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Endophytic fungi of decaying vegetable wastes

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Only 7% of the total estimated 1.5 million fungal species has been described so far and species documentation on new substrates will add up to our knowledge of fungal diversity in a region. The present study was conducted to evaluate the diversity of endophytic fungi on two decaying vegetable wastes viz., *Euryale ferox* Salisb. (Fox – Nut) and *Bambusa arundinaceae* Willd. (Edible Bamboo). A total of 32 fungal species including 3 sterile and 3 unidentified forms were isolated. Out of the total species isolated 12 were found to be common to both the substrates : *Aspergillus flavus, A. niger, Cladosporium cladosporioides, Fusarium oxysporum. F. semitectum, Paeceilomyces* sp., *Rhizopus nigricans, Penicillium expansum, Trichoderma koningii, T. virens* and sterile brown. Of which 9 were specific to *Euryale ferox : Alternaria alternata, Aspergillus candidus, A. saccharii, Fusarium poae, Mucor sylvaticus, Nigrospora oryzae, Penicillium brevicompactum,* Sterile yellow and Unidentified 1, while the remaining 11 species were specifically isolated from *Bambusa arundinaceae : Aspergillus fumigatus, Chaetomium globosum C. funicolum, Fusarium* sp., *Humicola* sp., *Nigrospora sphaerica, Pestalotia* sp., *Penicillium rugulosum, Trichoderma longibrachiatum,* Unidentified 2 and Unidentified 3.

Key words : Endophytic fungi, vegetable wastes : *Euryale ferox, Bambusa arundinaceae*

INTRODUCTION

The fungal kingdom is hyperdiverse and is estimated as representing 1.5 million species (Hawksworth, 2004). However, only 7% of this estimated figure has been described so far. Wastes like leaf litters, industrial wastes, crop residues etc. contain considerable quantity of organic matter and nutrients (Cooper and Golueke, 1977) and these house for excellent growth of microorganisms especially fungi. By far the greatest number of fungi are found on living and dead plants. Infact, the saprophytic fungi represent the largest proportion of fungal species in soil and they perform a crucial role in decomposition as well as nutrient cycling (Shukla and Tripathi, 2007). In addition, endophytic fungi also are the largest reservoirs of fungal species (Dreyfuss, 1989).

Studies on fungal diversity and succession on plant substrate have received renewed attention during recent years as they have immense potential in industrial mycology (Lodge, 1997). The basic objective being identifiction of as many rare species as possible for screening them for production of biologically active novel compounds (Hyde, 2001). Determining the magnitude and pattern of fungal species diversity has been an ongoing challenge for mycologists (Hawksworth, 1991; Hawksworth and Mueller, 2005; Schmit and Mueller, 2007) because they are often ephemeral and cryptic which makes them difficult to inventory.

Ecologists has also interest on the relationship between biotic diversity and ecosystem functional. Without an estimate of fungal diversity it is difficult to determine the level of redundancy in ecosystem functions provided by fungi. Therefore, the present study has been conducted to determine the diversity of endophytic fungi associated with selected decaying vegetable wastes in Manipur, North East India.

MATERIALS AND METHODS

Study site

The study was conducted in the experimental plot of the Department of Life Sciences, Manipur University, Canchipur, Imphal located at 24°45'259"N latitude and 90°55'690"E longitude and at an elevation of 768 msl (Etrex, 12 channel, GPS). The climate of the area is monsoonic with distinct rainy, winter and summer seasons in a year. The mean minimum temperature ranged between 5.6° to 20.9°C and mean maximum temperature varied between 23.1° to 31.6°C from Novermber, 2008 to June, 2009. The relative humidity varied from 47.1% to 78.9%. However, in this particular study period, much delayed monsoon was encountered with a total rainfall of 352.8 mm of which about 63% occurred in last two months of the study period i.e. May-June, 2009.

Study materials

Vegetable wastes viz. peels of *Euryale ferox* Salisb. (Fox-Nut) belonging to the family Nymphaceae and sheath of young shoot of *Bambusa arundianceae* Willd. (Edible bamboo) belonging to the family Poaceae were used for the present investigation.

Collection

The samples were collected from different households. These were sorted out and air dried.

Decomposition

Vegetables waste samples were allowed to decay using nylon mesh bag technique as described by Bocock *et al.,* (1960). A total of 70 nylon net bags (10×15 cm, 1 mm mesh) containing 5 g dried samples each @ 35 bags per sample were prepared and placed randomly in soil bed in the experimental plot on 15th of Novermber, 2008. Five bags per sample containing decaying samples were recovered at monthly intervals.

