Production and partial purification of cellulase from a novel fungus, *Aspergillus flavus* BS1

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Abstract This study describes the isolation and characterization of a novel fungus, *Aspergillus flavus* BS1 and its cellulolytic activities with special emphasis on endoglucanase production. Preliminary screening studies showed that *A. flavus* BS1 was a potent strain for the production of cellulase. To study the cellulolytic activities in detail by submerged fermentation (SmF), productions of endoglucanase, exoglucanase, and β-glucosidase were estimated from the basal salt medium (BSM) supplemented with 1 % carboxy methyl cellulose (CMC). CMC medium supported the maximum yield of endoglucanase (2,793 U/ml) on day 5 of incubation at 28 °C and 150 rpm, which was higher than that obtained with naturally available supplements (flour) from banana, tapioca, potato, or banana peel. During cellulase production by solid-state fermentation, 10 % (w/w) tapioca flour in sawdust (teak wood) moisturized with BSM (1:2, w/v) supported maximum cellulase yield (5,408 U/g dry substrate) on day 3 at 28 °C, which was 2-fold higher than that obtained during SmF. The active cellulase was qualitatively estimated by polyacrylamide gel electrophoresis (PAGE). Native-PAGE (0.25 % CMC impregnated on the 10 % gel) activity staining with congo-red showed a clear zone for CMCase activity, whereas SDS-PAGE showed a distinct band. In conclusion, this study showed that *A. flavus* strain BS1 is a potent strain for the production of cellulase on lignocellulosic media, the hot enzyme for bioethanol production from the lignocellulosic biomass by SSF.

Introduction

Lignocelluloses are the most abundant natural carbon source available on earth. Its polysaccharide components include celluloses and hemicelluloses, which account for 60–80 % of the total content. Lignocellulosic waste materials—which may be classified as agricultural, industrial or municipal wastes—are produced in large quantities in every part of the world. These solid wastes are usually discarded indiscriminately or dumped at various sites, where they are burnt, buried, or left to decompose, thereby causing enormous environmental pollution with serious health consequences (Ali et al. 1991; Abu et al. 2000). Organisms which utilize lignocellulosic materials for their carbon and energy sources could be exploited for the conversion of these wastes into products that are beneficial to mankind. Although bacteria and fungi can produce lignocellulolytic enzymes, the fungal enzymes are usually preferred over the others, because they are extracellular, adaptive, and usually secreted in large quantities (up to 2 % by weight) during growth. This is in sharp contrast to many bacterial enzymes which exist as tight multi-enzyme complexes, often membrane-bound, from which it is difficult to recover the individual active enzyme species (Berry and Paterson 1990; Pettersson and Porath 1963). Lignocellulosic materials provide ideal substrates for the growth of fungi, which can in turn degrade the fibrous wastes and add fungal protein to the spent substrates (Belewu and Banjo 1999; Banjo and Kuboye 2000; Belewu and Afolabi 2000).

Cellulases are inducible enzymes, synthesized by many microorganisms during their growth on cellulosic materials. Cellulose is a macropolymer of glucose with β-1-4-glycosidic

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bond, normally forming unbranched chains. It is the largest component of plant residues entering terrestrial ecosystems. Even though a large quantity of cellulose is formed annually, it does not accumulate in bulk due to the action of fungi and bacteria. They efficiently degrade cellulose to provide themselves with carbon and an energy source for growth and recycling carbon back into the ecosystem (Jun et al. 2009). Cellulase is a complex enzyme composed of cellobiohydrolases, endoglucanases, and β-glucosidases, which act synergistically on cellulosic biomass to ultimately and efficiently yield glucose (Holker et al. 2004), which can be used for producing many valuable products such as ethanol for biofuel and other various chemicals from the cellulosic feed stocks (Bhat 2000; Peng and Chen 2008; Tomas-Peja et al. 2009).

