



## Original Research Article

# Optimization of extracellular cellulase enzyme production from *Alternaria brassicicola*

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## A B S T R A C T

### Keywords

*Alternaria brassicicola*, Endo- $\beta$ -1,4-glucanase and exo- $\beta$ -1,4-glucanase, SDS-PAGE.

Cellulase is an enzyme has great industrial importance. Cellulase catalyzes the conversion of insoluble cellulose to simple, water-soluble products. Cellulases are produced from a wide variety of microorganisms including fungi and bacteria. The present study aimed to the screen phytopathogenic isolates of *Alternaria brassicicola* infecting crucifers for production of cellulase enzyme specifically exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase. Different physical and biotechnological parameters such as incubation time, temperature, pH, substrate specificity of carbon and nitrogen sources, were optimized. The exo- $\beta$ -1,4-glucanase activity of *A. brassicicola* was found more in comparison to endo- $\beta$ -1,4-glucanase activity. The mean cellulase (exo- $\beta$ -1,4-glucanase) activity of all thirty two isolates of *A. brassicicola* was found maximum on 5<sup>th</sup> and 6<sup>th</sup> day of incubation, at 35<sup>o</sup>C temperature and pH-6 in the presence different nutrient sources viz., citrus fruit peel, sucrose, cellulose, yeast extract and sodium nitrate. SDS-PAGE of partially purified cellulase revealed ~60kDa and ~52kDa of endo- $\beta$ -1,4-glucanase and exo- $\beta$ -1,4-glucanase respectively.

## Introduction

Cellulose is the most abundant component of plant biomass, exclusively in plant cell walls (Lynd *et al.*, 2002). Cellulose is totally insoluble in water (Lederberg, 1992). It is a linear, unbranched homopolysaccharide consisting of glucose subunit joined together via  $\beta$  1-4 glycosidic linkages. Individual cellulose molecules (polymer) vary widely in length and are usually arranged in bundles or fibrils (Walsh, 2002). Within the bundles, cellulose molecules can occur in crystalline or paracrystalline (amorphous) structures (Walter, 1998).

Cellulolytic enzymes are synthesized by a number of microorganisms. Fungi and bacteria are the main natural agents of cellulose degradation (Lederberg, 1992). However, fungi are well known agents of decomposition of organic matter, in general, and of cellulosic substrate in particular (Lynd *et al.*, 2002). The initial step in cellulose destruction is the enzymatic hydrolysis of polymers. The enzyme or enzyme-complex involved in the hydrolysis has been given name as cellulases. Cellulase

catalyzes the conversion of insoluble cellulose to simple, water soluble products (Alexander, 1961). The basic enzymatic process for the depolymerization of cellulose requires three types of enzymes: Endoglucanase (EG or CX), hydrolyses internal  $\beta$ -1,4 glucan chain of cellulose at random, primarily within amorphous regions and display low hydrolytic activity toward crystalline cellulose (Walsh, 2002; Grassin and Fauquembergue, 1996); Exoglucanase i.e., exoacting cellobiohydrolases (CBH), removes cellobiose from the non-reducing end of cello-oligosaccharide and of crystalline, amorphous and acid or alkali treated cellulose; Cellobiase or  $\beta$ -glucosidase (BGL), hydrolyses cellobiose to yield two molecules of glucose which completes the depolymerization of cellulose (Himmel *et al.*, 1994). Cellulases have enormous potential in industrial applications (Walsh, 2002), food processing, feed preparation, waste-water treatment, detergent formulation, textile production and in other areas. Additional potential applications include the production of wine, beer and fruit juice, biofuel (Philippidis, 1994).

Amongst fungal species, *Trichoderma* and *Aspergillus* are well known for having cellulolytic potential (Lynd *et al.*, 2002). *Alternaria* species has long been reported for their cellulolytic potential (Logan and Siehr, 1966) and some strains had previously been mutagenized for improved productivity (Macris, 1984). It has also been proved that some of the species of *Alternaria* induce plant invasion by elaborating the cellulolytic enzymes (Eshel *et al.*, 2000); and genes encoding endoglucanases from this organism have been characterized (Eshel *et al.*, 2002). The hydrolytic potential of an indigenous strain MS28 of *Alternaria* has been reported (Sohail *et al.*, 2009) and the production and partial characterization of cellulases are

described (Sohail *et al.*, 2011). Cellulase enzyme production and optimization study were reported from different *Alternaria* spp including *Alternaria helianthi*, *Alternaria triticina*, *Alternaria sesame* (Bhaskaran and Kandaswamy, 1978; Dawar and Jain, 2010; Jahangeer *et al.*, 2005; Jha and Gupta, 1988; Marimuthu *et al.*, 1974), *A. alternata* (Macris, 1984; Anand *et al.*, 2008; Hubballi *et al.*, 2011; Gautam *et al.*, 2012; Saleem *et al.*, 2013), *Alternaria brassicae* (Jain and Dhawan, 2008; Garg *et al.*, 1999).

