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Research Article

ISOLATION AND IDENTIFICATION OF MARINE FUNGAL SPECIES FROM SEA SHORE SOIL SAMPLES COLLECTED FROM CUDDALORE DISTRICT

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A B S T R A C T

Marine fungi are the potential and promising sources for biologically active secondary metabolite production. Secondary metabolites are the chemical compounds that are produced during the stationary phase of the organism. Many years of study revealed that fungi are excellent sources for novel bioactive secondary metabolites. In the present study physico-chemical characteristics of sea shore soil samples were analyzed and marine fungi were isolated from the samples collected from the coastal region of Cuddalore district, Tamilnadu, India, was performed. The marine soil were selected for the following boundaries like Soil texture, Calcium Carbonate, Electrical conductivity, Power of hydrogen, Macronutrients like (Organic carbon, Nitrogen, Phosphorus, Potassium), Micronutrients like (Iron, Manganese, Zinc, Copper) and others Caution exchange capacity, Magnesium, Sodium were studied. A total of 321 fungal colonies were isolated from sea shore soil samples. Among them, based on morphological and microscopic characteristics, nine genera of microfungi were identified namely, *Fusarium* (43%), *Aspergillus* (23%), *Curvularia* (10%), *Penicillium* (8%), *Neurospora* (6%), *Mucor* (5%), *Rhizopus* (2%), *Sarocladium* (1%), *Pestalotiopsis* (1%) and some unknown species.

INTRODUCTION

Sea shores are located at the junction between water and land which is defined by the sand, wave and tide regimes (Schlacher *et al*. 2008) and usually consist of loose deposits of sand, crushed shells, rocks, gravel and pebbles. The soil of sandy beaches supports a high population of organisms such as small invertebrates, bacteria, fungi, yeast, virus, algae and diatoms which can adapt in a constantly changing environment. These organisms live between the sand grains and they are involved in the food chain interactions (Larsen & Doggett 1990).

Microorganisms such as microfungi are significant components of sea shore soil and play an important role in decomposition of organic matter in sand and water, as well as in nutrient cycling and degradation of hydrocarbon (Moore-Landecker 1996). Microfungi in sandy soil can be a saprophyte, mutualist or pathogen on marine plants and invertebrates. The saprophytic microfungi play an important role as decomposers of cellulose in the form of washed-up leaves, algae and animal products such as chitin, keratin and calcium carbonate (Kohlmeyer *et al*. 2004).

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Marine fungi are an ecological rather than a taxonomic group and comprise a predictable 1500 species, excluding those that form lichens. Marine fungi are major decomposers of woody and herbaceous substrates in marine ecosystems. These fungi grow on a wide variety of substrata ranging from wood to sediments, mud's, soils, sand, algae and corals, alcareous tubes of mollusks, decaying leaves of mangroves, intertidal grasses and living animals, to those growing in the guts of crustaceans (Kohlmeyer and Kohlmeyer, 1979; Hyde, 1996). The distribution of fungi in the marine environment has not been well studied as compared with the studies on the fungi in freshwater and terrestrial ecosystems. They are poorly represented in the sea since the marine fungi account for only 5% of the total fungal flora. Although fungi occur widely in marine environment on dead organic matter and as parasites of living organisms still their distribution has not fully evaluated. Their number and species composition in the soil habitat differs from place to place depending upon the physical, chemical and biological factors of the particular habitat (Ainsworth *et al.,* 1973). The search for new biomedicals from marine organisms resulted in the isolation of more or less 10,000 metabolites, many of which endowed of pharmacodynamic properties. A broad spectrum of biological activities has been detected, such as: antibiotic, antifungal, toxic, cytotoxic, neurotoxic, antimitotic, antiviral, antineoplastic and CV activities. In more recent years, new targets have been added to the general screening, for example:

AIDS, immunosuppression, anti-inflammation, Alzheimer disease, ageing processes and some tropical diseases (Kelecom, 1999). Earth's surface provides the largest inhabitable space for living organisms, particularly microbes. Therefore, the present study has planned to determine the physic-chemical parameters and fungal distribution was documented from Cuddalore district, Tamilnadu, India.

