Current Research in Environmental & Applied Mycology 4 (2): 236–249(2014) ISSN 2229-2225

www.creamjournal.org **Article** CREAM

Online Edition

Doi 10.5943/cream/4/2/11

Optimization and characterization of cellulases from thermophilic strain of *Scytalidium thermophilum* SKESMBKU01

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Sujatha E, Santoshkumar S, ShivaKrishna P 2014 – Optimization and Characterization of cellulases from thermophilic strain of *Scytalidium thermophilum* SKESMBKU01.Current Research in Environmental & Applied Mycology 4(2), 236–249, Doi 10.5943/cream/4/2/11

Abstract

Fifteen fungal isolates were isolated from different thermogenic habitats(vegetable market compost, mushroom compost, horse dung, municipal waste, nests of birds, decomposing litter, soils from furnace area, cattle dung, zoo dump and industrial waste.) of Andhra Pradesh. All the fungal isolates were screened for their ability to produce cellulases. The results showed that *Scytalidium thermophilum* SKESMBKU01 found to have high cellulolytic activity at 45°C and pH–5.0. Optimization of enzyme production was studied in different carbon and nitrogen sources at the concentration of 1% and 0.2% respectively. The endo and exoglucanase activities are higher in media containing glucose as their carbon source followed by xylose and lactose. KNO_3 as good nitrogen source for endoglucanase and urea for exoglucanase activity. The organism showed maximum dry weight in malt extract and peptone. The culture filtrate of *S.thermophilum* was dialyzed against distilled water over night and used as enzyme source. The exo and endocellulases produced by the *Scytalidium thermophilum* SKESMBKU01 are highly stable at pH 8.0 and temperature of 85°C. The results indicate that the endo and exocellulases produced by *Scytalidium thermophilum* SKESMBKU01 are more stable at high temperature and alkaline pH.

Key words – *Scytalidium thermophilum* – cellulases – optimization – characterization

Introduction

Cellulose is one of the main components of plant cell wall material and is the most abundant and renewable nonfossil carbon source on the Earth. Degradation of cellulose to its constituent monosaccharides has attracted considerable attention for the production of food and biofuels (Sukumaran et al. 2010). The degradation of cellulose to glucose is achieved by the cooperative action of endocellulases (EC 3.1.1.4), exocellulases (cellobiohydrolases, CBH, EC 3.2.1.91; glucanohydrolases, EC 3.2.1.74), and beta-glucosidases (EC 3.2.1.21). Endocellulases hydrolyze internal glycosidic linkages in a random fashion, which results in a rapid decrease in polymer length and a gradual increase in the reducing sugar concentration. Exocellulases hydrolyze cellulose chains by removing mainly cellobiose either from the reducing or the non-reducing ends, which leads to a rapid release of reducing sugars but little change in polymer length. Endocellulases and exocellulases act synergistically on cellulose to produce cellooligosaccharides and cellobiose, which are then cleaved by beta-glucosidase to glucose (Vlasenko et al. 2010; Duo-Chuan Li et al. 2011). Cellulases

Submitted 11 June 2014, Accepted 12 September 2014, Published online 18 November 2014
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have been commercially available for more than 30 years, and these enzymes have represented a target for both academic as well as industrial research (Singh, 1999, Singh et al. 2007, Ramesh et al. 2011). Basic and applied studies on cellulolytic enzymes have demonstrated their biotechnological potential in various industries including food, animal feed, brewing and wine making, agriculture, biomass refining, pulp and paper, textile, and laundry. The current challenge in biomass conversion by cellulases concerns the degradation of cellulose in an efficient and cheap way. To increase cellulase efficiencies and to lower the cost, cellulases need to be improved to have higher catalytic efficiency on cellulose, higher stability at elevated temperatures and at nonphysiological pH, and higher tolerance to end-product inhibition (Percival et al. 2006). Currently, two main research approaches used in the improvement of cellulases through protein engineering are: structure-based rational site-directed mutagenesis and random mutagenesis through directed evolution. Site-directed mutagenesis requires detailed knowledge of the protein's 3D structure. On the other hand, the directed evolution approach is not limited by the lack of the protein's 3D structure but requires an efficient method for high throughput screening (Labrou, 2010). Thermophilic fungi are species that grow at a maximum temperature of 50°C or above, and a minimum of 20°C or above (Maheshwari et al. 2000). Based on their habitat, thermophilic fungi have received significant attention in recent years as a source of new thermostable enzymes for use in production of ethanol (Gnansounou, 2010), organic acids (Rao et al. 2001) and other chemicals. In this investigation, a cellulase producing strain of *Scytalidium thermophilum* SKESMBKU01, isolated from mushroom compost, were subjected to optimization of media and cultivation parameters for cellulase production.

