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Research Article
In vitro Optimisation of Fungal Cellulase Production from Fruit Waste for Handmade Paper Industries

Sweta Singh Meena, Vinay Sharma and Sarika Gupta

Department of Bioscience and Biotechnology, Banasthali Vidyapith, Vanasthali, India

Abstract

Background and Objective: Handmade paper industry utilises a large amount of commercially available enzymes for the bio-pulping process. Therefore the study focused on the estimation of cellulolytic fungi isolated from different fruit samples collected from Banasthali Vidhyapith for industrial application in the handmade paper industry sector. **Materials and Methods:** Nine fungal isolates of seven fungi were screened possessing cellulolytic activity as *Alternaria* sp., *Aspergillus niger* (*A. nigel*), *Aspergillus fumigatus* (*A. fumigatus*), *Cladosporium* sp., *Helminthosporium* sp., *Penicillium* sp., *Verticillium* sp. All the fungal isolates subjected to the primary and secondary production of the enzyme under submerged fermentation (SmF). **Results:** For the industrial implications out of the nine fungal, five isolates can be potentially exploited as *Alternaria* sp., *A. niger*, *Cladosporium* sp. and *Penicillium* sp. under acidic conditions and *Alternaria* sp., *A. niger*, *Helminthosporium* sp., *Penicillium* sp., under alkaline conditions. For the mass production utilising czapk dox broth at 28±2°C, pH of 5.0 with 2 days of incubation in the submerged fermentation process. **Conclusion:** In crude extract condition *Cladosporium* sp., *A. fumigates* and in 80% fraction *Alternaria* sp. and *Penicillium* sp. were found to be potent candidates for active cellulase production that can further be commercialized for handmade a paper industry.

Key words: Handmade paper industry, enzyme activity, fungal cellulase, *in vitro* optimisation, primary and secondary screening

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Corresponding Author: Sarika Gupta, Department of Bioscience and Biotechnology, Banasthali Vidyapith, Vanasthali, India Tel: +91-9414461423

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Handmade paper industry is one of the potent users for the commercially available enzymes as cellulase, pectinase, lignin peroxidases, etc. during various stages of pulping and bleaching[1]. Now-a-days 90% of paper pulp is made of wood and paper production account for nearly 35% of felled trees[2] it accounts for 1.2% of the global total economic output[3]. Most important application among these is the use of enzymes as an aid to pulping, bleaching and papermaking with the aim of reducing the requirement of bleaching chemicals, energy during pulping, refining, management of wood and waste minimization[4]. The enzyme solution (including a mixture of cellulases/hemicellulases) added to the hydrapulper and then kept for 2 h with intermittent mixing and the enzyme liquors collected from the hydrapulper for analysis of total solids and color, pH as given previously[4]. After the enzymatic processing of the pulp, it was washed thoroughly and further processed. The optimation involve a decline in pulp disintegration time, that was attributed to the action of enzymes on the inter fiber bonding areas through partial hydrolysis and partial depolymerization of carbohydrates on surface of the fibre[5]. Investigators have also found enzymatic hydrolysis of different categories of waste paper to reverse hornification and reclaim the fibre surface activity as per the original fibre[6]. Another study has also reported an augmentation in strength of secondary fibre through enzymatic treatment as the cellulolytic enzymes, act together to degrade cellulose[7]. Solubilization of cellulose represents a major challenge for enzymes. Also, the access to cellulose is more difficult, since it was embedded in other cell wall components, such as hemicellulose and lignin[8]. Cellulase (EG), alternatively known as endoglucanase, randomly attacks and hydrolyzes cellulose to produce cello-oligosaccharides[8]. The EG 1 is also active on xylan and xylo-oligosaccharides, but the activity is less than that on soluble derivatives and cello-oligosaccharides[9]. It has been known that the combined action of endo- and exo-acting enzymes is much greater than the sum of their individual actions. Although the synergistic action is poorly understood, many thoughts agreed that synergism is obvious on the crystalline domain, low or absent on amorphous cellulose and absent on soluble cellulase derivatives[10]. Due to eco-friendly nature, they are widely used in many industries, especially in genetics and protein engineering in research and development. The cellulase structure has a complexity and inflexibility of the cellulosic substrate[11]. Cellulase complex used in simultaneous scarification and fermentation systems (SSFS) as one of the necessary step in balance of opposing synthetic forces along with derivative forces in the carbon cycle and is a major limitation for the usage of wood, paper, pulp, cotton and cellophane[12]. Fungi are advantageous as the enzyme production rate is higher compared to other microorganism, more than a few fungi were produced such as *Aspergillus niger*, *Aspergillus fumigates*, *Alternaria* sp., *Cladosporium* sp., *Helminthosporum* sp., *Penicillium* sp., *Verticillium* sp.[13]. Cellulase enzyme related to industrial research, several researchers have been performing to increase the yield of cellulase production of recipient strain to rise. Cellulase enzymes were used in many industrial areas such as in the textile industry, wine and brewing industry, detergent industry, agriculture, etc.[1].

