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Isolation and Diversity of Endophytic Fungus Associated with Costus Speciosus

Wasim Akram* & Shriram Kunjam**
*Research Scholar, Department of Botany, Govt. V.Y.T. PG Autonomous College, Durg (Chhattisgarh)
**Assistant Professor, Botany, Department of Botany, Govt. V.Y.T. PG Autonomous College, Durg (Chhattisgarh)

Corresponding Author: **Wasim Akram**

Abstract: Endophytic fungi, which reside asymptomatically within plant tissues, play a crucial role in the ecological fitness and medicinal properties of their host plants. This study investigates the isolation and diversity of endophytic fungi associated with Costusspeciosus, a medicinal plant known for its wide range of pharmacological uses. Plant samples, including leaves and stems, were collected and subjected to surface sterilization, followed by fungal isolation on Potato Dextrose Agar (PDA). Morphological characterization was employed for fungal identification. A total of 09 fungal species were isolated, representing diverse genera such as Aspergillus, Penicillium, and Fusarium, among others. Diversity indices, including Shannon-Wiener and Simpson's indices, revealed a high fungal diversity within the host tissues, with a dominance of (specific endophytic taxa) in different plant parts. Phylogenetic analysis confirmed the distinct clustering of isolates, suggesting varying ecological roles. The results indicate that these endophytic fungi may contribute to the bioactive properties of Costus speciosus, potentially enhancing its medicinal value. Further research is needed to explore the bioactive compounds produced by these fungi and their potential applications in pharmaceuticals.

Keywords: Endophytic fungi, Costus speciosus, fungal diversity, bioactive compounds, medicinal plants

Introduction

Endophytic fungi are microorganisms that live inside plant tissues without causing immediate harm. They can promote plant growth, improve stress tolerance, and help in

pathogen resistance. Endophytic fungi that are residing asymptomatically in internal tissues of all higher plants are of growing interest as promising sources of biologically active agents. Endophytic fungi are one of the most creative groups of secondary metabolite producers that play important biological roles for human life.It also considers and their medicinal applications especially in the production of anticancer, antimicrobial, antioxidant, and antiviral compounds. (Selima et al, 2012). Two types of endophytic group's clavicipitaceous and non clavicipiatceous have been discriminated based on phylogeny and life history traits(Rodriguez et al., 2009).Endophytic microorganisms, such as the fungi inhabiting the inner tissues and/or organs of plants, are regarded as valuable ecological and agricultural resourcesgenerally;endophytic fungal species have a positive impact on their host by enhancing plant growth or inducing systemic resistance. Structure and composition of fungal endophytes communities are influenced by many factors and complex interactions (You et al., 2017).

Costusspeciosus (commonly known as crepe ginger) is a medicinal plant with several bioactive compounds. It is used in traditional medicine for various ailments such as diabetes, respiratory issues, and skin diseases. The association of endophytic fungi with this plant might influence its medicinal properties. Costusspeciosus a perennial herb is native to South East Asia, especially found in India, Srilanka, Indonesia and Malaysia. The name "Pramehaghna" described in Ayurveda and traditionally used by local people in different parts of Assam, Darjeeling, and Kerala for the treatment of diabetes. Various experimental studies around the world have explained its antidiabetic properties (Choudhury& Sharma., 2016). Costus is widely used for medicinal purpose and due to its extensive use in medicine and ill effective conventional method of propagation; it becomes one of the rare, threatened plants which are on danger of extinction. Therefore there is need to conserve genetic pool of this plant species (Pawar &Pawar, 2014).

The Dongargarh region in India, known for its rich biodiversity, offers a unique environment that influences the diversity of endophytic fungi. Dongargarh is situated in Rajnandgaon District, Chhattisgarh. Dongargarh with 21.1855951 Latitude and 80.7600562 Longitude.The specific ecological conditions of Dongargarh provide an exceptional opportunity to study the interaction between these fungi and their host plants. Despite the medicinal significance ofCostusspeciosus, there is limited research on the diversity and enzymatic potential of their endophytic fungi in this region.

Objective: To isolate, identify, and assess the diversity of endophytic fungi from Costusspeciosus and understand their role in the plant's ecology and medicinal properties.

Material and Methods

I. Sample Collection:

a. Collection of the Plant Material:

Costus speciosus was selected and authenticated on the basis of botanical characteristics. They are found growing throughout tropical India. Leaves and stems of healthy plants were collected from hills of Dongargarh, Rajnandgaon district of Chhattisgarhwith the help of sterile scalpel and collected in sterile polybags and stored at 4° C, till start of isolation.

b. Sampling Area and Collection Period:

The Healthy plant material was collected randomly from the hills of Dongargarh during December 2021. Sampling was done for Costus speciosus in triplicate for each plant parts (stem and leaves). Each sample was used within 48hrs from collection. Finally, 5 samples from each plant were analysed for isolation of endophytic fungi.

