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Optimization and characterization of thermostableβ-glucosidase by *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp.SKESMBKU03

S.Santosh Kumar, Edla Sujatha*, Kuraganti Guna Swetha

Department of Microbiology, Kakatiya University, Warangal-506009, Telangana, India

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Email: edlasujatha@yahoo.com

ABSTRACT

In the present study, β-Glucosidase production from *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03 was investigated and optimization of the cultural conditions to enhance production of enzymes has been reported. To enhance the production level of the enzyme, different cultural conditions were optimized and observed that optimum pH and temperature for β-Glucosidase production was pH-6.0 (*Scytalidium thermophilum* SKESMBKU01)pH-5.0(*Humicola* sp. SKESMBKU03) and 45oC respectively. Maximum enzyme production was recorded on 3rd day of incubation period in shake flask (100RPM) containing Mandel's Weber medium. Among nitrogen sources, yeast extract, peptone, beef extract and malt extract were found to be the best nitrogen source for β-glucosidase production. β-Glucosidase activities are higher in media containing glucose as their carbon source (1%) followed by xylose and lactose. The organism showed maximum dry weight in cellulose as carbon sources, yeast extract and malt extract as nitrogen source, pH of 9.0-10.0 and temperature of 45oC. β-glucosidaseproduced by the *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03 are highly stable at pH 8.0 and temperature of 75oC. The results indicate that β-glucosidaseproduced by *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03 are more stable at high temperature and alkaline pH.

INTRODUCTION

β-glucosidases (β-D-glucoside glucohydrolase, EC 3.2.1.21) are a heterogeneous group of enzymes that are capable of cleaving the β-glucosidic linkages of aryl and alkyl β-glucosides, β-linked oligoglucosides, and several other oligosaccharides, with the release of glucose, generally in the β configuration (Pitson et al.,1997; Hsieh and Graham,2001). This enzyme is also commonly referred to as gentiobiase and cellobiase. The enzyme β-glucosidase may be considered to be ubiquitous, occurring widely throughout the Microorganisms, plant and animal kingdoms, although its functions differ broadly according to the type of organism in which it occurs (Esen,1993).Cellulases break down cellulose to cellobiose ,β–glucosidases hydrolyze cellobiose to two glucose molecules. β–Glucosidase is inhibited by its end product, glucose; the substrate cellobiose accumulates and in turn inhibits the cellulase complex. For the enzymatic conversion of biomass to fermentative sugar on a commercial scale, it is necessary to have all cellulolytic components at the optimal level. Since β-glucosidases activity is low in many microbial preparations used usually for the saccharification process. It is necessary to supply additional β- glucosidases to such reaction. In order to optimize the use of different biomasses, it is important to identify new β-glucosidases with improved abilities on the specific biomasses as well as with improved abilities such as stability and high conversion rates (Gao et al., 2012).β-glucosidases have attracted considerable attention in recent years due to their important roles in various biotechnological processes from liberating flavours, aromas and isoflavoneaglycons to the synthesis of oligosaccharides and alkylglycosides, synthesize enzymes with high hydrolyzing activity, heat and glucose tolerance, acid resistance, and possible transglycosylase activity due to the properties of this enzyme to convert and to synthesize biomolecules of high added value (Singhaniaet al., 2013).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).In recent decades, there has been increasing interest in Beta-glucosidases 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 Beta-glucosidase. In this investigation, thermophilic strains of *Scytalidium thermophilum* SKESMBKU01, *Humicola* Sp. SKESMBKU03 isolated from mushroom compost and horse dung, were subjected to optimization of media and cultivation parameters for β-glucosidase production.

MATERIALS AND METHODS

Maintenance of culture

Thermophilic strains of *Scytalidium thermophilum* SKESMBKU01, *Humicola* sp. SKESMBKU03 used in this study were isolated from mushroom compost, horse dung manure respectively from Hyderabad, Telangana, India. It was grown and maintained at 45°C± 2°C on yeast-starch agar medium (YpSs: yeast extract-5gm, starch-15gm, K_2HPO_4-1gm, $MgSO_4$-0.5gm per 1000ml of distilled water)(Cooney and Emerson, 1964).Streptomycin and rose bengal were added to the molten medium after autoclave to inhibit the bacterial growth and the plates were incubated for 3-4 days and maintained on YpSs slants at low temperature (4°C) for further study.

