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Study of a Cellulase Producer, *Paenibacillus* 50-01

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Abstract A cellulase-producing bacterium designated 50–01 was isolated from a farm soil sample from Karnataka, India. The phenotypic and biochemical characteristics of this strain are presented here. The isolate is tentatively identified as belonging to the genus *Paenibacillus* based on the 16s rRNA gene sequence. The strain was found to be Gram positive, spore forming and rod shaped. Maximum cell growth was seen between 45–50°C and at pH 9. For endoglucanase from the culture supernatant, the optimum temperature was 50°C and optimum pH was 7.0. The enzyme was active over a wide range of pH, with almost 80% activity retained at pH 3 and about 70% activity retained at pH 10. The crude enzyme was found to be stable at 50°C for 24 h. Divalent ions such as Zn^{+2}, Co^{+2}, Cu^{+2}, Ca^{+2}, Mg^{+2} did not affect the enzyme activity to a great extent whereas, Hg^{+2}, Pb^{+2} and EDTA reduced the activity by about 50%. The isolate produces three endocellulases with molecular weights between 35–60 kDa. One of them was found to have a pI of less than 5.0, whereas the pI of the other enzyme was more than 8.8. The crude enzyme showed good activity with Avicel, acid-treated paddy straw and Carboxymethyl cellulose (CMC), releasing 4.07, 4.88 and 5.74 mg glucose / mL/h, respectively. Thus, the cellulase from the isolate 50–01 can be used for saccharification of plant biomass either alone or in combination with other fungal or bacterial cellulases.

Keywords Cellulase, *Paenibacillus*, Plant biomass, Acid pre-treatment, Alkali pre-treatment.

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Introduction

Cellulose is an unbranched polysaccharide, composed of linear chain of β- 1 - 4 linked D-glucose units. There are three major types of cellulose-degrading enzymes made by microbes namely Endo-β-1, 4-glucose (1, 4-β- D-glucan-4-glucanohydrolase ; EC 3.2.1.4), Exocello-bio-hydrolase CBH (1, 4-β-d-glucan glucohydrolase ; EC 3.2.1.74) and β-glucosidase (β-D-glucosideglucohydrolase ; EC 3.2.1.21). The endoglucanases randomly hydrolyze the β-1,4 bonds in the cellulose molecule. The exocello-bio-hydrolases in most cases release a cellobiose unit from the ends, which is subsequently converted to glucose by β-glucosidase (Béguin and Aubert 1994).

Cellulases have been found in a wide variety of organisms such as fungi, anaerobic and aerobic bacteria (Jutura and Wu 2014). The enzymes in these group differ from each other in many ways (Himmel et al. 2010), one such difference being the pH range in which the enzymes are active. Commercially used fungal cellulases have optimum pH between 4.5 to 6, beyond which their activity drops sharply. Neutral cellulases work between pH 6–8 and alkaline between pH 7.5–10. Neutral and alkaline cellulases have also found their way into certain specific application such as detergents and stone washing of denims (Karmakar

and Ray 2011, Akhtar et al. 2016). In this paper we describe the characterization of one such alkaliphilic bacterium capable of degrading cellulose and properties of the cellulases made by it.

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Materials and Methods

Bacterial strain and culture conditions

The isolation of *Paenibacillus* 50–01 was described in a previous study (manuscript in preparation). Strain 50–01 was cultivated in SOB-CMC medium at pH 10 that contained (L^{-1}) tryptone 20 g, yeast extract 5g, NaCl 500 mg, KCl 160 mg, $MgSO_4.7H_2O$ 2.5 g, CMC 0.2–0.5 g. All enzyme analysis were done with culture supernatants of isolate 50–01 grown in this medium at 37°C on a shaker for 3 days.

Identification of the isolate

Biochemical analysis was done using Biomerieux Vitek 2 cards. The isolate 50–01 has been identified previously using 16S rDNA sequencing (manuscript in preparation). The GenBank accession number for the 16S rDNA sequence of isolate 50–01 is MH675921.

