Research Article

A Method of Preservation of Marine Fungi in Sterile Marine Water

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ABSTRACT

Preservation of fungi is an essential practice of any mycologist. A method of preservation of marine fungi in sterile marine water was evaluated using 16 marine isolates. The marine fungi were isolated using Malt Extract Agar (MEA) medium at different regions of Nellore coast of Andhra Pradesh, India. The fungal strains were preserved in $12^{0}/_{00}$ sterile marine water. The preserved strains were subjected to recovery by sub culturing in MEA medium during 5 consecutive years (2015 to 2019). During all the years there was 100% recovery of the preserved fungi and moreover they were found to be morphologically identical to the original isolates. This study suggests that the modified castellany method by using marine water offers the cheap and alternative mode of preservation of marine fungi.

Key words: Marine fungi, Preservation, Long term, Modified Castellani method, Marine water.

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INTRODUCTION

Simple and economically cheap method of preservation is always a better choice for a mycologist. Maintenance and preservation of fungi in culture media is time consuming, requires a lot of attention. Media tends to be rapidly consumed and there are more chances of contamination during regular sub culturing process. Due to these difficulties, different methods for fungal conservation have been established viz., mineral oil, sand, silica gel, dried host tissues, distilled water, etc. Lyophilisation and conservation in liquid nitrogen are other alternatives, even though results are varied. Most of the laboratories are maintaining fungal cultures in mineral oil either by lyophilisation or by using liquid nitrogen (cryo preservation).[1]

Castellani method is one of the oldest method of preservation of fungi in distilled water. It uses only distilled water and do not require any special storage temperatures and can be stored at room temperature. Reports exist that the Castellani method is suggested for short term or long term preservation of fungi ranging from 1-20 years.[2,3]

In this study, the marine fungi isolates were preserved by using the modified Castellani method by means of sterile marine water in place of distilled water. The morphological stability, viability and purity of the 16 marine isolates were evaluated during the 5 years period (2015 to 2019).

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MATERIALS AND METHODS

Sampling area and sample collection

Samples were collected from the coast of Nellore District (Andhra Pradesh, Bay of Bengal). The sampling regions (SR) are Tummalapenta (SR1), Mypadu (SR2), Kodur (SR3), Muthukur (SR4), Thupilipalem (SR5) (Figure 1). Water and sediment samples were collected by using Niskin water sampler and Grab sampler respectively.[4] Water samples were preserved in labeled 500 mL sterile plastic bottles. The Sediment/soil samples were placed in distinctly labeled ziplock polythene bags. Both soil and water samples were maintained in ice-cold conditions and transported to laboratory. Samples were collected in triplicates during the months of January and February, 2015.

