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RESEARCH ARTICLE

Effect of physiological factors on production of cellulolytic enzymes by *Rhizoctonia bataticola*

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Abstract

Cellulose, an abundant carbohydrate, forms an integral part of the plant cell walls and provides structural integrity. Ability of any pathogenic fungus to degrade it depends largely on the extent of cellulolytic enzymes produced by a fungus. Therefore, present in vitro study was conducted to assess the effect of five each carbon and nitrogen sources on production of cellulolytic enzymes and growth of *Rhizoctonia bataticola* isolates the dry root rot pathogen of soybean. The results revealed that cellulolytic enzyme activity (µg of D-glucose/ml) of the 20 isolates of *R. bataticola* was strongly influenced and varied with the sources of carbon and nitrogen tested. Among five carbon sources tested, the cellulolytic enzyme activity was highest with Carboxy methyl cellulose (CMC) in the range of 0.629–1.286 µg, followed by Glucose (0.589–0.996 µg), Sucrose (0.549–0.961 µg), Starch (0.199–0.797 µg) and Pectin (0.152–0.293 µg) compared to control (0.107–0.187 µg). Similarly, all five nitrogen sources tested exhibited a wide range of cellulolytic enzyme activity among the test isolates. However, it was highest with Ammonium chloride (0.196–0.420 µg), followed by Potassium nitrate (0.170–0.425 µg), Glutamic acid (0.118–0.174 µg), Glutamine (0.109–0.171 µg) and Urea (0.100–0.167 µg), compared to control (0.080–0.107 µg).Various temperature regimes and pH of CMC broth medium also influenced the growth (mycelium dry weight) of the test isolates of *R. bataticola* and their cellulolytic enzyme activity. However, the temperature of 30 °C and 6.0 pH where found to be optimum.

Keywords *Rhizoctonia bataticola* · Cellulolytic enzyme · Carbon · Nitrogen · Temperature · pH

Introduction

Cellulolytic enzymes as digestive agents produced by fungi serve as invasive functionary which facilitates penetration of plant tissues by pathogens. The C_1 enzymes produced by an organism hydrolyze crystalline cellulose to simple and soluble anhydroglucose units, which further by C_x enzymes degraded to cellobiose and finally to glucose units. Cellulases hydrolyze cellulose polymer to smaller oligosaccharides and glucose. The model given by Wood et al. (1989) suggested that cellulose is degraded by the synergistic action of three types of enzymes viz. endo-β-glucanase (EC 3.2.1.4), exo-β-glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21). According to this model endo-β-glucanase (C_x) hydrolyze accessible intramolecular β-1, 4-glucosidic bonds of cellulase chains randomly to produce new chain ends. Number of plant pathogenic organisms are capable of producing multiple groups of enzymes, called cellulases which act to hydrolyze β-1, 4-D-glycosidic bonds within the cellulose molecules (Moreira et al. 2005). Cellulase, an abundant carbohydrate, forms an integral part of the plant cell walls and provides structural integrity. Major components of plant cell walls are cellulose, hemicellulose and lignin of which cellulose being the most abundant component. Fungi and bacteria are major natural agents of cellulose degradation. However, fungi play major role in decomposition of organic matter in general and of cellulosic substrate in particular (Lynd et al. 2002). A number of pathogenic fungi have been reported to produce cellulases in vivo and in vitro but relatively a small quantum of plant pathogens are able to degrade insoluble cellulose, even after it has been physically or chemically modified to make it more susceptible to enzyme action. The production of cellulase enzyme

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and degradation of cellulose by several fungi have been studied by many workers (Nagasathya et al. 2014). Production of cellulase enzymes by several pathogenic fungi and its role in causation and development of diseases has also been reported (Moussa and Tharwat 2013).

Materials and methods

Isolation and identification

Twenty isolates of *R. bataticola* were isolated from root rot (RR) infected soybean plants collected from various regions covering 19 districts of the state of Maharashtra, India (Table 1).