The present study was designed to isolate and optimize the production of cellulase specifically, exo- $\beta$ -1, 4-glucanase and endo- $\beta$ -1,4-glucanase enzymes from the phytopathogenic fungi, *Alternaria brassicicola*, dark leaf spot causing pathogen in crucifers. The respective enzymes were also characterized on SDS-PAGE.

## Materials and Methods

### Collection and maintenance of *Alternaria brassicicola* isolates

Thirty two isolates of *Alternaria brassicicola* were isolated and purified from *Alternaria* blighted leaf, stem and seed samples of cauliflower from seventeen different locations of India during 2009–2012 (Table 1). Blighted sample pieces (2mm) were surface sterilized with 0.1% Mercuric chloride ( $\text{HgCl}_2$ ) for one minute, rinsed in sterile water 3x for 10 min and placed on Potato Dextrose Agar (PDA) plates. Fungal growth was observed after 5–7 days of incubation at 25°C. *A. brassicicola* isolates growing on the leaf/stem pieces were confirmed microscopically (Ellis, 2001) and transferred onto other PDA plates until purification by single spore technique. Purified isolates were preserved as PDA slants at 4°C.

### Screening of *Alternaria brassicicola* isolates for cellulolytic activity

Plate assay was carried out by inoculating Cellulose Congo Red Agar medium with mycelial agar plugs (0.5cm) from 7 days old *Alternaria brassicicola* isolates grown on PDA. The inoculated plates were incubated for 7 days at room temperature. Cellulase production was observed by the clear zone around the fungal colonies (Gupta *et al.*, 2012).

### Cellulase (exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase) production by *Alternaria brassicicola*

For quantitative assay of production of cellulase from *A. brassicicola* isolates basal liquid media (modified Mandels Medium) was prepared (Jahangeer *et al.*, 2005; Mandel *et al.*, 1976) consisting of peptone (0.1%), urea (0.03%),  $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$  (0.0016%),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (0.0014%),  $(\text{NH}_4)_2\text{SO}_4$  (0.14%),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.03%),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (0.05%),  $\text{CaCl}_2$  (0.01%),  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (0.0029%),  $\text{KH}_2\text{PO}_4$  (0.2%) and cellulose (1%), pH-5.5. About 10ml of medium was poured in 100ml Erlenmeyer conical flasks and autoclaved at 15 lbs pressure for 20 minutes.

The flasks on cooling were inoculated separately with mycelial agar plugs (0.5cm) cut from 7 days old *Alternaria brassicicola* cultures grown on PDA plates. The flasks were incubated for 72 hrs at 150rpm and 30°C with diurnal periodicity of light.

On 7<sup>th</sup> day, the flasks were harvested by filtering the contents through Whatman filter paper no.1. The filtrate was centrifuged at 10000 rpm in 4°C for 10 minutes and the supernatants were used for enzyme activity assay as crude enzyme.

### Assay for cellulase activity by spectrophotometric method

**a. exo- $\beta$ -1,4-glucanase assay:** The exoglucanase activity was determined by incubating 1ml of crude enzyme with 1ml of 0.5% cellulose in 0.1M Citrate Phosphate buffer (pH-4.8) for 30 min at 50°C. The resulted reducing sugar was estimated using dinitrosalicylic acid (DNS) method (Jadhav *et al.*, 2013).

**b. endo- $\beta$ -1,4-glucanase/ CMCase assay:** The endoglucanase activity was determined by Jadhav *et al.*, 2013. 1ml of crude enzyme was incubated with 1ml of 1% Carboxy methyl cellulose (CMC) in 0.1M Sodium acetate buffer (pH-5) for 30 min at 50°C. The resulted reducing sugar was estimated using dinitrosalicylic acid (DNS) method.

The concentration of reducing sugar was estimated using dinitrosalicylic acid (DNS) method. 1ml of DNS reagent was added to the mixture and boiled for 5 min at 90°C. The reaction was stopped by adding 1ml of Rochelle's salt. The absorbance was measured spectrophotometrically at 540 nm. The amount of reducing sugars present in the sample was calculated using the standard graph, obtained using standard glucose solution, following the same procedure. One unit of cellulase activity was defined as the amount of enzyme which liberated 1 $\mu$ mol glucose per min.

### Effect of different parameters on cellulase enzyme production form *A. brassicicola* isolates

#### Effect of different day of incubation

Basal media was inoculated with agar plugs of *Alternaria brassicicola* culture and incubated at 30°C in a rotary shaker at 150 rpm. Culture filtrate was collected from the

flask at different period of incubation (after 1, 2, 3, 4, 5, 6 and 7 days). Absorbance was recorded spectrophotometrically at 540nm.

#### **Effect of different substrate (carbon and nitrogen source)**

To observe the effect of carbon sources, cellulose from the basal media was replaced by similar concentrations of agricultural wastes (rice husk, wheat bran, saw dust and citrus fruit peel) and synthetic chemicals (d-glucose, maltose, fructose, sucrose and cellulose). Similarly to observe the effect of nitrogen sources on Cellulase (exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase) production by *A. brassicicola* isolates the peptone component of the basal media was replaced with other nitrogenous substances viz., peptone, yeast extract, KNO<sub>3</sub>, NH<sub>4</sub>Cl and NaNO<sub>3</sub> etc). Flasks were incubated at 30<sup>0</sup>C in a rotary shaker at 150rpm. Culture filtrate was collected after 3<sup>rd</sup> day of incubation and absorbance was recorded spectrophotometrically at 540nm.

