel as solid substrate for the production of cellulase. Though banana stalk was tested for cellulase production by *Bacillus*

*Corresponding author. E-mail: hysun168@126.com or mmpeng_2000@yahoo.com. Tel: +86-898-66963161. Fax: +86-898-66890978.

Abbreviations: FPA, Filter paper activity; **CMCase,** carboxy methyl cellulase sodium activity; **BG,** β-glucosidase.

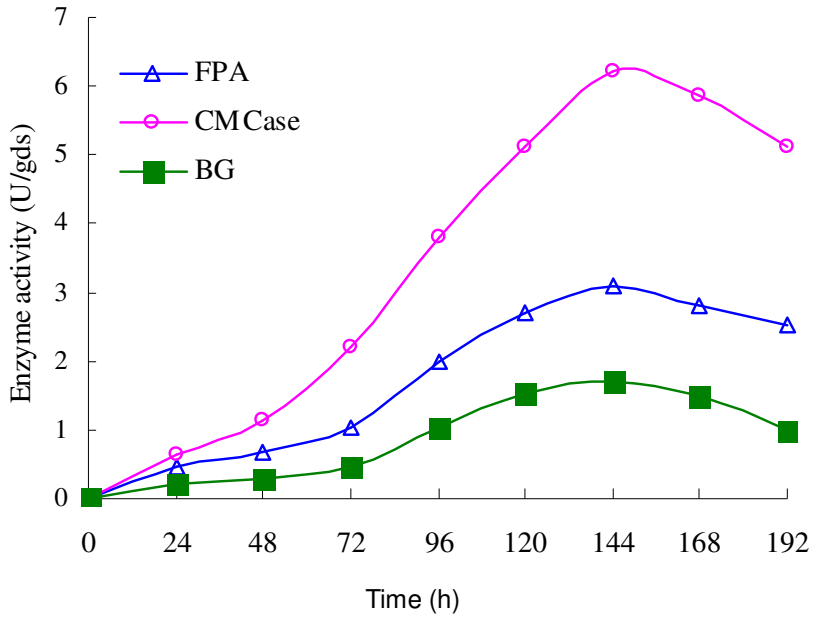

Figure 1. Time course of cellulases production by *T. viride* GIM 3.0010 using banana peel as substrate under SSF.

subtilis (Krishna 1999), there is still no evidence on the application of banana peel in cellulase production by *Trichoderma viride*. To the best of our knowledge, this is the first report on cellulase production by *T. viride* using banana peel as the substrate.

MATERIALS AND METHODS

Microorganisms

A novel cellulase producer was used in this study. It was identified and deposited in Guangdong Microbial Culture Collection Center in China as *T. viride* GIM 3.0010. It was preserved in potato dextrose agar medium at 4℃.

Substrate

Banana peel was obtained from local source. The peel from ripe banana fruit was dried in an oven at 80℃, crushed and sieved to an average size of 1 to 5 mm.

Solid-state fermentation

In the basal medium, 5 g dry banana peel moistened to the moisture level of 50% with distilled water in 250-ml Erlenmeyer flask was autoclaved at 121℃ for 40 min. One flask was inoculated with 1 ml spore suspension (10^9 spores/ml) and incubated at 30℃ for 192 h. During the process, the sample was withdrawn at regular intervals to determine enzyme activities.

Cellulase production under SSF was optimized by altering the medium composition or cultural conditions based on the basal medium. The optimal level of one factor was determined by varying its level, while keeping other factors in the medium constant. The effect of incubation time (24, 48, 72, 96, 120, 144, 168 and 192 h), incubation temperature (25, 30, 35, and 40℃), initial moisture content of the substrate (45, 50, 55, 60, 65 and 70%), and inoculum size (0.5, 1.0, 1.5, 2.0 and 2.5 ml) on cellulase production by *T. viride* GIM 3.0010 was investigated. Studies were also performed to evaluate the influence of different carbon sources (glucose, fructose, maltose, starch, sucrose, lactose, avicel and carboxy methyl cellulose at 2% w/v) and nitrogen sources (peptone, yeast extract, corn-steep solid, sodium nitrate, ammonium sulphate and ammonium nitrate at 1% w/v) on cellulase production by *T. viride* GIM 3.0010 when added to the fermentation medium.

Analytical methods

The filter paper activity (FPA), carboxy methyl cellulase sodium activity (CMCase) and β-glucosidase (BG) were assayed using Whatman No. 1 filter paper, 1% carboxy methyl cellulose sodium and 1% cellobiose in 0.05 M citrate buffer (pH 4.8) as substrate, respectively. The reaction was carried out at 50℃ for 30 min. One unit (U) of enzyme activity was defined as the amount of enzyme, which liberates 1 µmol of glucose equivalent from filter paper, carboxy methyl cellulose or cellobiose per min. Reducing sugars were estimated with 3,5-dinitrosalicylic acid (DNS), using glucose as standard. The enzyme activity was expressed as U per g dried substrate (U/gds). Dry weight of the samples was determined by drying them in a hot air oven at 80℃ to a constant weight.

