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Production and Optimization of Exo and Endocellulases from Thermophilic Fungi *Scytalidium thermophilum* SKESMBKU02

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Abstract

The study aimed at isolation and screening of thermophilic cellulase producer, optimization and stability studies for maximum cellulase production. Fifteen thermophilic cellulase producing fungi were isolated from 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 Telangana. All the fungal isolates were screened for their ability to produce cellulases. *Scytalidium thermophilum* SKESMBKU02 showed the highest cellulase activity in screening and was selected for further studies. The results showed that *S. thermophilum* SKESMBKU02 found to have high cellulolytic activity at 45 °C and pH 5.0 - 6.0. Optimization of enzyme production was studied in different carbon and nitrogen sources. The endo and exoglucanase activities were higher in media containing glucose as their carbon source followed by xylose. Yeast extract and peptone were good nitrogen sources for endoglucanase and exoglucanase activity respectively. The organism showed maximum dry weight in $(NH_4)_2SO_4$ and NH_4Cl. The exo and endocellulases produced by the *S. thermophilum* SKESMBKU02 were highly stable at pH 8.0 and temperature of 75 °C. The results indicate that the endo and exocellulases produced by the fungi were more stable at high temperature and alkaline pH.

Keywords: *Scytalidium thermophilum*, cellulases, optimization, characterization

Introduction

Cellulose composes the bulk of the plant cell wall materials and is the most abundant and renewable non-fossil carbon source on earth. Its degradation to the constituent monosaccharides has attracted considerable notice for the production of food and fuels. The degradation of cellulose to glucose is effected by the cooperative action of endocellulases (EC3.2.1.4), exocellulases (cellobio-hydrolase, CBH; (EC3.2.1.74) and β-glucosidases (EC3.2.1.21) [1]. Endocellulases randomly hydrolyse internal glycosidic linkages, which results in a rapid decrease in polymer length and a gradual increase in the reducing sugars [2]. Exocellulases hydrolyse cellulose chains by removing cellobiose either from the reducing or non-reducing ends [3], which results in rapid release of reducing sugars but little change in polymer length. Endocellulases and exocellulases act synergistically upon cellulose to produce cellobiose, which is then cleaved by β-glucosidase to glucose [4,5]. Municipal solid waste, decomposed organic matter, and cow dung are ideal habitats for the different species of cellulolytic fungi. Microorganisms isolated from these sources have been used to convert cellulosic materials in to valuable compounds such as ethanol and organic acids. Usually thermotolerant enzymes are more active at high temperature and more stable than the enzymes produced by mesophilic fungi. In recent decades, there has been increasing interest in cellulases from thermophilic fungi, which are expected to produce thermostable enzymes. Because of their rapid growth and high rate of cellulose decomposition, thermophilic fungi are an attractive potential

source of cellulases. Biodegradation of cellulose is the most important activity of living systems. Many cellulolytic waste products are converted into useful products by the microorganisms. In recent years, the cellulolytic enzymes have become industrially important enzymes that are used in various applications, such as in the textile industry [6,7], the food and feed industry [8] and the pulp and paper industry [9]. In order to make those large-scale applications economically feasible, the cost of the cellulolytic enzymes needs to be reduced [10,11]. In this investigation, a cellulase producing strain of *S. thermophilum* SKESMBKU02 isolated from cattle dung was identified based on their morphological and molecular characters and was subjected to optimization of media components and cultivation parameters for exo and endo cellulase production.

Materials and methods

Collection of samples

Samples were collected from different thermogenic habitats (cattle dung, zoo dump, industrial waste, vegetable market compost, mushroom compost, nests of birds, decomposing litter, soils from furnace area, horse dung and municipal waste) of Warangal, Telangana, India, for isolation of thermophilic fungi. Samples were collected in sterile polythene bags and brought to the laboratory for microbiological study.

Isolation and morphological identification of thermophilic fungi

Ten gram of sample was transferred to aliquots of 90 ml of distilled water in a conical flask, it was shaken vigorously for 15 min. The sample suspension was then subjected to serial dilution and poured on yeast extract-starch agar medium (YpSs: yeast extract-5 g, starch-15 g, K_2HPO_4-1 g, $MgSO_4$-0.5 g per 1 l of distilled water), yeast-extract glucose agar (yeast extract-5 g, glucose-15 g, K_2HPO_4-1 g, $MgSO_4$-50 g per 1 l of distilled water) media 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 in YpSs agar media at 4 °C until needed for further study [12-16].

