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Biodiversity and seasonal distribution of fungal species in some soils of Odisha

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ABSTRACT

In this study, various soil samples of Odisha were evaluated for presence of instinctive fungal community. Edaphic properties of the soil were evaluated from February to June, 2011. There was not much disparity evident in temperature and pH of the soil samples. The water content was found steadily diminished from February to May. Water holding capacity was 67 % and 66.9 %, respectively. Organic carbon, organic matter, total nitrogen and phosphorous contents were 0.26 %, 0.45 %, 0.2g/kg % and 0.38mg/kg respectively. Soil mycoflora enumeration revealed utmost numbers of fungi including *Aspergillus* species from soils of the botanical garden of P.G. Department of Botany (3×10^8 CFU/g) followed by Mancheswar Industrial Estate (1×10^8 CFU/g). The soils of Joda Industrial Estate and Barbil Industrial Estate of Keonjhar were also affluent in fungal community. Among all 35 fungal taxa, *Aspergillus niger* was the most predominant microflora followed by *Aspergillus terreus*. A detailed investigation of mycoflora was undertaken from the garden soil, Department of Botany, Utkal University. Other fungal species like *Alternaria alternata*, *Aspergillus candidus, A. flavus, A. fumigatus, A. stellatus* and *Aspergillus* sp. were more prevalent in summer. The utmost incidence of *Fusarium oxysporum, Aspergillus niger, Aspergillus terreus* and *Mucor* sp. in this environment was found in rainy season. Species like *Aspergillus terreus, A. oryzae, A. niger, A. fumigatus*, *A. awamori*, *Penicillium* sp., *Mucor* sp. and *Trichoderma* sp. were abundant in winter. However, throughout the year maximum numbers of *Aspergillus* spp., *Alternaria* spp. and *Penicillium* spp. were observed.

1. Introduction

Commencing from the poles to equators, arid region to tropical and subtropical zones, fungi are plentiful in environment and display various inimitable characters to the climatic changes. More than one million species of fungi are estimated and numerous of the species are yet to be identified (Hawksworth, 1991). India possesses 27,000 species, the most prevalent biotic community after insects (Sarbhoy *et al*., 1996). About 205 new genera have been illustrated from India, of which 32% were credited to C. V. Subramanian of the University of Madras. To that, 12 new genera, 60 new taxa and 500 new fungi were contributed by Manoharachary *et al*. (2005). India contributes one third of fungal diversity of the entire globe. From 1.5 million fungi, only 50 % are typified and only 5–10% of fungi can be cultured *in vitro*. Besides to their magnificence, fungi perform vital role in human welfare with their exploitation in industry, agriculture, pharmaceuticals, food industry, textiles, bioremediation, natural cycling, as biofertilizers, secondary metabolite production, industrial enzymes (e.g. amylases, cellulases, lipases, glucoamylases, pectinases, phosphatases and proteases) and in countless other ways. Now-a-days, mycobiotechnology has become an indispensable part of the human wellbeing (Manoharachary *et al*., 2005). The interactions of fungi with other organisms have also played a vital role in the evolution of microorganisms, plants and animals.

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In spite of their noteworthy significance, the taxonomy of fungi is still not absolutely resolved and the species distribution in soil is not completely reported, though many fungi particularly Aspergilli are acknowledged to biosynthesize diverse groups of bio-active metabolites and enzymes. Keeping in view of the significance of fungal community, present study was undertaken to isolate, enumerate and to report about the abundance of aboriginal fungal strains as candidates to explore their prospective potentials for industrial exploitations.

2. Materials and methods

2.1 Chemicals and media

All the chemicals and microbiological grade media components implemented in the present study were of analytical reagent grade and purchased from Sigma Chemicals Co. (USA), Hi-Media Limited, SRL Pvt. Limited and Merck India Limited (Mumbai, India).

2.2 Selection of the experimental field and Collection of the soil sample

Experimental fields (5×5 m^2) of Bhubaneswar (Patia, Mancheswar Industrial Estate, Ganga Nagar, BDA Colony and garden soil of P.G. Department of Botany, Utkal University) and Keonjhar (Joda and Barbil) were selected. Soil samples (1 Kg each) were collected during February to June, 2011 in two different parts. One part was used for soil analysis and other part for microbial analysis and enumeration as per Mueller and Durrell (1957) with slight modifications.

