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IDENTIFICATION AND ENZYME ACTIVITY OF FUNGUS FROM SITHANAVASAL, PIRANMALI AND KUNDRAKUDI

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ABSTRACT

Soil ecosystems provide excellent habitats for a large number of species of organisms from all domains of life including archaea, bacteria, fungi, protists, animals and plants. Abundant worldwide, most fungi are inconspicuous because of the small size of their structures, and their cryptic life styles in soil, on dead matter, and as symbionts of plants, animals, or other fungi. Different types of fungus are collected from three different hills station such as (Sithanavaasal, Piranmalai and Kundrakudi) and physicochemical parameters are evaluated especially pH and Temparature during January to March 2012. The enzyme tests are estimated from isolated fungus especially Aspergillus niger, pencillium *sp* and *Alternaria sp*.

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INTRODUCTION

A fungus is a member of a large group of Eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom. Fungi, which is separate to plants, animals and bacteria. One major difference is that fungal cell have all walls that contain chitin, unlike the cell walls of plants, which contain cellulose. For centuries, certain mushrooms have been predictable as a folk medicine in China, Japan, and Russia(Smith JE,et al.2002).Although the use of mushrooms in folk medicine is largely centered on the Asian continent, people in other parts of the world like the Middle East, Poland and Belarus have been documented using mushrooms for medicinal purposes (Sarfaraz,et al.2009 and Shashkina mla, et al.2006). These and other differences show that the fungi form a single group of related organisms, named the Eumycota (true fungi or Eumycetes), that share a common ancestor (a monophyletic group). This fungal group is distinct from the structurally similar Myxomycetes (slime molds) and Oomycetes (water molds). The discipline of biology devoted to the study of fungi is known as Mycology, which is often regarded as a branch of Biology, even though genetic studies have shown that fungi are more closely related to animals than plants. They may become noticeable when fruiting, either as mushrooms or molds. Fungi perform an essential part in the decomposition of organic matter and have fundamental roles in nutrient cycling and exchange. They have long been used as a direct source of food, such as mushrooms and truffles, as leavening agent for bread, and in fermentation of various food products, such as wine, beer, and soy sauce. Since the 1940s, fungi have been used for the production of antibiotics, and more recently, various enzyme produced by fungi are used industrially and in detergents. Fungi are also used as bio-pesticides to control weeds, plant diseases and insect pests. The fruiting structures of a few species contain psychotropic compounds and are consumed recreationally or in traditional spiritual ceremonies. Fungi occurs in desert areas can be grouped in some ecological groups like Terricolous, epi- and endolithic lichens with ascomycetous and less frequent basidiomycetous mycobionts in hot and cold deserts have been extensively studied and reported (Nienow & Friedmann 1993; Wirth 2010; Dojani et al. 2011) and Fungi associated with plants as phylloplane fungi or in mycorrhizal associations even truffles are able to grow in the Australian outback and the African Kalahari (Trappe et al. 2008, 2010).Fungi can breakdown manufactured materials and buildings, and become significant pathogens of humans and other animals. Losses of crops due to fungal diseases or food spoilage can have a large impact on human food supplies and local economics. The fungus kingdom encompasses an enormous diversity of taxa with varied ecologies, life cycle strategies, and morphologies ranging from single-celled aquatic chytrids to large mushrooms. However, little is known of the true biodiversity of kingdom fungi, which has been estimated at around 1.5 million species, with about 5% of these having been formally classified. Ever since the pioneering 18th and 19th century taxonomical works of Carl Hinnaeus, Christian Hendrik Persoon, and Elias Magnus Fries, fungi have been classified according to their morphology or physiology. Advances in molecular genetics have opened the way for DNA analysis to be incorporated into taxonomy, which has sometimes challenged the historical groupings based on morphology of mushrooms and molds, the root is also used in other

