ISSN : 0974 - 7435 Volume 7 Issue 3

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 7(3), 2013 [117-120]

Screening and production of cellulase by fungal culture isolated from soil contaminated with cattle dung

G.Nandana, A.Sridevi, G.Narasimha*

Applied Microbiology Laboratory, Department of Virology, Sri Venkateswara University, Tirupati-517502 Andhra Pradesh, (INDIA)

E-mail: gnsimha123@rediffmail.com; dr.g.narasimha@gmail.com

ABSTRACT

In this study a cellulolytic fungi was isolated from soil contaminated with cattle dung for cellulase production. The cellulolytic property of fungal culture was confirmed by plate screening assay based on formation of yellow zone surrounding of fungal culture on Congo red Agar medium plate. The cellulase enzyme was produced by fungal culture in submerged fermentation. The protein content in the fungal filtrate was estimated with 1660 g/ml. The cellulase activities, Filterpaperase (FPase, 1.79U), Carboxymethyl cellulase (0.68U) and β-glucosidase (0.2 U) was exhibited by fungal culture isolated from with cattle dung contaminated soil.

KEYWORDS

Cattle dung contaminated soil;
Cellulolytic fungi;
Cellulase.

INTRODUCTION

Cellulase is a complex enzyme which hydrolysis β-1-4 glycosidic linkages of cellulose or its derivatives. The bioconversion of cellulose to simple soluble reducing sugars by saccharification through biocatalyst cellulose derived from cellulolytic organisms has been suggested as a feasible process and offers potential to reduce use of fossil fuels and reduce environmental pollution[1]. Due to their importance in saccharification, cellulases are widely using in food bewarages, wine, animal feed, textile and laundry, paper and pulp industries as well as in agriculture and for research purpose[2,3]. In view of biotechnological importance of cellulase this enzyme was produced by wide variety of microorganism including bacteria, fungi, and actinomycetes. However, fungi are the most studied organisms because of their higher enzyme yields and capacities to produce complete cellulase production. Several studies were carried out to produce cellulolytic enzymes from bio waste degradation process by many fungal sps such as *Trichoderma, Penicillium, and Aspergillus sps*. These sps are reported to produce high cellulase activity[4-6]. About twenty three mould cultures belonging to the species of *Trichoderma, Aspergillus, Penicillium Rhizopus, and Fusarium* were isolated on a selective basal agar medium containing cellulose powder as main carbon source. In the present study the fungal cultures were isolates from soil contaminated with cattle dung and the cellulolytic property of fungal culture was determined by plate screening method. The cellulase enzyme was produced from isolated fungi and their individual activities were determined.

FULL PAPER

MATERIALS AND METHODS

Collection of soil

The compost soil composed with cattle dung and hay was collected from Coastal area of Andhra Pradesh, India. The compost soil was air-dried and mixed thoroughly to increase homogeneity and shifted through < 2 mm sieve. The collected soil was used for isolation of cellulolytic fungi.

Enumeration of fungi

The Fungal populations in cattle dung soil was enumerated on Czapeck-Dox agar medium with the following composition (g/L) Sodium nitrate: 2.0, Di potassium hydrogen phosphate; 1.0, Magnesium sulphate -0.5; Potassium chloride-0.5;Ferrous sulphate - 0.01;Sucrose-30. Agar -20.00. The pH of the medium was adjusted with PH-5.0. A 20 ml of sterile medium was aseptically transferred to sterile Petri plates and allowed for solidification. After solidification of the medium 0.1 ml aliquots of soil suspension was spreaded uniformly with the help of sterile glass spreader. The plates were incubated at room temperature (28°C±30°C) for 7 days. After incubated, fungal colonies grown on plates were counted. The fungal colonies grown on the medium are sub cultured on the Czapeck-Dox agar slants for further studies.

Screening of cellulolytic fungi

The cellulolytic property of fungal culture *was* confirmed by plate screening test. In this method 1% of CMC was amended with Czapeck -Dox agar media and the pH was adjusted to pH 5. The media was pored in sterile petri dishes, after solidification of media a small hole was put on centre of petri dish aseptically and the culture spores were added to this centre. The plates were incubated for 3days at 30^0 C and 2days at 50^0 C. After incubation the plates were stained with 1% Congo red solution for 15 minutes, after that the Congo red stain was neutralized with 1M Nacl solution. The yellow color zone formation concern the ability of cellulose utilization and enzyme activity of fungal culture.

Preparation of fungal spore inoculums for cellulase production

The mutant fungal culture was grown on Czapeck-Dox agar slants and they were incubated at room temperature for 7 days. After incubation 3ml of sterile distilled water was added for each slant. Fungal spore concentration was determined by haemocytometer. Inoculum density was 2 X 106 spores were used for cellulase production.

