INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com

Review Article

MARINE AND MANGROVE FUNGAL BIORESOURCES - A REVIEW

T.SIVAKUMAR*
Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi, Kanchipuram- 631551, Tamil Nadu, India

*Corresponding Author: shiva.fungi@gmail.com

Abstract

Fungal biotechnology has become an integral part of human welfare. Nature represents a formidable pool of bioactive compounds and is more than ever a strategic source for new and successful commercial product. Among the microorganisms, fungi are well recognized to produce a wide variety of most valuable pharmaceutical chemicals, agrochemicals and industrial products. Recent advances made in genomics, proteomics and combinatorial chemistry show that nature maintains compounds that are the essence of bioactivity, within the host and environment. So the major challenging task is to explore the unexplored fungal wealth in our country and reveal their potential applications.

Keywords: Fungal biotechnology, valuable pharmaceutical chemicals, agrochemicals and industrial products.

Introduction

Biodiversity of marine and mangrove fungi

Most early studies on fungi colonizing mangroves were taxonomic and confined mainly to cataloguing fungi and describing new taxa collected in a given area (Cribb and Cribb, 1955; Kohlmeyer and Kohlmeyer, 1964 – 1969, 1971, 1977; Kohlmeyer, 1966, 1969a, 1981, 1984, 1985; Kohlmeyer and Schatz, 1985). Until recently, there have been few ecological studies on manglicolous fungi. Recent studies on inertial mangrove fungi have provided information on (a) frequency of occurrence (b) vertical zonation, (c) host and substratum specificity, (d) succession, and (e) seasonal occurrence (Aleem, 1980; Jones *et al*., 1988; Hyde, 1988a, 1989c, 1990b, 1991; Leong *et al*., 1991; Poonyth *et al*., 1999). Of these, considerable effort has been spent investigating the frequency of occurrence of manglicolous fungi (Jones and Tan, 1987; Borse, 1988; Hyde, 1988a,b, 1989a,b,c; Hyde and Jones, 1988; Jones *et al*., 1988; Jones and Kuthubutheen, 1989; Tan *et al*., 1989; Tan and Leong, 1990,1992).

Early studies on marine fungi on mangroves have focused on taxonomy of marine fungi including descriptions of new species and new genera, lists of fungi and surveys. This includes the marine fungi occurring in mangrove environments. Excellent reviews and vast amounts of information on marine fungi have appeared in several texts. (Johnson and Sparrow, 1961; Jones, 1976; Moss, 1986; Hyde and Lee, 1995; Jones, 1995; Jones and Alias, 1996). For several accounts on various aspects of marine fungi the following works, among others, are referable (Chinnaraj, 1993a; Jones and Tan, 1987; Hyde and Jones, 1988; Hyde, 1990a; Hyde *et al*., 1990; Ravikumar, 1991; Leong *et al*., 1991; Kohlmeyer and Kohlmeyer, 1979; and Kohlmeyer *et al*., 1995). Ecological studies on manglicolous fungi are relatively recent i.e. from late 1980s onwards. A wealth of information is now available on different ecological aspects of fungi in mangroves including frequency of occurrence, vertical distribution, substrate preference, succession, seasonal occurrence and host specificity. However these are mainly from South East Asia (Hyde and Lee, 1995; Jones and Alias, 1996).

There are umpteen literatures on the ecology and taxonomy of soil fungi Most of the reports relate to the study of fungal flora from cultivated agricultural soils, uncultivated soil, pasturelands and forest soils. However, little is known about the microbial ecology of mangrove swamps. During the past several years, considerable work has been done on the taxonomy and ecology of mangrove swamp fungi in India (Padhye *et*

al., 1967; Pawar, *et al*., 1963; 1965 and Rai and Tewari, 1963).

Earliest studies on the ecology of mangroves fungi Kohlmeyer (1969a) encountered 3 common species of marine fungi in the mangrove habitat, namely *Lulworthia* spp (20% of all collections) *Leptosphaeria australiensis* (15%) and *Phoma* species (10%). 100 Mangrove species; only 8 have been examined for the occurrence of marine fungi and the latest Island research was conducted in Bermuda and collected 15 marine Ascomycetes, 1 Basidiomycetes, and 6 Deuteromycetes (Kohlmeyer and Kohlmeyer, 1977). Aleem (1980) reported that the Ascomycetes; *Halosphaeria viscidula*, *Rosellinia* sp and *Torpedospora radiata* were frequent on mangroves in sierra Leone and also found that the Mitosporic taxa. *Cirrenalia macrocephala*, *C. pygmea, C. tropicalis, Periconia* and *Zalerion* spp were abundant on mangrove wood. Kohlmeyar (1984) also reported that *L. australiensis* was a common species of mangroves. Although mangrove fungi of the West coast of India have been well studied, there have been few studies on the East coast (Bay of Bengal), despite the fact that its mangroves are more extensive compared to the west coast. (Untawale, 1987). Hyde and Jones (1988) observed the some fungi tend to occur configuration at certain levels and also reported that the greater species diversity occurred at the mid – littoral level.

Booth (1971b) observed on occurrence and taxonomy of aquatic fungi in saline habitats. Hyde (1989a) reported that the lignicolous materials were collected from 5 marine locations in Brunei; a rocky head land, a sandy beach, a man – made brackish lake, a healthy mangrove and an oil - polluted mangrove. Higher marine fungi present were identified and their percentage occurrence noted. There were significantly less diversity and number of fungi in the oil-polluted mangrove when compared to the healthy mangrove. Most attention to data has concentrated on assessment of fungal diversity, physiology and biochemistry (Kohlmeyer and Kohlmeyer, 1993). Hyde and Lee (1995) suggested that the diversity of marine fungi is greater in the tropics and attributed this to mangrove tree species richness. Jones *et al.* (1999) recorded all marine fungi from Marine habitats can be designated as micro fungi the "micro habitat predictor " model appear to the applicable in the marine environments.