Materials & Methods

Isolation and maintenance of thermophilic fungi

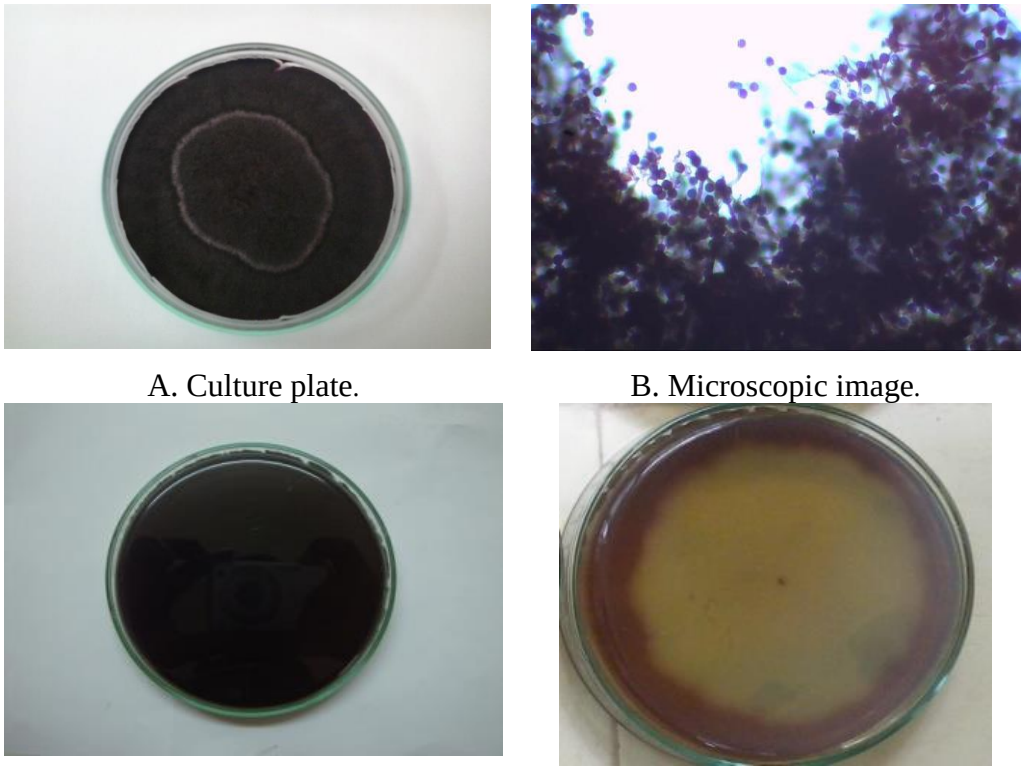
Thermophilic fungi were isolated from different thermogenic habitats (nests of birds, decomposing litter, soils from furnace area, cattle dung, zoo dump, industrial waste, vegetable market compost, mushroom compost, horse dung and municipal waste) of Warangal, Andhra Pradesh, India. Serially diluted samples poured on yeast-starch agar medium (YpSs: yeast extract-5gm, starch-15gm, K_2HPO_4-1gm, $MgSO_4$-0.5gm per 1000ml of distilled water), yeast-extract glucose agar (yeast extract-5gm, glucose-15gm, K_2HPO_4-1gm, $MgSO_4$-50gm per 1000ml of distilled water) medium and incubated at 45°C for 3–4 days. Colonies were picked and sub-cultured to obtain pure cultures and identified based on their morphological characters and maintained on YpSs agar media (Cooney & Emerson 1964).

Screening for cellulolytic activity

All the 15 isolates were screened for cellulolytic activity on selective carboxy methyl cellulose agar (CMC) containing gm/L: $NaN0_3$-2.0, KH_2PO_4-1.0, $MgSO_4.7H_2O$-0.5, KCl-0.5, carboxy methyl cellulose sodium salt-2.0, peptone-0.2, and agar17.0 (Sacin Talekar et al. 2011). Plates were spot inoculated with spores of pure culture and incubated at 45°C. After 3days of incubation plates were flooded with Grams iodine (2.0g KI and 1.0g iodine in 300ml distilled water) for 5 minutes, the diameter of zone of decolorization around each colony was measured. The fungal colony showing largest zone of decolorization was selected for cellulase production (Ramesh et al. 2008).

Identification of thermophilic fungi

Among the isolates screened for the cellulolytic activity, *Scytalidium thermophilum* was the most potent cellulolytic organism. Thermophilic fungi were identified based on the analysis of the ITS sequences of rDNA regions. DNA of the fungal isolate was extracted by the thermolysis method according to the protocols described by Zhang et al. (2010). The ITS regions were amplified by the polymerase chain reaction (PCR) with the universal primer pair ITS1 (5'-TCCGTAGGTGAACCTGCGG -3') and ITS4 (5′ TCCTCCGCTTATTGATATGC -3′) (Jingfeng et al. 2013). PCR products were purified and sequenced by Macrogen Laboratories



A. Culture plate. B. Microscopic image.

C. Control plate. D. Cellulolytic plate.

Fig.1 *Scytalidium thermophilum* SKESMBKU01.

(www.macrogen.com).The sequences were identified to genus and species level by querying the GenBank database, using the nucleotide-blast search option, available through the National Center for Biotechnology Information (website: http://blast.ncbi.nlm.nih.gov/Blast.cgi). Based on the sequence data analysis, the organism was identified as *Scytalidium thermophilum* and deposited in EMBL, accession number is HG738855.1 *Scytalidium thermophilum* SKESMBKU01).

Enzyme assay

(a) Carboxymethyl cellulase (CMCase) or endoglucanase activity: Production of cellulases was studied in Mandel and Weber media (containing mg/L: $(NH_4)_2SO_4$ -1,400, KH2PO_4-2,000, $CaCl_2 \cdot 2H_2O$-300, $MgSO_4 \cdot 7H_2O$-300, $FeSO_4 \cdot 7H_2O$-5.0, $MnSO_4 \cdot H_2O$-1.6, $ZnSO_4 \cdot 7H_2O$-1.4, $CoCl_2 \cdot 6H_2O$-2.0, Peptone-100, Tween-80-100). The pH was adjusted to 5.5 and carbon source were added at a concentration of 1 percent (Harinder et al. 2010). The carboxymethyl cellulase (CMCase) or endoglucanase activity determined by the method described by Ghose, (1987).The enzymatic reaction mixture contains 0.5ml of 1% carboxymethyl cellulose, 1ml of 0.05M phosphate buffer, (pH 5.5) and 0.5ml of enzyme filtrate and incubated at 60°C for 30 minutes. The enzyme reaction was stopped by adding 3ml of DNS regent and boiled for 10 minutes vigorously in boiling water bath. The amount of glucose liberated is measured at 575nm against heat killed enzyme as blank. One unit of carboxymethyl cellulase activity on cellulosic substrates was defined as 1µmol glucose (reducing sugars as glucose) per minute at 60°C during the hydrolysis reaction.

(b) Exoglucanase activity: Exoglucanase activity was performed on cellulose powder (Himedia). The reaction mixture (2 ml) contained 1 ml of 1% suspension of the substrate and 1ml of enzyme filtrate and incubated for 1hour at 60°C in water bath. The enzyme reaction was stopped by addition of 3ml of DNS regent and boiled for 10min in boiling water bath. The color developed was read at 575nm using heat killed enzyme as blank (Bhat & Ramesh Maheshwari, 1987). One unit of exoglucanase activity on cellulosic substrates was defined as 1 µmol glucose (reducing sugars as glucose) liberated per minute at 60°C during the hydrolysis reaction. Reducing sugars were determined by the dinitrosalicylicacid (DNS) method (Miller, 1959).