Screening for potent cellulase producing fungi (Carboxymethylcellulose agar method)

Carboxymethylcellulose (CMC) assay acts as a good indicator of cellulolytic ability since endoglucanase is generally produced in larger titers by fungi than cellobiohydrolases [17,18]. All the 15 isolates were screened for cellulolytic activity on selective carboxymethylcellulose agar (CMC) (containing 2.0 g/l of $NaN0_3$, 1.0 g/l of KH_2PO_4, 0.5 g/l of $MgSO_4.7H_2O$, 0.5 g/l of KCl, 2.0 g/l of carboxymethylcellulose sodium salt, 0.2 g/l of peptone, and 17.0 g/l of agar) [19]. Plates were spot inoculated with spores of pure culture and incubated at 45 °C for 3 days. After incubation period plates were flooded with Grams iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 5 min, the diameter of zone of decolorization around each colony was measured [20]. The fungal colony showing the largest zone of decolorization was selected for further studies.

Identification of thermophilic fungi

Genomic DNA was extracted by the thermolysis method described by Zhang [21] from cultures grown on YpSs media at 45 °C for 5 days. Mycelium was scraped from solid media with a sterile scalpel blade. DNA from the internal transcribed spacer (ITS) region was amplified by PCR, using primers ITS1 and ITS4 [22]. PCR products were purified and sequenced by Macrogen Laboratories (www.macrogen.com). The resultant fragment was searched against the GenBank database with nucleotide BLAST search option, available through the National Center for Biotechnology Information (website: http://www.ncbi.nlm.nih.gov). Aligned with reference sequences for construction of Phylogenetic tree using molecular evolutionary genetics analysis version 5.05 [23].

Enzyme assay

Carboxymethyl cellulase (CMCase)

The carboxymethyl cellulase (CMCase) or endoglucanase activity was determined by the method described by Ghose [24]. The enzymatic reaction contains 0.5 ml of 1 % carboxymethyl cellulose, 1 ml of 0.05M phosphate buffer (pH 5.5) and 0.5 ml of enzyme filtrate and the reaction mixture was incubated at 60 °C for 30 min and reducing sugars were estimated against blank at 575 nm using Miller's method [25].

Exoglucanase activity

Exoglucanase or cellobio-hydrolase activity was performed on cellulose powder (Himedia). The reaction mixture (2 ml) contained 1 ml of 1 % buffered suspension of the substrate and 1 ml of enzyme filtrate and incubated for 1 h at 60 °C in a water bath. The enzyme reaction was stopped by addition of 3 ml of DNS reagent and boiled for 10 min in a boiling water bath, color developed was read at 575 nm using heat killed enzyme as blank [26].

Enzyme units for Carboxymethyl cellulase, Exoglucanase activity

One unit (U) enzyme activity was defined as the amount of enzyme required to liberate 1 μmol of reducing sugar from the appropriate substrate per min under standard assay conditions. The results are shown in U/ml for submerged cultivation.

Effect of carbon and nitrogen sources on cellulases production

To evaluate the effect of carbon and nitrogen source on enzyme production, the fungi was grown on the basal medium described (containing g/L: (1.4 g/l of $(NH_4)_2SO_4$, 2.0 g/l of KH_2PO_4, 0.3 g/l of $CaCl_2 \cdot 2H_2O$, 0.3 g/l of $MgSO_4 \cdot 7H_2O$, 0.005 g/l of $FeSO_4 \cdot 7H_2O$, 0.0016 g/l of $MnSO_4 \cdot H_2O$, 0.0014 g/l of $ZnSO_4 \cdot 7H_2O$, 0.002 g/l of $CoCl_2 \cdot 6H_2O$, 0.1 g/l of Peptone and 80 - 100 g/l of Tween) [27]. The pH was adjusted to 5.5 and carbon sources were added at a concentration of 1 percent for all fermentations. The production medium was added with different carbon sources (1 %) such as glucose, xylose, cellulose, starch, cmc, sucrose, maltose, fructose and lactose for cellulase production. Nitrogen sources (peptone, yeast extract, malt extract, beef extract, urea, $(NH_4)_2SO_4$, $NaNO_3$, KNO_3, and NH_4Cl) were added at a concentration of 0.2 % to the Mandel and Weber fermentation medium. All the flasks were incubated at 45±2 °C in an orbital shaker incubator at 100 rpm. At regular intervals enzyme assay was carried out [28].

Effect of pH on cellulase production

To determine optimal pH, the cultures were cultivated in a 150 ml conical flask containing 25 ml of optimized media with different pH ranges from 3.0 to 10. The pH of the medium was adjusted by using 1-N HCl or 1-N NaOH. The flasks were kept in an orbital shaker incubator at 100 rpm at 45 °C for 3, 6, 9, and 12 days. After regular intervals enzyme assay was performed [29].