The present study focused on the isolation and screening of all the fungi for the production of cellulase enzyme. The objectives designed for the study involves isolation and screening of cellulase production fungi from fruit waste. *In vitro* production of cellulase enzyme by submerged fermentation and thereby *in vitro* optimisation of different parameters for better cultivation and production process through submerged fermentation.

MATERIALS AND METHODS

Sample collection and isolation of fungi: Four different samples collected from Banasthali Vidyapith, Banasthali, Rajasthan during the year 2016-17 from the fruit juice shop as the waste of the juice centre such as apple, banana, pineapple and orange. These samples were collected in a sterile zipup poly bags and analysed in the laboratory. Isolation of fungi from the samples was carried out on Sabouraud dextrose agar (SDA) and the plates incubated for 7 days at 28±2°C. From suspension (100 µL), spread onto SDA, the media was amended with streptomycin to prevent the growth of bacteria. The isolated fungi was being carefully sub-cultured on Sabouraud dextrose agar in plates and slant. The cultures were maintained at 4°C in agar slants for further use[14,15].

Identification of fungal isolates: The fungal isolates identified, based on cultural/macroscopic and microscopic characteristics using lacto-phenol cotton blue (LPCB) mount method[16].

Cellulase production by submerged fermentation: Fungal isolates were showing positive detection during primary screening selected for the quantitative determination of cellulase production by submerged fermentation. All the experiments were conducted in a set of three (triplicates) and the standard deviation was evaluated for each set, respectively.

Inoculum preparation and cellulase production: Uniform spore suspension prepared by mixing spores vigorously and their spore density was measured using Systronics UV-VIS spectrophotometer at 660 nm. The inoculum was transferred into an Erlenmeyer flask by taking 50 mL of Sabouraud media (pH 5.5) through submerged fermentation and incubated for 7 days at 28±2°C on an orbital shaker incubator at 120 rpm[17].

Primary and secondary screening for cellulase production: Nine fungal strains had been isolated and identified for cellulose production and subjected to primary and secondary screening analysis. Primary screening executed by inoculating the fungal isolates on CMC agar media with 1% (w/v) carboxy methyl cellulose as a carbon source. Congo red dye used in primary screening for the cellulolytic microorganism. Initially, carboxy methyl cellulose (CMC) media was used as a carbon source in screening of cellulase production. Isolated fungi inoculate on the CMC petri plates were incubated for 7 days at 28°C with 1N NaOH. A clear zone of hydrolysis observed around the fungus colony. The highest cellulose activity was implicit by clear zone ratio[12,18,19]. Secondary screening of cellulase production was performed accompanies the evaluation of the total protein content in the cell-free filtrate[20] as well as determining the activity of the cellulose enzyme[21].

Protein estimation: Protein estimation in which crude enzyme extracts and the enzyme activity were expressed as a specific activity which represented as U mg^{-1} of protein. The experiments were carried out in triplicates and were statistically analysed[20]. Calculation of the sample enzyme activity (U g^{-1}) by reading the equivalent glucose concentration on the standard curve for the sample and the reaction and then inserting them in the following equation:

$$\text{Cellulase activity (U g}^{-1}) = \frac{(C_G - C_{RB}) \times D}{W \times 10 \times V}$$

Determination of carboxy methyl cellulase (Endoglucanase) activity: The cellulase activity of the crude enzyme was determined using 1% carboxy methyl cellulose as a substrate, prepared in sodium acetate buffer (0.2 M, pH 4.8) for the standard. Enzyme activity expressed as specific activity that was then represented as U mg^{-1} of protein[21,22].

***In vitro* optimization of cellulase production:** Several factors were monitored for the cellulase enzymes production by fungal isolates as media and pH optimization in submerged fermentation (SmF). During the study, under *in vitro* condition five fungal isolates *Cladoporium cladosproides*, *Alternaria alternata*, *Penicillium chrysogenium*, *Helminthosporium cyclops* and *Aspergillus fumigatus* are used for production of cellulase enzyme. Culture filtrate and its purified fraction (60 and 80% ammonium salt precipitated) were collected from the flask and at every stage of optimizing the quantity of protein[20] were estimated along with the cellulase activity[21]. All the submerged fermentation vessels with 50 mL media were incubated at 28±2°C for 7 days at 120 rpm.