Optimization of Carbon and Nitrogen sources

Optimization of cellulase production was studied in different carbon sources (Glucose, Xylose, Cellulose, Starch, CMC, Sucrose, Maltose, fructose and Lactose) at a concentration of 1% to the production medium. The nitrogen sources such as peptone, yeast extract, malt extract, beef extract, urea. $(NH_4)_2$ SO_4, $NaNO_3$, KNO_3, NH_4Cl were added at a concentration of 0.2% to the Mandel and Weber fermentation medium. All the flasks were incubated at 45°C ± 2 in an orbital shaker incubator at 100 rpm. At regular intervals enzyme assay was carried out (Sujatha et al. 2006).

Effect of pH on cellulase production

Effect of pH on enzyme production was studied at different pH ranges from 3.0 to 10.0 of production medium. The pH of the medium was adjusted by using 1N HCl or 1N NaOH. After inoculation with *Scytalidium thermophilum* the flasks were kept in an orbital shaker incubator at 45°C at 100 rpm. After 3days of growth the culture filtrate was harvested and dialyzed against distilled water over night. The concentrated filtrate was used as enzyme source (Moses et al. 2012).

Effect of temperature on cellulase production

Effect of temperature on enzyme production was carried out by incubating the *Scytalidium thermophilum* at different temperatures 45°C, 50°C, 55°C in orbital shaker incubator at 100 rpm for three days of incubation. After regular intervals, enzyme assay was performed (Sadaf et al. 2005).

Effect of static and agitated conditions on cellulase production

There were two sets of flasks (*Scytalidium thermophilum*) containing media were prepared to check the effect of static and agitated conditions (100, 150, 200 rpm) on enzyme production (Mahdi et al.2011). In both sets, all the conditions (pH, temperature, inoculums size, substrate concentration) were kept similar. One set was kept in orbital shaker incubator at 100 rpm, while other was kept in static conditions. After regular intervals (3, 6, 9, 12 days) the culture filtrate was subjected to fractional ammonium sulphate precipitation (60–80%saturation). The resultant precipitated proteins were collected by centrifugation at 10000 rpm for 15 min. The precipitate was dissolved in phosphate buffer, (pH 5.5) and dialyzed overnight against the same buffer. The resultant dialyzed culture filtrate was used as enzyme source.

Determination of fungal biomass

After appropriate incubation period (3, 6, 9, 12 days) the contents of the flasks were aseptically passed through pre-weighed Whatman No.1 filter paper to separate mycelial mat from culture filtrates. The filter papers along with mycelial mat were dried at 70°C in an oven for overnight and weight was recorded. Difference between the weight of the filter paper bearing mycelia mat and weight of pre-weighed filter paper represented fungal biomass, which was expressed in terms of dry weight of mycelial mat in milligrams (Shilpi et al. 2011).

Effect of pH and temperature on the activity and stability of the enzyme

Cellulase activity at different pH and temperature were carried out under standard assay conditions to determine stability of enzyme activity. The pH stability of the enzyme was measured by incubating 1ml of enzyme for one hour, at 45°C, in a buffer of desired pH (3 to 10). The temperature stability was determined by incubating 1 ml of enzyme, at varying temperatures (30°C to 80°C) for one hour, and then estimated the residual enzyme activity under standard assay conditions (Quiroz et al. 2009).

Results

Screening for cellulolytic activity

All the 15 strains were screened for cellulolytic activity (Table 1). All the tested strains were capable of producing cellulase in varying degrees. The most potent strain was isolated from

Table 1 Fifteen isolates of thermophilic fungi and zone of cellulolysis in Centimeters**.**

S.No	Name of the organism	Zone of Cellulolysis in Centimeters
1.	*Chaetomium thermophile var. dissitum*	2.5
2.	*Humicola insolens*	2.0
3.	*Humicola* sp.	2.0
4.	*Malbranchea pulchella var. sulfurea*	2.0
5.	*Malbranchea* sp.	2.5
6.	*Penecillium duponti*	1.5
7.	*Penecillium* sp.	1.5
8.	*Rhizomucor pusillus*	2.5
9.	*Scytalidium thermophilum* SKESMBKU01	3.0
10.	*Sordariales* sp.AP-2012 strain	2.5
11.	*Sporotrichum thermophile*	2.5
12.	*Sporotrichum*	3.0
13.	*Thermoascus aurantiacus*	1.0
14.	*Torula thermophila*	2.5
15.	*Torula* sp.	2.0

mushroom compost with plate clearing zone of 3.0 cm in diameter. *Scytalidium thermophilum* SKESMBKU01 species was widely grown on YpSs agar at 45°C. Colonies on YpSs agar at 45°C are white at first but soon turns greyish to jet-black as spore maturation proceeds. Hyphae colourless, prostrate, branched, septate, 2–5 µm wide. Conidiogenous cells small, 8.7 × 3.7 µm, conidia dark brown, smooth walled, translucent, generally globose, 7–12.5 µm in diameter, or oval 11.2–14.6 × 7.5–10 µm, produced basipetally in chains on hyphal branches or developed intercalarily (Salar et al.2007) (Fig 1).

Optimization of carbon for cellulase production

Nine different carbon sources were chosen to elucidate the best of them which is suitable for maximal production of cellulases. Maximum endo and exoglucanase production was attained in glucose (0.897 U/ml^{-1}) followed by xylose (0.735 U/ml^{-1}) and lactose (0.540 U/ml^{-1}) by *Scytalidium thermophilum* SKESMBKU01 (Table 2, 3).

Table 2 Optimization of carbon sources.

