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Original Article

Screening for antimicrobial activities of endophytic fungi isolated from ripened fruit of *Capsicum Frutescence L.*

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ABSTRACT

This study is aimed to investigate potential antimicrobial activity of endophytic fungi isolated from ripened fruit of *Capsicum frutescence L*. Endophytic fungi (AVNR-1, AVNR-2, AVNR-3, AVNR-4, AVNR-5) isolated from red fruit of chill were screened for antimicrobial activity. Ethyl acetate extract of five isolates showed antimicrobial activity against two Gram positive and two Gram Negative bacteria. Five endophytic fungi showed variation in their antimicrobial activity against *Streptococcus pyogenes* (ATCC 12344), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC14028) and *Pseudomonas aeruginosa* (ATCC 27853) in terms of Zone of inhibition (mm).AVNR-2 isolate showed higher antimicrobial activity followed by AVNR-3,AVNR-1, AVNR-4 and AVNR-5.AVNR-1, AVNR-2, AVNR-3, AVNR-4, AVNR-5 were tentatively identified as *Aspergillus melleus* AVNR-1 (KM389203) *Emericella* sp AVNR2 (KM389204), *Aspergillus flavus* AVNR3 (KM389202),*Aspergillus flavus* AVNR4 (KM389205),*Penicillium capsulatum* AVNR5 (KM389207) by 18s rRNA partial sequence and deposited in GENBANK of NCBI with respective accession numbers. Ethyl acetate extracts of five endophytic fungi were screened for antimicrobial against two Gram positive and two Gram Negative bacteria.). Further, investigation of identification of lead molecule by fractionation will be needed for exploration of novel antibiotic principle from endophytic fungi.

Key words: antimicrobial, endophytic fungi, antibiotic-resistant, *Capsicum frutescence L.*

INTRODUCTION

World health problems caused by drug-resistant bacteria are increasing as a result an intensive search for newer and effective antimicrobial agents is needed [1]. Exploitation of medicinal plants for extraction of antimicrobial agents of plant origin and limitations of plant resources due to various factors like requirement of land for cultivation, environmental competence of plants, seasonal specificity etc. and screening of the natural products has become costly and laborious process. Search for new and effective antimicrobial agents is becoming a necessity [2]. Therefore worldwide there is an increased interest in searching novel bioactive compounds having high effectiveness, low toxicity and negligible environmental impacts. Microbes have been an abundant source of novel chemo-types and pharmacophores from thousands of years. Various traditionally used medicinal plants are being studied worldwide for their ability to host endophytic fungi having antimicrobial potential. Most of the endophytes are known to possess biosynthetic capabilities greater than the host plant due to their long co evolution and genetic recombination [3].Distinctly from plants, endophytic fungi can be cultured quickly and the production of bioactive compounds can be increased by biotechnology of endophytic fungi in order to meet demands while keeping biodiversity and sustainable ecosystem [4].Endophytic fungi have received attention of the scientific community due to their capacity to produce novel bioactive compounds [5, 6,7,8]. A recent comprehensive study has indicated that 51% of biologically active substances isolated from endophytic fungi were previously unknown. Many endophytic fungi have the ability to produce antimicrobial substances [9, 10, 11, 12, 13, 14, 15, 16, 17]. Endophytes are the microorganisms which are present in living tissues of various plants, establishing mutual relationship without apparently any symptoms of diseases [16, 18]. Endophytic fungal strains are also found to be potentially useful in the production of pigments, bioactive metabolites, immunosuppressant, anticancer compounds and bio-control agents [19,

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20, 21]. Therefore screening of antimicrobial compounds from endophytes is a promising way to meet the increasing threat of drug-resistant strains of human and plant pathogens. This study aimed to evaluate antimicrobial activity of endophytic fungi isolated from ripened fruit of *C frutescence L*.

MATERIALS AND METHODS

Collection of plant material: Ripened *C frutescence L.* fruits were collected from field at Bapatla, Guntur district, A.P. India and fresh fruits were used for isolation of endophytic fungi.

Isolation of endophytic fungi: Endophytic fungi were isolated as per the procedure [22] with modification and the whole process of endophytic fungi be carried out aseptic condition [23]. Samples were washed in running tap water to remove dust and debris dried in air and then cut into 1 cm segments. Surface sterilization was done with 95% ethanol, 4% sodium hypochlorite, 0.1% HgCl2, HCHO, 20% H2O2 respectively as follows. Sample segments were soaked in 95% ethanol for 1 min. and washed in sterile distilled water three times and dried in a laminar air flow chamber [24] Segments were soaked 10% sodium hypochlorite solution for 3 min, subsequently washed in sterile distilled water three times and dried in a laminar air flow chamber. Segments were soaked in 0.1% mercuric chloride the samples were subsequently washed in sterile distilled water three times and dried in a laminar air flow chamber. Segments were soaked in formalin for one min and samples were subsequently washed in sterile distilled water three times and dried in a laminar air flow chamber. Segments were soaked in 20% hydrogen peroxide for one min and were subsequently washed in sterile distilled water three times and dried in a laminar air flow chamber. After sterilization fruit tissue is homogenized and homogenized sample is diluted according to the standard serial dilution method and cultured on Sabouraud Dextrose Agar medium by spread plate method.