Enzyme Production Medium

The optimization of cultural conditions was done by shake flask-culture method. The submerged fermentation medium described by Mandel and Weber (1969)containing milligrams / liter : $(NH_4)_2SO_4$ - 1,400,KH2PO4 - 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, pH was adjusted to 5.5 carbon source added at 1%) was used to investigate the effects of physical and nutritional factors on fungal growth and β-glucosidase enzymes production. The medium was modified according to the conditions and factors that were studied. The 25ml submerged fermentation broth medium was prepared in 150ml Erlenmeyer flask and autoclaved at 121°C for 15 min. The fungal spores were inoculated to the sterilized culture broth and the flasks were incubated at 45°C± 2°C in an orbital shaking incubator (100 rpm). At regular 3-days intervals culture medium (one flask, 25 ml) was used for fungal dry weight determination and enzyme extraction by Whatman no.1 filter paper and the clear supernatant was used as crude enzyme source. Fermentation was carried out in duplicate and average values from duplicates are presented in this work.

Assay of β-D-Glucosidase activity

Activity of β-D-glucosidase in the culture filtrate was quantified according to the method of Herr (1979). Its activity was measured in assay mixture containing 0.2 ml of 5 mM para-nitrophenyl β-D-glucopyranoside (pNPG) solution and 0.7 ml of citrate phosphate buffer (0.05 M, pH 5.5) and 0.1 ml of diluted enzyme solution with appropriate controls. After incubation for 30 min at 60°C, the reaction was stopped by adding 4 ml of 0.25 M NaOH-glycine buffer (pH 10.6). The yellow colouredp-nitrophenol liberated was determined at 420 nm. One unit of β-glucosidase activity is defined as the amount of enzyme liberating 1 µmole of p-nitrophenol per min under standard assay conditions.

Effect of initial and final medium pH on β-glucosidase production

To examine the effect of initial and final medium pH on β-glucosidase production, fermentation media containing glucose (1%) as carbon source, adjusted to a pH of 3.0 to 10.0 with NaOH (0.1M) or HCl (0.1M), was seeded with fungal biomass grown on YpSs agar for 4-5 days, and fermentation was conducted at 45°C± 2°C under shaking (100 rpm) incubator for 3, 6, 9,12days (Roberto et al.,2005).

Effect of temperature on β-glucosidase production

In order to determine the effective temperature for β-glucosidase

production by the *Scytalidium thermophilum* SKESMBKU01, *Humicola* Sp. SKESMBKU03 fermentation was carried out at temperature of 35°C,45°C, 50°C, 55°C ± 2°C for 3, 6, 9,12days in shaking (100 rpm) incubator(Gomes et al.,2000).

Effect of carbon source on β-glucosidase production

To test the effects of different types of carbohydrate as the sole carbon source on the β-glucosidaseproduction, nine different carbon sources were incorporated separately into the production medium incubated at 45°C± 2°C in an orbital shaking incubator (100 rpm) for 3,6, 9,12days (Coronel et al.,1991).

Effect of nitrogen source on β-glucosidase production

The effect of nine nitrogenous (0.2 % w/v) compounds on the enzyme production in Mandel and Weber medium was determined under optimum conditions by two thermophilic fungi. In all cases, the pH of the medium was adjusted to 5.5. Upon incubation time, the amount of β-glucosidase produced was estimated (Gautam et al., 2010).

Determination of fungal biomass

At regular intervals of time (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. 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 milligrams (Shilpi et al., 2011).

Characterization of crude enzyme

The crude enzyme was characterized for stability studies, substrate concentration, and enzyme concentration. The thermal stability was investigated by measuring the enzyme activity after keeping the aqueous enzyme solution for 1 hour at temperatures between 35 ºC and 80 °C in the absence of substrate and at constant pH 5.5. Remaining enzyme activity was determined by enzyme assay. The pH stability (pH 3.0 to10.0) of the crude enzyme was evaluated by mixing the enzyme and buffer to give final proportion of 0.5:1 (v/v). These solutions were incubated at 45 ºC for 1 hour and remaining activity was determined by enzyme assay (Quiroz et al., 2011).Substrate concentration varied from 2-10µM of para-nitrophenyl β-D-gluco-pyranoside (pNPG) and enzyme concentration was done by varying the concentration of enzyme from 50-500 µl after incubation, enzyme activity was determined by enzyme assay (Sujatha et al., 2005).

RESULTS

Effect of initial and final medium pH on β-glucosidase production

There exists a strong influence of initial pH of the medium on enzyme production. The pH profiles of the cultivations which were conducted in this study are presented in Table 1, illustrates that the production of β-glucosidase by *Scytalidium thermophilum* SKESMBKU01and *Humicola* sp.SKESMBKU03 was found between pH of 5.0 and 6.0,while further increase in pH showed decreasing trend in enzyme activity. It has been reported that the optimal pH for fungal cellulases varies from species to species.

Table 1: Effect of initial and final medium pH on β-glucosidase production.

