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Full Length Research Paper

Exploring *Penicillium* Diversity in Coastal Sand Dunes: A Study of Cashew Plantations in Odisha, India

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The abundance and diversity of *PENICILLIUM* species were studied from soils in coastal sand dunes of Odisha, India for a period of two years covering three distinct seasons. A total of 41 species were enumerated, of which the surface soil of the site without vegetation had 23 species while its sub-surface soil contained 24 species. In the site with *ANACARDIUM OCCIDENTALE* (cashew) plantation, the surface soil contained 31 species while sub-surface soil contained 20 species. More species were recorded from the sites with an *ANACARDIUM* plantation which may be due to less competition with other fungi. The diversity index varies from 0.954 to 1.058 (Shannon) and 0.0096 to 0.0106 (Simpson). Similarity indices show that, surface layers were more akin than the subsurface soils. The evenness index varies from 0.337 to 0.396, an indication that species showed a fairly even distribution. The surface soils of both sites have the highest species richness while sub-surface soils of barren sand dunes show the lowest richness.

Key words: Coastal sand dune, diversity indices, fungi, *Penicillium*.

INTRODUCTION

Microbes are especially important components of biodiversity. Particularly fungi and bacteria are crucial, as they change and release many nutrients playing important roles in nutrient cycling (Parotta, 1999; Sall et al., 2003) and sustenance of vegetation. Coastal sand dunes are unproductive and sterile habitats of the world where the rate of primary productivity is quite low; but they support many different soil organisms. The plantations established on coastal sand dunes vary in structure and composition and these variations may alter soil microbial communities (Warren and Zou, 2002). The efficiency of fungi in decomposition and their potentiality depend upon their abundance and composition. *Penicillium* is an important cellulose decomposing fungus common in tropical forest soils. It utilizes the simplest carbohydrates and thereby plays a pivotal role in the initiation of cellulose decomposition in a soil ecosystem (UmaDevi and Manoharachary, 1987; Mamtaz and Mishra, 1991; UmaDevi and Manoharachary, 1991; Panda, 2010).

Although, there have been many publications on the occurrence and distribution of soil fungi of forest soils, some of these have dealt with the influence of plant community type (Mohanty and Panda, 1991; Mohanty and Panda, 1994a; Mohanty and Panda, 1998; Manoharchary et al., 2005; Manoharchary et al., 2008), while others have examined the effect of soil depth (Behera et al., 1991; Behera and Mukherji, 1985; Mohanty and Panda, 1994b) and a few have attempted to examine the diversity of these fungi (Nilima et al., 2007). There have, however, been few studies in coastal sand dunes (Panda, 2010). As a result of the very limited knowledge of the abundance and diversity of soil fungal flora in coastal sand dunes of Odisha, the study reported here was undertaken to examine the role of beach plantation in determining the abundance and diversity of soil fungi in general and genus *Penicillium* in particular with tree plantation of the cashew nut plant *Annacardium occidentale*.

MATERIALS AND METHODS

The study site was in Ganjam district of Odisha (19°15'N and

84°50'E) having 60 km of coastline along the Bay of Bengal at a height of 6 to 8 m above mean sea level. The climate of the region is monsoonal with coastal characteristics. The atmospheric temperature ranges from 37°C in summer to 13°C in winter. The annual rainfall is about 130 cm.Some of the unproductive uplands and coastal sand dunes are extensively covered by *Casuarina equisetifolia* and cashew (*A. occidentale*) plants. Cashew plantation at the inner belt of the study site covers an area of about 1500 ha extending 4 to 5 km with a width of 250 to 450 m, varying from place to place and having a shelter belt cum wind break vegetation of *Casuarina* about 30 to 40 rows covering 15 to 20 m in the outer coastal belt along the sea. The cashew plant has been preferred over many others because of its physiological adaptation and tolerarance to extreme drought conditions, its good growth in nutritionally poor soils; it is extensive and near surface lateral roots and its dense canopy is due to broad leaf and horizontal growth.

Two sites each of about 1 ha were selected for the present investigation for a period of two years. The first site was a 6 to 8 year old plantation of *A. occidentale* without any undergrowth and the second site was a big patch of sand dune with a vegetation of just a few grasses situated adjacent to an *Anacardium* plantation. Soil samples from surface and sub-surface (15 cm depth) were collected from the two sites in sterilized test tubes by randomly sampling at monthly intervals. The samples were temporarily stored in an ice chest prior to isolation of microbes. The micro fungi were isolated by dilution (Waksman, 1927) and pour plate (Warcup, 1950) techniques using PDA medium. Fungi were studied after 3 to 7 days of incubation. Fungi were identified using standard procedures (Barnett and Hunter, 1972; Ellis, 1971, 1976; Gilman, 1966; Subramanian, 1971). Physico-chemical properties of soils were estimated as per Jackson (1967).