2.3 Edaphic properties of selected soil samples

Physical parameters studied constituted soil texture, temperature, water content, water holding capacity and pH while chemical parameters of soil such as organic carbon, organic matter, total nitrogen and available phosphorous were recorded.

2.4 Isolation and identification of potent microorganisms by morphological analysis

A total of 35 samples from approximately 7 kg of different polluted and garden soil were collected from the selected experimental fields. Samples were serially diluted; pour-plate technique (Sohail *et al*., 2009) was performed for isolation and the plates incubated for 5 days separately on Potato dextrose agar (PDA), Sabouraud's dextrose agar (SDA) and Czapek's Dox agar (CZA) amended with sefixime (50 mg/100ml) under sterile conditions at 25 °C and 37 °C for 7 days. Isolated fungi were identified as per Alexopoulos and Mims (1979) and Watanabe (2002) based on macro and microscopic characteristics. The isolates were characterized and reconfirmed by National Center for Fungal Taxonomy, New Delhi, India. All isolates were maintained as pure cultures on PDA slants.

2.5 Statistical analysis

All experiments were carried out in triplicates and repeated three times. The samples collected from each replicate were tested for isolation of microorganisms. Each value is an average of three parallel replicates. Data were analyzed using analysis of variance (ANOVA) for a complete randomized design. Duncan's New Multiple range test (DMRT) (Gomez and Gomez, 1984) was used to indicate means with significant differences at $p \leq 0.05$.

3. Results and discussion

3.1 Edaphic properties of soil and fungal isolates

Surface soil up to one foot depth is very prolific precinct that holds highest number of soil dwelling microorganisms and all the microbial processes are performed at this stratum. Hence, all the soil samples were collected in between one foot depth.

Prior to the analysis, the temperature of the soil was recorded twice in a month and average of each month is presented in Table 1 depicting February with minimum temperature (24 °C) and June with maximum temperature (39 °C). There was not much disparity recorded in temperature of the soil as no rain fall occurred during the investigation period. The value of water content was least in the month of June and maximum in February. Water holding capacity was only determined twice during the total study period and it was found to be 67 and 66.9 % respectively (Table 1). The pH of the soil was recorded in beginning of every month with no variations between the values from February to June. Organic carbon and organic matter content of the soil were found to be 0.26 % and 0.45 %, respectively. Total nitrogen and phosphorous content were 0.2 g/Kg and 0.38 mg/Kg respectively.

Highest numbers of fungi including *Aspergillus* species were isolated from the botanical garden soil of P.G. Department of Botany (3×10^8 CFU/g) followed by Mancheswar Industrial Estate (Table 2; Fig. 1) by employing the dilution plate techniques. Lower counts in different garden soils were reported by Fleet and Mian (1987). The soils of Joda Industrial Estate and Barbil Industrial Estate of Keonjhar were rich in fungal community. All the fungal isolates were identified by the assistance of laboratory experiences, consultation of certain monographic books and NCFT, New Delhi (Thom and Raper, 1945; Alexopoulos and Mims, 1979; Watanabe, 2002).

Table 1
Physical and chemical properties (edaphic properties) of soil[a] taken in the study

Soil parameters	February	March	April	May	June
Texture	Sandy loam				
Temperature (°C)[b]	24	29	31	36	39
Water content (%)	6.0	5.7	5.6	5.5	5.0
Water holding capacity (%)		67.0		66.9	
pH[b]	5.2	5.1	5.2	5.2	5.3
Organic carbon (%)			0.26		
Organic matter (%)			0.45		
Total nitrogen (g/Kg)			0.2		
Phosphorus content (mg/Kg)			0.38		

[a] Represents the botanical garden soil of Post Graduate Department of Botany, Utkal University, Bhubaneswar, India.

[b] Temperature and pH of the soil samples were recorded at 11: 00 am in the beginning of every month.

Table 2
Colony forming units (CFU/g) in polluted soils of eastern and north-east regions of the state-Odisha

Soil collection sites	Total number of soil samples collected	Positive samples with maximum isolation	Percentage of positive samples	Colony forming units in original sample (CFU/g)
BHUBANESWAR				
Patia Industrial Estate	35	23	65.7[d]	5×10^7
Mancheswar Industrial Estate	35	27	77.1[c]	1×10^8
Ganga Nagar	35	29	82.9[b]	2×10^7
BDA Colony, CS Pur	35	26	74.3[c]	2×10^7
Botanical garden[a]	35	34	97.1[a]	3×10^8
KEONJHAR				
Joda Industrial Estate	35	24	68.6[d]	2×10^7
Barbil Industrial Estate	35	30	85.7[b]	7×10^7

[a] Botanical garden represents the botanical garden soil of Post Graduate Department of Botany, Utkal University, Bhubaneswar, Odisha, India. Data pooled from a total of 3 separate experiments each comprising of 3 replicates. Means within a column with different superscripts are significantly different at p d≤ 0.05 tested through Duncan's New Multiple Range Test.

