

Original Research Article

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Evaluation of Jasmonic Acid Production by *Lasiodiplodia theobromae* under Submerged Fermentation

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ABSTRACT

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Present study was conducted to evaluate the jasmonic acid (JA) production capacity of the isolated pathogenic fungus *Lasiodiplodia* sp. causing leaf blight in coconut and pod rot in cocoa. The fungal cultures were isolated from the diseased coconut and cocoa using PDA (Potato dextrose Agar). Morphological confirmation was done by examining the isolates under microscope, having obvoidal shape conidia. Jasmonic acid production was compared with standard culture and evaluated for higher JA production using four different media (Potato dextrose medium, Potato carrot medium, Nutrient medium and Minimal salt medium). The results depicted that potato dextrose media recorded the maximum in terms of growth and basal salt medium recorded higher jasmonic acid (JA) production under optimized growth conditions.

Introduction

Jasmonic acid (JA) play a vital role in molecular plant pathogen interaction and act as a elicitor for induced systemic resistance (ISR) and induces series of defense signaling cascade when pathogen attack occurs. Similar defense mechanism also present during insect and nematode attack. This JA produces in nano and picomolar amount during plant pathogenic interaction. Different fungi are reported as producers of jasmonates such as *Botryodiplodia theobromae* (*Lasiodiplodia theobromae*) and *Gibberella fujikuroi*. JA was first isolated from the culture filtrate of *Lasiodiplodia theobromae* (Alderidge *et al.*, 1971). *G. fujikuroi* was reported as producer of a mixture of N-jasmonyl-isoleucine and N-

dihydrojasmonyl-isoleucine (Miersch *et al.*, 1987). *Fusarium oxysporum* was reported as producer of more than 20 jasmonates and *Aspergillus niger* grown on liquid medium could form more than 25 jasmonate compounds upon application of JA, 9,10-dihydro-JA and their methyl esters (Wasternak, 2007).

Lasiodiplodia theobromae was first described from *Theobromae cacao* fruit in Ecuador in the late 1800s (Patouillard and Lagerheim, 1892). It is recognized as an important plant pathogen of many woody and herbaceous hosts, especially in the tropical and subtropical region and symptoms include

dieback, canker, gummosis, leaf blight, root and collar rot of woody plants and agricultural crops (Sinclair and Lyon, 2005). *L. theobromae* is predominantly a latent pathogen, frequently found as an endophyte in healthy plant tissue, but that can become a virulent pathogen when the host is weakened or stressed (Mullen *et al.*, 1991; Sinclair and Lyon, 2005). *L. theobromae* can be distinguished based on their conidia size (Burgess *et al.*, 2006). *Lasioidiplodia* has slightly wider and more obovoid conidia. A study of Dhandhukia and Thakkar (2006) reveals that optimum temperature and pH for growth of *L. theobromae* were found as 30°C and 5.5, respectively. Regardless of its pathogenic nature *L. theobromae* have the potential to induce jasmonate mediated defense signaling against plant pathogen. Hence present investigation envisage to explore the defense potential of this pathogen and to maximize the Jasmonic acid production by altering the nutrition and other growth conditions.

Materials and Methods

Isolation and morphological characterization of fungus

The fungal cultures were isolated and purified from the diseased coconut (leaf blight) and cocoa (pod rot, dieback) collected from Pollachi region, Tamil Nadu, India using PDA. Isolated cultures were confirmed by amplification of ITS 1 and ITS 4 primers. Standard culture was obtained from MTCC (*L. theobromae*: MTCC 3068) and maintained in PDA. Morphological observations of conidia were done under microscope. Mycelial tips were placed into the PDA medium and were incubated for thirty days at room temperature. After fungal germination occurs, conidia were collected and observed under a light microscope. The conidial morphology was studied and their shape,

color and presence of septation were recorded.

Fermentation and optimization of cultural conditions

L. theobromae was grown in 100 ml potato dextrose broth in 250 ml conical flask by transferring a disc containing actively growing fungal mycelia and incubated for 3 days at 25 ± 2°C in dark. After that the fungus was cultured in potato dextrose broth, potato carrot broth, nutrient broth and minimal salt medium with the addition of 2% mother culture. pH of the culture was set as 5.5 and fermentation was carried out in 250 ml Erlenmeyer flasks containing 100 ml sterile medium at 25 ± 2°C in dark for 7 days.

Growth determination

Biomass production was determined by dry weight after broth filtered on pre-weighted Whatman No.1 filter paper followed by drying at 60°C for 24 h.

Extraction and measurement of JA

At the end of incubation period, mycelia were separated from the culture filtrate using muslin cloth, and filtrates were separated by adding equal volume of ethyl acetate. Organic phase was removed by separating funnel and condensed using rotary evaporator. The organic extracts were resuspended in 1 ml of HPLC grade methanol, filtered through 0.22 µ filters (Hi-media) and analyzed using high performance liquid chromatography. The mobile phase consisted of acetonitrile: methanol: 2-propanol (95:3.5:1.5) and the flow rate was 1 ml /min.