Effect of media on cellulase production under SmF: Three different types of culture media were used as Sabouraud dextrose broth, CMC broth and semi-synthetic czapk dox broth. These media were used for optimize cellulase production. These media were prepared and inoculated as discussed before in the 50 mL media and incubated at 28±2°C for 7 days at 120 rpm.

Effect of different pH on cellulase production under SmF: Three different pH containing vessels of Sabouraud dextrose media 5.0, 7.0 and 9.0 was used for cellulase enzyme production. Prepared inoculums transferred to 50 mL media of different pH. The broth culture (Media, pH) were filtered off and transfer into the eppendorf tube to centrifuge at 10,000 rpm for 5 min to take out cell debris. The pallet was used to assay cellulose enzyme quantity by using lowery's method and activity by using DNS method, the OD measured at 575 nm[23].

Effect of different day of incubation on cellulase production under SmF: The optimal media with optimized pH was inoculated with all the five fungal isolates, observations were recorded after 1, 2, 3, 4, 5, 6 and 7 days of incubation.

RESULTS AND DISCUSSION

Collection and microbiological analysis of sample: The entire fruit samples collected were analyzed for diversity of fungus. Isolation of fungi was carried out by raw and suspension method using Sabouraud dextrose agar media. Individual colonies of nine fungal colonies obtained (Fig. 1). Nine fungal isolates of seven fungi were randomly selected based on the difference in the plate morphology and were subjected to routine subculturing, preservation and subjected to identification. The fungal isolates *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Helminthosporium* sp., *Verticillium* sp., show higher enzyme specific activity and quantity. The cellulase enzyme activity can be enhanced by

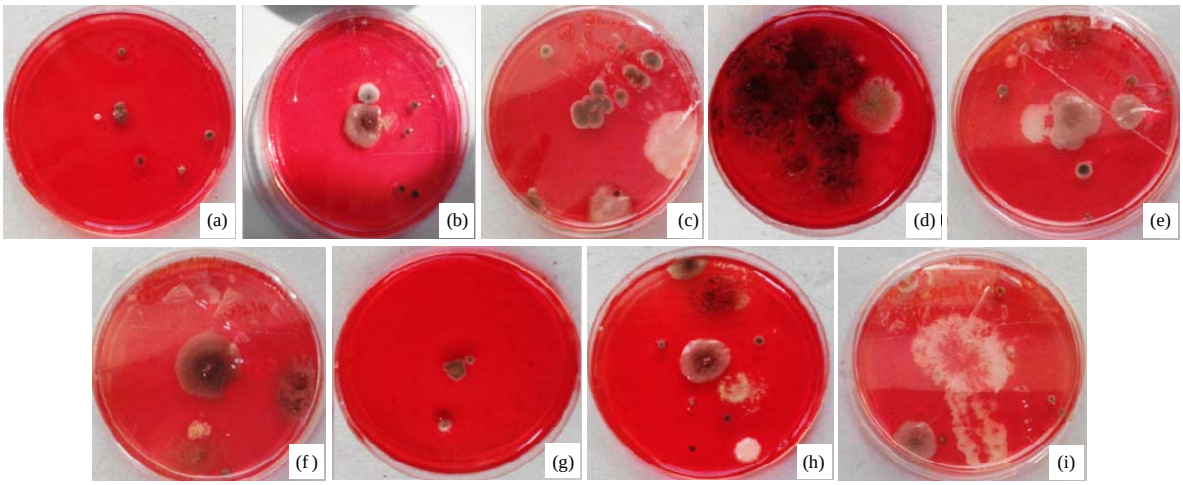

Fig. 1: Primary screening of fungal isolates (n = 9) for cellulase activity by chromogenic reaction in media containing CM-cellulose and congo red dye, (a) *Cladosporium* sp., (b) *Alterneria* sp., (c) *Penicillium* sp., (d) *Aspergillus niger*, (e) *Helminthosporium* sp., (F) *Alternaria* sp., (g) *Verticillium* sp., (h) *Alternaria* sp., (i) *Aspergillus fumigates*

different *in vitro* optimization of media, pH and days of incubation for better cultivation under submerged fermentation.