Effect of pH	Days of Incubation	*Scytalidiumthermophilum* SKESMBKU01 pH	Dry. Wt. (mgs)	β-glucosidase activity U/ml	*Humicolasp.* SKESMBKU03 pH	Dry. Wt. (mgs)	β-glucosidase activity U/ml
	3	3.00	30	ND	3.05	60	ND
	6	2.18	50	0.008	2.50	70	ND
	9	2.00	60	0.010	2.00	90	ND
pH 3	12	1.87	80	ND	1.80	110	ND
	3	4.00	30	ND	3.95	30	ND
	6	3.86	40	0.025	3.50	50	0.008
	9	3.46	50	0.011	3.20	60	ND
pH 4	12	3.00	70	ND	3.00	80	ND
	3	4.95	30	0.034	4.60	40	0.063
	6	4.20	40	0.017	3.86	50	0.012
	9	3.56	60	0.008	3.60	50	0.010
pH 5	12	3.00	70	0.003	3.20	80	ND
	3	5.04	40	0.038	5.20	40	0.033
	6	4.10	60	0.015	4.54	50	0.015
	9	3.86	80	0.012	4.24	60	0.008
pH 6	12	3.46	90	0.005	3.98	80	0.003
	3	4.78	30	0.019	4.80	40	0.030
	6	3.80	60	0.010	4.20	60	0.019
	9	3.69	100	0.006	3.96	90	0.010
pH 7	12	3.25	130	0.003	3.56	120	ND
	3	4.53	30	0.018	4.53	60	0.010
	6	3.79	50	0.007	4.00	70	0.009
	9	3.55	60	0.005	3.80	100	0.003
pH 8	12	3.20	90	ND	3.20	120	ND
	3	4.45	60	0.008	4.40	60	ND
	6	3.92	100	0.006	3.98	80	ND
	9	3.76	130	0.003	3.59	120	ND
pH 9	12	3.42	150	ND	3.15	160	ND
	3	4.62	60	0.008	4.30	40	ND
	6	4.20	80	0.006	3.58	70	ND
	9	3.87	110	ND	3.28	120	ND
pH 10	12	3.56	140	ND	3.03	160	ND

ND= No activity detected

Effect of temperature on β-glucosidase production

Enzyme activity recorded at different temperatures revealed that β-glucosidase production by *Scytalidiumthermophilum* SKESMBKU01 and Humicola sp. SKESMBKU03 was maximum at 45˚C (Table 2). The result shows that the enzyme activity was decreased with increase in temperature above 45˚C.

Table 2: Effect of temperature on β-glucosidase production.

Effect of temperature in °C	Days of Incubation	*Scytalidiumthermophilum* SKESMBKU01			*Humicolasp.*SKESMBKU03		
		pH	Dry. Wt. (mgs)	β-glucosidase activity U/ml	pH	Dry. Wt. (mgs)	β-glucosidase activity U/ml
35°C	3	5.30	30	0.016	5.40	30	0.010
	6	4.90	50	0.010	5.18	60	0.006
	9	3.00	80	0.006	3.80	80	ND
	12	2.83	110	ND	3.20	100	ND
45°C	3	4.67	40	0.040	4.65	50	0.066
	6	4.10	50	0.015	4.30	70	0.020
	9	4.00	80	0.005	3.60	80	0.013
	12	3.56	120	ND	2.80	100	0.010
50°C	3	4.50	50	0.017	5.25	20	0.025
	6	4.20	60	0.010	4.30	70	0.012
	9	4.00	150	0.003	3.50	120	0.006
	12	3.80	190	ND	2.60	160	ND
55°C	3	4.40	40	0.010	5.30	50	0.020
	6	4.50	60	0.012	5.00	60	0.011
	9	3.50	100	ND	3.70	80	0.003
	12	2.00	110	ND	3.00	100	ND

Effect of carbon source on β-glucosidase production

To select suitable carbon source for β-glucosidase production, *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03 were cultivated in the medium containing various carbon sources (Table 3). *Scytalidium thermophilum* SKESMBKU01 and Humicola sp.SKESMBKU03 was grown in liquid culture with 1% (w/v) carbon source at 45°C for 3, 6,9,12 days. Among the carbon sources tested, highest enzyme activities were detected in glucose, xylose whereas very low yields were achieved with starch and CMC with both the organisms.

Effect of nitrogen source on β-glucosidase production

The effects of the nitrogen sources on enzyme activity are shown in Table 4. The β-glucosidase production was greatly affected by the nitrogen source used in the culture medium. Among the nitrogen sources tested, yeast extract was the nutrient that enhanced the highest production of β-glucosidase, where cultures supplemented with other nitrogen source yielded low activity. The results reported that good β-glucosidase yield can be obtained with organic nitrogen source when compare to the inorganic nitrogen sources.

Table 3: Effect of carbon source on β-glucosidase production.