Effect of pH and temperature on growth of the isolate

To determine the optimum pH for growth of the strain, sterile SOB-CMC broth of pH of 3, 4, 5, 6, 7, 8, 9, 10 and 11 were inoculated with overnight grown culture of 50-01 such that OD_{540nm} was < 0.05. The assay was set up in a 96-well plate where 200 uL of each of the above-inoculated medium were placed in nine wells. Sterile medium was used as blank. The plate was incubated at 37°C with shaking for 48 h. OD_{540nm} were taken every hour using EPOCH2 (BioTek Instruments, USA) instrument. The results are presented as averages of two independent experiments of nine replicates. To determine the optimum temperature, overnight grown culture of 50–01 was used to inoculate SOB-CMC at pH 9.0. The flasks were kept shaking at 30, 40, 50, 60 and 70°C. OD_{540nm} of the culture were taken every hour for about 8 h and the final reading after 24 h.

Optimum pH for enzyme activity

All enzyme assays were carried out as described by Ghose (1987). Optimum pH for endoglucanase activity of 50-01 supernatant was checked with 2% CMC made in 50 mM buffers at pH 3, 4.8, 7, 8, 9 and 10. Release of reducing sugars in 60 min at 50°C was measured by DNSA method (Miller 1959). FPase activity was assayed in a manner similar to that used to determine CMCase activity (Mandels et al. 1976). Whatman No 01 filter paper (50 mg) was suspended in 1 mL of 50 mM buffers at different pH to which 0.5 mL of the supernatant was added, mixed and incubated in a 50°C water bath for an hour, followed by reaction with DNSA reagent. A unit of activity is defined as the amount of enzyme required to liberate 1 µmol of glucose per min under assay conditions. Protein content of culture filtrates was also determined by Folin-Ciocalteu reagent (Lowry et al. 1951) using Bovine Serum Albumin (BSA) as standard. Buffers used were glycine-HCl buffer pH 3, Na-citrate buffer pH 4.8, phosphate buffer pH 7, Tris buffer pH 8, glycine-NaOH buffer pH 9 and carbonate-bicarbonate buffer at pH 10.

Optimum temperature for enzyme activity

Culture supernatants were mixed with 2% CMC made in Na-phosphate buffer pH 7 and incubated at 22°C, 30°C, 40°C, 50°C, 60°C, 70°C and 80°C for an hour. DNSA assay was carried out as mentioned above.

β-glucosidase activity

β-glucosidase activity was determined by measuring the hydrolysis of p-nitrophenyl b-D-glucopyranoside (PNPG) as described previously (Cai et al. 1998). The reaction mixture (1mL) contained 5 mM PNPG (Sigma) in 0.1M sodium acetate buffer (pH 5) and an appropriately diluted enzyme solution. After incubation at 50°C for 30 min, the reaction was stopped by adding 4 mL of 400 mM glycine-NaOH buffer. The color formed was measured at 430 nm. One unit of β-glucosidase activity is defined as the amount of

enzyme liberating 1 µmol of p-nitrophenol per minute under the assay conditions.

Activity of crude enzyme on various substrates

Paddy straw and coir were soaked in 1.0 N HCl and 1.5% NaOH, respectively, for a day. They were washed with three volumes of water thrice and dried. For the assay, 100 mg of these treated substrates were incubated with 1ml of the culture supernatant. The tubes were incubated at 50°C for an hour followed by reaction of 0.5 mL of the clear supernatant with 3 mL DNSA reagent (Mandels et al. 1976). In separate tubes, Whatman filter paper (50 mg) suspended in 1 ml phosphate buffer pH 7 was mixed with 0.5 mL supernatant ; similarly, 2% CMC in the same buffer, was mixed with 0.5 mL supernatant. The tubes were incubated at 50°C for 1 h and 3 mL of DNSA reagent was added to it.

Effect of additives on 50-01 endoglucanase activity

Constant volume of culture supernatant was mixed with appropriate amounts of different effector compounds and incubated at 50°C for 30 min. CMC (2%) made in 50 mM phosphate buffer pH 7 was then added to each tube. After mixing, the tubes were incubated at 50°C for an hour after which DNSA assay was carried out. The compounds tested were $CaCl_2$, $MgSO_4$, $K_2Cr_2O_4$, $CuCl_2$, $MnCl_2$, $HgCl_2$, Pb $(NO_3)_2$, Citric acid, $CdSO_4$, EDTA, $ZnSO_4$ and $CoSO_4$. The final concentrations used were between 1 mM to 100 mM.