Mass production of cellulase can be than explored for industrial implication. R and D along with the commercialization of enzymes are extensively used in the biotransformation and with the importance of enzymes in the synthesis of a chiral molecule of pharmaceutical significance, a separate section on enzymes are involved in the biotransformation[24]. Another study suggested three isolates of *Aspergillus* sp., AMA, *Aspegillus terreus* AN1 and *Myceliophthora fergusil* T41, exhibited 53, 52.7 and 40.32% de-inking with an augmentation in brightness by 4.32, 3.58 and 3.01% ISO[25]. Another study reported cellulase from the genus *Trichoderma* was reported to be used for recycling plants convert waste paper into pulp and subsequently into the paper (cartoon), mainly tissue paper products[26]. Study demonstrated cellulase so as to hydrolyze producing glucose, which can be used for the production of ethanol, organic acids in food, beverages, textile, laundry and other chemicals producing industries[27].

Cellulase production by submerged fermentation and primary and secondary screening for cellulase production: The fungal isolates were screened for their cellulolytic activity by visual detection of the clear zone around the fungal isolates formed on plates containing CMC agar media in presence of Congo red dye. On the basis of appearance of the area of clearance, it was found that nine fungal isolates were showing positive test for cellulolytic activity to variable extent and therefore further selected for cellulase production (Fig. 1). The isolates showing cellulase production during screening were from genera *Alternaria* sp., *Aspergillus niger*, *Aspergilus fumigates*, *Cladopsorium* sp., *Helminthosporium* sp., *Penicillium* sp., *Vertcillium* sp. All the fungal isolates (n = 9) were inoculated into production media with uniform spore suspension. On the 7th day, the growth of fungal biomass was observed. The cell-free filtrate was processed for determination of enzyme specific activity. A variable pattern of activity was observed from the enzymes of cellulolytic fruit mycotic flora from different environment. The enzyme purity is most important which can be assessed by calculating enzyme activity and specific activity (activity per unit mass). In the present study harvested filtrate processed under optimum conditions followed by DNS method for enzyme assay. The specific activities (U g^{-1}) of cellulase for isolates were calculated (Fig. 2). Another study performed selection of fungal cultures isolated through forest litter soil for cellulases production and their activity were determined by FPA (Filter paper assay), CMCase (carboxy-methyl cellulase assay) and β-D-glycosidase assay[28]. Whereas, study employed it on currency waste as a raw material and it's processing through enzymatic route might help in improving the cost competitiveness and environmental status of the Indian handmade paper industry[4]. Whereas, report suggests that when the culture medium supplemented with banana leaves, rice husk, millet husk, coir waste, wheat bran, sawdust, Rice straw and ground nut banana leaves (725 units) shows optimum production[29]. Another study indicated the cellulase and xylanase activity of the organisms was evaluated by

