



Isolation of fungal endophyte from *Bixa orellana* L and screening for its extracellular enzyme production

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Abstract

Fungal endophytes are the group of microorganisms that occupy parts of higher plants and colonize them without causing any noticeable injury to the host. Due to the increased application of enzymes in various sections of industries, there are high demands of finding some novel sources of enzymes especially from microbial origin. This study focusses on enzymatic activities of endophytic fungus isolated from *Bixa orellana* L. Young, healthy and uninfected leaves were used to isolate the endophytic colony. The colony morphology was screened for the formation of spores/ conidia or fruiting bodies were also noticed under light compound microscope after staining. The endophyte was screened for the presence of various enzymatic activities like amylolytic, lipolytic, cellulolytic, proteolytic and catalase activity. Results showed that the isolated endophyte was a potential source of amylolytic and cellulolytic activities whereas it showed negative result for lipolytic, catalase and proteolytic activities. Hence, this study showed that fungal isolate from *Bixa Orellana* L could be a potential source for enzymes with biological activities. Further, molecular characterisation of this endophyte and application of the enzymes need to be carried out.

Keywords: endophytes, fungi, *Bixa orellana* L leaf, Isolation, enzymatic activity

Introduction

In the endophytic community, fungal endophytes are very significant as they occupy more than 41% of the total community. They are endo-symbiotic microorganisms that lives in healthy living cells of plant species and are known for their beneficial activities to the host [1]. Some of the highlighted beneficial activities include protective functions against the pathogenic microorganisms to the host, promotes growth, provide stress resistance and so on [2, 3]. Therefore, the use of endophytic fungi opens up novel avenues for their biotechnological exploitation, which gradually leads to the isolation and cultivation of these organisms [4].

Bixa orellana L is a plant originally belonging to Brazil but it has also been grown in other tropical countries like Peru, Mexico, Ecuador, Indonesia, India, Kenya, and East Africa. This plant is also distributed to Central and South America [5]. The plant is widely popular in terms of economic importance as it has a wide application in the field of cosmetic industries, textile industries and paint industries. The plant has also gained attraction as natural colouring agent as it produces a dye which is used worldwide [6].

Endophytic fungus are extensively studied as a potential source of enzyme. This enzyme production can be of extracellular and intra cellular types [7]. Microorganism produced enzymes always have a wide interest as they are applied in food processing industries, tissue industries, leather industries, textile industries and pharmaceutical industries [8]. Endophytes produce enzymes like amylase, catalase, protease, lipase etc. which are having several applications like biodegradation, hydrolysis and protection against invading pathogens [7, 8]. Hence, identification of enzyme secreting fungal endophytes are gaining interest of researchers as they could be the potential alternatives to some synthetic enzymes.

Methods and Materials

Source of Endophyte

Fresh and young leaves were used to isolate fungal endophyte. The leaves of *Bixa orellana* L were collected from the garden of Assam down town University, Panikhaiti, Kamrup (M) District and was identified based on its vernacular name.

Surface Sterilization

The collected samples were processed through standard sterilization techniques [1, 9]. The healthy and young leaves were washed in tap water sequentially dipped in ethanol for 2 minutes which was followed by 0.5% sodium hypochlorite solution for 1 minute. The leaves were then washed thoroughly with distilled water and allowed to surface dry under sterile environments.

Sample Inoculation and Incubation

A measureable diameter of 0.5mm of the leaves were punched with the help of a sterile puncture and placed into prepared plated of Potato Dextrose Agar (PDA) under sterile condition [10]. The plates were incubated at room temperature for 4 days to observe the growth of fungal colonies. Plates were observed twice a day for the growth of the fungal endophyte. Plates were maintained in triplicates.

Fungal Staining with Lactophenol Cotton Blue Stain

The incubated plates showing single colony of endophyte was selected for fungal staining under sterile condition. A small tuft of the fungal colony was collected with the help of a sterile forcep and placed on to the glass slides and few drops of 70% alcohol was added. One drop of Cotton blue stain was added to the slide. Using two flame cooled needles the fungal tuft was gently teased. The slide was observed

under bright field microscope using a cover glass [11]. The slides were prepared in triplicates.

Evaluation of Enzymatic Activity

The isolated fungal endophyte was subjected to qualitative enzyme assay. It was screened for the presence of following enzymatic activity using standard methods [12, 13, 14].

Amylolytic Activity

The assessment of amylase enzyme activity was done by growing the fungal endophyte on Potato Dextrose Agar (PDA) media containing 1% soluble agar. The plates were incubated for 5 days at room temperature and plates were flooded with 1% iodine stock solution. The plates were observed for the presence of clear zone surrounding the colony.

Cellulolytic Activity

For cellulase activity, the fungi were cultured in Potato Dextrose Agar (PDA) media and 0.5% Na- carboxymethyl cellulose (CMC) was added. After incubation in room temperature, the plates were flooded with aqueous Congo red and destained with 1M NaCl solution for 15 min. The presence of clear zone surrounding the colony indicated the presence of cellulase activity.

