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Isolation, characterization and identification of predominant microorganisms from Puducherry region

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ABSTRACT

Fungi were ubiquitous in nature. Fungi are eukaryotic, spore producing achlorophyllous, heterotrophic organisms. Saprophytic and facultative parasitic fungi present in the coastal and adjacent areas of the Puducherry. Different fungi were so far isolated from soil of different regions of Puducherry (India). Isolation of fungi from soil samples were carried out by serial dilution plate technique followed by their identification through microscopic and macroscopic method. Sabourard Dextrose Agar (SDA) media was used for the growth of fungi. In our findings, following fungi were isolated and identified which include: Aspergillus niger, Aspergillus flavus, Aspergillus terreus, Penicillium sp. Among all these isolated fungi, Aspergillus niger was more dominant and shows the fast and highest growth pattern at temperature 28^{0}C and pH 5.6.

Key words: Puducherry, fungi, *Aspergillus, Penicillium*

INTRODUCTION

Studies on the prevalence of soil mycoflora in five different regions of Pondicherry were carried out by serial dilution method on June 2015. Composition and concentration of fungal spores considerably varied from these regions (Bahour, Kalapet, Madagadipet, Murungapakkam and Veerampattinam). Bahour harbored maximum number of species it's very high when compared to four other areas. Bahour considered as rice bowl of Puducherruy, it has predominant fungus in soil due to agriculture land and Madagadipet also the fertile land which carries intensive agriculture of mixed crops. other three areas are saline and river-bed soils.

Fungi play very important role in various biogeochemical cycles [7, 14] and are responsible for the cycling of organic compounds. Soil microorganisms mainly influence the soil ecosystems by contributing to plant nutrition [6, 15] plant health [4, 5] and soil structure and soil fertility [16].

MATERIALS AND METHODS

Study site and location

Pondicherry region is situated on the Coromandel Coast between 11^0 46' and 12^{0}30' N latitudes and 79^{0}36' and 79^{0}53' E longitudes. The region is bounded on the north, south and west by Marakkanam, Cuddalore and by Villupuram districts of Tamilnadu, and on the east by Bay of Bengal. It covers an area of 29377 ha, according to village revenue records and consists of 179 villages. The present study was carried out in three different localities of

Puducherry region such as Bahour(S1), Kalapet(S2), Madagadipet(S3), Murungapakkam(S4) and Veerampattinam(S5).

Methods for collection of soil samples

The soil samples were collected from three different localities of Bahour(S1), Kalapet(S2), Madagadipet(S3), Murungapakkam(S4) and Veerampattinam(S5) on June 2015. The samples were collected 3 inches below the soil surface using the sterile spatula and carefully collected in the containers. The soil samples were collected randomly from the each place within the radius of 1 km. The sealed containers were brought to the Microbiology Laboratory, Botany Department, KMCPGS, Puducherry for further investigation.

Determination of physicochemical properties of soil samples

The pH values, electrical conductivity, soil moisture, organic carbon, nitrogen, phosphorous, potassium, iron, manganese, copper and zinc were analyzed (Table 1).

Data collection and analysis:

Soil physic-chemical properties The physico-chemical properties of experimental soil: texture, pH, organic carbon content, total nitrogen content, available phosphorus content and available potassium content, were estimated by combined glass electrode pH meter method, Walkley and Black's rapid titration method, modified macro Kjeldahl method, Olsen's method and flame photometer method, respectively[8].

Sterilisation

Glassware and culture media were sterilised in an autoclave for 15 mins. and used for the isolation, characterization and identification of organisms which are responsible for the degradation of different organic waste.

Preparation of samples

Dispensed one gram of organic sample in 10 ml of distilled water, mixed well by Vortexing and transferred one ml of suspension to another test tube to make 10^{-5} dilution. Dilution procedure was continued up to 10^{-6}.