Carbon sources	Days of Incubation	*Scytalidiumthermophilum* SKESMBKU01			*Humicolasp.* SKESMBKU03		
		pH	Dry. Wt. (mgs)	β-glucosidase activity U/ml	pH	Dry. Wt. (mgs)	β-glucosidase activity U/ml
Glucose	3	4.67	30	0.040	4.63	30	0.066
	6	4.10	50	0.016	4.28	60	0.016
	9	4.00	80	0.009	4.10	120	0.008
	12	3.34	120	0.003	3.53	90	0.003
Xylose	3	4.50	30	0.038	5.03	30	0.043
	6	4.30	60	0.022	5.00	60	0.020
	9	4.13	80	0.010	4.50	80	ND
	12	3.03	120	ND	4.00	100	ND
Cellulose	3	4.90	20	0.027	4.41	20	0.035
	6	4.80	60	0.016	4.16	60	0.035
	9	3.60	100	0.010	4.05	140	0.010
	12	2.10	130	ND	3.86	200	ND
Starch	3	4.30	30	0.030	5.20	30	0.012
	6	4.25	60	0.010	3.46	60	0.019
	9	3.68	120	0.007	3.00	110	0.005
	12	2.85	140	ND	2.96	120	ND
CMC	3	4.80	20	0.006	4.32	20	0.015
	6	4.35	40	0.003	4.10	30	0.026
	9	3.59	50	0.003	4.00	50	0.010
	12	3.00	60	ND	3.73	50	0.005
Sucrose	3	5.10	80	0.019	4.87	100	0.076
	6	5.00	90	0.012	4.15	110	0.038
	9	4.59	100	ND	4.00	120	0.002
	12	4.15	110	ND	3.63	140	ND
Maltose	3	5.15	80	0.038	5.24	70	0.021
	6	4.84	90	0.006	4.45	80	0.015
	9	4.50	110	ND	4.15	100	0.006
	12	4.00	120	ND	4.00	120	ND
Fructose	3	4.76	90	0.001	4.27	80	0.019
	6	4.42	100	0.003	4.00	130	0.015
	9	4.04	120	ND	3.83	160	0.010
	12	3.83	140	ND	3.50	170	0.003
Lactose	3	5.05	80	0.032	5.20	70	0.024
	6	4.93	90	0.035	4.55	80	0.016
	9	4.50	100	0.010	4.23	110	0.010
	12	4.10	110	0.006	4.03	130	0.006

ND= No activity detected

Table 4: Effect of Nitrogen source on β-glucosidase production.

Nitrogen sources	Days of Incubation	*Scytalidiumthermophilum* SKESMBKU01			*Humicola sp.* SKESMBKU03		
		pH	Dry. Wt. (mgs)	β-glucosidase activity U/ml	pH	Dry. Wt. (mgs)	β-glucosidase activity U/ml
Peptone	3	5.25	110	0.016	4.87	100	0.038
	6	4.67	130	0.036	4.70	110	0.035
	9	4.15	140	0.019	4.56	130	0.025
	12	4.07	160	0.009	4.38	160	0.015
Yeast extract	3	5.35	60	0.048	5.15	120	0.037
	6	5.15	70	0.208	5.00	130	0.185
	9	5.00	80	0.062	4.38	150	0.055
	12	4.57	110	0.026	4.00	160	0.020
Malt extract	3	5.15	100	0.020	5.20	100	0.038
	6	4.84	120	0.015	5.00	120	0.076
	9	4.63	130	0.012	4.90	140	0.065
	12	4.15	140	ND	4.40	160	0.005
Beef extract	3	5.10	70	0.025	5.14	60	0.039
	6	4.33	90	0.011	5.00	80	0.068
	9	4.03	110	ND	4.70	90	0.026
	12	4.00	130	ND	4.50	110	0.012
Urea	3	5.20	60	0.030	4.90	30	0.026
	6	5.00	80	0.022	4.73	40	0.012
	9	4.90	90	ND	3.84	60	0.011
	12	4.50	110	ND	3.50	100	0.006
$(NH_4)_2SO_4$	3	5.30	70	0.025	4.63	60	0.036
	6	4.82	90	0.020	3.93	80	0.024
	9	3.83	110	ND	3.50	90	0.020
	12	3.53	130	ND	3.00	110	ND
$NaNO_3$	3	5.25	50	0.019	5.20	50	0.030
	6	4.50	70	0.010	3.73	70	0.020
	9	4.00	90	0.003	3.00	90	0.020
	12	3.83	110	ND	2.72	110	ND
KNO_3	3	4.30	60	0.010	5.15	70	0.020
	6	4.00	90	0.003	4.10	80	0.019
	9	3.68	100	ND	3.87	90	0.016
	12	3.00	120	ND	3.00	100	0.003
NH_4Cl	3	4.80	100	0.012	5.30	90	0.035
	6	4.35	110	ND	4.73	100	0.006
	9	4.00	130	ND	4.15	120	0.010
	12	3.59	160	ND	3.60	130	0.005

