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# Extracellular enzyme activities of endophytic fungus associated with Costus speciosus

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**Abstract**
*Costus speciosus* (Koen ex. Retz.) related to family *Costaceae* and believed to originated natively from the southeast Asia, distributed to India and Sri lanka. Endophytic fungi are microorganisms that have established oneself inside the higher plant asymptomatically and these are promising source of biologically active metabolites. These metabolites have been used from ancient time by human in various field like agriculture, as remedy for various ailments. The multiple beneficiary action which has been demonstrated as anticancer, antimicrobial, anti-helminthic, anti-inflammatory, cytotoxic are the result of assistance of these fungal endophytes The isolation was done with freshly prepared PDA media by inoculating healthy tissue of leaf and stem of *Costus*, inoculation done after surface sterilization by sodium hypochlorite (5%). Some of the fungal genera found as endophytes belongs to *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Colletotrichum*, *Phoma*, *Curvularia*commonly found in nature. This study included the extracellular enzyme activities such as amylase, pectinase, cellulase, L-asparaginase on freshly isolated fungal isolates.

**Keywords**: Endophytic fungus, fungal endophytes, *Costus speciosus,* Extracellular enzyme activity

## Introduction

Endophytic fungus inhabits the healthy tissue of higher plants asymptomatically and these are of growing interest as help to produce biologically active agents. Endophytic fungi are the microorganisms which are producers of most innovative groups of secondary metabolites that play vital biological roles in human life. It also considers the benefit and their medicinal applications especially in the production of antiviral, antioxidant, anticancer, antimicrobial, antioxidant, compounds (Selima *et al.*, 2012). Endophytic fungi have symbiotic relationships with all higher plants naturally. These endophytes have an intense impact on plants not only in production of some valuable secondary metabolites but also to aid at biotic and abiotic stress conditions, biomass production, and to overcome competition. The phylogenetic characters and life history are important criteria according to which endophytes have been described as clavicipitaceous and non clavicipitaceous (Rodriguez *et al.*, 2009).

Endophytes also augment the ecological and economical value of plants via symbiotic interaction. Endophytes have a worthwhile impact by enriching the plant growth, development and induced systemic resistance. Structure and composition of fungal endophytes communities are influenced by many factors and complex interactions (You *et al*., 2017). *Ophiopogonin japonicas* used as traditional medicine in china for the treatment of cough, diabetes mellitus, to eliminate phlegm and in case of latent heat in lungs apart from that the fungal endophytes help to produce biologically active compound with prominent antibacterial activity. Endophytic fungi have been recognized as an abundant reservoir supporting novel compounds with significant biological activities. (Hanqio Liang *et al.*, 2012).

Endophytes are microorganisms colonizing healthy plant tissue without causing any apparent symptoms and noticeable injury to the host.Both fungi and bacteria are the most common microbes existing as endophytes.Endophyteshave the

ability to produce interesting and novel classes of secondary metabolites which have been used for various purposes by humans.Several decades of research and numerous articles on endophytic fungi in plants have resulted in a surfeit of knowledge of the group (Padhi *et al*., 2013). Isolation of plumbagin (5 hydroxy-2- methylnapyhalene-1, 4-dione) obtained from endophytic fungi *Cladosporium delicatum*from endemic medicinal plants viz., *Pterocarpus santalinus, Rhynchosiabeddomei, Terminalia paliida*, which shows antimicrobial activity (Venkatneswarulu*et al*., 2018).

Phytochemical investigation of endophytic fungi *Phoma*sp. recovered from *Senecio kleinii* resulted in isolation of sclerodin (antifungal activity), atrovenetinone and sclerodione both showed antifungal activity towards *Ustilagoviolacea* (Hussain *et al*., 2015). Two napthaloquinones, anhydrofusarubin andmethyl ether of fusarubin isolated from *Cladosporaium* sp.associated with *Rauwolfia seprntina.* These compounds were assayed against human leukaemia cells which showed potential cytotoxicity (Khan *et al*., 2016).

The genus *Penicillium* is ubiquitous and its species are commonly recovered from every kind of substrate and environmental condition. This fungal genus was isolated from Coffea arabica and Theobroma cacoa (Nicoletti et al., 2014) and its role was evaluated for the production of bioactive metabolites ). Uzma *et al*., (2016) described a total of 112 endophytic fungi belonging to 26 genera and were isolated from six wild medicinal plants belonging to Bisle region, western ghat of Karnataka among which *Hedychium flavescens* and *Hedychium coronarium* are listed as endangered plants species. The fungal isolates from these plants were screened for production of extracellular enzyme activity like amylase, cellulase, pectinase, asparaginase.