Effect of Temperature on Enzyme Production

In order to determine the effective temperature for cellulase production by the *S. thermophilum* SKESMBKU02 the fermentation was carried out at 35, 45, 50, and 55 °C for 3, 6, 9, and 12 days in an orbital shaker incubator at 100 rpm [30].

Effect of static and agitated conditions on cellulase production

The effect of agitation speed on exo and endocellulases activity was determined by incubating fermentation media at 4 different conditions (static, 100, 150 and 200 rpm) using a shaker incubator. The fermentation medium was prepared with appropriate pH that had been optimized and inoculated with *S. thermophilum* SKESMBKU02 overnight. All 4 conditions of the set up for the agitation effect were run in duplicate at once. Finally, samples were harvested after the optimal time for enzyme assay [31].

Determination of fungal biomass and pH

After the incubation period (3, 6, 9, and 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 overnight and their weight recorded. The 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 mycelia mat in micrograms [32]. The initial pH of the medium was adjusted at 5.5, with the progress of the incubation period 3, 6, 9, and 12 days, and change in medium pH was observed and noted [33].

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

Exo and endocellulases activity at different pH and temperature were carried out under standard assay conditions to determine the stability of enzyme. The pH stability of the enzyme was measured by incubating 1 ml of enzyme for 1 h at 45 °C in a buffer of desired pH (3 to 10). The temperature stability was determined by incubating 2 ml of enzyme at varying temperatures (30 to 80 °C) for 1 h and then estimating the residual enzyme activity under standard assay conditions [34].

Results and discussion

Isolation, screening of thermophilic fungi for cellulolytic activity

A total of 15 thermophilic fungi belonging to 10 different genera were isolated from various thermogenic habitats of Warangal, Telangana, India. The fungal species were identified and characterized based on their morphological characters and microscopic analysis by using taxonomic guides, referring relevant literature and standard procedures. All the 15 strains were screened for cellulolytic activity. All the tested strains were capable of producing cellulases in varying degrees. The potent species for cellulase production was *S. thermophilum* SKESMBKU02 isolated from cattle dung compost with a plate clearing zone of 2.5 cm in diameter (**Figure 1**). Microscopic techniques were not found sufficient enough to reveal taxonomic details of the isolated strain (*S. thermophilum* SKESMBKU02). Therefore, the modern molecular technique (r-DNA sequencing) was used for identification of the isolated strain. The obtained sequences of isolates were aligned with reference sequences showing sequence homology from the NCBI database using the multiple sequence alignment program of MEGA 5.05. Phylogenetic trees were constructed by using the neighbor joining method (**Figure 2**) and the strain was identified as *S. thermophilum* which was deposited in EMBL, accession number is HG934776.1 (**Figure 2**) (*S. thermophilum* SKESMBKU02). The pure culture of the fungi were made by the hyphal tip method and maintained at 4 °C for further use.

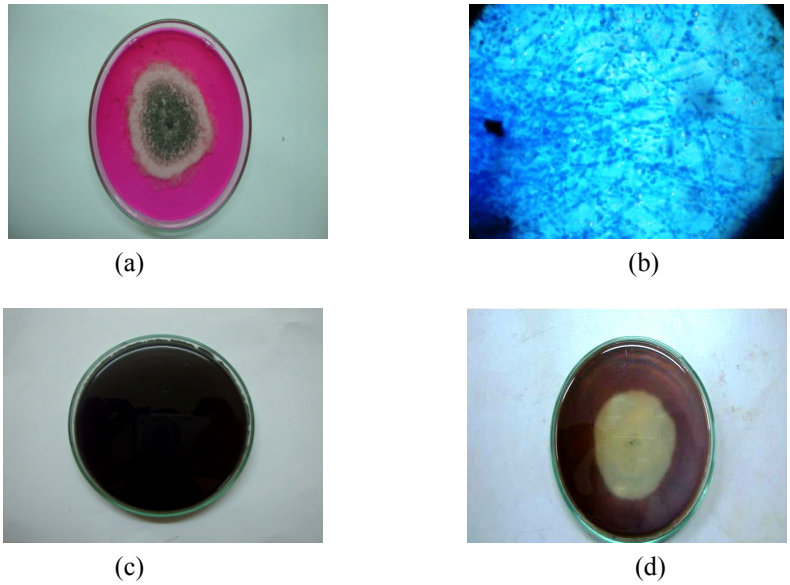

Figure 1 *Scytalidium thermophilum*, strain SKESMBKU02 (a) culture plate (b) microscopic image (c) control plate (d) culture plate showing cellulolytic zone.