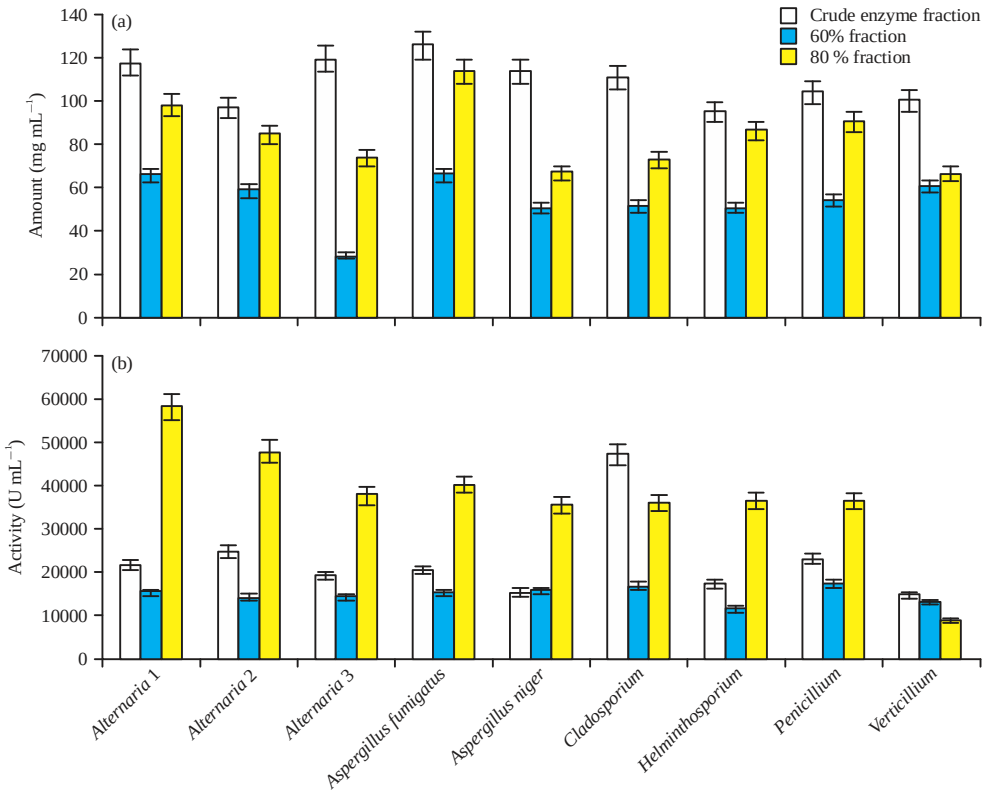

Fig. 2: Histogram represent the specific activity of cellulase as Unit per mg of protein in different fungal isolates (n = 9) of fruit samples. The data is represented (a) The quantity of enzyme (mg g^{-1}) and (b) Activity of enzyme (U g^{-1}) of different fractions

The error bars indicating the standard deviation

determining the diameter of the clear-zone around the colony indicating the hydrolytic value on cellulose congo red agar media[12]. A study reported the production of cellulolytic enzymes by staining with 1% congo red from *T. harzianum*, *T. viride*, *T. koningii*, *A. japonicus*, *A. nidulans* ver. dentatus *P. lanosum*, *P. expansum* and *P. oxalicum* gave the utmost cellulase activity on the other hand *A. flavus*, *A. raperi*, *A. acculeatus*, *A. tamarri*, *A. niger*, *A. terreus*, *A. nidulans*, *P. citrinum* and *P. simplicissimum* showed lowest or nil enzyme activity[30].

Determination of quantity and activity of cellulase enzyme: The specific cellulolytic quantity of the isolated fungi was determined by the fungi when grown in Sabouraud dextrose broth, CMC broth and semi-synthetic czapk dox broth. The quantity and activity of cellulase enzyme estimated in the crude fraction (cell free extract) and 80% partially purified salt precipitated fraction.

***In vitro* optimisation of cellulase production:** Out of the three media used czapk dox broth was found to be most optimum for cellulase production in SmF. In czapk dox broth, utmost active enzyme (29.56-135.94 U g^{-1}) demonstrated by *Verticillium* sp. (135.94 U g^{-1}) followed by *A. niger* (111 U g^{-1}) and *Alternaria* sp. (108.611 U g^{-1}), respectively in a crude fraction. Whereas, in Sabouraud dextrose broth enzymatic activity was found to be in the range 18.22-106.78 U g^{-1}, but the maximum recorded by *Cladosporium* sp. (106.78 U g^{-1}) followed by *A. niger* (85.94 U g^{-1}) (Table 1, Fig. 3a, b). In 80% partially purified salt precipitated fraction, the activity ranged from 17.17-82.72 and 11.89-77.39 U g^{-1} from czapk dox broth and Sabouraud dextrose broth, respectively. On the other hand, CMC broth is the least effective for cellulose production (Table 2, Fig. 3c, d). Another study[31] uses the cost-effective substrates for the isolation of the enzymes producing micro-organisms that inturns significantly cut down the cost of isolation and screening. Study suggests cellulase from *Aspergillus niger* reported maximum titers of FPase, CMCase and BGL obtained with this combination were 2.632, 2.478 and 2.984 U mL^{-1} in SmF and 29.81, 25.2 and 32.18 U g^{-1} DS in SSF[32].

During *in vitro* optimisation of 5.0, 7.0 and 9.0 pH activities were used to analyze cellulase production in acidic and alkaline conditioned media. It was observed that under

