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RESEARCH ARTICLE

ISOLATION AND PARTIAL PURIFICATION OF FUNGAL CELLULASES FROM FOREST SOILS OF BHADRA WILDLIFE SANCTUARY, WESTERN GHATS OF SOUTHERN INDIA

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ABSTRACT

Present study was followed with the aim that selection of more efficient strains of fungal species from the forest soils for the production of industrially important fungal cellulase under submerged fermentation (SmF). Isolation and characterization of cellulolytic fungal strains from the nutrient enrichment method yields 22 fungal species. More efficient eight fungal strains were selected based on the rate of zone of clearance on the CMC agar plates by Congored test. Production of cellulase was studied in Czapek broth supplemented with 2% CMC at an optimal conditions of temperature 28±1ºC, pH-7.0 and 5th days of incubation under shake culture. Maximum Corboxymethyl cellulase activity showed by *Penicillium pallidum* (0.059±0.001 U/mL), *Aspergillus terreus* (0.056±0.001 U/mL) and *Trichoderma viridae* (0.052±0.003 U/mL). Filter Paperase activity by *Penicillium pallidum* (0.057±0.001 U/mL), *Trichoderma viridae* (0.054±0.002 U/mL) and *Aspergillus terreus* (0.050±0.005 U/mL) than the other isolates. Soluble crude proteins were maximum by *Penicillium* sp. (170±5 µg/mL), *Penicillium pallidum* (170±2.64 µg/mL) and *Aspergillus niger* (170±2 µg/mL) and partial purified protein was maximum by *Penicillium pallidum* (115±2 µg/mg), *Cunninghamella echinulata* (80±2 µg/mg) and *Trichoderma viridae* (75±2.64 µg/mg) than the other isolates at 5th day of incubation.

INTRODUCTION

Cellulose is one of the most abundant renewable polymers in earth, it is a linear polysaccharide composed of β-1, 4-linked glucose molecules. Cellulolytic microorganisms produce a complex array of glycosyl hydrolases during growth on cellulosic substrates. Filamentous fungi, typically *Trichoderma* and *Aspergillus* species, are the well known and efficient producers of plant cell wall-degrading systems and also preferred sources of industrial cellulase preparations because their capacity for extracellular protein production is greater than that of cellulolytic bacteria (Berlin *et al.,* 2005). These organisms consist of three classes of enzymes endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21). Members of all these classes are necessary to degrade cellulose (Bhat, 2000; Coral *et al.,* 2002). Successful saprophytic survival of a fungus largely depends upon its cellulolytic ability and they are rich source for the production of high amount of extracellular enzymes (Garrett, 1966; Banakar and Thippeswamy, 2011). The fungi colonizing plant litter play very important role in degrading organic matter which remains in a bound and complex form, and in releasing energy rich compounds in the soil (Dwivedi and Singh, 1974). Studies of physiological variation among fungi that degrade wood, cotton, and various food products as well as soil-inhabiting microorganisms, it often is desirable to determine and compare the cellulolytic activity of various species. Conventional methods to determine such activity involve either assessment of activity in culture filtrates or direct study of growing organisms (Rautela and Cowling, 1966). Mc Beth and Scales (1913) first measured cellulolytic ability by determining the magnitude of a clear zone formed in a cellulosic medium. Carboxymethyl cellulases are predominantly endoglucanases able to catalyze the hydrolysis of glycosidic bonds in the soluble, substituted cellulose, Carboxymethyl cellulose (CMC). These enzymes, formerly called CMCase, are important components of the cellulase complex that catalyzes the degradation of crystalline cellulose. Several studies were carried out to produce cellulases under suitable conditions from different cellulolytic organisms including fungi such as *Trichoderma, Penicillium, Aspergillus, Myrothecium, Fusarium* and *Chaetomium* species etc. (Hari Krishna *et al.,* 2000; Hayat *et al.,* 2001; Milala *et al.,* 2005; Singhania *et al.,* 2006; Chandra *et al.,* 2007; Kapoor *et al.,* 2010). Filamentous fungi are preferred for cellulase production in either submerged fermentation (SmF) or solid-state fermentation (SSF) on several carbon sources (Krishna, 2005).