Name of thermophilic fungi	Carbon sources	Endoglucanase activity/ U/ml^{-1} Incubation period (days)				Exoglucanase activity/ U/ml^{-1} Incubation period (days)			
		3rd	6th	9th	12th	3rd	6th	9th	12th
Scytalidium thermophilum SKESMBKU01	Glucose	0.897	0.366	0.340	0.300	0.203	0.143	0.106	0.017
	Xylose	0.735	0.436	0.418	0.152	0.045	0.078	0.164	0.014
	Cellulose	0.012	0.066	0.012	0.006	0.014	ND	ND	ND
	Starch	0.067	0.311	0.077	0.062	0.014	0.036	0.027	ND
	CMC	0.007	0.022	0.116	0.048	0.007	0.016	0.020	0.013
	Sucrose	ND	0.003	0.005	ND	0.014	0.008	0.007	ND
	Maltose	ND	0.118	0.270	0.390	0.007	0.037	0.134	0.014
	Fructose	0.029	0.059	0.222	0.077	0.029	0.060	0.149	0.055
	Lactose	0.540	0.699	0.418	0.070	0.029	0.051	0.048	0.016

Note: ND = No enzyme activity was detected

Optimization of nitrogen for cellulase production

Nitrogen sources which supported the growth and enzyme production by *Scytalidium thermophilum* SKESMBKU01 were studied (Table 4, 5). There was a distinct variation on utilization of nitrogen sources for enzyme production. The results revealed that KNO_3 (0.366 U/ml^{-1}) was the suitable nitrogen source which exerted the highest endoglucanase followed by NH_4Cl (0.27 U/ml^{-1}).

Table 3 Dry weight of mycelium (*Scytalidium thermophilum* strain SKESMBKU01.) in milligrams (mgs) on different carbon sources

Name of thermophilic fungi	Carbon sources	Dry weight of mycelium in milligrams (mgs) Incubation period (days)			
		3rd	6th	9th	12th
Scytalidium thermophilum SKESMBKU01	Glucose	30	50	80	120
	Xylose	30	60	80	120
	Cellulose	20	60	100	130
	Starch	30	60	120	140
	CMC	20	40	50	60
	Sucrose	80	90	100	110
	Maltose	80	90	110	120
	Fructose	90	100	120	140
	Lactose	80	90	100	110

Table 4 Optimization of nitrogen sources for the enzyme production by *Scytalidium thermophilum* strain SKESMBKU01**.**

Name of thermophilic fungi	Nitrogen sources	Endoglucanase activity / U/ml^{-1} Incubation period (days)				Exoglucanase activity / U/ml^{-1} Incubation period (days)			
		3rd	6th	9th	12th	3rd	6th	9th	12th
Scytalidium thermophilum SKESMBKU01	Peptone	ND	0.214	0.222	0.088	ND	0.053	0.044	0.007
	Yeast extract	0.059	0.211	0.179	0.003	0.019	0.058	0.055	0.004
	Malt extract	ND	ND	ND	0.179	0.056	0.019	0.016	0.007
	Beef extract	ND	0.168	0.168	0.118	0.007	0.040	0.038	0.010
	Urea	ND	0.233	0.214	0.220	ND	0.053	0.055	0.103
	$(NH_4)_2SO_4$	ND	0.185	0.196	0.222	ND	0.007	0.007	0.038
	$NaNo_3$	0.011	0.240	0.222	ND	ND	0.007	0.007	0.053
	KNO_3	0.035	0.240	0.281	0.366	ND	0.025	0.029	0.007
	NH_4Cl	0.118	0.270	0.240	0.118	ND	0.048	0.049	0.072

Note: ND = No enzyme activity was detected

Table 5 Dry weight of mycelium (*Scytalidium thermophilum* strain SKESMBKU01) in milligrams (mgs) on different nitrogen sources.

Name of thermophilic fungi	Nitrogen sources	Dry weight of mycelium in milligrams (mgs) Incubation period (days)			
		3rd	6th	9th	12th
Scytalidium thermophilum SKESMBKU01	Peptone	110	130	140	160
	Yeast extract	60	70	80	110
	Malt extract	100	120	130	140
	Beef extract	70	90	110	130
	Urea	60	80	90	110
	$(NH_4)_2SO_4$	70	90	110	130
	$NaNo_3$	50	70	90	110
	KNO_3	60	90	100	120
	NH_4Cl	100	110	130	160

Effect of pH

(Fig 2, 3) shows that the highest production of endo and exoglucanase of *Scytalidium thermophilum* SKESMBKU01 opted at pH 5.0 and 6.0. However the enzyme production was meager at pH 9.0 and 10.0 the fungus showed good growth at these pH values.

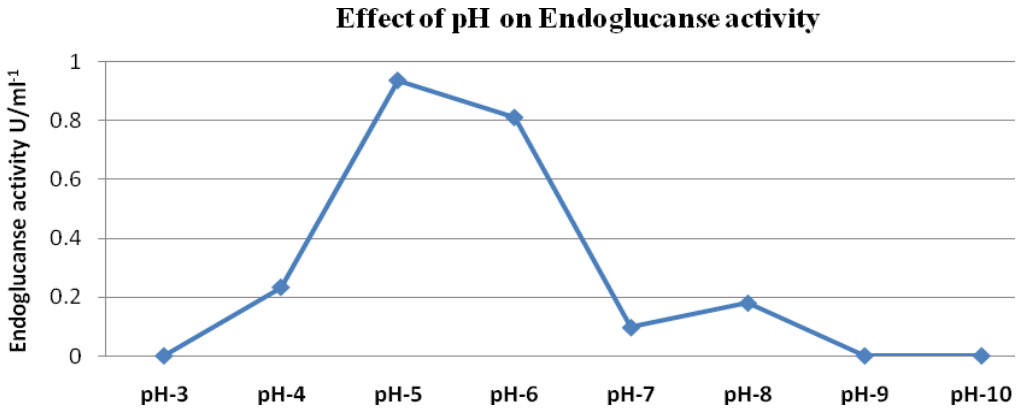


Fig. 2 – Effect of pH on Endoglucanase activity by *Scytalidium thermophilum* SKESMBKU01.