Stability studies

The culture supernatant was incubated at temperatures ranging from 20°C to 90°C. Aliquots were taken at intervals and the enzyme assay was carried out with 2% CMC in 50 mM phosphate buffer at pH 7. To assess the stability of the enzyme in the presence of ionic detergent, SDS was added to the supernatants to get a final concentration of 0.1%. Aliquots of this mixture were incubated at 22°C, 40°C, 60°C, 80°C and 100°C for 10 min. Untreated controls were also kept under similar conditions.

Induction of cellulases

To analyze the effect of carbon sources on the induction of cellulases made by 50-01, various carbon sources were added in the growth medium. Stock solutions of the sugars (10–40%) were sterilised separately and added to the SOB medium to get a final concentration of 1–4%. Carbon sources tested include maltose, cellobiose, avicel, lactose, CMC, xylose, sucrose, fructose, galactose, ribose and dextrose. Supernatants were harvested after 48 h of growth at 37°C and assayed for FPase (total cellulase) and endoglucanase activity using DNSA method as described previously.

SDS-PAGE analysis of the supernatant

Four-day-old supernatant of 50-01 was concentrated 10 times using ethanol (final concentration 80%) and loaded on SDS-polyacrylamide gels, one containing 0.2% CMC and one without. The gels were run with pre-stained and unstained molecular weight ladders (10kDa-200kDa). The enzyme samples were run without any heat treatment at 25 mA constant current. The gel containing the CMC was incubated at 37°C for 3 h followed by staining with 0.1% Congo red and destaining with 1M NaCl Mazeau and Wyszomirski (2012). The gel without CMC was stained with 0.1% Coomassie Brilliant Blue R-250.

Purification using column chromatography

The binding of the cellulase from the supernatant to DEAE-sepharose, Q-sepharose, SP-sepharose and Phenyl-sepharose was studied. DEAE-sepharose (100 mg) was allowed to swell overnight in 50 mM Tris pH 8.0. Phenyl-sepharose and SP-sepharose were washed with 50 mM Tris with 1M NaCl. Supernatant was loaded on DEAE and Q-sepharose as it is. DEAE- and Q-sepharose were eluted successively with 50 mM Tris containing 0.2, 0.4, 0.8 and 1M NaCl. The supernatant mixed with NaCl to get a final concentration of 1M was loaded on Phenyl- and SP-sepharose. These were eluted progressively with 50mM Tris containing 0.8, 0.4, 0.2, 0.0M NaCl. The eluted fractions were spotted on CMC assay plates and also run on 10% SDS PAGE.

Table 1. Biochemical tests with Isolate 50–01 (+positive reaction, –negative, (–negative, (-) variable).

Test	50-01	Test	50-01
B-Xylosidase	+	D-Mannitol	–
L-lisine-Arylamidase	–	D-Mannose	–
L-apartate Arylamidase	+	D-Melizitose	(–)
Luecine Arylamidase	+	N-acetyl-D-glucosamine	–
Phenylalanine Arylamidase	+	Palatinose	–
L-proline Arylamidase	–	L-Rhamnose	–
Beta-Galactosidase	+	Beta-glucosidase	+
L-Pyrrolydonyl arylamidase	+	Beta-mannosidase	+
Alpha-Galactosidase	+	Phosphoryl choline	–
Alanine Arylamidase	+	Pyruvate	+
Tyrosine Arylamidase	+	Alpha-glucosidase	+
Beta-N-acetyl-glucosaminidase	–	D-Tagatose	–
Ala-Phe-Pro-Arylamidase	+	D-Trehalose	–
Cylcodextrin	–	Inulin	–
D-galactose	–	D-glucose	–
Glycogen	–	D-Ribose	–
Myo-inositol	–	Putrescine assimilation	–
Methyl-A-D-Glucopyranoside acidification	–	Growth in 6.5% NaCl	–
Ellman	+	Kanamycin resistance	(–)
Methyl-D-Xyloside	–	Oleandomycin resistance	–
Alpha-Mannosidase	+	Esculin hydrolysis	+
Maltotriose	–	Tetrazolium red	+
Glycine Arylamidase	–	Polymixin B resistance	–
Glycine Arylamidase	–	Polymixin B resistance	–