The biotechnology of cellulase and hemicellulases began in the early 1980s, first in animal feed and then in food applications. Subsequently, these enzymes were used in the textile, laundry and pulp and paper industries (de-inking and recycling of paper), as well as in bioconversion of agricultural

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waste and waste cellulose to compounds of economic importance such as glucose, cellobiose and bioethanol (Smith, 1996; Cavaco-Paulo, 1998; Bhat, 2000; Kapoor *et al.,* 2010). Now a days, these enzymes account for approximately 20% of the world enzyme market, mostly from *Trichoderma* and *Aspergillus*. Cellulases are relatively costly enzymes, and a significant reduction in cost will be important for their commercial use in biorefineries. Cellulase based strategies that will make the biorefinery processing more economical includes, increasing commercial enzyme volumetric productivity, producing enzymes using cheaper substrates, producing enzyme preparations with greater stability for specific processes, and producing cellulases with higher specific activity on solid substrates (Zhang *et al.,* 2006). Cellulases and related enzymes from certain fungi are capable of degrading the cell wall of plant pathogens and controlling the plant disease (Bhat, 2000). Present study was taken up to isolate and characterize fungi from forest soils, screen them for cellulase enzyme production and selection of more efficient isolates for industrial applications. The selected strains were studied for the production of cellulase at different time of incubation and they were partially purified.

MATERIALS AND METHODS

Study area and sample collection

Forest soil sample was collected from Bhadra Wildlife sanctuary, organic soil sample was collected in a depth of 5-10 cm by random mixed sampling method in forest by removing upper litter layer. Bhadra Wildlife Sanctuary (493 km^2) is a hot spot biological diversity in the Western Ghats, with a wide range of tree vegetation such as dry and moist deciduous, semi-evergreen and evergreen forests (Champion and Seth 1968). Temperatures in the sanctuary ranges between 9°C (mean minimum temperature in December) and 36°C (mean maximum temperature in March), the mean annual precipitation of 2000 - 2500 mm most of which occur during the southwest monsoon (July to September) (Raju and Hegde 1995).

Isolation and characterization of more efficient cellulolytic fungal species

Enrichment technique: Isolation of cellulolytic fungi was done by enrichment technique (Reese and Mandels, 1963). Inoculated moist chopped rice straw (5 g.) with soil sample (5 g.) and farmyard manure, incubated the samples at 30ºC in incubator for one week.

Qualitative screening: Isolation of effective microorganisms were done by using inoculums from the enrichment technique, they were plated on Czapek agar plates supplemented with 2% Corboxymethyl cellulose sodium salt (CMC) (HiMedia) as a carbon source and Tetracycline (10-25 µg/mL) to control the bacterial contamination (pH 7.0), incubation was done at 28 ± 1ºC for 5-7 days. After incubation fungal species were purified and subcultured on CMC agar and Czapek agar plates and used for the subsequent purpose. Secondary screening was done for the selection of more potent colonies for the production of extracellular cellulase by culturing on CMC agar. Actively growing mycelium (3 days old) were removed from the growing edge of the fungal isolates by using sterile cork borer of 6 mm dia., the discs were inoculated to the pre-welled CMC agar (pH 7.0) plates and incubated at 28±1ºC for 5-7 days. After incubation plates were flood with Congo red solution (1mg/ml in distilled water) for 20 min, decant the dye and flooded with 5 M NaCl for 20-30 min and decanted it. Carboxymethyl cellulase (CMCase) producing colonies were seem to be surrounded by the pale orange to clear against the background (Teather and Wood, 1982).

Characterization of cellulolytic fungi: The fungal species were grown on the CzA plates, identified based on Cultural and morphological characteristics using standard manuals (Pitt 1979; Domsch *et al.,* 1980; Ellis and Ellis 1987; Gilman 2001; Nagamani *et al.,* 2006).