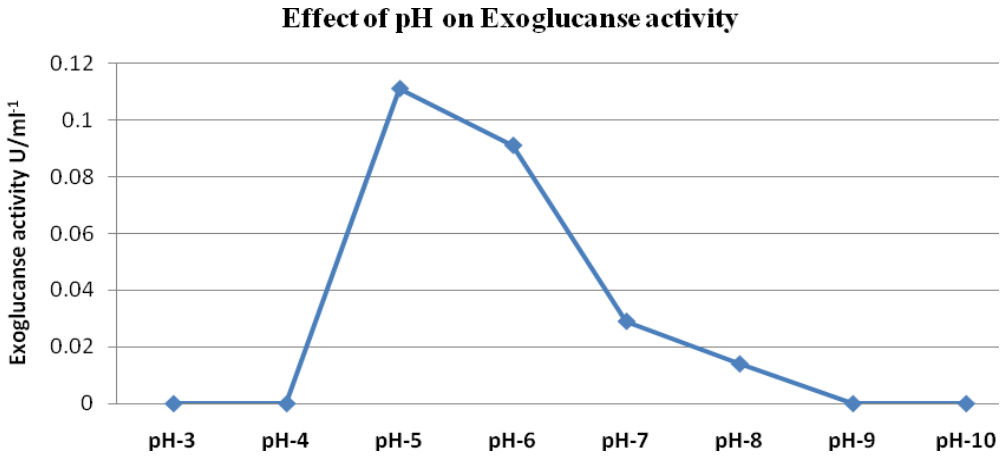


Fig. 3 – Effect of pH on Exoglucanase activity by *Scytalidium thermophilum* SKESMBKU01.

Effect of Temperature

The effect of temperature on the enzyme production was studied at 45–55°C. The optimal temperature for endo and exocellulases was 45°C (Fig.4, 5). Meager amount of enzyme produced at 50°C on 3rd day of incubation.

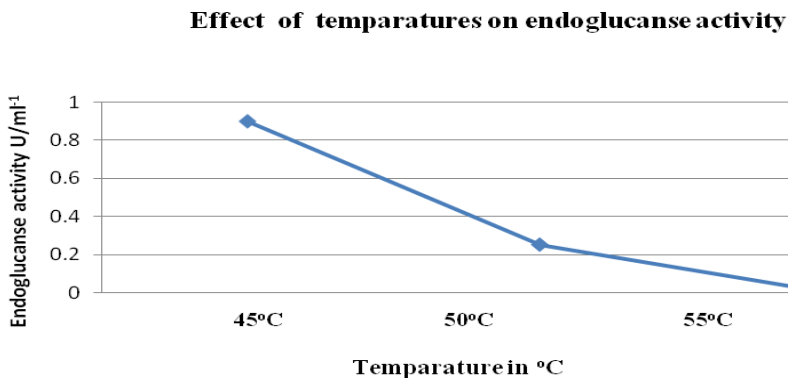


Fig. 4 – Effect of Temperature on Endoglucanase activity by *Scytalidium thermophilum* SKESMBKU01

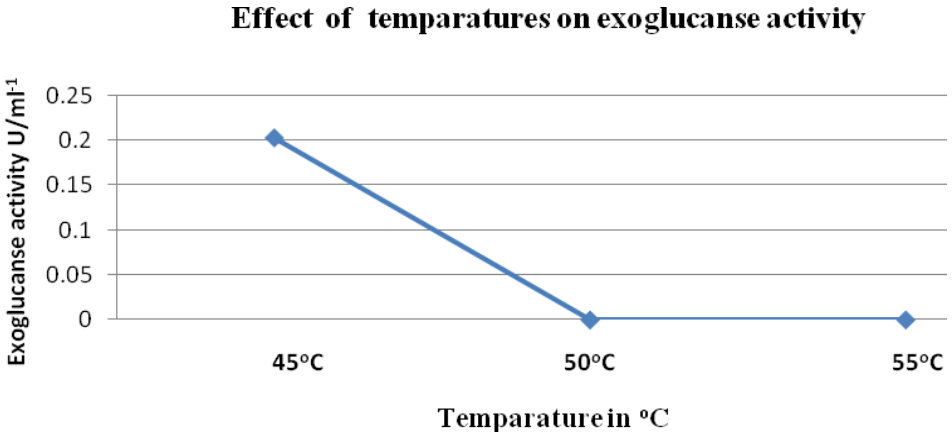


Fig. 5 – Effect of Temperature on Exoglucanase activity by *Scytalidium thermophilum* SKESMBKU01

Effect of static and agitation condition on cellulase production

The influence of static and different RPM on endo and exoglucanase activity by *Scytalidium thermophilum* was studied (Fig.6, 7, 8). The 100 RPM was more favorable for endo and exo cellulases, whereas at 150-200 RPM the enzyme production was nil (Fig.9, 10, 11).

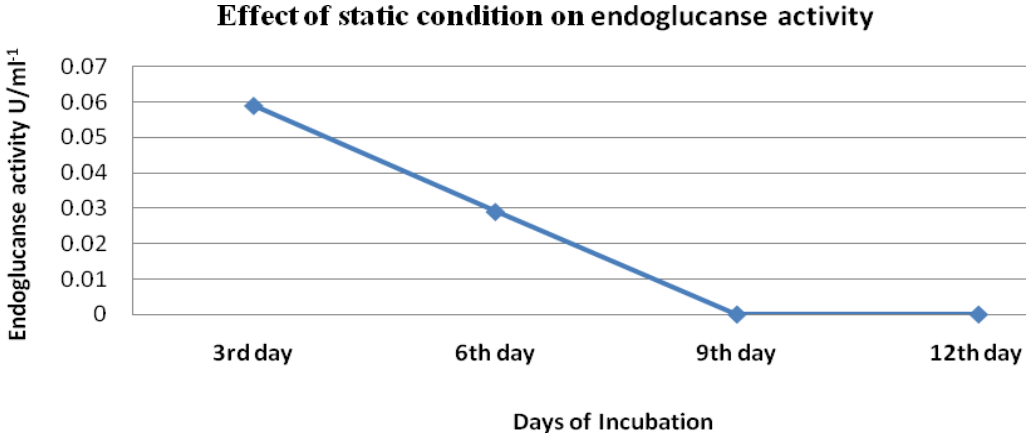


Fig. 6 – Effect of Static Condition on Endoglucanase activity by *Scytalidium thermophilum* SKESMBKU01