Fig. 1. Mixed fungal cultures isolated from different soil samples.

In all soil samples, *Aspergillus niger* was the most dominated species followed by *Aspergillus terreus* (data not shown). A total of 35 fungal taxa were isolated belonging to 9 genera (Table 3; Fig. 2). *Curvularia* and *Alternaria* have represented by two taxa, *Fusarium* by two taxa, *Penicillium* by four taxa, *Rhizopus* by 3 taxa and *Trichoderma* by only one species were recorded (Fig. 3). There were respectively two and three isolates of *Mucor* sp. and *Rhizopus* sp. recorded from the sub division Zygomycotina. Even after a short treatment with U.V. radiation, only one fungal species was found never sporulated in the agar medium and was in white sterile mycelium. The observations were not sufficient for the identification up to the species level, but substantiated their affiliation up to the genus level. A specific identification is also required, which is underway. Fungi represent an array of microorganisms that are extensively spread in environment including soil (Boer *et al.*, 2005). Previously, Duarte and Costa-Ferreira (1994) reported that *Aspergillus* was the most copious genus of soil fungi.

In this finding, *Alternaria alternata*, *Aspergillus candidus, A. flavus, A. fumigatus, A. stellatus* and *Aspergillus* sp. were more frequent in summer. The highest occurrence of *Fusarium oxysporum, A. niger, A. terreus* and *Mucor* sp. in soil of garden environment was recorded in rainy season. *Penicillium* sp., *Mucor* sp., *Trichoderma aureoviride, Aspergillus terreus, A. oryzae, A. niger, A. fumigatus* and *A. awamori* were more abundant in winter. Maximum numbers of isolations were of genus *Aspergillus, Alternaria,* and *Penicillium* during all three seasons (Table 3; Fig. 3). de Ana *et al*. (2006) reported similar findings while investigating on seasonal distribution of *Alternaria, Aspergillus, Cladosporium* and *Penicillium* species in homes of fungal allergic patients.

Table 3
Prevalence frequency and seasonal variation of fungal isolates in soil collected from botanical garden of the department

Name of the fungus	Seasonal Variation		
	Summer	Rainy	Winter
Alternaria alternata	+++ [ac]	++	+
Alternaria tenuis	+	+	++
Aspergillus awamori	-	+	+++ [a]
Aspergillus brevipes	-	+	+
Aspergillus candidus	+++ [b]	++	++
Aspergillus clavatus	+	+	++
Aspergillus flavus	+++ [b]	++	+++
Aspergillus fumigatus	+++ [bc]	++	+++ [a]
Aspergillus japonicus	+	-	++
Aspergillus kanagawaensis	-	++	+
Aspergillus niger	++	+++ [d]	+++ [d]
Aspergillus nidulans	-	+	++
Aspergillus parasiticus	-	-	++
Aspergillus oryzae	-	++	+++ [ac]
Aspergillus stellatus	+++ [ac]	+	++
Aspergillus sydowii	+	++	++
Aspergillus tamarii	-	++	++
Aspergillus terreus	++	+++ [ac]	+++ [d]
Aspergillus versicolor	-	+	++
Aspergillus species	+++ [d]	++	++
Curvularia lunata	-	+	++
Curvularia pallescens	+	+	+

Fusarium oxysporum	-	+++ [ac]	-
Fusarium sp.	-	+	+
Mucor hiemalis	-	++	+++ [a]
Mucor sp.	+	+++ [c]	+++ [b]
Penicillium sp.1	+	++	+++ [d]
Penicillium sp.2	+	++	+++ [d]
Penicillium sp.3	+	++	+++ [d]
Penicillium sp.4	+	++	+++ [d]
Rhizopus nigricans	-	+	++
Rhizopus sp.1	-	+	+
Rhizopus sp.2	-	+	++
Trichoderma aureoviride	-	++	+++ [ab]
White sterile mycelium *	-	+	++

-: absent; +: less; ++: moderate; +++: abundant. [a] Beginning of the season; [b] mid of the season; [c] end of the season; [d] through out of the season. * The species was not sporulated in the agar medium even after a short treatment with UV radiation and was noted as white sterile mycelium.