#### **Effect of different pH**

A set of basal media were prepared with varying pH (3, 4, 5, 6, 7, 8 and 9). Media were autoclaved and inoculated with *A. brassicicola* culture. Flasks were incubated at 30<sup>0</sup>C in a rotary shaker at 150rpm. Culture filtrate was collected after 3<sup>rd</sup> day of incubation and absorbance was recorded spectrophotometrically at 540nm.

#### **Effect of different temperature**

A set of basal media were prepared with constant pH 5.0 and inoculated with *A. brassicicola* culture. Flasks were incubated in a rotary shaker at 150rpm at different temperatures (25<sup>0</sup>C, 30<sup>0</sup>C, 35<sup>0</sup>C, 40<sup>0</sup>C and 45<sup>0</sup>C). Culture filtrate was collected after 3<sup>rd</sup> day of incubation and absorbance was recorded spectrophotometrically at 540nm.

#### **Protein assay**

The protein content of the crude enzyme was determined by the Bradford assay using Bovine Serum Albumin (BSA) as standard.

#### **Statistical analysis**

The data of cellulase activity for all the isolates were statistically analyzed using PRISM software version 3.0. The data for plate assay was analyzed at p<0.05 by performing one way ANOVA and least significant difference was calculated by student's t-test while the cellulase activity at different parameters was analyzed by performing two-way ANOVA at p<0.0001.

#### **Partial purification of cellulase enzymes from *A. brassicicola***

The supernatant/crude enzyme from *A. brassicicola* isolate (CaAbcT4) was partially purified using the following two sequential steps (Klug-Santner *et al.*, 2006).

#### **Ammonium sulphate precipitation**

The supernatant was brought to 60% saturation by mixing ammonium sulphate (pH 7.0) slowly with gentle agitation and allowed to stand for 24hrs at 4<sup>0</sup>C in the cold room. After the equilibration, the precipitate was removed by centrifugation (10,000 rpm at 4<sup>0</sup>C for 20 min). The precipitate obtained was dissolved in 10ml of 0.5ml phosphate buffer (7.0).

#### **Desalting by dialysis**

The precipitate was desalted by dialysis following the standard protocol, the 10cm pretreated dialysis bag was taken and activated by rinsing in double distilled water. One end of the dialysis bag was tightly tied and the precipitate recovered was

taken inside the bag. The other end of the dialysis bag was tightly tied to prevent the leakage. After that, dialysis bag was suspended in a beaker containing phosphate buffer (pH 7.0).

### **SDS-Polyacrylamide gel electrophoresis (PAGE)**

Twelve percent SDS-PAGE was performed on the partially purified cellulase both exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase enzymes from *A. brassicicola* isolate (CaAbcT4) by the method described by Laemmli (1970) using Bio-Rad electrophoresis apparatus. The gel was run on a constant voltage of 50 V. The gel was stained by the coomassive brilliant blue stain.

### **Results and Discussion**

Fungi and bacteria are the main natural agents of cellulose degradation (Lederberg, 1992), however, fungi are well known agents of decomposition of organic matter, in general, and of cellulosic substrate in particular (Lynd *et al.*, 2002). Cellulase catalyzes the conversion of insoluble cellulose to simple, watersoluble products (Alexander, 1961). The basic enzymatic process for the depolymerization of cellulose requires three types of enzymes: Endoglucanase, Exoglucanase and Cellobiase or  $\beta$ -glucosidase (Himmel *et al.*, 1994). Cellulases have enormous potential in industrial applications (Walsh, 2002), food processing, feed preparation, wastewater treatment, detergent formulation, textile production and in other areas. Additional potential applications include the production of wine, beer and fruit juice, biofuel (Philippidis, 1994). In the present study production of exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase were evaluated from thirty two isolates of *A. brassicicola*.

Screening of all thirty two isolates of *A. brassicicola* on cellulose congo red agar media showed clear zones after 7 days of incubation indicating the cellulolytic ability of the fungi (Fig. 1). Among all the isolates, the *A. brassicicola* isolate from Orissa (CaAbcO2) was found to be producing highest cellulase in the form of largest clearance zone of 1.13cm while the isolate from Uttar Pradesh (CaAbcUP2) was producing minimum clearance zone of 0.17cm (Table 1). Statistical analysis showed plate assay for cellulase production by *A. brassicicola* isolates were significant ( $p < 0.05$ ).