Statistical analysis

The following indices of diversity were calculated based on species level identification (Ludwig and Reynolds, 1988). Shannon – Weaver index

$H = -\sum_{i=0}^{s} Pi \ln Pi$

Where Pi is the proportion of the individual found in the i^{th} species, $\ln$ denotes natural logarithm and H is the Shannon –Weaver index.

Simpson's index $D = \sum_{i=0}^{s} (Pi)^2$

Where Pi is the proportion of the individual found in the i^{th} species and D Simpson's index.

Evenness index (E) = $H/\ln S$

Where H is the Shannon –Wiener index of diversity, S total number of species and $\ln$ is the natural logarithm.

Jacquard's index, $S_{ab} = S_{AB} / (S_A + S_B - S_{AB})$

Where S_{AB} is the number of species shared by two locations (A and B), S_A the total number of species in location A and S_B the total number of species in location B. S_{ab} is the extent of similarity between the species in location A and B.

Richness index (Margalef, 1963) $R = S-1/\ln N$

Where S is the total number of species and N is the sampling number.

RESULTS AND DISCUSSION

Coastal offshore and onshore habitats have great significance on the survival of the coastal microflora and microfauna especially the micro-fungi. Diversity of fungi is related to particular habitats, different vegetation systems and to both environmental and edaphic factors (Behera and Mukherji, 1985; Gentry, 1988; Behera et al., 1991; Mohanty and Panda, 1994b; Mohanty and Panda, 1998). There was a difference between the fungal communities in the two sites when they were compared using composition and nutrient status. Soils from the site with an *Anacardium* plantation because of low temperature, high moisture and better nutrient status harbored more fungi (Table 1). Micro-fungi of both soils showed a positive correlation with soil moisture and total organic carbon but were negatively correlated with soil temperature. The qualitative and quantitative differences of genera and species at the two sites indicated that surface vegetation, as well as nutrient composition influenced micro-fungal inhabitants of the soil (Gentry, 1988; Mohanty et al., 1991; Nilima et al., 2007; Panda, 2010). Similar results have been obtained from the soils at lower depth in all sampling sites. The higher population associated with plantation site may be ascribed to the greater surface area available for microbial colonization. Fungal number of two sites differed significantly (t-test 5.34<p 0.01). Analysis of variance (ANOVA) clearly indicated significant seasonal difference between the samples of soil (Table 2).

A distinct pattern of fungal community structure was observed in all the samples during the study period. The percentage composition and rank abundances of different fungal species fluctuated (Table 3). The majority was from the genus *Aspergillus* and the next two in order of dominance were *Penicillium* and *Trichoderma*. Earlier reports have indicated that, these genera appeared abundantly in soils (Mohanty and Panda, 1994b, Panda et al., 2007; Rai and Kumar, 1988). This may be due to the faster growth rate of these fungi in addition to their better intrinsic prolific sporulating capacity to utilize the substrate. Considering the dominant species, it is clear that fungal succession in the plantation site greatly differed from without plantation. The species composition in soil showed marked differences with a change in habitat and surface vegetation (Table 4). A total of 177 species of fungi belonging to 71 genera were enumerated. The genus Deutoromycotina had the highest number of species followed by the genera Zygomycotina and Ascomycotina. Their occurrence might be due to the ability of these groups of fungi to survive in adverse conditions, as well as their ability to the environment. Fifty two fungal species were detected

Table 1. Edaphic factors and fungal population of study site.

Sites		Temperature (°C)	Moisture content (%)	pH	Total organic carbon (%)	Total nitrogen (%)	C/N ratio	Phosphorous (mg/100 g)	Potassium (mg/100 g)	Fungal number x10^4
Site without vegetation	Surface soil	34.1	0.699	6.2	0.21	0.016	13.8	0.45	1.53	54
	Sub-surface soil	32.3	1.56	6.1	0.131	0.01	12.7	0.74	0.99	40
Site with Anacardium plantation	Surface soil	30.28	1.26	6.9	0.403	0.0246	17.2	0.2	1.7	66
	Sub-surface soil	28.74	2.03	6.3	0.275	0.0195	14.97	0.31	1.1	45

Average of 2 years data.

Table 2. ANOVA.