Submerged state fermentation

Cultures were grown in 250 ml Erlenmeyer flask containing 100 ml of Czapek broth (pH 7.0), contains (gl^{-1}) 3 g of $NaNO_3$, 1 g of K_2HPO_4, 0.5 g of $MgSO_4.7\ H_2O$, 0.5 g of KCl, 0.01 g of $FeSO_4.\ 7\ H_2O$, 30 g of Sucrose and 20 g of Corboxy methyl cellulose (CMC) were used for cellulase enzyme production. After the sterilization of the Erlenmeyer flask containing fermentation medium at 121ºC for 15 min., young fungal myceliums of 3 day old cultures were used to inoculate the flask culture media. Cultures were incubated in the incubator shaker operating at 120-180 rpm at 28±1ºC, aseptically transferred 10 ml of incubated broth from the culture flasks at different time intervals (24, 48, 72, 96 and 120 hrs). Mycelia and spores were harvested by centrifugation under 4˚C at 10000 rpm for 10 min., the supernatants obtained from centrifugation were used as the crude enzyme sources for the quantification of CMCase and Filter Paperase enzyme activity and soluble protein content (Irfan *et al.,* 2010).

Protein estimation: The protein concentration was determined by the Lowry's method, as described by Lowry's (1951) using bovine serum albumin (BSA) as a standard, absorbance was read at 660 nm using JENWAY-6305 UV-VIS Spectrophotometer. The culture filtrates from the culture flasks at different time of incubation and lyophilized proteins were used for the quantification of proteins.

Estimation of reducing sugars: The glucose concentration was determined by DNS method, as described by Miller (1959) using glucose as a standard. The color developed was measured at 540 nm using JENWAY-6305 UV-VIS Spectrophotometer.

Enzyme Assay

Determination of Carboxymethyl Cellulase (CMCase) (Endoglucanase) activity: Supernatants from the incubated shake culture flasks at different time intervals (24, 48, 72, 96 and 120 hrs) were used as crude CMCase, activity was assayed using a method described by Wood and Bhat (1998). The CMCase activity was determined using 2% CM-cellulose as substrate, prepared in sodium citrate buffer (50 mM, pH 5.0). The reaction mixture containing equal amounts of enzyme (0.5 mL) and substrate (0.5 mL) by maintaining a blank containing enzyme (0.5 mL) with buffer instead of substrate was incubated at 50˚C for 30 min. The reactions were stopped by addition of 1 mL of DNS reagent followed by

boiling for 5 min. and cooled in ice water then add 10 mL of distilled water and its optical density was read at 540 nm against blank. A standard curve of glucose (1 mg/mL) was developed under identical conditions to determine the reducing sugars formed. The enzymatic activity of filtrate was expressed as Unit per ml (U/ml), which is defined as the amount of enzyme which liberates 1 µmole of reducing sugar per mL per minute under assay conditions.

Determination of Filter Paperase (FPase) activity: The activity of FPase or endo β-1,4 glucanase was measured according to the method of Ghose (1987) and Wood and Bhat (1998). Supernatants from the incubated shake culture flasks at different time intervals (24, 48, 72, 96 and 120 hrs) were used as crude Filter Paperase (FPase), activity was assayed by using Whatman No. 1 filter paper as substrate, the reaction mixture contained a strip of Whatman No. 1 filter paper (1x6 cm) approximately 50 mg was soaked in 0.5 mL of sodium citrate buffer (50 mM, pH 5.0). The reaction mixture containing equal amounts of enzyme (0.5 mL) and substrate (0.5 mL) by maintaining a blank enzyme (0.5 mL) with buffer instead of substrate was incubated at 50˚C for 60 min. The reaction was stopped by adding 1.0 mL of 3, 5-Dinitro salicylic acid (DNS) reagent, the mixture was boiled under water bath for 5 min. and cooled in ice water then add 10 mL of distilled water and its optical density was determined at 540 nm against blank. A standard curve of glucose (1 mg/mL) was developed under identical conditions to determine the reducing sugars formed. The activity is expressed as filter paper units (FPU) per mL, where one unit of FPase activity (U) was defined as the amount of the enzyme that released 1 µmol of glucose per mL per min under assay conditions.

Partial purification of Enzymes: The contents of the culture flasks were filtered through Whatmann's filter paper after the incubation and centrifuged under refrigerated condition at 8000 rpm for 10 min to remove the fungal cells and spores. The crude enzyme was precipitated under magnetic stirrer at 4ºC in different concentrations of ammonium sulfate solution up to the saturation level from 20-90%, protein precipitate was collected by refrigerated centrifugation at 10000 rpm for 10 min. The pellet was suspended in sodium citrate buffer (pH 5.0) and dialysed against 2-3 changes of buffer for overnight under refrigerated condition. The dialysed crude enzyme preparation was lyophilized and used for the subsequent studies. Quantification of powdered forms of proteins was done by following Lowry's method.