ND= No activity detected

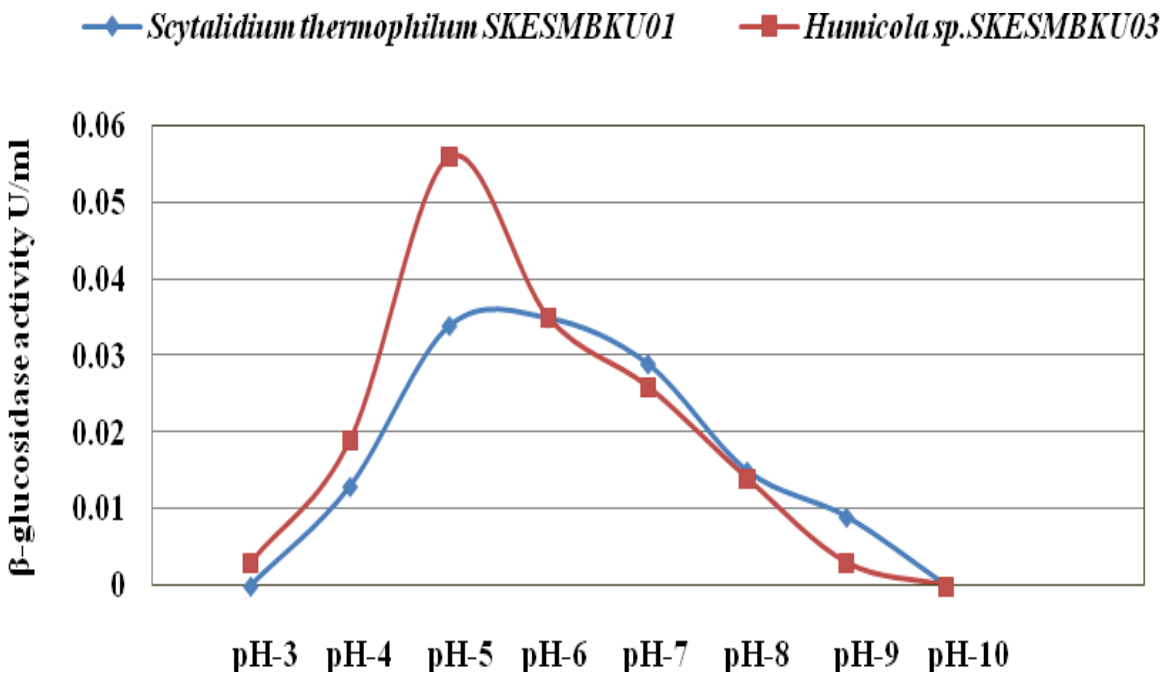

Figure 1: Stability of enzyme at different pH by Scytalidiumthermophilum SKESMBKU01 and Humicola sp. SKESMBKU03

Table 5. Effect of still and shake flask condition on β-glucosidase production

RPM	Days of Incubation	*Scytalidiumthermophilum* SKESMBKU01			*Humicola sp.* SKESMBKU03		
		pH	Dry. Wt. (mgs)	β-glucosidase activity U/ml	pH	Dry. Wt. (mgs)	β-glucosidase activity U/ml
100	3	4.60	40	0.036	4.60	50	0.066
	6	4.10	50	0.016	4.30	70	0.020
	9	4.00	80	0.010	3.60	80	0.013
	12	3.50	120	0.003	2.80	100	0.010
150	3	5.25	50	0.015	4.30	20	0.015
	6	4.30	60	0.010	3.86	60	0.010
	9	3.50	80	0.005	3.20	80	0.006
	12	2.60	100	ND	2.78	110	ND
200	3	4.50	40	ND	4.30	50	0.015
	6	4.00	60	0.010	3.90	60	0.010
	9	3.59	100	ND	3.40	80	0.006
	12	2.83	110	ND	2.88	100	ND
Static condition	3	5.20	80	0.015	5.00	60	0.021
	6	4.90	90	0.010	4.56	80	0.006
	9	3.00	120	0.0003	3.80	100	0.003
	12	2.83	150	ND	3.20	110	ND