II. Isolation of Endophytic Fungi:

a. **Surface Sterilization:** All the collected samples were removed aseptically and washed with proper care in running tap water followed by double distilled water before processing for isolation. To eliminate epiphytic microorganisms sample were initially surface sterilized by following method proposed by Petrini et al. 1992. After washing with distil water, samples were immersed in 70% ethanol for 1-2 minutes and then sterilize with aqueous sodium hypochlorite (4%) for 2-5 minutes, then rinsed in 70% ethanol for 30s. The material was then rinsed thrice in sterile double distil water and allowed to surface drying in sterile conditions properly.

b. **Isolation:** The sterile leaves and stems were then cut into small pieces using sterile cutter into 0.5 × 0.5cm square. 3 leaf tissues were placed PDA (Potato Dextrose Agar) media Petri plate added with streptomycin, sealed (Venkatneswaruluet al.,2018) and incubated at 28 ± 1°C for two weeks. A total of 3samples of each plant parts were incubated to isolate and report diversity of endophytic fungi.

The incubated plates were regularly observed at interval of 2 days for actively growing fungi which were then transferred to fresh PDA plate. Each fungal culture was checked for purity and transferred to another plate by hyphal tip method.

Specific Fungi observed were grown on separate media plates.

The leaf and stem sample without sterilization was processed as negative control to check the contaminated fungi.

All the isolates obtained were purified by serial dilution technique till axenic strains were obtained.

c. **Maintenance & Preservation:** The selected endophytes were maintained in cryovials layered with mineral oil at room temperature and also stored in deep freezer. All the samples were deposited in the Department of Botany, Govt. VYTPG Autonomous College, Durg, and Chhattisgarh.

d. **Colonization frequency and Isolation rate:** Colonization frequency and Isolation rate were calculated using formula and observation details(Petrini, P.J. Fisher, 1986)

$$\mathbf{CF}\% = \frac{\text{No. of segments colonized}}{\text{Total no. of segments}} \times 100$$

$$\mathbf{IR}\% = \frac{\text{No. of isolates obtained}}{\text{Total no. of segments}} \times 100$$

Colonization and isolation rate of endophytic fungi associated with Costus speciosus

Item	Stem	Leaf	Total	Average
Number of inoculated tissue blocks	25	25	80	25
Number of infected tissue blocks	22	23	45	22.5
Number of isolated strains	15	20	35	17.5
Colonization rate %	88	92	180	90
Isolation rate %	60	80	140	70
Proportion to isolated strain out of total	42.84	57.16	100	50

III. Identification of Isolated Endophytic Fungi:

a. **Morphological identification:** The purified axenic strains were identified on the basis of their macroscopic and microscopic characteristics such as colony colour/morphology, fruiting and spore morphology.

b. **Microscopy:** Microscopic slides were prepared, stained by lactose phenol cotton blue (Cappicchino & Sherman, 1996) and were observed under

microscope (10X, 40X), and photographs were taken by using microscopic image projection system (Magnus) camera.

c. Standard taxonomic manuals were used to identify the fungal genera and species (Ainsworth et al.1973; Ellis 1976; Barnett & Hunter 1998).

IV. Diversity Analysis:

Assessment of fungal diversity was done using diversity indices such as Shannon-Wever and Simpson's index.

		Mean ± SE			
		Enzymatic activity			
Code	**Fungal sp.**	**Amylase**	**Pectinase**	**Cellulase**	**L-asparaginase**
CLD1	Fusarium sp.	39.00 ± 0.58^{e}	0.00^{a}	39.67 ± 0.33^{c}	0.00^{a}
CLD2	Aspergillus sp.	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 sp.	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
CLD7	Aspergillus fumigatus	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
CSD1	Fusarium sp.	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}

Simpson's index

Species	**(n)**	**n*(n-1)**
1. Fusarium sp. 99*(9-1)= 72		
2. Aspergillus sp. 118*(8-1) = 56		
3. Penicillum sp.129*(9-1) = 72		
4. Pacilomyces sp.1		**1(1-1)= 0**
5. Aspergillus fumigatus22(1-1)= 2		
Total (N)	**35**	**202**

Simpson index D = ∑ n*(n-1) / N(N-1)

Simpson index D = 202/ 1190 = 0.1697 = 0.2

Shannon-Wever index

H' = ∑ -Pi lnPi

Endophyte	Abundance	Pi	Pi* lnPi
Fusarium sp	9	9/35= 0.25	0.25*(-1.4)= -0.35
Aspergillus sp	11	11/35= 0.31	0.31*(-1.2)= -0.372
Penicillumsp	12	12/35=0.34	0.34*(-1.1)= -0.374
Pacilomyces sp	1	1/35=0.02	0.02*(-3.9) = -0.078
Aspergillus fumigatus	2	2/35=0.05	0.05*(-2.99) = -0.149
			∑ - 1.323

H' = ∑ -Pi lnPi = - ∑ - 1.323 = 1.323

Results and Discussion:

1. **Colonization and Isolation Rates**:

Colonization rate was calculated based on the proportion of infected tissue blocks to the total inoculated blocks. The stem showed an 88% colonization rate and the leaf 92%, leading to an overall rate of 90%.

