

Diversity of sand dune fungi in the coastal areas of Puducherry and Karaikal region

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ABSTRACT

Sand dune soil of seven different coastal areas of Puducherry and Karaikal were collected. The soil mycoflora were analyzed by serial dilution method by implementing SDA agar plates. A total number of 29 species representing 15 genera were isolated and identified from all the sand dune soils. Physicochemical analysis of the sand dune soil was carried out in which, pH was found between 7.2 to 8.2, EC between 0.5 to 1.9, available nitrogen between 20 to 60mg/g, available phosphorus between 0.14 to 0.51mg/g, available potassium between 1020 to 3050mg/g and micronutrients such as copper, zinc, manganese, iron were recorded at different concentrations. Similarity coefficient of fungal diversity was found significant, but the frequency and occurrence of mycoflora of each soil were varied at some extent. The greatest similarity in soil fungi occurred between S3 and S6 (83.8%), followed by S1 and S3 (76.4%) and S1 and S6 (74.2%).

Keywords: Diversity of sand dune fungi, Serial dilution, Similarity coefficient, Coastal region.

INTRODUCTION

Sand dune provides an interesting niche for the study of rare and endangered microbes. Fungi are the most adaptable organism in all types of habitats [1]. Fungi are one of the important microbial components of the soil, crucial in global ecological processes and play a major role in the cycling of elements in the biosphere and soil ecosystem functioning [2,3]. There are approximately 1.5 million fungal species on earth reported by previous authors [4]. Among these, 3000 species are associated with aquatic habitats [5]. The filamentous fungi contribute major in soil, degrade the toxic substance and play important role in soil formation, soil fertility, soil structure and improvement [6,7,8]. Fungi involve in organic matter decomposition and elemental release by mineralization and useful in the production of pharmaceutical products such as antibiotics, organic acids, enzymes, pigments and secondary metabolites used in food industry and fermentation [9,10]. Till today, there is no work related to the above matter was undertaken in the Pondicherry coastal areas to find out the diversity of soil fungi. The present investigation has been undertaken to assess the isolation, identification and compare the composition, abundance and diversity of sand dune fungi in 7 different ecological soil types of Puducherry and Karaikal.

MATERIALS AND METHODS

Collection of samples

The soil samples were collected from 7 different coastal areas of Puducherry and Karaikal, from 3 inches below the surface of the sand dunes, using sterilized metal spatula. The samples were collected from the coastal sand dunes of Ganapathichetikulam (S1), Puducherry beach (S2), Ariyankuppam (S3), Murthikuppam (S4), Karaikalmedu (S5),

Karaikal beach (S6), Karaikal estuary (S7) (Table1). At each station 3 samples were collected randomly from different sand dunes within the radius of 1 km. The samples were transferred in sterilized polythene bags, sealed properly and brought to the Microbiology laboratory, Department of Botany, KMCPGS (Autonomous), Pondicherry for further studies.

Table1. Sand dune samples collected from different areas.

Sample No.	Coastal area	Place
S1	Ganapathichetikulam	Puducherry
S2	Puducherry beach	
S3	Ariyankuppam	
S4	Murthikuppam	
S5	Karaikalmedu	Karaikal
S6	Karaikal beach	
S7	Karaikal estuary	

Physico-chemical analysis of soil

The physico-chemical parameters of soil such as pH, salinity, micronutrients (Cu, Zn, Mn, Fe) and macronutrients (N, P, K) were analyzed at Soil testing laboratory, Thattanchavady, Dept. of Agriculture, Puducherry.

Isolation of sand dune fungi

The fungi were isolated by dilution plating technique described [11]. The sand dune samples weighing 1gm were subjected to serial dilution in 10 ml of distilled water and transferred 1 ml of microbial suspension, aseptically into agar containing plates like Sabouraud dextrose agar (SDA) with addition of antibiotics such as amoxicillin and streptomycin to the medium (Spread plate method). The petridishes were then incubated at 25°C in dark, and observed up to three days.

Identification of isolated fungi

Colony color and morphology were noted besides hyphae structure, spore size, shape and spore bearing structure. The microscopic identification was done by staining with Lactophenol cotton blue. The fungi were identified with the help of textbooks available in the laboratory [12, 13, 14] and expertise of the authors.