MATERIALS AND METHODS

Sample collection

Totally ten locations belonging to sea shore soil environs was selected in Cuddalore district, Tamil Nadu, India (Lat. 11o43' N; Long. 79o49' E), Kodiyampalayam, Kellai, MGR thittu, Parangipettai, Puthupettai, Chinnur, Pudhukuppam, Velingarayanpettai, Samiyarpettai, Kumarapettai. The soils were taken by scraping off the surface and subsurface to a depth of 10 cm. Approximately 1.5 kg of soils were collected from each site and put in plastic bags and labeled based on the collection sites. All the soil samples were air-dried at room temperature (27±1°C) for 48 to 72 h. Approximately 1g of the soil samples was dissolved in 9 ml of sterile Phosphate Buffer Saline (PBS) buffer (pH 7.4) to make soil suspension. The supernatant from the soil solution was pipette and spread over ten PDA plates. The plates were left at room temperature for 5 days to calculate and record the fungal colonies. Then, the plates were kept at 4^{o}C for 2 days to delay the growth of soil microorganisms.

Physico-Chemical analysis of Soil sample

The soil samples were analyzed for their texture, pH and macro and micro nutrients. Soil texture was determined using the feel method described by Brady and Weil (2008) and the soil texture was classified based on Biondo and Lee (1997).

pH Analysis

Soil pH was measured by weighing 30 g of the soil samples in a 100 ml beaker and 75 ml of distilled water, was added and mixed well. The mixture was then incubated at room temperature (27±1°C) for 24 h to ensure all substances in the soil sample were diluted. The pH reading was taken by using a pH meter (Mettler Toledo Delta 320, Greif-ensee, Switzerland) and recorded after 24 h of incubation. Power of hydrogen value as calculates of the hydrogen ion action of the soil water and state the acidity and alkalinity of the soil. It is an important property of soil as it fixes the accessibility of nutrients and physical condition of soil, Jackson (1967); Smith and Doran (1996).

Soil Texture

The percentage of the soil particles were determined by 10 g air dried soil sample was mixed with 20 ml distilled water. The mix was heated for 3 min to break the large sized soil. 5 ml hydrogen peroxide was added to the mix. The solution was filtered through common filter paper. The soil balance of filter paper was then treating with 125 ml of 3 Normality HCl and left it for immediately. The solution was filtered again and soil filtrate dried in air. The dried soil was passed through 0.2 mm pore sized technical filter. Filter soil was transferred into a flask and sufficient amount of 1 Normality sodium hydroxide was added to make the inside alkaline. After shaking the container for 6 h on the mechanical shaker, the content was move to 500 ml measuring jar and the volume was made up to 250 ml by distilled water. It was stopper and was allowed to settle after shaking. The supernatant was free into a separate beaker and soil was washed again and again till the delay was clear. This was transferred to another tarred dish, heated and weighted, which was the reading of fine sand, Piper (1964; Jackson (1973); Jackson (1967); Smith and Doran (1996).

Analysis of EC and Cation Exchange Capacity

Calcium carbonate was extracted with neutral 1 N ammonium acetate and the available calcium carbonate in the extract was determined by Smith and Doran Smith and Doran, 1996. Electrical conductivity (EC) of the soluble salts in the soil contents of the ion current fixing reveals. If any one of the electrical conductivity of the soil samples is included in 20 g soil suspension 40 ml distilled water and stirred for 30 min after the soluble salts had been fully used by an Equiptronics digital electrical conductivity was detected and recorded. Cation exchange capacity (CEC) of the soil was determined by using 1 Normality ammonium acetate solution as give details for (Jackson, 1973).