ND= No activity detected

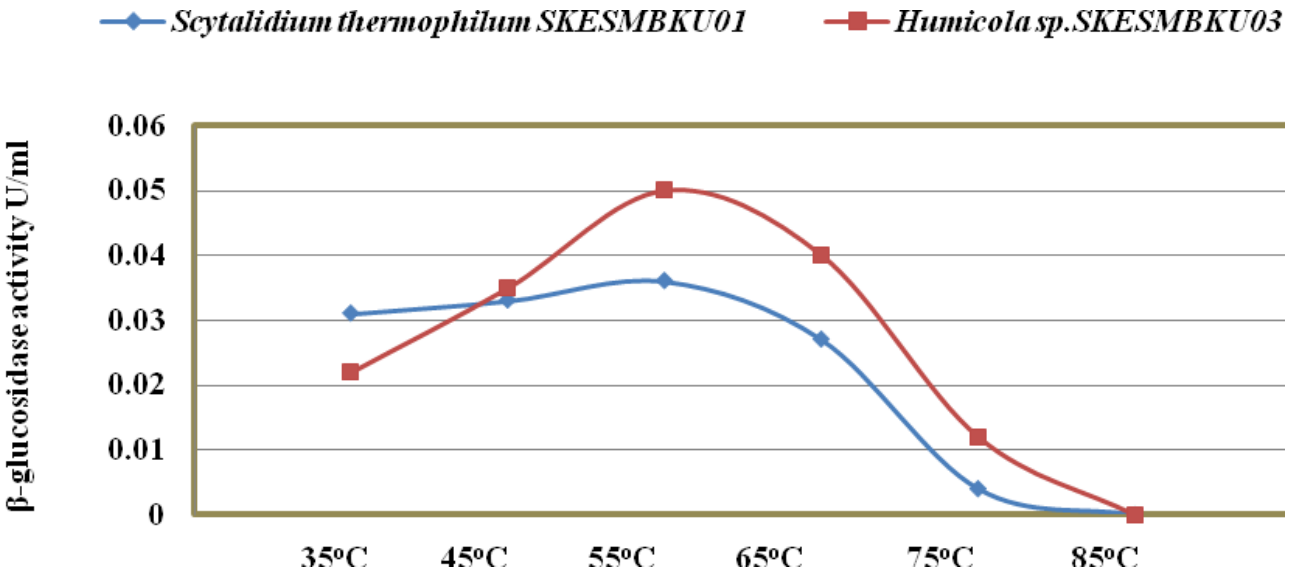

Fig 2: Stability of enzyme at different temperature by *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03

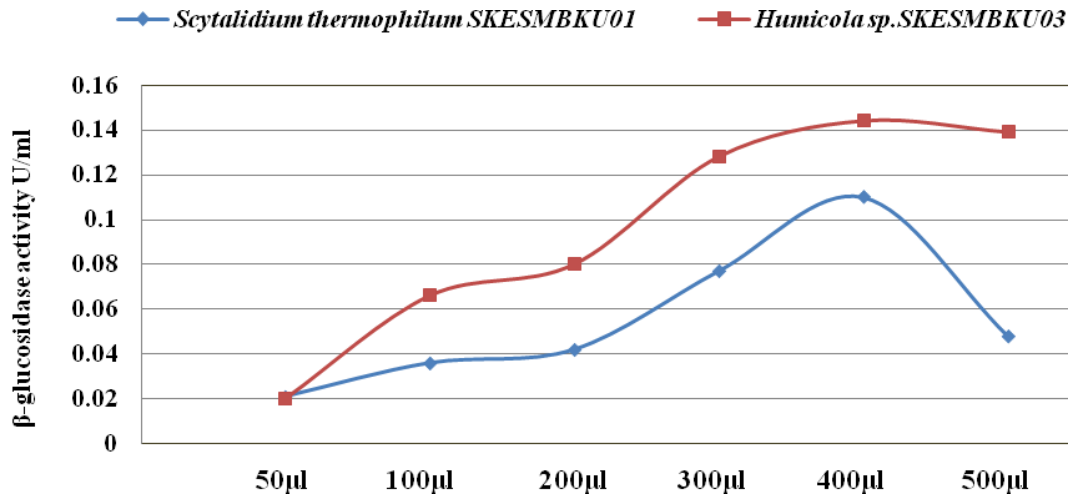

Fig 3: Effect of enzyme concentration on activity by *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03