Sources	DF	SS	MSS	F value		p Value	
Varieties	3n	1164	388	7.8	4.8*	9.8**	23.7***
Season	2n	614	307	6.2	5.1*	10.9**	27.0***
Error	6n	298	49.7				
Total	11						

*p<0.05, ** p<0.01, *** p<0.001.

common to both the site without vegetation and the site with *Anacardium* plantation.

During this study, 41 *Penicillium* species were isolated, of which surface soil without vegetation had 24 species, sub-surface 23 species while surface soil with plantation contained 31 species and sub-surface had 20 species .The annotated list of different species is presented in Table 5. *Penicillium citrinum, P. javanicum, P. minio-leuteum, P. oxalicum and P. verruculosum* were the dominant species in all the soils under study. They have frequently been isolated in various soils in India (Behera and Mukherji, 1985; Mamtaz and Mishra, 1991; Mohanty and Panda, 1994a; Manoharachary et al., 2005; Panda et al., 2007;Behera and Mukherji, 1985; Mamtaz and Mishra, 1991; Mohanty and Panda, 1994a; Manoharachary et al., 2005; Panda et al., 2007). Their role as decomposers has also been reported (Umadevi and Manoharachary, 1987; Mohanty and Panda, 1994a).

Of the 41 species isolated, 13 were common to all the sites while a few were restricted in their distribution.

In fact, the numbers of restricted species were more in the plantation site than the soil without plantation. It was observed that more species of *Penicillium* were present in the site with vegetation than the sand dunes site without vegetation. It may be due to low competition with other categories of fungi which are less abundant in monoculture plantations of *A. occidentale* compared to barren sand dunes. The diversity values (H and R) are higher in the sub-surface soil of the site with plantation than in the surface soil of the site without plantation (Table 6).

The evenness values however show a different trend. It is highest in sub-surface soil without plantation and lowest in soil with plantation. The evenness index indicates that, species were of fairly even distribution. The surface soil of both sites has the highest species richness whereas

Table 3. Percentage contribution and ranks of some dominant fungi isolated from samples at study sites.

Fungi	Soil from site without vegetation						Soil from site with *ANACARDIUM* plantation					
	Surface			Subsurface			Surface			Subsurface		
	No. of colony	%	Rank	No. of colony	%	Rank	No. of colony	%	Rank	No. of colony	%	Rank
Absidia butleri	14	1.99	21	15	2.27	19	45	5.32	4	38	5.12	4
A. glauca	-	-	-	-	-	-	23	2.72	10	19	2.56	15
A. spinosa	-	-	-	-	-	-	17	2.01	20	22	2.96	10
Alternaria alternata	13	1.85	22	-	-	-	-	-	-	-	-	-
Aspergillus awamori	56	7.98	1	47	7.11	1	57	6.74	1	48	6.47	1
A. flavus	24	3.2	8	21	3.18	13	24	2.84	9	18	2.43	16
A. fonsecoeus	-	-	-	-	-	-	21	2.48	11	-	-	-
A. fumigatus	28	3.99	6	27	4.08	7	25	2.96	8	21	2.83	12
A. luchuensis	18	2.56	14	21	3.17	14	18	2.13	16	13	1.75	24
A. niger	43	6.12	2	42	6.35	2	49	5.79	3	46	6.2	2
A. terreus	19	2.71	13	25	3.78	8	16	1.89	21	16	2.16	18
Chaetomium homopilatum	22	3.13	10	24	3.63	9	14	1.66	25	14	1.89	22
C. murorum	-	-	-	14	2.12	20	13	1.54	27	-	-	-
Cladosporium cladosporoides	18	2.56	15	28	4.24	5	20	2.36	12	26	3.5	9
C. oxysporum	15	2.14	19	18	2.72	16	16	1.89	22	30	4.0	8
Curvularia eragrostidis	27	3.85	7	23	3.48	10	-	-	-	16	2.16	19
C. lunata	17	2.42	16	12	1.82	21	15	1.77	23	15	2.02	20
C. pallescens	12	1.71	23	18	2.72	17	-	-	-	14	1.89	23
Drechslera australiensis	16	2.28	17	13	1.97	23	-	-	-	12	1.62	26
Fusarium species	16	2.28	18	17	2.57	18	20	2.36	13	18	2.43	17
Mucor species	-	-	-	-	-	-	13	1.66	26	-	-	-
Penicillium citrinum	30	4.27	4	28	4.24	6	44	5.2	5	33	4.45	5
P. cyaneum	-	-	-	-	-	-	-	-	-	15	2.02	21
P. javanicum	39	5.56	3	39	5.9	3	32	3.78	7	32	4.3	6
P. minio-leuteum	22	3.13	11	23	3.48	11	19	2.25	15	21	2.8	13
P. nigricans	11	1.57	24	12	1.82	22	18	2.13	17	13	1.75	25
P. oxalicum	20	2.85	12	19	2.87	15	15	1.77	24	22	2.96	11
P. rubrum	15	2.14	20	11	1.66	24	18	2.13	18	20	2.7	14
P. rugulosum	-	-	-	-	-	-	19	2.25	14	-	-	-
P. verruculosum	30	4.27	5	29	4.39	4	52	6.15	2	44	5.93	3
Rhizopus nigricans	-	-	-	-	-	-	18	2.13	19	12	1.62	27
Trichoderma viride	23	3.28	9	22	3.33	12	44	5.2	6	32	4.3	7