Presentation of data

Population density was referred as CFU/g. frequency occurrence was calculated as follows in order to identify their existence in the soils.

$$\% \text{ Frequency} = \frac{\text{No. of samples in which a particular fungus occurred}}{\text{Tot. no. of samples examined}} \times 100$$

Based on the frequency occurrence the fungi were grouped as rare (0.25% freq.), occasional (26-50% freq.), frequent (51-75% freq.), and common (76-100% freq.) species.

Similarity coefficient index

The similarities in fungal community composition between two areas were calculated by

Sorensen's index of similarity is shown in Table 4.

$$\text{Similarity coefficient} = \frac{2W}{a+b} \times 100$$

Where, a: Total no. of species isolated from one site, b: Total no. of species isolated from other site, W: Common no. of species.

RESULTS AND DISCUSSION

Fungal diversity in sand dune samples

The fungal diversity in all the 7 stations was recorded. Totally 29 fungal species were isolated. Of them, 4 species belong to 3 genera were Zygomycetes, 3 species belong to 2 genera were Ascomycetes, 22 species belong to 10 genera were Deuteromycetes (Table 2).

Species occurrence

The variation among the fungal diversity was recorded. The maximum number of 20 species was recorded in Ganapathichetikulam (S1), followed by 16 species recorded in Ariyankuppam (S3), Karaikalmedu (S5), and Karaikal beach (S6), 14 species recorded in Murthikuppam (S4), 10 species in Karaikal estuary (S7) and the minimum number of 9 species in Puducherry beach (S2)(Fig.1)

Species Composition

The *Aspergillus* species contributed the more number (11 species) among the 15 genera. This indicates that *Aspergillus* was the dominant fungal genus of sand dune soils as reported by some authors[15]. Followed by *Penicillium*, *Trichoderma*, *Mucor*, *Colletotrichum* (2 species each). All other genera were represented by one species each (Table2).

Percentage frequency

The percentage frequency calculated, showed variation in occurrence of fungal species in all the stations. The highest frequency was recorded by *Aspergillus niger*, *A.flavus*, *A.terreus*, *A.fumigatus* (100% each) followed by *Cladosporium herbarum*, *Penicillium chrysogenum*, *Trichoderma harzianum* (71.4% each), *Rhizopus sp.*, *A.nidulans*, *A.ochraceus*, *Fusarium oxysporum*, *Penicillium sp.*, *T.viridea* (57.1% each), *Mucor hiemalis*, *Syncephalstrum racemosum*, *A.austus*, *Verticillium lateritium*, *White sterile mycelia* (42.8% each), *Mucor.sp.*, *Colletotrichum capsici*, *C.coccodes*, *A.flavipes*, *A. sydowii*, *A.versicolor*, *A.oryzae*, *Dreschlera sp.*, *Geotrichum candidum* (28.5% each), *Scopulariopsis brevicaulis*, *Bipolaris sorokiniana* (14.2% each). The occurrence of species like *A.niger*, *A.terreus* was found to be highest and *A.sydowii* contributed less as reported in the Tamilnadu coast [15] (Table2).

Frequency class

The frequency class for all the species was classified. In this, *A.niger*, *A.flavus*, *A.terreus*, *A.fumigatus*, *C.herbarum*, *P.chrysogenum*, *T.harzianum* as common; *Mucor hiemalis*, *Mucor.sp.*, *S.racemosum*, *C.capsici*, *C.coccodes*, *A.flavipes*, *A.austus*, *A. sydowii*, *A.versicolor*, *A.oryzae*, *Dreschlera sp.*, *G.candidum*, *V.lateritium*, *White sterile mycelia* were classified as occasional; *Rhizopus sp.*, *A. nidulans*, *A.ochraceus*, *F. oxysporum*, *Penicillium sp.*, *T.viridea* as frequent; *B.sorokiniana*, *S.brevicaulis*, as rare.

Similarity coefficient study

The results showed that the greatest similarity in soil fungi occurred between S3 and S6 (83.8%), followed by S1 and S3 (76.4%) and S1 and S6 (74.2%). The similarities of soil fungi between these three pairs of land uses were considered high (> 70%). The pairs between which the similarity in soil fungi was considered as medium were S1 and S7 (57.2%), S2 and S3 (58.3%) and S6 and S7 (56%). The low similarity in soil fungi between S2 and S6 (48%), S1 and S5 (47%), S2 and S5 (41.6%), S3 and S4 (44.4%) was observed.