Cultivation of fungal cultures for cellulase production

Fungal cultures was cultivated in basal medium with following ingredients in g/L. KH_2PO_4, 2.0; $MgSO_4.7H_2O$, 0.3; $CaCl_2.2H_2O$, 0.3%; $NaNO_3$, 1.4; $NH_4H_2PO_4$, 0.8; Protease peptone, 0.5; yeast extract, 0.3; $NaNO_3$ 1.4; Casamino acids, 0.4; Tween-80, 1 ml and trace elements (mg/litre), $FeSO_4.7H_2O$, 5.0; $MnSO_4.4H_2O$, 1.6; $ZnSO_4.2H_2O$, 1.4 and $CuSO_4.5H_2O$, 1.0. The pH of the medium was adjusted to 5.0. Sterile fifty milliliters of the above growth medium in 250 ml Erlenmeyer flasks were amended with 0.5% W/V cellulose. Inoculums' of mycelial suspension was prepared by flooding the slant with 2 ml of sterile distilled water and was used to inoculate basal medium in the flasks. Inoculated fresh fungal cultures were incubated at 28 ± 20°C on a rotary shaker (180 rpm). The filtrate obtained after removal of mycelial mat by filtration through Whatman Filter paper No. 1, was used as an enzyme source.

Total protein determination

Total Protein content after pretreatment and fermentation was determined according to Lowry method[7].

Enzyme assays

FPase activity (filter paper activity) and CMCase activity (carboxy methyl cellulase activity) in the culture filtrate was determined by Ghose method[8]. β-glucosidase activity was assayed by using the Herr method[9]. Units (IU) of FPase and CMCase were defined as the one micro mole of glucose liberated per minute per milliliter under assay conditions. One unit of β-glucosidase was defined as the amount of enzyme liberating one micro mole of p-nitro phenol per minute per milliliter.

RESULTS AND DISCUSSION

Screening of cellulolytic fungi

The fungal culture isolated from compost soil was

screened for of cellulolytic activity. The cellulolytic nature of the fungal culture was confirmed by clear zone formation on CMC agar plate shown in Figure 1. Maximum yellow zone was formed around the agar well consisiting of fungal culture (spores) from cattle dung soil formation of yellow zone surrounding the fungal culture is a indication of cellulolytic property of fungal culture further this culture was used for cellulase production in submerged fermentation.

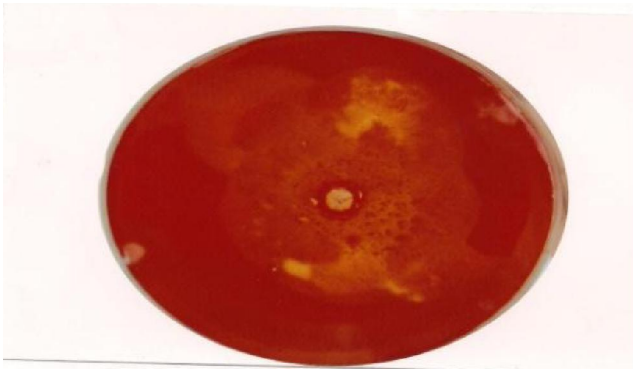

Figure 1 : Plate screening assay for cellulase production.

Production of cellulase by fungal isolate

The fungal isolate was grown on Czapek- Dox medium amended with 1% cellulose as a substrate[10]. Production of individual cellulases like exoglucanse with FPU 1.79 U/ml., CMCase was 1.68 and 0.12U/ml respectively shown in TABLE 1. The enzyme cellulase activity was measured in terms of liberation of micromoles of glucose from substrate per ml /min. whereas for glucosidase activity liberagtion of para nitrophenol from PNPG substrate per ml.hr. Similarly the cellulase from fungal culture *Trichoderma argizienem grown* on micro crystalline cellulose at 10 grams per litre yeilded 3000U/L of CMCase and 400 U/L of filter paper activity and 4U/L cotton activity[11]. Rajasekhar Reddy et al 1998[12] isolated four fungal cultures from soil contaminated with cotton ginning mill effluents and screened for cellulase production. Among the four fungal cultures isolated *Aspergillus niger* showed maximum cellulolytic activity interms of 1.7 U of glucosidase on submergd medium. Similarly Reddi Pradeep and Narasimha 2012[13] also produced cellulase from fungal culture Mutant and native fungal cultures *Aspergillus Niger* with maximum cellulases on both submerged and solid state fermentation. The fungal culture exhibited total protein concentration with 1666 Ug/ml at 7thday of fermentation. Similar reports made Narasimha et al 2006[10] maximum protein content excreated at 7th day of incubation of fungal culture in liquid medium. Similar Reddi Pradeep and Narasimha 2012[13] reported that the Mutant *Aspergillus niger* strain GNEB1 showed highest protein concentration of 2.23 mg/ml, which was more than that of the parent strain (0.60 mg/ml). Simarly the cellulolytic fungal culture was isolated from forest litter soil and cellulase enzyme was isolated from the isolated fungal strain[14].

