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Bacterial endophyte from *Hevea brasiliensis* antagonistic against *Phytophthora meadii* and bio-prospecting of its antifungal metabolites

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INTRODUCTION

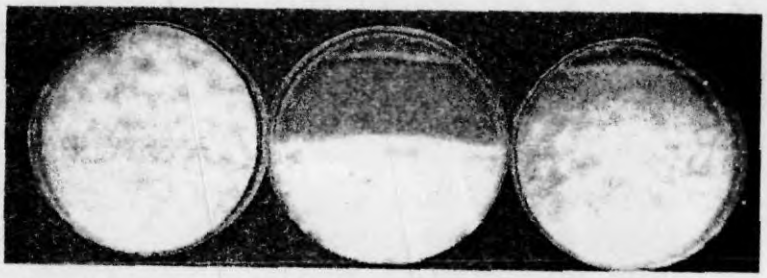
Hevea brasiliensis accounts for 99% of the world's total natural rubber production. The most destructive disease of rubber in India is Abnormal Leaf Fall (ALF) caused by *Phytophthora* sp. and results in yield loss of 38-56%. Plant protective microbial symbionts determine the ecological success of plants. Bacterial endophytes in plants reflect a huge genetic and metabolic biodiversity and offer a very high application potential as biocontrol agents (Sturz *et al.*, 2000). In the present study, the antagonistic potential bacterial endophytes from *H. brasiliensis* against *P. meadii*, was evaluated for the development of an eco-friendly and sustainable disease management strategies in rubber ecosystem.

MATERIALS AND METHODS

Samples were collected from root, petiole and leaf tissues of RRII 105 & RRIM 600 clones of rubber in ALF disease free, moderately disease prone and highly disease prone areas in India. Endophytic bacteria were isolated by disinfection and trituration method and estimated the total population count (Colony Forming Units/g fresh tissue). Isolated endophytes were tested for their ability to inhibit the growth of the pathogen *P. meadii* by dual culture test. Selected bacterial endophytes showing antagonism against *P. meadii* were characterized by 16s rDNA sequencing. *In vitro* bioassay of antifungal activity of selected antagonistic endophyte *Alcaligenes faecalis* against *P. meadii* was evaluated by detached leaf technique. *In vivo* bioassay of antifungal activity of *A. faecalis* against *P. meadii* was evaluated in polybag plants. Antifungal compound was extracted from potato dextrose broth culture supernatant of *A. faecalis* by liquid-liquid extraction using diethyl ether as solvent. The antifungal property of extracted culture supernatant of *A. faecalis* and PDB broth were examined by poison food technique. Thin layer chromatography and silica column chromatography were used for the separation of compounds in ether extracts and the extract is fractionated in to five portions. Each fraction was incorporated in filter paper discs and verified the antifungal activity against *P. meadii*. The fraction showing antifungal activity was characterized by GC-MS and reverse phase HPLC analysis.

RESULTS AND CONCLUSIONS

Two hundred and forty root, leaf and petiole tissues of rubber clones RRII 105 and RRIM 600 from five locations (Palippally Estate, Thrissur; RRII farm, Kottayam; Ambadi Estate ,Nagarcovil; RRS Padiyoor and Taranagar farm, Agarthala) were collected for bacterial isolation. Root tissues showed higher bacterial population compared to petiole and leaf tissues. A total of 252 morphologically different bacterial endophytes were collected from these locations. Out of 252, 42 isolates showed growth inhibition of *P. meadii* (Fig.1).



Control plate | Endophyte without any antagonism | *Invitro* antagonism by endophyte

Fig.1. Antifungal activity bacterial endophytes in dual culture plate

Eleven isolates showing more than 50% growth inhibition of pathogen and morphological difference were selected for further study. Out of the eleven endophytes, eight showed similarity to *Pseudomonas aeruginosa* and others were *Bacillus subtilis, Klebsiella oxytoca* and *Alcaligenes faecalis* respectively (Fig.2). *In vitro* bioassay of selected antagonist against *P. meadii* showed less infection in leaf tissues of antagonistic endophyte treated plants (Lesion size, 3.30mm in RRII 105 and 4.34 in RRIM 600) compared to media sprayed control (Lesion size, 5.89 mm in RRII 105 and 6.28 mm in RRIM 600) and untreated control (Lesion size, 6.30 mm RRII 105 and 6.57 in RRIM 600) (Fig.3). As per data on *in vivo* bioassay, *A. faecalis* treated plants showed an enhanced disease tolerance against *P. meadii* in susceptible clone RRIM 600.