Determination of the PI of the enzymes

Agarose gels (1%) with 0.2% medium viscosity-CMC was made in 50 mM Na acetate buffer pH 5.0 and Tris buffer pH 8.8, separately. Concentrated supernatant (10 µL) was mixed with 2 µL of gel-loading dye. (The gel loading dye was made by mixing 250 µL of saturated Bromophenol blue with 750 µL of glycerol). The gels were run at 100V, 50 mA for an hour in the respective buffers. After the run, they were incubated at 37°C overnight, followed by staining with 0.2% Congo red and destaining with IM NaCl.

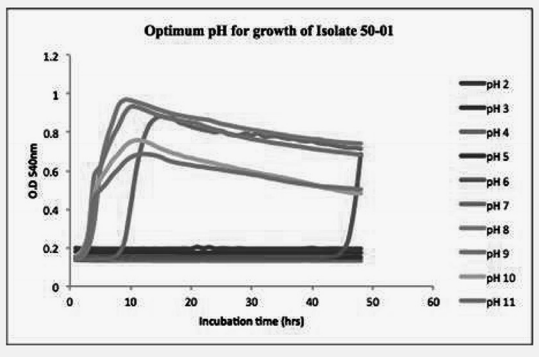

Fig. 1. Growth curves of isolate 50-01 at different pH. Growth curves were set up in micro titre plates with SOB-CMC medium at different pH. Optimum pH was around 9. At pH 7 a long lag was seen following which the culture grew to fairly good density.

Results

Identification of 50-01

The isolate 50-01 is gram positive, motile, sporulating bacillus. The colonies of 50-01 are flat, smooth and transparent. It forms motile micro colonies on agar plates. Its biochemical characterization indicated that it does not utilise D-glucose, D-mannose, galactose, sucrose, lactose, maltotriose, D-melizitose, D-mannitol, Cyclodextrin and glycogen (Table 1). The 16S rDNA analysis of the isolate showed 99% similarity with *Paenibacillus campinasensis*. The GenBank accession number for the 16S rDNA sequence of isolate 50-01 is MH675921. MALDI-TOF-MS analysis of the culture based on lipid and protein profiles indicated the strain to be *Brevibacillus*.

Optimum pH and temperature for cell growth

The isolate failed to show grow in SOB medium between pH 3 to 6. A long lag period was seen at pH 7 (Fig. 1). Whereas fastest growth was seen in medium at pH 9. Time course studies for cellulase production showed that maximum cellulase synthesis occurs at pH 9 between 40–72 h. The isolate 50-01 managed to grow well at pH 12 and day 3 supernatants at this pH showed 85% of the maximum activity. Growth curves carried out at 30°, 40°, 45°, 50°, 60° and 70°C