After recovery, the bags were brought to the laboratory in separate sterile polythene bags where the samples of each bag after brushing carefully to remove the adhering soil particles were processed for the isolation and evaluation of endophytic fungi.

Isolation, purification and identification

Two surface sterilization techniques were employed for isolation of endophytic fungi associated with the decaying vegetable samples : (i) surface sterilization using 15% H_2O_2 and 70% ethanol (Kinkel and Andrews, 1988), and (ii) surface sterilization using 1% $AgNO_3$ and 1% NaCl (Wildman and Parkinson, 1979).

Five surface sterilized sample bits were placed equidistant in each Petriplate containing 20 ml solidified PDA (supplemented with streptomycin 150 mg/l) medium. The plates were incubated at 25±1°C for 7 days and the fungal colonies developing on the sample bits were isolated, purified and identified at least up to the genus using standard literatures (Thom and Raper, 1945; Raper and Thom, 1949; Gilman, 1957; Rifai, 1969; Subramanium, 1971; Barnett and Hunter, 1972; Ellis, 1971 & 1976; Ellis and Ellis, 1985; Watanabe, 2002; Leslie and Summerell, 2006, etc.) and were confirmed at the Agarkhar Research Institute, Pune Five replicates were maintained in each case.

Calculation

Mean % frequency of occurrence of the fungal species were calculated using the following formula :
Frequency of Occurrence (%) =

$$\frac{\text{No. of sample bits on which a fungal species occurred}}{\text{Total no. of sample bits observed}} \times 100$$

RESULTS AND DISCUSSION

The % frequency of occurrence of fungal species isolated from the study samples by two surface sterilization techniques has been presented in Tables 1, 2, 3 and 4. Overall 32 fungal species belonging to 13 general mostly representing the Hyphomycetes group were isolated. Out of the 32 species 3 were sterile forms designated as white sterile, brown sterile and yellow sterile while another 3 spp. remains unidentified. Of the total species isolated 12 were found to be common to both the study materials : *Aspergillus flavus, A. niger, Cladosporium cladosporioides, Fusarium oxysporum, F. semitectum, Paeceilomyces* .sp., *Rhizopus nigricans, Penicillium expansum, Trichoderma koningii, T. virens* and sterile brown; 9 were specific to *Euryale ferox : Alternaria alternata, Aspergillus candidus, A. sacchari, Fusarium poae, Mucor sylvaticus, Nigrospora oryzae, Penicillium brevicompactum,* Sterile yellow and Unidentified 1, while the remaining 11 were specifically isolated

Table 1 : Monthly variation in mean frequency of occurrence (%) of endophytic fungi on decaying *Euryale ferox* waste isolated by surface sterilization using 1% $AgNO_3$ and 1% NaCl

Fungi	Nov'08	Dec'08	Jan'09	Feb'09	Mar'09	Apr'09	May'09	Jun'09
Aspergillus candidus				20	4			
A. flavus		92						
A. niger	8				4	80	100	100
A. sacchari	16				4			
Cladosporium cladosporioides		100	80				80	100
Fusarium oxysporum				80	100	100	96	100
F. poae		16	100					
F. semitectum					100	100	80	40
Mucor sylvaticus				100				
Nigrospora oryzae			30					
Paeceilomyces sp.		4	4					
Penicillium brevicompactum		8						
Rhizopus nigricans	100	100	100	100	100	8		
Sterile white						72	80	
Sterile yellow							100	100
Trichoderma koningii						32	32	68
T. virens						100	100	100
Unidentified 1	36							
Total no. of fungal Species	4	6	5	4	6	7	8	7

Table 2 : Monthly variation in mean frequency of occurrency (%) of endophytic fungi on decaying *Euryale ferox* waste isolated by surface sterilization using 15% H_2O_2 and 70% ethanol

Fungi	Nov'08	Dec'08	Jan'09	Feb'09	Mar'09	Apr'09	May'09	Jun'09
Alternaria alternata					20			
Aspergillus candidus	12	–	–	20	4	–	–	
A. flavus	–	44						
A. niger		4	4	4	72	100	100	100
A. sacchari	24					4		
Cladosporium cladosporioides		100	100				72	100
Fusarium oxysporum				80	80	100	100	100
F. poae		100	100					
F. semitectum					100	100	68	32
Mucor sylvaticus	100							
Nigrospora oryzae							96	12
Paeceilomyces sp.		12	4					
Penicillium brevicompactum				4				
Rhizopus nigricans		100	100	100	92	16		
Sterile brown						40	32	
Sterile white							100	100
Sterile yellow						100	100	100
Trichoderma koningii				100				
T. virens	12				100	100	100	100
Total no. of fungal Species	4	6	5	5	8	8	9	8