Utilization of waste lingocellulosic materials for the production of useful products has been an increasing trend in recent years. Microbial production of cellulase through solid-state fermentation (SSF) has received more attention, as it is generally regarded as cost-effective for high volume products (Pandey et al. 1999). SSF has successfully been employed for large-scale production of fungal metabolites and bioconversion of plant and animal wastes into useful products (Pandey et al. 1999). As a cost-effective technology, SSF is rapidly gaining interest for its higher yields of cellulase, compared to liquid cultures (Singhania et al. 2006). In recent years, many researchers are focusing on systematic studies, especially the screening of fungal isolates for novel bioactive compounds. Most such isolates are reported from places where lignocellulosic materials such as wood, grasses, palms, and seeds are available in quantity (Rubeena et al. 2013). Of late, increased interest has been seen for enzymes involved in the degradation of lignocelluloses, with special emphasis on their ecological roles and potential in cleaning and biotechnology (Rubeena et al. 2013). For efficient hydrolysis, different lignocellulosic substrates require different enzyme complexes. Such specific enzyme complexes occur most often in microorganisms that grow on specific substrates (niches) in nature. A screening program for taking into consideration such substrate specificity may yield new wild strains that are particularly suitable for the optimal hydrolysis of the target substrate. It is expected that such wild strains could be improved further by genetic manipulation (Szakacs and Tengerdy 1997).

Fungi such as *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., etc. are mainly exploited for the production of various industrial enzymes. In this study, we used a novel strain of *Aspergillus flavus,* a saprophytic fungus capable of growing on a variety of nutrient sources, mainly on debris of plants on soil, for the production of cellulase. Thus, the specific objectives of this study were: to isolate and characterize novel mycelial fungi from the centuries-old woodyards on the Kallai river belt; screen them for the cellulolytic activities; explore the potentials of SmF and SSF for the production of cellulase; and to partially purify the cellulase produced.

Materials and methods

Chemicals

All chemicals and reagents used were of analytical grade. Potato-dextrose agar (PDA) and Carboxymethyl-cellulose (CMC) were procured from Hi-Media Laboratories, India. All other chemicals and reagents were procured from Sigma (USA), Hi-Media (India), or Merck India.

Media preparations

Details of media used in this study are given in Table 1. Basal mineral salt medium (BSM) with composition (g/l): ($NaNO_3$, 2; K_2HPO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; and protease peptone, 2; pH 6) was the basic medium (synthetic) used throughout the study. For this study, BSM was supplemented with raw carbon sources, as written in the respective sessions (Table 1). Prior to inoculation, all the media were autoclaved at 1 atm and 121 °C for 20 min.

Isolation of fungi

Samples were collected from different localities from the woodyards on the Kallai river belts as described by Gautam et al. (2010), suspended in sterile double-distilled water (ddH$_2$O) and serially diluted. Briefly, 10 ml ddH$_2$O was added to 10 g sample (wood bark) and centrifuged at 800 g at 4 °C for 5 min, and the supernatant (1 ml) obtained as above was serially diluted (up to 10^{-7}) in ddH$_2$O (Priji et al. 2013). Suspensions after serial dilution were spread on PDA medium containing (g/l): potato infusion, 200; dextrose, 20; and agar, 15. The purity of the isolated fungal cultures was confirmed by repeated sub-culturing and then preserved on PDA slants.

Morphological characterization

The isolate was macro-morphologically characterized by observing colony characteristics such as color, texture, and spore structures according to the handbook for the identification of fungi (Alexopoulos et al. 1996), and micro-morphologically

Table 1 Media used with detailed composition

Cultivation strategy	Basal salt medium (BSM)	Supplements
Submerged Fermentation (SmF)	10 ml	0.1 g CMC (1 %, w/v)
		0.1 g flour of banana, tapioca, potato or banana peel (1 %, w/v)
Solid-State Fermentation (SSF)	10 ml	5 g sawdust
		4 g sawdust +1 g flour of banana, tapioca or potato (i.e. 10 %, w/w flour in sawdust)

by employing conventional lacto-phenol cotton blue technique (LPCB). For this technique, a small tuft of fungus with spores and spore-bearing structures was transferred to a drop of LPCB on a clean glass slide using a sterile needle. The material was gently teased using the mounted needle and a cover slip was placed carefully over the preparation which was observed under binocular microscope connected with image analyzer (Nikon Eclipse E400; Towa Optical, Japan) fitted with Nikon digital camera (DXM1200F; Japan).

Molecular characterization

The isolates were confirmed at molecular level by the PCR-amplification using internal transcribed spacer 1, 5.8S ribosomal RNA gene; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence with 8 F and 1492R primers using BDT v.3.1 cycle sequencing kit on ABI 3730XL genetic analyzer (Xcelris Labs, Ahmedabad, India).

Screening for cellulase activity

Cellulolytic activity of the culture on CMC medium was determined qualitatively by iodine plate assay (Kasana et al. 2008). Five-day-old cultures from PDA slants stored at −20 °C were cultured on BSM agar plates enriched with 0.5 % CMC. After 4 days of incubation, the plates were flooded with iodine solution containing 1 % iodine crystals and 2 % potassium iodide, then incubated at 30 °C for 15 min, and excess stain was washed off for visual observation, i.e., a clear zone around the mycelial growth.