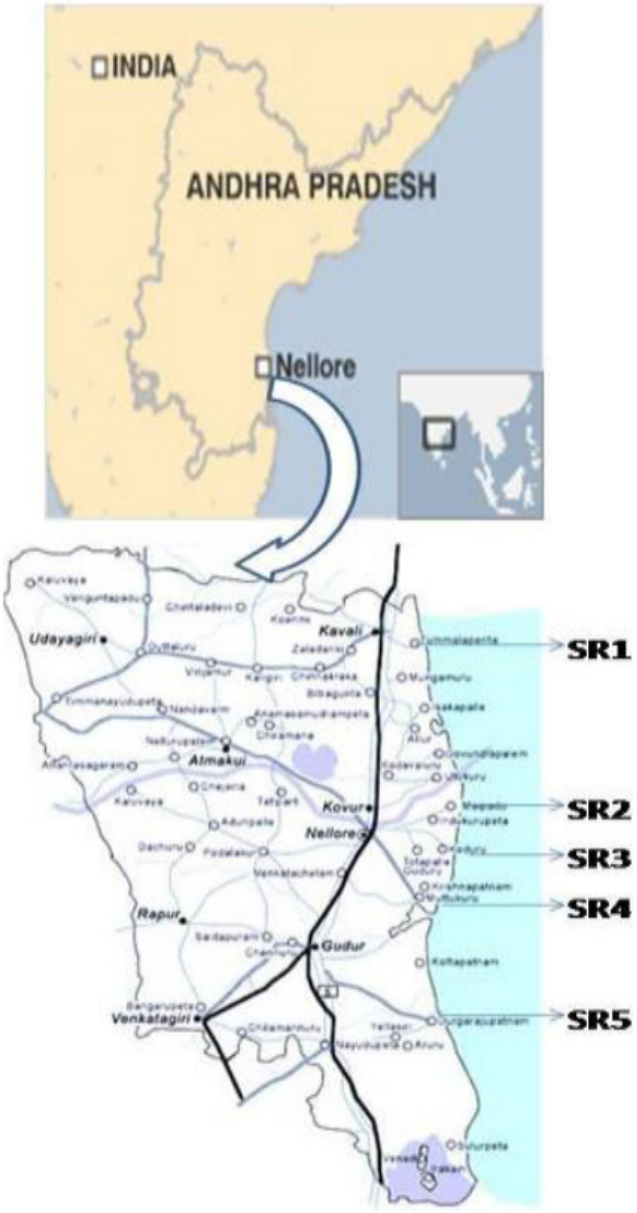

Figure 1: Sampling regions Tummalapenta (SR1), Mypadu (SR2), Kodur (SR3), Muthukur (SR4), Thupilipalem (SR5).

Isolation of fungi

Sample processing was started within the day of sample collection. Malt Extract agar (MEA) medium (Himedia, Mumbai) containing, Malt extract 30 g/L, peptone 5 g/L, agar 15 g/L in filtered and sterile marine water ($12^0/_{00}$), chloramphenicol 100mg/L was used.Sediment samples were subjected to serial dilutions (10^{-1} – 10^{-5}) and water samples (0.1 mL) were aseptically transferred to MEA plates by spread plate method. Inoculated plates were kept for incubation at 28°C for 5-6 days.[5]

Identification of fungi

The isolated fungi were sub cultured on ME agar medium and pure cultures were used for the identification studies. Slide culture method was adopted for the preliminary identification of fungi. The fungi grown on the slide cultures were stained using lacto-phenol cotton blue.[6] Morphology of the fungal hyphae, sporulation patterns and mycelial structures were studied under compound microscope (400 X). All the isolated fungi were identified by using fungal identification keys.[7]

Preservation of fungi in sterile Marine water

All the isolates were preserved in sterile marine water; for this, 12 $^0/_{00}$ marine water was prepared by diluting and filtering the marine water. The filtered marine water was subjected to sterilization. The spores of the fungi together with mycelium from the original fungal cultures were carefully scraped and introduced aseptically into the screw cap vials containing sterile marine water (4mL). The vials were swirled and stored at room temperature in a sterile container.

Recovery of marine fungi

The fungi were recovered from the preserved vials by aseptically transferring a fragment of the fungi into the petridish containing the culture media (MEA). The inoculated plates were incubated at 28°C for 5-6 days. For the strains which did not grow, the inoculation and incubation process was repeated. All the fungal strains were identified and were confirmed by slide culture method. The morphological features of the recovered fungi were compared with the initial descriptions of the strain. The recovery of the preserved fungi was done in the month of December from 2015 to 2019.

RESULTS

The marine fungi belonging to different genera were isolated and enumerated by plating techniques. Well isolated colonies were obtained on MEA Medium. Colonies were subcultured, purified and lactophenol cotton blue mount was carried out for morphological evaluation (Figure 2). A total of 16 different fungal isolates were obtained from sediment and water samples collected from five different places of Nellore coast, A.P, India. The identified isolates were classified based on the arrangement of genera under their respective orders and families. The isolated marine fungi was preserved in sterile vials containing 12 $^0/_{00}$ sterile marine water. The marine fungi were recovered by sub culturing in ME Agar medium periodically from 2015 to 2019 ie., five years (Table 1). All the marine fungi were successfully recovered from the preserved vials.