Pure cultures of these isolates were maintained on agar slant tubes in refrigerator (4 °C) for further studies. Based on morphological and microscope observations (Kanchan and Biswas 2009), the test fungus was identified as *Rhizoctonia bataticola* and further confirmed by pathogenicity test.

Table 1 *R. bataticola* isolates from diverse geographical locations in Maharashtra state

Locations	District	Code assigned
MPKV, Rahuri	Ahmednagar	Rb-1
Jamkhed Tahsil	Ahmednagar	Rb-3
KVK, Mohal	Solapur	Rb-5
DOGR, Rajgurunagr	Pune	Rb-6
ARS, Digras	Sangali	Rb-8
College of Agriculture, Kholapur	Kholapur	Rb-10
Patoda Tahsil	Beed	Rb-13
VNMKV, Parbhani	Parbhani	Rb-15
KVK, Pokharni, Nanded	Nanded	Rb-17
KVK, Jalna	Jalna	Rb-18
KVK, Aurangabad	Aurangabad	Rb-21
ORS, Latur	Latur	Rb-23
PDKV, Akola	Akola	Rb-24
KVK, Buldana	Buldana	Rb-27
COA, Nagpur	Nagpur	Rb-30
RRC, Amravati	Amravati	Rb-32
Jain Irrigation, Jalgaon	Jalgaon	Rb-33
KVK, Nandurbar	Nandurbar	Rb-36
COA, Dhule	Dhule	Rb-38
Hingoli	Hingoli	Rb-40

Effect of various carbon and nitrogen sources on production of cellulolytic enzymes

The method of Chapman et al. (1975) was used. The carbon-free basal medium containing 1 g K_2HPO_4, 0.3 g $MgSO_4$·7H2O, 1 g peptone, 0.1 g yeast extract and 0.1 g $NaNo_3$ solution made up to 1 litre with sterile distilled water (Kasana et al. 2008). For the effect of carbon sources, 1 g each of the carbon sources—starch, glucose, pectin, sucrose and carboxymethyl cellulose (CMC) were suspended separately in 10 ml distilled water in 250 ml flasks and autoclaved for 15 min. The pH was adjusted to 6.0 with 0.2 M NaOH after sterilization. The effect of nitrogen sources was determined using a nitrogen-free sterilized basal medium containing 1 g K_2HPO_4, 0.3 g $MgSO_4$·7H2O, 1 g peptone, 0.1 g yeast extract, 0.1 g $NaNo_3$ and solution made up to 1 litre with sterile distilled water. Solutions (1.0%) of nitrogen sources KNO_3, urea, NH_4CL, glutamic acid and glutamine, were dissolved separately in distilled water in 250 ml flasks, sterilized and incorporated separately into the basal medium as nitrogen source and the pH of each treatment was adjusted to 6.0.

About 20 ml nitrogen or carbon source was added to 20 ml sterile basal medium in 125 ml in each flask and these flasks were seeded separately with a mycelial disc (5 mm diam.) of the test isolates obtained from a 5-days old pure culture and incubated for 10 days at 30 °C. At end of the incubation period, the cultures were filtered separately through Whatman No. 1 filter paper.

Effect of temperature and pH on production of cellulolytic enzymes

The method of Chapman et al. (1975) was used. The utilization of an insoluble (native) form of cellulose was investigated with oven dried and weighed filter paper (Whatman no. 1, 11 cm). The basal medium composed of 1 g K_2HPO_4, 0.3 g $MgSO_4·7H_2O$, 1 g peptone, 0.1 g yeast extract, and solution made up to 1 litre with sterile distilled water. Utilization of soluble cellulose by the organism was determined by growing organism in medium composed of the same ingredients as that for insoluble cellulose except, 1.2 g $(NH_4)_2HPO_4$, 1.4 g urea, and 10 g carboxymethyl cellulose (CMC). CMC was deleted in some experiments to determine whether the cellulolytic enzymes were produced in its absence. pH of the media was adjusted to 7 with 0.2 M NaOH, after sterilization. The sterilized medium (30 ml per flask) was inoculated with agar-mycelial discs (5 mm dia.) obtained from 5-day-old pure culture of the test fungus and incubated at 30 °C for 10 days. Three replicates per treatment were maintained in CRD and mean

values were recorded. The filtrate was cleared by centrifugation at 3000 g for 20 min at 4 °C and used supernatant as culture filtrate. The mycelial pads used to harvest the cultures were oven-dried at 60 °C for 24 h and weighed.

