Malaysian Journal of Microbiology, Vol 10(3) 2014, pp. 215-218
http://dx.doi.org/10.21161/mjm.56513

Malaysian Journal of Microbiology
Published by Malaysian Society of Microbiology
(In SCOPUS since 2011)

SHORT COMMUNICATION

Identification and assessment of frequency distribution in fungi isolated from coastal Andhra Pradesh

Jangala Swathi, Katta Meera Sowjanya, Kumara Narendra, Kalakota Venkata Naga Rathnakar Reddi, Mokkapati Jaya Sravanthi, Chitta Padmavathi, Alapati Krishna Satya*

Department of Biotechnology, Acharya Nagarjuna University, Guntur-522510, Andhra Pradesh, India.
Email: akrishnasatya78@gmail.com

Received 14 August 2013; Received in revised form 31 December 2013; Accepted 20 January 2014

Aims: Marine micro flora has a wide range of importance in view of its diversity and distribution. Fungi have a large number species, and each species has their own specified characters. The main objective of the work is to isolate and identify marine fungi collected from different habitats.
Methodology and results: The samples were collected and stored in tight sterile bottles and transferred to lab carefully. Fungal isolates were screened from marine water sample at different dilutions and its genus were identified and studied. All the colonies of each plate are counted by colony counter, further those pure colonies were isolated and studies are carried out to identify them. The site for the isolate collection had been studied, the regions in this area is highly diversified with different marine ecosystems. The water sample is collected at a distance 1 km away from the edges of beaches. Thus sample is analysed for taxonomical evaluation and species identification. The taxonomical study evaluated nearly for 15 spp. out of 22 isolates. Among those some isolates were identified as *Aspergillus* sp., *Curvularia* sp., *Fusarium* sp., and *Microascus* sp. These species have more diversified in its spore formation and its arrangement.
Conclusion, significance and impact study: Throughout the study we tried to list out all the methodologies that will help a fresh researcher to identify a marine isolate through microscopic and macroscopic studies. Thus the coastal line of Andhra Pradesh had significant studies in isolating a valuable sp. for further development. Our study reveals, among all isolates *Microascus* sp., had provoked a good literature for the future research and till now its taxonomical studies are less evaluated. This would be encouraged for scientists to identify rare species to increase the evolution.

Keywords: Marine sample, fungal isolates, screening, identification

INTRODUCTION

Fungi are more in terrestrial environment and they are mainly involved in wide functions like drives nutrient cycles in detritus environment and as well as decomposers, parasites and symbionts (Richards *et al*., 2012). Among the three major habitat of the biosphere, the marine realm which covers 70% of the earth's surface provides the largest in habitable space for living organisms, particularly microbes.

Among all the organisms fungi is predominant in worldwide environment. Literature reports that fungi are the major microbial component of soil. Initially from 1860's scientists isolated fungi from soil of forests, drift wood, grass lands, polar region, deserts and recently from marine, mangrove habitat and coastal sand of various parts of the world (Thennarasu *et al*., 2011). Fungi are eukaryotic and colonise with diverse habitats in terrestrial, aquatic as well as marine ecosystem. Marine fungi are having a special group of filamentous ascomycetes (Burgaud *et al*., 2009). Among all sources, a lot of work is being carried out on fungi from aquatic and marine habitats towards their diversity, ecological role, and distribution (Shearer *et al*., 2007). The fungal kingdom has a wide diversity with about 1.5-1.6 million species. The study of cultured fungal isolates from terrestrial environments paved an opportunity to understand the ecology and evolutionary complexity to explore fungi (Hawksworth, 1991; 2001).

In modern days researchers are using powerful molecular methods to investigate and understand the fungal evolutionary complexity and its environmental diversity (Jumpponen *et al*., 2009). Global fungal diversity is studied by sequencing internal transcribed sequence (ITS) marker. From soil DNA samples, 1.5 million to 3.5-5.1 million species were identified (O'Brien *et al*., 2005). But only ~75,000 fungi culture isolates according to Kis-Papo (2005) and 64,000 ascomycete isolates and 32,000 basidiomycete isolates according to Kirk *et al*. (2008) were maintained and cultured. Accuracy of these

***Corresponding author**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

estimates is likely less than 5% of the diversity of fungal species. Further, the use of environmental DNA (eDNA) methods are available to expand our understanding towards the diversity of fungi at the highest taxonomic levels (Jones *et al*., 2011), suggesting that these estimates are conservative and that culture collections are in no way representative of natural diversity.