There have however been no efforts to study the marine fungi on mangroves until recently when systematic studies on manglicolous fungi in India were initiated. A detailed investigation of fungi on mangroves of west coast was made by Patil and Borse (1985a,b) and Chinnaraj (1993a,b). However vast tracts of mangroves on the east coast remained virtually unexplored except for the studies of Ravikumar and Vittal (1996).

Quantitative data on the occurrence of tropical marine fungi have been published by Raghukumar (1973); Koch (1986); Kohlmeyer (1984); Zainal and Jones (1984, 1986). However all of these reports were on driftwood in the sea, along with driftwood on the mangrove floor or panels belonging to various timbers submerged near jetties.

Marine fungi have been classified into three geographical groups by Kohlmeyer and Kohlmeyer (1979): i) cosmopolitan species; ii) temperate – water species and iii) species from tropical and subtropical waters. Mangrove fungi have been incorporated in biogeographical maps by Jones (1993), Kohlmeyer (1981, 1984), Kohlmeyer and Volkmann – Kohlmeyer (1987) and Volkmann - Kohlmeyer and Kohlmeyer (1993). Based on the distribution in Atlantic Ocean, Indian Ocean, South – East Asia and Pacific Ocean. Hyde and Lee (1995) revised geographical distribution of representative mangrove fungi (*Halosarphaeria fibrosa, H. marina, Lignincola laevis* and *Lulworthia grandispora*). Geographical and seasonal distribution of *Asteromycetes cruciatus, Stigmoidea marina* and *Varicosporina ramulosa* correlated with their growth patterns under different temperature regimes (Boyd and Kohlmeyer, 1982).

Predominantly mangrove species: the Ascomycetes *Dactylospora haliotrepha, Halorosellinia oceanica, Lignincola leavis, Lulworthia grandispora, Saagaromyces abonnis* and *Verruculina enalia*;the basidiomycete *Halocyphina villosa* and anamorphic fungi *Cirrenalia pygmea* and *Zalerion varium* (Kohlmeyer 1984; Jones and Alias, 1996; Sarma and Hyde, 2001; Abdel-Wahab and El- Sharouney, 2002; Jones and Abdel-Wahab, 2005). Other species are more characteristic of open ocean waters: *Antennospora quadricornuta, A. salina, Periconia prolifica, Torpedospora radiata*, or wood associated with sand: *Corollospora maritima, Trichocladium melhae*. Mangrove fungi of the east coast of India have been well studied, there have been few studies on the east coast (Bay of Bengal), despite the fact that its mangroves are more extensive compared to the west coast (Untawale, 1987).

The marine fungi of Hong Kong and Thailand have been studied intensively over the past 15 years, and include not only random collections of drift material, but also the exposure and recovery of bait samples (exposure of bait in Hong Kong (Vrijmoed *et al.,* 1986; Sadaba *et al.,* 1995; Abdel- Wahab, 2000; Thailand: Pilantanapak *et al.,* 2005), collection of drift and attached mangrove samples in Hong Kong (Abdel-Wahab and El-Sharouny, 2002; Jones and Vrijmoed, 2003), Thailand (Hyde *et al.*, 1993; Sakayaroj *et al.*, 2004). Schmidt and Shearer (2004) analysed the geographical distribution data published on lignicolous mangrove fungi, and found that different oceans supported varying numbers. The number of fungi at each site varied: Atlantic Ocean: 12-46 per site (14 sites: mean 25.6); Indian Ocean: 12-64 (14: 42.9) and the Pacific Ocean: 17-87 (16: 44). The Pacific Ocean has the highest recorded number of fungi,

again the result of repeated collections over many years: Hyde (1988c) in Brunei; Jones and Kuthubutheen (1989), Alias *et al.* (1995), Tan *et al*. (1989) and Leong *et al.* (1991) in Singapore, and the greater diversity of mangrove tree species in this region. The paucity of marine fungi from the Atlantic has been attributed to low mangrove tree diversity, for example three in Florida mangroves and four in the Bahamas (Jones and Abdel-Wahab, 2005; Jones and Puglisi, 2006). However, more intensive collections yielded 81 species for Florida mangroves from 250 collected samples (previously only 28: Jones and Puglisi, 2006) and 112 for the Bahamas from 600 collected samples, where only 31 had previously been recorded (Jones and Abdel-Wahab, 2005).

Diversity most simply can be expressed as species richness, that is the number of species (Magurran, 1988). However, since richness increase in direct relation to number of individuals, area and variety of habitats sampled. Ecological variation over the temporal and spatial dimensions of the sample may augment diversity because of the increased number of areas, habitats or seasons included. Hyde (1990c) recognized the different in the common species at study sites, a core group of fungi occurring in the mangrove ecosystem. The Majority of the species *Dactylospora haliotrepha*, *Leptosphaeria avicenniae* were also reported from Brunei and other tropical mangroves (Hyde 1989a). Alias *et al*. (1995) reported that more than 60 fungal species can be recorded as common to mangrove ecosystems of the West Indo Pacific region.

Chinnaraj (1993a,b) had earlier reported 63 species of higher marine fungi from the Andaman and Nicobar Islands, which are approximately 1000 km away from the mainland on the East coast. Ravikumar and Vittal (1996) reported 48 species belonging to 37 fungal genera on *Rhizophora apiculate* at Pichavaram.

As diverse vegetation exists in mangroves, it is considered as a major niche of fungal repository. Mangrove fungal diversity is dependent on the age of mangrove, diversity of mangrove plant species and the physicochemical features of mangrove habitat (temperature, salinity and tidal range) (Hyde and Jones, 1988; Jones, 2000). Twenty-eight mangrove tree species yielded 120 higher marine fungi (Hyde, 1990b). Fifty-five mangroves and their associates yielded about 200 higher marine fungi (Jones and Alias, 1996). *Rhizophora apiculata* among the mangrove tree species harboured a maximum of 63 higher marine fungi (Sarma and Vittal, 2000).