Effect of temperature

The flasks containing medium for cellulolytic synthesis as earlier described were inoculated with the test fungus and incubated at 5, 10, 15, 20, 25, 30, 35 and 40 °C for 10 days. Three flasks were removed from each temperature value, filtered the contents and enzyme activity of the filtrate determined as described earlier.

Effect of pH

The effect of pH on stability of the enzymes from two preparations was studied by adjusting enzyme preparations to pH 2-8. The medium for enzyme assay was prepared as previously described and divided into seven portions with the pH adjusted to 2, 3, 4, 5, 6, 7 and 8, using appropriate buffer solutions as previously described (Amadioha and Oladiran 1993).

Enzyme Assay

The cellulolytic activity of filtrates was determined by the dinitrosalicylic acid method (Miller 1959). The reaction mixture consisted of 1 ml 1.0% (w/v) CMC in 0.1 M citrate buffer (pH 5.0) and 0.4 ml enzyme preparation. The reaction mixture was incubated in a hot water bath at 30 °C for 3 h. Boiled enzyme preparation was used as control. One ml of filtrate reaction mixture was transferred into a test tube. To this added 3 ml DNSA reagent (prepared by adding 1 g DNSA to 20 ml of 2 M NaOH and mixed with 20 g potassium sodium tartarate dissolved in 100 ml distilled water). The mixture was boiled for 5 min in a water bath at 100 °C and cooled under running tap water. The amounts of sugars released from the substrate were determined Spectrophotometrically at 570 nm using an SP 600 spectrophotometer. Reducing sugars released by the action of the culture filtrate on CMC were estimated quantitatively by refereeing to a standard curve constructed with standard aqueous solutions of D-glucose (0–10 mg/l). One unit was defined as the amount of enzyme per ml of reaction mixture that, under assay conditions, catalyzed the release of reducing sugars from 1 ml of CMC solution in 2 h. Three replicates per treatment were maintained and recorded mean value.

Results and discussion

Effect of different carbon and nitrogen source on production of cellulolytic enzyme

The results revealed that five each sources of carbon and nitrogen (Table 2) influenced strongly the cellulolytic enzyme activity (µg of D-glucose/ml) of the test isolates of *R. bataticola*. Among the carbon sources (Table 2), the cellulolytic enzyme activity was highest with CMC (0.629–1.286 µg), followed by Glucose (0.589–0.996 µg), Sucrose (0.549–0.961 µg), Starch (0.199–0.797 µg) and Pectin (0.152–0.293 µg) and compared to control (0.107–0.187 µg). CMC as a carbon source, induced maximum cellulolytic enzyme production by the isolate Rb-24 (1.286 µg), followed by Rb-3 (1.187 µg) and Rb-32 (1.032 µg) and minimum in isolate Rb-27 (0.629 µg). With Pectin, it was maximum in isolate Rb-8 (0.293 µg), followed by Rb-10 (0.276 µg) and Rb-15 (0.274 µg) and minimum in isolate Rb-18 (0.152 µg); with Glucose, it was maximum in isolate Rb-24 (0.996 µg), followed by Rb-27 (0.939 µg) and Rb-10 (0.935 µg) and minimum in isolate Rb-17 and Rb-30 (0.589 µg); with Starch, it was maximum in isolate Rb-21 (0.794 µg), followed by Rb-23 (0.775 µg) and Rb-36 (0.634 µg) and minimum in isolate Rb-13 (0.199 µg) and with Sucrose, it was maximum in isolate Rb-13 (0.961 µg), followed by Rb-10 (0.885 µg) and Rb-15 (0.806 µg) and minimum in isolate Rb-5 (0.549 µg). Whereas, in control (no carbon source), overall cellulolytic enzyme activity was minimum, which ranged from 0.107 µg (Rb-3) to 0.187 µg (Rb-32).