Analysis of macro and micro nutrients

The primary macro nutrients like total N was determined by the Micro-Kjeldahl method (Jackson, 1973) and available P and K was analyzed by the Brays P1 method (Bray and Kurtz, 1945). Exchangeable bases (K, Ca, Na and Mg) and micronutrients (Cu and Zn) of the soil were extracted with a Mehlich-3 solution (Mehlich, 1984). Organic carbon (O) was determined using wet Walkely Black dichromate digestion method (Nelson and Sommers, 1982). Iron (Fe) was determined using the standard method described by Havlin and Sultanpour (1981). Organic matter (OM) was determined by the method of Walkely (1947).

Isolation and Identification of marine Microfungi

Dilution plating technique described by Warcup (1950) was used to isolate the fungi from soils. Soil sample weighing 1g was diluted in 10 ml of 50% seawater (1:1 v/v seawater (30 ppt): distilled water). Serial dilutions were obtained to 10^{-2} and 100 µl of each dilution was inoculated on Potato dextrose agar medium was prepared using artificial sea water (ASW: Vita-Marques et al., 2008): artificial sea water agar (ASW: 1 L of ASW; agar 15 g). Growths in Petri dishes were performed at 25^{o}C.

Rifampicin was added in media used for inoculation at a concentration of 300 mg/L in order to prevent bacterial contamination. After inoculation, plates were regularly examined in order to verify the growth of filamentous fungi. Isolation of strains was performed as previously reported (Vita- Marques et al., 2008). Pure fungal colonies obtained by successive purification steps were photographed, morphologically described, subjected to microscopic analysis and deposited at the Microbiology Laboratory.

Identification was based on colony morphology and spore characters. Spore morphology was studied by microscopic observation. A slide culture technique was also used for this study. The organisms were grown on agar blocks covered with cover slips. After 5 days of incubation periods at 27 ^{0}C the agar was removed and the organisms stained with the help of Cotton Blue- Lactophenol solution and observed under microscope. The isolates were then identified using morphological characteristics according to the methods and

descriptions of Ellis (1971), Barron (1972), Nelson *et al*. (1983), Barnett and Hunter (2006).

RESULT AND DISCUSSION

Physico-chemical parameters

The marine sea shore soil has significant issue in the marine atmospheres that influence the growth, replica and metabolic actins of microbes. In the present study the physic chemical factor including soil texture, p^H, (Tamizhazhagan and Pugazhendy,2016). Calcium carbonate, Electrical conductivity, macronutrients such as Organic carbon, Nitrogen, Phosphorus and Potassium. Micronutrients Iron, manganese, Zinc, copper and other cautious exchange capacity, sodium and magnesium of the sea shore soil samples colle4cted from different places of Cuddalore district coastal region Tamilnadu, India shown in Table 1.

Puthupettai and Velingarayanpettai soil samples were recorded. The minimum content of Calcium carbonate (4.79), Magnesium (2.55), CEC (21.21C.Mole proton/kg) and Sodium (1.46), was recorded at Parangipettai sea shore soil sample.

Macronutrients such as Organic carbon (46%), Nitrogen (33.2kg/ha), Phosphorus (8.12kg/ha) and Pottasium (387kg/ha) were maximum at Puthupettai, Velingarayanpettai, Samiyarpettai and MGR thittu sea shore soil. Minimum Organic carbon (0.11%), Nitrogen (23.8kg/ha), Phosphorus (3.23kg/ha) and Potassium (126kg/ha) were recorded at Kellai, Chinnur, Kumarapettai and Puthukuppam respectively (figure 1).

Micronutrients such as Iron (8.31 ppm), Manganese (9.18ppm), Zinc (1.50 ppm) and Copper (2.56 ppm) the present investigated were recorded maximum at Kumarapettai soil.