RESULTS

Isolation and characterization of more efficient cellulolytic fungal species

A total of 22 cellulolytic fungal species were isolated on the 2% CMC agar plates from the forest soils by nutrient enrichment method, cultural and morphological characters of isolates were examined and identified. The strains were further screened for qualitative cellulolytic activity on CzA plates supplemented with CMC based on the rate of zone of clearance around the fungal colony. They were differentiated as less (+), moderate (++) and good (+++) cellulolytic activity (Table 1). The good eight cellulolytic fungal strains were selected among the isolated species, the selected fungal species like, *Penicillium* sp., *Aspergillus chevalieri, Aspergillus terreus, Penicillium*

Table 1. Isolated fungal species showed different rate of cellulolytic activity on CMC agar.

Sl. No.	Fungal Isolates	Cellulolytic Activity
1	*Penicillium documbens*	++
2	*Penicillium westlingi*	+
3	*Trichoderma viridae*	+++
4	*Penicillium restrictum*	++
5	*Aspergillus terreus*	+++
6	*Penicillium javanicum*	++
7	*Penicillium* sp.	++
8	*Bipolari sacchari*,	+
9	*Penicillium lilacinum*	+
10	*Cordona pauciseptata*	+
11	*Eupenicillium* sp.	+
12	*Cladosporium herbarum*	++
13	*Penicillium* sp.	+++
14	*Penicillium pallidum*	+++
15	*Penicillium* sp.	++
16	*Mucor hiemenis*	++
17	*Mucor* sp.	+
18	*Verticillium* sp.	+
19	*Aspergillus chevalieri*	+++
20	*Trichoderma* sp.	+++
21	*Cunninghamella echinulata*	+++
22	*Aspergillus niger*	+++

Note **:** + = Less, ++ = Moderate and +++ = Good

pallidum, Aspergillus niger, Cunninghamella echinulata, Trichoderma viridae and *Trichoderma* sp. The good cellulolytic cultures were used for extracellular cellulase production using submerged fermentation.

Table 2. Carboxymethyl Cellulase (CMCase), Filter paperase (FPU) activity and specific activity along with protein content of fungal species at 5th day of incubation

Sl. No.	Fungal Species	CMCase*		Filter Paperase*		Protein content*	
		Activity (IU/mL)	Specific Activity (IU/mg)	Activity (IU/mL)	Specific Activity (IU/mg)	Crude (µg/mL)	Partially purified (µg/mg)
1	*Penicillium* sp.	0.027±0.001	0.16±0.02	0.043±0.002	0.25±0.01	170±5	40±2
2	*Aspergillus chevalieri*	0.030±0.004	0.19±0.01	0.043±0.001	0.27±0.02	156±2	35±1.73
3	*Aspergillus terreus*	0.056±0.001	0.38±0.01	0.050±0.005	0.34±0.02	146±2	30±2
4	*Penicillium pallidum*	0.059±0.001	0.35±0.01	0.057±0.001	0.34±0.01	170±2.64	115±2
5	*Aspergillus niger*	0.030±0.004	0.17±0.01	0.041±0.002	0.24±0.02	170±2	35±1
6	*Cunninghamella echinulata*	0.033±0.001	0.31±0.02	0.043±0.001	0.40±0.02	106±2	80±2
7	*Trichoderma viridae*	0.052±0.003	0.31±0.01	0.054±0.002	0.32±0.02	166±2.64	75±2.64
8	*Trichoderma* sp.	0.035±0.001	0.21±0.01	0.041±0.001	0.25±0.01	166±2	40±2

*Mean of three replicates ± standard deviation (SD)