All five nitrogen sources tested, also encouraged the cellulolytic enzyme production/activity of the 20 test isolates of *R. bataticola* (Table 2). However, it was conclusively highest with Ammonium chloride (0.196–0.420 µg), followed by Potassium nitrate (0.170–0.425 µg), Glutamic acid (0.118–0.174 µg), Glutamine (0.109–0.171 µg) and Urea (0.100–0.167 µg), compared to control (0.080–0.107 µg). Among the test isolates, Potassium nitrate induced maximum cellulolytic enzyme activity in isolate Rb-32 (0.425 µg), followed by Rb-21 (0.414 µg) and Rb-33 (0.363 µg) and minimum in isolate Rb-5 (0.170 µg); Ammonium chloride, maximum in isolate Rb-32 (0.420 µg), followed by Rb-24 (0.366 µg) and Rb-6 (0.325 µg) and minimum in Rb-8 (0.196 µg); Glutamic acid, maximum in isolate Rb-13 (0.174 µg), followed by Rb-32 (0.157 µg) and Rb-3 (0.142 µg) and minimum in Rb-15 and Rb-30 (0.118 µg); Glutamine, maximum in isolate Rb-32 (0.171 µg), followed by Rb-40 (0.147 µg) and Rb-33 (0.141 µg) and minimum in Rb-5 (0.109 µg) and Urea, maximum in isolate Rb-32 (0.167 µg), followed by Rb-10 (0.132 µg) and Rb-30 (0.129 µg) and

Table 2 Effect of various carbon and nitrogen sources on cellulolytic enzyme production by *R. bataticola* isolates

Isolates no.	Cellulolytic enzyme production (µg/ml)											
	Carbon sources						Nitrogen sources					
	CMC	Pectin	Glucose	Starch	Sucrose	Control	Urea	Potassium Nitrate (KNO_3)	Ammonium Chloride (NH_4CL)	Glutamine	Glutamic acid	Control
Rb-1	0.975	0.184	0.627	0.218	0.627	0.144	0.109	0.212	0.279	0.129	0.126	0.102
Rb-3	1.187	0.210	0.731	0.466	0.579	0.107	0.122	0.251	0.312	0.134	0.142	0.096
Rb-5	0.957	0.208	0.730	0.226	0.549	0.136	0.115	0.170	0.213	0.109	0.122	0.080
Rb-6	0.901	0.187	0.649	0.434	0.597	0.125	0.125	0.312	0.325	0.122	0.136	0.107
Rb-8	0.730	0.293	0.910	0.276	0.655	0.116	0.122	0.158	0.196	0.129	0.122	0.083
Rb-10	0.913	0.276	0.935	0.405	0.885	0.155	0.132	0.273	0.231	0.112	0.135	0.100
Rb-13	0.791	0.192	0.839	0.199	0.961	0.122	0.107	0.289	0.282	0.139	0.174	0.096
Rb-15	0.958	0.274	0.809	0.351	0.806	0.129	0.112	0.228	0.285	0.118	0.118	0.100
Rb-17	0.878	0.244	0.589	0.331	0.708	0.120	0.105	0.240	0.228	0.119	0.123	0.099
Rb-18	0.780	0.152	0.916	0.279	0.600	0.128	0.100	0.269	0.286	0.129	0.128	0.097
Rb-21	0.859	0.170	0.669	0.794	0.711	0.141	0.116	0.414	0.308	0.115	0.128	0.106
Rb-23	0.727	0.195	0.698	0.775	0.752	0.128	0.106	0.296	0.287	0.131	0.135	0.100
Rb-24	1.286	0.212	0.996	0.245	0.575	0.131	0.116	0.334	0.366	0.119	0.128	0.099
Rb-27	0.629	0.179	0.939	0.366	0.569	0.135	0.116	0.193	0.226	0.112	0.138	0.103
Rb-30	0.832	0.192	0.589	0.510	0.640	0.129	0.129	0.286	0.282	0.122	0.118	0.099
Rb-32	1.032	0.235	0.695	0.620	0.698	0.187	0.167	0.425	0.420	0.171	0.157	0.107
Rb-33	0.724	0.192	0.647	0.255	0.742	0.138	0.112	0.363	0.276	0.141	0.126	0.102
Rb-36	0.746	0.180	0.610	0.634	0.668	0.160	0.123	0.241	0.271	0.113	0.119	0.105
Rb-38	0.946	0.258	0.631	0.630	0.658	0.139	0.112	0.283	0.293	0.123	0.129	0.102
Rb-40	0.780	0.218	0.681	0.300	0.687	0.132	0.109	0.283	0.271	0.147	0.131	0.105
SE ±	0.06	0.05	0.10	0.05	0.05	0.03	0.03	0.02	0.02	0.03	0.03	0.03
C.D.@5%	0.18	0.14	0.29	0.15	0.16	0.10	0.09	0.07	0.07	0.08	0.08	0.09