**Material and method**

Sample of C*ostus speciosus* is collected and cultivated for the purpose of this study from Dongargarh area of Chhattisgarh. To complete the objective of isolation of endophytic fungus, the fresh samples of leaves and stem were collected, washed withdistilled water for 3- 5 times then washed with 70% ethyl alcohol and surface sterilized with 5% sodium hypochlorite (NaOCl) solution to avoid contamination from other source. Following the sterilization step, the plant parts were dried on sterilized filter paper. After completing the sterilization process samples of leaves and stem were inoculated on a petri plate of 90mm containing PDA media, plates were placed for the period of 4-5 days at 26 ºC temperature by standard method (Uzma *et al*., 2016). After incubation,the colonies were observed on a regular basis, separate plates were used to prepare pure culture of the endophytic fungus. Identification of endophytic fungal isolates were performed on the basis of morphology with the help of standard identification manuals ( mention the name of manual used).

**Discernment of extracellular enzyme activity**

The fungal endophytes can be screened with the help of qualitative techniques in large numbers relatively quickly. Screening for extracellular enzymes depends upon growth of endophytes on different solid media as reported by Hankin and Ananostakis, 1975 andSunitha *et al.*, 2013. Evaluation of extracellular enzyme activity of fungal endophytes was done by growing these endophytes on solid PDA media and fresh colonial plugs of 5mm of pure culture were placed on different media having dissolved substrate. After 3-5 days of incubation the enzyme activity zone was measured.

**Amylolytic activity**: The glucose yeast extract peptone agar media (GYP), having a composition of - glucose-1gm, Yeast extract-0.1gm, peptone-0.5gm, agar-16gm, soluble starch 0.2% in one litre of distilled water at pH 6 was used for the detection of amylase production. Following the post incubation period, the plates were flooded with 1% iodine. . The production of amylase degrades the starch, which results in formation of clear zones, while the dark blue colour represents the absence of enzyme amylase.

**Pectinolytic activity**: for the detection of pectinolytic activity, the fungal endophytes were grown on pectin agar media (PAM) with concentration of pectin- 5gm, yeast extract-1gm, agar-15gm for one litre of volume at PH 5. After, post incubation period, the plates were flooded with 1% aqueous solution of hexadecyl trimethyl ammonium bromide (C-TAB). The clear zone formed around the fungal colony indicated the of degradation of pectin by pectinolytic enzyme.

**Cellulase activity**: For the screening of cellulase activity, the fungal isolates were cultured on glucose yeast extract

peptone agar (YPA)medium having 0.5% carboxy methyl cellulose.After incubation,plates were flooded with 0.2% solution of Congo red dye and destained with 1M NaCl for 15 minutes. The yellow area emerged around the fungal colony confirms the presence of cellulase activity.

**L-asparaginase activity:** Modified Czapekdox's media with constituent of glucose-2.0gm, L -asparagine-10gm, KH2PO4 1.52gm, KCL-0.52gm, MgSO4.7H2O- in trace amount, CuNO3.3 H2O- in trace amount, Zn MgSO4.7 H2O- in trace amount, agar-20 gm per litre of volume along with 0.3ml of 2.5% phenol red dye solution (0.2gm phenol red, ethyl alcohol 95% 500ml, distilled water 500ml for 1 litre of volume) was employed for the screening of fungal endophyte.

## RESULTS AND DISCUSSION

Endophytic fungi were isolated from plant *Costus speciosus*. A total of 9 strains of endophytic fungal isolates were recultured, screened and were tested for extracellular enzymatic activity viz., amylase, pectinase, cellulase and L-asparaginase (Table 1 ). After 3-5 days of incubation, it was observed that none of the strains exhibited positive results for all four enzymes, simultaneously. Maximum strains showed positive results for amylase (66.66%) followed by cellulase (55.55%) and pectinase (44.44%) producing ability. However, none of the isolates were found positive for L-asparagine activity. Among the group of *fusarium* sp.(CSD 1) showed the highest amylolytic activity whereas *Aspergillus* sp. (CLD 4) showed the minimum degradation of amylase followed by some *Aspergillus* sp. and *Pacilomyces* sp. The *fusarium* sp.(CSD1) was again found to be the most potent strain for the production of pectinase enzyme as compared to the other strains. Fungal strains belonging to genus *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. and *Pacilomyces* sp. were also found negative for this enzyme activity. Similarly, the cellulolytic activity was observed maximum in *penicillin* sp. (CSD2) as compared to other positive strains, while some strains belonging to *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. and *Pacilomyces* sp. showed the negative results. The similar results were shown by (Uzma *et al*., 2016).

