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Screening, Characterization and Biosynthesis of Cellulase from Trichoderma Viride on Rice Bran under Solid State Fermentation

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Abstract: Although a large number of microorganisms are capable of degrading cellulose, only a few of these microorganisms produce significant quantities of cell-free cellulase enzymes capable of completely hydrolyzing crystalline cellulose *in vitro.* In the present study nearly 25 different strains of *Trichoderma viride* on CMC agar media were isolated and identified and all are having very good capacity to degrade Cellulose enriched media based on their enzymatic properties and clear zone appears around the each colony. The cellulase synthesis was screened by employing different substrate-rice bran, wheat bran, cotton waste, groundnut husk and saw dust by fermentation parameters. The supplement of lactose and corn-steep solid to the media e favored the enzyme formation markedly. The nitrogen sources such as corn steep solid and ammonium nitrate enhanced the production of cellulase enzyme.

Key words: Cellulase · Rice bran · Ammonium Nitrate · Wheat Bran · Cotton Waste · Groundnut Husk · *Trichoderma viride*

INTRODUCTION

Cellulase production is the most important step in the economical production of ethanol, single cell protein and other chemicals from renewable cellulosic materials. Rice bran is the main solid waste generated in Agriculture practices. It accounts for between 15 to 45% of the dry weight of the processed raw material. Worldwide, several million metric tonnes of this residue is produced annually [1]. Rice bran act as an animal food because its high cellulose content. It does not find any significant commercial application till now and most of this byproduct is generally disposed of in open areas, leading to 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.

Plant cell walls are the most abundant renewable source of fermentable sugars on earth [2, 3] and are the major reservoir of fixed carbon in nature [4]. The major components of plant cell walls are cellulose, hemicellulose and lignin, with cellulose being the most abundant component [5]. Plant biomass comprises on average 23% lignin, 40% cellulose and 33% hemicellulose by dry weight [6]. Annually 830 Gt of renewable plant biomass is formed consisting mainly of cellulose and hemicelluloses [7]. It has been reported that solid-state fermentation is an attractive process to produce cellulase economically due to its lower capital investment and lower operating expenses [8]

The objective of the present study was to screen efficient *Trichoderma viride* strain and to examine the potential of using rice bran as a substrate for the production of cellulase by efficient strain of *Trichoderma viride.*

MATERIALS AND METHODS

Collection of Fruits and Vegetables Sample: The decaying fruits and vegetables samples were collected in different localities of Bellary and adjoining areas. Decaying fruits and vegetables samples (50g each) were collected from fruit and vegetable from city market in Bellary and the center of the field from various places. The composite fruit and vegetable samples were collected from a particular field in the polythene bag and labeled.

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Enumeration of Fungi from Fruits and Vegetables Samples: A large number of cellulose degrading fungi of different groups are found in decaying fruits and vegetables. The fruits and vegetables borne fungi can be isolated by serial dilution methods on potato dextrose agar (PDA) medium, incubate the plates at 25-30° C for 5 to 6 days and their total population enumerated by the standard methods.

Isolation and Identification of Trichoderma Viride: Fungal colonies of different sizes and colors grow on potato dextrose agar (PDA) medium. In the present investigation, Many *Trichoderma viride strains* were identified from various infected fruits and vegetables.

Small amount of mycelium grown was taken by sterile needle and transferred to glass slide containing one drop of cotton blue stain and observed under the microscope.

Cultures were identified according to conidiophores, shape of the phialides and emergence of phialophores and phialospores. The purified and identified cultures of *Trichoderma viride* were maintained on PDA medium and stored at 4°C for further use.

Screening of Cellulase Producers on CMC Medium: All these fungal strains are screened for Qualitative test. Fungal colonies were grown on Carboxy methyl cellulose (CMC) agar media composition (g/l): CMC-5.0g, $NaNo_3$-1.0g, K_2HPo4-1.0g, Kcl-1.0g, $MgSo_4$-0.5g, Yeast extract-0.5g, Glucose-1.0g, Agar agar 17.0g and pH -5.5.

Screening of Agro Industrial Wastes for the Production of Cellulase by Trichoderma spp. (SSF): Cellulase production experiments were carried out in 250 ml flasks containing 10 g wheat bran, rice bran, cotton waste, ground nut husk, coir pith and saw dust are moistened with distilled water to a moisture level of 70%. All flasks were sterilized at 121°C for 30 min, inoculated (10^8 spores/flask) and then incubated at 30°C for 6 days. The samples were withdrawn at regular intervals to determine enzyme activities.

