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RESEARCH ARTICLE

Bioprospecting and characterisation of cellulase producing endophytic fungus, *Fusarium annulatum* from *Caryota urens*

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Abstract

Cellulases are a complex system of three enzymes, including endoglucanases, exoglucanases and β-glucosidases, that work together in synergic fashion to degrade lignocellulosic biomass into simple sugars. Despite their enormous applications, they imply high production costs, which represent a significant bottleneck limiting the industrial utilisation. This study therefore investigated the purification and characterisation of cellulase producing endophytic fungal strain, *Fusarium annulatum*, isolated from *Caryota urens* (Jaggery palm), identified by morphological characteristics and ITS sequencing. Crude cellulase enzyme was obtained from *F. annulatum* solid-state fermentation and partial purification of the enzyme was achieved through ammonium sulphate precipitation and dialysis. The partially purified enzyme sample exhibited significant activities of endoglucanase (1.2358 ± 0.0055 IU/mL), β-glucosidase (0.708 0.017 IU/mL) and cellobiohydrolase (0.244 ± 0.019 IU/mL). The filter paper assay of the partially purified enzyme demonstrated significant cellulase activity of 0.7406 ± 0.0258 IU/mL. The optimal pH and temperature of the partially purified enzyme were determined to be 6.0 and 50.0 °C, respectively. These results demonstrate the cellulase producing capacity of *F. annulatum*, anticipating its industrial production for various purposes, such as biomass conversion and biofuel production. This is one of the first reports of cellulase activity by *F. annulatum*.

Keywords: cellulase; enzyme production; endophytic fungus; *Fusarium annulatum*

Introduction

The global energy crisis, driven by the depletion of fossil fuel reserves and growing environmental concerns, has intensified the search for sustainable alternative fuel sources (1, 2). Lignocellulosic biomass (LCB) from plants offers a viable alternative for converting into biofuels using microbial fermentation and enzymatic hydrolysis. LCB is composed of cellulose, β-1,4-glucan polymer, which is highly crystalline in nature, responsible for forming cellular structural microfibrils and a heterogeneous, amorphous matrix of branched polysaccharides called hemicellulose, which plays a major role in the flexibility and porosity of the cell wall. Additional components, such as lignin, provide hydrophobicity and mechanical strength, which, in turn, contribute to recalcitrance during the bioconversion process (3, 4). Renewability, abundance and carbon neutrality are the attractive features of LCB (5). However, the recalcitrance of LCB poses challenges for its conversion into glucose (saccharification) for eventual fermentation and ethanol production in biofuels. Microbial cellulases help convert pre-treated LCB into glucose and have been explored for development and utilisation in industrial processes for biofuel production. Fungal cellulases are preferred over bacterial ones due to their better infiltration and substrate consumption abilities (6).

The main challenges faced by the biofuel industry are the non-availability of an ideal microbial cellulase with optimum performance and a broad substrate activity. Apart from enzyme efficiency, factors such as composition, pH, temperature, the presence of inhibitors and enzymatic deactivation can also adversely affect the downstream process. The main fungal strains used in industries for cellulase production are *Trichoderma reesei, Aspergillus niger, Aspergillus oryzae, Penicillium oxalicum, Penicillium funiculosum* etc. Additional significant producers of the cellulase enzyme complex include *Phanerochaete chrysosporium, Fusarium solani, Talaromyces emersonii, Trichoderma koningii* and multiple Rhizopus species, all of which demonstrate importance in biotechnological applications. A considerable portion of the fungal community remains to be explored, including the endophytic fungi that have unique mechanisms for interacting with their plant hosts. Endophytic fungi live inside plant tissues asymptomatically and produce numerous phytochemicals called plant growth promoters (PGP), which promote the growth of the host plant. They produce several extracellular enzymes, which play a crucial role in multiple biological activities within the host system. These extracellular enzymes have various functions, including cell wall degradation, tissue penetration, plant colonisation, nutrient acquisition and disease suppression in the host system (7). The highest endophytic fungal cellulase produces include; *Penicillium oxalicum* R4, *Candida stellimalicola* (7) and *Colletotrichum gloeosporioides* (8). Endophytes have an intimate association with the plant cell, exposing them to plant cell wall components such as cellulose and hemicellulose, which makes them adaptable to environments rich in cellulase. Moreover, they are more metabolically diverse and possess

additional abilities, such as the capacity to degrade lignin. Currently, many endophytes are being studied for their enzymatic profile, especially their ability to produce industrially important enzymes.

An endophytic lifestyle can be interpreted as a coevolutionary adaptation, with a complementation of bioactive compounds between the endophyte and the host as long as both the endophyte and the host maintain the relationship (9). Their long-term interaction with plant polymers enables them to evolve into more effective cellulase producers and this coevolutionary adaptation can be seen as a powerful tool for genetic diversity (10). The colonisation of endophytes on host plants is mainly non-pathogenic and sometimes mutualistic, providing the host plant with additional advantages, such as better tolerance of abiotic and biotic stresses, which can be achieved by the presence of protective compounds like osmolytes through proABC (proline synthesis), otsA/otsB (trehalose synthesis), betA/betB (glycine betaine pathway) and mtlD (mannitol synthesis) (11-14). Even though the cellulase enzymes produced by the endophytes are primarily used for plant tissue penetration and infection establishment, they later serve as a defence mechanism against other plant pathogens by degrading their cell wall components, ultimately protecting the host (12, 13). In addition to cell wall-degrading components, they also produce bioactive compounds, including polyketides, indole derivatives and fumaric acid, with antifungal and antimicrobial properties (11). The tissue-specific colonisation properties of endophytes, in turn, enhance the quality and function of the enzymes they produce. Additionally, the enzymatic arsenal in their process enables them to participate in multiple biochemical transformations both within themselves and in the environment they thrive in (13).

Large-scale cellulase enzyme production typically employs fermentation processes. Although both submerged and solid-state fermentation (SSF) methods are used for cellulase production, SSF is generally preferred for filamentous fungi due to its lower moisture requirements. Nevertheless, submerged fermentation effectively manages scale-up challenges and minimises by-product formation (15). Given the current demand for this enzyme and anticipated future growth, there is a critical need to identify new sources and develop more efficient production processes. Hence, this research focuses on the isolation and screening of novel endophytic fungal cellulase producers, anticipating the unique advantages of endophytic fungi to be a potential candidate, such as; their ability to establish themselves in a plant host system without any damage and to utilise plant-derived polysaccharides like cellulose (16), their ability to produce diverse enzymes in the host system (17) and the biochemical potential of the enzymes synthesized by them in terms of stability and activity (7). Natural diversity among endophytes is also a positive attribute for strain selection and optimisation of cellulolytic potential. Additionally, endophytic cellulases are the best components for designing a sustainable technology for biomass conversion and biofuel production (18).

Materials and Methods

Sample collection and endophyte isolation

The present study was undertaken to identify an excellent cellulase producing fungal endophyte by bioprospecting various plant species. An overview of the work is depicted in Fig. 1. Leaf samples from 10 different plant species were collected from 2 Indian states: 2 from Karnataka and 8 from Kerala (Table 1). The samples were transferred to the laboratory in sealed polythene bags within 24 hr of collection and stored in the refrigerator at 4 °C for 2 hr for further processing. Herbarium sheets were prepared for these plant samples for further identification and authentication (Fig. 2).

Sample preparation involved multiple cleanings of each specimen with distilled water, followed by blot-drying. Surface sterilisation was achieved through a two-stage chemical treatment: first, a 60-sec immersion in 70 % ethanol, followed by rinsing; then, a three-minute exposure to 3.5 % sodium hypochlorite solution was performed, followed by rinsing in distilled water. Samples were then spread onto blotting paper and dried under sterile laminar airflow. Later, the plant samples were sectioned into 1 × 1 cm pieces using sterile surgical blades and transferred to Potato dextrose (Himedia, pH 6.0) agar supplemented with streptomycin sulfate (100 mg/L) to inhibit bacterial contamination (19). Subsequently, the prepared leaf segments were cultured on Potato Dextrose Agar medium (Himedia,