The best definition for a marine fungus was given by Kohlmeyer and Kohlmeyer (1979): Obligate marine fungi are those that grow and sporulate exclusively in a marine or estuarine habitat; facultative marine fungi are those from fresh water and terrestrial milieus able to grow and possibly sporulate in the marine environment**.**

Importance of marine fungi

Marine fungi play an important role in the biogeochemical cycling. Recently marine fungi have proved to be a rich source of bioactive natural products such as, novel anticancer, antibacterial, anti-plasmodial, anti-inflammatory and antiviral agents.

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 accounts for only 5% of the total fungal flora (Thennarasu *et al*., 2011).

Data count

Number of species is referred as species diversity. Population density is expressed in terms of colony forming unit (CFU) per gram of soil with dilution factor. In order to assess the dominance of individual species in each site percentage contribution was worked out as follows.

$$\% \text{ contribution} = \frac{\text{Number of colonies of fungus in a sample}}{\text{Total number of colonies of all the species in a sample}} \times 100$$

Frequency of occurrence was calculated as follows in order to identify their existence in the soils collected from different areas.

$$\% \text{ frequency} = \frac{\text{Number of samples in which a particular fungus occurred}}{\text{Total number of samples examined}} \times 100$$

Based on the frequency of occurrence, the fungi were grouped as rare (0-25% frequency), occasional (26-50%. frequency), frequent (51-75% frequency) and common (76-100% frequency) species.

MATERIALS AND METHODS

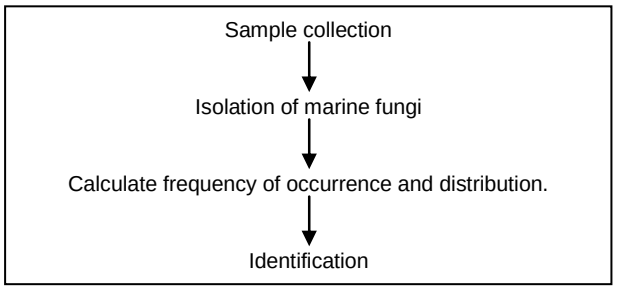

Figure 1: Evaluation and identification (Sakayaroj *et al.,* 2011).

Descriptions of sample collection site

Suryalanka Beach is located 9 km from Bapatla in Guntur District of Andhra Pradesh. It is located 50 km south of Guntur City. The Suryalanka Beach overlooks the crystal blue waters of the Bay of Bengal.

Sampling and isolation of fungi

Sampling was carried out four times from October 2006 to July 2007. Over 1,900 samples of decaying mangrove wood, leaf blades, seaweeds, sea grasses, fruits and seeds of mangrove plants were randomly collected. Samples were washed using sterile seawater and examined immediately after returning to the laboratory and up to three weeks after incubation in a damp chamber at 25 °C. Sterile seawater was sprayed periodically to maintain the moisture in the incubation chambers. All fungi were isolated using single spore technique (Choi *et al*., 1999) and maintained on seawater sabouraud dextrose agar (SDA).

Macroscopic studies

A loop full of the selected strain is placed at the centre of Saboured dextrose agar and incubated to obtain colony for morphological identification (Swathi *et al*., 2013).

Slide culture technique / Microscopic tests

To identify genus level of fungi slide culture method was done. This method is also known as moist chamber method. Wet filter paper is placed at the bottom of chamber in order to maintain moist condition all the time during experiment. Two caps are used for support at the center of chamber. Sterile slide is placed over the caps. Two square agar blocks are placed at the each end of slide. Spore culture was inoculated at four corners of agar blocks. Sterile cover slips were kept over agar blocks. The plate was incubated till growth and sporulation occurred. After incubation the cover slip was removed. The growth on cover slip was observed and stained with Lactophenol cotton blue on clear sterile glass slide. The slide left overnight to dry. After slide was dried, observed

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

under microscope at 100X oil immersion (Swathi *et al*., 2013).

Identification

To identify the genus of the fungi morphological texture, colour and size of the colony were noted along with its arrangement of hyphae, spore formation, spore size and its shapes. All these characters were compared with standard manuals to report the genus level identification.

RESULTS

Fungi isolation

The marine sample is serially diluted by serial dilution method. Different dilutions include 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} were inoculated in five different plates with 50 µL of desired dilution and it is spreader with L shaped glass rod. The sampling plates were incubated, after incubation isolated colonies were observed in table below:

Table 1 : Dilution plate count.