Among the different geographical locations; South East Asia has been sampled most thoroughly (Hyde and Lee, 1995; Jones and Alias, 1996). There seem to be no discernible difference between mangrove fungi reported in the subtropics as compared to those found in tropical areas. Among 900 known marine fungi, 358 are recorded from the mangrove ecosystem (Jones and Alias, 1996; Jones and Mitchell, 1996). Out of 54 mangrove tree species and 60 mangrove associate plant species, up to 55 species have been studied for fungi (Jones and Alias, 1996). Studies on mangrove fungi from the Indian Ocean are limited compared to the Atlantic Ocean and Pacific Ocean and South – East Asian region. Although the Indian peninsula possesses about 6700 km^2 of mangroves (Natarajan, 1998) only a few studies dealt with fungal richness and diversity in Gujarat (Borse *et al*., 2000; Patil and Borse, 2001), Maharashtra (Borse, 1988), Karnataka (Ananda and Sridhar, 2003), Tamil Nadu (Ravikumar and Vittal, 1996) and Andra Pradesh (Sarma and Vittal, 2000).

Chandralata (1999) and Raghukumar and Raghukumar (1998) reported adaptation and activity of terrestrial fungi under marine/ mangrove ecosystem as facultatives or indwellers or residents. Terrestrial fungi are common in mangrove water and mud (Chowdhery *et al*., 1982; Garg, 1983), mangrove leaves (Raghukumar *et al*., 1995), wood (Aleem, 1980), standing senescent stems (Sadaba *et al*., 1995), decomposing mangrove palm (*Nypa fruticans*) (Hyde and Alias, 2000). Terrestrial fungi in deep – sea region of Arabian Sea were recovered (Raghukumar and Raghukumar, 1998). Seawater, sea foam and beach soil of Arabian Gulf Coast, Saudi Arabia yielded terrestrial fungi, typical marine and freshwater fungi (Bokhary *et al*., 1992). Sampling of the leaf litter from the Nethravathi mangroves, India revealed the occurrence of many freshwater Hyphomycetes (Sridhar and Kaveriappa, 1988).

Sarma and Vittal (2000) investigated the fungal diversity of proproots, seedlings and wood of *Rhizophora apiculata* and wood, roots and pneumatophores of *Avicennia* spp in deltaic mangroves of Godavari and Krishna rivers in the east coast of India. The number of fungi recorded on proproots (61) was much greater when compared to wood (24) and seedling (21).

Prasannarai and Sridhar (2001) reported the diversity of marine fungi on intertidal wood collected from 13 locations in the West coast of India was assessed out of 3327 wood samples scanned, of which, 72%, posses sporulating fungi. Altogether 88 species belonging to 47 genera were uncounted. The species richness and diversity was highest in Islands than in beaches and harbour locations. It has been predicted that Islands adjacent to the West coast of India provide critical habitat for marine fungi.

Borse (2002) reported that the distribution and substratum range of 166 species (13 Labyrinthulomycota, 4 Chytridiomycota, 20 Oomycota, 1 excluded sp., 120 Ascomycota, 3 Basidiomycota and 23 mitosporic fungi) of marine fungi recorded so far from India on animal substratum, driftwood, intertidal wood, algae, mangroves, sea grasses, salt marsh plants and as propagules in the sea foams samples. Maria and

Sridhar (2002) studied the richness and diversity of filamentous fungi on woody litter of mangrove along the West coast of India. Diversity of fungi in the roots of mangrove species of West coast of India (Ananda and Sridhar, 2002).

Prasannaraj and Sridhar (2003) reported fungi from intertidal wood collected from four coastal locations of the West coast of India. Of the 59 taxa identified, 43 Ascomycetes, 3 Basidiomycetes and 13 anamorphic fungi.

Detritus and live parts of mangrove vegetation have surveyed for the occurrence of higher fungi. In recent – past (up to 2000), 625 fungi encompassing 279 Ascomycetes, 277 mitosporic fungi, 29 Basiomycetes, 3 Chytridiomycetes, 3 Myxomycetes, 14 Oomycetes, 9 Thraustochytris and 12 Zygomycetes have been reported from mangrove forests worldwide (Schmidt and Shearer, 2003). Maria and Sridhar (2003) studied fungal diversity on decomposing biomass of five mangrove plant species from the South West coast of India.

Typical marine fungi were not dominant in root endophytes of coastal sand dunes halophytes (Beena *et al*., 2000), roots of mangrove plant species (Ananda and Sridhar, 2002). The assemblage and diversity of filamentous fungi on leaf and woody litter accumulated on the floor of two mangrove forests (Nethravathi and Udyavara) in the South West coast of India. In their study, yielded 78 taxa belonging to 32 ascomycetes and 46 mitosporic fungi (Ananda and Sridhar, 2004). Sridhar (2005) attempted to deal with occurrence, distribution and diversity of filamrntous fungi in mangrove ecosystem.

Jones *et al.* (2006) reported marine fungal diversity of Thailand was investigated and 116 Ascomycota, 3 Basidiomycota, 28 anamorphic fungi, 7 Stramenopiles recorded, with 30 tentatively identified. These species have primarily been collected from driftwood and attached decayed wood of mangrove trees. The holotype number of 15 taxa is from Thailand and 33 are new records from the country.

Hyde and Sarma (2006) Biodiversity and ecology of higher filamentous fungi on *Nypa fruticans* in Brunei were examined during 1999. Forty-six taxa were recorded including 33 ascomycetes and 13 anamorphic taxa in 25 genera. *Linocarpon* was the most species genus (6 species) followed by *Aniptodera* and *Astrosphaeriella* (4 each). More diversity was found on fronds than on leaves. *Linocarpon appendiculatum, L*. *bipolaris*, *Neolinocarpon globosicarpum* and *Oxydothis nypae* were more frequently recorded on fronds than other fungi, while *Linocarpon bipolaris* (13.5%), *Astrosphaeriella striatispora* (12.2%), *Trichocladium nypae* (8.1%) and *Linocarpon appendiculatum* (8.1%) were more frequently recorded on leaves.