Table 4. Total count of fungi isolated during the study period.

Sites	Total genera	Total species	*Penicillium* species
Site without vegetation			
Surface soil	51	112	23
Sub surface soil	37	87	24
Site with *Anacardium* plantation			
Surface soil	45	114	31
Sub surface soil	41	93	20
Total	71	177	41

Table 5. Penicillous fungi isolated from different sites.

Penicillum species	Soil with plantation of *Anacardium*		Soil without plantation	
	Surface	Subsurface	Surface	Subsurface
Penicillium atromerotosom	-	-	-	+
P. adametezi	+	-	-	-
P. brefaldianum	+	+	+	+
P. canadens	+	-	-	-
P. charlesi	-	-	+	-
P. chermesinum	+	-	+	+
P. chrysogenum	+	-	+	-
P. citrinum	+	+	+	+
P. citroviride	+	-	-	+
P. corrylophylum	+	+	+	+
P. cyaneum	+	+	+	+
P. decumdens	+	+	+	+
P. diversum	+	+	-	-
P. ehrlichii	+	-	-	-
P. fellutatum	+	-	+	-
P. funiculosum	+	-	-	-
P. glabrum	+	-	-	-
P. harvei	-	-	-	+
P. implicatum	+		+	-
P. islandicum	-	+	-	-
P. janthinelum	+	-	+	+
P. javanicum	+	+	+	+
P. lanosum	+	+	+	+
P. leuteum	+	-	-	+
P. lepidosum	-	-	-	+
P. levitum	-	+	-	-
P. minio-luteum	+	+	+	+
P. minutissima	+	-	-	-
P. nigricans	+	+	+	+
P. oxalicum	+	+	+	+
P. purpurogenum	+	+	+	+
P. resticulosum	-	-	+	-
P. restrictii	-	+	-	+
P. roseo-purpureum	-	-	+	+
P. rubrum	+	+	+	-
P. rugulosum	+	+	+	+

Table 5. Contd.

P. spinulosum	-	-	-	+
P. turbatum	+	-	-	-
P. variable	+	+	+	-
P. verruculosum	+	+	+	+
P. waksmani	+	+	+	-

(+) =Presence, (-) = absence.

Table 6. Dominance, diversity, evenness and richness indices of *Penicillium* in different samples at study sites.

Sites	Samples	D	1-D	H	E	R
Site without vegetation	Surface soil	0.0096	0.99	0.954	0.337	5.03
	Sub Surface soil	0.0103	0.99	0.982	0.396	3.46
Site with Anacardium plantation	Surface soil	0.0104	0.989	1.089	0.358	6.29
	Sub Surface soil	0.0106	0.99	0.977	0.393	3.46

D= Simpson dominance index, H = Shannon diversity index, E = evenness, R = richness.

Table 7. Comparison of different samples by Jacquard's coefficients of comparison.

Samples	A_1	A_2	B_1	B_2
A_1	1.0	0.59	0.62	0.46
A_2		1.0	0.59	0.54
B_1			1.0	0.52
B_2				1.0

sub-surface soil of barren sand dunes shows the lowest richness. The β diversity (Jacquard's index) value indicates changes in species composition from one location to another (Table 7). It is similar to the findings of Wilson and Shimda (1984) and Aparajita (2007).

Conclusion

The results of my study on coastal sand dunes of Odisha demonstrate the importance of soil microorganisms in controlling the biological soil properties. It has been observed that a change in the habitat variable can affect species composition and diversity. The above ground vegetation in these coastal sandy sites is essential, to maintain a productive environment to enhance microbial growth and activity. Thus, vegetation cover of the sand dunes should be protected for proper management of the biodiversity in Odishan coastal belts.

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