Spread plate methods

Sabourard Dextrose Agar plates were prepared and 0.1 ml of suspension was pipetted from each dilution on the agar surface. The L rod was dipped in 95% alcohol which was taken in the beaker. The glass rod was removed from the beaker and the bent position was sterilized in the Bunsen burner flame. The rod was cooled for 10-15 sec. and softly touched on the agar and spread the suspension on the agar surface. The procedure was repeatedly carried out to prepare up to 10-6 and then the plates were incubated in an inverted position at 25 0c for 24 to 48 hrs.

Enumeration of colonies

The method, Most Probable Number (MPN), was used for the enumeration of cultured colonies. The different colonies in the plate were counted manually. For each sample the counting was carried out and the count of fungi was tabulated.

Identification of organisms

After the growth of microbial colonies in the spread plates the various colonies were differentiated by colony morphology. Then the colonies are streaked onto the different agar slants by taking a loop full of culture. From those slants a single colony was inoculated into the sterile broths and incubated for 4 to 6 hrs. These were used for further experiment.

Table 1: Physico-Chemical parameters of five regions of Puducherry

Sample	pH	EC	Lime	Soil texture	Macronutrient			Micronutrient			
					N	P	K	Cu	Zn	Mn	Fe
S1	7.1	1.12	N	S	120.65 L	16.87 L	33.45 L	0.96 L	1.6 M	3.6 M	24.5 M
S2	7.4	0.17	N	S	64.90 L	19.57 L	8.97 L	1.2 0 L	1.3 L	121 VH	21.3 M
S3	6.8	0.22	N	S	78.96L	19.01L	7.25L	1.12L	1.1L	74VH	19.1M
S4	7.4	0.17	N	S	64.90 L	19.57 L	8.97 L	1.2 0 L	1.3 L	121 VH	21.3 M
S5	7.1	1.1	N	S	118.65 L	16.7 L	33.14 L	1.10 L	1.6 M	3.6 M	24.5 M

Bahour(S1), Kalapet(S2), Madagadipet(S3), Murungapakkam(S4) and Veerampattinam(S5)

Isolation of soil mycoflora

The soil micro fungi were enumerated by two methods, namely, Soil dilution [20], and Soil plate method [21] on different media such as Potato Dextrose Agar and Sabourard Dextrose Agar at pH 6.5. All the Petri dishes were incubated at room temperature 27 ± 3°C for a period of 4 – 7 days and then examined. The first set of observations were made at the end of two days to make sure that the fast growing flocculent types such as Rhizopus, Mucor and Trichoderma, etc., has grown excessively to interfere with observations of other species. Second observation was made when these had come to an advanced stage to enable identification. Finally, the slow growing organisms has to be sub- cultured in different media for the purpose of further growth to save them from being overrun by the more aggressive types. The number of colonies per plate in 1 g of soil was calculated.

Identification

Identification of the organisms was made by microscopic analysis using taxonomic guides, standard procedures and relevant literature[10,11,4,5]. While presenting the data two terms, viz; periodicity of occurrence and 'percent contribution and statistical analysis were used. The percent contribution of each isolate was calculated by using the following formula:

Total no. of CFU of an individual species
X 100 Total no. of CFU of all species

Table 2: Temperature & Humidity of five regions of Puducherry

Location	Temperature	Humidity
Bahour(S1)	33.5^{0}C	48%
Kalapet(S2)	34.5 ^{0}C	49%
Madagadipet(S3)	34 ^{0}C	46%
Murungapakkam(S4)	32 ^{0}C	48 $^{\%}$
Veerampattinam(S5)	34 ^{0}C	50%

Bahour(S1), Kalapet(S2), Madagadipet(S3), Murungapakkam(S4) and Veerampattinam(S5)