Average of three replication, control = basel media with no carbon and nitrogen source, *CMC* carboxymethyl cellulose

minimum in isolate Rb-18 (0.100 µg). Whereas, in control (no nitrogen source), overall cellulolytic enzyme activity was minimum, which ranged from 0.080 µg (Rb-5) to 0.107 µg (Rb-6 and Rb-32). Thus, an appreciable amount cellulolytic enzyme activity/production was exhibited by maximum number of the isolates of *R. bataticola*, with carbon sources in the order of merit CMC > Glucose > Sucrose > Starch > Pectin and nitrogen sources in the order of merit Ammonium chloride > Potassium nitrate > Glutamic acid > Glutamine > Urea. Among 20 test isolates of *R. bataticola*, which responded better to both carbon and nitrogen sources and exhibited appreciable amount of cellulolytic enzyme production, in the order of merit were Rb-32 > Rb-10 > Rb-13 > Rb-24 > Rb-5 > Rb-2.

Similarly, these results are in consonance with Bhattacharya (2013), who reported an appreciable cellulose enzyme activity in the culture filtrate of *Fusarium* grown on the culture medium amended with Pectin and CMC, as carbon sources. Singh et al. (2012) also reported maximum cellulolytic enzymes production by *F. oxysporum* f.sp. *pisi*, in the culture medium amended with carbon sources Sucrose and Glucose, and it was poor with maltose. Ramos et al. (2016) reported that the isolates of *Macrophomina phaseolina* produced maximum amount of cellulolytic enzymes, when grown in the broth culture medium supplemented with carbon sources viz., Glucose, Pectin and Carboxy methyl cellulose or xylan and/or glutamic acid as nitrogen source. Mallikharjuna et al. (2016) also reported that the isolates of *Trichoderma* spp. Produced maximum amount of extracellular enzymes in the broth culture medium amended with Fructose and Glucose, as carbon sources and Ammonium nitrate as nitrogen source.

Effect of temperature regimes on growth and production of cellulolytic enzyme

The results (Table 3) revealed that various temperature regimes influenced both growth (mycelium dry weight) and cellulolytic enzyme production by *R. bataticola* test isolates and both the parameters were maximum at optimum temperature of 30 °C, in all of the test isolates. The quantum of mycelium dry weight and cellulolytic enzyme

Table 3 Effects of various temperatures on cellulolytic enzyme production and mycelium dry weight of *R. bataticola* isolates grown in CMC medium