An overview on the diversity and ecology of fungi colonizing litter of mangroves in Bay of Bengal region (mangroves of Godavary and Krishna deltas of Andhra Pradesh, Pichavaram of Tamil Nadu, and Andaman and Nicobar Islands). A total number of 131 species belonging to 77 genera have so far been reported from the three regions. *Verruculina enalia* showed highest percentage occurrence at all the sites and on different hosts. The fungi exhibited vertical zonation in their occurrence with more number occurring in the intertidal zone. While some fungi occurred throughout the tidal range many showed affinity to a particular level. Ascomycetes with immersed or semi-immersed fruit bodies occurred in water inundated niches (Vittal and Sarma, 2006).

Sridhar and Maria (2006) studied that the pattern of colonization and diversity of filamentous fungi on naturally deposited and deliberately introduced *Rhizophora mucronata* Lamk. wood during monsoon and summer in a mangrove of southwest India and compares overall occurrence with three species co-occurrence. Among 17 core-group fungi (≥10 %), *Aigialus mangrovei, Cirrenalia pygmea, Lignincola laevis, Lulworthia grandispora, Passeriniella mangrovei, Trichocladium linderi, Tirispora* sp., *Zalerion maritimum* and *Z. varium* were highly dominant (≥20 %). On wood showing co-occurrence of three fungi, *A. mangrovei, Cirrenalia tropicalis, L. grandispora* and *T. linderi* were highly dominant core-group fungi. Even though *A. mangrovei, C. pygmea, C. tropicalis, Halosarpheia cincinnatula, L. grandispora, P. mangrovei, Verruculina enalia* and *Z. maritimum* are typical marine or mangrove fungi, they were core-group fungi on deliberately introduced wood in monsoon season indicates their high colonization activity on wood even under low salinity. Several terrestrial mitosporic fungi (*Alternaria, Arthrobotrys, Aspergillus, Penicillium, Phoma* and *Tetracrium*) were found particularly in monsoon season, but none of them belonged to core-group.

The distribution of fungi in Muthupettai mangroves along the East coast of Tamil Nadu, India was studied in terms of species diversity, seasonal variation, and frequency of occurrence in five sampling stations at two different seasons. In this study, total of 118 species of fungi isolated, of which maximum 94 species from sediment samples followed by water with 83 species. Among the fungal isolates *Aspergillus* was the common genus followed by *Penicillium, Curvualria* and *Alternaria* (Sivakumar *et al*., 2006).

Fungal biodiversity in freshwater, brackish and marine habitats were estimated based on reports in the literature. The taxonomic groups treated were those with species commonly found on submerged substrates in aquatic habitats: *Ascomycetes* (exclusive of yeasts), *Basidiomycetes*, *Chytridiomycetes*, and the non-fungal *Saprolegniales* in the Class *Oomycetes*. Based on presence/absence data for a large number and variety of

aquatic habitats, about 3,000 fungal species and 138 saprolegnialean species have been reported from aquatic habitats. The greatest number of taxa comprise the *Ascomycetes*, including mitosporic taxa, and *Chytridiomycetes*. Taxa of *Basidiomycetes* are, for the most part, excluded from aquatic habitats. The greatest biodiversity for all groups occurs in temperate areas, followed by Asian tropical areas (Shearer *et al.,* 2007).

The screening of marine fungi for novel bioactive compounds has yielded several novel metabolites, some of which are being commercially developed for medicinal or agricultural use. Sadly the data generated by pharmaceutical companies in screening for bioactive compounds if often 'lost' to science due to the need for industrial secrecy. Fungal enzymes are widely used in industry and, many vitamins and food supplements rely on fermentation processes using terrestrial fungi. Due to their slow growth rates it is unlikely that marine fungi will replace their faster – growing terrestrial counterparts in this respect.

Many important industrial products are now produced from fungi using fermentation technology. A wide range of enzymes are excreted by fungi and play an important role in the breakdown of organic materials and many of these enzymes are now produced commercially. Most of these enzymes are used in food processing. Fungi are good candidate for employing them in degrading refractory substrates, cellulose, lignin, chitin, keratin and other substrates. Fungi like *Aspergillus niger* and *A. oryzae* are regarded as safe by the food and drug administration.

Microbial cells produce a variety of enzymes and help in microbial growth and respiration including other cellular activities. At times, these enzymes may themselves become fermentation products, so that one of them is specifically interested in obtaining high level of the enzymes (Bell *et al*., 1972). Qualitative screening of degrading enzymes in marine fungi was reported by Rohrmann and Molitoris (1992).

The use of enzymes in food preservation and processing predates modern civilization. Fermentation of common substrates such as fruits, vegetables, meat and milk provide a diverse array of food in the human diet. Beer, wine, pickles, sausage, salami, yogurts, cheese and buttermilk are all fermented products. Irrespective of their origin, these fermented food products are, in fact, result of the enzymatic modification of constituents in the substrate. The use of enzymes in food industry also involves a range of effects including the production of food quality attributes such as flavors and fragrances and control of colour, texture, and appearance besides affecting their nutritive value.

Molecular studies and phylogeny

Protein profile of fungi

Gel electrophoresis of soluble proteins, particularly isozymes, has proves a powerful tool in the investigation of a wide range of questions concerning the taxonomy, evolution and population genetic structure of a large number of animals and plant species. Isozyme variations were analyzed between different species and formae speciales of the genus *Puccinia* (Burdon and Marshall, 1981). Similar isozyme studies have been carried out for *Ceratocyatis coerulescens* (Harrington *et al*., 1996), the Harpellales (Grigg and Lichtwardt, 1996) and *Ganoderma* species (Gottlieb *et al.*, 1998). To date, no isozyme studies have been reported for marine fungi but Pointing *et al*. (1999) has studied the production of extracellular enzymes of five lignicolous mangrove fungi.