Table 3: Fungi population of five regions of Puducherry

S. No.	Fungal species	S 1	S 2	S 3	S 4	S 5	
1	*Absidia spp*	2	1	-	2	-	5
2	*Acromonium spp*	-	-	1	-	-	1
3	*Alternaria spp*	2	3	3	2	1	11
4	*Aspergillus flavus*	4	15	7	7	12	45
5	*A. versicolor*	-	2	-	-	-	2
6	*A. fumigatus*	2	2	3	2	5	14
7	*A. niger*	7	1	8	21	13	50
8	*A.terreus*	4	-	6	2	4	16
9	*Cladosporium spp*	-	2	-	-	-	2
10	*Curvularia sp.*	*4*	4	-	-	-	8
11	*Fusarium sp.*	*2*	3	7	2	-	14
12	*Penicillium spp*	4	2	4	6	2	18
13	*Mucor Spp.*	1	-	5	-	-	6
14	*Rhizopus spp*	2	-	3	-	2	7
15	*Torula herbarum*	-	4	-	-	-	4
16	*Trichoderma spp*	2	6	-	-	-	8
17	*Paecillomyces spp.*	1	1	-	-	5	7
18	*Pythium sp.*	2	-	3	2	2	9
19	White sterile mycelia	4	5	3	7	3	22
20	Grey sterile mycelia	2	2	3	-	1	8
		45	53	56	53	50	257

CONCLUSION

Physico-chemical parameters of soil samples:

Physico-chemical parameters of five different soil samples revealed high saline conditions as indicated by low concentrations of micronutrients, alkaline pH and high Electrical Conductivity. Some of the samples indicated low electrical conductivity as they were taken from areas colonized by shrubs or tress growing in the saline areas. The soils samples were showing neutral pH due to its saline nature. The results were also reported by [9] in tropical soils

characterized by low nutrient status. Similarly, have observed the variation in soil nutrient availability with space and time [18].

The temperature and humidity level all the five regions show slight variations in accordance with its distance from sea. Among the filamentous fungi isolated from soil samples in Bahour, *Aspergillus niger* is the most prevalent ascomycetous fungus and also dominant species that was isolated from five soil samples of the five different regions, followed by *Rhizopus stolonifer*, *Penicillium Chrysogenum* and *Mucor spp.* , *Aspergillus flavus* and *Aspergillus fumigates* are also prevalent in the soil samples. The results found also agree with who reported similar result in the study of distribution of fungi in the five soil series [17]. It is of interest to note that most of the ascomycetous fungi obtained in this study correlated with the physico-chemical characteristics of the soil from where they were isolated. Report the compendium of soil fungi from various soils which show that *Aspergillus niger* was the most frequently isolated [3]. The soil moisture has a direct effect on the population of fungi positively hence, at higher moisture, the tolerance and colonization by fungi is badly affected [19]. Excessive moisture leads to inadequate oxygen diffusion. In this study, dump soil of Murungapakkam has relatively high moisture content and consequently the fungal was high [13] which also agree with the reported made on soil analysis. However, drainage site soil of veerampattinam had the highest moisture content but the fungal plate count was lower compared with refuse dump soil. Fungi as a group will tolerate a wide pH range, but some fungi are more tolerant to acidic soils. As compared to bacteria they can tolerate a wide range of pH 4-8 [12] which also correlates with the findings in this research study. Any unfavourable alkaline soil often inhibits the development of *Penicillium spp* correlates with the findings [12].

Scientist reported that humus (organic matter) rich soils have large fungal population than soil poor in humus [1]. This finding is in consonance with the work done [2]. There are basic groups of fungi encountered in this data obtained from Puducherry correlates with the other data which obtained by previous workers, in other parts of the world.

CONCLUSION

Aspergillus spp. (especially *Aspergillus niger)* was found to be much in the isolated. This could possibly be due to the fact that *Aspergillus niger* is a common contaminant on various substrates while others include *Fusarium spp, Penicillium spp, Mucor spp, Cladosporium* and *Rhizopus spp*. Their common occurrence could also be due to their high sporulating nature and this is also coupled with their ability to grow well on laboratory media.

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