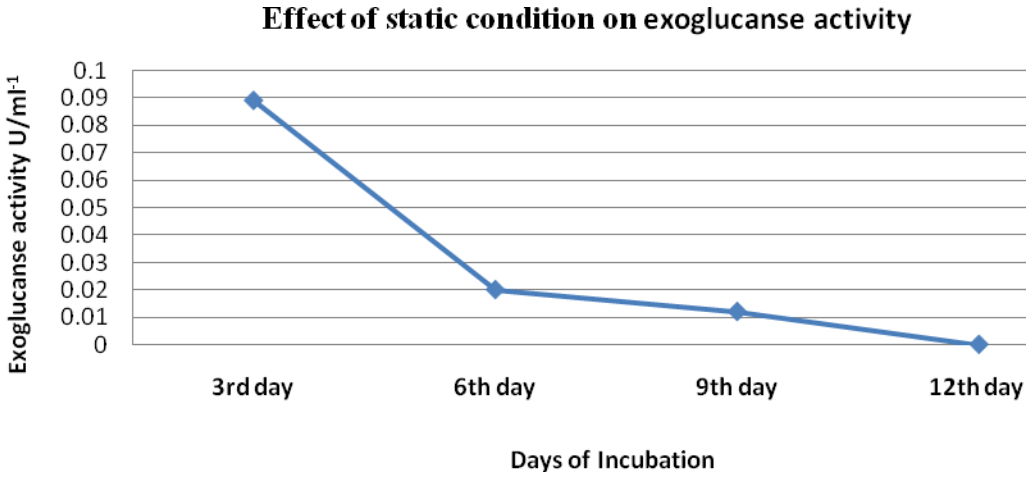


Fig. 7 – Effect of Static Condition on Exoglucanase activity by *Scytalidium thermophilum* SKESMBKU01

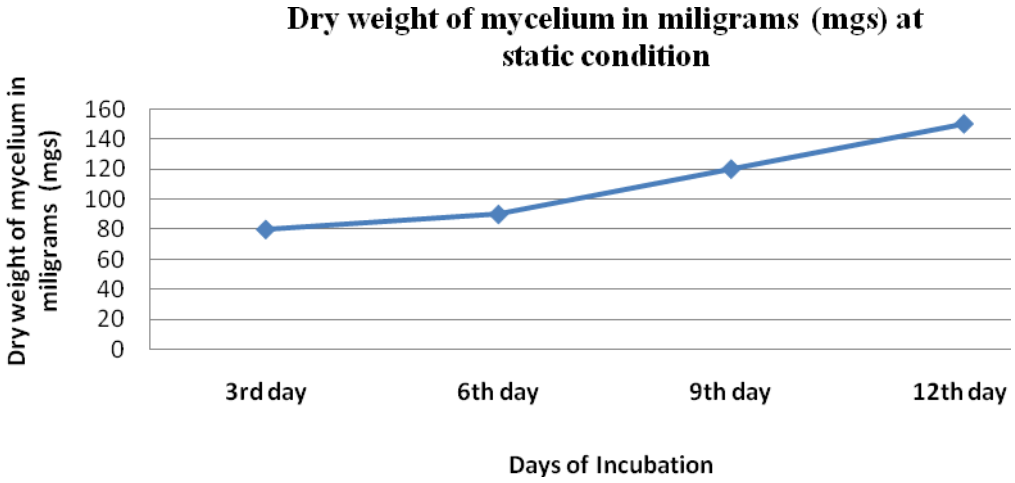


Fig. 8 – Effect of Static Condition on dry weight of mycelium by *Scytalidium thermophilum* SKESMBKU01.

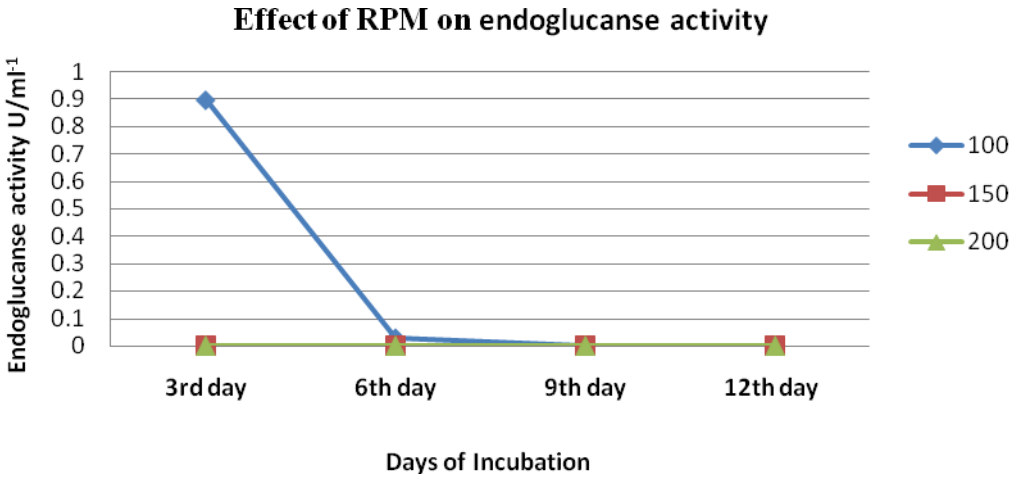


Fig. 9 – Effect of shake culture on Endoglucanase activity by *Scytalidium thermophilum* SKESMBKU01

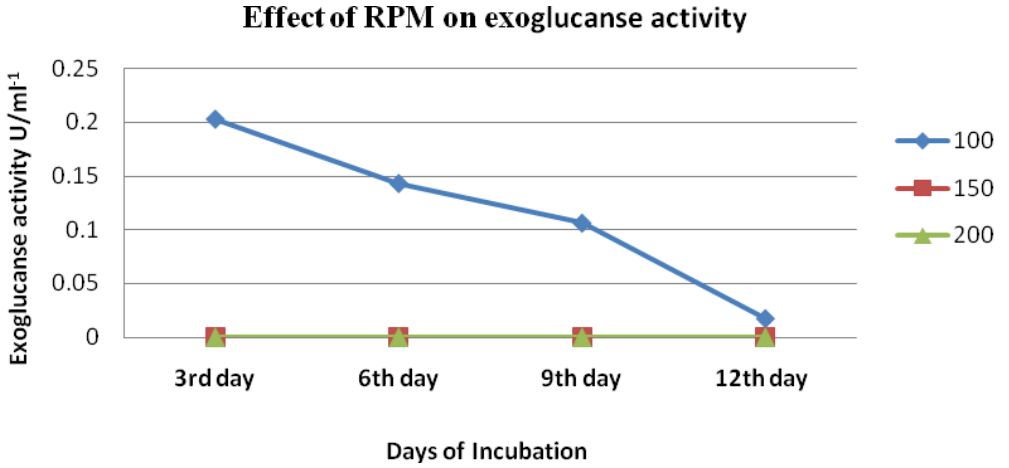


Fig. 10 – Effect of shake culture on Exoglucanase activity by *Scytalidium thermophilum* SKESMBKU01