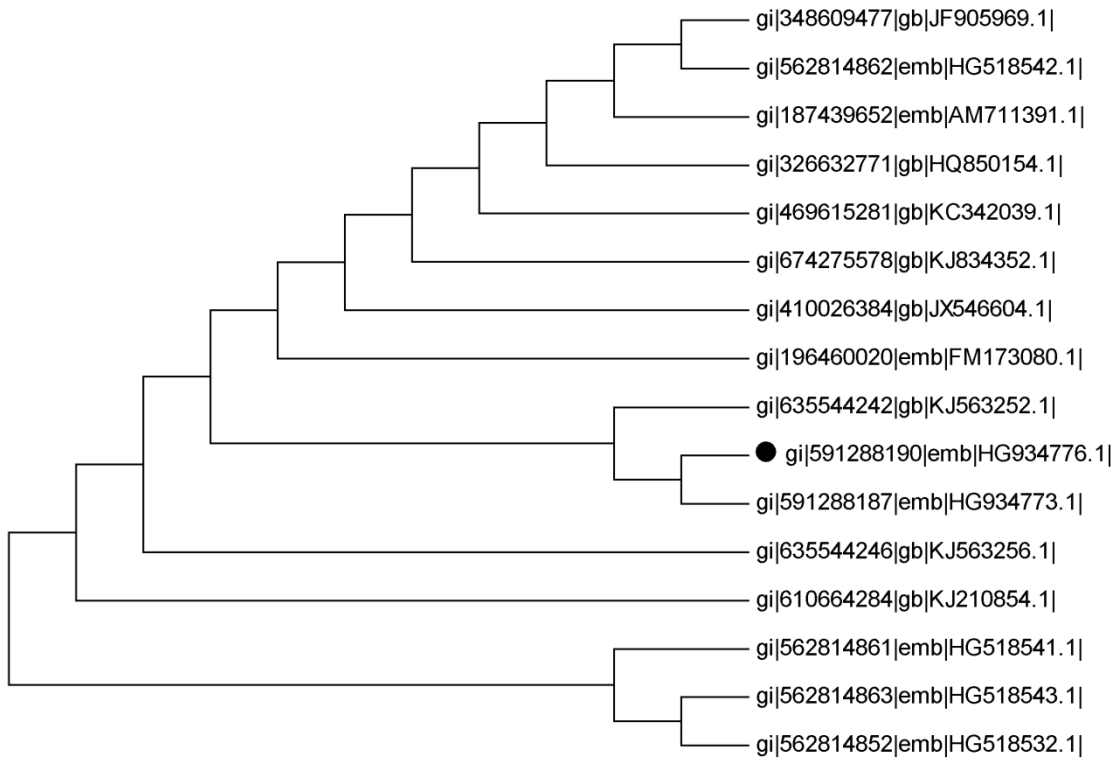

Figure 2 Phylogenetic tree constructed by using MEGA-5 (Molecular Evolutionary Genetics Analysis) software using the neighbor joining method.

Effect of carbon and nitrogen sources on endo and exoglucanase production

Carbon sources play an important role in fungal metabolism and production of enzymes. The *S. thermophilum* SKESMBKU02 was investigated for both endo and exoglucanase productions in shake flasks containing Mandel and Weber media optimized with different carbon sources (1 %) (**Table 1**). In this investigation, the fungi produced maximum endo (0.884 U/ml) and exoglucanase (0.274 U/ml) in glucose containing production media. Further advancement of the incubation period there was a decrease in endo and exoglucanase production. This was due to an increase in the pH values from favorable to unfavorable conditions and nutritional depletion. *Trichoderma viride* VKF3 showed maximum cellulase activity with glucose as sole sources of carbon which is in agreement with the investigation [35]. The present findings are good compared to the results of *A. niger* where it has recorded highest CMCase activity of 0.48 U/ml with carboxymethylcellulose as carbon source [36].

Table 1 Effect of carbon sources on endo and exoglucanase production.

Carbon source	Days of incubation	*Scytalidium thermophilum* SKESMBKU02			
		pH	Dry Wt. (mg)	Endoglucanase activity (U/ml)	Exoglucanase activity (U/ml)
Glucose	3	4.82	30	0.884	0.075
	6	4.20	70	0.340	0.274
	9	3.83	80	0.214	0.028
	12	3.15	100	ND	ND
Xylose	3	5.21	40	0.732	0.075
	6	4.82	60	0.625	0.168
	9	4.00	80	0.436	0.072
	12	3.83	90	0.059	0.063
Cellulose	3	5.30	20	0.022	0.023
	6	5.00	60	0.070	0.019
	9	4.84	130	0.012	0.017
	12	4.00	150	0.003	0.002
Starch	3	5.03	30	0.066	0.251
	6	3.81	60	0.144	0.104
	9	3.00	80	0.053	0.06
	12	2.91	100	0.041	ND
CMC	3	4.50	20	0.003	0.026
	6	4.20	30	0.059	0.009
	9	4.00	40	0.029	0.006
	12	3.30	80	0.007	ND
Sucrose	3	5.13	70	ND	0.014
	6	4.93	80	0.118	0.042
	9	4.85	90	0.388	0.067
	12	4.55	100	0.088	0.098
Maltose	3	5.00	70	0.029	0.050
	6	4.86	90	0.118	0.057
	9	4.73	90	0.666	0.171
	12	4.36	100	0.070	0.073
Fructose	3	4.56	70	0.150	0.064
	6	4.30	80	0.029	0.067
	9	4.05	100	0.029	0.032
	12	4.00	130	0.011	0.026
Lactose	3	5.08	70	0.077	0.002
	6	4.87	90	0.059	0.012
	9	4.36	100	0.055	0.013
	12	4.00	120	0.011	0.014

