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Biodiversity and antagonistic activity of fungi isolated from soil samples of Point Calimere, east coast of Tamilnadu

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ABSTRACT

Totally 20 isolates of fungi were isolated from two soil samples of Point Calimere, coastal area of Tamil Nadu, India. Among them *Aspergillus* was the dominant genus. Out of 20 species, 6 species were dominantly isolated which were tested for antagonistic activity against plant pathogen *Curvularia senegalensis.* Of them *Aspergillus ustus* exhibited effective inhibitory activity against phytopathogen. Thus the results revealed that *Aspergillus ustus* was able to inhibit the growth of *curvularia senegalensis* to the maximum extent possible. The bioactive compounts *of Aspergillus ustus* have been analyzed by using Gas chromatography mass spectrometry.

Marine fungi are a world wide ecological group, but distinct in their geographical distribution and the substrata on which they are found[4,5]. Analysis of fungal biodiversity also helps in isolating and identifying new and potential fungi having high specificity for recalcitrant compounds.

Biological control of plant pathogens is one of the important plant protection methodologies exploited as an ecofriendly approach and is an important integrated disease management strategy[6,8,9].

Curvularia senegalensis is a plant pathogen which causes leaf spot disease in cashew seedlings[11] and cause disease in sugarcane, seedling foliage blights[13]. The antibiotic ability and potential strains (5) of *Trichroderma viride* were isolated from five different field soil against brown spot disease and its tolerance to antibiotics were studied[7].

Therefore, the present investigation was carried out for the isolation of fungi from marine environment and their antagonistic activity against plant disease causing fungus *Curvularia senegalensis* and also determining the structure of antifungal substances from the fungal strain having maximum antifungal activity against plant pathogen.

Sample collection:

The marine sediment soil samples were

collected from Bird's View Point and Old Light House area in Point Calimere.

Isolation of fungi :

One gram of marine soil sample was diluted serially in distilled water. Potato Dextrose Agar medium (PDA) was prepared and sterilized in an autoclave at 121°C for 15 minutes. The medium was incorporated with Streptomycin Sulphate Solution (1%) and poured in to the Petriplates. After solidification 0.1 ml of serially diluted (10^{-3}, 10^{-4}) soil samples were inoculated into the medium. The inoculum was uniformly spread and kept undisturbed in dust free chamber at 28 ± 2°C for a period of 3-5 days. The fungal colonies were counted. Pure cultures were maintained in potato dextrose agar medium.

Isolation of plant pathogen :

The plant pathogen *Curvularia senegalensis* was isolated from infected leaves of cashew plant. The pure culture of plant pathogen was maintained in PDA medium for future purpose.

Identification and photomicrography :

Semi permanent slides were prepared using lactophenol and Cotton blue. Morphological features of fungi were photographed using Nikon Microscope. All the fungi were identified with referring the standard manual of soil fungfi[3].

Dual culture experiment[16]*:*

Colony interaction between the test pathogen *Curvularia senegalensis* and the soil fungi were studied in *in vitro* dual culture experiments. The test pathogen *C. senegalensis* and the soil fungi viz., *Aspergillus ustus, A. wentii, A. sulphureus, A. terreus, A. variecolor, A. granulosis* were grown separately on PDA medium. The position of the colony margin on the back of the disc was recorded daily. The measurement was taken on the fifth day. The percentage inhibition of growth was calculated as follows.

$$\text{Percentage of inhibition of growth} = \frac{r - r^1}{r} \times 100$$

r = growth of the fungus was measured from the centre of the colony towards the centre of the plate in the absence of antagonistic fungus.

r^1=growth of the fungus was measured from the centre of the colony towards the antagonistic fungus.

Gas chromatography-Mass spectrometry Analysis of fungal filtrate:

The selected isolates were inoculated separately into 500ml conical flasks containing PDA broth, and shaken at 28 ±2°C and 250 rpm for seven days, after incubation. The staling substances were filtered through filter paper. The mycelial mat was collected and crushed by using methanol in the pestle and mortar. Then it was vortexed for 30min and centrifuged at 20,000g. The supernatant was collected and the compounds present in the filtrates were analysed in GC-MS technique[14].

Biodiversity of fungi :

In this study, totally 20 species of fungi were isolated by dilution plating technique. Out

Table-1. Mycoflora isolated from the soil samples of Point Calimere

S.No.	Fungal species	Bird's View Point	Old light house
	DEUTEROMYCETES		
1.	*Aspergillus flavus*	+	+
2.	*A. granulosis*	+	+
3.	*A. koeningii*	+	-
4.	*A. niger*	+	+
5.	*A. ochraceous*	+	+
6.	*A. luchensis*	-	+
7.	*A. sulphureus*	+	+
8.	*A. terreus*	+	+
9.	*A. ustus*	+	+
10.	*A. variecolor*	+	+
11.	*A. versicolor*	+	+
12.	*A. wentii*	+	+
13.	*Penicillium citrinum*	+	-
14.	*P. funiculosum*	+	+
15.	*P. janthinellum*	+	-
16.	*P. purpurescens*	+	-
17.	*Trichoderma koeningii*	-	+
18.	*T. viride*	+	+
19.	*Verticillium sp.*	-	+
	PHYCOMYCETES		
20.	*Choanephora cucubitarium.*	+	-

+: Present, - :Absent

of 20 species, the maximum numbers of fungi belonged to Deutromycetes (4 genera, 19 species) and only one Phycomycete was recorded (Table-1).