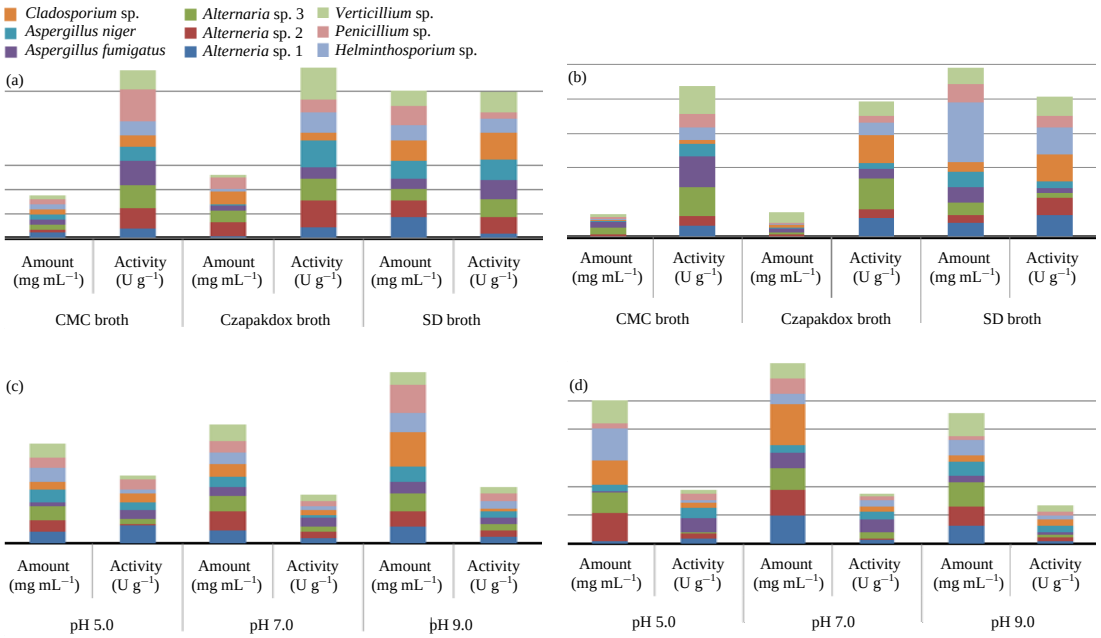

Fig. 3(a-d): *In vitro* optimization of fungal cellulase production in SmF: Culture media optimization, (a) Crude enzyme fraction, (b) 80% enzyme fraction, (c) pH optimization-crude enzyme fraction and (d) 80% enzyme fraction in sabouraud dextrose broth

Table 1: Media optimization of crude fungal cellulase in Czapk dox broth and Sabouraud dextrose broth

	CMC broth		Czapk dox broth		SD broth	
Fungus	Amount (mg mL^{-1})	Activity (U g^{-1})	Amount (mg mL^{-1})	Activity (U g^{-1})	Amount (mg mL^{-1})	Activity (U g^{-1})
Alternaria sp. 1	22.65	39.44	8.72	46.44	86.28	18.22
Alternaria sp. 2	11.99	83.56	60.36	108.611	66.63	69.44
Alternaria sp. 3	22.14	95.00	44.39	88.39	48.72	69.56
Aspergillus fumigatus	17.91	95.83	21.07	47.17	45.10	81.61
Aspergillus niger	21.43	64.50	7.65	111.00	71.48	85.94
Cladosporium sp.	22.19	43.89	48.673	29.56	80.66	106.78
Helminthosporium sp.	22.24	58.83	8.72	87.39	68.32	56.28
Penicillium sp.	18.01	132.22	49.54	46.50	77.45	30.67
Verticillium sp.	18.37	75.28	9.23	135.94	61.89	81.44

Table 2: Media optimization of 80% fraction fungal cellulase in czapk dox broth and Sabouraud dextrose broth

	CMC broth		Czapk dox broth		SD broth	
Fungus	Amount (mg mL^{-1})	Activity (U g^{-1})	Amount (mg mL^{-1})	Activity (U g^{-1})	Amount (mg mL^{-1})	Activity (U g^{-1})
Alternaria sp. 1	2.04	30.83	2.80	55.67	42.24	63.83
Alternaria sp. 2	5.00	30.22	4.80	22.22	22.14	49.00
Alternaria sp. 3	18.52	80.94	5.71	91.00	37.35	15.00
Aspergillus fumigatus	19.13	92.00	9.49	26.50	41.07	11.89
Aspergillus niger	3.01	33.56	4.23	17.17	46.84	23.00
Cladosporium sp.	2.70	14.33	5.61	82.72	29.18	77.39
Helminthosporium sp.	2.30	35.22	4.78	37.28	173.47	75.60
Penicillium sp.	4.80	40.50	3.62	18.89	54.03	36.94
Verticillium sp.	8.27	80.17	29.29	43.67	45.15	55.94

acidic conditions (pH 5.0) *Alternaria* sp., performed best with 99.44U g^{-1} followed by *Penicillum* sp. (66.11 U g^{-1}) active cellulose in crude extract (Table 3) and 80% fraction *A. fumigates* and *A. niger* produced maximum cellulase 49.17 and 33.94 U g^{-1} (Table 4), respectively. On the other hand, under alkaline conditions in crude preparation *Helminthosporium* sp. (48.89 U g^{-1}) and *Penicillium* sp. (41.67 U g^{-1}) was the potent candidates (Table 3). In partially