ND = No enzyme activity detected

To detect the appropriate nitrogen source for cellulase production by *S. thermophilum* SKESMBKU02, the fermentation medium was supplemented with 4 inorganic $(NH_4)_2SO_4$, $NaNO_3$, KNO_3, NH_4Cl) and 5 organic (urea, yeast extract, beef extract, malt extract and peptone) nitrogen sources. The nitrogen sources (2.0 g/l) were separately added to the fermentation medium for endo and exoglucanase production. The results (**Table 2**) indicate that yeast extract favours the production of maximum amount of endo and exoglucanases (0.555, 0.196 U/ml) and generally it was observed that organic nitrogen sources gave better endo and exoglucanase activity than inorganic nitrogen sources. These results are in agreement with the results of [37] who found that urea caused maximum CMCase production and inorganic nitrogen sources did not exhibit any significant effect on an increase in enzyme production. On the contrary, *Aspergillus flavus* showed the highest production of cellulase enzyme utilizing ammonium sulfate as a nitrogen source than yeast extract [38]. These results revealed that the variations of cellulolytic activity of microorganisms were determined by the nature of the nitrogen sources and the respective organisms.

Table 2 Effect of nitrogen sources on endo and exoglucanase production.

Nitrogen sources	Days of incubation	*Scytalidium thermophilum* SKESMBKU02			
		pH	Dry Wt. (mg)	Endoglucanase activity (U/ml)	Exoglucanase activity (U/ml)
Peptone	3	4.85	80	ND	0.060
	6	4.55	90	ND	0.025
	9	4.2	90	ND	ND
	12	3.00	100	0.436	ND
Yeast extract	3	5.20	30	0.555	0.029
	6	5.00	50	0.196	0.044
	9	4.86	70	0.035	0.196
	12	4.20	80	0.007	0.007
Malt extract	3	4.13	100	0.118	0.035
	6	4.00	110	0.214	0.016
	9	4.00	130	0.162	0.013
	12	3.79	140	ND	0.007
Beef extract	3	5.00	60	ND	0.042
	6	4.15	80	0.214	0.040
	9	4.00	90	0.179	0.033
	12	3.56	110	0.035	0.007
Urea	3	4.83	40	ND	ND
	6	4.50	60	0.337	ND
	9	4.30	80	0.222	ND
	12	4.00	100	0.029	0.058
$(NH_4)_2SO_4$	3	4.93	100	0.359	ND
	6	4.50	110	0.179	0.008
	9	4.30	120	ND	0.056
	12	4.00	130	ND	0.042
$NaNO_3$	3	5.00	60	ND	ND
	6	4.83	80	0.144	ND
	9	4.55	100	0.150	0.012
	12	4.00	110	0.174	0.026
KNO_3	3	5.15	60	ND	ND
	6	4.53	80	0.116	0.058
	9	4.20	100	0.118	0.060
	12	3.40	110	0.233	0.079
NH_4Cl	3	5.20	70	ND	ND
	6	5.00	90	0.140	0.037
	9	4.65	110	0.179	0.033
	12	4.15	130	0.007	0.033

ND = No enzyme activity detected

Effect of pH on endo and exoglucanase production

There was a strong influence of pH on the enzyme production. To evaluate the influence of pH on enzyme production, the *S. thermophilum* SKESMBKU02 was cultured in Mandel and Weber media with glucose (1 %) and $(NH_4)_2SO_4$ (1.4 %) as the carbon and nitrogen sources respectively. The optimal temperature for enzyme production was kept at 45 °C and pHs (3 - 10) were adjusted by the addition of HCl and NaOH (1N). The highest endo and exoglucanase activities were observed at pH 5.0 (0.840 U/ml) and 6.0 (1.075 U/ml) (**Table 3**). However, the enzyme production was meager at pH 9.0 and 10.0, the fungus showed good growth at these pH values. Whereas thermostable enzyme produced by *S. thermophilum* SKESMBKU02 was active at both acidic and alkaline conditions. The effect of pH on cellulase production by these fungi supports the findings of [39] who reported that cellulase by thermophilic fungus *Humicola* sp. SKESMBKU03 exhibited maximum cellulase activity at pH 5.0 - 6.0.