RESULTS AND DISCUSSION

Time course of cellulase production by *T. viride* GIM 3.0010 on basal medium

SSF was carried out on banana peel with the initial moisture content of 50% at 30℃. As shown in Figure 1, after 144 h of incubation, the enzyme activity of FPA, CMCase

Table 1. Effect of incubation temperature on cellulase production by *T. viride* GIM 3.0010 on banana peel.

Temperature (℃)	Enzyme activity (U/gds)		
	FPA	CMCase	BG
25	1.52	3.21	0.84
30	3.10	5.86	1.69
35	2.24	4.25	1.41
40	0.86	1.43	0.52

Table 2. Effect of initial moisture content on cellulase production by *T. viride* GIM 3.0010 on banana peel.

Initial moisture content (%)	Enzyme activity (U/gds)		
	FPA	CMCase	BG
45	2.53	5.36	1.88
50	3.10	5.86	1.69
55	3.87	7.00	1.68
60	4.25	8.54	2.44
65	4.68	9.66	2.67
70	4.12	7.85	1.96

and BG reached the maximum of 3.08, 6.23 and 1.70 U/gds, respectively. Incubation beyond 144 h resulted in decreased enzyme activity. Many workers have reported similar trends (Pietersen, 1977; Allen and Roche, 1989; Muniswaran and Charyulu, 1994). The reason for this might have been due to the denaturation of the enzyme caused by the interaction with other components in the medium (Ramesh and Lonsane, 1987).

Effect of incubation temperature on cellulase production by *T. viride* GIM 3.0010

Temperature is one of the important factors, which strongly affect the SSF process (Pandey et al., 2000). Cell growth, production of enzymes and metabolites are usually sensitive to temperature. As shown in Table 1, 30℃ proved to be the best temperature for the enzyme synthesis. Incubation at higher temperature affected the fungus harmfully.

Effect of initial moisture content of the medium on cellulase production by *T. viride* GIM 3.0010

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 2, the results in the present study revealed that the optimum initial moisture content was 65% for cellulase production by *Trichoderma* sp. GIM 3.0010. Lower or higher than 65%, both decreased the cellulase production. The possible reasons are as follows: 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 particle structure, promotes development of stickiness, decreases diffusion, lowers oxygen transfer or increases formation of aerial hyphae.

Table 3. Effect of inoculum size on cellulase production by *T. viride* GIM 3.0010 on banana peel.

Inoculum size (10^9 spores)	Enzyme activity (U/gds)		
	FPA	CMCase	BG
0.5	2.52	4.43	0.89
1.0	4.68	9.66	2.67
1.5	5.56	10.31	3.01
2.0	3.52	5.70	1.66
2.5	2.77	4.23	1.15

Effect of inoculum size on cellulase production by *T. viride* GIM 3.0010

The inoculum size also plays a significant role in the enzyme production. As shown in Table 3, maximum enzyme activity was obtained when the inoculum size was 1.5 ml spore suspension (with the cell count of 10^9/ml) per flask. A lower level of inoculum may not be sufficient for initiating growth and enzyme synthesis. An increase in inoculum size ensures a rapid proliferation of biomass and enzyme 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 substrate material would yield maximum enzyme.

Effect of supplementation of carbon source and nitrogen source on cellulase production by *T. viride* GIM 3.0010

The exogenous addition of various nutrients to the solid medium may improve the growth of organism and enzyme production (Pandey et al., 2004). The results from this study indicated that among various carbon sources tested, none of them could enhance enzyme yield. Supplementation with monosaccharides (glucose or fructose) inhibited cellulase significantly. Similarly, all the nitrogen sources tested had little or negative effect on cellulase production (data not shown). These results indicated that natural banana peel provided all the nutrients needed by the organism for cell growth and enzyme production. The exogenous addition of various nutrients is needless. This is of great interest for industrial production of cellulase, for the cost of the addition of nutrients would be saved.

Conclusion

The data obtained in this study indicated that banana peel provided necessary nutrients for the microorganism to grow and synthesize cellulase. It can be used as a potential substrate for cellulase production by *viride* GIM 3.0010 under SSF. When banana peel was moistened to the moisture content of 65% with the inoculum size of 1.5 × 10^9 spore / flask and incubated at 30°C for 144 h, the maximum activities of FPA, CMCase and BG obtained were 5.56, 10.31 and 3.01 U/gds, respectively.

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