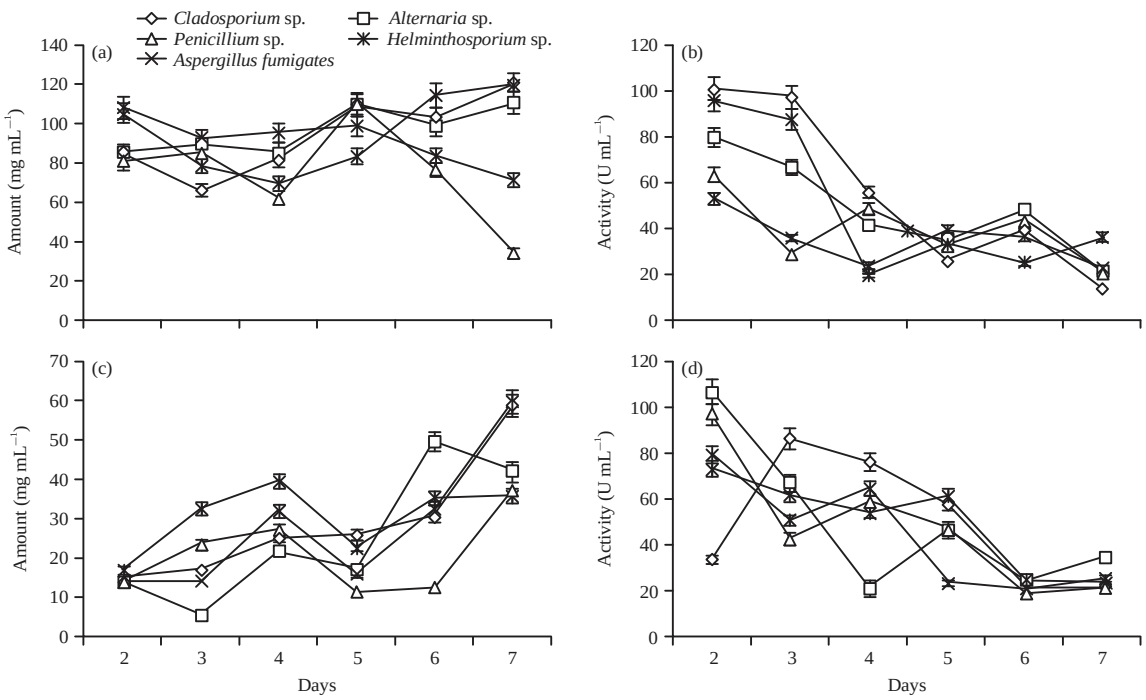

Fig. 4(a-d): *In vitro* optimization of fungal cellulase production in SmF; Days of incubation (a) Crude enzyme fraction with enzyme quantity and (b) Crude enzyme fraction with enzyme activity, (c) 80% enzyme fraction with enzyme quantity and (d) 80% enzyme fraction with enzyme activity
The error bars indicating the standard deviation

Table 3: pH Optimization of crude fungal cellulase in Sabouraud dextrose broth

	pH 5.0		pH 7.0		pH 9.0	
Fungus	Amount (mg mL^{-1})	Activity (U g^{-1})	Amount (mg mL^{-1})	Activity (U g^{-1})	Amount (mg mL^{-1})	Activity (U g^{-1})
Alternaria sp. 1	67.81	99.44	75.97	26.11	97.96	36.67
Alternaria sp. 2	65.61	15.00	106.28	40.00	85.15	39.44
Alternaria sp. 3	79.18	28.89	93.72	31.67	104.49	37.22
Aspergillus fumigatus	31.28	49.00	50.77	51.22	70.61	36.11
Aspergillus niger	64.34	51.33	61.12	13.78	89.54	35.72
Cladosporium sp.	52.91	46.67	79.44	28.33	200.56	14.89
Helminthosporium sp.	73.42	21.67	63.52	26.11	108.01	48.89
Penicillium sp.	69.24	66.11	68.72	31.11	173.15	41.67
Verticillium sp.	78.42	16.67	94.90	31.67	70.26	37.78