Table 3 Effect of pH on endo and exoglucanase production.

Effect of pH	Days of incubation	*Scytalidium thermophilum* SKESMBKU02			
		pH	Dry Wt. (mg)	Endoglucanase activity (U/ml)	Exoglucanase activity (U/ml)
pH 3	3	3.00	60	ND	ND
	6	2.65	80	0.192	0.040
	9	2.20	100	0.179	0.048
	12	2.00	120	0.029	0.029
pH 4	3	3.99	60	ND	ND
	6	3.32	70	0.022	0.005
	9	3.00	100	0.070	0.033
	12	2.56	120	0.011	0.029
pH 5	3	5.26	40	0.840	0.097
	6	4.67	50	0.447	0.111
	9	4.35	60	0.088	0.082
	12	4.00	80	0.035	0.022
pH 6	3	5.63	40	1.075	0.075
	6	4.38	60	0.155	0.056
	9	4.15	100	0.155	0.038
	12	3.82	120	0.144	0.029
pH 7	3	5.03	30	0.227	0.029
	6	4.26	50	0.144	0.022
	9	3.00	90	0.118	0.007
	12	2.88	110	ND	ND
pH 8	3	4.80	30	0.3	0.066
	6	4.10	40	0.233	0.038
	9	3.86	60	0.118	0.037
	12	3.48	90	0.118	0.036
pH 9	3	5.04	80	0.203	0.052
	6	4.20	110	ND	0.035
	9	3.65	130	ND	0.013
	12	3.19	210	ND	ND
pH 10	3	5.16	50	ND	ND
	6	4.46	100	0.118	ND
	9	4.00	140	ND	0.019
	12	3.69	180	ND	ND

ND = No enzyme activity detected

Effect of Temperature on endo and exoglucanase production

Temperature is an important environmental factor that most markedly influence the enzyme production. The effect of temperature on the enzyme production was studied at 35 - 55 °C (pH 5.5). The production of endo and exoglucanase was increased at 45 °C and thereafter a drastic reduction in the yield of cellulases was observed at 50 and 55 °C (**Table 4**). Thus 45 °C was considered an optimum temperature for both endo and exoglucanase. According to earlier studies by Sujatha *et al*. [40], *C. thermophile var. dissitum*, *T. thermophila*, *M. pulchella var. sulfurea*, opted for 45 °C for endo and exoglucanase production, as these results were in harmony with our the finding.

Table 4 Effect of temperature on endo and exoglucanase production.

Effect of temperature	Days of incubation	*Scytalidium thermophilum* SKESMBKU02			
		pH	Dry Wt. (mg)	Endoglucanase activity (U/ml)	Exoglucanase activity (U/ml)
35 °C	3	5.35	60	0.275	0.055
	6	5.15	70	0.074	0.034
	9	3.50	90	0.028	0.030
	12	2.65	110	ND	ND
45 °C	3	4.82	50	0.884	0.075
	6	4.25	60	0.340	0.274
	9	3.35	80	0.214	0.028
	12	3.00	100	ND	ND
50 °C	3	5.40	50	0.244	0.007
	6	4.95	60	0.452	0.060
	9	3.30	70	ND	0.075
	12	3.00	120	ND	0.056
55 °C	3	5.30	50	ND	ND
	6	5.25	60	ND	0.033
	9	3.80	80	ND	0.044
	12	2.85	110	0.203	0.067

ND = No enzyme activity detected

Effect of static and agitation condition on endo and exoglucanase production

Shaking improves the oxygen penetration in the medium and ensures equal distribution of nutrients, thus helping in fungal growth. Shaking cultures are convenient for rapid growth and for maximizing the yield of endo and exoglucanase. Maximum activity of endo and exoglucanase was observed under agitation. The endoglucanase activity was around 0.884 U/ml at 100 rpm on 3 days of incubation period. Exoglucanase activity was around 0.274 U/ml on 6^{th} day of incubation respectively. In static condition, maximum endo and exoglucanase activity was 0.118, 0.093 U/ml on 3^{rd} day of incubation, respectively. This clearly explains that endo and exoglucanase production was more in agitated conditions than the static condition (**Table 5**). Shaking cultures gave higher yields of cellulases compared to that of static ones by *Sclerotium rolfsii* which were in good agreement with the results of [41].

Table 5 Effect of static and agitation conditions on endo and exoglucanase production.