Further all the thirty two isolates of *A. brassicicola* were evaluated for cellulase (exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase) production in submerged fermentation with good amount of reducing sugars in the cellulose/carboxy methyl cellulose (CMC) reaction mixture after treatment with culture filtrates. The mean cellulase (exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase) activity of all thirty two isolates showed significant difference ( $p < 0.0001$ ) due to the effect of different parameters (Fig. 2) while all the thirty two isolates showed similar results individually with no significant difference (Fig. 3). While studying the effect of different parameters (incubation time, pH and temperature) on the cellulase enzyme activity, the exo- $\beta$ -1,4-glucanase activity of *A. brassicicola* was found more in comparison to endo- $\beta$ -1,4-glucanase activity. The mean cellulase (exo- $\beta$ -1,4-glucanase) activity of all thirty two isolates of *A. brassicicola* was found maximum on 6<sup>th</sup> day (64.81U/ml) of incubation, at 35<sup>o</sup>C temperature (43.13U/ml) and pH-6 (27.18U/ml), whereas the endo- $\beta$ -1,4-glucanase activity of all thirty two isolates of *A. brassicicola* was found maximum on 5<sup>th</sup> day (12.50U/ml) of incubation, at 35<sup>o</sup>C temperature (35.93U/ml) and pH-6 (15.55U/ml).

In order to study the effect of carbon sources on cellulase (exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase) production in the fungi *A. brassicicola* isolated from crucifers, different carbon sources including agricultural wastes (rice bran, wheat bran, saw dust and citrus fruit peel), monosaccharide (d-glucose), disaccharides (maltose, fructose and sucrose) and polysaccharides (cellulose) were added to the basal media separately. There was no such significant difference in between the exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase activity of all the *A. brassicicola* isolates. Among agricultural wastes Citrus fruit peel helped in maximum mean cellulase (exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase) activity (86.43U/ml and 86.18U/ml respectively) followed by rice bran (78.49U/ml and 78.07U/ml respectively). Among synthetic carbon sources sucrose produced maximum exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase activities viz., 120.71U/ml and 120.33U/ml respectively followed by cellulose (96.83U/ml and 96.42U/ml respectively).

To study the effect of nitrogen sources, organic nitrogen in the form of peptone and yeast extract were incorporated separately in the basal medium while inorganic nitrogen in the form of nitrate and ammonium forms were incorporated separately. Basal medium containing peptone served as the control. Among organic nitrogen sources, yeast extract was found to increase the mean exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase activities viz., 59.01U/ml and 58.72U/ml) as compared to peptone which produced 48.29U/ml exo- $\beta$ -1,4-glucanase and 47.51U/ml endo- $\beta$ -1,4-glucanase respectively. Among inorganic nitrogen source sodium nitrate (106.93U/ml and 106.78U/ml) was found to enhance mean exo- $\beta$ -1,4-glucanase and endo- $\beta$ -1,4-glucanase activities respectively.

Similar results were reported by different workers on different *Alternaria* spp. including *Alternaria helianthi*, *Alternaria triticina* and *Alternaria sesame* (Bhaskaran and Kandaswamy, 1978; Jha and Gupta, 1988; Marimuthu *et al.*, 1974). Screening of cellulolytic ability of fungi from native environmental source including soil, air and infected plant were done, in which *Alternaria* spp also found to possess cellulose degrading ability. Highest yield of enzyme was noted at 37°C while maximum activity in the range of pH 4–4.8 was observed after 7 days. Cellulase synthesis increased by ~10 folds in the presence of cellulose while it repressed in the presence of glucose (Jahangeer *et al.*, 2005). Cellulose production also reported from *A. alternata* causing fruit rot in chilli (Anand *et al.*, 2008). Hubballi *et al.*, (2011) finds that the activity of cellulolytic enzymes increased with the increase in the age of the culture of *A. alternata* when infected to Noni plant. *Alternaria* spp and *A. alternata* were found among the cellulase producers by helping in biodegradation of municipal solid waste (Gautam *et al.*, 2012). Similarly *Alternaria alternata* along with several other fungal species isolated from anise and cumin seeds in Upper Egypt were found to produce cellulases (exo- and endo- $\beta$ -1,4-glucanase) on solid media (Saleem *et al.*, 2013).

Macris (1984) also reported similar result in *Alternaria alternata*, Carboxy methylcellulase and extracellular,  $\beta$ -glucosidase functioned optimally at pH 5 to 6 and 4.5 to 5 and at temperatures of 55 to 60 and 70 to 75°C, respectively. Both temperature optima and thermo stability of P-glucosidase were among the highest ever reported for the same enzyme excreted from cellulase and  $\beta$ -glucosidase hyper producing microorganisms. To understand the mechanism of *Alternaria brassicae* induced changes in the activities of cell wall

degrading enzymes viz. polygalacturonase (PGU), cellulase and  $\beta$ 1-3 glucanase, was studied by Jain and Dhawan (2008) and finds that role of both PG and cellulase in pathogenesis. Garg *et al.*, (1999) studied the cell wall degrading enzymes in *Alternaria brassicae* and finds that polygalacturonase and cellulase decreased in leaf blight resistant cultivar RC-781 and increased in the susceptible cultivar Varuna up to 3 days.