Population density

The variations in population density among the different stations were observed. Mean fungal population density recorded the minimum of 9×10^{-1} CFU/g and the maximum of 15.3×10^1 CFU/g in the sand dunes collected from Pondicherry beach (S2) and Ganapathichetikulam (S1) respectively. The samples from Ariyankuppam(S3), Karaikalmedu (S5), Karaikal beach (S6), Murthikuppam (S4), Karaikal estuary (S7), recorded 14.3, 13.3, 11.6, 11.3, 10.6×10^1 CFU/g(Fig. 2).

Physico-chemical properties of sand dune soil

The variations in Physico-chemical properties in different stations were recorded. The pH was alkaline and neutral in all the sand dune soils of different stations. The maximum pH was 8.3 were recorded in the sample collected from Pondicherry beach (S2).The minimum pH of 7.2 was observed in Ganapathichetikulam (S1) among the different stations. The variation in Electrical conductivity showed from 0.5-1.9 in the samples collected from Pondicherry

beach(S2) and Ganapathichetikulam (S1) respectively. The variations in Macronutrient and Micronutrient content were recorded. The available nitrogen content was as low as 30 mg in the soils collected from Ganapathichetikulam(S1) and Karaikal beach (S6). The maximum of 60mg/g was recorded in the soils collected from Ariyankuppam (S3), and the available phosphorus showed variations with minimum of 0.14mg/g in the samples collected from Ganapathichetikulam (S1), Pondicherry beach (S2), Murthikuppam (S4), Karaikal estuary (S7) respectively. The maximum of 0.51mg/g was recorded in Karaikal beach (S6). The available potassium from 1020mg/g in samples of Murthikuppam (S4), and the maximum of 3050mg/g recorded in Karaikal beach (S6). The variations in the micronutrients such as copper, zinc, manganese, iron were recorded in all the stations (Table 3).

Relationship between physicochemical parameters and fungal population

There is no significant relationship with pH; macronutrient and micronutrient were recorded by the correlation analysis of physico chemical parameters and fungal population as reported in the sand dunes soils of Tamilnadu coast [15].

Table 2: Occurrence, Percentage, Frequency class of different fungal species in sand dune soils of Puducherry and Karaikal

Sl. No.	Fungi	Puducherry				Karaikal			% frequency	F. Class
		S1	S2	S3	S4	S5	S6	S7		
Zygomycotina										
1.	<i>Mucor hiemalis</i>	+	-	-	+	-	-	+	42.8	O
2.	<i>Mucor</i> sp.	-	-	-	+	-	-	+	28.5	O
3.	<i>Rhizopus stolonifer</i>	+	+	+	-	-	+	-	57.1	F
4.	<i>Syncephalastrum racemosum</i>	+	-	+	-	-	+	-	42.8	O
Ascomycotina										
5.	<i>Colletotrichum capsici</i>	-	-	-	-	+	+	-	28.5	O
6.	<i>Colletotrichum coccoides</i>	+	-	-	+	-	-	-	28.5	O
7.	<i>Scopulariopsis brevicaulis</i>	+	-	-	-	-	-	-	14.2	R
Deuteromycotina										
8.	<i>Aspergillus niger</i>	+	+	+	+	+	+	+	100	C
9.	<i>A. flavus</i>	+	+	+	+	+	+	+	100	C
10.	<i>A. terreus</i>	+	+	+	+	+	+	+	100	C
11.	<i>A. fumigatus</i>	+	+	+	+	+	+	+	100	C
12.	<i>A. flavipes</i>	-	-	+	-	+	-	-	28.5	O
13.	<i>A. ustus</i>	+	+	+	-	-	-	-	42.8	O
14.	<i>A. nidulans</i>	+	-	+	-	+	+	-	57.1	F
15.	<i>A. sydowii</i>	-	-	-	-	+	+	-	28.5	O
16.	<i>A. ochraceus</i>	+	+	-	+	-	-	+	57.1	F
17.	<i>A.versicolor</i>	-	-	+	-	+	-	-	28.5	O
18.	<i>A. oryzae</i>	+	-	-	+	-	-	-	28.5	O
19.	<i>Bipolaris sorokiniana</i>	-	-	-	-	+	-	-	14.2	R
20.	<i>Cladosporium herbarum.</i>	+	-	+	-	+	+	+	71.4	C
21.	<i>Dreshlera</i> sp.	-	-	-	-	+	-	-	28.5	O
22.	<i>Fusarium oxysporum</i>	+	+	+	-	-	+	-	57.1	F
23.	<i>Geotrichum candidum</i>	+	-	-	+	-	-	-	28.5	O
24.	<i>Penicillium chrysogenum</i>	+	-	+	+	+	+	-	71.4	C
25.	<i>Penicillium</i> sp.	+	-	+	+	-	+	-	57.1	F
26.	<i>Trichoderma harzianum</i>	+	-	-	+	+	+	+	71.4	C
27.	<i>Trichoderma viridea</i>	-	-	+	-	+	+	+	57.1	F
28.	<i>Verticillium lateritium</i>	-	+	-	+	+	-	-	42.8	O
29.	White sterile mycelia	+	-	+	-	-	+	-	42.8	O