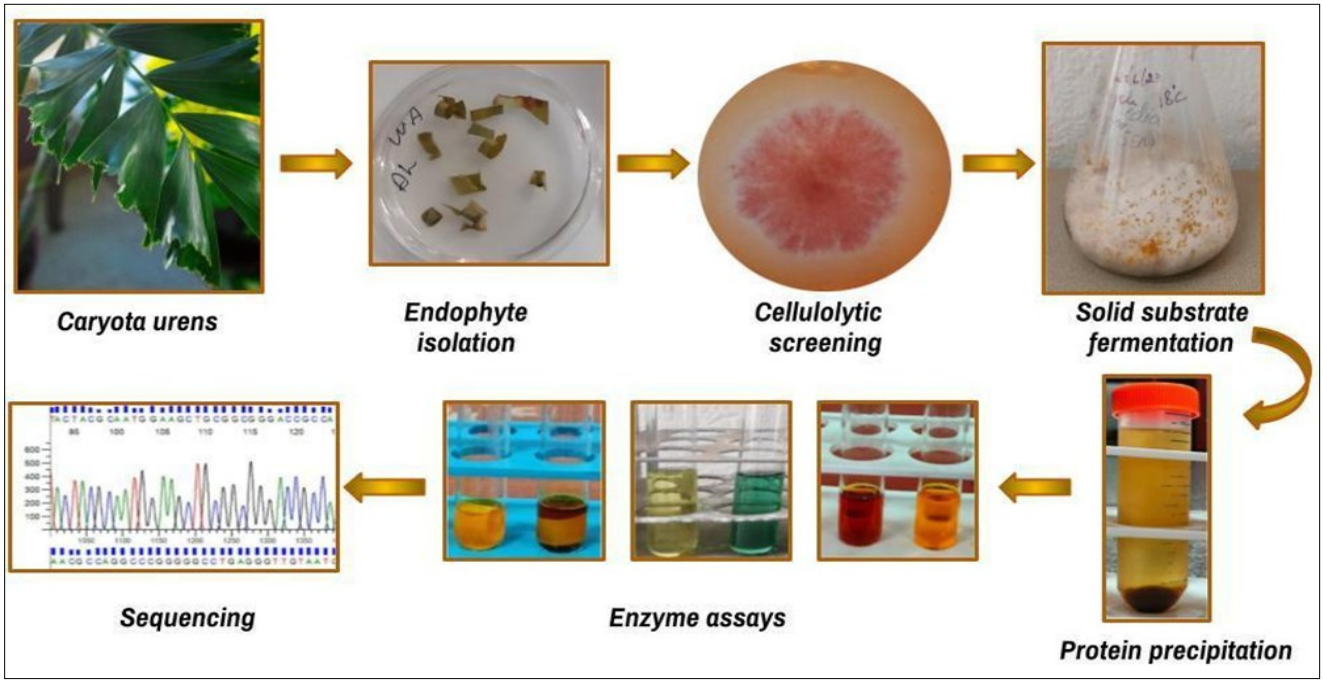

Fig. 1. Overview of the work from bioprospecting to characterisation of the strain of *F. annulatum* isolated from the plant *Caryota urens*.

Table 1. Plant sources for bioprospecting for cellulases and the locations where they were collected

Sl. No	Sample Code	Scientific name	Location	Coordinates
1	CA 01	*Centella asiatica*	Bengaluru, Karnataka	13°03'29.6″N 77°38'33.6"E
2	AP 02	*Alpinia purpurata*	Bengaluru, Karnataka	13°03'29.6″N 77°38'33.6"E
3	EC 03	*Elettaria cardamomum*	Idukki, Kerala	10°00'27.0″N 77°06'38.8"E
4	CM 04	*Cinnamomum malabatrum*	Kottayam, Kerala	9°44'12.6″N 76°43'23.2"E
5	PN 05	*Piper nigrum*	Kottayam, Kerala	9°44'12.6″N 76°43'23.2"E
6	RR 06	*Rhynchostylis retusa*	Kottayam, Kerala	9°44'13.3″N 76°44'01.6"E
7	CU07	*Caryota urens*	Kottayam, Kerala	9°44'12.6″N 76°43'23.2"E
8	SP 08	*Spathoglottis plicata*	Kottayam, Kerala	9°40'10.8″N 76°48'54.3"E
9	CO 09	*Corypha umbraculifera*,	Kottayam, Kerala	9°40'10.8″N 76°48'54.3"E
10	MA 10	*Musa acuminata*	Alappuzha, Kerala	9°29'31.2″N 76°23'29.1"E

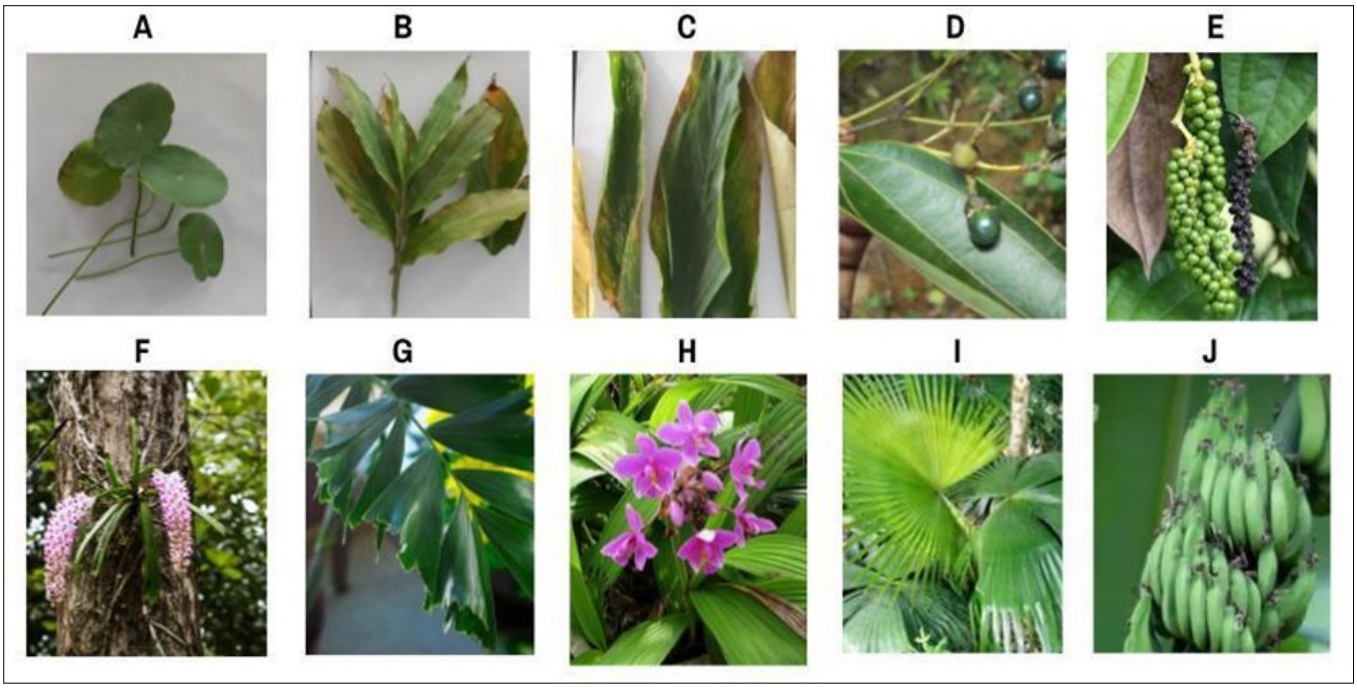

Fig. 2. Plant species used for bioprospecting for cellulase producing fungi. A- *Centella asiatica* (Indian Pennywort), B- *Alpinia purpurata* (Red Ginger), C- *Elettaria cardamomum* (Cardamom), D- *Cinnamomum malabatrum* (Wild Cinnamon), E- *Piper nigrum* (Pepper), F- *Rhynchostylis retusa* (Foxtail Orchid), G- *Caryota urens* (Jaggery palm), H- *Spathoglottis plicata* (Ground Orchid), I- *Borassus flabellifer* (Toddy palm), J- *Musa acuminata* (Cavendish Banana).

pH 6.0) for fungal isolation. The inoculated plates were incubated at 20 °C until the fungal colonies developed (20).

Screening of cellulase-producing isolates using CMC agar plates

The cellulolytic potential of the fungal isolates was assessed using the Carboxymethylcellulose (CMC) agar medium. The growth medium was formulated by incorporating the following components: $NH_4H_2PO_4$ (1 g), KCl (0.2 g), $MgSO_4$ $7H_2O$ (1 g), yeast extract (1 g), carboxymethyl cellulose (26 g) and agar (3 g), dissolved in 500 mL of distilled water. The prepared medium underwent standard sterilisation procedures, cooled and then inoculated with the isolated fungal cultures. It was incubated for 4-5 days at 20 °C to screen for cellulase production (20). After the incubation period, the plates were flooded with a 0.1 % Congo Red solution for 15 min and the dye was removed using 1 M NaCl (21). The highest cellulase-producing colony was identified by measuring the clear zones formed on CMC agar Petri plates and calculating the cellulose Degradation Coefficient (CDC) by respective fungal species using the formula;

Cellulose degradation coefficient (CDC) = (dh/dc) (Eqn. 1)

Where, dh: degradative halo and dc: colony diameter (21)

The superior cellulase-producing isolate was selected and sub-cultured onto fresh medium, with pure cultures preserved on potato dextrose agar slants.