From the Deuteromycetes *Aspergillus* (12 species) was the dominant genera followed by *Penicillium* (4 species), *Trichoderma* (2 species) and *Verticillium* (1 species). Dominance of *Aspergilli* and *Penicillia* are universal features of various marine habitats. Studies of Souza *et al.*,[16] also confirmed the present study.

The leaf spot, associated with *C. senegalensis* was preceded by interveinal chlorosis manifested on 8 week -old cashew seedlings were studied by Olunloyo[10].

All the tested fungi showed the ability to inhibit the test pathogen but at the same time they showed variation. Based on the percentage inhibition of growth of the pathogen, the antagonistic

Table-2 Colony interaction between *Curvularia senegalensis* and marine fungi in dual culture experiment

Growth response of the antagonistic and test fungus (mm)	Antagonistic fungi tested					
	Aspergillus granulosis	*A. sulphureus*	*A. terreus*	*A.ustus*	*A.variecolor*	*A.wentii*
Colony growth of pathogen towards antagonist	13	20	17	11	12	15
Colony growth of pathogen away from the antagonist	11	23	22	12	11	11
% growth inhibition of pathogen in the zone of interaction	71.1	55.5	62.2	75.5	73.3	66.6
Colony growth of antagonist in control *(i.e)* growth towards the centre of the plate in the absence of the pathogen	60	18	29	60	60	60
Colony growth of antagonist towards the pathogen	27	15	29	35	29	26
Colony growth of antagonist away from the pathogen	28	18	20	21	21	20
% of growth inhibition in the zone of interaction	55	16.6	38	41.6	51.6	56.6

potential of the soil fungi were assessed. The soil fungi tested can be arranged based on their antagonistic potential as below *Aspergillus ustus* (75.5%), *A. variecolor* (73.3%), *A. granulosis* (71.1%), *A. wentii* (66.6%), *A. terreus* (62.2%) and *A. sulphureus* (55.5%). (Table-2).

In the present study, *A. ustus* (75.5%) showed efficient antagonistic activity against *Curvularia senegalensis* than the other *Aspergillus* isolates. *Aspergillus* and *Trichoderma* isolates have high antagonistic activity against *Sclerotium* was studied by Panneerselvam and Saravanamuthu[11].

GC - MS analysis :

Totally 21 bioactive compounds were identified in the filtrate *of Aspergillus ustus* through GC-MS technique (Table-3).

This GC-MS analysis revealed that maximum percentage of peaks was covered by n-hexadecanoic acid (36.96 %), followed by Oleic acid (20.18 %) and remaining compounds contributed below 10% each.

Marine derived fungi have been shown to produce interesting bioactive metabolites

Table-3. Compounds identified in the culture filtrate of *A. ustus* by GC-MS analysis

No.	Retention Time	Name of the compound	Molecular Formula	Molecular Weight	Peak Area (%)
1.	3.95	Diglycerol	$C_6H_{14}O_5$	166	7.17
2.	4.69	2,6-Pyridinedicarboxaldehyde, 4-hydroxy-, bis(methyl(2-pyridly) hydrazone)	$C_{19}H_{19}N_7O$	361	1.31
3.	7.07	Isosorbide	$C_6H_{10}O_4$	146	2.51
4.	7.44	d-Mannitol, 1,4-anhydro-	$C_6H_{12}O_5$	164	2.95
5.	10.66	2-Dimethyl(isopropyl)silyloxymeth-yltetrahydrofurane	$C_{10}H_{22}O_2$ Si	202	6.60
6.	11.65	Undecanoic acid	$C_{11}H_{22}O_2$	186	1.56
7.	12.52	D-Galactose, 6-deoxy-	$C_6H_{12}O_5$	164	0.28
8.	12.74	3 ,4-Dihydroxypropiophenone	$C_9H_{10}O_3$	166	0.47
9.	14.09	Pyrrolo(l ,2-a)pyrazine- 1,4-dione, hexahydro-	$C_7H_{10}N_2O_2$	154	3.05
10.	14.34	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.11
11.	14.99	2-Undecene, (Z)-	$C_{11}H_{22}$	154	0.65
12.	15.79	1 ,2,3,4,5-Cyclopentanepentol	$C_5H_{10}O_5$	150	0.24
13.	16.70	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	1.85
14.	17.36	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	36.96
15.	17.73	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256	3.37
16.	19.43	9-Dodecenoic acid, methyl ester, (E)-	$C_{13}H_{24}O_2$	212	2.43
17.	20.14	Oleic Acid	$C_{18}H_{34}O_2$	282	20.18
18.	20.36	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	2.42
19.	20.45	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	3.88
20.	25.12	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	7.17
21.	26.17	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	1.31

including some potential antibiotics[1,2,12,17].

On the basis of this study it is concluded that the *Aspergillus* isolates showing antagonistic activity through the presence of bioactive compounds which has been proved to be effective against plant pathogen. This study used to develop *Aspergillus* isolates as a potential biological control agent against the plant pathogen *Curvularia senegalensis*.

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