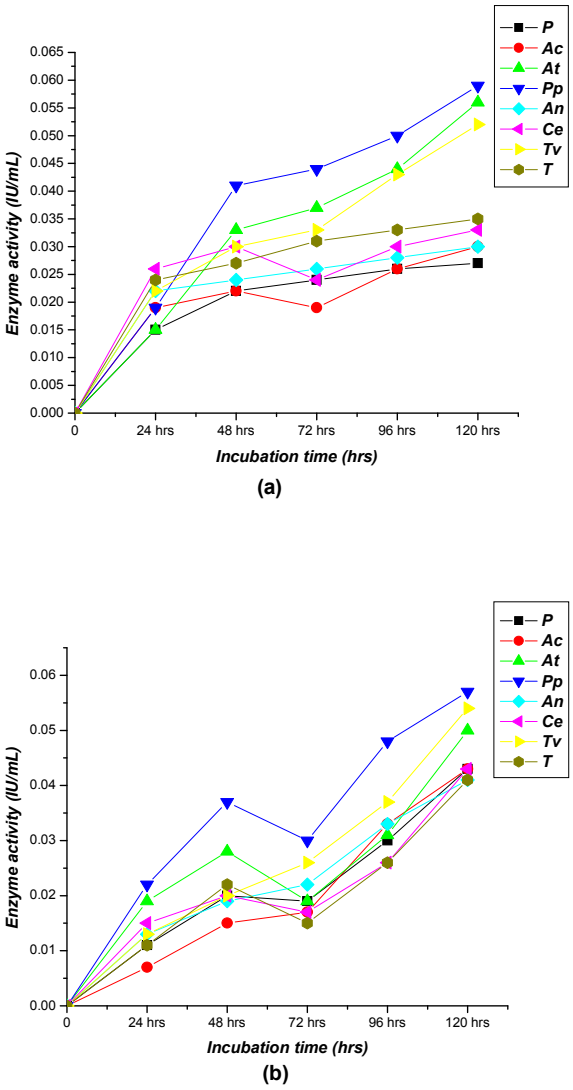

Fig. 1. CMCase (a) FPase (b) activity of fungal species at different time of incubation (P - *Penicillium* sp., Ac - *Aspergillus chevalieri*, At - *Aspergillus terreus*, Pp - *Penicillium pallidum*, An - *Aspergillus niger*, Ce - *Cunninghamella echinulata*, Tv - *Trichoderma viridae* and T - *Trichoderma* sp.)

Enzyme assays

More efficient cellulolytic soil fungal species were assayed for extracellular endogluconase and filter paperase activity at different time of incubation (24, 48, 72, 96 and 120 hrs) and they were showed varied levels of enzyme activities among the species investigated. All eight investigated species were showed very high cellulolytic and filter paperase activity at 120 hrs of incubation and some species were showed slight increased or decreased rate of activity at 2nd an 3rd day of incubation (Fig. 1(a) & (b)).

Carboxymethyl Cellulase (CMCase) activity: Culture filtrates were assayed for endogluconase (CMCase) activities, the following fungal species showed different rate of activities under assay conditions. *Penicillium pallidum* showed maximum activity (0.059±0.001 U/mL) at 5th day of incubation followed by *Aspergillus terreus* (0.056±0.001 U/mL), *Trichoderma viridae* (0.052±0.003 U/mL),