Identification of cellulase-producing strains

The fungal isolate demonstrating the highest cellulose-degrading capability was identified by morphological characterisation and mycelial/spore examination using lactophenol-cotton blue staining. Furthermore, the pure culture of the selected organism, which exhibited the highest cellulase activity, was inoculated into sterile Czapek Dox medium and incubated for 10 days at 20 °C. QIAGEN DNeasy Ultra Clean Microbial Kit (Cat. No. 12224-50, QIAGEN, Hilden, Germany) was used to extract the Genomic DNA from the fungal culture where the manufacturers protocol was exactly followed. 1.0 % (w/v) agarose gel in 1× TAE buffer, stained with ethidium bromide was used to assess the quality of the extracted DNA sample and was visualized under a UV transilluminator where, a single band of high-molecular-weight DNA confirmed successful extraction and genomic DNA integrity. Universal fungal primers; ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') were used to amplify the ITS regions of the genomic DNA sample (22). PCR amplification was performed using approximately 50 ng of

the DNA sample, 1.25 U Taq polymerase and 0.5 µM of each primer. Other components include: 1× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix and the volume was made up to 25 µL. The following thermal cycling conditions were used for the amplification: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 52 °C for 30 sec and extension at 72 °C for 45 sec, with a final extension at 72 °C for 3 min. The amplified product was analysed by electrophoresis, where a single, clear band of approximately 700 bp was observed, consistent with the fungal ITS region. Purification of the amplified PCR product was done using QIAGEN QIAquick PCR Purification Kit (Cat. No. 28104, QIAGEN, Hilden, Germany). The above-mentioned ITS primers were used for bidirectional DNA sequencing of the PCR amplicon and the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) was used. Cycle sequencing reactions were conducted on an Applied Biosystems™ MiniAmp™ Plus Thermal Cycler and the analysis of the genetic product was carried out using an ABI 3730xl Genetic Analyser (Applied Biosystems) with POP-7™ polymer according to the manufacturer's specifications. Further, the forward and reverse sequences were assembled, edited and analysed using the BLASTn algorithm against the GenBank database (http://blast.ncbi.nlm.nih.gov) (23). A maximum likelihood tree was constructed using the Molecular Evolutionary Genetics Analysis software (MEGA version 12.0.11). Selected nucleotide sequences obtained through a BLAST search using the fungal ITS sequence as a query were used to infer phylogeny. The obtained sequences were aligned using the ClustalW algorithm. Based on the aligned sequences, a phylogenetic tree was constructed using the Maximum Likelihood (ML) method in MEGA, with 1000 bootstrap replicates (20, 23).

Solid State Fermentation (SSF) for enzyme production

SSF was carried out in sterilised large, transparent, airtight plastic containers. Finely ground wheat bran was sterilised and used as a substrate for the production and Czapek-Dox modified medium containing ferrous sulfate (0.01 g), potassium chloride (0.5 g), dipotassium phosphate (1 g), sodium nitrate (2 g), magnesium sulfate (0.5 g) and sucrose (30 g) with pH adjusted to 7.3± 0.2 enriched with 1 % CMC (carboxymethyl cellulose) used as a moistening agent. The incubation conditions were set at 24 °C for 10 days (24).

Crude enzyme extraction and partial purification of the enzyme

The crude extraction was performed using a 50 mM citrate buffer (pH 4.8), with 400 mL of buffer used per 40 g of mouldy bran. The process was carried out in a shaker incubator at room temperature, 200 rpm for 60 min. A sieve was used to filter the slurry, removing the larger sediments and a second level of filtration was performed using Whatman filter paper No. 1. Partial purification of the enzyme was carried out using ammonium sulphate (80 %) precipitation followed by dialysis. 614 g of ammonium sulfate in 1000 mL of crude extract was used to attain 80 % saturation. The temperature and duration were set to 20 °C overnight, respectively and a magnetic stirrer (operating at 1500-1800 rpm) was used to ensure complete dissolution. The entire content was centrifuged at 10000 rpm for 30 min at 4 °C. The pellet was collected and resuspended in 5 mL of 50 mM acetate buffer for further dialysis procedures. Dialysis of the precipitated and resuspended extract was performed using a dialysis membrane (Hi Media, pore size: 2.4 nm, molecular weight cut-off between 12000 and 14000 Da). The dialysis process was carried out at room temperature against a 30 mM Acetate buffer in a 2 L beaker, placed on a magnetic stirrer at 320 rpm. Buffer replacement was performed twice, with a 2 hr gap between procedures and the entire process was completed overnight.

Assay for endoglucanases

Dinitrosalicylic acid (DNS) assay

The DNS assay was used to determine the amount of reducing sugars in the reaction mixture. The amount of reducing sugars was equivalent to the amount of the cellulase enzyme available in the partially purified sample. 1 mL of 1 % CMC was used as a substrate and 0.5 mL of partially purified enzyme was added to each tube. The tubes are then incubated for 20 min at 50 °C to allow the colour to develop. The colour intensity was measured using a spectrophotometer at 540 nm (25).

Nelson-Somogyi assay

The Nelson-Somogyi Assay was another method used to estimate the presence of reduced sugars, utilising the intensity of a blue-coloured complex that forms when arsenomolybdic acid reacts with copper ions (26). Alkaline copper tartrate and the Folin Ciocalteu (FC) reagent were used in the reaction mixture, followed by incubation in the boiling water bath until the colour changes. The copper ions formed were equal to the amount of reducing sugars released by the enzymatic activity and their concentration was measured at 620 nm using a spectrophotometer.

Assay for cellobiohydrolase (CBHs) using

pNPC (p-nitrophenyl-β-D-cellobioside) assay

The pNPC assay (p-nitrophenyl-β-D-cellobioside assay) was used to estimate the CBH activity of the partially purified extract (27). A stock solution of 10 mM p-nitrophenyl β-D-cellobioside (pNPC) in acetate buffer was used as the reaction substrate. The pNP was later protonated to form a coloured complex during incubation and the complex was measured calorimetrically at 410 nm. A 1 M Na_2CO_3 solution (1 mL) was used to arrest the entire reaction before recording the absorbance values.

Assay for β-glucosidases

pNPG (p-nitrophenyl-β-D-glucopyranoside) assay

To estimate the activity of β-glucosidase enzymes, 2 mM p-nitrophenyl-β-D-glucopyranoside (pNPG) was used as a substrate along with sodium acetate buffer (50 mM, pH 5.0) as the reaction buffer. The incubation was conducted for 30 min at 50 °C. The reaction was arrested using 1.0 mL of 1 M sodium carbonate solution and the absorbance values were measured at 410 nm (28).

Filter paper assay

The filter paper assay was developed in earlier reports and was standardised by Ghose T K to analyse the overall cellulolytic activity of the content. Whatman No. 1 filter paper strips were cut to 1 x 6 cm (~50 mg) as a substrate for this assay. 1 mL of Citrate buffer (50 mM, pH 4.8) was used as the reaction buffer and 0.5 mL of partially purified enzyme solution was added. The mixture was then incubated for 1 hr at 50 °C. The amount of reducing sugar units was estimated by the DNS method, as described above (29).

Determination of optimum conditions for enzyme activity

Optimum temperature

The optimum temperature of the partially purified enzyme activity was found using CMCase assay method (DNS) at 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C and 80 °C. The determination of reducing sugars

proposed by Miller (1959) was used, where different aliquots of citrate buffer (50 mM) and 1 mL CMC (1 %) were added along with partially purified enzyme (25 to 500 µL) (25). Incubation was done for 20 min at 50 °C and DNS (1 mL) was added to stop the reaction and the mixture was placed in a boiling water bath for 5 min. The absorbance was calculated at 540 nm using a spectrophotometer (30).

Optimum pH

Phosphate buffer was used to determine the optimum pH for enzymatic activity. One of the limitations of phosphate buffer is that it is effective only within the pH range of 5.8-7.8. To extend the buffer range, 3 buffer combinations were used: citrate buffer (pH 3-6.2), phosphate buffer (pH 5.8-7.8) and glycine buffer (pH 8.6-10) (31). The values were compiled to form a complete graph of pH optima. The cellulase activity was determined using the DNS assay (25, 27). An overview of the work done is depicted in Fig. 1.

Statistical analysis

All enzyme assay measurements were performed in triplicate (n = 3 per group). Data were presented as mean ± standard error of the mean (SEM). Statistical analysis was conducted using IBM SPSS Statistics Version 28. Differences between the control and test groups were analysed using unpaired two-tailed Student's T-tests, assuming equal variances and significance levels were set at $\alpha = 0.05$. Statistical significance was defined as: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Results and Discussion

Sample collection, initial screening and characterisation of cellulase-producing endophytic fungi

The general fungal isolation process, using PDA supplemented with Streptomcin (50 µg/mL) to restrict bacterial growth, yielded 63 fungal endophytic cultures from the collected plant samples after 10 days of incubation. Ten cultures exhibited significant cellulase activity, as indicated by the formation of distinct orange halos after the addition of a 1 % Congo red solution. The B/A ratio in Fig. 3 indicates cellulolytic enzyme production efficiency relative to colony size, where higher ratio values represent superior cellulolytic efficiency. Among the tested isolates, CU 07 showed the highest B/A ratio, indicating the highest cellulase-degrading activity. From the calculation of the ratio between the diameter of the colony and the diameter of the clear zone formed, the fungal strain from CU 07 isolated from *Caryota urens* demonstrated higher cellulolytic activity with a clear zone ratio of 4.5.

CU 07 exhibited rapid growth, characterised by abundant white aerial hyphae with a distinctive cotton-like appearance and subtle pink pigmentation at the colony base when grown on PDA agar plates. Microscopic examination using Lactophenol cotton blue (LCB) stain revealed the presence of multiple spore types, such as macroconidia (slender, slightly oblong structures characterised by the presence of a single septum), microconidia (distinctive dagger/ovoid-shaped spore morphology) and sporodochia (compact hyphae) (Fig. 4).

Species level identification was achieved through ITS sequencing and subsequent phylogenetic analysis. Based on the comprehensive morphological and molecular analyses, CU 07 was identified as *Fusarium annulatum*, which depicts the clear cellulolytic zone when cultured on CMC agar medium (Fig. 5 A and B). Numerous studies have focused on *Fusarium* sp., specifically their ability to produce cellulolytic enzymes as exemplified by *F. nygamai* (32), *F. graminearum* (33), *F. solani* (34) and *F. oxysporum* (35), which exhibit significant cellulolytic activity when grown under optimised conditions.