Isolation rate was determined from the total number of strains isolated. Stem tissue exhibited 60% isolation efficiency, while leaf tissue demonstrated 80%, averaging 70%.

2. **Proportion of Isolated Strains**:

Strain distribution between stem and leaf showed that 42.84% of strains were from the stem and 57.16% from the leaf.

Diversity Assessment

1. **Identified Genera**:

Fungi were identified morphologically and microscopically, revealing five genera: Fusarium sp., Aspergillus sp., Penicillium sp., Paecilomyces sp., and Aspergillus fumigatus.

2. **Diversity Indices**:

Simpson's Index (D):

$$D = \frac{\sum n(n-1)}{N(N-1} = \frac{202}{1190} \cong 0.2$$

A low value indicates a diverse fungal population.

Shannon-Weaver Index (H'):

$H' = \sum(Pi \cdot \ln Pi) = 1.323 H'$

The index reflects high fungal diversity, with Penicillium sp. being the most abundant.

Discussion

Ecological Role

Endophytic fungi associated with Costus speciosus likely play critical roles in maintaining plant health, improving stress resistance, and contributing to secondary metabolite production:

1. **Plant Health and Stress Resistance**:

Endophytes such as Fusarium sp., Penicillium sp., and Aspergillus sp. are known to enhance plant resilience against biotic (pathogens) and abiotic (drought, salinity) stresses (Arnold et al., 2003; Redman et al., 2002). These fungi may achieve this by producing bioactive compounds, inducing systemic resistance, and improving nutrient uptake.

The ability of some isolates (e.g., Fusarium sp. and Aspergillus sp.) to produce cellulase and pectinase suggests their involvement in modifying the plant cell wall structure, which may aid in stress mitigation and nutrient assimilation (Schulz et al., 2002).

2. **Secondary Metabolite Production**:

Endophytes contribute to the production of metabolites such as alkaloids, flavonoids, and phenolics, enhancing the pharmacological potential of Costus speciosus. These fungi may act as biocatalysts or directly produce metabolites with antifungal, antibacterial, or antioxidant properties (Strobel et al., 2004).

Medicinal Relevance

The fungal endophytes isolated from Costus speciosus may significantly impact the plant's bioactive compound profile:

1. **Augmenting Phytochemical Production**:

Many endophytes produce secondary metabolites that complement or enhance the host plant's compounds. For example, Penicillium sp. and Fusarium sp. are well-documented producers of bioactive molecules like terpenoids and polyketides (Kharwar et al., 2011; Kaul et al., 2012).

These fungi may contribute to Costus speciosus's known antidiabetic, anti-inflammatory, and antimicrobial properties by boosting bioactive metabolite synthesis (Das et al., 2010).

2. **Novel Compound Discovery**:

Some endophytic genera (e.g., Aspergillus sp.) have a reputation for producing novel bioactive compounds with potential medicinal applications, including anticancer and immunomodulatory agents (Gunatilaka, 2006).

Comparison with Other Medicinal Plants

The diversity of endophytic fungi in Costus speciosus is comparable to other medicinal plants but has unique features worth noting:

1. **Higher Diversity in Costus speciosus**:

With a Shannon-Weaver diversity index of 1.323 and the presence of five fungal genera, Costus speciosus exhibits a moderately high diversity of endophytes, suggesting its potential for a wide array of ecological and medicinal applications (Present Study).

In comparison, studies on other medicinal plants, such as Azadirachta indica (neem) or Tinospora cordifolia, report similar or slightly lower diversity indices but with overlapping genera like Aspergillus sp. and Penicillium sp. (Kusari et al., 2013).

2. **Species-Specific Variations**:

Unlike Costus speciosus, endophytes from other plants, such as Catharanthus roseus (known for vincristine production), are often dominated by fewer fungal genera but with

distinct functional roles tied to the plant's unique bioactive compound profile (Kumar & Kaushik, 2013).

3. **Functional Diversity**:

The enzymatic activities (amylase, cellulase, and pectinase) observed in Costus speciosus endophytes suggest their ecological flexibility, comparable to the diverse metabolic capabilities of fungi in plants like Curcuma longa (turmeric) (Verma et al., 2009).

Conclusion

The endophytic fungi of Costus speciosus play essential ecological roles in enhancing plant health, providing stress resistance, and contributing to the plant's medicinal potential through secondary metabolite production. Their diversity aligns with that of other medicinal plants but highlights unique enzymatic and metabolic capacities that may be leveraged for both ecological and pharmaceutical advancements. Further exploration of these fungi may reveal novel bioactive compounds that amplify the therapeutic value of Costus speciosus.

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