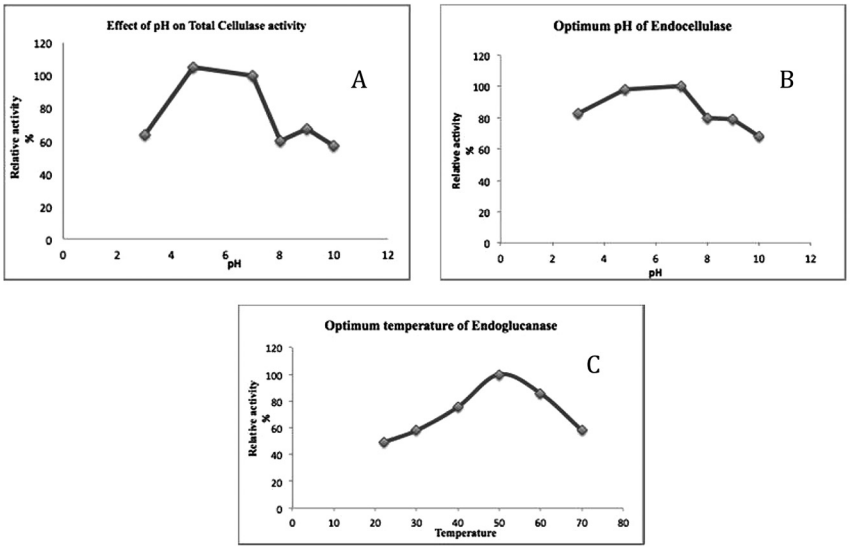

Fig. 2. Optimum pH of total cellulase in 50-01 supernatant. Optimum pH for endocellulase activity and Optimum temperature of endoglucanse activity.

showed that isolate 50-01 grew more rapidly in the first three hours at 50°C as compared to 45°C, but as the time progressed, the culture at 45°C grew better, eventually giving slightly higher cell densities at the end of 7 h and after overnight incubation. The isolate did not grow at temperatures of 55°C and above (data not shown).

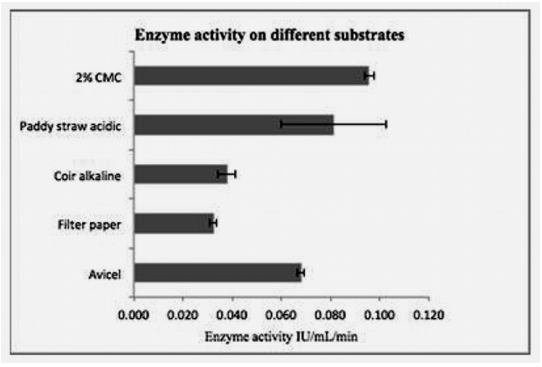

Fig. 3. Activity of the crude enzyme on various sub-strates. Filter paper and Avicel without treatment and pretreated coir and paddy straw were incubated with the culture supernatants and the sugars released were quantified using the DNSA assay. The assays were carried out in triplicates and error bars represent standard deviations.

Optimum pH and temperature of the *Paenibacillus* 50-01 cellulases

Substrates made in buffers at different pH were used to determine the optimum pH for the cellulase activity. Endoglucanase activity on CMC preferred a pH of 7.0 and 75% of the activity was retained at pH 9 (Fig. 2). Total cellulase activity, or FPase activity, showed a preference for pH 4.8 (Fig. 2) and 67% activity was retained at pH 9.0. Optimum temperature for the endoglucanase activity was 50°C (Fig. 2). ß-glucosidase activity of the isolate was measured after three days of growth on SOB-CMC medium and was found to 14.8 IU/mL/min.

Activity of crude enzyme on various substrates

The crude extract contained both exo-as well as endoglucanases, as it exhibited activity not only with CMC but also with Avicel , acid-treated paddy straw and alkali-treated coir (Fig. 3). The culture supernatant showed the following enzyme activities (in IU/ml L/min) against CMC–0.096 ; filter paper–0.032, Avicel –0.068 ; acid-treated paddy straw–0.081 and alkali-treated coir–0.038.

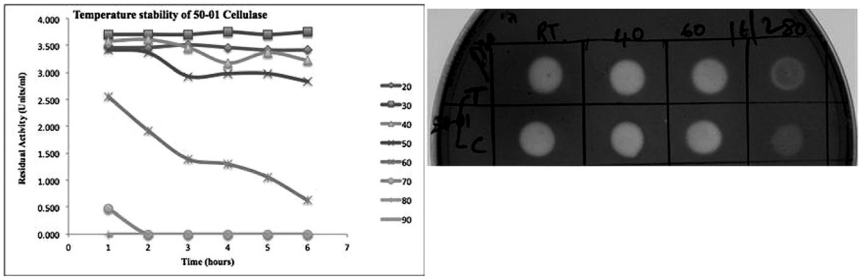

Fig. 4. Temperature stability of 50-01 cellulase . On storage at 50°C for 24 h, the endocellulase activity dropped by about 25%. At 60°C, there was a sharp drop in activity after 3 h. Stability of the enzyme for 10 minutes at 22°C, 40°C, 60°C, 80°C, 100°C in presence of 0.1% SDS. The supernatant after incubation at the above temperatures were spotted on CMC-assay plate and incubated for 3 h at 37°C, followed by staining with 0.1% Congo red and destaining with 1M NaCl. In presence of 0.1% SDS, the 50-01 cellulase was stable for 10 min at 60°C.