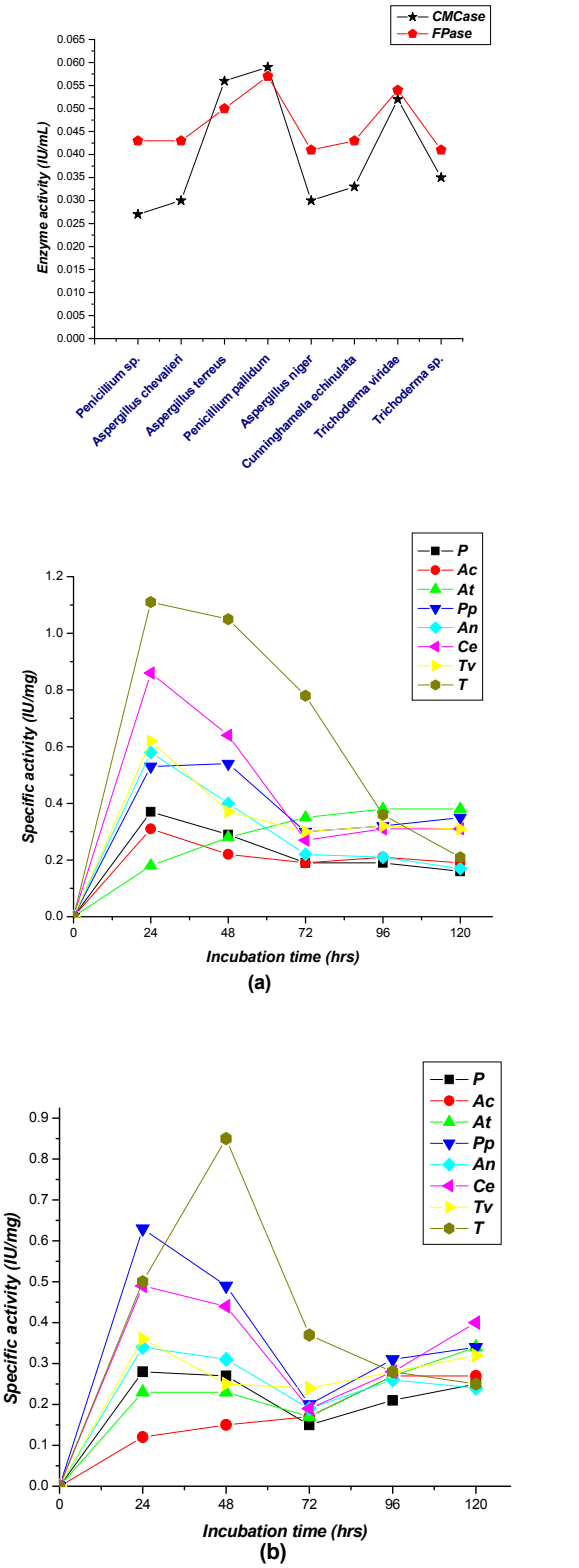

Fig. 3. Specific activity of CMCase (a) and FPase (b) at different time of incubation (P - *Penicillium* sp., Ac - *Aspergillus chevalieri*, At - *Aspergillus terreus*, Pp - *Penicillium pallidum*, An - *Aspergillus niger*, Ce - *Cunninghamella echinulata*, Tv - *Trichoderma viridae* and T - *Trichoderma* sp.)

richoderma sp. (0.035±0.001 U/mL), *Cunninghamella echinulata* (0.033±0.001 U/mL), *Aspergillus chevalieri and Aspergillus niger* (0.030±0.004 U/mL) and *Penicillium* sp. (0.027±0.001 U/mL) (Fig. 1(a), & 2) (Table 2). Specific activity of endogluconase (CMCase) at 5th day of incubation from *Aspergillus terreus* was maximum (0.38±0.01 U/mg) at 5th day of incubation followed by *Penicillium pallidum* (0.35±0.01 U/mg), *Trichoderma viridae* (0.31±0.01 U/mg), *Cunninghamella echinulata* (0.31±0.02 U/mg), *Trichoderma* sp. (0.21±0.01 U/mg), *Aspergillus chevalieri* (0.19±0.01 U/mg), *Aspergillus niger* (0.17±0.01 U/mg) and *Penicillium* sp. (0.16±0.02 U/mg) (Fig. 3(a)) (Table 2).

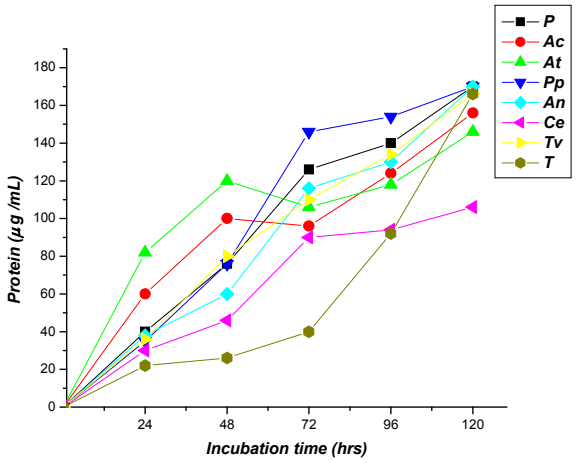

Fig. 4. Soluble crude protein content at different time of incubation (P - *Penicillium* sp., Ac - *Aspergillus chevalieri*, At - *Aspergillus terreus*, Pp - *Penicillium pallidum*, An - *Aspergillus niger*, Ce - *Cunninghamella echinulata*, Tv - *Trichoderma viridae* and T - *Trichoderma* sp.)