The obtained sequence was deposited in GenBank under accession PV393838. A phylogenetic tree was constructed to illustrate the evolutionary relationships among the strains (Fig. 5). A dendrogram was created using eleven *Fusarium* spp. to investigate

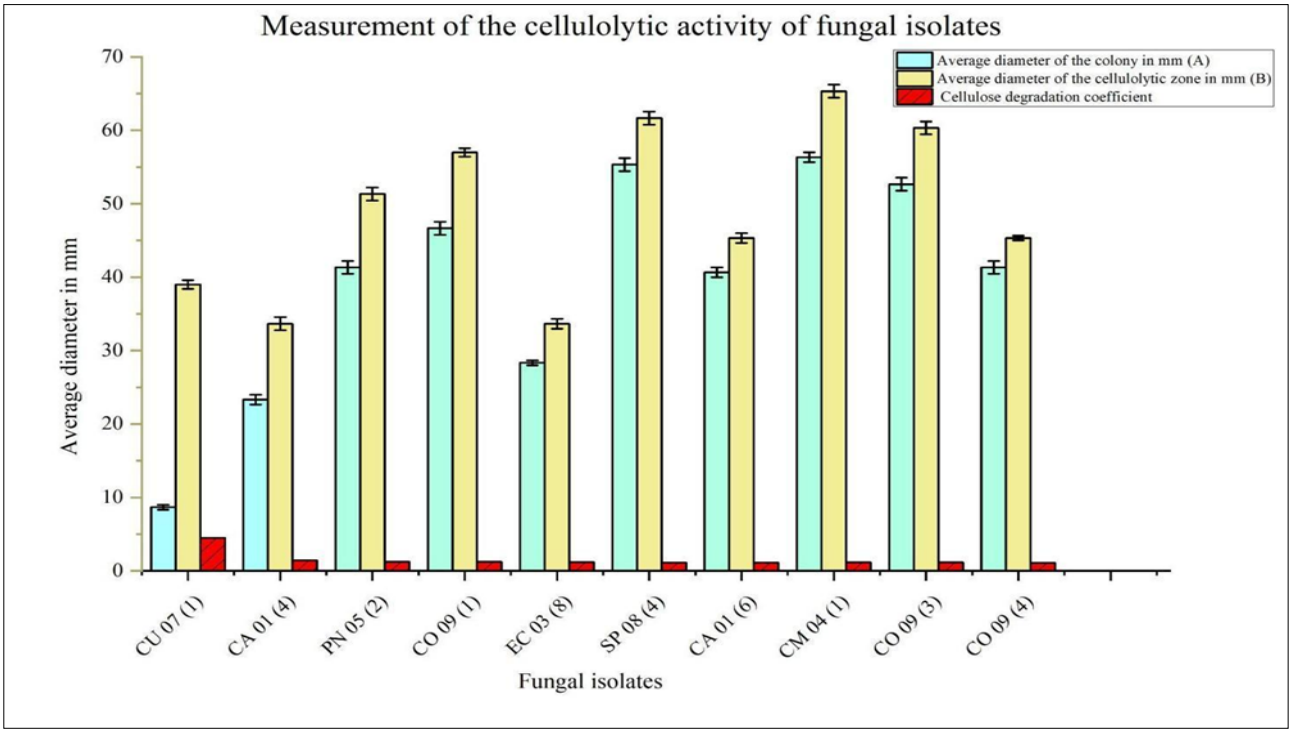

Fig. 3. Comparative analysis of the cellulolytic zone diameter of the selected fungal isolates having significant cellulolytic activity. The graph represents 3 bars in a group, indicating average colony diameter, cellulolytic zone diameter; their ratio (B/A) is depicted as Cellulose Degradation Coefficient.

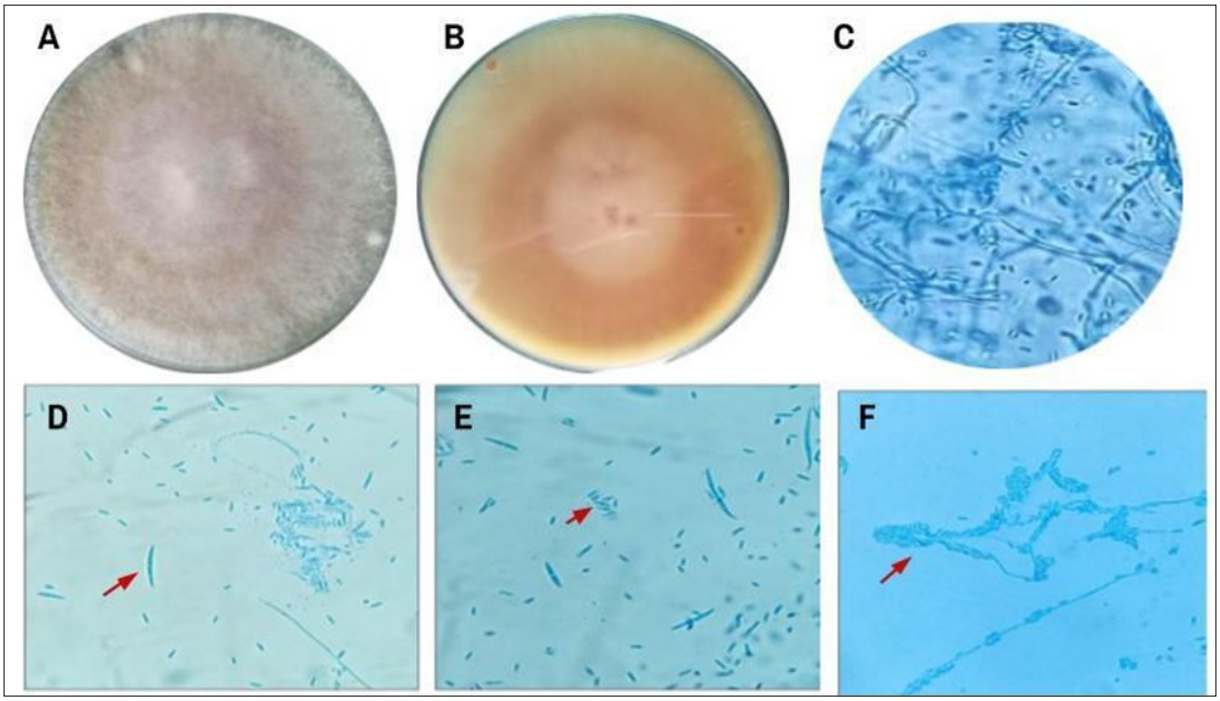

Fig. 4. Mycelial growth of *Fusarum annulatum* in potato dextrose agar medium: A: Top view; B: Bottom view; C: Lactophenol cotton blue (LCB) staining; D: Macroconidia (in red circle) with 3-5 septa and slightly curved foot-shaped basal cells; E: Microconidia with distinctive dagger/ ovoid-shaped spore morphology; F: Sporodochia with clustered conidiophores and mass spore production.

evolutionary relationships, with a scale of 0.01 substitutions per site. The analysis demonstrated clear taxonomic groupings within the genus, characterised by distinct evolutionary relationships. There were two clades obtained, which showed close relatedness to *F. annulatum*, such as a major clade with a bootstrap support value of 43 % having nine species in it (*F. hechiense, F. pantonense, F. concentricum, F. acutatum, F. dlamini, F. annulatum, F. phyllophilum, F. fujikuroi and F. elaeagni*). The second clade comprises three species (*F. hostae, F. nyagarai* and *F. aquaticum*) with exceptionally high bootstrap support of 96 %. Even within the major clade, well-organised subclades were observed, such as *F. hechiense* and *F. pantonense* (bootstrap value 11 %) and a moderately supported cluster containing *F. concentricum*, *F. acutatum, F. dlamini* and *F. annulatum* (bootstrap values ranging from 59 % to 66 %). This data is supported by various studies done by previous researchers (36. 37), which also reported similar scenarios, especially in agricultural pathogenic environments where host-pathogen coevolution drives genetic differentiation.