Inoculum preparation

Fungal cultures were grown on PDA slants and the spores were harvested aseptically from 5-day-old PDA slants (stored at −20 °C). Spores were mixed in the required quantity of sterile ddH$_2$O and shaken vigorously for preparing a uniform suspension, which was used as inoculum. The number of spores in 10 µl of suspension ($\sim$6.62×10^5) was calculated by counting the spores using a hemocytometer.

Culture conditions for submerged fermentations (SmF)

Cellulase production by CMC as carbon source

To investigate cellulase activity in liquid medium, microorganisms were cultured in BSM with 1 % CMC as carbon source. SmF was carried out in Erlenmeyer flasks. After sterilization and cooling, media were inoculated with 10 µl spore suspension ($\sim$6.6×10^5 colony-forming units, CFU) under sterile conditions. All flasks were incubated at 28 °C in an environmental shaker for 8 days, and samples were withdrawn

(whole flask) at 24-h intervals. After centrifugation (9,000 g), the supernatant was used as crude enzyme extract for endoglucanase, exoglucanase, and β-glucosidase assays.

Cellulase production by natural flours as carbon source

To investigate the cellulase activity in liquid medium, microorganisms were cultured in BSM with banana flour (BF), tapioca flour (TF), potato flour (PF), or banana peel flour (BPF) as carbon source. SmF was carried out in Erlenmeyer flasks. After sterilization and cooling, media were inoculated with 10 µl spore suspension ($\sim$6.6×10^5 CFU) under sterile conditions. All flasks were incubated at 28 °C in an environmental shaker for 8 days, and samples were withdrawn at 24-h intervals. The supernatant was used as the crude enzyme extract for endoglucanase assay.

Culture conditions for solid state fermentations (SSF)

For SSF, sawdust (from the heart wood of teak) was mixed with 10 % (w/w) flours (BF, PF, or TF). BPF was independently used without mixing with sawdust. SSF was carried out in 100-ml Erlenmeyer flasks. One gram of substrate was taken in an individual flask and 10 ml BSM was added (pH 6) to give moisture content and nutrients. The flasks were sterilized by autoclaving at 121 °C and 1 atm for 15 min. After cooling, the flasks were inoculated with 10 µl spore suspension ($\sim$6.6× 10^5 CFU) in sterile condition. The contents in the flask were mixed thoroughly to ensure uniform distribution of the inoculum. All flasks were incubated at 28 °C for 15 days under static condition. The samples were withdrawn at 2-day intervals, and extracted with 10 ml citrate buffer (pH 4.8) by stirring for 10 min under chilled condition. The supernatant was used as the crude enzyme extract, after centrifugation (9,000 g, 10 min, 4 °C).

Quantification of cellulase

Production of cellulolytic enzymes , i.e., endoglucanase , exoglucanase, and β- glucosidase was quantified using CMC (Himedia), Filter paper (Whatman, GE Healthcare UK) and salicin (Himedia), respectively, as substrates by the dinitrosalicylic acid (DNS) method of assay (Miller 1959). For the estimations of endoglucanase and β-glucosidase, the reaction mixture used contained 0.5 ml of 1 % (w/v) substrate in 0.1 M citrate buffer (pH 4.8) and 0.5 ml of culture supernatant. The mixture was incubated at 50 °C for 30 min, which was stopped by adding 3 ml DNS. For exoglucanase activity assay, a rolled filter paper strip (1×6 cm, 50 mg) was saturated with 0.5 ml of 0.1 M citrate buffer (pH 4.8) and equilibrated for 10 min at 50 °C, followed by the addition of 0.5 ml culture supernatant. Then, the reaction mixture was incubated at 50 °C for 60 min, and the reaction was stopped by adding 3 ml DNS. Subsequently, the mixtures were incubated for

5 min in a boiling water bath and were cooled rapidly. The absorbance was measured against the reagent blank at 540 nm using UV-vis spectrophotometer (Shimadzu UV-1601; Japan). The amount of reducing sugar liberated was quantified using glucose as standard. One unit of cellulase is defined as the amount of enzyme that liberates 1 μmol of glucose equivalents per minute under the assay conditions.