languages, such as the German Schwamm ("Sponge") and Schimmel ("mold"). The use of the word mycology, which is derived from the Greek Mykes (mushroom) and logos (discourse), to denote the scientific study of fungi is thought to have originated in 1836 with English naturalist Miles Joseph Berkeley's publication The English Flora of Sir James Edward Smith. Before the outline of molecular methods for phylogenetic analysis, taxonomists considered fungi to be members of the plant kingdom because of similarities in lifestyle, both fungi and plants are mainly motionless, and have similarities in general morphology and growth habitat. Like plants, fungi often grow in soil, and in the case of mushrooms from fruiting bodies, which sometimes bear resemblance to plants such as mosses. The fungi are now deliberated a separate kingdom, different from both plants and animals, from which they appear to have diverged around one billion years ago. Some morphological, biochemical, and genetic features are shared with other kingdoms. Equally other eukaryotes, fungal cells contain membrane bound nuclei with chromosomes that contain DNA with non-coding regions called Introns and coding regions called exons. In addition, fungi possess membrane-bound cytoplasmic organelles such as mitochondria, sterol-containing membranes, and ribosomes of the 80S type. They have a characteristic range of soluble carbohydrates and storage compounds, including sugar alcohols (e.g., mannitol), disaccharides, (e.g., trehalose), and polysaccharides (e.g., glycogen, which is also found in animals). In animals, Fungi lack chloroplasts and are heterotrophic organisms, requiring preformed organic compounds as energy sources. In plants, Fungi posses a cell wall and vacuoles. They reproduce by both sexual and asexual means, and like basal plant groups (such as ferns and mosses) produce spores. Similar to mosses and algae, fungi typically have haploid nuclei. Fungi could not grow well without oxygen (Widawti and Suliasih, 2001).

MATERIALS AND METHODS:

Materials Required:

Test tubes, conical flask, petriplate, pipette, measuring cylinder, cover slip, glass slide, cotton, Lacto phenol cotton blue stain, Inoculation loop, cork borer.

Sample Collection:

Soil sample was collected from different hills regions of Sithanavasal, Piranmali And Kundrakudi. During the month of January-March 2012 three hills soil samples were collected from the different regions of the hills. All the samples were collected by using sterile polythene bags. The fungus are isolated by serial dilution method.

Media Composition:

PDA (Potato Dextrose Agar)

Potato	-	200g
Dextrose	-	20g
Agar	-	15g (dissolved in 1000ml of distilled water)
pH	-	5.6

Media Preparation:

Take 14.04g of Potato Dextrose Agar medium in a conical flask. Add 360ml of sterile distilled water, close the conical flask using cotton plug. Then the medium will be sterilized in autoclave of 15lb for 30 mins.

Plating Techniques:

The PDA media was prepared and poured in to sterile Petri plates and allowed to solidify. The serial dilutions 10^{-2} – 10^{-6} was selected 1ml of each dilution from 10^{-2} – 10^{-6} was added in sterile plate containing PDA media plates were incubated at 28°C for 3-5 days.

Identification of Fungi:

The isolated fungi were identified to the species level. When possible on the basis of micro-morphological characteristics using slide cultures (Obtained by inoculating micro fungi directly on a small square of agar medium). Small square of agar medium was taken on the slide, add one drop of lacto phenol cotton blue on the slide, and close the culture using cover slip. Then observe under the microscope.

Screening for Extracellular Enzymes:

Aspergillus niger and *Penicillium* were taken for enzymatic screening for amylase & cellulose activity. Isolates were grown on starch agar & CMC medium. After seven days incubation at 28°C the culture plates were tested. Around the fungal inoculums when Grams Iodine solution (for amylase and cellulose) is added the cultures making zone of clearance were selected.

RESULTS:

The typical characteristics of soil samples from different sites and microbial population at three hills station. Fungi were studied and summarized in Table 3. A total of 7 fungal genera were recorded in all the three samples. In Sithanavaasal Sample (S1) important fungal genera like *Aspergillus niger, Aspergillus sp., Penicillium sp.*, were recorded. In Piranmalai Sample (S2) important fungal genera like *Alternaria sp, Aspergillus sp., Penicillium sp.*, were recorded. In Kundrakudi Sample (S3) important fungal genera like *Aspergillus sp., Penicillium* was recorded. *Aspergillus niger*, *Penicillium* and *Alternaria sp.*, were screened by plate test method for amylase and cellulase activity showed in the table.2. Based on the plate test assay the amylolytic activity which was reflected by wide clear zone formation around the colony on the solid starch agar medium. Physico chemical analyses showed that pH range of soil conditions ranging from 7.16-7.85 and average temperature is 28 in three hills station. The soil textures were fine sand, coarse sand, silt and clay. The soil textures were determined depending upon the percentage of sand, dust and clay. The stability of an aggregates depending upon both the content of organic matters in each type of soil samples and the nature conditions of microbes which tied the soil particles to become one. Thus, soil acidity, soil fertility, soil textures, and soil colors can influence the variety and population of microbes in Rhizosphere. According to Widawati and Suliasih (2001) the number of microbes at Halimun Mountain was influenced by the different vegetation type, soil pH and the elevation of area. The composition of population and soil microbes activity were influenced by the different climate and vegetations (Jha *et al.*, 1992). On the other hand, the activities of microorganisms are constantly changing with temperature, moisture, pH, food supply and other environmental conditions. So, different species prefer different conditions. So, microbes are generally assumed that of the major microbial group's soil, fungi are tolerant of acidity. The microbial populations are mostly present in rhizosphere soil than non rhizosphere soil samples. Each type of microbe filled a special niche and played a different role in the nutrient cycle. Microbes, which were potential as bio-fertilizers were often found in rhizosphere. Because Rhizosphere is rich with biological activity as microbes feed on the carbon compounds exudedby root, while organic and inorganic materials released by the plants into the areas (in the form of exudates), will be useful for life continuity of soil microbes (Rosch *et al.*, 2002).