Filter Paperase (FPase) activity: Culture filtrates were assayed for Filter paperase (FPase) activities, the following fungal species showed different rate of activities under assay conditions. *Penicillium pallidum* showed maximum activity (0.057±0.001 U/mL) at 5th day of incubation followed by *Trichoderma viridae* (0.054±0.002 U/mL), *Aspergillus terreus* (0.050±0.005 U/mL), *Penicillium* sp. (0.043±0.002 U/mL), *Aspergillus chevalieri* and *Cunninghamella echinulata* (0.043±0.001 U/mL), *Aspergillus niger* (0.041±0.002 U/mL) and *Trichoderma* sp. (0.041±0.001 U/mL) (Fig. 1(b) & 2) (Table 2). Specific activity of Filter paperase (FPase) at 5th day of incubation from *Cunninghamella echinulata* was maximum (0.40±0.02 U/mg) at 5th day of incubation followed by *Penicillium pallidum* (0.34±0.01 U/mg), *Aspergillus terreus* (0.34±0.02 U/mg), *Trichoderma viridae* (0.32±0.02 U/mg), *Aspergillus chevalieri* (0.27±0.02 U/mg), *Penicillium* sp. and *Trichoderma* sp. (0.25±0.01 U/mg) and *Aspergillus niger* (0.24±0.02 U/mg) (Fig. 3(b)) (Table 2).

Protein Content

Soluble crude proteins were more in *Penicillium* sp., *Penicillium pallidum* and *Aspergillus niger* (170±2 µg/mL) followed by *Trichoderma viridae* (166±2.64 µg/mL) *Trichoderma* sp. (166±2 µg/mL), *Aspergillus chevalieri* (156±2 µg/mL), *Aspergillus terreus* (146±2 µg/mL) and *Cunninghamella echinulata* (106±2 µg/mL) they were obtained from the culture filtrates of fungal species grown at 5th day of incubation (Fig 4) (Table 2.). Powdered forms of partially purified proteins were obtained from the dialysed proteins of fungal culture filtrates at 5th day of incubation, maximum concentration of protein was showed by *Penicillium pallidum* (115±2 µg/mg), *Cunninghamella echinulata* (80±2 µg/mg), *Trichoderma viridae* (75±2.64 µg/mg), *Penicillium* sp. and *Trichoderma* sp. (40±2 µg/mg), *Aspergillus chevalieri* (35±1.73 µg/mg) *Aspergillus niger* (35±1 µg/mg) and *Aspergillus terreus* (30±2 µg/mg) (Table 2.).

DISCUSSION

The present study showed that forest soil is rich source of biodiversity of lignocellulolytic fungal species, those were plays an important role in the biogeochemical cycles in the environment. The indigenous microflora from the forest soils was more potent for the production of industrially important cellulolytic enzymes. Cellulose is the most abundant and renewable source of energy on earth (Suto and Tomita, 2001; Guedon *et al.,* 2002), its conversion to soluble sugars is preferably enzymic hydrolysis and involves cellulases that provide a key opportunity for achieving the tremendous benefits of biomass utilization (Himmel *et al.,* 1999). cellulases are produced by a number of microorganisms, the yields are still low due to catabolite repression and end-product inhibition (Yoshihiko and Takahisa, 2002; Nairn and Jarnil, 2007). Nutrient enrichment technique and solid medium providing rapid assays were very helpful for the direct enumeration and isolation of more efficient extracellular cellulase producing fungal strains from the natural environments (Hankin and Anagnostakis, 1977; Banakar and Thippeswamy, 2011). During our study more number of filamentous fungal species showed the goood cellulase activity among the other species. During our study we found that maximum endogluconase activity was observed in *Penicillium pallidum* followed by *Aspergillus terreus*, *Trichoderma viridae*, *Trichoderma* sp., *Cunninghamella echinulata*, *Aspergillus chevalieri, Aspergillus niger* and *Penicillium* sp. Maximum FPase activity was showed by *Penicillium pallidum* followed by *Trichoderma viridae*, *Aspergillus terreus*, *Penicillium* sp., *Aspergillus chevalieri* and *Cunninghamella echinulata*, *Aspergillus niger* and *Trichoderma* sp.