Cellulase activities from SmF and SSF were calculated using the formula, $\Delta E \times V_f / \Delta t \times \sum \times V_s \times d$ and $\Delta E \times V_f \times V_s / \Delta t \times \sum \times g \, ds \times d$, respectively. Where, ΔE = absorbance at 540 nm, V_f = final volume of reaction mixture including DNS, V_s = crude supernatant (ml) containing cellulase used, Δt = incubation time for of hydrolysis, $\sum$ = extinction coefficient of glucose (0.0026), $g \, ds$ = grams dry substrate, and d = diameter of cuvette.

Partial purification and characterization of cellulase

Crude enzyme extract (after SSF with sawdust-TF-BSM) was precipitated by 60 % saturation with ammonium sulfate and then dialyzed against 20 mM sodium citrate buffer (pH 4.8) for 24 h at 4 °C. After dialysis, the precipitate was removed by centrifugation (9,000 g for 10 min at 4 °C) and supernatant was used for native PAGE (polyacrylamide gel electrophoresis) and SDS-PAGE (sodium dodecyl sulphate-PAGE) for the determination of approximate molecular weight. The protein content was estimated by the method of Lowry et al. (1951). For the native PAGE, CMC (0.25 %) was impregnated with separating gel (10 %), and stacking gel was 5 %. After electrophoresis, the gel was immersed in 100 mM sodium citrate buffer (4.8) for 30 min at 28 °C. Congo-red (0.1 %) was used for staining the gel and 1 M NaCl was used for destaining, which helps in visualizing the yellow active zone (hallo) on the gel (Zhang et al. 2009). SDS-PAGE was performed by the method of Laemmli (1970), in which 12 % separating and 5 % stacking gels were used. Reference marker proteins (Bioscience, Merck, India) were used. After electrophoresis, the gel was stained by coomassie-brilliant blue R 250 (0.1 %).

Statistics

All experiments were conducted in triplicate and the values were given as mean ± SE. Microsoft Excel was used to draw the graph. Images were set using Adobe Photoshop CS5.

Results

Culture identification

In the present study, we screened three pure fungal isolates obtained on PDA for cellulolytic activity, and among them one showed high cellulolytic activities. This culture was identified as *Aspergillus flavus* strain BS1 (Gen Bank Accession Number: HQ645940) by morphological and molecular characterizations, which was used as the fungal culture for this study.

During the initial 3–4 days of incubation, the fungus grew as yellowish-green patches on PDA and later spread all over the surface of plate as green mycelial mat. The isolated colony was granular and velvety in appearance (Fig. 1a). Growth was rapid and micro-morphology of the culture showed long conidiophores beneath the globose vesicle. Phialides arose circumferentially and conidia were round and smooth (Fig. 1b). The preliminary identification was confirmed at species level by molecular characterization using PCR-amplification of D1/D2 region of LSU (large subunit of the) 28S rDNA gene from the isolated genomic DNA. Its similarity to other related taxa was also analyzed (Fig. 2).

Cellulolytic activity

As inferred from the plate assays, the fungus *A. flavus* BS1 we described here showed cellulolytic activity. *Aspergillus flavus* BS1 on BSM-CMC agar plate showed a clear zone around the mycelial colony on flooding with Gram's iodine, indicating cellulolytic activity (Fig. 3).
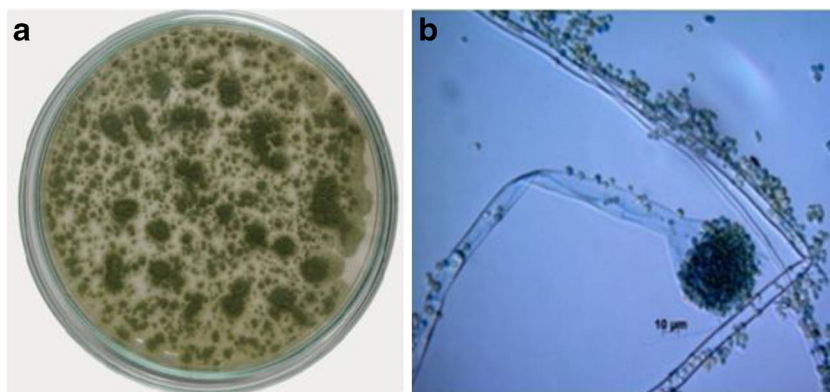
Cellulase production by SmF

Production of cellulase (endoglucanase, exoglucanase and β-glucosidase) by *A. flavus* BS1 was estimated in BSM supplemented with 1 % synthetic carbon source (CMC), among them the highest activity was for endoglucanase as displayed in Fig. 4. Hence, for the remaining studies, endoglucanase production was given emphasis. Production of endoglucanase was quantified by replacing CMC with a natural carbon source (TF, BF, PF, or BPF) in BSM is detailed in Fig. 5. CMC is the selective carbon source used rampantly for enriching the production of cellulase by the fungus.