DISCUSSIONS:

All fungi have mycelium thread, which are organized from individual hypha. So, a fungi colony can dominate all soil types (Haili *et al.*, 2008). There are seven fungal seven genuses present in 3 soil types from 3 hillsstation. Among 7 genus of fungi, two strains namely *Aspergillus niger* and *Aspergillus flavus* were dominated in several places. The fungus is one of the most important microbes in the soil ecosystem dynamics, because they function in the decomposition, mineralization and organize the migration of soil elements to plant root (Widawati and Suliash, 2001). A fungi colony is microbes which is more resistant to soil acidity, their live hood still depends on the availability of organic materials and is much influenced by climate, especially soil moisture content (Widawati *et al.*, 2004). Fungal diversity values are determined by very variable local species richness but in general by high evenness of abundance and high overall richness which indicates considerable space-time heterogeneity. Diversity has a temporal variation which suggests a permanent feed-back process in the community structure. Following experimental disturbance, these communities recover quickly under the conditions imposed by the climate, indicating the existence of internal mechanisms which maintain their diversity fluctuating around a high level (5 bits), and a notable complexity of the community. The sequence of disturbances produced by itinerant agriculture sudden changes in the diversity of soil fungi, mainly in ferrallitic soils. However, abandoning cultivation facilitates a rapid recovery of the pre-existing community organization. Ferrallitic soils, which are affected by disturbance more than hydromorphic soils, have a notably greater capacity for recovery.

Table: 1.

S.No	Hills Soil	Places	Average pH	Average Temperature
1	Sample – 1	Sithanavaasal	7.16	23.9
2	Sample – 2	Piranmalai	7.45	23.7
3	Sample – 3	Kundrakudi	7.80	24.8

Table:2.

FUNGUS	ZONE FORMATION(MM)	
	Amylase	Cellulase
Aspergillus niger	16	14
Pencillium sp	14	12
Alternaria sp	12	13

GRAPH FOR ENYME ACTIVITY:

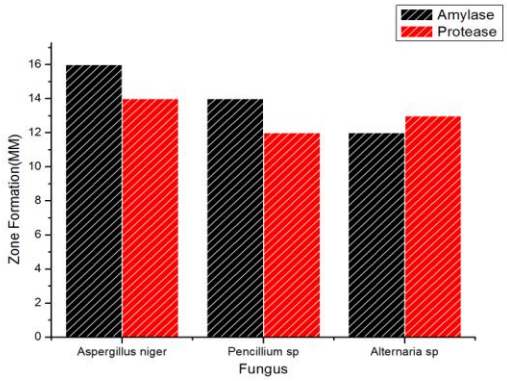

Table:3.

S.No	Places	Total Number of Colonies	2012		
			January	**February**	**March**
1	Sithanavaasal	*Alterneria* sp	-	-	-
		Aspergillus niger	+	+	+
		Aspergillus *sp*	+	+	+
		Penicillium sp	+	+	+
		Penicillium sp	-	+	-
		Aspergillus sp	+	+	-
		Aspergillus sp	+	+	+
2	Piranmalai	*Alterneria* sp	-	+	-
		Aspergillus niger	+	+	+
		Aspergillus *sp*	+	+	+
		Penicillium sp	+	+	+
		Penicillium sp	+	-	-
		Aspergillus sp	+	-	+
		Aspergillus sp	+	+	+
3	Kundrakudi	*Alterneria* sp	-	-	-
		Aspergillus niger	+	+	+
		Aspergillus *sp*	+	+	+
		Penicillium sp	+	+	+
		Penicillium sp	+	+	-
		Aspergillus sp	+	+	-
		Aspergillus sp	+	+	+

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