ABSTRACT

ANTIMICROBIAL ACTIVITY OF FUNGAL ENDOPHYTES FROM SOME PLANTS

RAKSHITH D

Under the guidance of Dr. S. Satish

Department of Studies in Microbiology, University of Mysore, Manasagangotri,

Mysore- 570 006

Present study was undertaken with the aim of isolation and characterization of antimicrobial metabolite from fungal endophytes isolated from medicinal plants. Two hundred thirty eight fungal endophytes were isolated from stem and leaf tissues of *Ficus pumila* Linn. (Moraceae) and *Mirabilis jalapa* Linn. (Nyctaginaceae) in 3 different seasons during summer, monsoon and winter during 2010-2011 from Mysore, Karnataka, southern India. Isolated endophytic fungal isolates were categorized into 23 taxa, comprising 3 ascomycetes genera (species of *Chaetomium Sporormia* and *Xylaria*), 5 coelomycetes genera (Species of *Colletotrichum*, *Pestalotiopsis*, *Phoma*, *Phomopsis* and *Phylllosticta*), 11 hyphomycetes genera (species of *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Myrothecium*, *Nigrospora*, *Penicillium* and *Trichoderma*), 2 Zygomycetes genera (species of *Mucor* and *Rhizopus*), 2 morphospecies of *Mycelia sterilia*. The recovery of endophytes was higher in leaf tissue than in stem tissues. The diversity was more in monsoon and winter, where as species richness was higher in *Mirabilis jalapa* during summer season.

Species of *Fusarium* and *Phomopsis* were predominant in stem tissues of *Mirabilis jalapa*, whereas species of *Fusarium*, *Pestalotiopsis* and *Xylaria* were predominant genera in stem and leaf tissues of *Ficus pumila*.

Twenty five antimicrobially active endophytic fungal isolates were selected out of 238 isolates by dual agar culture assay during primary screening. Five potent endophytic fungal isolates (3 species of *Xylaria*, and one species each of *Pestalotiopsis* and *Phomopsis*) were selected from *Ficus pumila* due to their broad spectrum antimicrobial activity against human and phytopathogenic bacteria and fungi during secondary screening.
To confirm the reliability of morphological identification, selected bioactive endophytic fungal strains were subjected to molecular identification based on amplification of rDNA-ITS fragment and phylogenetic analysis. The characterization of bioactive endophytic fungi by traditional techniques and keys, coupled with a DNA based molecular approach led to the identification of 5 bioactive endophytes belongs to 3 different genera such as Pestalotiopsis, Phomopsis and Xylaria.

A functional gene-based molecular screening strategy was used to target nonribosomal type I polyketide synthase (PKS) genes in five bioactive fungal endophytes by employing three pairs of degenerate primers. Polyketide synthase gene related to melanin synthase and non reduced polyketide synthase was detected in Phomopsis and three species of Xylaria. Bioinformatics analysis of these biosynthetic pathways facilitated the inference of the potential bioactivity of endophyte natural products suggesting that the isolated endophytes are may be capable of producing of bioactive secondary metabolites of polyketide origin.

Based on genome screening and bioautography, two potent Xylaria species viz., FPL-10(S) and FPL-25(M) were selected for chemical investigation. Two fractions at R_f value 0.45 and 0.78 showed zone of inhibition for both bacteria and fungi on thin layer chromatogram developed from bioactive fraction obtained by chromatographic purification by column and TLC. LC-MS analysis of two fractions exhibited major ESI-MS molecular ion peaks at m/z 181 and 213. IR spectral data indicated strong signals for ketones in the range 1600 -1700 cm⁻¹, hydroxyl functional groups (3000-3600 cm⁻¹), O-CH₂ (methoxy) and O-C₂H₅ stretching at 1100-1200 cm⁻¹, for CN stretching at 2336.34 cm⁻¹ and for ester (C=O) at 1636 cm⁻¹ revealed aromatic and aliphatic nature of the metabolites. Based on the prominent number of protons and carbons from ¹H and ¹³C NMR spectral data together with above mentioned HPLC, LC-MS and IR analyses, the antimicrobial metabolites were identified as benzoic acid derivatives viz., 4-cyanomethoxy benzoic acid and a xylobovide 9-methyl ester. Detection of polyketide synthase gene in the genome of bioactive endophytic fungal species of Xylaria might explain biosynthetic origin of isolated antimicrobial metabolites via polyketide pathway.
Minimum inhibitory concentrations (MIC) were between 3.13 to 25.0 µg/ml for Gram-negative bacteria, opportunistic pathogen (*Candida albicans*) and dermatophytes which is closer to the co-assayed standard drugs nystatin (0.10-3.13 µg/ml) and gentamicin (0.10-0.78 µg/ml) for 4-cyanomethoxy benzoic acid metabolite of *Xylaria* sp. FPL-10(S).

Similarly, minimum inhibitory concentrations were 3.13-100.0 µg/ml against Gram-negative bacteria, opportunistic pathogen (*Candida albicans*) and dermatophytes which is closer to the co-assayed standard drugs nystatin (0.10-3.13 µg/ml) and gentamicin (0.10-0.78 µg/ml) for xylobovide 9-methyl ester isolated from *Xylaria* sp. FPL-25(M).

Significance of the present investigation includes the first report on the isolation and identification of endophytic *Xylaria* sp. from *Ficus pumila*. Present study is also a first report on the antimicrobial activity of Xylobovide 9-methyl ester isolated from *Xylaria* and antimicrobial activity of Xylobovide 9-methyl ester and 4-cyanomethoxy benzoic acid against human pathogenic fungi. Detection of PKS gene during genome mining responsible for the production of polyketides (Xylobovide 9-methyl ester and 4-cyanomethoxy benzoic acid) in *Xylaria* sp. which is a significant finding also represents the first report. The sequences were deposited in Gen Bank with an accession number KC579364 and KF147932.

Antimicrobial potentialities of both the compounds isolated from *Xylaria* sp. were comparable and almost nearer to the MIC of the standard drugs co-assayed and hence the compounds isolated could be of useful in exploitation for drug development.