Solid-state fermentation of the fungal isolation and partial purification of the enzyme

Solid-state fermentation of the fungus was carried out and partial purification was conducted using ammonium sulphate (80 %) method and dialysis procedures. The obtained enzyme extract was subjected to various assays and the results were compared (Fig. 6). The whole cellulase enzyme complex is composed of 3 major parts:

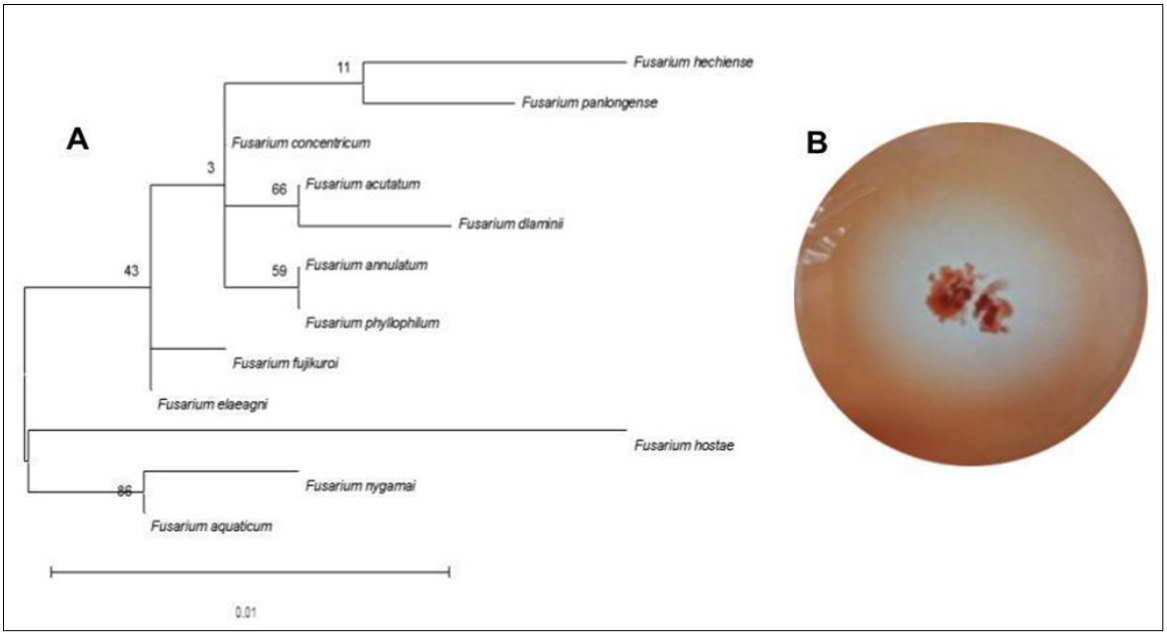

Fig. 5. A: Phylogenetic tree of Fusarium species based on molecular sequence analysis. Dendrogram showing evolutionary relationship among various *Fusarium species*, including our study isolate *Fusarium annulatum*. The scale bar represents 0.01 substitutions per nucleotide position. *F. annulatum* clusters closely with *F. phyllophilum* with 59 % bootstrap support; B: Cellulolytic ability of the fungal isolate *F. annulatum* (CU 07) on Carboxymethyl cellulose agar enriched with Congo red solution.

endoglucanases (mainly determined using the DNS assay or NS method), Exoglucanases (determined using the pNPC assay) and β-glucosidases (determined using the pNPG assay).

Assay for endoglucanase

The endoglucanase activity of the partially purified enzyme was measured after 10 days of SSF using wheat bran as the substrate and the extraction procedures. DNS assay demonstrated an activity of 1.236 ± 0.006 IU/mL, where one unit (IU) is defined as the amount of enzyme that releases 1 µmole of reducing sugar (as glucose equivalent)/min under assay conditions (50 °C, pH 5.0, 1 % CMC). In contrast, the Nelson-Somogyi NS assay yielded 0.715 ± 0.016 IU/mL; both the yields were compared to the control group for the tested concentration (T1 vs Control: $p < 0.001$). It has been observed that the NS activity is 57.85 % lower than the activity shown by the DNS assay. This considerable difference between the values of the two assays can be justified by the overestimation that occurs in the DNS assay. When cellulase activity on CMC was measured, the DNS assay yielded values typically 40–50 % higher than those obtained with the NS assay (38, 39).

While some studies have reported very high values for endoglucanase activity, such as 23.7IU/mL by *Candida stellimalicola* (7), most of the fungal endoglucanase activities fall within the same range observed in this study. The same author also reported a different isolate, *Fusarium oxysporum luffae*, which exhibits endoglucanase activity of 5.73 IU/mL. Other related findings include *Trichoderma viridae*, which shows 1.23 U/mL of endoglucanase activity (40), *Trichoderma reecei* (2.849 IU/mL) (41), *Chaetomium globosum* (3.07 IU/mL) (42), *Penicillium* sp. (1.9 IU/mL) (43) and *Penicillium oxalicum*: 5.27 U/mL (43). Lower CMCase readings were also reported, with Trichoderma isolates showing cellulase activities ranging from 0.08 to 1.23 U/mL, consistent with our findings (44).

Assay for β-glucosidases (BGLs)

BGLs are responsible for degrading oligosaccharides into individual glucose units, thus completing the cellulase reaction system. The BGL assay reported 0.708 ± 0.01 IU/mL of enzymatic activity for the tested concentration (T1 vs Control: $p < 0.001$) at an optimum pH of 5.0 where one unit (IU) is defined as the amount of enzyme that releases 1 µM of p-nitrophenol per minute from 1 mM pNPG under assay conditions aligning with findings from other studies. In former studies various Fusarium species, including *F. acuminatum, F. graminaerum, F. Mesoamericanum* and *F. asiaticum*, which exhibited high β-glucosidase activity were reported (45). Previous researchers reported on *F. proliferatum* showing 3.31 U/mL of BGL activity (46). Other mentions include *Aspergillus fumigatus* (0.87 IU/mL) (47), *Humicola grisea* (0.459 IU/mL) (48) and *Aspergillus niger* (0.93 IU/mL) (47), which match our results. *Aspergillus saccharolyticus* displayed superior BGL activity compared to the industrially available BGL cocktail, Novozyme 188, which has a standard pNPG Assay value of 96.1 IU/mL were reported earlier (49). In previous reports, optimal BGL activity can be observed at pH 5.0 at 50 °C, which is the ideal situation for most fungal β-glucosidases (28, 50).

Assay for cellobiohydrolase (CBHs)

CBHs, also known as exoglucanases, catalyse the hydrolysis of 1,4 β-D-glucosidic linkages in cellulose, specifically releasing cellobiose units from the non-reducing ends of cellulose chains. The pNPC assay with the partially purified enzyme showed significant CBH activity compared to the control group at the tested concentration (T1 vs Control: $p < 0.001$). The enzyme activity was found to be 0.486 ± 0.027 IU/mL for a 50 µL enzyme volume. OD values were converted to IU/mL using a pNP standard curve, where one unit (IU) is defined as the amount of enzyme that releases 1 µM of p-nitrophenol /min from 1 mM pNPC under assay conditions (50 °C, pH 5.0). Although CBH studies, in particular, are rarely conducted, there are some reports, such as the exoglucanase activity of *Penicillium glabrum*, which shows 48.3 U/mL (51) and the exoglucanase activity of *Chaetomium globosum*, which ranges from 3.5 to 5.96 IU/mL (42).

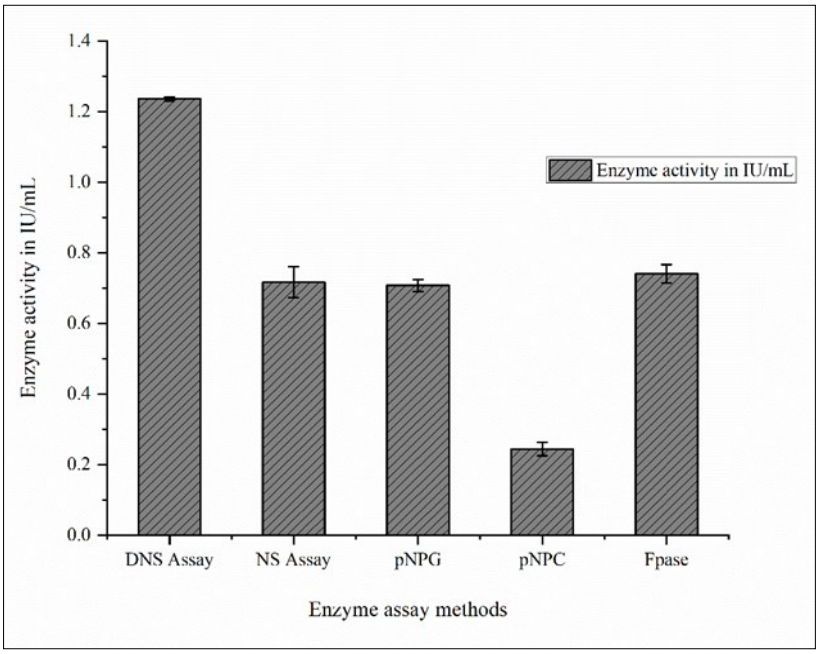

Fig. 6. Enzymatic profile of *Fusarium annulatum* (CU 07) showing cellulase production. Total reducing sugar release was assessed using the DNS method (1.236 ± 0.0055 IU/mL), which provides an overall measure of cellulase activity. The NS assay represents the total release of reducing sugars, indicating overall cellulase activity (0.717 ± 0.044 IU/mL). Individual activities were assayed through pNPG for β-glucosidase (0.708 ± 0.017 IU/mL) and pNPC for exoglucanase (0.244 ± 0.019 IU/mL). The filter paper assay measured total cellulase activity by monitoring the degradation of filter paper strips. The total degradation was estimated using the DNS method (0.7406 ± 0.0258 IU/mL).

Filter Paper Assay (FPA) for cellulase activity

FPA quantifies the overall cellulase activity by measuring the collective enzymatic function of endoglucanases, exoglucanases and β-glucosidases, the three enzyme components responsible for cellulose degradation. The method is commonly employed due to its ease of execution, despite having limited accuracy in predicting cellulase effectiveness against complex lignocellulosic substrates (46, 47). The enzyme activity was recorded as 0.7406 ± 0.0258 IU/mL (T1 vs Control: $p < 0.001$), which is comparable with those of a few fungi as *Chaetomium globossum*: 0.58 IU/mL (42), Penicillium sp.: 1.2 IU/mL (43) and *Talaromyces thermophilus*: 0.91 U/mL (44).