Filamentous fungi are considered to be one of the most efficient hyper producers of cellulase that is used in industry. Many microorganisms have been classified as cellulolytic but, only few possess a complete cellulase complex capable of efficient depolymerization of crystalline cellulose. *Trichoderma, Penicillium, Aspergillus, Myrothecium, Fusarium* and *Chaetomium* are common soil fungus, which produces cellulolytic enzymes. Cellulase activity was varied widely between the species. Generally the fungi were more active against water soluble derivatives than on filter paper cellulose. Carboxymethylcellulose (CMC), a water-soluble cellulose derivative, is a useful substrate for detection of CMCase production because it is degraded quickly by microorganisms (Mandels *et al.,* 1976). To achieve maximum cellulase enzyme, researchers have studied various process parameters during fermentations that use filamentous fungi. Ahamed and Vermette (2008) studied the influence of

inoculum size and media composition on *T. reesei* RUT-C30 morphology and cellulolytic enzyme activity. Cellulose consists of long insoluble chains of covalently bonded glucose molecules, which are too large to be transported through cell walls. However, through the use of microorganisms such as, filamentous fungal cells, enzymes known as cellulases are secreted and these enzymes hydrolyze or depolymerise the cellulose into its monomeric glucose components, which can be readily transported through cell wall and subsequently metabolized. Hankin & Anagnostakis (1977) reported the development of media with CMC as the cellulose substrate for use with fungi. Various parameters relating the degree of substitution to production of Corboxymethyl Cellulase (CMCase) and also the rates of hydrolysis of different CMC by cell-free culture filtrates. Cellulase production on Czapek medium, as measure by endogluconase and Filter Paperase was far superior compared to that produced on Mandels medium. The Mandels medium was designed originally designed for cellulase production by *Trichoderma* species (Odegaard *et al.,* 1984). Simple and inexpensive media, Czapek medium is one of the most recommended media suitable for routine work with *Aspergillus* species it was used for the cellulase production experiments (Taj-Aldeen and Alkenany, 1993).

The cellulase is an inducible enzyme system in which several carbon sources have been tested to find the best inducer (Muthuvelayudham, *et al.,* 2005). Cellulose itself has been recognized as one of the best inducer for the complete cellulase complex and the other most important inducers include saphorose and lactose (Mandels *et al.,* 1962; Mandels, 1975; Hari krishna, *et al.,* 2000; Ahamed and Vermette, 2008). Strategies to improve performance typically involve random mutagenesis of fungal strains, genetic engineering of individual 1,4-β-glucanases, and optimization of the ratio of the various 1,4- β -glucanase components, followed by screening for increased specific activity or stability. Generally, screening of cellulase complexes for improved activity uses a standard assay proposed by the International Union of Pure and Applied Chemistry (IUPAC) to measure hydrolysis of filter paper (Ghose 1987). Recently this assay has been automated for high throughput screening (Decker *et al.,* 2003; Berlin *et al.,* 2005). In future, one of the important potential applications of cellulases will be the production of fuel ethanol from lignocellulosic biomass (Duff and Murray, 1996; Olsson *et al.,* 2003), which is a good substitute for gasoline in internal combustion engines.

CONCLUSION

The results from our study indicated that the screening and isolation of indigenous microorganisms will yields maximum quantity at optimum condition. Forest soil is rich source for the isolation of industrially important microorganisms. The 22 isolates were obtained from the nutrient enrichment method, they were able to grow in the medium containing carboxymethyl cellulose as a sole carbon source. Eight species of fungi were selected as potential extracellular cellulolytic enzyme producers by screening and isolation methods. Among them *Penicillium pallidum* showed maximum cellulolytic activity followed by *Aspergillus terreus* and *Trichoderma viridae*. Further investigations will be required to achieve a maximum amount of cellulolytic enzymes by providing optimum conditions.

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