Table 3 : Monthly variation in mean frequency of occurrence (%) of endophytic fungi on decaying *Bambusa arundinaceae* waste isolated by surface sterilization using 1% $AgNO_3$ and 1% NaCl

Fungi	Nov'08	Dec'08	Jan'09	Feb'09	Mar'09	Apr'09	May'09	Jun'09
Aspergillus candidus		4						
A. fumigatus	100	100	100					
A. niger				20	96	100	100	100
Chaetomium globosum				8				
Cladosporium cladosporioides				4	16	4		
Fusarium oxysporum							100	92
F. semitectum						80	12	
Nigrospora sphaerica							100	64
Penicillium expansum					32		4	
P. rugulosum							36	100
Rhizopus nigricans			24	100				
Sterile brown		8						
Sterile white					100	60	4	
Trichoderma koningii								32
T. longibrachiatum			80	64	100	100	100	100
T. virens				24				
Total no. of fungal Species	1	3	3	6	5	5	8	6

Table 4 : Monthly variation in mean frequency of occurrence (%) of endophytic fungi on decaying *Bambusa arundinaceae* waste isolated by surface sterilization using 15% H_2O_2 and 70% ethanol.

Fungi	Nov'08	Dec'08	Jan'09	Feb'09	Mar'09	Apr'09	May'09	Jun'09
Aspergillus fumigatus	100	100		16				
A. niger					32	100	100	100
Chaetomium funicolum		4						
Cladosporium cladosporioides							4	8
Fusarium oxysporum					100	100	100	100
F. semitectum			24	64			20	4
Fusarium sp.				20				
Humicola sp.	8							
Penicillium expansum			4					16
P. rugulosum			20					32
Paeceilomyces sp.						4	24	
Pestalotia sp.				16				
Rhizopus nigricans			100					
Sterile white					48			
Trichoderma longibrachiatum					100	100	92	
T. virens					100	100	100	100
Unidentified 2				20				
Unidentified 3				20				
Total no. of fungal Species	2	2	4	6	5	5	7	7

from *Bambusa arundinaceae* : *Aspergillus fumigatus, Chaetonium globosum, C. funicolum, Fusarium* sp., *Humicola* sp., *Nigrospora sphaerica, Pestalotia* sp., *Penicillium rugulosum, Trichoderma longibrachiatum,* Unidentified 2 and Unidentified 3. Fungal composition in the two substrates, thus showed quite a significant variation in the present investigation. Host specificity may be due to the fact that generally different plant species have a different chemical composition and this may have affected the microbial community composition and biomass (Mille – Lindbolm *et al.,* 2006). Again, fungal species isolated by the two sterilization methods showed only slight variation with *Penicillium brevicompactum* and Unidentified 1 having isolated from *E. ferox* in surface steriliztion using 1% AgNO, while *A. alternata, P. expansum* and Sterile brown were isolated when the same substrate was sterilized with 15% H_2O_2. On the other hand 1% $AgNO_3$ surface sterilization of *B. arundinaceae* could be tolerated by species like *A. flavus, C. globosum, N. spherica, T. koningii* and Sterile brown mycelium while species like *C. funicolum, Fusarium* sp., *Humicola* sp., *Paecilomyces* sp. along with the two Unidentified spp. were isolated when the substrate surface was sterilized with 15% H_2O_2.

In terms of % occurrence *A. niger, F. oxysporum* and *R. nigricans* were the most frequently encountered species during the 8 months study of *E. ferox* decay while *A. niger* and *T. longibrachiatum* occurred most frequently in *B. arundinaceae.* Effectiveness of the sterilization techniques used in the study could not be established as the pattern of occurrence of the fungal species in both the substrates in both the sterilization methods remains vague. A single isolation method, is therefore, not preferred because a significant section of the fungal population may be missed when a single isolation method is adopted. Development of techniques to assess the presence of microorganisms within the substrate that do not rely on direct observation of fruiting bodies and culturability are essential to make accurate estimates of fungal biodiversity (Jones and Hyde, 2002). In order to gain a better understanding of fungal diversity, we should continue to concentrate on studying the fungal communities in selected habitats and substrates, especially those that appear to support high diversity and also explore understudies or unstudied habitats and substrates (Tang *et al.,* 2007). The patterns of biodiversity among endophytic fungi are complex both in space and time and these patterns cannot be fully resolved within one vegetation period (Unterseher *et al.,* 2007). Since very little is known about the diversity of fungi, especially endophytes inhabiting decaying vegetable wastes more detailed study is needed before a concrete conclusion is drawn.

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