In fungi, the synthesis of heat shock proteins is a rapid process. In *Fusraium oxysporum* began to synthesis after 10 minutes of heat treatment (Freeman *et al*., 1989), while for *Saccharomyces cerevisiae*, a peroid of 20 to 30 minutes was required. The synthesis of HSPs peaked at 60 minutes after heat treatment of *Achyla* (Silver *et al*., 1983) and *Neurospora crassa* (Plesofsky – Vig and Brambl, 1985b). However, under high temperature treatments, fungi differ from plants and animals in needing a long recovery time before synthesizing normal proteins (Plesofsky – Vig and Brambl, 1985a).

Species within the genera *Penicillium* and *Aspergillus* have been used in several phylogenetic studies to establish whether the taxa within these individual genera belong to their respective genera and whether they form separate monophyletic groups (LoBuglio *et al*., 1993; Berbee *et al*., 1995; Verweij *et al*., 1995). Studies on fungal molecular systematics have proved the way for studies of fungi in fresh water and marine habitats (Chen *et al*., 1995; Spatafora *et al*., 1995; Fallah *et al*., 1997; Spatafora *et al*., 1998; Chen *et al*., 1999; Ranghoo *et al*., 1999). Differences in strains within species and geographical variations of single species have also been investigated for *Phytophthora striiformis* (Chen *et al*., 1993), *Fusarium oxysporum* (Appel and Gordon, 1995,1996).

Electrophoretic Karyotyping of Fungal DNA

A recent advance in gel electrophoresis technology has allowed megabase – sized DNA molecules to be efficiently separated. The successful separation of the chromosomes within *Saccharomyces cerevisiae* (Schwartz and Cantor, 1984; Carle and Olson, 1985) had led the way for similar electrophoretic karyotyping studies of other fungi. By the beginning of this decade, more than 25 species of fungi have been successfully karyotyped (Mills and McCluskey, 1990).

DNA relatedness of the species of *Aspergillus* section Flavi was studied by Kurtzman *et al*. (1986) with the DNA reassociation method. The cot values calculation results showed 100% relatedness between *A. flavus* and *A. oryzae*. Similarly *A. parasiticus* and *A. sojae* were 91% related. The homology between these two groups was 70%.

Electrophorectic karyotyping of certain species of fungi such *as Tilletia, Ustilago* and *Phytophthora* show distinctive patterns when viewed by Pulse Field Gel Electrophoresis (PFGE) as their chromosome have variable base numbers and hence allows species to be easily distinguished. It has been considered to be wiser to use a combination of Electrophorectic karyotyping and other molecular techniques like RFLP to make taxonomic interferences (Mills and McCluskey, 1990). Taga *et al*. (1998) compared different ways to study the karyotype of *Nectria haematococca* and found that PFGE was the most effective tool

Species within the genera *Penicillium* and *Aspergillus* have been used in several phylogenetic studies to establish whether the taxa within these individual genera belong to their respective genera and whether they form separate monophyletic groups (LoBuglio *et al*., 1993; Berbee *et al*., 1995; Verweij *et al*., 1995). Phylogenetic analyses within specific genera include those of *Gliocladium* (Rehner and Samules, 1994), *Colletotrichum* (Sheriff *et al*., 1994), *Fusarium* (O' Donnell, 1993; Appel and Gordon, 1995,1996; Waalwijk *et al*., 1996), *Puccinia* (Zambino and Szabo, 1993), *Phytophthora* (Lee and Taylor, 1992; Cooke *et al*., 1996; Crawford *et al*., 1996), *Pythium* (Chen, 1992), *Ganoderma* (Moncalvo *et al*., 1995) and *Laccaria* (Gardes *et al*., 1991).

Fungal biotechnology

Amylase from fungi

The decomposition of starch by marine fungi was demonstrated by Barghoorn (1944) for the representative of the genera *Ceriosporopsis, Corollospora, Lulworhtia, Phialophorophoma* and *Zalerion* and by Nilsson (1974b) for *Humicola alopallonella*. Denaturation of bacterial and fungal α-amylase by heat acid and urea was also investigated in the presence of added calcium ions (Hagihara *et al*., 1956).

Iqbal and Zafar (1994) reported a new matrix petiolat felt- sheath of palm (*Livistona chinesis*) immobilize the cells of *Aspergillus niger* for the production of aplha amylase. Growth of immobilized culture was 19% greater than free cells. Different types of immobilized cell systems have also been used for Glucoamylae (GA) production (Li *et al*., 1984; Fiedurek and Szczdrak, 1995; Shimada *et al*., 1998; Ariff and Webb, 1996). Mycelia of *A. niger* were immobilized on various seeds such as wheat rye, barley, mustrad etc., for GA production. Enzyme productivity was 1.6 times higher in immobilized system than by free cells (Fiedurek and Szczdrak, 1995). Shimada *et al*. (1998) described a system using immobilized cells of *Saccharomyces cerevisiae* for the production of GA. The system was capable of accumulating high quantity of GA. Ariff and Webb (1996) compared the influence of different fermenter configuration and modes of operation on GA production by *Aspergillus awamori*.

Goto *et al.* (1998) studied the amylase from fungi easy to manufacture then the amylases from the bacteria and *Streptomyces.* Among the fungi *Aspergillus oryzae* and *Aspergillus niger* have been well studied. Arora *et al*. (2000) studied that the potato waste was fermented by *Rhizopus oryzae* under solid substrate fermentation and yielded a dry biomass of 25 g containing 17- 18% protein and 70% dry matter digestibility (*in - vivo*) and 3.2 g crude alpha amylase enzyme. Mangrove derived fungi (35 sp.) was screened for amylase activity using starch agar and all the fungi showed zone of clearance on starch agar plates (Sivakumar and Ravikumar, 2006c).