Polygalacturonase (PG) and cellulase activities increased progressively upon infection with *Alternaria helianthi* in sunflower leaves of susceptible cultivar upto 7 and 9 days respectively. In contrast, a decline in activities of PG and cellulase was discerned in leaves of resistant cultivar upto 9 days after inoculation (DAI). The results suggest that PG and cellulase may play a role in pathogenesis and  $\beta$  1,3 glucanase in expression of resistance (Dawar and Jain, 2010).

The protein content for cellulase enzyme among the *A. brassicicola* isolates varies from 15.5 to 30 $\mu$ g/ml. Crude and partially purified cellulase enzymes from *Alternaria brassicicola* (CaAbcT4) isolate was electrophoresed on 12% SDS-PAGE. Two bands showing cellulolytic activity were detected having molecular weights of ~60kDa & ~52kDa representing endo and exo glucanase enzymes respectively (Fig. 4). Similarly two bands (proteins) representing the isoenzymes or subunits of the cellulase enzyme were also reported by different workers in *Aspergillus niger* (Devi and Kumar, 2012).

Use of fungi for enzyme production have

many advantages such as, the enzymes produced are normally extracellular, making easier for downstream process. In the present study cellulase production was found from phytopathogenic *Alternaria brassicicola* isolates which were screened by cellulose congo red plate assay method.

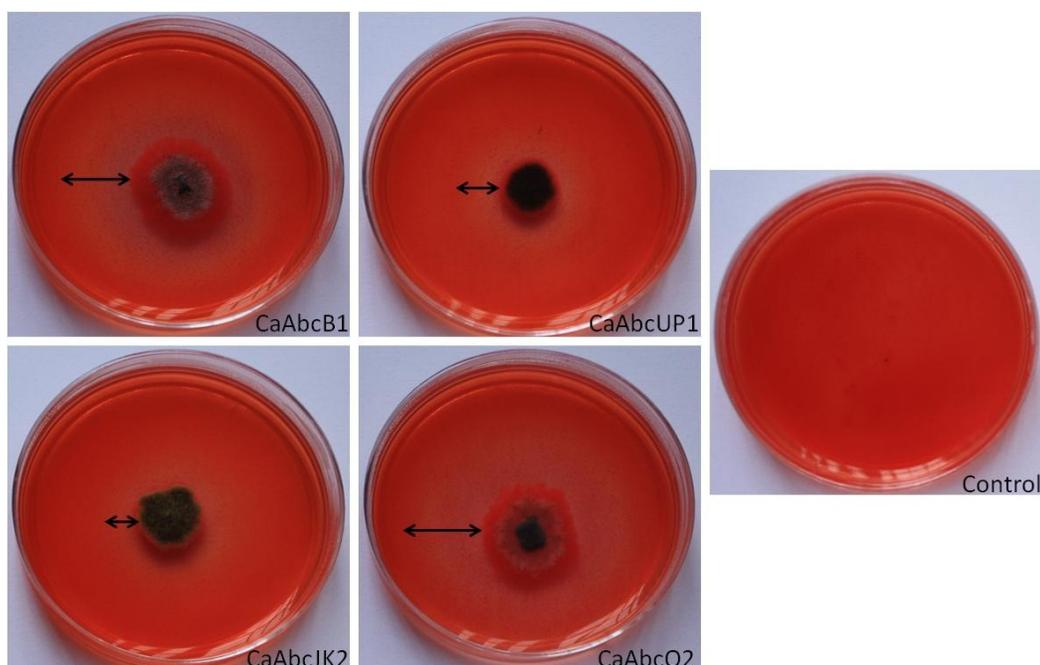
The process development is the key step in fermentation processes. The study related to process development involves optimization of different fermentation conditions (physical and nutritional) towards enhancement of cellulolytic enzymes production. Shake flask cultural conditions (physical and nutritional factors) for cellulolytic enzymes production by the *Alternaria brassicicola* isolates were optimized by Dinitrosalicylic acid (DNS) method. The exo- $\beta$ -1,4-glucanase activity of *A. brassicicola* was found more in comparison to endo- $\beta$ -1,4-glucanase activity.

The mean cellulase (exo- $\beta$ -1,4-glucanase) activity of all thirty two isolates of *A. brassicicola* was found maximum on 5<sup>th</sup> and 6<sup>th</sup> day of incubation, at 35<sup>o</sup>C temperature and pH-6 in the presence different nutrient sources viz., citrus fruit peel, sucrose, cellulose, yeast extract and sodium nitrate. The partial purification of cellulolytic enzymes from the culture filtrate was performed by ammonium sulphate salt precipitation followed by desalting through dialysis. SDS-PAGE revealed two bands representing endo and exo glucanase enzyme subunits respectively. The cellulase enzyme isolated from *Alternaria brassicicola* infecting crucifers can also be utilized further for industrial purpose along with cellulases from other microbial sources.