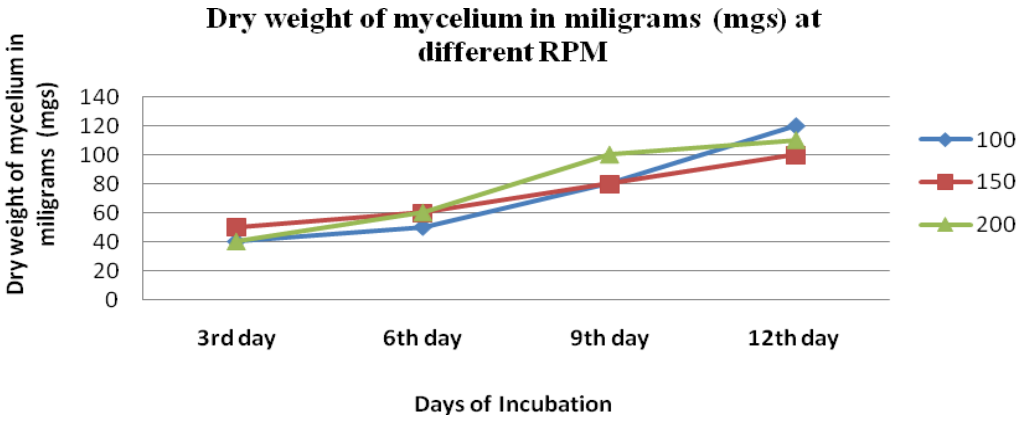


Fig. 11 – Effect of shake culture on dry weight of mycelium by *Scytalidium thermophilum* SKESMBKU01.

Effect of pH and temperature on the activity and stability of the enzyme

The pH stability results (Fig.12, 13) showed that enzyme was stable at both acidic and alkaline pH.The enzyme retained its activity at pH as low as 4.0 and as high as 8.0.Temperature stability study reveals that these endo and exo cellulases were stable at 75–85°C for 1 hrs (Fig.14, 15).

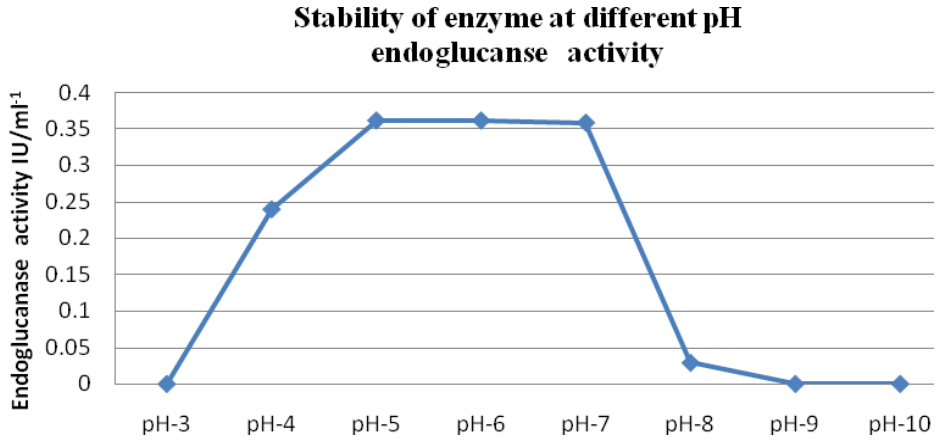


Fig. 12 – Stability of Endoglucanase activity by *Scytalidium thermophilum* SKESMBKU01 at different pH**.**

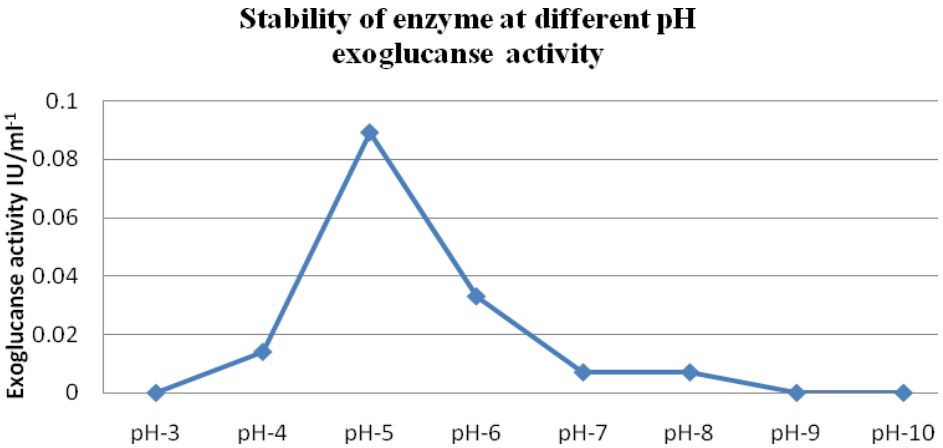


Fig. 13 – Stability of Exoglucanase activity by *Scytalidium thermophilum* SKESMBKU01 at different pH**.**