Protease from fungi

Pisano *et al*. (1964) screened 14 marine fungi for their geletinase activity and found such activity in the culture filtrate of 13 isolates, *Halosphaeria mediosetigera* produced the highest level of gelatinase. Sguros *et al*. (1973) concluded that the *Culcitalana achtaspora, Halosphaeria mediosetigera* and *Humicola alopallonella* were probably insignificantly proteolytic, lipolytic, nucleolytic or ligninolytic.

The extracellular protease production was studied in many *Aspergillus* species. Klapper *et al*. (1973a,b) reported about this enzyme production and the factors affecting their synthesis and release from *A. oryzae* NRRL 2160. Fukushima *et al*. (1989) studied continuous protease production in a carbon limited chemostat salt tolerant *A. oryzae*. In the same way Battaglino *et al*. (1991) also reported the culture requirements for the production of protease by *Aspergillus oryzae* in solid-state fermentation.

Alkaline protease production from *Aspergillus niger* was also reported by Singh *et al*. (1973); Bathomeuf *et al*. (1992). Monod *et al*. (1991) also studied the enzyme production in *Aspergillus fumigatus, A. sojae* by Nasuno and Ohara (1971), *A. nidulans* by Stevens (1985), Cohen (1973) and *A. melleus* (Luisetti *et al*., 1991). Malathi and Chakraborty (1991) reported about the production of alkaline protease from new *Aspegillus flavus* strain by solid substrate fermentation.

Dahot (1993) reported that *Penicillium expansum* was grown on 1% rice husk fine powder medium and along with 1% glucose, raffinose, maltose and molasses and corn steep liquor for the production of protease. The

other *Penicillium* species like *P. lilacinces* and *P. griseofulvin* was also known to produce alkaline protease reported by Kitano *et al*. (1992). Dozie *et al*. (1994) studied the production of alkaline protease by *Chrysosporium keratinophilum*.

Aspergillus ustus (NIOCC 20) producing the highest amounts of the enzyme was selected for further studies. The growth yield was substantial at 30°C and 50°C at 1 bar and elevated hydrostatic pressures. The fungus produced alkaline, cold-tolerant protease when grown at 30°C and 1 bar pressure. The enzyme was active at combinations of 30°C and 50°C and 300 bar pressure. The enzyme was totally inhibited in the presence of 2 mM PMSF suggesting it to be a serine protease (Damare, *et al*., 2006).

Cellulase from fungi

Barghoorn (1944) was first to use marine Ascomycetes and Deuteromycetes to demonstrate their ability to grow on wood flour and regenerated cotton cellulose by measuring the rate of radial growth on agar medium. The clearing of cellulose – contianing agar by 14 marine fungi was also used by Henningsson (1976) as a measure of cellulase and xylanase production. Nilsson (1974a) employed several methods to assay the enzymatic activities 0f 36 wood inhabitating fungi, among them one marine species, namely*, Humicola alopallonella*. 12 of these fungi unable to degrade pure cellulose substrates in culture but produced characteristic soft-rot patterns, namely cavities in the secondary cell walls of wood.

A number of fungal species are known to produce cellulase enzymes. Among these are the Ascomycetes such as *Neurospora* and *Trichoderma.* Shoemaker *et al*. (1983) reported a variety of cellulase produced from *Trichoderma.* These enzymes are also produced *by Sporotrichum, Humicola, Thermoascus, Trichoderma ressei* and *T. koningii. Penicillium funiculosum* is a potent cellulase producer, has been studied earlier for various applications including cellulolysis of various cellulose substrates (Dighe *et al*., 1987; Betrabet and Paralikar, 1977).

Cellulolytic enzyme system can be produced by a number of different fungi, such as white rot fungi (Uzcategui *et al*., 1991; Thompson *et al*., 1998) soft rot - fungi (Kubicek *et al*., 1990) and anaerobic fungi (Barichievic and Calza, 1990).

Pectinase from fungi

Raghukumar *et al*. (1994) have investigated degradative enzyme pectin lyase production by fungi isolated from detritus of the leaves of the mangrove *Rhizophora apiculata*. Twenty-one higher filamentous fungi isolated from *Spartina alterniflora* and other salt march substrata were shown to capable of degrading cellulose, lipids, starch including pectin compounds (Gessner, 1980).

Lipase from fungi

Lawrence (1967) and Barockerhoff and Jensen (1974) have presented its comprehensive reviews. These lipases are being exploited due to their low cost of extractions, thermal and pH stability, substrate specificity, and activity in organic solvents. The chief producers of commercial lipases are *Candida cylindracea, Humicola lanuginose, Rhizopus delemar, R. japonicus, R. niveus and R. oryzae* (Godfredson, 1990).

In 1981, one group highlighted the lipolytic activity of thermophilic fungi of paddy straw compost (Satyanarayana and Johri, 1981). Systematic screening strategies were employed by (Bhaduria, 1989). This study reported *Aspergillus niger, Aspergillsus flavus, A. fumigatus* and *Penicillium glaucum* as the potential lipase producers isolated form the kernels of Chironji and Walnut. Yadav *et al*. (1997) purified and characterized of a regiospecific lipase from *Aspergillus terreus.* The purified enzyme showed excelled temperature tolerance and was highly thermo stable. The enzyme showed good pH tolerance. Ionic detergents inhibited enzyme activity where as non-ionic detergents stimulated enzyme activity.

Lazer and Schroder (1992) investigated fungal lipases, which degrade lipids from palm oil. Among Mucorales, the lipolytic enzymes of the moulds *Mucor hiemalis, Mucor miehei, Mucor lipolyticus, Mucor pusillus, Rhizopus japonicus , R. arrhizus, R. delear, R. nigricans, R. microsporus* and *R. chinesis* have been studied. Kamini *et al.* (1997) studied the fungal strain isolated from curd. The fungal strain was identified as *Aspergillus niger.* Tributyrin was the substrate for examining lipase production on agar plates. A holozone of 9mm diameter around a colony in the tributyrin agar plate clearly indicated the production of lipase. The initial lipase activity was 8U ml^{-1} at 72 hrs in the culture supernatant of the basal medium, which indicated the extra cellular nature of the lipase.