Morphological and Molecular identification: The Isolate was inoculated and incubated at 35°C for 7 days. Colonies were compared for their overall color and color of conidia, reverse color, texture, zonation and sporulation. Further the isolate was also subjected to microscopic analysis for it characterization and identification [25, 26]. Genotypic identification was carried out by PCR amplification and partial sequencing of the rDNA for the confirmation of morphological identity. ITS regions were amplified by PCR with primers forward (ITS1-5'-TCC GTA GGT GAA CCT GCG G-3') and reverse (ITS4-5'-TCC TCC GCT TAT TGA TAT GC-3') primers [32]. Comparative study of other rDNA sequences with rDNA sequence of isolates was done using BLAST algorithm at the website http://www.ncbi.nlm.nih.gov. The nucleotide sequence of isolated has been assembled and submitted at the NCBI GenBank [27].

Extraction of Ethyl acetate extract: Endophytic fungi isolated. were grown in 250ml Erlenmeyer flasks containing 100ml of Sabouraud Dextrose medium then incubated for one week days at 35° C. After incubation the fermented whole broth was filtered through three layers of cheesecloth to separate supernatant and mycelia. The supernatant was used for the extraction of secondary metabolites by addition of equal amount of ethyl acetate. Culture filtrates with the ethyl acetate were transferred to separating funnel and then it was kept for 24hrs. Intermediate shaking of the culture filtrate with solvent enhances the better extraction of secondary metabolites. The ethyl acetate extract was separated from broth medium and crude extract was concentrated by using a rotary evaporator under reduced pressure. Concentrated crude extract which was then dissolved in dimethyl sulfoxide (DMSO) and stored at 4°C to be used as stock solution for antimicrobial assay [28].

Screening the Antimicrobial activity of endophytic fungi: Ethyl acetate extracts of selected five endophytic fungi were subjected to screening for antimicrobial activity against the human pathogenic bacteria *S. pyogenes* (ATCC 12344), *S. aureus* (ATCC 25923), *S. typhimurium* (ATCC14028), *P. aeruginosa* (ATCC 27853) was performed by using well diffusion method [25]. The test was done in triplicates.

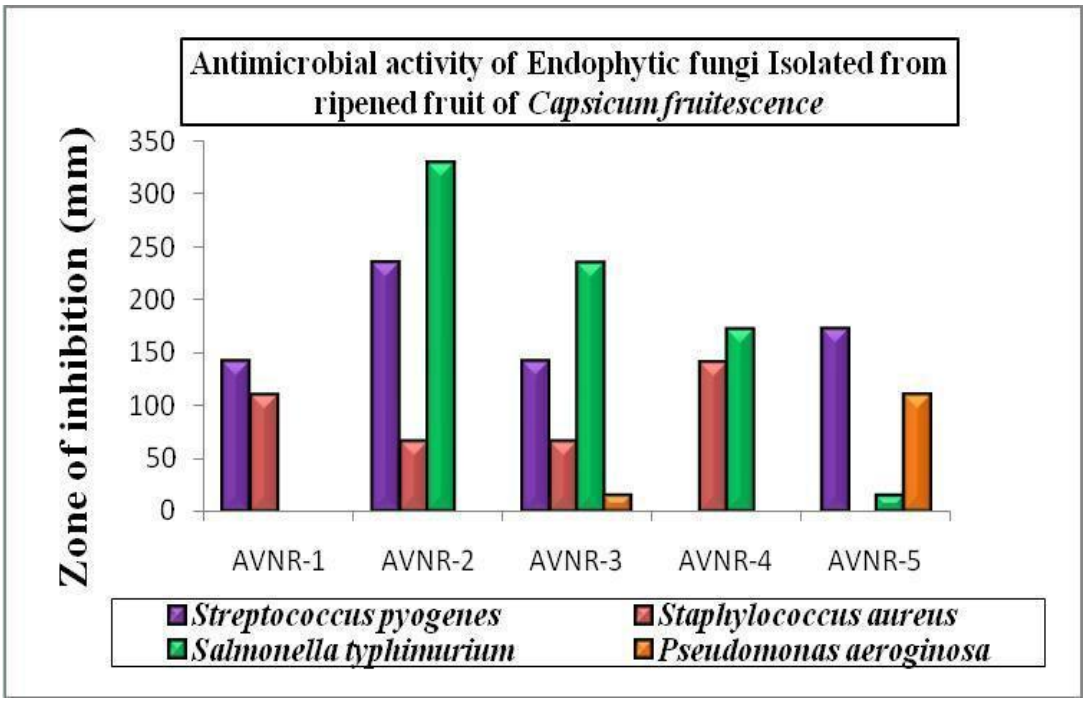
Statistical analysis: Statistical analysis was performed using one way ANOVA followed by statistical software package Statistica 5.5, Studentis t test and $p < 0.05$ was used as the level of significance. All samples were prepared in triplicate.