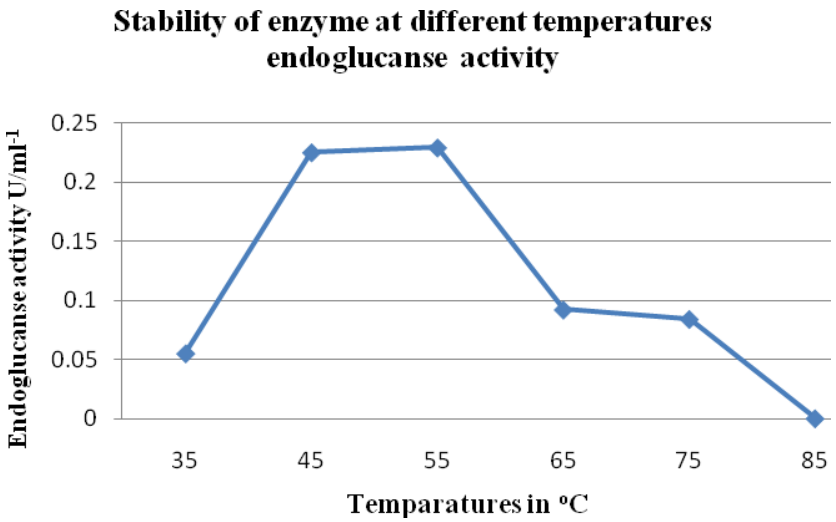


Fig. 14 – Stability of Endoglucanase activity by *Scytalidium thermophilum* SKESMBKU01 at different temperatures**.**

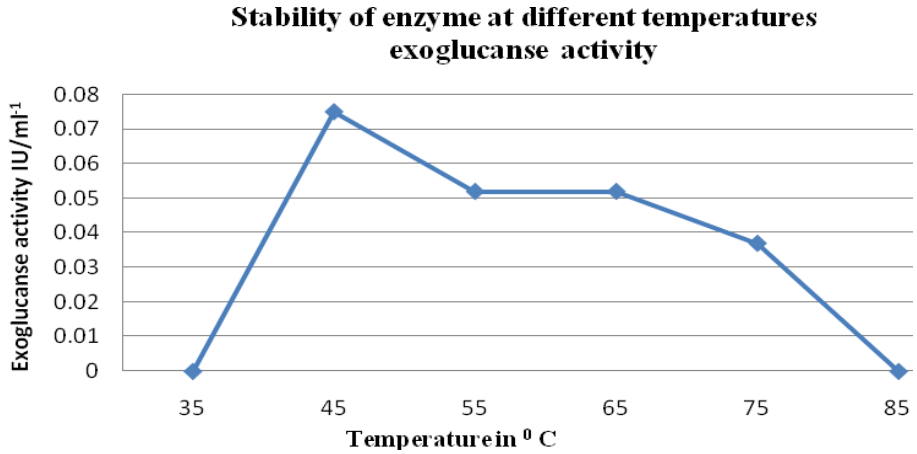


Fig. 15 – Stability of Exoglucanase activity by *Scytalidium thermophilum* SKESMBKU01 at different temperatures.

Discussion

Most of the Earth's renewable carbon exits in the form of cellulose that mainly composed of insoluble fibers of *β*-1-4 glucose, and its hydrolysis into fermentable sugars have the potential of biofuels generation. The cellulose hydrolysis into sugars essentially need cocktail of cellulolytic enzymes such as cellobiohydrolases, glucanohydrolases, and beta-glucosidases (Sunil et al. 2010). In our investigation, an attempt was made to understand the effect of various cultural parameters for maximum production of cellulases produced by *Scytalidium thermophilum* strain SKESMBKU01. The *Scytalidium thermophilum* strain SKESMBKU01 was isolated from mushroom compost showed highest production of cellulase in CMC plate assay. The strain was identified morphologically and molecularly by analyzing the ITS sequences of rDNA regions and identified as *Scytalidium thermophilum* strain SKESMBKU01.

The maximum amount of endo and exocellulases production was shown in glucose as its best carbon sources by *Scytalidium thermophilum* strain SKESMBKU01followed by xylose. Whereas Gautam et al. (2010) reported that cellulase production was maximum *by Trichoderma viride* in sucrose. These results reveal that the variation of cellulolytic activity of microorganisms was determined by nature of carbon sources and the respective organisms. *Scytalidium thermophilum* SKESMBKU01 showed high endo and exocellulases activity at beginning of the fermentation (3 days) because of the presence of suitable pH (5.5), further advancement of incubation period, endo

and exocellulases activity decreased due to pH drift towards highly acidic condition (pH 2.0–3.0), this may not be favorable for endo and exocellulases activity. There is no correlation between the enzyme activity and dry weight of the fungi (Table 2, 3).

Scytalidium thermophilum SKESMBKU01 showed good endo and exocellulases activity in KNO_3 (0.366 U/ml^{-1}), This result is in agreement with those obtained by Roberto et al. (2010) who found that ammonium sulfate was the best nitrogen source for cellulase production by *Penicillium funiculosum*. The optimum pH required for the maximum production of cellulase by *Scytalidium thermophilum* SKESMBKU01 is pH 5.0 and 6.0. This situation is in contrast to other fungal cellulases reported, *Aspergillus niger* opted pH 4.0–4.5 which were only active at acidic conditions for enzyme production (Acharya et al. 2008). Whereas enzymes produced by *Scytalidium thermophilum* SKESMBKU01 is active at both acidic and alkaline conditions.

Most of the fungal organisms exhibit optimal temperature in the range of 25 to 37°C for production of cellulase as shown by *Aspergillus fumigatus* (Gilna & Khaleel, 2011).where as *Scytalidium thermophilum* SKESMBKU01 a thermophile opted 45°C for maximum production of cellulases. The use of agitation or shaken flask culture method has enhanced the production of endo and exocellulases activity by more times compare to that of static condition. This data seem consistent with results from other studies, *Aspergillus niger* produced maximum levels of enzyme at 120 rpm for 96 hrs (Irfan et al. 2011).

A systematic characterization of cellulases from thermophilic fungi is necessary to better understand their thermostability. The stability studies reveals that the cellulases which are produced by *Scytalidium thermophilum* SKESMBKU01 more stable at both acidic and alkali conditions (pH 3–8) and stable at high temperatures (45–85°C). The greater stability of the exo and endo glucanases could be more useful for many biotechnology applications. The results are in agreement with that of enzyme produced by *Thermoascus aurantiacus* which were most active under acidic conditions with optimum pH at 4.0–6.0, temperatures of 60°C to retain maximum activity even after 24 hr of incubation (Mikio et al. 1987). The results suggest that the thermostable fungal cellulases have versatile biotechnological applications.

Acknowledgement

This work was supported by UGC-MRP, New Delhi, We are very thankful for providing the financial support.

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