During study period a total number of 214 fungal CFUs were isolated, among these 20 species belonged to 12 genera were isolated from Ganapathichetikulam (S1), 10 species belong to 4 genera were from Puducherry beach (S2), 16 species belong to 8 genera were from Ariyankuppam (S3), 14 species belong to 7 genera were from Murthikuppam (S4), 17 species belong to 8 genera were from Karaikalmedu (S5), 16 species belong to 9 genera were from Karaikal beach (S6), 10 species belong to 5 genera were from Karaikal estuary (S7). The maximum fungal species belong to Deuteromycotina consist 22 colonies, Zygomycotina 4 colonies, Ascomycotina 3 colonies were observed. The percent frequency of mycoflora in 7 stations was statistically analyzed. This showed frequent occurrence of 6 species, common occurrence of 7 species, occasional occurrence of 14 species, and rare occurrence of 2 species. The soil pH were found to be alkaline and neutral in all the samples, in which the samples of Pondicherry beach (S2) showed pH of 8.3 and Ariyankuppam (S3) showed pH of 8.2. The fungal species were not

influenced by the pH, as reported by previous workers [15]. Even though the samples of Pondicherry beach, recorded few species (9 species), the samples of Ariyankuppam recorded more number of species (16 species) compared to Pondicherry beach. The organic content was analyzed and the nutrients levels were recorded. Hence, the correlation analysis did not bring any relationship with the fungal population [15]. The result indicates that *Aspergillus niger*, *A. flavus*, *A. terreus*, *Penicillium* spp., *Cladosporium* were of high occurrence in all areas and fungi like, *Scopulariopsis*, *Colletotrichum coccoides*, *Bipolaris* were negligible. Among the 29 species recorded in the present study the genus *Aspergillus* was represented by 9 species. This indicates that *Aspergillus* was the dominant fungal genus of sand dune soils. Dominant occurrence of *Aspergillus* was reported [17, 18] from various marine soils. This shows *Aspergillus* grow in high saline concentration.

Table 3: Physico-chemical parameters of the sand dune soils collected from two regions

Sample	pH	E.C. status	Lime status	Soil texture	Macronutrient			Micronutrient			
					N	P	K	CU	ZN	MN	FE
S1	7.2	1.9	N	S	25 VL	0.14VL	1450VH	0.207 L	3.717 H	0.423 L	0.462 L
S2	8.3	0.5	N	S	30 VL	0.14 VL	1450 VH	0.169 L	1.941 M	0.343 L	0.219 L
S3	8.2	1.6	N	S	60 VL	0.26 VL	1305 VH	0.220 L	2.876 H	0.913 L	0.377 L
S4	7.3	1.8	N	S	55 VL	0.14 VL	1020 VH	0.168 L	1.637 M	0.667 L	0.401 L
S5	7.9	1.2	N	S	30 VL	0.26 VL	1850 VH	0.233 L	1.0304 L	0.814 L	0.224 L
S6	7.4	1.4	N	S	20 VL	0.51 VL	3050 VH	0.056 L	0.620 L	0.208 L	0.159 L
S7	7.5	1.5	N	S	55 VL	0.14 VL	1750 VH	0.151 L	1.709 M	0.681 L	0.583 L

VL-VERY LESS; L-LESS; M-MEDIUM; H-HIGH; VH-VERY HIGH.