Based on the optimum culture time, moisture level, culture temperature and inoculum size, different carbon sources (glucose, fructose, maltose, starch, sucrose, lactose, avicel, carboxy methyl cellulose at 2% w/w) and nitrogen sources (peptone, yeast extract, corn-steep solid, sodium nitrate, ammonium sulphate, ammonium nitrate at 1% w/w) were added to the fermentation medium, respectively, to evaluate the influence of different carbon sources and nitrogen sources on cellulase production.

Assay of Cellulase: During the process of cellulase 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 minutes. One unit (U) of enzyme activity was defined as the amount of enzyme, which liberates 1µmol of glucose equivalent from carboxy methyl cellulose per minute. 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

In the present study, 25 different strains of *Trichoderma viride* grown on potato dextrose agar (PDA) medium which were isolated from various infected fruits and vegetables in Bellary District. Further all these fungal strains are screened for Qualitative test. It was noticed that most of the strains had shown cellulase degradation activity.

The maximum cellulase activity was observed by strains DLW-23 isolated from *Dolichos lab lab* and GSG-12 from *Gossypium herbaceum*. Another strain DLG-22, isolated from *Dolichos lab lab* was also exhibited maximum cellulose activity. Considerable cellulase activity was noticed by GSW-14 isolated from *Gossypium herbaceum* and MPG-10 from *Malus pumila. T. viride* DLW23 was the efficient strain in the cellulase production and it was used for the cellulase enzyme production. (Table 2).

Cellulase production under solid state fermentation was checked with different substrates. Maximum cellulase production was observed on rice bran and cotton substrates. Moderate production was observed on ground nut husk. Least production was noticed in saw dust and wheat bran(Table 3).

Cellulase production with supplementation to various carbon sources was checked (Table 4). Lactose supplementation to the medium enhanced maximum production of cellulase. Moderate increase was observed with addition of carboxy methyl cellulase and mannitol. The least production was noticed in the medium supplemented with glucose and fructose.

Table 1: Common fruits and vegetables selected for experimental purposes

Fruits and Vegetables		
Sl. No	Scientific name	Common name
1	*Annona squamosa.* Linn	Custard apple
2	*Malus pumila* Mill	Apple
3	*Gossypium herbaceum* Linn	Cotton
4	*Pisum sativam* Linn	Garden pea
5	*Dolichos lab lab* Linn	Country bean
6	*Hibiscus cannabinus* Linn	Deccan hemp

Table 2: Enumeration of Fungal colonies from various fruits and vegetable by Serial dilution methods

Fruits and Vegetables			Cellulase activity
Sl. No	Scinetific name	No. of Isolates	Inhibition zone (mm)
1	*Annona squamosa*	*T.viride* ASG1	10.0
		T.viride ASG2	7.5
		T.viride ASG3	11.0
		T.viride ASW4	7.5
		*T.viride A*SW5	10.0
		T.viride ASG6	12.0
		T.viride ASW7	8.0
2	*Malus pumila*	*T.viride* MPG8	7.5
		T.viride MPG9	10.0
		*T.viride MP*G10	15.0
		T.viride MPG11	12.5
3	*Gossypium herbaceum*	*T.viride GSG 12*	20.0
		T.viride GSG 13	12.0
		T.viride GSW 14	15.0
		T.viride GSG 15	14.0
		T.viride GSG16	10.0
4	*Pisum sativam*	*T.viride* PSY17	12.5
		T.viride PSG18	10.0
		T.viride PSG19	12.5
		T.viride PSG20	10.0
		T.viride PSG21	5.0
5	*Dolichos lab lab*	*T.viride DLG22*	17.5
		T.viride DLW23	20.0
6	*Hibiscus cannabinus*	*T.viride HCG 24*	10.0
		T.viride HCG 25	7.5

Table 3: Production of cellulose with different substrate sources under solid state fermentation

Sl.No	Treatments	Enzyme activity (U/gds)
1	Control	3.20
2	Rice bran	6.60
3	Cotton waste	5.20
4	Wheat bran	3.32
5	Ground nut husk	4.54
6	Coir pith	3.61
7	Saw dust	3.21

Table 4: Effect of supplementation of rice bran with different carbon sources.