Table 1 Physico-chemical Analysis of marine soil samples

S.No	Parameters	Kodiyampalayam	Kellai	MGR thittu	Parangipettai	Puthupettai	Chinnur	Puthukuppam	Velingarayanpettai	Samiyarpettai	Kumarapettai
1	Soil texture	sand	loamy sand	loamy sand	sand	sand	loamy sand	sand	sand	sand	loamy sand
2	$CaCO_3$(mg/kg)	6.44	7.42	6.5	4.79	7.79	7.56	7.25	6.72	8.2	6.6
3	EC (dsm-1)	2.8	2.10	6.12	3.10	2.88	2.9	2.10	6.13	3.10	2.88
4	p^H	7.56	7.25	6.72	8.1	6.62	6.44	7.42	7.33	8.3	8.16
5	Organic carbon (%)	22	11	35	32	46	22	44	35	32	28
6	Nitrogen (kg/ha)	24.4	26.3	32.2	24.1	33.2	23.8	25.3	33.2	29.1	27.98
7	Phosphorus (kg/ha)	6.12	7.14	5.9	8.10	5.9	6.12	7.14	3.23	8.12	3.23
8	Potassium (kg/ha)	155	150	387	170	167	155	126	386	170	167
9	Iron (ppm)	4.7	4.6	7.47	4.87	8.25	4.4	4.3	7.47	4.62	8.31
10	Manganese(ppm)	3.74	4.34	9.23	4.18	2.98	3.71	4.54	2.93	4.91	9.18
11	Zinc (ppm)	0.30	1.6	0.28	1.34	0.34	1.30	1.66	0.22	1.23	1.50
12	Copper (ppm)	2.13	0.43	1.46	1.93	2.1	2.13	2.23	1.56	2.13	2.56
13	Magnesium (mg/kg)	11.41	6.62	3.48	2.55	4.61	12.47	5.62	3.48	6.14	5.11
14	CEC (C.Mol Proton + /Kg)	46.61	22.21	42.14	23.32	31.41	46.61	53.71	35.22	25.43	31.43
15	Sodium (mg/kg)	2.15	1.36	3.1	1.46	2.31	2.04	2.14	3.17	1.49	2.33

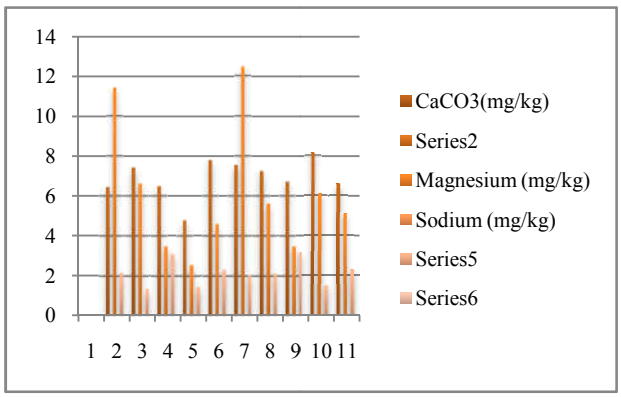
The texture of the collected soil samples categorized into two types such as Sands and Sandy loams. Both of these soil types have a gritty feeling and will form crumbled ball when moistened and is regarded as sandy soil (Brady & Weil 2008).

The majority of the soil contents are sand particles up to 85 and about 70–90 percentage in loamy sand. In sand, there are only about 15% of clay and 10 percentages of silt particles. However, the silt and clay contents are relatively higher in loamy sand which contains about 35% of silt particles and 17 percentages of clay particles (Brady & Weil 2008)

The maximum p^H (8.2dsm-1), Electrical conductivity (6.13) were recorded in Samiyarpettai and Velingarayanpettai, The minimum p^H (6.6 dsm-1), Electrical conductivity (2.8) were recorded in Kumarapettai and Kodiyampalayam respectively. Although the optimal pH that favours the growth of different types of fungi varies greatly, majority of fungi seem to grow best at pH 4 to pH 7 (Alexopoulos *et al.* 2002). However, *Fusarium* sp. tolerate a wide pH range and thus variation in pH does not seem to affect species prevalence and density except possibly in extreme conditions (Mandeel 2006).

