# Biochemical Variability in *Lasiodiplodia theobromae*, the Incitant of Fruit rot in Eriophyid Mite Infested Coconut

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## Abstract

Three virulent isolates representing three major groups of *Lasiodiplodia theobromae* causing fruit rot and immature nutfall in eriophyid mite infested coconut collected from eight southern districts of Kerala state varied in the biochemical constituents such as total mycelial proteins, sugars free amino acids and also in protein profile. The SDS polyacrylamide gel electrophoresis of soluble mycelial proteins revealed intra-specific variations within *L.theobromae* isolates. All the isolates showed a common protein band and also protein bands specific to each group of isolates.

**Key words:** *Lasiodiplodia theobromae*, *protein* profile, intraspecific variations

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Coconut (*Cocos nucifera* L.) is cultivated throughout Kerala state of India covering an area of 9.06 lakhs ha with a productivity of 6379 nuts/ha which is below the annual potential yield. Diseases and pests are major causes for this low productivity. With the wide spread occurrence of eriophyid mite (*Aceria guerreronis* Keifer) infestation, severe incidence of rotting of nuts and immature nutfall have been observed throughout the year at different locations in the southern districts of Kerala, the areas which receive well distributed rainfall. In India, the incidence of mite attack on coconut palm was first noticed in 1998 in Ernakulam district of Kerala (Sathiamma et al 1998). The pathogen *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. enters the tissue through the wound caused mainly by eriophyid mite in the soft tissue beneath the perianth. The tissue at this region being soft may be very conducive for the fungus to penetrate through the wounds, ramify and multiply causing extensive rotting (Chandramohanan and Baby 2004; Venugopal and Chandramohanan 2006).

Studies on cultural, morphological and pathogenic characters of 373 isolates of *L.theobromae* collected from different localities in disease endemic area in Kerala revealed the existence of a wide variability within isolates. Based on the studies the isolates were mainly classified into three groups viz, dark gray type, grayish black type and white type depending on colony color (Venugopal et al 2008). Since there was a marked variability in cultural and morphological characters of *L.theobromae* isolates collected from different locations, further studies were conducted to find out the biochemical diversity among the selected isolates of *L.theobromae.*

## Materials and Methods

To study the biochemical variability within *L.theobromae*, three highly pathogenic isolates representing each of three main groups of isolates classified based on earlier studies were selected. The isolates selected were APA/L-172 (dark gray type colony), EKM/L-307 (grayish black type colony) and TPM/L-36 (white type colony). They were grown in 250 ml conical flasks containing 50 ml potato dextrose broth. Three flasks were used for each isolate. Each flask was inoculated with five discs each of 7 mm dia cut from the periphery of an actively growing 5 d old culture grown on PDA. The inoculated flasks were incubated at 28±2C for 14 d. The mycelial mat was harvested by filtering through Whatman No.1 filter paper, washed with phosphate buffer (pH 7.0) and damp dried. Soluble protein concentrations in the extracts of mycelium were determined by Lowry's method (Lowry et al 1951). Soluble sugar concentrations in the extracts of mycelium were determined by Dubois et al (1956). The free amino acids were estimated by ninhydrin method (Moor and Stein 1948).

**Electrophoresis of native proteins.** The proteins were fractionated by electrophoresis according to the method of Laemmli (1970). Electrophoresis of native protein preparations was carried out on a discontinuous system using 2.5% stacking gel and 7.5% separating gel in a vertical slab mould of 30 x 22 x10 cm size.

Electrophoresis buffer was tris-glycine (pH 8.3). Sample loading buffer was mixed with soluble protein preparations in the ratio 1:1 and aliquot containing 20 µl of protein of each *L. theobromae* isolate was placed in the wells of the gel. Standard protein marker (Sigma low molecular weight protein marker) was also loaded in one of the wells. Electrophoresis was performed at 60 V for stacking gel and at 120 V for separating gel at 40 C. The gels were removed when the run was over as indicated by the tracking dye, bromophenol blue. The protein patterns were visualized by staining gels, for 7 h, with Coomassie brilliant blue G in water: methanol: perchloric acid (15:1:4) mixture and destained with several changes of a mixture of water: methanol: acetic acid (7:2:1). The gels were then photographed and used for studying the difference in banding pattern of the isolates of *L. theobromae.*

## Results and Discussion

**Biochemical studies.** Variations among three isolates were observed in the biochemical constituents such as total mycelial proteins, sugars and free amino acids. The protein contents were comparatively low but varied among three isolates (Table 1). Gupta and. Neema (1997) studied the quantitative analysis of sugar and nitrogen contents of mycelia of *Colletotrichum papayae* and *L. theobromae*. The analysis revealed that the levels of polysaccharides and nitrogen were high in mycelium of *C. papayae* and considerably low in *L. theobromae.* Among the three isolates, the protein content was low in grayish black isolate and high in dark gray isolate.

**Table 1. Total protein, carbohydrates and free amino acids in mycelial extracts of dark gray (APA/L-172), grayish black (EKM/L307) and white (TPM/L-36) isolates of *L.theobromae***

| Isolate type and no. | Total protein (mg/g) | Total carbohydrates (mg/) | Free amino acids (mg/g) |
|---|---|---|---|
| APA/L-172 | 1.19 | 1.13 | 0.924 |
| EKM/L307 | 0.598 | 1.11 | 0.842 |
| TPM/L-36 | 0.957 | 1.51 | 0.801 |

The total carbohydrate contents of dark gray and grayish black isolates did not vary whereas the carbohydrate content of the white isolate was higher as compared to other two isolates indicating its variation from other two isolates. Amino acids contents of three isolates did not vary much. However, among the isolates, dark gray isolate contained higher amino acid content than other two isolates of *L.theobromae*. Many workers (Devay 1954; Tandon 1960; Chandra and Tandon 1964; Bisen 1974; Crossan and Lynch 1958) correlated the amino acid contents with the pathogenic nature of certain organisms.

**Electrophoretic studies.** Mycelial protein profile has been used by several workers to differentiate many fungal species (Hall et al 1969; Gill and Zentmyer 1978 and Agarwal et al 2001). Studies on *L.theobromae* revealed that mycelial protein of approximate molecular weight of 25.5KD was common to all the three isolates and protein band of 74 KD was common to dark gray and white isolates. The protein band of 30 KD was common to grayish black and dark gray isolates only. Thus all the isolates showed a common protein band (25.5KD) and also a protein band specific to a group of isolates obtained from coconut. The present investigation has demonstrated diversity among the isolates of *L.theobromae*, the causal organism of immature nutfall and fruit rot of coconut. The results obtained with electrophoresis support its use as one of the aids in distinguishing intra-specific variation in *L.theobromae* isolates encountered on coconut.

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