Stability studies

The enzyme in the crude extract was found to be stable at 50°C for 24 hs with decrease in acitivity of about 25% (Fig. 4). At 60°C, there was a 32% drop in activity seen after one hour of incubation. In the presence of 0.1% SDS, isolate 50-01 was found to be stable for 10 min at 60°C (Fig. 4).

Effect of additives on activity of the Paenibacillus 50-01 endoglucanase

Effect of divalent ions, heavy metals, chelators and surfactants was tested on the endoglucanase activity of isolate 50-01. Divalent ions, like Zn^{+2}, Co^{+2}, Cu^{+2}, Ca^{+2}, Mg^{+2} at 10 mM did not show any effect on the enzyme activity. Upto 10–20% reduction in activity was seen with Hg^{+2} 1mM, Pb^{+2} 1 mM, EDTA 10 mM and citric acid 100 mM. Hg^{+2} at 10 mM and EDTA at 100 mM reduced the enzyme activity by 50%. SDS at 0.1% did not affect the activity of the enzyme at 50°C (Fig. 5). At 100 mM Mg^{+2}, enzyme activity increased by 20%, while 10 mM Zn^{+2} showed an 8% increase in activity.

Effect of carbon sources on induction of cellulase

Cellulases were constitutively synthesised at a low level by 50-01 and the amount of enzymes increased

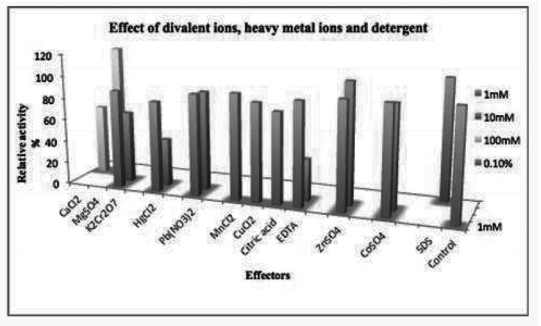

Fig. 5. Effect of additives on *Paenibacillus* 50-01 endoglucanase. $ZnSO_4$ (10 mM, 108.7%), $MgCl_2$ (100 mM, 119.3%), SDS (0.1%, 109.6%) enhanced endoglucanase activity.

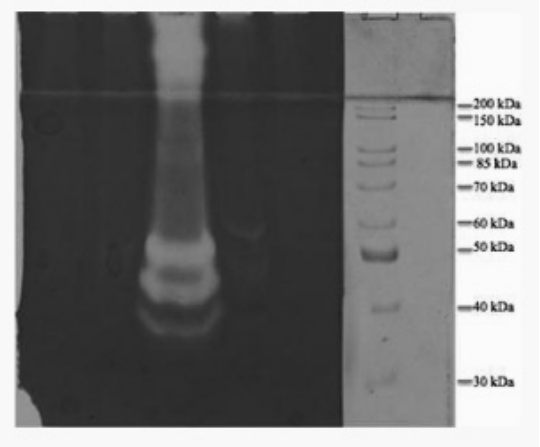

Fig. 6. Zymogram and protein staining on SDS-PAGE of ethanol concentrated supernatant of isolate 50-01. Three bands of enzyme activity corresponding to 40-60 kDa were seen.