TABEL 1 : Cellulolytic activity* of fungal culture isolated from cattle dung soil.

Substrate	Fpase[a] (U/ml/h)	CMCase[b] (U/ml/h)	β-glucosidase[c] (U/ml/h)	Protein content (μgs/ml)
Cellulose	1.79	1.68	0.12	1660

***Values represented in the table are mean of two separately conducted experiments.**

a. Filter paperase (Fpase) is expressed in terms of filter pare units. One unit is the amount of enzyme in the filtrate that releasing 1μmole of redusing sugar from filter paper/hour.
b. Corboxymethyl cellulose (CMCase) is expressed in terms of units. One unit is the amount of enzyme releasing 1μmole of redusing sugar from corboxymethyl Cellulose /hour.
c. One unit of β-glucosidase activity is defined as the amount of enzyme liberating 1μmole of p-nitrophenol/hour.

CONCLUSION

A fungal culture was isolated from soil contaminated with cattle dung and the cellulolytic activity of fungal culture was confirmed by plate screening assay method. The fungal isolate produced cellulase enzyme in submerged fermentation. Maximum production of protein content and cellulase is an indication of cellulolytic property of fungal cultures isolated from cattle dung contaminated soil.

REFERENCES

[1] B.E.Dale; Biobased industrial products: Bioprocess engineering when cost really counts, Biotechnol. Prog., **15**, 775-776 **(1999)**.
[2] M.K.Bhat, S.Bhat; Cellulose degrading enzymes

and their potential industrial applications. Biotechnol.Adv., **15**, 583-620 **(1997)**.

[3] M.K.Bhat, S.Bhat; Cellulases and related enzymes in biotechnology, Biotechnology Adv., **18**, 355-383 **(2000)**.

[4] M.Mandels; E.T.Reese; Fungal cellulase and microbial decomposition of cellulosic fibers. Dev.Ind.Microbiol, **5**, 5–20 **(1985)**.

[5] R.M.Hoffman; Wood TM isolation and partial characterization of a mutant penicillum for the saccharification of straw. Biotechnol.Sci.Tech., **3(1)**, 25-34 **(1985)**.

[6] K.Lakshmikant, S.N.Mathur; Cellulolytic activities of chaetomium globosum on different cellulosic substrates. World.J.Microbiol.Biotechnol., **11**, 23-26 **(1990)**.

[7] O.H.Lowry, N.T.Rosebrough, A.L.Farr, R.J.Randall; Protein measurement with the folin phenol reagent, J.Biol.Chem., **193**, 265-275 **(1951)**.

[8] T.K.Ghose; Measurement of cellulase activities, Pure& Appl.Chem., **59**, 257-268 **(1987)**.

[9] D.Herr; Secretion of cellulases and β-glucosides by trichoderma viridae TTCC 1433 in submerged cultures on different substrates. Biotechnol.Bioeng., **21**, 1361-1363 **(1979)**.

[10] G.Narasimha, A.Sridevi, Buddolla Viswanath, M.Subhosh Chandra, B.Rajasekar Reddy; Nutrient effects on production of cellulolytic enzymes by aspergillus niger. African Journal of Biotechnology, **5(5)**, 472-476 **(2006)**.

[11] S.Rousses, M.Raimbault; Hydrolysis of cellulose by fungi. II Cellulase production by trichioderma harzianum in liquid medium fermentation. Ann.Microbiol.Paris, **133B**, 465-474 **(1982)**.

[12] B.Rajasekhar Reddy, G.Narasimha, G.V.A.K.Babu; Cellulolytic activity of fungal cultures, J.Scientific & Indus.Research, **57**, 617-620 **(1998)**.

[13] M.Reddi Pradeep, G.Narasimha; Production of cellulase enzyme by mutant fungal strain A. Niger In submerged fermentation. Journal of Biotechnology, **5(3)**, **(2011)**.

[14] A.Sri Lakshmi, G.Narasimha; Production of cellulases by fungal cultures isolated from forest litter soil. Ann.For.Res., **55(1)**, 85-92 **(2012)**.