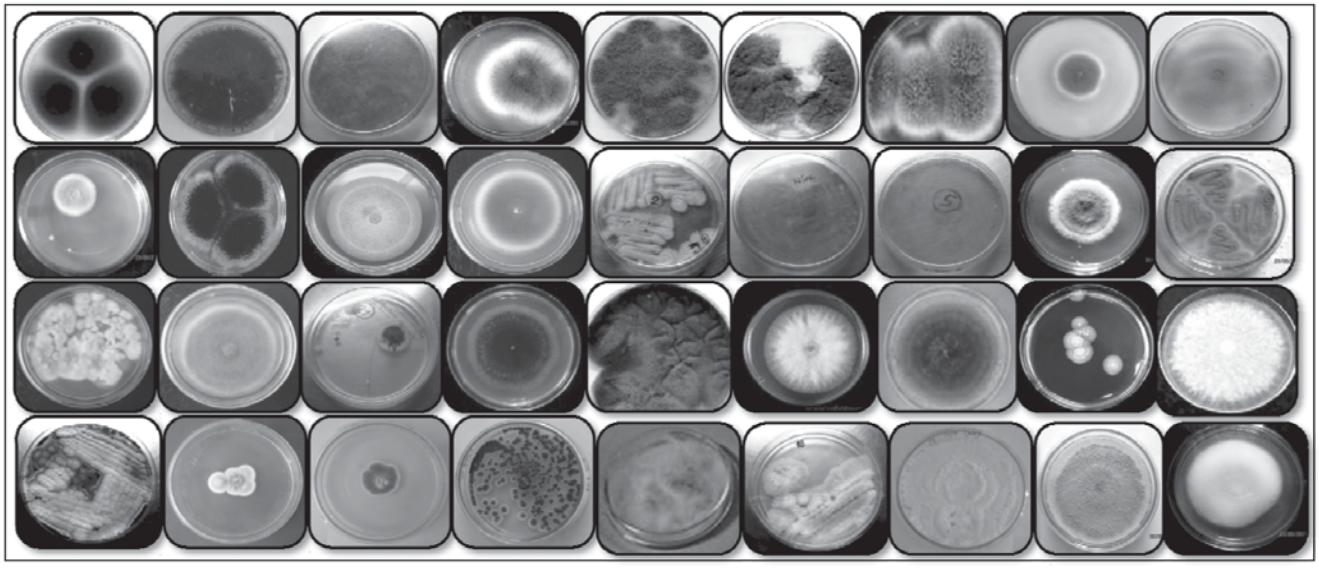

Fig. 2. Pure culture plates of isolated fungi; from left to right *Alternaria alternata, A. tenuis, Aspergillus awamori, A. candidus*, *A. clavatus*, *A. flavus, A. fumigatus, A. glaucus, A. japonicus, A. kanagawaensis, A. niger, A. nidulans, A. parasiticus*, *A. oryzae, A. stellatus, A. sydowii, A. tamari, A. terreus, A. versicolor, Aspergillus* sp., *Cladosporium* sp., *Curvularia lunata, C. pallescens, Fusarium oxysporum, Fusarium* sp., *Mucor hiemalis, Mucor* sp., *Penicillium* sp.1, *Penicillium* sp.2, *Penicillium* sp.3, *Penicillium* sp.4, *Rhizopus nigricans, Rhizopus* sp.1, *Rhizopus* sp.2, *Trichoderma aureoviride* and white sterile mycelium.