RPM	Days of incubation	*Scytalidium thermophilum* SKESMBKU02			
		pH	Dry Wt. (mg)	Endoglucanase activity (U/ml)	Exoglucanase activity (U/ml)
100	3	4.82	50	0.884	0.095
	6	4.25	60	0.029	0.274
	9	3.35	90	ND	0.028
	12	3.00	120	ND	ND
150	3	4.80	50	0.862	ND
	6	4.42	90	ND	ND
	9	3.70	100	ND	ND
	12	3.00	120	ND	ND
200	3	4.30	50	ND	ND
	6	3.90	60	ND	ND
	9	3.40	90	ND	ND
	12	2.88	110	ND	ND
Static conditions	3	5.25	50	0.118	0.093
	6	5.00	60	0.050	0.075
	9	3.50	90	ND	ND
	12	2.65	110	ND	ND

ND = No enzyme activity detected

Determination of fungal biomass and pH

Biomass of the fungal strain was determined by measuring the dry weight of the mycelium. The strain showed the same trend with all the parameters as gradual increasing the dry weight as the days of incubation proceeds. The maximum dry weight was found in cellulose (200 mg/ml) containing medium as carbon source. For the nitrogen sources it was found in $(NH_4)_2SO_4$ and NH_4Cl (130 mg/ml). The pH of 9.0 - 10 favored the growth of the fungi but not enzyme production (210, 180 mg/ml). The maximum dry weight was obtained at 50 °C. Agitation conditions showed a good dry weight (120 mg/ml) rather than static conditions (110 mg/ml). The result suggests that an increase in mycelium density is not directly related to an increase in enzyme production, and may be a result of complex repressing factors (incubation time, pH, temperature, aeration, toxicity of end products etc.). Transportation of various chemicals across the cell membrane, including movement of enzymes and their activity is importantly influenced by pH of the medium. There are general reports showing that different pH have different influence on extracellular enzyme production by different strains. Current findings also showed that the pH of the production medium was an important factor affecting endo and exoglucanase production (**Tables 1 - 5**). The change in the pH value from slightly acidic (pH-5.5) to more acidic (pH 2 - 3) condition is unfavorable for the production of endo and exoglucanase activity.

Effect of pH and temperature on the activity and stability of endo and exoglucanases

The pH stability results (**Figure 3**) showed that the 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 endo and exo cellulases produced by *S. thermophilum* SKESMBKU02 were stable at 75 - 85 °C for 1 h. (**Figure 4**). The greater stability of the exo and endo glucanases could be more useful for many biotechnology applications. Enzymes from *P. sanguineus* were more robust resisting one hour incubation at high temperatures (up to 80 °C), and exhibiting cellulolytic activity and stability at a pH range from 2.0 to 8.0 [42].