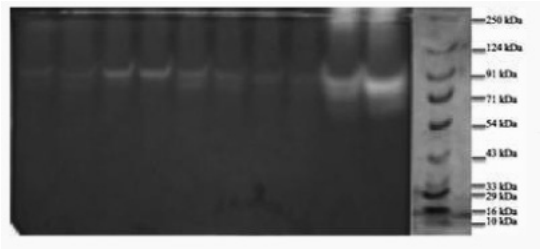

Fig. 7. SDS-PAGE and Zymogram of Phenyl-sepharose fractions. Lane 1 and 2 : 0.8 M NaCl fractions a, b, Lane 3 and 4: 0.4M. NaCl fractions b, c ; Lane 5 and 6: 0.2 M NaCl fractions a, b ; Lane 7 and 8 : 50 mM Tris fractions a, b ; Lane 9 :Crude sup and Lane 10 : flow-through. Some enzyme activity was seen in the flow-through indicating excess protein load. One enzyme can be seen eluting with 0.4 M NaCl.

multifold in the presence of substrates such as Avicel and CMC. As expected, presence of easily utilisable sugars like glucose, cellobiose, fructose, xylose strongly repressed synthesis of the cellulolytic enzymes.

SDS-PAGE analysis of the supernatant

Ethanol-concentrated supernatant of 50-01 was run on 10% SDS PAGE containing 0.2% CMC. Thezymogram gel showed presence of 5 activity bands. Three of them between 35–60 kDa (Fig. 6). Two bands were seen in the stacking gel. These could correspond to protein aggregates since the sample was not subjected to heat treatment.

Purification using column chromatography

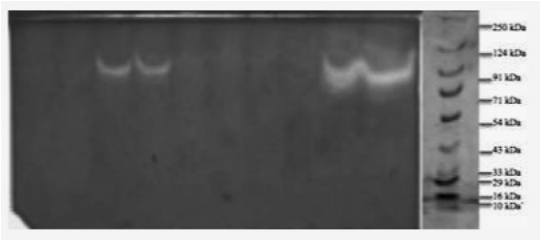

Fig. 8. SDS-PAGE and Zymogram of Q-sepharose fractions. Lane 1 and 2: 0.2 M NaCl fractions d, e ; Lane 3 and 4 : 0.4 M NaCl fractions d, e ; Lane 5 and 6: 0.8 M NaCl fractions c, d ; Lane 7 and 8: 1 M NaCl fractions c, d ; Lane 9:Crude sup and Lane 10: Flow-through. Crude sup shows two bands, of which one can be seen eluting out with 0.4 M NaCl.

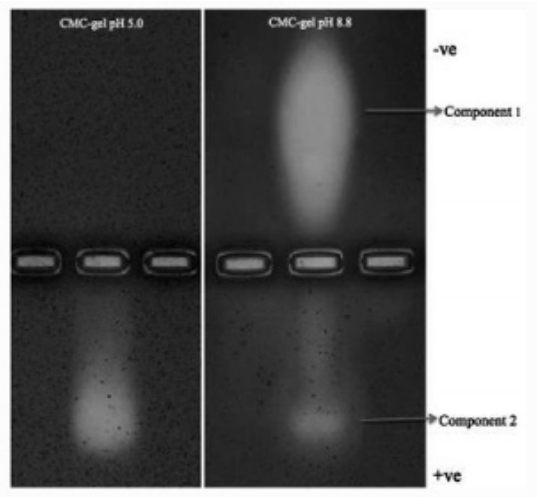

Fig. 9. (L) CMC-agarose gel pH 5, (R) CMC-agarose gel pH 8.8. The crude supernatant has two cellulases, one with pI less than 5 and other, more 8.8.

DEAE - and SP-sepharose did not bind to the enzyme effectively. Phenyl - and Q-sepharose were used to purify the cellulases in the culture supernatant of isolate 50-01. The bound enzymes were eluted successively with 50 mM Tris containing different concentrations of NaCl. The eluted fractions were spotted on CMC-assay plates and also run on 10% SDS-PAGE containing 0.2% CMC. In Phenyl-sepharose, one enzyme was found to elute with 0.4 M NaCl (Fig. 7), while another band of cellulase eluted with 0.2 M and 0M NaCl. With Q-sepharose, the enzyme was seen eluting in the buffer with 0.4 M NaCl (Fig. 8).