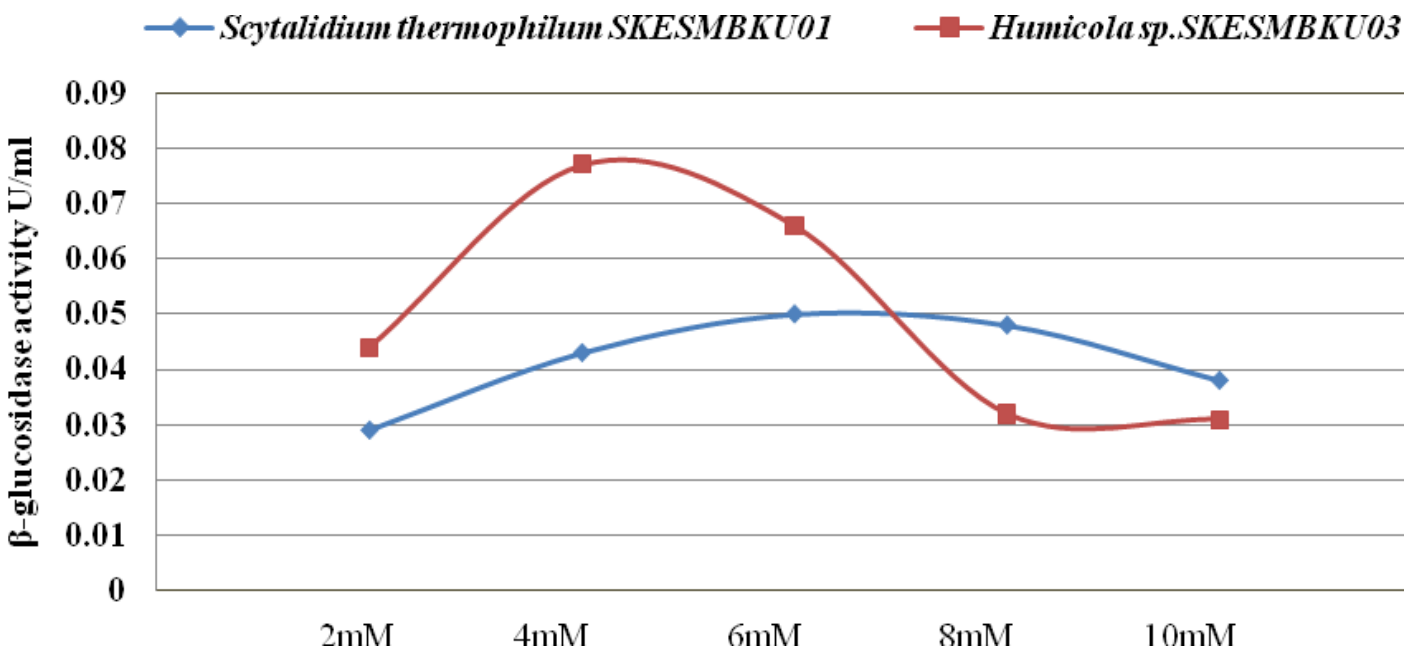

Fig 4: Effect of substrate concentration on activity by *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03

Effect of still and shake flask condition on β-glucosidase production

β-glucosidaseproduction was studied under still and shake flask conditions (100, 150,200 RPM) for 3, 6,9,12 days at 45˚C. The β-glucosidase production by *Scytalidium thermophilum* SKESMBKU01 *Humicola* sp. SKESMBKU03 was found to be high in agitation condition in comparison with that of static conditions. It was noticed that the optimum level of rotation needed for the maximum production for β-glucosidase at 100 RPM (Table 5).

Determination of fungal biomass

Although all of the liquid media evaluated were able to support mycelial growth of *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03, the biomass production was greatly affected by the culture medium used. The biomass production of fungi denotes that biomass increased with incubation period. The results revealed that the *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03 showed increased biomass at pH of 5.0 – 6.0 (Table 1) and temperature of 45°C (Table 2). Cellulose found to be best carbon sources for biomass production followed by fructose and lactose (Table 3). Among nitrogen sources peptone, yeast extract and malt extract were found to produce maximum fungal biomass (Table 4). Compare to the static, maximum fungal biomass was produced in the shake flask culture at 100RPM (Table 5).There is no co-relationation between β-glucosidaseproduction and biomass production.

Characterization of crude enzyme

Results of characterization of crude enzyme shows that the β-glucosidase of *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03 were completely active at a large range of pH (4 – 8) and presented an optimum pH stability at a pH value of 5.0 (Figure 1).The experiment was conducted to determine the effect of different treatment temperatures (30 - 80˚C) on the thermal stability of crude enzyme. The crude β-glucosidase was incubated under different temperatures. After 1hr of incubation β-glucosidase was assayed to determine the effect of temperature on stability of enzyme activity with the same procedure as mentioned previously. Thermal stability of crude enzyme by both the fungi was observed at 55˚Cfor one hour (Figure 2). The β-glucosidase activity was characterized at different enzyme concentrations (50-500 µl), and the maximum activity was observed in 400 µl (Figure 3), which was found to be 0.110 U/ml (*Scytalidium thermophilum* SKESMBKU01) and 0.144 U/ml (Humicola sp. SKESMBKU03).β-glucosidase activity was characterized at different substrate concentrations (2-10µM of para-nitrophenyl β-D-gluco-pyranoside (pNPG)), and the maximum activity was observed in 6 µM ofpNPG, which was found to be 0.050 U/ml (*Scytalidium thermophilum*SKESMBKU01) and 0.077 U/ml (*Humicola* sp. SKESMBKU03). Initially, as the concentration of substrate was increased there was a significant increase in enzyme activity, but as soon as all the substrate binding sites were filled there was a decline in enzyme activity (Figure 4).