The maximum level of Calcium carbonate (3.17 mg/kg), Magnesium (12.47mg/kg), CEC (53.71C.Mole proton/kg) and Sodium (3.17 mg/kg) was present in Samiyarpettai, Chinnur, Puthukuppam and Velingarayanpettai soil samples. The minimum contents of Iron (4.3 ppm), Manganese (2.93 ppm), Zinc (0.22 ppm) and Copper (0.43 ppm) were documented at Puthukuppam, Velingarayanpettai, and Kellai soil (figure 2). Sea, soil, characteristics, properties, pH-acidic nature have been reported previously (Vaijayanthi and Vijayakumar 2014).

It is clear that the threat of ocean acidification on marine ecosystems and species represents a priority for future investigations and large-scale investments in green-energy sources.



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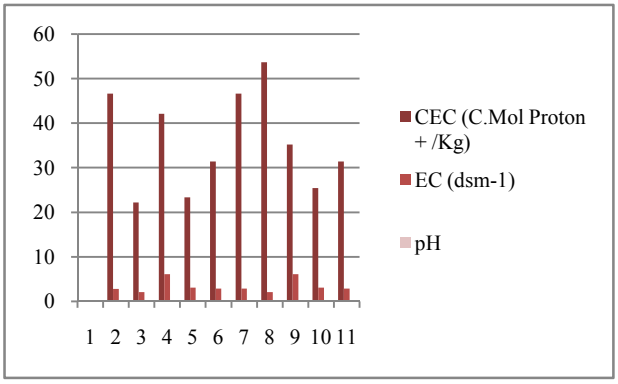

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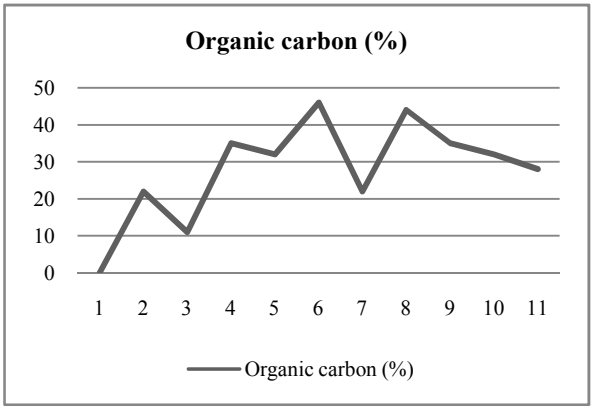

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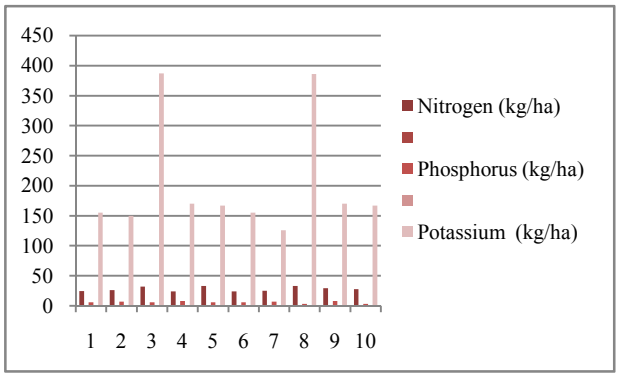

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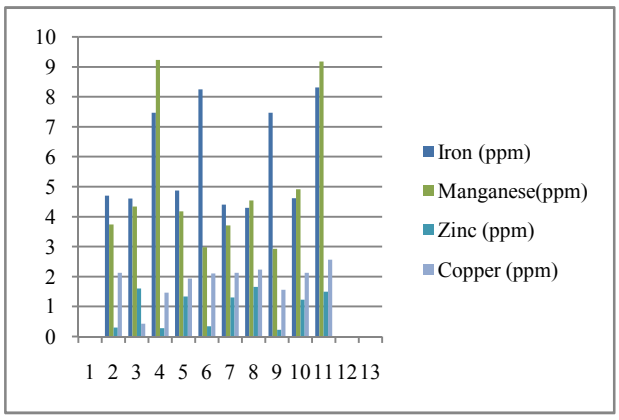

e

Figure 1 Graphical structure of marine soil parameters from Palk Strait (a) CaCO3, Mg, Sodium, (b) CEC, pH, EC, (c) Organic carbon, (d) N, P, K, and (e) Fe, Mn, Zn, Cu.