Determination of the optimum temperature for enzyme activity

The cellulase production was analysed at different temperatures ranging from 20 to 80 ℃ and the extracted crude enzyme was partially purified (using ammonium sulfate and dialysis) for the assays. The enzyme activity was measured by incubating the partially purified enzyme at different temperatures using CMC as substrate and allowing the reaction to proceed. Maximum enzymatic activity was measured at 50 ℃ (Fig. 7). The results were consistent with recent research on cellulolytic endophytes. Previous researchers reported that *Penicillium sp*. exhibited the maximum crude enzymatic activity at 40-50 ℃ (52). The highest enzymatic activity at 50 ℃ for *F. oxysporum* isolated from soil was reported earlier (53). A new strain of *Trichoderma reesei* was obtained by subjecting *T. reesei* spores to UV treatment, which has improved the tolerance towards the end-products of enzymatic cellulose digestion, with an optimum temperature of 60 ℃ (54). The short-term temperature optima for *A. niger* for cellulase production as 46.5 ℃ was studied earlier (55). Temperature is a crucial factor in the growth of an organism, where higher temperatures compared to the optimal temperature facilitate the denaturing of proteins and therefore reduce activity. Conversely, lower temperatures affect enzyme activity due to reduced substrate-enzyme interaction efficiency (56).

Determination of the optimum pH for enzyme activity

pH is an important factor that determines the optimum activity of enzymes produced by stabilising the enzyme's three-dimensional structure and ensuring the active site binds to the substrate. Altered or high pH ranges distort the binding site and also denature the protein structure. Assessment of optimum pH was done using three buffers (citrate buffer, pH3-6.2, phosphate buffer, pH 5.8-7.8 and glycine buffer, pH 8.6-10) for covering the pH range from 3.0 to 10. Maximum enzyme activity (1.98 ± 0.05 IU/mL) was achieved at pH 6.5. Enzyme production peaked in a slightly acidic to neutral pH range of 6.0-6.5 (Fig. 8). Low activity was observed at acidic pH (3.0-5.0) and a significant decline in activity was observed at alkaline pH (7.0-10.0). Similar optimal pH changes (6.0-7.0) have been reported for cellulase production by various fungi including *Aspergillus sp*. (56), Ectomycorrhizal fungal species like 'ammonia fungi' (57), *Aspergillus niger* and *Trichoderma sp*. (58). Previous reports indicate that an exponential increment in saccharification yielded up to 26.2 % when the optimal pH was maintained throughout the production process (59).

Relevance of endophytic microbes in agriculture

Microbial endophytes have been associated with many plant species for millions of years and confer multiple benefits to plants. Being one of the world's eight biodiversity hotspots, the Western Ghats is home to over 5000 endemic plant species and countless microorganisms, representing vast genetic diversity. Considerable variations in the elevations of this region create multiple climate zones, which in turn support unique ecological niches and force organisms to produce specialised enzymes for survival. These microorganisms, including fungi, bacteria and actinomycetes, are prolific producers of enzymes and represent largely untapped sources of novel biocatalysts. The majority of this region remains unexplored and is home to millions of organisms with highly unique metabolic pathways and enzymatic specificities. The flora of Western Ghat region is rich in such endophytic associations (60). Both bacterial and fungal endophytes have been found to produce enzymes and pigments useful in agriculture and industry (61-63, 34). In our earlier studies we have

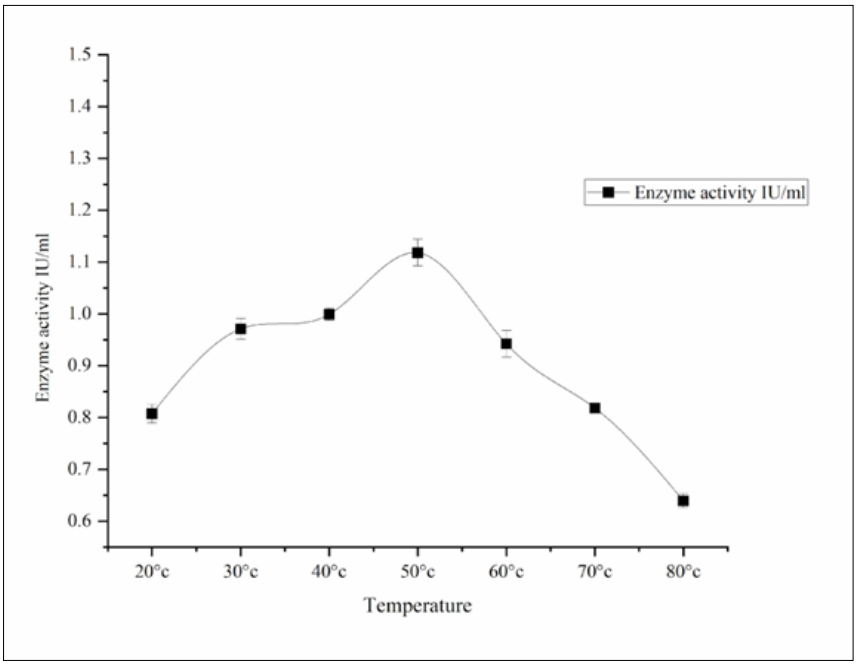

Fig. 7. Effect of temperature on the activity of partially purified cellulase enzyme from *Fusarium annulatum* (CU 07). Data points represent the mean values with standard error bars (n = 3). Maximum enzyme activity (1.12 ± 0.03 IU/mL) was observed at 50 °C, indicating the optimal temperature for the crude enzyme production.

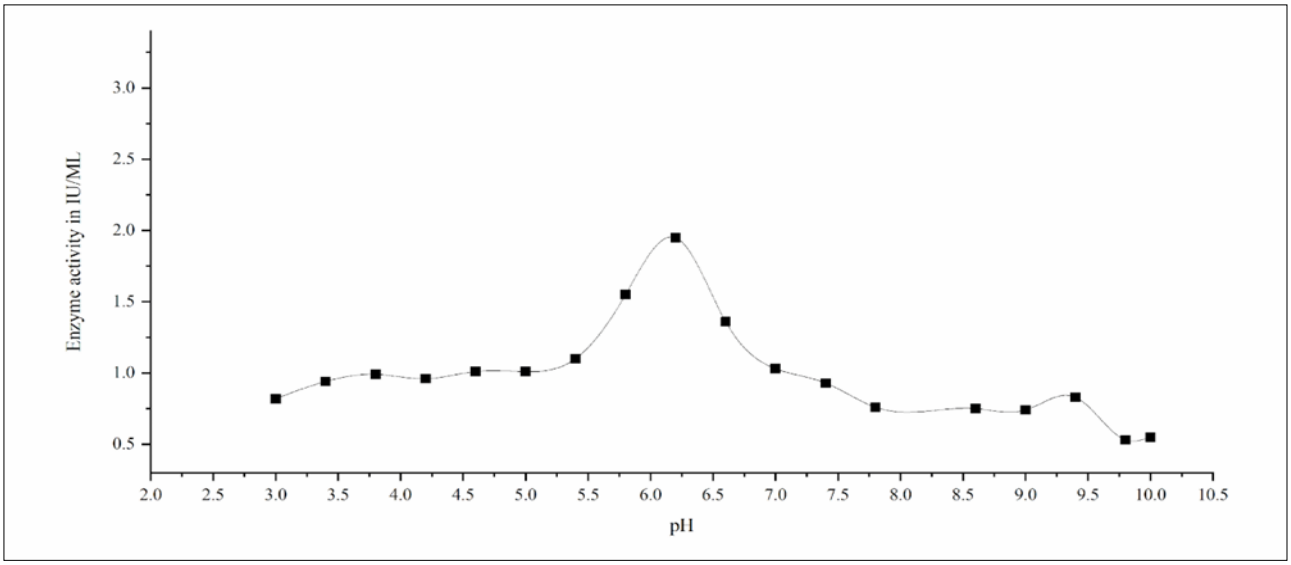

Fig. 8. Effect of pH on the activity of partially purified cellulase enzyme from *Fusarium annulatum* (CU 07) using different buffer systems.

found, a few endophytic *Fusarium sp*. providing plant growth-promoting advantages (64). The present study unveiled a cellulase producing *F. annulatum* species from *Caryota urens* after bioprospecting a few diverse plant species. Such natural sources of agriculturally and industrially important microbes that yield potentially useful enzymes align with the SDG goals.