Lipolytic Activity

To determine the lipase activity, the fungi was grown on Potato Dextrose Agar (PDA) media which was added with 1% Tween20 (v/v). After incubation, the plates were observed for the presence of precipitation around the colony indicates the lipase activity.

Proteolytic Activity

The fungi grown on the Potato Dextrose Agar (PDA) media was supplemented with 1% milk and incubated at room temperature. The plates were screened for the presence of clear zone around the colony which the presence of proteolytic activity by the fungus.

Catalase Activity

The fungal isolate was transferred to clean glass slide with the help of a sterile wire loop and 3% hydrogen peroxide was added. The slide was observed for the presence of bubbles which indicated the presence of catalase activity.

Results and Discussion

A fungal endophytic colony was isolated from fresh leaves of *Bixa orellana* L. The isolate, R1 appeared as cottony, dense white mat on PDA plate after a period of 5-6 days (Figure 1a). It was found to be slow growing and it covered only less than half of the culture plate thus, confirming the endophytic nature. However, it was noticed that after 10 days, the colony turned slight greenish in colour from the other side of the plate (Figure 1 b). Similar studies by Kannan KP *et al*, 2017 revealed presence of fungal endophytes isolated from leaves of *Bixa orellana* l., collected from Western Ghats of Sathyamangalam [15]. Morphological characterisation was done based on Lactophenol Cotton Blue staining. The colony characters exhibited branched, filamentous hyphae under bright field microscope (Figure 1 c).



Fig 1: a) White dense cottony mat fungal endophytic isolate (R1) from *B.orellana* b) White colony turns greenish at the backside of the PDA plate after a period of 10 days c) Branched, filamentous hyphae of R1 upon staining with lactophenol cotton blue

The isolated fungal endophyte R1 was screened for its enzyme secretion bioactivities (Table 1). In this preliminary screening of amylase, cellulase and lipase activities, the isolate recovered from *B.orellana* was found to exhibit positive substantial amylolytic, cellulolytic and lipolytic activities. R1 isolate showed presence of a clear zone around the colony when treated with congo red (Figure 2 a). This can be attributed to degradation of CMC that signifies R1 as a potent producer of cellulase. Studies by Shubha J *et al*, 2017 also reported high cellulolytic activity of 77.77% among the leaf fungal endophytes from *C. aloifolium* [16]. In context to lipase secretion, R1 again proved a good producer of lipase enzyme as it showed a good clear zone on PDA plate supplemented with Tween 20 (Figure 2 b). Similar studies by [16] correlated with our study in terms of significant lipolytic activity by *C. falcatum* from *B.orellana*. This higher lipase potentiality can be explored in their fat

utilization ability [17] Results of amylolytic activity revealed a prominent clear zone around the R1 colony upon flooding the plates with 1% iodine (Figure 2 c). Our results thus highlighted strong amylolytic activity which was unique as against studies carried by Sunitha VH *et al*, 2013 where endophytic isolates from *B.orellana* were weak producers of amylase enzyme [18]. Whereas R1 endophytic isolate showed negative results for proteolytic and catalase enzymes. Other studies on *B.orellana* endophytic isolates highlighted moderate protease activity [18].

Table 1: Enzymatic activity of the isolated endophyte

Amylase enzyme	Cellulase enzyme	Lipase enzyme	Protease enzyme	Catalase enzyme
+	+	+	-	-

+ve indicates Present, -ve indicates absent

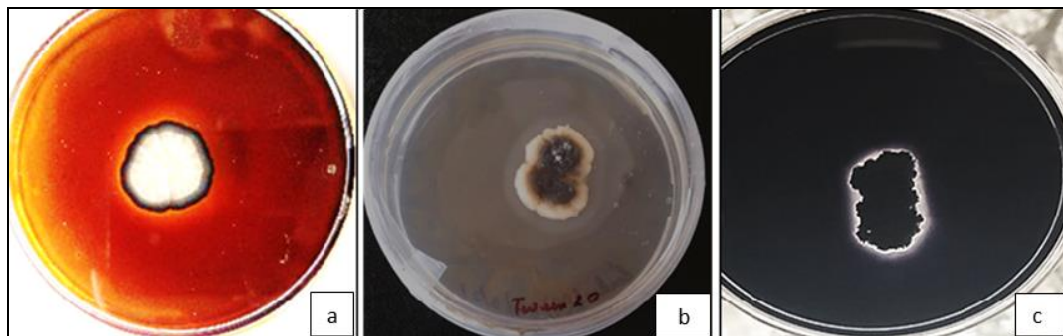


Fig 2: Clear zone around R1 isolate showing a) Cellulolytic activity b) Lipolytic activity and c) Amylolytic activity b)

Conclusion

From our study, it can be concluded that *B.orellana* leaves from Guwahati, Assam harbours fungal endophyte R1. The isolate proved very efficient as producers of extracellular enzymes. It showed promising amylolytic, lipolytic and cellulolytic activities which emerged as new sources of these industrially active enzymes. However, molecular identification of the isolate needs to be carried out and further quantification of these enzymes could open up new arenas for exploitation of these enzymes in various industrial sectors.

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Conflict of Interest

The authors declare no conflict of interest.

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