The most important challenge for research is to identify the vulnerability of some physiological processes of key marine species but also rate of tolerance and adaptation capability to the global climate change.

Diversity of microfungi from marine soil

Totally, 321 fungal colonies were isolated from various sea shore soil samples of Cudalore district, Tamilnadu. Among them, the maximum (55 CFU/g) was recorded at Putthupettai soil, followed by MGR thittu (40 CFU/g), Parangipettai (35 CFU/g), 30 CFU/g each were isolated from Chinnur, Pudhukuppam and Samiyarpettai, 28, 27, 25 and 21 CFU/g from Velingarayanpettai, Kellai, Kodiyampalayam and Kumarapettai respectively (Table1) . From these 321 colonies of marine fungus, morphologically variying colonies were purified, sub-cultured and stored at 4^{o}C on PDA medium for further studies.

Among the 321 fungal colonies, only 40 isolates were morphologically distinct (Table 2). From these 40 isolates, Velingarayanpettai sea shore soil samples contributed maximum of 7 isolates, followed by Kodiyampalayam (n=6), (n=5) isolates from Pudhukuppam and Kumarapettai, 4 isolates from Chinnur , (n=3) contributed from MGR thittu, Puthupettai and Parangipettai, 2 isolates from Kellai and Samiyarpettai (Table-2).

Table 2 Fungal populations of different sea shore soil sampling station

S. No	Soil collection area(s)	Number of CFU/g				Total CFU/g/ isolates
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	
1	Kodiumpalayam	9	5	6	5	25 (6)
2	Kellai	8	9	6	4	27 (2)
3	MGR Thittu	12	10	10	8	40 (3)
4	Parangipettai	15	11	6	3	35 (3)
5	Puthupettai	TNTC	TNTC	TNTC	55	55 (3)
6	Chinnur	17	9	4	-	30 (4)
7	Pudhukuppam	15	9	6	-	30 (5)
8	Velingarayanpettai	11	8	8	1	28 (6)
9	Samiyarpettai	13	10	7	-	30 (2)
10	Kumarapettai	8	8	4	1	21 (6)
	Total	108	79	57	77	321 (40)

CFU: Colony forming unit; TNTC: Too numerous to count; Data in parentheses are morphologically distinct isolates

Based on morphological characteristics, nine genera of microfungi were identified namely, *Fusarium* (43%), *Aspergillus* (23%), *Curvularia* (10%), *Penicillium* (8%), *Neurospora* (6%), *Mucor* (5%), *Rhizopus* (2%), *Sarocladium* (1%), *Pestalotiopsis* (1%) and some unknown species. From the present study, it appears that the sandy beach contains a microfungi reservoir comprising of a variety of genera which contributes significantly to the ecological functioning of a marine ecosystem.

In general, the occurrence and diversity of microfungi in Cuddalore district sandy beach soils were similar with other study in different parts of the world. The differences in species composition may be due to different sampling strategies, different isolation techniques, and salinity of the seawater, temperature and nutrient status as indicated by Jones (2000). Although many species of microfungi isolated from different types of soils are saprophytic, some species are biotrophic mutualists and parasites of plants, animals and other fungi (Bills *et al*. 2004).

CONCLUSION

The physico-chemical analysis of soil samples under evaluation showed variable concentrations of various boundaries. Irregular distribution of macronutrients and micronutrients were recorded. During the present investigation which may be recognized to the further fertilizers for the period of the product development. The survey of Marine sea shore soil displays of micronutrients in the soil occasionally an able to assess the qualitative and quantitative of the metal concentrations. Thus the present study gives an idea about the *fungal* biodiversity in sea shores soil of Cuddalore district, Tamil Nadu. Although marine fungal are ubiquitous, they vary in their morphological, physiological and genetical characteristics depending on the physico-chemical environment of the habitats.

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