Presence of 1 % (w/v) CMC in the BSM enabled the fungus for the maximum yield of endoglucanase, exoglucanase, and β-glucosidase on day 5 of incubation, which were about 2,793, 659, and 540 U/ml, respectively. When CMC was replaced with natural substrates, moderate production of endoglucanase was observed (Fig. 5). Upon comparison with CMC, the natural carbon source supported less endoglucanase production, i.e., the highest (1,631 U/ml) with TF (on day 2). Cellulase production with BF, PF, or BPF was still lesser, i.e., 539, 243, and 917 U/ml, respectively. These results suggested that presence of natural carbon sources can also induce *A. flavus* BS1 for cellulase production, though less.

Cellulase production by SSF

To investigate the cellulase activity in solid medium, *A. flavus* BS1 was grown in BSM-supplemented raw sawdust or BPF

Fig. 1 Morphology of *A. flavus* BS1: **a** *Aspergillus flavus* BS1 growing on PDA medium (Digital image, DSLR Canon 450D, Japan); **b** Fruiting body (Phase Contrast Microscope, Leica M80, Germany); *scale bar* 10 μm

alone or BSM-sawdust-flour (90 % sawdust + 10 % TF, PF, or BF; w/w) combination. Proper ratios (w/v) between the solid content to the moisture (BSM) (1:1, 1:2, or 1:3) were also tested for optimum cellulase production. Of these, the maximum cellulase production was observed at the 1:2 combination of the solid substrate (sawdust alone, BPF alone, or a combination of sawdust + flour as above) to BSM, i.e., 1 part solid substrate and 2 parts BSM (Fig. 6). *Aspergillus flavus* BS1 spread on the substrate in 3 days of incubation.

Aspergillus flavus strain BS1 secreted extracellular cellulase in sawdust from the day 3 onwards and showed maximum production (1,438.8 U/g ds) on day 12 of incubation, but BPF supported low cellulase activity (1,293 U/g ds), which was on day 6 of incubation. For enhancing the production of cellulase substrates like 10 % TF, BF or PF was mixed with sawdust (10:90; W/W). Among these combinations, one with TF supported maximum production (5,408.5 U/g ds) cellulase yield, which was on day 3 of incubation, whereas BF and PF showed maximum production of cellulase on the days 9 and 3 of incubation, which were 4,687 and 1,061 U/g ds, respectively.

Partial purification and characterization of cellulase

Native PAGE and SDS-PAGE were performed to characterize the partially purified enzyme qualitatively. A band showing cellulolytic activity was detected on CMC-native PAGE (Fig. 7). The molecular weight of this band was estimated approximately as 23 kDa by SDS-PAGE which was observed as single band in native gel (Fig. 7).

Discussion

This study intended to isolate novel microbes from the woodyards on the Kallai river belt producing efficient lignocellulolytic enzymes and characterize such enzymes to explore their potentials for industrial applications. It was expected that the centuries-old woodyards would be a hotspot for lignocellulolytic microbes. Thus, many bacteria and fungi from this locality with high potentials for lignocellulolytic activities were identified. One of these fungi (*A. flavus* strain BS1) which showed high efficiency for cellulase production, especially by SSF, was focused in this study. Cellulolytic enzymes can qualitatively be screened by plate assay, which is an important tool for rapid screening at low cost (Devi and Kumar 2012a).