Isolates no.	Cellulolytic enzyme (µg/ml)								Mycelium dry weight (mg/30 ml)							
	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
Rb-1	0.000	0.000	0.232	0.639	1.261	2.453	0.884	0.000	0.00	0.00	19.20	34.60	79.33	96.97	26.77	0.00
Rb-3	0.000	0.000	0.251	0.655	1.235	2.401	1.025	0.000	0.00	0.00	20.00	34.37	77.37	92.13	39.90	0.00
Rb-5	0.000	0.000	0.357	0.703	1.327	2.527	0.868	0.000	0.00	0.00	19.23	38.63	81.53	97.43	26.37	0.00
Rb-6	0.000	0.000	0.380	0.726	1.328	2.768	0.948	0.000	0.00	0.00	21.10	39.63	81.07	99.83	34.63	0.00
Rb-8	0.000	0.000	0.196	0.684	1.250	2.865	0.912	0.000	0.00	0.00	18.53	35.73	78.73	108.53	30.27	0.00
Rb-10	0.000	0.000	0.196	0.780	1.221	2.616	0.890	0.000	0.00	0.00	18.20	43.33	77.07	97.37	27.10	0.00
Rb-13	0.000	0.000	0.414	0.687	1.263	2.368	0.893	0.000	0.00	0.00	21.87	36.47	78.20	91.93	27.40	0.00
Rb-15	0.000	0.000	0.184	0.787	1.190	2.394	0.810	0.000	0.00	0.00	15.57	43.97	74.57	93.27	25.13	0.00
Rb-17	0.000	0.000	0.196	0.726	1.216	2.427	0.791	0.000	0.00	0.00	15.90	40.17	75.70	92.73	24.67	0.00
Rb-18	0.000	0.000	0.229	0.749	1.357	2.482	0.761	0.000	0.00	0.00	18.53	40.03	85.13	95.17	23.43	0.00
Rb-21	0.000	0.000	0.328	0.745	1.318	2.363	0.797	0.000	0.00	0.00	20.03	41.97	80.03	92.47	24.13	0.00
Rb-23	0.000	0.000	0.324	0.708	1.346	2.539	0.903	0.000	0.00	0.00	19.43	39.30	81.33	97.27	26.37	0.00
Rb-24	0.000	0.000	0.383	0.719	1.356	2.847	0.946	0.000	0.00	0.00	20.70	39.53	80.03	105.50	30.73	0.00
Rb-27	0.000	0.000	0.216	0.780	1.211	2.353	0.708	0.000	0.00	0.00	18.17	42.83	74.83	90.80	19.27	0.00
Rb-30	0.000	0.000	0.196	0.729	1.254	2.395	0.925	0.000	0.00	0.00	17.57	40.97	78.80	92.53	30.83	0.00
Rb-32	0.000	0.000	0.372	0.704	1.338	2.880	0.974	0.000	0.00	0.00	19.13	37.97	79.53	108.47	33.80	0.00
Rb-33	0.000	0.000	0.424	0.800	1.386	2.960	0.977	0.000	0.00	0.00	21.63	45.53	80.03	111.90	32.37	0.00
Rb-36	0.000	0.000	0.424	0.719	1.305	2.854	0.836	0.000	0.00	0.00	20.10	41.50	79.90	105.77	28.90	0.00
Rb-38	0.000	0.000	0.462	0.772	1.402	2.929	0.945	0.000	0.00	0.00	22.37	42.83	82.07	107.97	31.50	0.00
Rb-40	0.000	0.000	0.456	0.701	1.399	2.836	0.904	0.000	0.00	0.00	22.87	40.27	83.07	103.27	27.63	0.00
SE ±	–	–	0.04	0.05	0.08	0.27	0.18	–	–	–	2.54	2.70	3.31	3.92	2.48	–
C.D.@5%	–	–	0.12	0.16	0.24	0.77	0.51	–	–	–	7.27	7.73	9.47	11.22	7.09	–