The statistical analysis of the data revealed that there is a significant difference between the amylase producing isolates ($P<0.001$) as most of the strain produces amylase among which *Fusarium* sp. (CLD1) showed the maximum degradation of starch followed by CLD3, CLD1, CLD2, CLD11, and CLD7 showed no degradation. (Graph 1)

The statistical analysis of the data revealed that there is a significant difference between the pectinase producing isolates ($P<0.001$) as some of the strain produces pectinase among which *Fusarium* sp. (CSD1) showed the maximum degradation of starch followed by CLD4, CLD3, ,CLD2, whereas CLD1, CLD7, CLD11, and CSD2, CSD3 showed no degradation. (Graph 2)

The statistical analysis of the data revealed that there is a significant difference between the Cellulase producing isolates ($P<0.001$) as some of the strain produces Cellulase among which *Penicillium* sp. (CSD2) showed the maximum degradation of starch followed by CLD4, CSD3,CLD1, CSD1 whereas CLD2, CLD3, CLD7, CLD11 showed no degradation. (Graph 3) Statistical analysis was done by SPSS version 2.0.

## CONCLUSION

*Fusarium* sp. exhibited maximum enzymatic activity for amylase and pectinase whereas *Penicillium* sp. demonstrated maximum enzymatic activity for cellulase. None of the isolates were found to have L-asparaginase activity. The present investigation showed that the production of enzymes may vary from one species to another or even differences also occur between the strains of similar species.The possible reason for the variation in enzyme production efficiency of different fungal endophytes could be the environmental conditions on which they grew. By the knowledge of these extracellular enzyme producing fungal strains we may use them for industrial purposes where large amounts of enzymes are needed.

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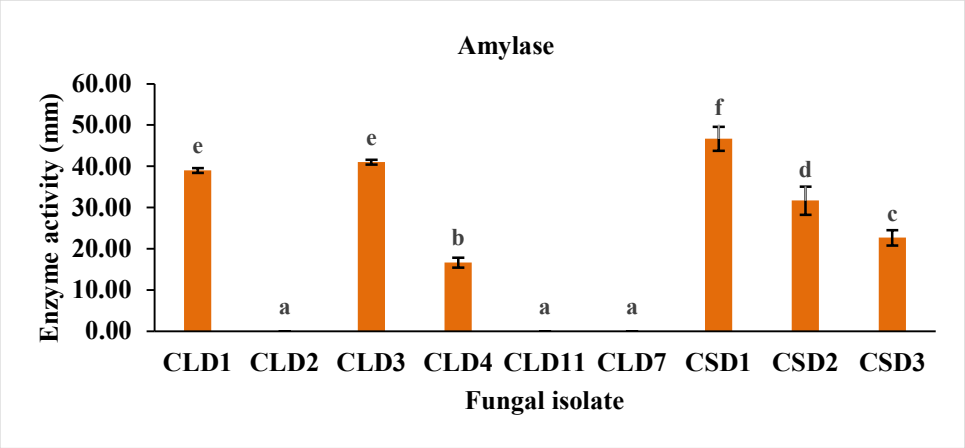
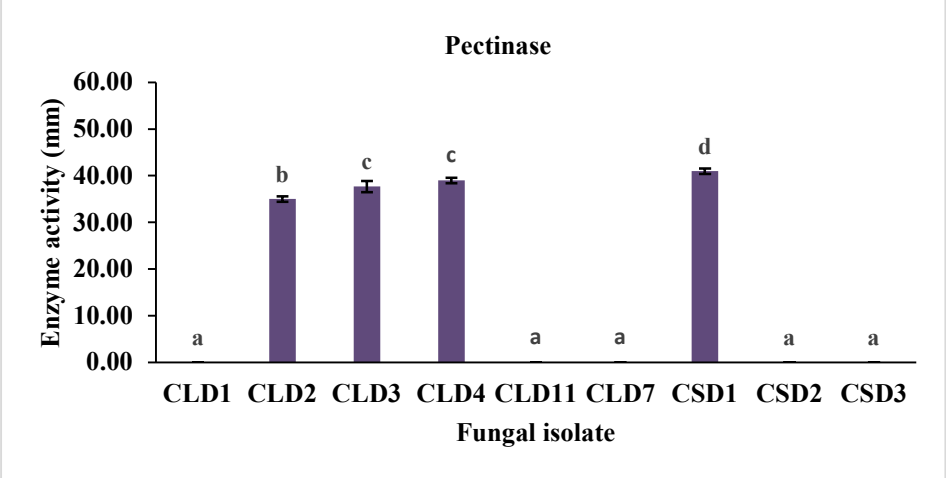
**Table 1-Endophytic fungal strains isolated s exhibiting varied enzymatic activity**