The major factors to be considered for the exploitation of the commercial potential of cellulases are the yield, stability and cost of cellulase production. The latter could be reduced by the use of cheap lignocellulosic materials. We showed that
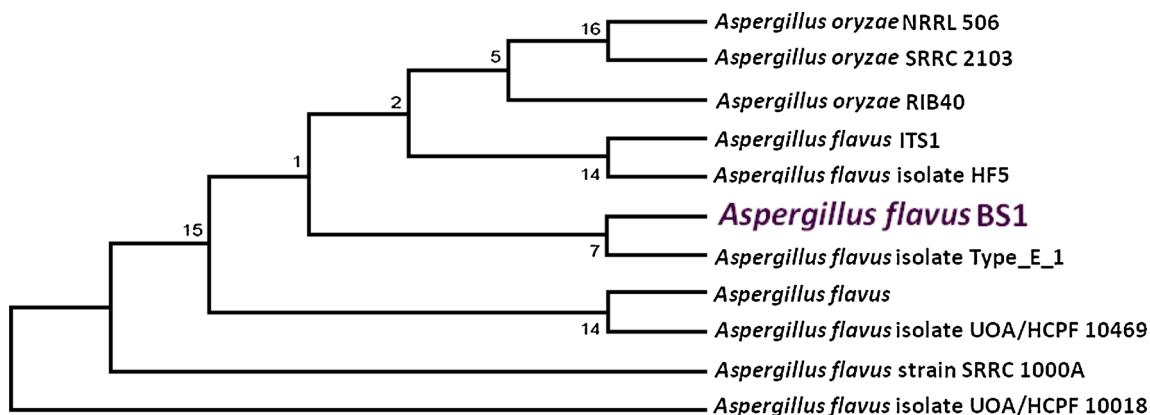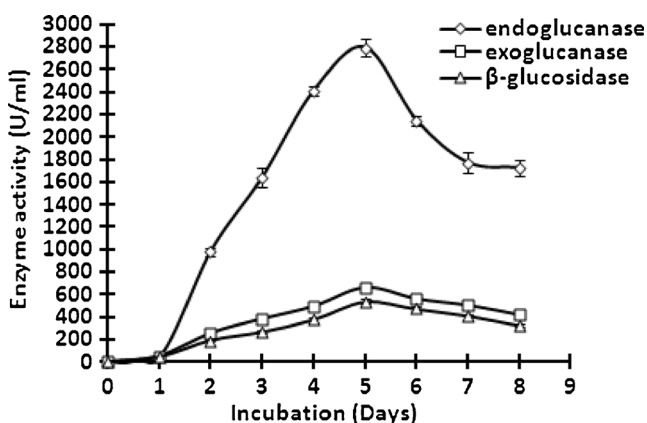

Fig. 2 Evolutionary relationships of *A. flavus* BS1 with another 10 related strains
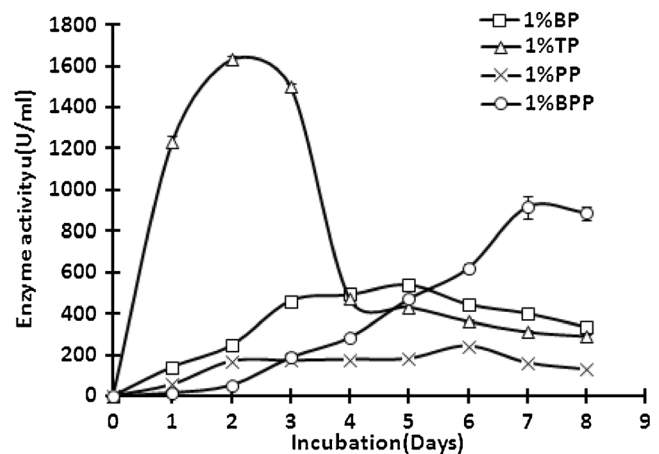
Fig. 3 Cellulolytic activities of *A. flavus* BS1: iodine plate assay showing clear zone around the colony indicating cellulase activity on day 4 of incubation at 28 °C

Fig. 5 Cellulase production profile of *A. flavus* BS1 in SmF: production of endoglucanase in BSM supplemeted with 1 % of different substrates (BF, TF, PF, BPF) at 28 °C. Assay was done by DNS method spectrophotometrically (Shimadzu UV-1601, Japan) at 540 nm