Average of three replication

production by the test isolates were found to be influenced drastically and inversely proportional to the temperature regimes. However, the test isolates could grow better and exhibit maximum cellulolytic enzyme activity, in the temperature range of 15 °C to 35 °C, but couldn't showed both of these traits below 15 °C (e.g. 5 and 10 °C) and above 35 °C (e.g. 40 °C) temperatures. The temperature of 30 °C was found optimum, at which mycelium dry weight (mg/30 ml broth) and cellulolytic enzyme activity (µg of D-glucose/ml) by the test isolates were highest in the range of 90.80–111.90 mg and 2.353–2.960 µg, respectively, followed by 25 °C (74.57–85.13 mg and 1.190–1.402 µg, respectively), 20 °C (34.37–45.53 mg and 0.639–0.800 µg, respectively), 35 °C (19.27–30.90 mg and 0.708–1.025 µg, respectively) and lowest at 15 °C (15.57–22.87 mg and 0.184–0.462 µg, respectively). At optimum temperature of 30 °C, among the test isolates, Rb-33 yielded highest mycelium dry weight (111.90 mg) and cellulolytic enzyme activity (2.960 µg), followed by Rb-8 (108.53 mg and 2.865 µg, respectively), Rb-32 (108.47 mg and 2.880 µg, respectively), Rb-38 (107.97 mg and 2.929 µg, respectively) and Rb-27 (90.80 mg and 2.353 µg, respectively).

Similarly, the temperature of 30 °C was reported optimum for cellulose enzyme production by several fungi including *Alternaria citri* and *Cochliobolus spicifer*. However, 30–35 °C was optimum for cellulose enzyme production by *Chaetomium globosum* (El-Said et al. 2014). The 30 °C temperature was reported optimum for the maximum production of cellulose enzyme by *Sclerotium rolfsii* (Chaurasia et al. 2015). Gautam et al. (2011) reported the optimum temperature and pH of the medium for cellulase production by *A. niger* as 40 °C and 6–7, respectively; whereas, those for the production of cellulase by *Trichoderma* sp. were 45 °C and 6.5, respectively. Mallikharjuna et al. (2016) evaluated the isolates of *Trichoderma* spp. for their ability to produce extracellular enzymes, which was significantly influenced by acidic pH and was optimum at 30 °C.

Effect of CMC broth pH on the growth and production of cellulolytic enzyme

The results (Table 4) revealed that various pH levels of the CMC broth greatly influenced both growth (mycelium dry weight) and cellulolytic enzyme production by

Table 4 Cellulolytic enzyme activity and mycelium dry weight of *R. bataticola* isolates influenced by various pH levels of CMC medium