Determination of pI

The isolate 50-01 showed presence of enzyme component 1 which migrated towards anode at pH 8.8 and component 2 which moved towards the cathode (Fig. 9).This indicates that there are two different cellulases made by the bacterium, component 1 with pI more than 8.8 and component 2 with less than 5.0. Component 1 is active at alkaline pH but not acidic, whereas, component 2 shows activity at both acidic and alkaline pH, though the activity is more at acidic pH.

Discussion

The last decade has seen a lot of focused effort on-

searching for bacteria which can degrade cellulose (Koeck et al. 2014). This is largely due to the fact that fungal cellulases, though very efficient, work only in the acidic pH range, thus falling short in some industrial applications (Jorgensen et al. 2007). Quite a few bacteria capable of secreting alkaline cellulases have been found and some have made their way in to the detergent industry (Ben Hmad and Gargouri 2017). An inducible alkaline CMCase from alkalophilic *Bacillus* sp. no. 1139 was characterized by Fukumori et al. (1986) Kotchoni et al. (2006). Ito et al. (1989) isolated alkaliphilic *Bacillus* sp. KSM-635 which secretes two constitutive alkaline CMCases which are now used as a part of laundry additive.

We have studied one isolate, namely, 50-01, which is capable of growth and cellulase production upto pH 12. The ability of a cellulolytic bacterium to grow at such extremely alkaline pH has not been reported before. To identify and classify the isolate, biochemical characterization and 16s rDNA sequencing was carried out. The 16S rDNA analysis of the isolate showed 99% similarity match with *Paenibacillus campinasensis*.

However, Yoon et al. (1998) who originally isolated *Paenibacillus campinasensis* noted that it could grow between 10° and 45°C, the optimum temperature being 40°C. Their strain was alkaliphilic showing growth at pH 7.5–10.5, with an optimum at 10, but did not grow at pH 7. Our strain, 50–01, grew luxuriously at 50°C, though the optimum temperature for growth was 45°C. It did grow at pH 7, though with a long lag. The optimum pH was 9. Furthermore, their strain grew in 7% NaCl, while 50-01 failed to show growth in 6.5% NaCl.

Paenibacillus campinasensis BL11 isolated by Ko et al. (2007) and studied extensively for its xylanase activities showed white colonies on solid agar and lacked protease and lipase activities. In contrast, our strain produces completely transparent motile micro colonies and is protease and lipase-positive.

Identification based on the MALDI-TOF analysis indicated that the strain 50-01 belongs to the genus *Brevibacillus*. Taking into consideration all these factors, the taxonomic status of the isolate 50-01 needs to be evaluated in detail at a later date.

In the present work, the biochemical characterization of its cellulase enzymes and the feasibility of using these for industrial applications was also studied. It is very clear that the bacterium secretes at least two different types of enzymes, one very active at alkaline pH and the other at acidic pH.

We observed that Mg^{+2} and Zn^{+2} enhanced endoglucanase activity, though Ca^{+2} did not show any effect. When Obeng et al. (2017) reported the effects of additives on the activities of endoglucanase, exoglucanase and β-glucosidase from *T. reesei*, they showed that $CaCl_2$ and non-ionic detergents remarkably improved the exo and endocellulase synergism on cellulosic biomass. This difference seen in the behavior of enzymes from the two organisms could be attributed to the differences between the bacterial and fungal cellulolytic systems.

Effect of non-ionic detergents was not investigated by us. An increase was seen with the ionic detergent SDS, which probably promotes mobility of the enzyme. Inhibition by Hg^{+2} indicated the importance of-SH groups in enzyme function, as observed in other microbial cellulases.

As predicted by Horikoshi in 1999, alkaliphilic cellulose degraders are now very much in demand, in the detergent industry, food industry, waste management and bioremediation, where they can be used alone or as partners to work with fungal cellulases.

The 50-01 supernatant digested alkali-treated coir and acid-treated paddy straw in a short time interval. Other substrates and pre-treatment methods need to be evaluated for effective degradation of raw materials using 50-01 cellulases. It can then be used in the biofuel industry for conversion of cellulosic plant biomass to sugars (Yeoman et al. 2010).Therefore, isolate 50-01 holds promise for the future.

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