Conclusion

This study presents one of the first reports on the cellulolytic ability of *Fusarium annulatum*, a less-studied plant endophyte in the context of industrially important enzymes. The initial methodology for sample collection, primary screening and isolation primarily focused on plant endophytes, assuming their cellulolytic potential to degrade the plant cell wall during infection and related pathogenic cycles. Among the isolated and screened endophytes, CU 07, isolated from *Caryota urens* (Jaggery palm), exhibited the highest potential on CMC agar media for cellulase degradation and ITS sequencing confirmed the organism's identity as *F. annulatum*. The enzymatic assay reports of *F. annulatum* revealed significant endoglucanase (1.2358 ± 0.0055 IU/mL), β-glucosidase (0.708 ± 0.017 IU/mL), cellobiohydrolase (0.244 ± 0.019 IU/mL) and FPA (0.7406 ± 0.0258 IU/mL) activities. The optimal pH and temperature of the partially purified enzyme were determined to be 6.0 and 50.0 °C, respectively, indicating significant potential for cost-effective cellulase enzyme production. Compared with the other established fungi, the enzymatic results for *F. annulatum* are moderate but consistent with the ecological role of endophytic fungi. *F. annulatum* is an endophytic fungus and its lifestyle primarily involves establishing a non-pathogenic relationship with its plant host. In this mutualistic/symbiotic relationship, the main function of an enzyme like cellulase is not lignocellulosic decomposition as performed by a typical non-endophytic fungi, but rather plant growth promotion, stress tolerance enhancement, pathogen protection and nutrient exchange. Comparatively, less intense cellulase gene expression is expected for these functions, in contrast to the intense cellulase gene expression in the fungus, which lives and feeds on organic matter rich in cellulose and hemicellulose. The enzyme estimation procedures used in this study mainly focused on the extracellular enzyme, but not on the total enzyme production, which includes both extracellular and intracellular. Future studies focusing on optimising the Growth medium composition (carbon/nitrogen ratio), pH levels, temperature, incubation period and inoculum concentration will definitely increase the efficiency and activity of the enzyme. Despite moderate cellulolytic activity, *F. annulatum* exhibits balanced endoglucanase, exoglucanase and β-glucosidase activities, warranting further investigation for biotechnological applications and optimisation.

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Authors' contributions

SS conceived the idea and designed the experiments. DJ conducted the experiments, analysed the data, performed the statistical analysis and drafted the manuscript. SS supervised the work and revised the manuscript. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Schmitz R, Flachsbarth F, Plaga LS, Braun M, Härtel P. Energy security and resilience: Revisiting concepts and advancing planning perspectives for transforming integrated energy systems. Energy Policy. 2025;207:114796-6. https://doi.org/10.1016/j.enpol.2025.114796
2. Smal T, Wieprow J. Energy security in the context of global energy crisis: Economic and financial conditions. Energies. 2023;16(4):1605. https://doi.org/10.3390/en16041605
3. Basera P, Chakraborty S, Sharma N. Lignocellulosic biomass: Insights into enzymatic hydrolysis, influential factors and economic viability. Discover Sustainability. 2024;5(1). https://doi.org/10.1007/s43621-024-00543-5

4. Sarojam PJ, Patel AK, Shruthy NSS, Shalu S, Dong CD, Singhania RR. Sustainability through lignin valorization: Recent innovations and applications driving industrial transformation. Bioresources and Bioprocessing. 2025;12(1). https://doi.org/10.1186/s40643-025-00929-x
5. Kumar B, Bhardwaj N, Agrawal K, Chaturvedi V, Verma P. Current perspective on production and applications of microbial cellulases: A review. Bioresources and Bioprocessing. 2021;8:95:1-41. https://doi.org/10.1186/s40643-021-00447-6
6. Zhang Z, Xing J, Li X, Lu X, Liu G, Qu Y, et al. Review of research progress on the production of cellulase from filamentous fungi. Int J Biol Macromol. 2024;277(4):134539. https://doi.org/10.1016/j.ijbiomac.2024.134539
7. Mwendwa PK, Omwenga GI, Maingi JM, Karanja AW. Evaluation of cellulase production by endophytic fungi isolated from young and mature leaves of medicinal plants using maize cob substrate. Sci Rep. 2025;15(1). https://doi.org/10.1038/s41598-025-94864-8
8. Venkatesagowda B, Ponugupaty E, Barbosa AM, Robert. Diversity of plant oil seed-associated fungi isolated from seven oil-bearing seeds and their potential for the production of lipolytic enzymes. World J Microbiol Biotechnol. 2011;28(1):71-80. https://doi.org/10.1007/s11274-011-0793-4
9. Prajapati C, Rai SN, Singh AK, Chopade BA, Singh Y, Singh SK, et al. An update of fungal endophyte diversity and strategies for augmenting therapeutic potential of their potent metabolites. Appl Biochem Biotechnol. 2025. https://doi.org/10.1007/s12010-024-05098-9
10. Bhadra F, Gupta A, Vasundhara M, Reddy MS. Endophytic fungi: A potential source of industrial enzyme producers. 3 Biotech. 2022;12(4). https://doi.org/10.1007/s13205-022-03145-y
11. Nazir A, Puthuveettil AR, Hasnain F, Hamed KE, Munawar N. Endophytic fungi: Nature's solution for antimicrobial resistance and sustainable agriculture. Front Microbiol. 2024;15. https://doi.org/10.3389/fmicb.2024.1461504
12. Xia Y, Liu J, Chen C, Mo X, Tan Q, He Y, et al. The multifunctions and future prospects of endophytes and their metabolites in plant disease management. Microorganisms. 2022;10(5):1072. https://doi.org/10.3390/microorganisms10051072
13. Ali MA, Ahmed T, Ibrahim E, Rizwan M, Chong KP, Wan J. A review on mechanisms and prospects of endophytic bacteria in biocontrol of plant pathogenic fungi and their plant growth-promoting activities. Heliyon. 2024;10(11):e31573-3. https://doi.org/10.1016/j.heliyon.2024.e31573
14. Wippel K. Plant and microbial features governing an endophytic lifestyle. Curr Opin Plant Biol. 2023;76:102483. https://doi.org/10.1016/j.pbi.2023.102483
15. Nazir M, Iram A, Cekmecelioglu D, Demirci A. Approaches for producing fungal cellulases through submerged fermentation. Front Biosci Elite. 2024;16(1):5. https://doi.org/10.31083/j.fbe1601005
16. Hawar SN. Extracellular enzyme of endophytic fungi isolated from *Ziziphus spina* leaves as medicinal plant. Int J Biomater. 2022;1-9. https://doi.org/10.1155/2022/2135927
17. Raghav D, Jyoti A, Siddiqui AJ, Saxena J. Plant-associated endophytic fungi as potential bio-factories for extracellular enzymes: Progress, challenges and strain improvement with precision approaches. J Appl Microbiol. 2022. https://doi.org/10.1111/jam.15574
18. Sopalun K, Iamtham S. Isolation and screening of extracellular enzymatic activity of endophytic fungi isolated from Thai orchids. S Afr J Bot. 2020. https://doi.org/10.1016/j.sajb.2020.02.005
19. Wang X, Pecoraro L. Analysis of soil fungal and bacterial communities in Tianchi Volcano Crater, Northeast China. Life (Basel). 2021;11(4):280. https://doi.org/10.3390/life11040280
20. Basha MA, Shetaia YM, Mehaya FM, Abdelzaher FH. Solid-state fermentation and optimization of cellulase production using local fungal isolate. Egypt Pharm J. 2023;22(3):456-65. https://doi.org/10.4103/epj.epj_30_23
21. Guder DG, Krishna MSR. Isolation and characterization of potential cellulose degrading bacteria from sheep rumen. J Pure Appl Microbiol. 2019;13(3):1831-9. https://doi.org/10.22207/JPAM.13.3.60
22. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols. 1990:315-22. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
23. Díaz GV, Coniglio RO, Chungara CI, Zapata PD, Villalba LL, Fonseca MI. *Aspergillus niger* LBM 134 isolated from rotten wood and its potential cellulolytic ability. Mycology. 2020:1-14. https://doi.org/10.1080/21501203.2020.1823509
24. Zhao S, Wang JX, Hou R, Ning YN, Chen ZX, Liu Q, et al. Novel transcription factor CXRD regulates cellulase and xylanase biosynthesis in *Penicillium oxalicum* under solid-state fermentation. Appl Environ Microbiol. 2023;89(6):e00360-23. https://doi.org/10.1128/aem.00360-23
25. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31(3):426-8. https://doi.org/10.1021/ac60147a030
26. Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem. 1944;153(2):375-80. https://doi.org/10.1016/S0021-9258(18)71980-7
27. Sharrock KR. Cellulase assay methods: A review. J Biochem Biophys Methods. 1988;17(2):81-105. https://doi.org/10.1016/0165-022X(88)90040-1
28. Santa-Rosa PS, Souza AL, Andrade EVR, Astolfi-Filho S, Mota AJ, et al. Production of thermostable β-glucosidase and CMCase by *Penicillium* sp. LMI01 isolated from the Amazon region. Electron J Biotechnol. 2018;31:84-92. https://doi.org/10.1016/j.ejbt.2017.11.005
29. Ghose TK. Measurement of cellulase activities. Pure Appl Chem. 1987;59(2):257-68. https://doi.org/10.1351/pac198759020257
30. Sulyman AO, Igunnu A, Malomo SO. Isolation, purification and characterization of cellulase produced by *Aspergillus niger* cultured on *Arachis hypogaea* shells. Heliyon. 2020;6(12):e05668. https://doi.org/10.1016/j.heliyon.2020.e05668
31. Zverlov VV, Volkov IY, Velikodvorskaya TV, Schwarz WH. Highly thermostable endo-1,3-β-glucanase LamA from *Thermotoga neapolitana*: Nucleotide sequence of the gene and characterization of the recombinant gene product. Microbiology. 1997;143(5):1701-8. https://doi.org/10.1099/00221287-143-5-1701
32. Arif S, M'Barek HN, Bekaert B, Aziz MB, Diouri M, Haesaert G, et al. Lignocellulolytic enzymes production by four wild filamentous fungi for olive stones valorization. J Microbiol Biotechnol. 2024;34(5):1017-28. https://doi.org/10.4014/jmb.2312.12048
33. Azzaz HH, Murad HA, Aboamer AA, Alzahar H, Fahmy M. Cellulase production by *Fusarium graminearum* and its application in ruminant diets degradation. Pak J Biol Sci. 2019;23(1):27-34. https://doi.org/10.3923/pjbs.2020.27.34
34. Poornamath BP, Sarojini S, Jayaram S, Biswas S, Kaloor A, Umesh M. Solid-state fermentation of pigment producing endophytic fungus *Fusarium solani* from Madiwala Lake and its toxicity studies. J Appl Biol Biotechnol. 2024;12(2):264-72. https://doi.org/10.7324/JABB.2024.143030
35. Najjarzadeh N, Matsakas L, Rova U, Christakopoulos P. Effect of carbon source and degree of oligosaccharide polymerization on production of cellulase-degrading enzymes by *Fusarium oxysporum* f. sp. *lycopersici*. Front Microbiol. 2021;12:652655. https://doi.org/10.3389/fmicb.2021.652655
36. Rahnama M, Condon B, Ascari JP, Dupuis JR, Del Ponte EM, Pedley KF, et al. Recent co-evolution of two pandemic plant diseases in a multi-hybrid swarm. Nat Ecol Evol. 2023;7(12):2055-66. https://doi.org/10.1038/s41559-023-02237-z
37. Zaffarano PL, McDonald BA, Linde CC. Rapid speciation following recent host shifts in the plant pathogenic fungus *Rhynchosporium*.