| | | **Mean ± SE** | | | |
|---|---|---|---|---|---|
| | | **Enzymatic activity** | | | |
| **Code** | **Fungal sp.** | **Amylase** | **Pectinase** | **Cellulase** | **L-asparaginase** |
| CLD1 | *Fusarium oxysporum* | 39.00±0.58$^{e}$ | 0.00$^{a}$ | 39.67±0.33$^{c}$ | 0.00$^{a}$ |
| CLD2 | *Aspergillus flavus* | 0.00$^{a}$ | 35.00±0.58$^{b}$ | 0.00$^{a}$ | 0.00$^{a}$ |
| CLD3 | *Penicillum sp.* | 41.00±0.58$^{e}$ | 37.67±1.20$^{c}$ | 0.00$^{a}$ | 0.00$^{a}$ |
| CLD4 | *Aspergillus sp.* | 16.67±1.20$^{b}$ | 39.00±0.58$^{c}$ | 48.33±0.88$^{e}$ | 0.00$^{a}$ |
| CLD11 | *Pacilomyces javanicus* | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ |
| CLD7 | *Aspergillus* | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ |

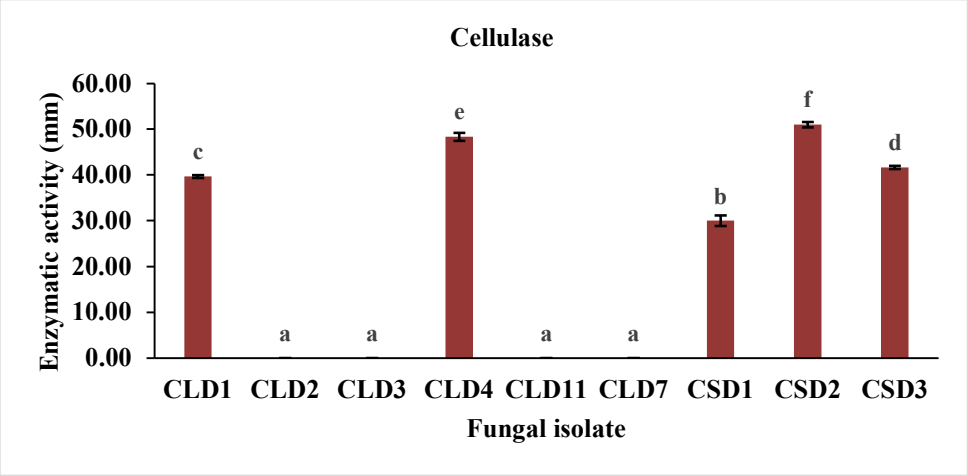
| | | | | | |
|---|---|---|---|---|---|
| | *fumigatus* | | | | |
| CSD1 | *Fusarium oxysporum* | 46.67±2.91$^{f}$ | 41.00±0.58$^{d}$ | 30.00±1.15$^{b}$ | 0.00$^{a}$ |
| CSD2 | *Penicillium sp.* | 31.67±3.38$^{d}$ | 0.00$^{a}$ | 51.00±0.58$^{f}$ | 0.00$^{a}$ |
| CSD3 | *Penicillium sp.* | 22.67±1.86$^{c}$ | 0.00$^{a}$ | 41.67±0.33$^{d}$ | 0.00$^{a}$ |