Table 4: pH optimization of 80% fraction fungal cellulase in Sabouraud dextrose broth

	pH 5.0		pH 7.0		pH 9.0	
Fungus	Amount (mg mL^{-1})	Activity (U g^{-1})	Amount (mg mL^{-1})	Activity (U g^{-1})	Amount (mg mL^{-1})	Activity (U g^{-1})
Alternaria sp. 1	6.43	19.00	100.46	12.22	60.00	11.67
Alternaria sp. 2	97.96	15.56	88.67	7.22	69.64	9.39
Alternaria sp. 3	73.37	5.55	78.32	20.00	86.63	9.44
Aspergillus fumigatus	7.96	49.17	51.33	43.06	23.06	11.67
Aspergillus niger	21.58	33.94	27.04	27.61	45.15	19.00
Cladosporium sp.	84.95	18.33	145.15	17.78	26.73	23.50
Helminthosporium sp.	109.03	8.89	36.08	22.72	51.73	11.11
Penicillium sp.	20.71	24.44	49.39	11.67	13.78	18.05
Verticillium sp.	83.37	16.11	55.61	11.11	81.76	20.00

purified preparation *Cladosporium* sp. (23.5 U g^{-1}) and *Penicilium* sp. (18.05 U g^{-1}) were utmost producers of active cellulase (Table 4). Inferred that under acidic conditions *A. niger* and *Penicillium* were preferred and under alkaline conditions *Alternaria* sp., *A. fumigates* and *Penicillium* were identified to be better performers (Table 3, 4; Fig. 3c, d; Fig. 4).

During *in vitro* optimisation studies for the days of incubation (2-7 days) for five different isolates *Cladosporium* sp., *Alternaria* sp., *Penicillium* sp., *Helminthosporiun* sp., *Aspergillus niger* using optimised conditions as 28±2°C, pH of 5.0 on Czapk dox broth (Fig. 4). Maximum active cellulase found to be produced after 2 days of incubation 53.17-100.72 and 72.94-106.22 U g^{-1} in 80% in the crude and partially purified fraction by all the fungal isolates, respectively. Cellulase activity continued to decrease till 7th day. After purification, the overall activity of the enzyme significantly enhanced and all the five isolates considered as potential candidates for cellulase production. In crude extract maximum activity was reported by *Cladosporium* sp. (100.72 U g^{-1}) and *A. fumigates* as 95.67 U g^{-1}. On the other hand, *Alternaria* sp., produced 106.22 U g^{-1} and *Penicillium* sp., 96.97 U g^{-1} (Fig. 3, 4). Another group of research demonstrated to Jung *et al.*[33] optimise FPase activity was at pH 5.0 and 50°C in simultaneous saccharification and fermentation (SSF) process, which employed the yeast Kluyveromyces marxianus and *Penicillium* sp.[32] proposes best cellulolytic activity (GFPase and CMCase) from the crude extract of *Aspergillus niger*, *Aspergillus flavus* and the least was *Trichoderma* sp., with 5.0 g of the substrate at 25-30°C and pH 5.0-6.0 with 72 h of incubation. Another study reported *Trichoderma viride* and *Aspergillus niger* were a record as cellulase producing species as 1.76 U mL^{-1} at 30°C, pH of 5.0 on the 5th day of incubation[34]. Fungal cellulase enzyme were related to industrial implications with special reference to hand made a paper industry that has been performed to increase the yield of cellulase production of recipients strain to rise. The data present in the study represents that five fungal isolates *Alternaria* sp., *Aspergillus fumigatus*, *Cladosproium* sp., *Helminthosporium* sp., *Penicillium* sp. have shown to be suitable for cellulase production under *in vitro* condition as they also have higher specific productivities.

CONCLUSION

It is concluded that the study signified that in crude extract condition *Cladosporium* sp., *A. fumigates* and in 80% fraction *Alternaria* sp. and *Penicillium* sp. was found to be potent candidates for active cellulase production these isolates may be further suggested to develop a cellulolytic activity process for industrial implications with special reference to the handmade paper industry.

SIGNIFICANCE STATEMENT

The present study focused on the isolation and screening of all the fungi for the production of cellulase enzyme. The objectives designed for the study involves isolation and screening of cellulase production fungi from fruit waste. *In vitro* production of cellulase enzyme by submerged fermentation and thereby *in vitro* optimisation of different parameters, for better cultivation and production process through submerged fermentation. This study will help the researcher, to uncover the critical areas of fungal cellulase production to be suitable for industrial implications that many researchers were not able to explore. Thus this study will be helpful for the handmade paper industries at large.

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