Sl.No	Carbon source	Enzyme activity (U/gds)
1	Control	4.2
2	Glucose	0.8
3	Fructose	1.2
4	Maltose	3.8
5	Sucrose	4.1
6	Lactose	5.8
7	Starch	3.9
8	Mannitol	4.5
9	Carboxy methyl cellulose	4.8

Table 5: Enzyme activity influenced by the supplementation of different nitrogen sources.

Sl.No	Nitrogen source	Enzyme activity (U/gds)
1	Control	3.6
2	Peptone	4.2
3	Yeast extract	0.8
4	Corn-steep solid	7.6
5	Sodium nitrate	3.8
6	Ammonium sulphate,	4.1
7	Ammonium nitrate,	5.8

As shown in Table 4, addition of monosaccharide (glucose and fructose) significantly inhibited the production of cellulase. The possible reason is that glucose usually acted as a catabolite repressor and repressed the enzyme formation. Addition of sucrose and maltose do not enhances the production cellulase. Lactose was considered as a good inducer for cellulase production. The exogenous addition of various nutrients to the medium may improve cell growth and enzyme production [9].

Fig. 1: Plate assay of *T. viride* for cellulase production on CMC agar media

Enzyme production with the supplementation of different nitrogen sources was assayed. The maximum cellulas production was observed in the medium supplemented with corn steep solid followed by ammonium nitrate. Enzyme production was considerably decreased in the medium added with yeast extract. It was reported that good cellulase yield can be obtained with ammonium compound as the nitrogen source [10].

CONCLUSION

Maximum cellulase production was observed on rice bran and cotton substrates. Lactose supplementation to the medium enhanced maximum production of cellulase. The maximum cellulase production was observed in the medium supplemented with corn steep solid followed by ammonium nitrate.

REFERENCES

1. Beatriz G., Y. Remedios, J.L. Alonso and J.C. Parajo 2008. L-Lactic acid production from apple pomace by sequential hydrolysis and fermentation. Bioresource Technol., 99: 308-319.
2. 2.Himmel M.E., M.F. Ruth and C.E. Wyman, 1999. Cellulase for commodity products from cellulosic biomass. Curr. Opin. Biotechnol. 10: 358-364 Kasing Apun, Sarawak., 1995. Cellulase production. PRACTICAL BIOTECHNOLOGY, National Centre for Biotechnology Education, Malaysia.
3. Saleem, F., S. Ahmed and A. Jamil. 2008. Isolation of a xylan degrading gene from genomic DNA library of a thermophilic fungus *Chaetomium thermophile* ATCC 28076. Pak. J. Bot., 40: 1225-1230.
4. Yang, C.H., S.F. Yang and W.H. Liu. 2007. Produciton of xylooligosaccharfides from xylans by extracellular xylanases from *Thermobida fusca*. J. Agri. Food Chem., 55: 3955-3959.
5. Han, S.O., H. Yukawa, M. Inui and R.H. Doi. 2003. Regulation of expression of cellulosomal cellulase and hemicellulase genes in *Clostridium cellulovorans*. J. Bacteriol., 185: 6067-6075.
6. Sa-Pereira, P., H. Paveia, M. Costa-Ferreira and M.R. Aires-Barros, 2003. A new look at xylanases: An overview of purification strategies. Mol. Biotechnol., 24: 257-281.
7. Rauscher, R., E. Wurleitner, C. Wacenovsky, N. Aro, A.R. Stricker, S. Zelinger, C.P. Kubicek, M. Penttila and R.L. Mach. 2006. Trancriptinal regualtion fo xyn1, encoding xylansse 1 in *Hypocrea jecorina*. Eukaroytic Cell., 5: 447: 456.
8. Cen, P.L. and L.M. Xia, 1999. Production of cellulase by solid-state fermentation, Adv. Biochem. Engin. Biotechnol., 65: 68-92.
9. Seiboth, B., B.S. Pakdaman, L. Hartl and C.P. Kubicek, 2007. Lactose metabolism in filamentous fungi: how to deal with an unknown substrate. Fungal Biology Reviews, 21: 42-48.
10. Sun, T., B.H. Liu, Z.H. Li and D.M. Liu, 1999. Effects of air pressure amplitude on cellulase productivity by Trichoderma viride SL-l in periodic pressure solid state fermenter, Process Biochem., 34: 25-29.