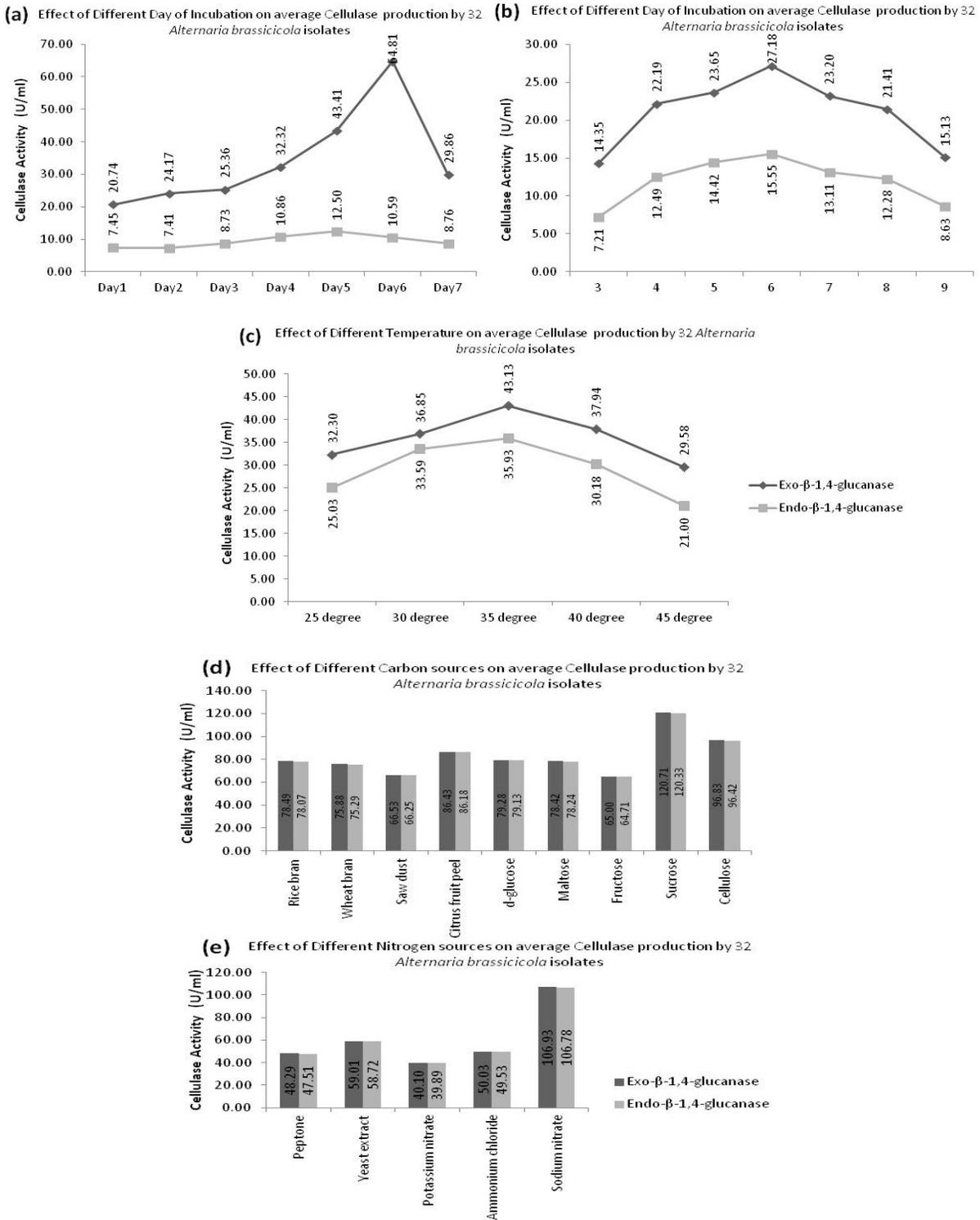
**Table.1** Qualitative plate assay for screening of enzyme production from thirty two *A.brassicicola* isolates

Isolates	Place of Collection	State	Mean width of clearance zone (cm)	Isolates	Place of Collection	State	Mean width of clearance zone (cm)
CaAbcR1	Jaipur	Rajasthan	0.23	CaAbcJr1	Ranchi	Jharkhand	0.37
CaAbcH1	Faridabad	Haryana	0.33	CaAbcMH1	Pune	Maharashtra	0.33
CaAbcJK1	Kathua	Jammu & Kashmir	0.57	CaAbcWB3	Durgapur	West Bengal	0.33
CaAbcB1	Samastipur	Bihar	0.33	CaAbcP1	Moga	Punjab	0.37
CaAbcUP1	Ghaziabad	Uttar Pradesh	0.37	CaAbcO2	Cuttack	Orissa	1.13
CaAbcAs1	Jorhat	Assam	0.7	CaAbcR3	Jaipur	Rajasthan	0.47
CaAbcO1	Bhubaneswar	Orissa	0.37	CaAbcD1	IARI field	Delhi	0.57
CaAbcR2	Kota	Rajasthan	0.27	CaAbcUP3	Meerut	Uttar Pradesh	0.43
CaAbcHP1	Solan	Himachal Pradesh	0.23	CaAbcR4	Jaipur	Rajasthan	0.53
CaAbcG1	Ahemadabad	Gujarat	0.47	CaAbcR5	Samode	Rajasthan	0.43
CaAbcWB1	Nadia	West Bengal	0.73	CaAbcR6	Kota	Rajasthan	0.37
CaAbcWB2	Kolkata	West Bengal	0.37	CaAbcD7	Yamuna Bank	Delhi	0.33
CaAbcJK2	Jammu	Jammu & Kashmir	0.57	CaAbcT2	Palani	Tamil Nadu	0.4
CaAbcUP2	Bulandsahar	Uttar Pradesh	0.17	CaAbcT3	Dindigul	Tamil Nadu	1.1
CaAbcUK1	Dehradun	Uttarakhand	0.27	CaAbcT4	Kannavadi	Tamil Nadu	1.1
CaAbcH2	Shikohpur	Haryana	0.23	CaAbcK1	Bengaluru	Karnataka	0.9
LSD	0.09						
CV (0.05%)	53.92						