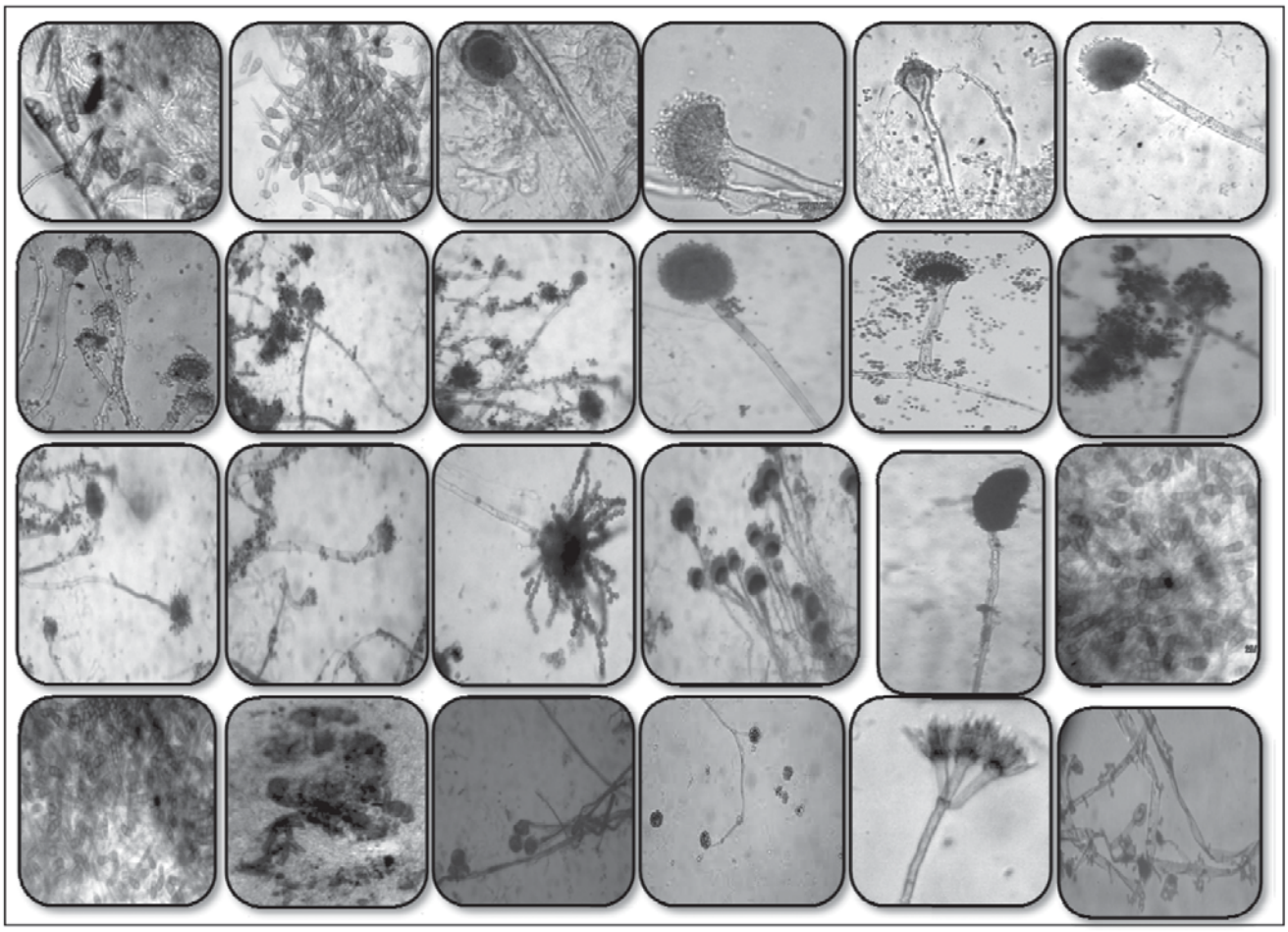

Fig. 3. Phase contrast microscopic view of isolated fungi (450 times magnified). From left to right : *Alternaria alternata, A. tenuis, Aspergillus awamori, A. candidus*, *A. flavus, A. fumigatus, A. glaucus, A. japonicus, A. kanagawaensis, A. niger, A. nidulans, A. parasiticus*, *A. stellatus, A. sydowii, A. tamari, A. terreus, Aspergillus* species, *Curvularia lunata, C. pallescens, Fusarium* sp., *Mucor hiemalis, Mucor* sp., *Penicillium* sp.1 and *Rhizopus* sp.2.

4. Conclusion

The genus *Aspergillus* is a very high flying and diverse community among all innate soil dwelling fungi. Affiliates of this genus are renowned for their impending and prospective attributes in biosynthesizing cluster of enzymes and metabolites at bountiful quantity by degrading various squanders and cheap substrates such as plant biomass, agro-industrial wastes and play pivotal place in managing ecosystem. Implementing these natural isolates, high valued-low cost products can be achieved from cheap substrates (wastes and effluents) addressing the wellbeing of mankind.

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