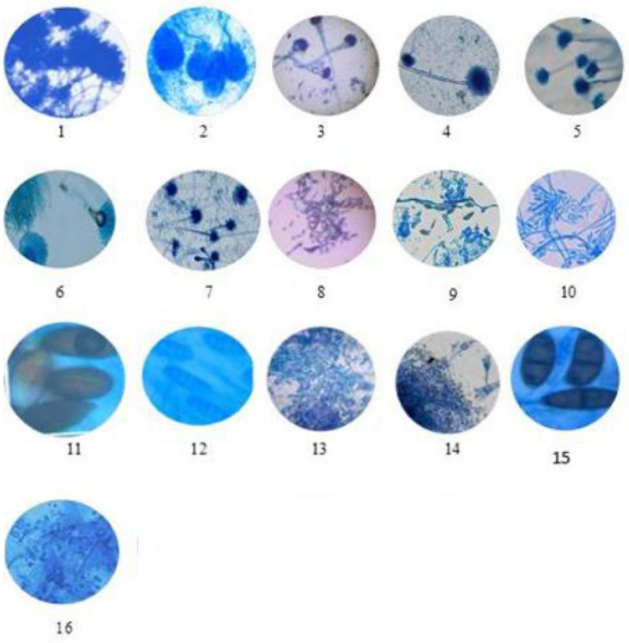

Figure 2: Lactophenol cotton blue mounts of different fungi isolated from different sampling regions.
(1. *Aspergillus awamori,* 2. *Aspergillus clavatus* 3. *Aspergillus fumigatus*, 4. *Aspergillus granulosus, 5. Aspergillus nidulans,* 6. *Aspergillus terreus,* 7. *Aspergillus versicolor,* 8.*Cladosporium* sp.1, 9. *Cladosporium* sp.2, 10. *Fusarium* sp. 11. *Halorosellinia* sp., 12. *Leptosphaeria* sp.13. *Penicillium* sp., 14. *Penicillium expansum,* 15. *Savoryella* sp., 16. *Trichoderma* sp.)

Table 1: Recovery of fungi preserved in Sterile Marine water.

S.No	Name of the fungi	Period of recovery of fungi by subculture				
		2015	2016	2017	2018	2019
	Aspergillus awamori	+	+	+	+	+
	A. clavatus	+	+	+	+	+
	A. fumigatus	+	+	+	+	+
	A. granulosus	+	+	+	+	+
	A. nidulans	+	+	+	+	+
	A. terreus	+	+	+	+	+
	A. versicolor	+	+	+	+	+
	Cladosporium sp.1	+	+	+	+	+
	Cladosporium sp.2	+	+	+	+	+
	Fusarium sp.	+	+	+	+	+
	Halorosellinia sp.	+	+	+	+	+
	Leptosphaeria sp.	+	+	+	+	+
	Penicillium sp.	+	+	+	+	+
	P. expansum	+	+	+	+	+
	Savoryella sp.	+	+	+	+	+
	Trichoderma sp.	+	+	+	+	+

DISCUSSION

Preservation of fungal cultures is an essential part during fungal biodiversity studies. Selection of right preservation technique is an important factor to be considered during fungal biodiversity studies. Preservation technique should not alter morphological and physiological and genetic nature of the fungi. The most widely used preservation method of spore producing fungi is drying. Soil or Silica gel are the common substrata that are used for preservation of fungi by drying method. The maximum duration of storage using drying method was reported to be 11 years.[8] Another alternative method of preservation of fungi is cryopreservation using liquid nitrogen. Microbank system is the advanced cryopreservation technique where fugal species are stored in liquid nitrogen vapour up to 8 years.[9] Lyophilization is another technique in which fungi are freeze dried by applying vacuum at -80°C by using ultra freezers.[10] Claudia *et al.* recommended the Castellani method of preservation of fungi in distilled water. They recovered the 20 years old strains of different species of fungi preserved in distilled water.[11] Hilda *et al.* evaluated the efficacy of the method of preservation of fungi in distilled water for preservation of 43 species of fungi for over a period of 12 months and concluded that the method was reliable.[12] As the marine fungi naturally occur in high salinity conditions, preserving in marine water could retain their morphological and functional characteristics. In the current study, modified Castellani method was used for the preservation of fungi by using sterile marine water. The recovered fungi showed identical morphological characteristics like that of their original isolates.

CONCLUSION

This study concluded that sterile marine water ($12\ ^{0}/_{00}$) can be used to preserve marine fungi. The preserved fungi were viable and there was 100% recovery during all the 5 years of study. This method of preservation of marine fungal strains is simple, cheaper and is less laborious. The isolates can be kept at room temperature and transported easily. However it should be taken into account that the number of fungi selected for the study was limited and further research using more diversified marine fungi is needed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

MEA: Malt Extract Agar; **SR:** Sample Region, **‰:** Parts per million.

SUMMARY

A simple and economical method of preservation of marine fungi by modified Castellani method was evaluated during the study. 12 0/00 sterile marine water was used as preservation medium. 16 marine fungal isolates were obtained and preserved at room temperature using screw cap vials filled with sterile marine water. All the fungi were subjected to recovery at specific intervals of time during 5 years. It was found that all the fungal isolates were successfully recovered from the preserved vials during the study period. This study suggests that sterile marine water can be used for preservation of marine fungi. Further study is required to apply the method to more diversified marine fungi and for different time intervals of preservation.

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