Prabhakar *et al.* (2002) reported the effect of cultural conditions on the production of lipase by Fungi. They found that selected organism *Aspergillus niger, A. flavus, A. japonicus* and a fungi isolated from the contaminated ghee belonging to the genus, *Aspergillus* spp. were tested for the production of lipase on four different media by submerged fermentation technique.

Xylanase from fungi

Nilsson (1974a) demonstrated a xylanase in *Humicola alopallonella*, whereas mannose was absent. Barghoorn (1944) found that *d* –xylose, produced by the hydrolysis of xylan, was used by the 8 species of marine fungi tested. Pectin was used as a carbon by the same

species Barghoorn (1944). Leightley and Eaton (1977) determined the ability to degrade wood cell wall components of several marine fungi belonging to the genera *Cirrenalia, Culcitalana, Halosphaeria, Humicola, Nia* and *Zalerion*. They compared fresh water and terrestrial fungi and found production of cellulase, xylanase and mannanase in all species tested.

Neurospora crassa has also the ability to ferment D – glucose, D- xylose and treated cellulosic substrates directly to ethanol (Deshpande *et al*., 1984). Most microbial hemicellulolytic system contain beta xylosidase, which has been purified and characterized from many fungi *Aspergillus niger* (Rodonova *et al*., 1983), *A. fumigatus* (Kitpreechavanich *et al*., 1986), *Trichoderma viride* (Matsuo and Yasui, 1984b), *Emericella nidulans* (Matsuo and Yasui, 1984a) and *Chaetomium trilaterale* (Uziie *et al*., 1985). Screening and production of xylanase enzyme required in the hydrolysis of different xylan was investigated using strains of 35 species of fungi isolated from mangrove samples (Sivakumar and Ravikumar, 2006a).

Phosphatase from fungi

Phosphatases fall into the category of "extracellular enzymes" which are secreted and actively pass through the cytoplasmic membrane, and are associated with the producers. So, their function is involved in chemical communication of microorganisms with the surrounding microenvironment. Both alkaline and acid phosphatases have been found as external and internal enzymes in microorganisms (Siuda, 1984). There exists a relationship between pH and synthesis and release of phosphatase congregation of organisms producing the enzymes and phosphatase stability and conformation (Herbien and Neal, 1990).

Lignin degrading enzymes from fungi

Lignin is an amorphous high molecular – mass composed of phenylpropane subunits interconnected by variety of non – hydrolysable bonds. The relatively few groups of microorganisms that can degrade the macromolecule. The most efficient degraders are the white rot fungi (Orth and Tien, 1995; Paul and Clark, 1989). Safari Sinegani *et al*. (1999) assessed the production of lignin – degrading enzymes by the imperfect fungi *Aspergillus terreus* and *Trichoderma reesei* and yeast in the N-ethyl alanine, benzyl alcohol and benzaldehyde. In contrast to low biomass of the yeast, the Mnp- and LiP activiites of this fungus were much higher of the Deuteromycetes *A. terreus* and *T. ressei*. Among the fungi *A. terreus* reduced pH of its culture media significantly. Laccase activity of *A. terreus* was higher than 2 and 1.35 time of *T. ressei* and the yeast respectively. All the fungi had the highest MnP and LiP activities and fungal biomasses were significantly low in the benzaldehyde treated media.

DeSouza-Ticlo *et al*. (2006) studied that the carbon and nitrogen sources in the growth medium play an important role in the production of lignin-degrading enzymes in the white-rot Basidiomyceteous fungi. The role of nutrient nitrogen sources in growth media on production of lignin-degrading enzymes namely laccase, lignin peroxidase and manganese peroxidase as well as on the decolorization of industrial effluents like black liquor, molasses spent wash and textile mill effluents was studied using the Basidiomycetous fungus NIOCC No.2a isolated from mangrove wood. The amount of extracellular peroxidases increased by several fold in the presence of effluents whereas in their absence they were of negligible quantity. Some of the effluents had an inhibitory effect on laccase production.

Fungal metabolites

Amino acids, Lipids, and Fatty acids from fungi

Schafer and Lane (1957) demonstrated 12 amino acids in *Lulworthia* sp. and Perters *et al*. (1975) found the following common to 10 species of marine fungi; alanine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine,serine, threonine, tyrosine and valine.

Lipids are important fungal components both in terms of structure and membrane constitution. Many studies have demonstrated the importance of lipids for development, sporulation and germination and their involvement in various physiological process (Rattray, 1975;van Etten and Gottlies, 1965, Weete, 1980, 1981; Weete *et al*., 1973).

Most fungi contain 5 to 32% lipids depending on culture conditions, developmental stage and species. The lipid content of spores of many fungi ranges from 5 to 17% dry weight, but spores some species, such as rusts contain up to 35% lipid (Shen, 1966). The major factors influencing the extent of lipid production are the nature and proportion of carbon (C) and nitrogen (N) as nutrients sources in the medium. In fungi in general, lipids have been reported to be important for germination, in addition to having other functions (Cochrane *et al*., 1963; Owens, 1955; Turain and Bianchi, 1972). Smith and Silverman (1973) reported a 30 to 40% decrease in lipids during the early phase of germination. In a study on *Rhizopus stolonifer* (Weete *et al*., 1973) was observed that spore having a low concentration of lipid required a new synthesis of lipid during the early stages of spore germination compared to spores with a high concentration of lipid.

Linoleic acid has also been detected in large amounts in *Penicillium atrovenetum*, where it represents 66% of all fatty acids (van Etten and Gottlies, 1965). The study of fatty acid composition has been used for the identification of species of entomopathogenic fungi (Latge and Bievre, 1980; Tyrrell and Weatherston, 1976; Tyrrell, 1967,1968, 1969). However, studies on lipid and

fatty acids at the physiological and genetic level may permit the selection of strains for environmental persistence and expression at the epizootic level and may provide information for their large-scale production and utilization.