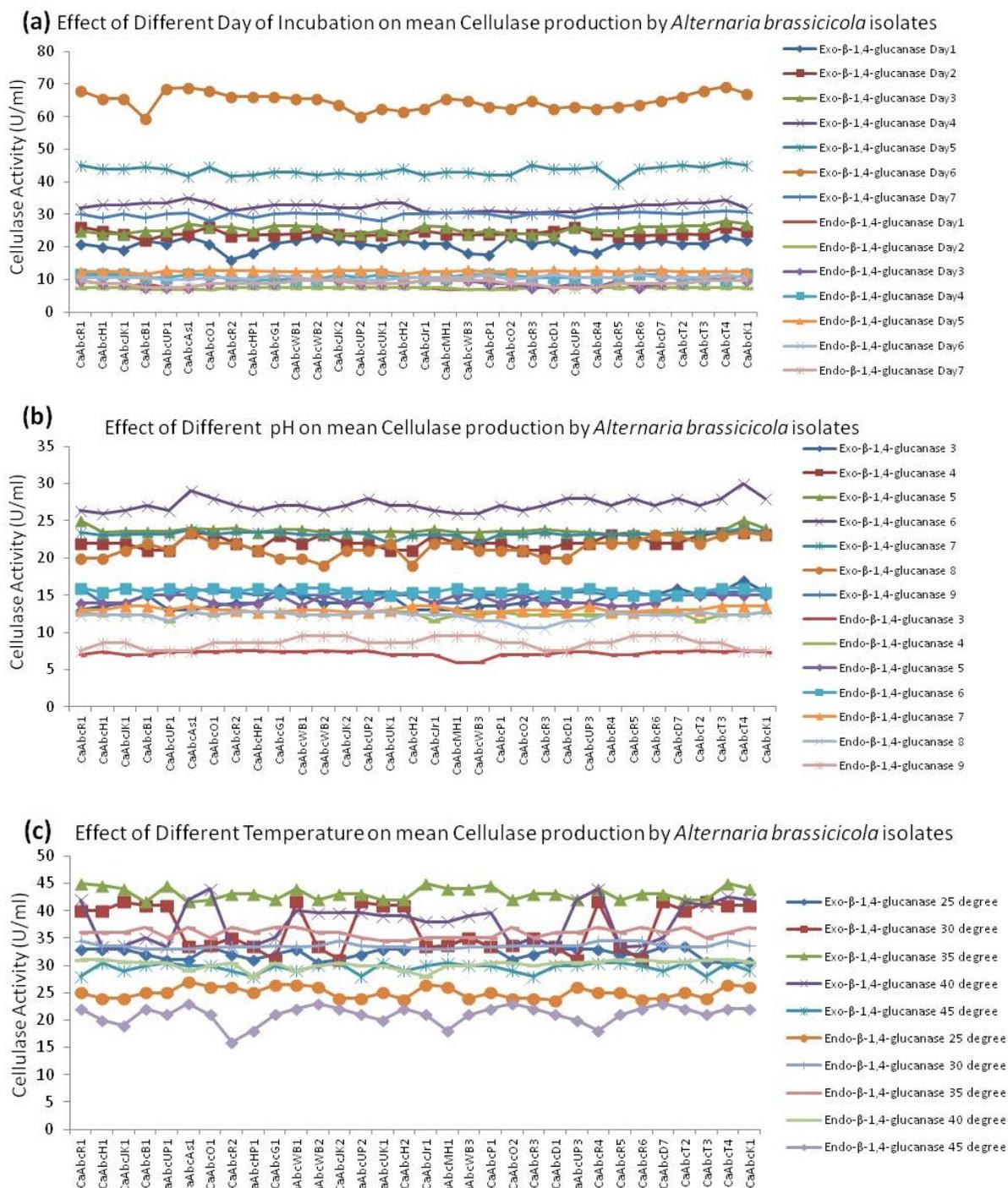
**Fig.1** Qualitative plate assay showing release of cellulase enzyme through zonation in plate by *Alternaria brassicicola* isolates.