Table 4: Similarity coefficient of fungal species distributed in sand dune soil of Puducherry and Karaikal coastal area

	S1	S2	S3	S4	S5	S6	S7
S1	20 [#]	8/57.1	13/76.4	12/70.9	8/47	13/74.2	8/57.2
S2	8 [*] /57.1 ^{SS}	11	7/58.3	6/57.1	5/41.6	6/48	5/55.5
S3	13/76.4	7/58.3	16	6/44.4	10/60	13/83.8	6/50
S4	12/70.9	6/57.1	6/44.4	14	7/51.8	7/50	8/66.6
S5	8/47	5/41.6	10/60	7/51.8	17	11/70.9	7/50
S6	13/74.2	6/48	13/83.8	7/50	11/70.9	16	7/56
S7	8/57.2	5/55.5	6/50	8/66.6	7/50	7/56	12

^{SS}: Similarity coefficient, ^{*}: Common no. of species, [#]: Total no. of species*

Fig 1: Fungal diversity in different sampling stations

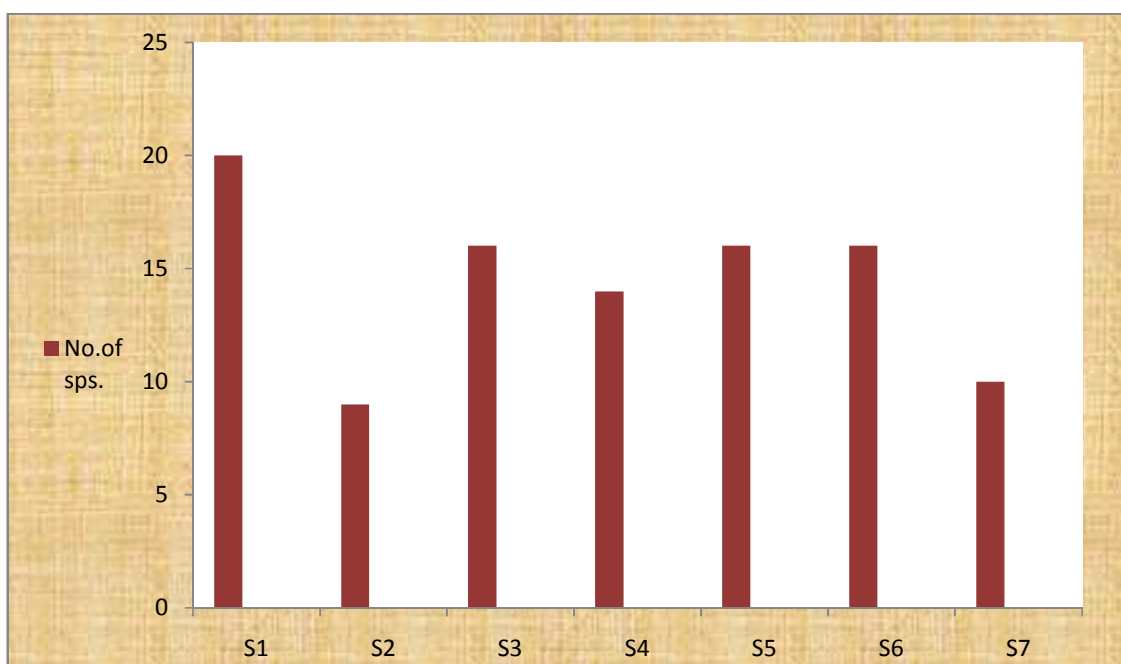


Fig 2: Population density in different sampling stations

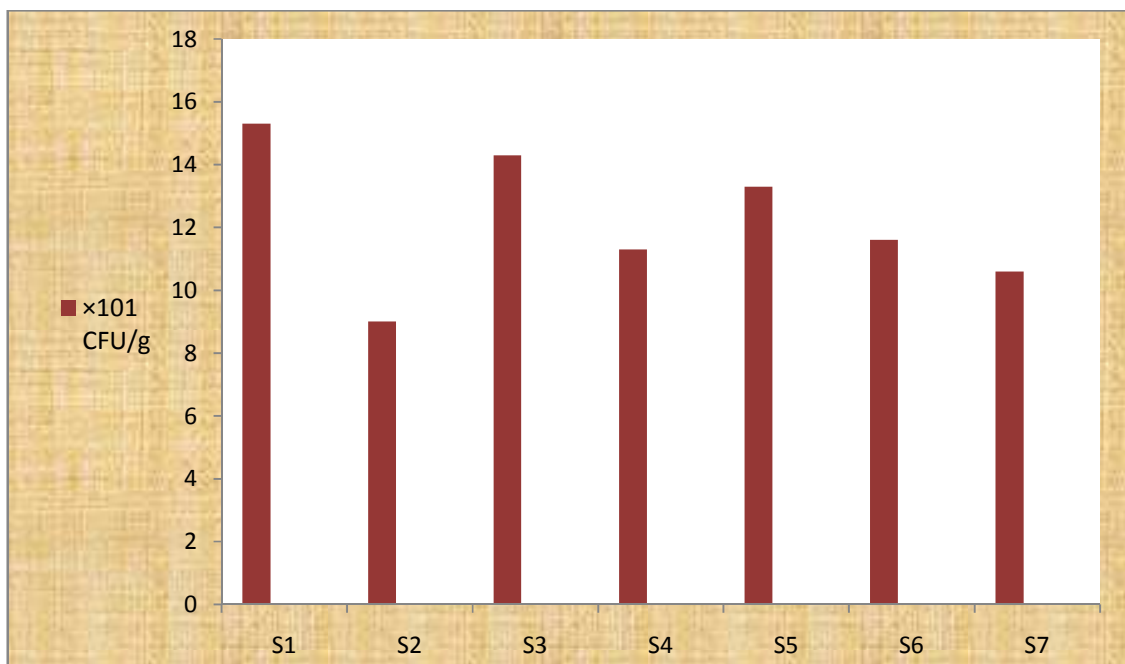
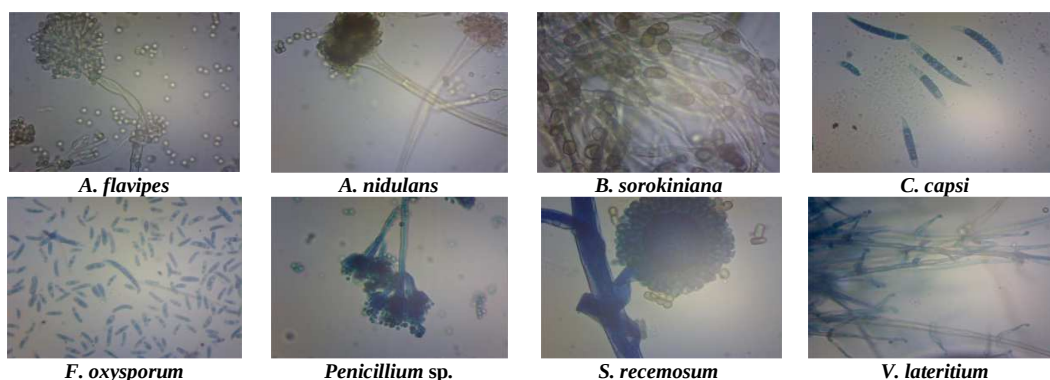


Fig 3: Microscopic structure of few fungi isolated from sand dunes: (10x40X)



The high similarity indices of soil fungi were found in between S1 and S3, S1 and S6 and S3 and S6 sand dune soils. This indicates the high similarity between the soil environmental factors in these three pairs of areas and the similarity in the soil environmental factors such as soil moisture, organic matter, pH and electrical conductivity showed similar in fungal community composition prevailing in the areas confirmed with the previous authors [16]. Our present study did not support the high fungal similarity indices due to the high variation among the mean values of the soil environmental factors. Therefore, to improve the statistics available for analysis, the number of soil samples per land use type should be increased in future research and many other soil environmental factors, should be measured as reported [9]. In the present study it was shown that the fungal diversity, population density and frequency occurrence of sand dunes and the role of physico-chemical conditions, in changing the species diversity in all the 7 different stations was in an order.

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