A separation of the two conjugated isomers may be obtained using the ability of lipases produced by fungus *Geotrichum* to selectively hydrolyse the cis – 9, trans – 11 –18; 2 methy ester (Hass, 1999). Glyceride fatty acids, in particular, oleic, palmitic and linolic acids were isolated from *Corollospora maritima* and *Zalerion maritimum* by Block *et al*. (1973) and Kirk *et al*. (1974). A number of these and other fatty acids were also determined in *Buergenerula spatinae* and *Dendtyphiella salina* (Schultz and Quinn, 1973). Szaniazlo and Mitchell (1971) compared the hyphal wall compositions of marine and terrestrial species of the genus *Leptosphaeria* and found qualitatively identical compositions in both groups. The walls consisted of glucose, mannose, galactose, glucosamine, amino acids, and traces of galactosamine.

Conclusion

From this investigation, we have concluded that the fungal biodiversity in Muthupet mangrove ecosystem, *Aspergillus* and *Penicillium* was the common fungal genera among the isolated from the study period. Fungi play an important role in decomposition of natural substrates in mangrove ecosystem. The fungi isolated from mangroves are mainly used in enzyme technology, biochemical, agricultural, pharmaceutical, molecular biology and other applied research fields.

References

Ariff, A.B., and Webb, C. 1996. The influence of different fermenter configuration and methods of operation on glucoamylase production by *Aspergillus awamori*. Asia Pacific J. Mole. Biol. Biotechnol. 4: 183-195.

Arora, M., Sehgal, V.K. and Thapar, V.K. 2000. Production of fungal protein and amylase by soild substrate fermentation of potato-waste. Indian. J. Microbial. 40: 259-262.

Barichievic, E.B. and Calza, R.E. 1990. Supernatant protein and cellulase activities of the anaerobic ruminal fungus *Neocallimastrix fronalis* EB 188. Appl. Environ. Microbiol.56: 43-48.

Bathomeuf, C., Pourrat, H. and Pourrat, A. 1992. Collagenolytic activity of new semi-alkaline protease from *Aspergillus niger*. J. Ferment. Bioeng. 73: 233.

Battaglino, R.A., Huergo, M., Pilosuf, A.M. and Bartholomi, G.B. 1991. Culture requirement for the production of proteses by *Aspergillus oryzae* in solid state fermentation. Appl. Microbiol. Biotechnol. 35: 292-296.

Betrabet, S.M. and Paralikar, K.M. 1977. Effect of cellulase on the morphology and fine structure of cellulosic substitutes part II.wheat straw pulp. Cellulose chem. Technol.11: 615-625.

Cohen,ZB.L. 1973. The neutral and alkaline protease of *Aspergillus nidulans*. J. Gen. Microbiol. 77: 521-528.

Dahot, M.V. 1993. Cultivation of *Penicillium expansum* on rice husk powder for protease production. J. Islamic. Acad. Sci.6: 3.

Damare, S., Raghukumar, C., Muraleedharan, U. and Raghukumar, S. 2006. Deep-sea fungi as a source of alkaline and cold-tolerant proteases. Enzyme. Microb. Technol.: 39(2): 172-181.

Fiedurek, J. and Szcudrak, J. 1995. Immobilization of *A. niger* mycelium on seeds for glucoamylase production. Starch/Starke. 47: 196-199.

Gallo, B.J., Andreotti, R., Roche, C., Rye, D. and Mandels, M. 1978. Cellulase production by a new mutant strain of *Trichoderma ressei* MCG 74. Biotechnol. Bioeng. Symp. 8: 89 –101.

Kitano, K., Morita, S., Kuriyama, M. and Maejima, K. 1992. Alkaline protease gene from a *Fusarium* species. Eur. Pat. Appl. Ep. 0519229.

Kitpreechavanich, V., Hayashi, N. and Nagai, S. 1986. Purification and characterization of extracellular beta-xylosidase and beta-glucosidase from *A. fumigatus*. Agr. Biol. Chem. 50: 1703 - 1711.

Malathi, S. and Chakraborthy, R. 1991. Production of alkaline protease by new *Aspergillus flavus* isolate under solid substrate fermentation conditions for use as adepilation agent. Appl. Environ. Microbiol. 57: 712-716.

Nausho, S. and Ohara. T. 1971. Hyperproduction of proteinase and some hydrolytic enzymes by mutant of *Aspergillus sojae*. Agri. Biol. Chem. 25: 829-835.

Pederson, A. and Neilson, G. 2000. Sources of amylase enzyme production. J. Biochem. 42: 223.

Rai, J.N., and Chowdhery, H.J. 1976. Cellulolytic activity and salinity relationship of some mangrove swamp fungi. Nova Medwigia. 27: 609-617.

Romana, T., Rajoka, M. I. and Malik, K. A. 1990. Production of cellulase and hemicellulase by an anaerobic mixed culture from lignocellulosic biomass. W. J. Microbiol. Biotechnol. 6: 39 – 49.

Sivakumar, T. and Ravikumar, M. 2006a. Screening and production of Xylanase enzyme from fungi. Indian. J. Appl. Microbiol. 6(1): 54- 56.

Sivakumar, T. and Ravikumar, M. 2006c. Preliminary screening of enzymes from fungi. Indian. J. Appl. Microbiol. 6 (1): 66 - 68.

Stevens, L. 1985. Regulation of proteinase activity in *Aspergillus nidulans.* Trans. Biochem. Soc. 13: 283-285.

Stewart, J.C., Lester, A., Milburen, B. and Parry, J.B. 1983. Xylanase and cellulase production by *Aspergillus fresenius*. Biotech. Lett. 6: 543-548.

Uzcategui, E., Ruiz, A., Montesino, R., Johansson, G. and Patterson, L.G. 1991. The 1-4,beta - D glucan cellobiohydrolase. A system of synergistically acting enzymes homologous to *Trichoderma reesei*. J. Biotechnol. 19: 271-286.