Evolution. 2008;62(6):1418-36. https://doi.org/10.1111/j.1558-5646.2008.00390.x

38. Breuil C, Saddler JN. Comparison of the 3,5-dinitrosalicylic acid and Nelson-Somogyi methods of assaying for reducing sugars and determining cellulase activity. Enzyme Microb Technol. 1985;7 (7):327-32. https://doi.org/10.1016/0141-0229(85)90111-5

39. Gusakov AV, Kondratyeva EG, Sinitsyn AP. Comparison of two methods for assaying reducing sugars in the determination of carbohydrase activities. Int J Anal Chem. 2011;2011:283658. https://doi.org/10.1155/2011/283658

40. Ja'afaru MI. Screening of fungi isolated from environmental samples for xylanase and cellulase production. ISRN Microbiol. 2013;2013:283423. https://doi.org/10.1155/2013/283423

41. Pirzadah T, Garg S, Singh J, Vyas A, Kumar M, Gaur N, et al. Characterization of actinomycetes and *Trichoderma* spp. for cellulase production utilizing crude substrates. SpringerPlus. 2014;3 (1):622. https://doi.org/10.1186/2193-1801-3-622

42. Munyasi KM, Omwenga GI, Mwamburi FM. Production and characterization of cellulolytic enzymes by *Chaetomium globosum* for biomass saccharification and ethanol production. Discovery. 2024;60 (334):1-21. https://doi.org/10.54905/disssi.v60i334.e14d1425

43. Syed S, Riyaz-Ul-Hassan S, Johri S. A novel cellulase from an endophyte, *Penicillium* sp. NFCCI 2862. Am J Microbiol Res. 2013;1 (4):84-91. https://doi.org/10.12691/ajmr-1-4-4

44. Abdullah R, Tahseen M, Nisar K, Kaleem A, Iqtedar M, Saleem F, et al. Statistical optimization of cellulases by *Talaromyces thermophilus* utilizing *Saccharum spontaneum*. Electron J Biotechnol. 2021;51:79-87. https://doi.org/10.1016/j.ejbt.2021.03.007

45. Kwon HW, Yoon JH, Kim SH, Hong SB, Cheon YA, Ko SJ. Detection of extracellular enzyme activities in various *Fusarium* spp. Mycobiology. 2007;35(3):162-5. https://doi.org/10.4489/MYCO.2007.35.3.162

46. Gao Z, Van Hop D, Yen LTH, Ando K, Hiyamuta S, Kondo R. Production of β-glucosidases by *Fusarium proliferatum* NBRC109045 isolated from Vietnamese forest. AMB Express. 2012;2 (1):49. https://doi.org/10.1186/2191-0855-2-49

47. Saroj P, Manasa P, Narasimhulu K. Characterization of thermophilic fungi producing extracellular lignocellulolytic enzymes under solid-state fermentation. Bioresour Bioprocess. 2018;5(1):31. https://doi.org/10.1186/s40643-018-0216-6

48. Filho EXF. Purification and characterization of a β-glucosidase from solid-state cultures of *Humicola grisea* var. *thermoidea*. Can J Microbiol. 1996;42(1):1-5. https://doi.org/10.1139/m96-001

49. Sørensen A, Lübeck PS, Lübeck M, Teller PJ, Ahring BK. β-Glucosidases from a new *Aspergillus* species can substitute commercial β-glucosidases for saccharification of lignocellulosic biomass. Can J Microbiol. 2011;57(8):638-50. https://doi.org/10.1139/w11-052

50. Tiwari P, Misra BN, Sangwan NS. β-Glucosidases from the fungus *Trichoderma*: An efficient cellulase machinery in biotechnological applications. BioMed Res Int. 2013;2013:203735. https://doi.org/10.1155/2013/203735

51. Cabezas L, Calderon C, Medina LM, Bahamon I, Cardenas M, Bernal AJ, et al. Characterization of cellulases of fungal endophytes isolated from *Espeletia* spp. J Microbiol. 2012;50(6):1009-13. https://doi.org/10.1007/s12275-012-2130-5

52. Toghueo RMK, Ejiya IE, Sahal D, Yazdani SS, Boyom FF. Production of cellulolytic enzymes by endophytic fungi isolated from Cameroonian medicinal plants. Int J Curr Microbiol Appl Sci. 2017;6 (2):1264-71. https://doi.org/10.20546/ijcmas.2017.602.142

53. Dutta SD, Tarafder M, Islam R, Datta B. Characterization of cellulolytic enzymes of *Fusarium* soil isolates. Biocatal Agric Biotechnol. 2018;14:279-85. https://doi.org/10.1016/j.bcab.2018.03.011

54. Silva JCR, Salgado JCS, Vici AC, Ward RJ, Polizeli MLTM, Guimarães LHS, et al. A novel *Trichoderma reesei* mutant RP698 with enhanced cellulase production. Braz J Microbiol. 2020;51(2):537-45. https://doi.org/10.1007/s42770-019-00167-2

55. Kabir MF, Ju LK. On optimization of enzymatic processes: Temperature effects on activity and long-term deactivation kinetics. Process Biochem. 2023;130:734-46. https://doi.org/10.1016/j.procbio.2023.05.031

56. Boondaeng A, Keabpimai J, Trakunjae C, Vaithanomsat P, Srichola P, Niyomvong N. Cellulase production under solid-state fermentation by *Aspergillus* sp. IN5. Heliyon. 2024;10(5):e26601. https://doi.org/10.1016/j.heliyon.2024.e26601

57. Yamanaka T. Effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi *in vitro*. Mycologia. 2003;95(4):584-9. https://doi.org/10.1080/15572536.2004.11833062

58. Gautam SP, Bundela PS, Pandey AK, Khan J, Awasthi MK, Sarsaiya S. Optimization for the production of cellulase enzyme from municipal solid waste residue by two novel cellulolytic fungi. Biotechnol Res Int. 2011;2011:810425. https://doi.org/10.4061/2011/810425

59. Li C, Yang Z, Zhang R, Zhang D, Chen S, Ma L. Effect of pH on cellulase production and morphology of *Trichoderma reesei* and application in cellulosic material hydrolysis. J Biotechnol. 2013;168 (4):470-7. https://doi.org/10.1016/j.jbiotec.2013.10.003

60. Mahalakshmi V, Venkatesan G. Characterization of Endophytic Fungi Associated with Pteridophytes from the Western Ghats. Int J Biosci. 2024;25(4):320-9.

61. Philip I, Sarojini S, Biswas S, Jayaram S. Plant growth promotion properties of bacterial endophytes isolated from *Alternanthera philoxeroides* and *Eichhornia crassipes*. S Afr J Bot. 2023;162:588-603. https://doi.org/10.1016/j.sajb.2023.09.028

62. Biswas S, Philip I, Jayaram S, Sarojini S. Endophytic bacteria *Klebsiella* spp. and *Bacillus* spp. from *Alternanthera philoxeroides* exhibit additive plant growth-promoting and biocontrol activities. J Genet Eng Biotechnol. 2023;21(1):153. https://doi.org/10.1186/s43141-023-00620-8

63. Jayaram S, Biswas S, Philip I, Umesh M, Sarojini S. Differential laccase production among diverse fungal endophytes in aquatic plants of Hulimavu Lake, Bangalore, India. J Pure Appl Microbiol. 2023;17(1):298-308. https://doi.org/10.22207/JPAM.17.1.19

64. Biswas S, Sarojini S. Fungal endophytic species *Fusarium annulatum* and *Fusarium solani*: Identification, molecular characterization and plant growth promotion properties. Plant Sci Today. 2024;11(1):466-72. https://doi.org/10.14719/pst.2688

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