RESULTS

Five endophytic fungi (AVNR1, AVNR2, AVNR-3, AVNR-4, AVNR -5) showed variation in their antimicrobial activities (Figure-2). AVNR-2 showed highest inhibition against *Streptococcus pyogenes* followed by AVNR-1, AVNR-3 and AVNR- 5, AVNR - 4 showed negative response against this Gram +ve pathogen. AVNR-4 showed highest inhibition against *Staphylococcus aureus* than AVNR1 whereas AVNR2 and AVNR-3. AVNR -5 showed negative response. Highest antibiotic potential was observed in AVNR-2, AVNR – 3, AVNR- 4against*Salmonella*

typhimurium, a gram –ve bacteria. Antibiotic activity was not observed in AVNR-1. AVNR-1, AVNR-2, AVNR-4 has not shown inhibition against on *Pseudomonas aeruginosa* gram –ve bacteria whereas AVNR-3 and AVNR-5 showed potential antibiotic response against *Pseudomonas aeruginosa*. Streptococcus pyogenes inhibition was observed to be highest in response toAVNR-5 [Figure 1].

Figure 1: Antibacterial activity of ethyl acetate extracts of endophytic fungal Isolates



Based on Colony morphology and Morphological characters of mycelium, conidia and conidiophores Five Endophytic fungi[AVNR-1, AVNR-2, AVNR-3, AVNR-4 and AVNR-5] isolated from red fruit of chilli.AVNR-1, AVNR-3, AVNR-4 were putatively identified as genus *Aspergillus*,AVNR-2 as genus *Emericella* and AVNR-5 as *Penicillium.* Based on 18s DNA partial gene sequence analysis and similarity studies these five endophytic fungi were identified and deposited in GEN BANK of NCBI as shown in Table-1

S.No	Genus	18s DNA partial sequence		
		Similarity	Name	Accession Number
AVNR-1	*Aspergillus*	99%	*Aspergillus melleus*	EF661426.1
AVNR-2	*Emericella*	99%	*Emericella Sp*	JN672587.1
AVNR-31	*Aspergillus*	99%	*Aspergillus flavus*	EF4098071
AVNR-4	*Aspergillus*	99%	*Aspergillus flavus*	LN482516.1
AVNR-5	*Penicillium*	99%	*Penicillium capsulatum*	JX841240

DISCUSSION

Discovery of endophytic fungi in plant tissues opened up new possibilities in the search for metabolically active compounds. Endophytic fungi represent an immense source of bioactive substances with immune-stimulatory response make them very potent natural supplements in cancer therapy. The activities of various endophytic fungal species are worth investigation in order to establish potent bioactive compounds. In this study preliminary assessment was performed for the

antibacterial activity of endophyte fungi isolated from ripened fruit of *C. frutescence L*. The bioactive abilities of endophytic fungi were not identical, even though they were isolated from the same host species. Ethyl acetate extracts of endophytic fungi isolated from same ecological niche showed antibiotic activity with different degree of variation (Fig 1). It was observed that AVNR-2, AVNR-3, and AVNR-5 have shown broad Spectrum antibiotic activity and assumed to be highly potential with novel antimicrobial secondary metabolites. Except AVNR-4 remaining four isolates (AVNR-1, AVNR-2, AVNR-3, AVNR-5) showed antimicrobial activity against *Streptococcus pyogenes*. It is also observed that except AVNR-5, remaining four isolates (AVNR-1, AVNR-2, AVNR-3, AVNR-4) all endophytes showed positive inhibition against *Staphylococcus aureus*. In case of gram-ve bacterial pathogens except AVNR-1, rest of the four endophytes showed potential activity against *Salmonella typhimurium* and AVNR-3 and AVNR- 5 showed positive inhibition on *Pseudomonas aeruginosa*. Antibiotic activity of Aspergillus melleus [AVNR-1] is the first time reports as per our knowledge. Antimicrobial activity of AVNR-2 is similar to the study of previous reports [30].AVNR-3 and AVNR-4 showed highest potential of antibiotic activity than Aspergillus flavus reported earlier [29]. Similarly AVNR-5 also showed potential antibiotic response against Gram +ve and Gram –ve bacteria so far studied [30, 31, 32, 33, 34, 35, 36].

CONCLUSION

Antibiotic activity of endophytic fungi isolates from chilli red fruit are highly specific in response to Gram +ve and Gram –ve bacteria. Further investigation of identification of lead molecule by fractionation will be needed for exploration of novel antibiotic principle from endophytic fungi.

Conflict of interests: The authors declare that there is no conflict of interest regarding the publication in this paper

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