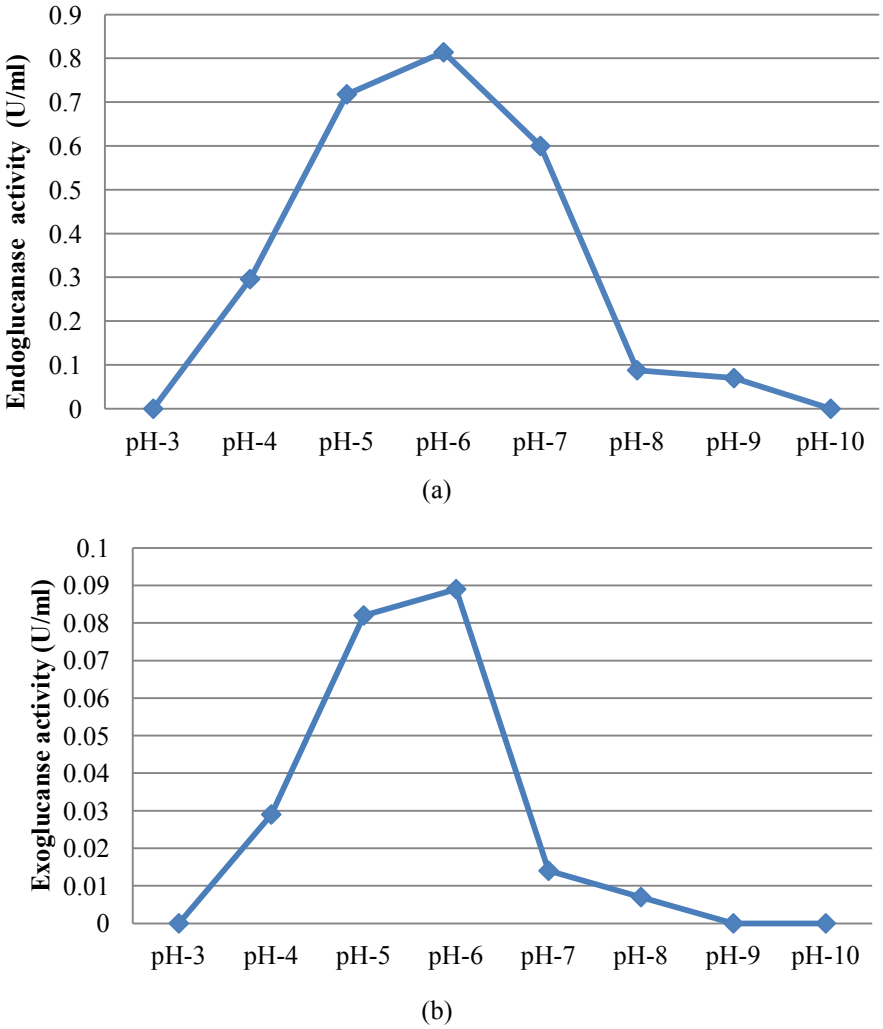

Figure 3 Stability of enzyme at different pH; (a) endoglucanse (b) exoglucanse.

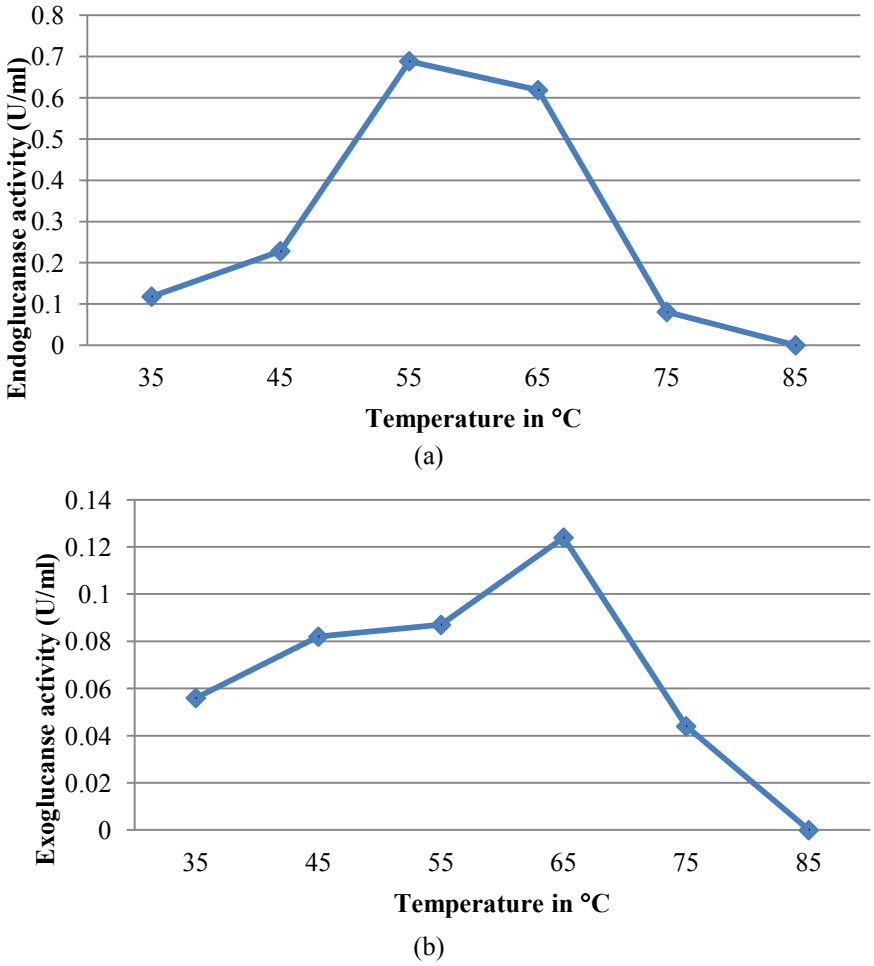

Figure 4 Stability of the enzyme at different temperatures; (a) endoglucanse (b) exoglucanse.

Conclusions

From the above investigations it was evident that the cellulase production by *S. thermophilum*, strain SKESMBKU02, isolated from the cattle dung compost, Warangal, Telangana India, was influenced by various nutrient supplements in the production media. The maximum production of endo and exoglucanase was achieved in glucose and xylose as their carbon sources and yeast extract and $(NH_4)_2SO_4$ as nitrogen sources. Further process parameters like incubation temperature at 45 °C, pH 5.5, and shaking condition (100 rpm) was found to be optimum for the maximal production of endo and exoglucanases. The stability studies revealed that the cellulases produced by *S. thermophilum* SKESMBKU02 was more stable at both acidic and alkali conditions (pH 3 - 8) and at high temperatures (45 - 75 °C) will be of more useful in various industrial and biotechnological applications.

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