Isolates no.	Cellulolytic enzyme production (µg/ml)							Mycelium dry weight (mg/30 ml)						
	2	3	4	5	6	7	8	2	3	4	5	6	7	8
Rb-1	0.000	0.158	0.642	1.076	2.199	0.717	0.347	0.00	39.43	70.90	84.00	111.73	70.97	42.70
Rb-3	0.000	0.177	0.623	1.035	2.259	0.761	0.266	0.00	41.43	69.37	81.67	116.57	71.50	41.40
Rb-5	0.000	0.119	0.659	1.003	2.240	0.685	0.241	0.00	37.83	71.33	80.27	114.53	67.40	40.97
Rb-6	0.000	0.144	0.687	0.917	2.106	0.698	0.334	0.00	38.33	73.17	77.90	106.90	69.10	42.20
Rb-8	0.000	0.195	0.685	1.041	2.214	0.634	0.290	0.00	42.13	72.07	82.17	111.77	66.30	41.57
Rb-10	0.000	0.165	0.694	0.926	2.125	0.730	0.228	0.00	40.93	72.30	78.93	107.43	70.97	40.07
Rb-13	0.000	0.110	0.669	0.957	2.163	0.714	0.212	0.00	37.50	71.47	79.53	109.27	69.47	39.03
Rb-15	0.000	0.170	0.547	0.935	2.177	0.668	0.370	0.00	41.20	66.63	78.57	111.23	67.73	44.87
Rb-17	0.000	0.100	0.668	0.957	2.134	0.631	0.340	0.00	36.17	71.70	79.20	107.40	66.10	42.27
Rb-18	0.000	0.155	0.566	1.013	2.093	0.601	0.196	0.00	39.50	67.30	81.73	103.17	65.03	38.80
Rb-21	0.000	0.174	0.557	0.986	2.176	0.645	0.216	0.00	39.93	66.20	80.17	110.07	66.03	40.47
Rb-23	0.000	0.160	0.679	1.044	2.147	0.681	0.279	0.00	40.93	72.77	82.10	108.40	67.20	41.30
Rb-24	0.000	0.210	0.716	1.099	2.286	0.784	0.382	0.00	44.83	74.10	85.33	117.97	74.30	45.47
Rb-27	0.000	0.165	0.549	1.032	2.132	0.620	0.287	0.00	41.50	66.60	81.87	107.33	65.67	41.13
Rb-30	0.000	0.142	0.572	1.060	2.108	0.687	0.335	0.00	38.20	68.67	83.33	106.37	68.07	42.37
Rb-32	0.000	0.195	0.682	1.054	2.250	0.791	0.354	0.00	42.67	72.23	82.97	115.00	74.40	43.97
Rb-33	0.000	0.216	0.697	1.108	2.221	0.759	0.395	0.00	45.17	73.27	85.53	114.17	72.33	45.77
Rb-36	0.000	0.184	0.540	0.973	2.119	0.682	0.354	0.00	40.43	65.83	80.47	107.63	67.80	42.07
Rb-38	0.000	0.168	0.533	1.013	2.138	0.703	0.322	0.00	40.33	65.80	81.00	108.77	69.40	41.47
Rb-40	0.000	0.150	0.607	1.042	2.180	0.663	0.234	0.00	39.83	69.37	82.17	110.80	67.20	40.37
SE ±	–	0.04	0.06	0.09	0.22	0.09	0.07	–	2.53	3.10	2.78	4.19	3.13	2.35
C.D.@5%	–	0.11	0.19	0.28	0.63	0.27	0.21	–	7.24	8.87	7.95	11.99	8.95	6.72

Average of three replication

R. bataticola test isolates and both the parameters were maximum at optimum pH of 6.0, in all of the test isolates, but there was no any growth or cellulolytic enzyme activity at pH 2.0. However, the test isolates could grow better and exhibit optimum cellulolytic enzyme activity, in the pH range of 3.0–8.0. The pH of 6.0 was found optimum, at which mycelium dry weight (mg/30 ml broth) and cellulolytic enzyme activity (µg of D-glucose/ml) by the test isolates were highest in the range of 103.17–117.97 mg and 2.093–2.28 µg, respectively, followed by the pH 5.0 (77.90–85.53 mg and 0.917–1.108 µg, respectively), pH 4.0 (66.20–74.10 mg and 0.540–0.716 µg, respectively), pH 7.0 (65.03–74.40 mg and 0.601–0.791 µg, respectively) pH 8.0 (38.80–45.77 mg and 0.196–0.395 µg, respectively) and lowest at pH 3.0 (36.17–45.17 mg and 0.100–0.216 µg, respectively). At optimum pH 6.0, among the test isolates, Rb-24 yielded highest mycelium dry weight and cellulolytic enzyme activity, respectively of 117.97 mg and 2.286 µg, followed by Rb-3 (116.57 mg and 2.259 µg, respectively), Rb-32 (115.00 mg and 2.250 µg, respectively) and lowest was Rb-18 (103.17 mg and 2.093 µg, respectively).

These results are in conformity with the results of Oyeleke et al. (2012) who reported maximum cellulase and pectinase enzymes production by *Aspergillus niger,* in the pH range of 4–6, of the culture medium. Adeleke et al. (2012) reported maximum production of cellulose and pectinase enzymes from orange peels by fungi within 5–5.5 pH. Gautam et al. (2011) reported optimum pH of 5.5 for cellulase enzyme production by *Trichoderma harziarum* and *T. viride*. The pH 5.0 was reported optimum for maximum production of cellulase enzyme by *Sclerotium rolfsii* (Chaurasia et al. 2015). Similar results were also reported by *Chaetomium globosum* (El-Said et al. 2014).

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