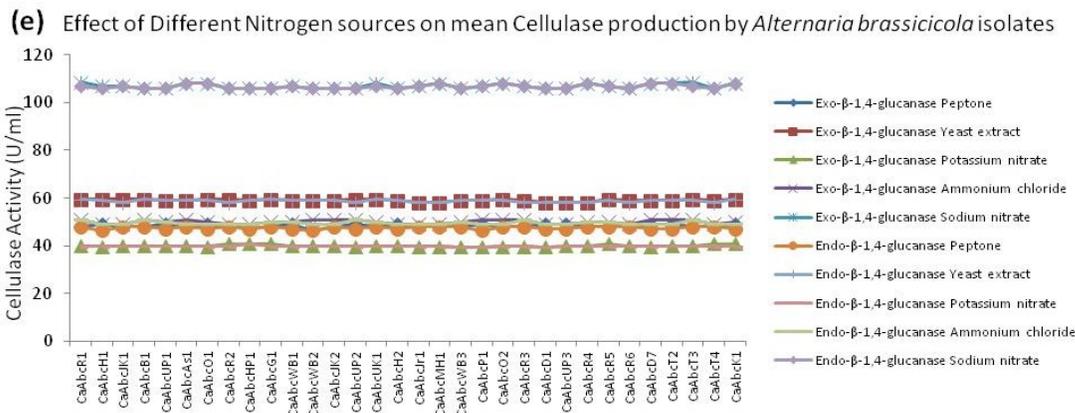
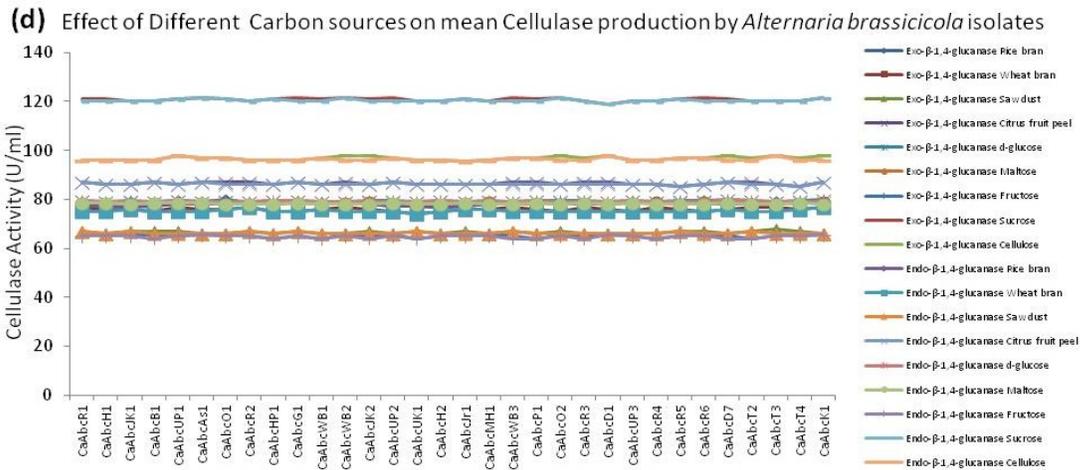


**Fig.2** Effect of different (a) day of incubation, (b) temperature, (c) pH, (d) supplementation of different carbon source and (e) nitrogen source on the mean cellulase activity of 32 *A.brassicicola* isolates.

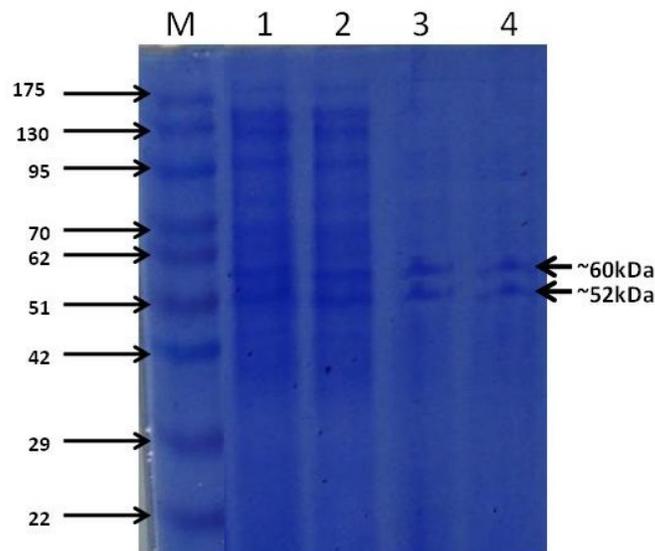


**Fig.3** Mean cellulase activity of 32 *A.brassicicola* isolates each at different (a) day of incubation, (b) temperature, (c) pH of the culture media supplemented with different (d) carbon source and (e) nitrogen source.





**Fig.4** SDS-PAGE of Cellulase from *Alternaria brassicicola* (CaAbcT4) isolate on 12% gel. M: molecular weight protein standards (mid range, prestained, Gene Direx), lane 1 & 2: crude cellulase, lane 3 & 4: partially purified endo (~60kDa) & exo glucanase (~52kDa) enzyme.



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