various natural carbon sources (whose average market price is 0.15 USD/kg) induce cellulase production with variable yields. In the present study, six different natural supplements were used as major carbon source. By SmF, *A. flavus* BS1 showed highest cellulase yield with the commercial CMC. Gomathi et al. (2012) demonstrated maximum CMCase yield (40 U/mg) from *A. flavus* under optimized culture conditions on day 3 in Czapek Dox medium using wheat bran as supplements. Alam et al. (2005) investigated cellulase production by *Streptomyces omiyaensis* in liquid Winsted's medium having 1.2 % CMC as supplement and beef extract as nitrogen source. CMCase activity was 269 U/ml at pH 6.5 and temperature 45 °C, it was concluded that CMC was the best cellulosic supplement for the induction of extracellular cellulolytic enzymes. In comparison, the culture described here showed over 10-fold more (2,793 U/ml) endoglucanase (crude) activity in the presence of 1 % CMC during SmF. Milala et al. (2005)
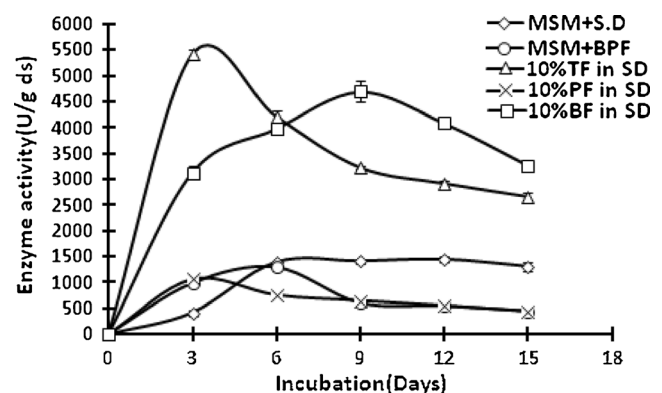
used millet, guinea corn straw, rice husks, and maize straw as supplements for the production of cellulolytic enzymes by *A. niger* in SmF. Among these supplements, the optimal cellulase production was 102 U/ml (72 h) in the presence of maize straw which was quite negligible, compared to the cellulase activity of *A. flavus* BS1 in TF as we described. *Rhizopus oryzae* produced 450 U/ml cellulase in an optimized cultivation condition utilizing water hyacinth in the medium as supplement (Karmakar and Ray 2011). Our group showed that *Trichoderma viride* produced 173 U/ml of cellulase in BSM supplemented with 1.25 % of CMC at 28 °C at pH 4 (Neethu et al. 2012), while *T. harzianun* produced 146 U/ml cellulase in the presence of 0.5 % CMC in BSM (Rubeena et al. 2013). From this, it is evident that the 2,793 U/ml cellulase produced by *A. flavus* BS1 (SmF) in the presence of CMC (1 %), as reported in the present study is highly advantageous. Furthermore, the type of strain, culture conditions,

Fig. 4 Cellulase production profile of *A. flavus* BS1 by 1 % CMC in SmF: production of endoglucanase, exoglucanase and *β*- glucosidase in BSM supplemeted with 1 % CMC at 28 °C. Assay was done by DNS method spectrophotometrically (Shimadzu UV-1601, Japan) at 540 nm

Fig. 6 Cellulase production profile of *A. flavus* BS1 in SSF: production of endoglucanase in SD, BPF, and SD supplemented with 10 % TF, 10 % PF, and 10 %,BF, respectively, and moistened with BSM in the ratio of 1:2 at 28 °C
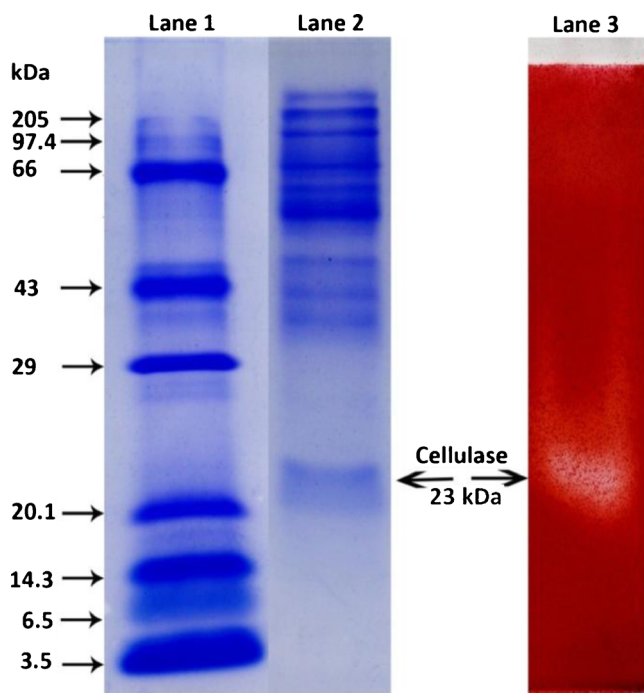
Fig. 7 SDS-PAGE profile of partially purified cellulase from *A. flavus* BS1: *lane 1* reference molecular weight marker; *lane 2* SDS-PAGE analysis of partially purified cellulase in 12 % gel; *lane 3* native PAGE analysis of partially purified cellulase in 0.25 % CMC impregnated 10 % gel stained with 0.1 % congo-red

nature of the supplement, and availability of nutrients are the important factors affecting enzyme production by microbes (Benjamin and Pandey 1998).