Dilution factor	Number of colonies
10^{-1}	23
10^{-2}	18
10^{-3}	11
10^{-4}	9
10^{-5}	4

Fungal identification

All the isolated fungi were identified based on their morphology texture and its spore study. The *Curvularia* sp., appears as puffy ash colour, *Micro ascus* sp., appears as greenish colony. *Aspergillus* appears as black color and identified based on hyphae it formed *Fusarium* appears as yellowish colony.

Species composition

Among the 15 genera recorded, the genus *Aspergillus* (4 species) was dominant followed by *Curvularia* (3 species), *Fusarium* (3 species), *Cladosporium*, *Microascus* (2 species each). All other genera were represented by one species each.

Frequency of distribution

Based on this frequency among the isolates *Aspergillus* and *Fusarium* species were commonly distributed but *Curvularia* and *Microascus* species are rarely distributed in marine ecosystem.

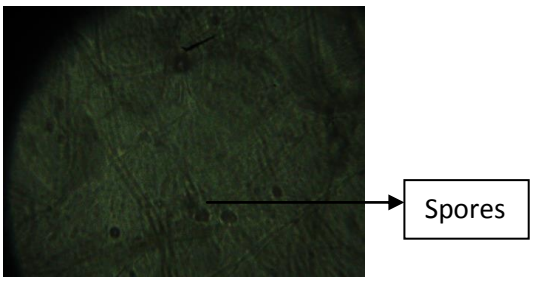

Figure 2: Microscopic observation of *Curvularia* sp. at 100X.

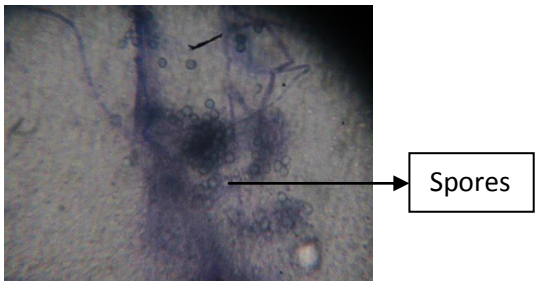

Figure 3: Microscopic observation of *Microascus* sp. at 100X.

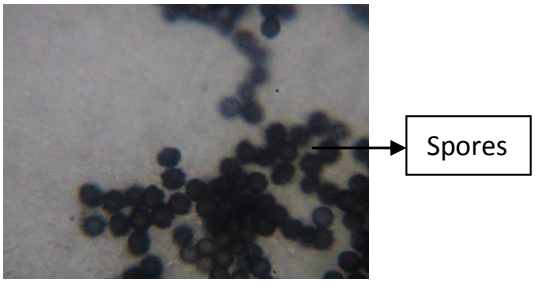

Figure 4: Microscopic observation of *Aspergillus* sp. at 100X.

DISCUSSION AND CONCLUSION

Over last few decades, Pang *et al.* (2010) researches and reviewers have been isolating and evaluating more marine fungi from Asia. Apart from this, our work is targeting the taxonomical studies of fungi from coastal line of Andhra Pradesh, India. Probably this would be one of the best research in coastal line of Andhra Pradesh. Marine fungi are potent sources for many commercial products. Among microbial population, fungi play a prominent role in decomposition of organic matter and cycling of nutrients. The main objective of our work is to study the occurrence, species, richness, diversity,

ISSN (print): 1823-8262, ISSN (online): 2231-7538

distribution of marine fungi from India to explore in specified areas. In our present research work, number of colony isolation depends on dilution factor of sample, among all together dilutions, 22 marine fungi has been recorded, among those 15 fungal isolates were reported. Those isolates were reported as *Curvularia* sp., *Microascus* sp., *Aspergillus* sp., *Fusarium* sp., considering macroscopic studies and microscopic studies in to criteria, genus level of these fungal isolates were reported with reference to desired books.

The diversity and its distribution pattern depend on its geographical regions and its required conditions. Those conditions include salinity, origin, nature of substrate, pH and its region. Alkaline conditions are more preferable for the growth of marine fungi.

Rani and Panneerselvam (2010) reported the distribution of marine fungi based on the physical and chemical properties of marine soil. The site is well examined and selected for the fungal isolation. Among the isolates *Microascus* and some species of *Curvularia* are rare distributers, while *Aspergillus* and *Fusarium* species are found to be commonly distributed. There are many remaining fungi in work to evaluate further by molecular studies. Hence, further this research will help us to document unidentified fungal species in marine environment.

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538