Result and Discussion

The isolated cultures were identified as *Lasioidiplodia* by its ovoidal shape conidia

observed under stereo zoom microscope and confirmed by ITS1 and ITS 4 primer amplification. The isolate and standard were subjected to further fermentation studies. Dry matter production was higher in potato dextrose broth (1.97g/0.1 l) followed by minimal salt broth (1.89g/0.1 l) and least value (were recorded in nutrient broth 1.53g/0.1 l). Similarly higher JA production

was recorded in minimal salt broth (50.77 $\mu\text{g}/0.1\text{ l}$) followed by potato dextrose broth (50.71 $\mu\text{g}/0.1\text{ l}$) and least value were recorded in nutrient broth (49.32 $\mu\text{g}/0.1\text{ l}$). The results has close conformity with the results of Grolamys *et al.*, (2014), shows JA was found at concentrations between 48 and 100 $\mu\text{g mL}^{-1}$ in fermentation broths of *Botryodiplodia* strains.

Table.1 JA production in submerged fermentation

Medium	Isolate DMW(g/0.1 l)	Standard	Isolate JA ($\mu\text{g}/0.1\text{ l}$)	Standard
Potato dextrose broth	1.97	1.73	50.71	52.12
Potato carrot broth	1.82	1.82	50.21	52.12
Nutrient broth	1.53	1.56	49.32	51.21
Minimal salt broth	1.89	1.88	50.77	53.31

Fig.1a&b Chromatogram of JA (standard) and Chromatogram of culture filtrate

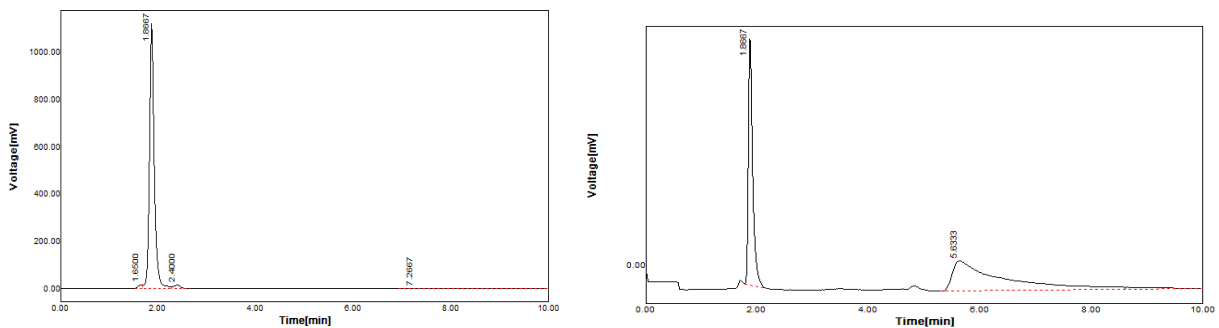
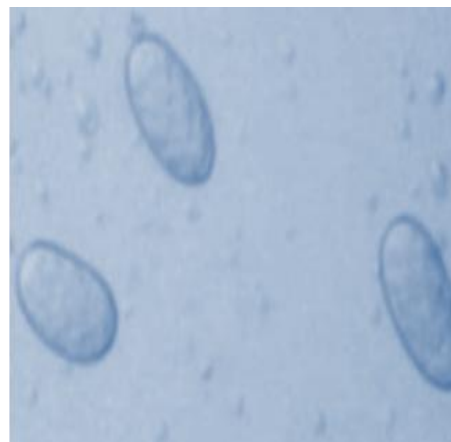


Plate.1a&b *Lasiodiplodia theobromae* and microscopic observation of conidia



A study of Dhandhukia and Thakkaar (2007a,b) reveals that 5 and 10 μL of samples, JA was found to be 14.157 and 30.357 μg , respectively and JA produced by *L. theobromae* on the seventh day was 30.357 mg/L of FCF. JA production of isolate was comparable with standard culture also. Negligible amount of differences existed between standard and isolate (Table 1). Extracted jasmonic acid was analyzed through HPLC along with standard JA.

Jasmonic acid was extracted and separated from *Lasiodiplodia theobromae* and analyzed through HPLC. Standard jasmonic acid (Sigma) was run at different concentrations and retention time (RT) of JA was near 2 minute (1.8667) and the sample (extracted JA from *Lasiodiplodia theobromae*) peak also obtained near 2 minute (1.866), which confirms the presence of jasmonic acid (JA) in the sample. Negligible amount of peaks were also recorded from sample chromatogram which shows *Lasiodiplodia* contains other metabolites along with JA (Fig. 1).

In conclusion, the fungus *B. theobromae* has been studied by different authors (Eng *et al.*, 1998; Dhandhukia and Thakkar, 2007a,b, Saha *et al.*, 2008; Jernerén *et al.*, 2012) in order to identify and quantify JA and related compounds. In this study, submerged fermentation of the fungus evaluated for higher levels of JA using four different media.

The JA concentrations found in our fermentation broths are similar to those obtained in other studies with the same fungus species (Dhandhukia and Thakkar, 2007a). Exogenous application of JA in millimolar concentrations increases the resistant against plant pest and diseases. Hence this bioproduct will have the potentiality to become an effective bio control agent. Since it is a plant pathogen genetic modification also needed for effective utilization by the end users.

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