Shankar and Isaiarasu (2011) showed that agro-industrial solid media are considered best for enzyme production. Some newly developed agro-industrial wastes such as banana wastes, rice straw, corncob residue, rice husk, wheat straw, banana fruit stalk, coconut coir pith, etc. have been used as base media for cellulase production. Cellulase production by microorganisms is conventionally made by SmF. But nowadays, SSF is being practised, because it offers some apparent economic and engineering advantages over the classical SmF (Karmakar and Ray 2011). SSF is advantageous to produce more stable products, requiring less energy, in smaller fermenters, and with easier downstream processing measures (Robinson et al. 2001). In recent years, important agro-industrial residues such as sugarcane bagasse, sugar beet pulp or husk, orange bagasse, oil cakes, apple pomace, grape juice, grape seed, coffee husk, wheat bran, cereals, straw, leaves, corncobs, etc. have been used as base media for cellulase production by SSF (Shankar and Isaiarasu 2011). Cellulase production by *T. reesei* NRRL 11460 in pretreated sugarcane baggase was the maximum (155 U/g ds) at 66 % moisture, pH 4, and 28 °C, which was negligible compared to our study (Singhania et al. 2006). Ojumu et al. (2003) showed that *A. flavus* (isolate NSPR 101) produced 0.0743, 0.0573, and 0.0502 IU/ml cellulase in sawdust, bagasse and corncob,

respectively, at 12 h of incubation, almost negligible yields. Murad and Azzaz (2013) showed that maximum cellulase activity of *A. flavus* NRRL 5521 was 0.11 IU/ml on the medium containing 10 % rice straw as carbon source and 0.33 % yeast extract as nitrogen source at pH 7 at 48 h of incubation. From this, it is evident that various strains of *A. flavus* showed very low yields, compared with the strain reported in this study.

Chandra et al. (2007) compared the production of cellulolytic enzymes by *A. niger* on lignocellulosic solid media such as groundnut fodder, wheat bran, rice bran, and sawdust by SSF at the laboratory level. Czapek Dox liquid broth with 0.5 % cellulose was used to moisten the substrates. From the results, wheat bran (3.24 U/g CMCase) was the best solid support, followed by groundnut fodder (1.36 U/g) for cellulolytic enzyme production. Abostate et al. (2010) showed that the standard *T. viride* produced highest CMCase on wheat straw (555 U/ml), and a new isolate of *Aspergillus* strain MAM-F35 also showed comparable CMCase activity on wheat straw (487 U/ml). The present study clearly showed that sawdust supplemented with 10 % TF was the most suitable combination for cellulase production using *A. flavus* BS1, which in comparison with cited studies showed the highest yield of cellulase (5,408 U/g ds).

We further showed that the MW of the cellulase produced by *A. flavus* BS1 is about 23 kDa. Very recently, Devi and Kumar (2012b) showed that the apparent MWs of the cellulase isoforms produced by *A. niger* were 33 and 24 kDa. Similarly, the partially purified enzyme from a strain of *A. flavus* had a MW of approximately 30.2 kDa (Ajayi et al. 2007). Onsori et al. (2005) described as *Aspergillus* sp. with over-production of endo-1,4-glucanase, which revealed three protein bands with approximate MW of 18.5, 23, and 28 kDa on PAGE. The MW of the single cellulase from *A. flavus* BSI described in this study is very much comparable to these cellulases, and it suggests that these enzymes may have similar active domains.

In conclusion, compared to commercial strains of *Trichoderma* spp., *A. flavus* BS1 showed higher cellulolytic activity, which is the first report from a species of *Aspergillus*, regarding higher yield. *A. flavus* BS1 cultured on liquid medium showed cellulase yield with CMC, a good inducer as also reported by many other authors. Upon SSF, though sawdust or banana peel supported cellulase production, a more than 4-fold increase was noticed when 10 % natural carbon source (flour) was supplemented to the sawdust, indicating their inducing role. From the results, it is further evident that SSF is an efficient strategy for the exploitation of *A. flavus* BS1 on a large scale for the production of industrially significant cellulase. Generation of large quantities of cellulosic waste materials has been the major concern in the recent years, which could be solved to a certain extent if such 'wastes' are judiciously managed for SSF with a view for the production of

industrially useful enzymes. Thus, this study has suggested a suitable method for utilizing waste cellulosic materials (sawdust) as a low cost carbon base material for the large-scale production of cellulase from mycelial fungi like *A. flavus* BS1 by SSF.

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