DISCUSSION

The enzymatic conversion of cellulose is catalyzed by a multiple enzyme system. Beta-glucosidase (glucohydrolase, EC 3.2.1.21) is one of the essential enzymes in the enzymatic conversion of cellulose. It is an important component of cellulase system and acts synergistically with endogluconase and cellobiohydrolase for complete degradation of cellulose (Szengyel et al.,2000).Thus, Beta-glucosidase not only produces glucose from cellobiose, but also reduces cellobiose inhibition, allowing endoglucanase and exoglucanase enzymes to function more efficiently (El-Bondkly et al., 2010; Harhangi et al., 2002).In the present study production and characterization of β-glucosidase by *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03 isolated from mushrrom compost and horse dung was investigated.

The pH value of the fermentation medium for β-glucosidase production by fungi is considered the most important factor. Current finding also shows that pH of production medium was an important factor affecting -glucosidase production. *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp.SKESMBKU03 shows the highest production of beta-glucosidase at pH 5.0 and 6.0 on third day of incubation as the incubation proceeds the beta-glucosidase activity is decreased due to the change in the pH value from slightly acidic (pH-5.5) to more acidic (pH 2.0-3.0) condition which is unfavorable for the production of beta-glucosidase activity (Table 1). Fareeha Razaet al., (2011) found that Effect of initial pH on the production of β-glucosidase by co-culture of A. *niger* and A. *oryzae* opted pH of 5.5 which is in relation with the current findings.

The incubation temperature of the fermentation medium is one of ultimate factor influencing the production of enzymes. Enzymes have an optimum temperature at which their activity is maximum and at higher or lower temperatures, their activity decreases. Optimization of temperature was achieved by incubating the inoculated flasks containing fermentation medium at different temperatures (35, 45, 50 and 55 °C). The results shows that both the fungi as opted a temperature of 45°C for maximum -glucosidase production. The results are in bond with the findings of B aig, (2005).

β-glucosidase production is affected by the nature of the substrate used in fermentation, hence the choice of an appropriate inducing substrate is highly important. Production of enzyme was observed with all the tested sugars. Among the carbon sources examined, glucose found to be the best inducer in SmF on third day of incubation next to this is xylose. Further advancement of incubation period there was a decreased -glucosidase production this was due the increase in the pH values from favorable condition to un-favorable conditions and nutritional depletion. Park et al., (2015) observed that glucose was found to be the best carbon source for production of -glucosidase

The effect of nitrogen sources on β-glucosidase production by these fungi supports the findings of Mahapatraet al., (2016) who found microbes from shellfish waste when grown on yeast extract showed maximum BGL activity, while other nitrogen sources were poor sources of nitrogen.

The maximum production of β-glucosidase is achieved at 100RPM. Further increase in RPM level, there was decrease in enzyme activity, this could be due to the fact that the increase in RPM level has resulted in the coagulation of the organism to form clumps and decrease in enzyme production. Tarek et al., (2013) who establish that rate of β-glucosidase was seven times more in shaking cultures than in static ones in *Sclerotiumrolfsii*.

Thermostability profile of the enzyme revealed that the β-glucosidase enzyme was stable at temperature up to 55 °C for 1 h of pre-incubation period. But higher temperature up to 85 °C causes denaturation of the enzyme. This thermostability of the enzyme at 55 °C for 1 h is very beneficial for enzymatic hydrolysis of cellulose. Ahmed et al.,(2013) also found that thermostability of free and immobilized enzyme of *Aspergillus niger* showed same pattern activity.

The pH stability curves for β-glucosidase are shown in (Figure 2). It exhibited an optimal activity at pH 5.0, by both the fungi. The results are in consensus with findings Yan and Lin, (1997) who reported that *Aspergillus niger* CCRC 31494 exhibited pH stability at 5.0 for 24hours.

The enzyme concentration-response was measured by varying the enzyme concentration at a fixed substrate concentration. The enzyme activity increased linearly with an increase in β-glucosidase enzyme concentrations from (50 to 500μM) (Figure3).Substrate concentration was determined by varying the concentration of substrate and it as found that *Scytalidium thermophilum* SKESMBKU01 shown maximum activity at 6mM where as *Humicola* sp. SKESMBKU03 has showed 4Mm(Figure. 4).Kuar et al.,(2007) found the same trend of activity in *Melanocarpus* sp. MTCC 3922 strain with increase in concentration of substrate there is increase in the enzyme activity.

CONCLUSION

The result has enabled the ideal formulation of media composition for maximum β-glucosidase production by *Scytalidium thermophilum* SKESMBKU01 and *Humicola* sp. SKESMBKU03. The high activity and stability of β-glucosidase between neutral and alkaline pH and high temperature will be of use in various industrial and biotechnological applications.

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