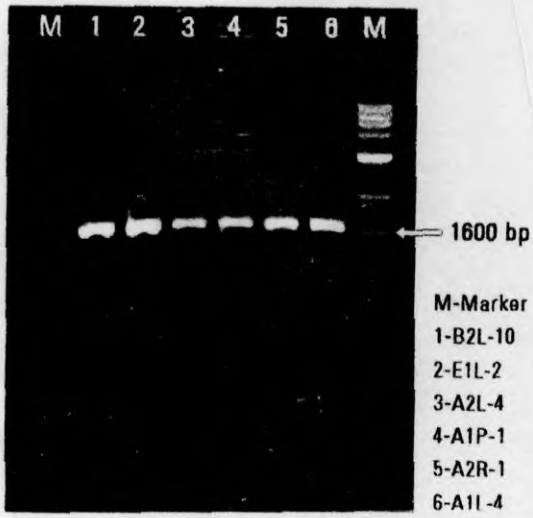


Fig.2. 16S rDNA amplified from bacterial endophytes of *H. brasiliensis* antagonistic against *P.meadii*

Control | *A. faecalis* treated

Fig.3. Lesion size of *P. meadii* infection in *A. faecalis* treated and control RRIM 600 leaves

Antifungal compound extracted from culture supernatant of *A. faecalis* showed inhibition of *P. meadi* growth in petriplate. PDB broth extract and ether incorporated control plates did not show any growth inhibition of *P. meadii*. The present study indicates that *A. faecalis* is producing certain extra cellular metabolites, having antifungal activity against *P. meadi*. Antifungal compound was extracted in diethyl ether and used for further characterization. Five spots were observed in extract of *A. faecalis* culture supernatant in TLC. The extract was fractionated into five fractions by flash chromatography using silica column and among the five fractions separated on silica column, only one fraction showed antifungal activity against *P. meadi*. The compounds in antagonistic fraction was

characterized by GC-MS and Reverse Phase HPLC Analysis and identified as Diethyl and Dibutyl Pathalates, Phenanthridine, Propylaraben, Phenylpyridine, 1,2-Benzenedicarboxylic Acid, Bis Ester and Phenazine (Fig.4).

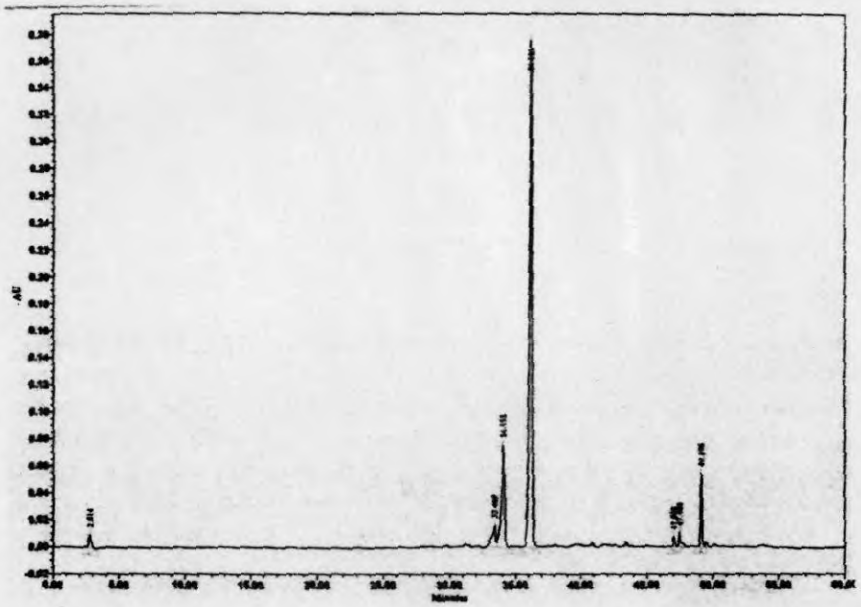


Fig.4 HPLC Chromatogram of antagonistic fraction from ether extract of endophytic bacterial culture supernatant

In this study, antagonistic endophyte from *Hevea brasiliensis* against *P. meadii* was isolated and showed its antagonistic potenial in *in vitro* and *in vivo* bioassays. The ether extract of antagonistic endophyte also showed growth inhibition of *P. meadii* and the antagonistic fraction of the extract contains a cocktail of antimicrobial compounds. This study clearly indicated that endophytic bacteria of *H. brasiliensis* offer great potential as antagonistic agents against *P. meadii*.

REFERENCES

Sturz AV, Christie B R and Novak J (2000).Bacterial endophytes: Potential role in developing sustainable systems of crop production. *Crit Rev Plant Sci.*, 19:1-30.