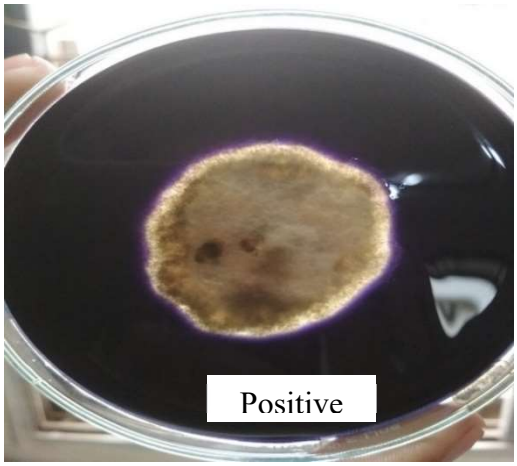

Graph 1A

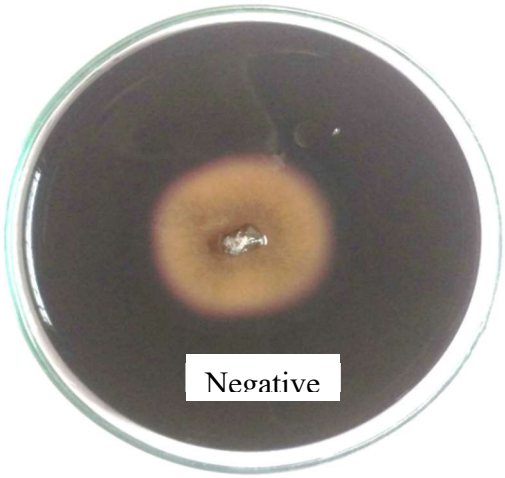



Graph 1B

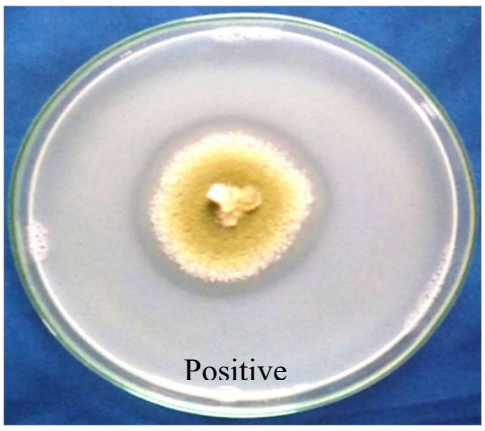


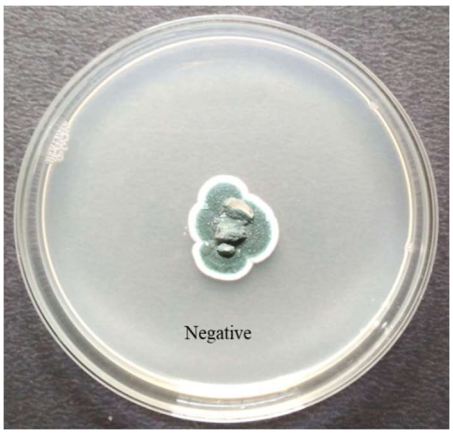

Graph 1C



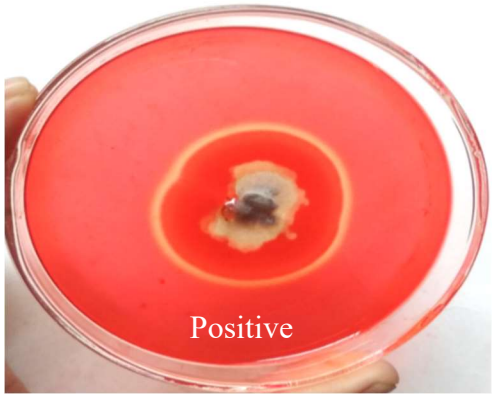



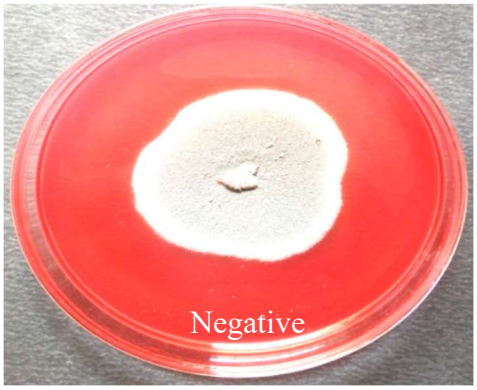

**Amylolytic activity**



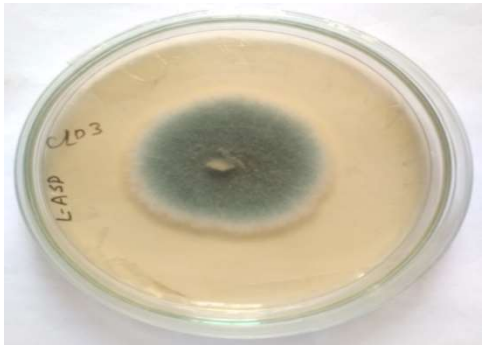




**Pectinolytic activity**




**Cellulolytic activity**




**L-Asparagine activity**