e-ISSN: 2582-7278
https://doi.org/10.59265/mycoasia.2024-01

Original Research

Exploring fungal diversity and their ecological roles in the coastal waters of Ramakrishna Beach, Visakhapatnam, India

Mayuri Chopra[1, *], Swarnaprava Behera[1], Loganathan Jagadeesan[1], Vivek Rachuri[1, **], Belle Damodara Shenoy[1, #]

[1]CSIR-National Institute of Oceanography Regional Centre, 176, Lawson's Bay Colony, Visakhapatnam 530017, Andhra Pradesh, India, [*]Present address: Department of Biology, 11a Mansfield Road, Oxford, United Kingdom, [**]Present address: Fish Welfare Initiative, Door No. 108, Narayanapuram Colony, Poranki, Krishna District, Vijayawada 521137, Andhra Pradesh, India, [#]Corresponding author, email: belleshenoy@nio.org, shenoynio@gmail.com

Abstract

The coastal waters of Ramakrishna Beach, Visakhapatnam, India, present a unique ecosystem for exploring fungal diversity and ecological roles, yet have been underexplored in mycological studies. This study utilizes amplicon sequencing targeting the fungal ITS region from environmental DNA to fill this knowledge gap. Our findings reveal a predominant presence of Ascomycota, with *Candida* and *Aspergillus* being the most abundant genera. Notably, *Candida tropicalis* emerged as the most prevalent species, followed by *Candida hyderabadensis* and *Aspergillus penicillioides*. This study not only contributes new fungal records for the marine environments of Visakhapatnam but also offers insights into the ecological functions of these fungi, as interpreted from the FUNGuild database. By highlighting the abundance, diversity, and potential ecological impacts of fungi in the coastal waters of Ramakrishna Beach, this research provides valuable insights into coastal ecosystem dynamics and the contributions of fungal communities to marine biodiversity.

Keywords: Amplicon sequencing, Ascomycota, Coastal ecosystem dynamics, Environmental DNA, FUNGuild Database

Chopra M, Behera S, Jagadeesan L, Rachuri V, Shenoy BD (2024) Exploring fungal diversity and their ecological roles in the coastal waters of Ramakrishna Beach, Visakhapatnam, India. **MycoAsia 2024/01**.

Received: 27.01.2022 | **Accepted**: 22.03.2024 | **Published**: 23.03.2024 | **Handling Editor**: Dr. Nalin Wijayawardene | **Reviewers**: Dr. Nattawut Boonyuen, Dr. Bandarupalli Devadatha

Introduction

Visakhapatnam, situated on the East Coast of India, is a city of industrial significance that frequently attracts hundreds of thousands of tourists every year. Visakhapatnam has four prominent beaches, namely Bheemli, Ramakrishna, Rushikonda, and Yarada. Of these, Ramakrishna Beach stands out, drawing an estimated 300,000 visitors at its peak, as cited by the Times of India on 28th May 2018. Yet, there have been concerning reports of elevated pollution levels in its seawater (Clark et al. 2003, Babu et al. 2014). These escalated pollution indices are largely attributed to rampant human-driven activities, encompassing aspects like tourism and industrial pursuits, among others.

In marine environments, microorganisms have been established as reliable indicators of water pollution, and the beach and coastal waters of Visakhapatnam have been the subjects of numerous studies investigating bacterial diversity (Sailaja et al. 2013, Chakravarty et al. 2015, Sudha Rani et al. 2018, Khandeparker et al. 2020). Despite these extensive investigations, the fungal diversity of these waters remains relatively unexplored, with only a few studies performed to date. Notably, Shanti and Kondalarao (2019) reported significant fungal abundance in seawater samples from the Visakhapatnam fishing harbour. Reportedly, the mean density of fungi grown on Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) media was 26.92×10^5 cfu/ml and 25.85×10^5 cfu/ml, respectively.

e-ISSN: 2582-7278
https://doi.org/10.59265/mycoasia.2024-01

Maruthi et al. (2012) documented the presence of *Alternaria* sp., *Aspergillus* sp., *Chrysosporium* sp., *Cladosporium* sp., *Geotrichum* spp., *Microsporum gypseum*, *Penicillium* sp., *Rhizopus* sp., and unidentified yeasts in the sewage sludge entering the seawater of Ramakrishna Beach. Therefore, this study aimed to comprehensively analyze the fungal diversity in Ramakrishna Beach's seawater, addressing the existing knowledge gap.

Amplicon sequencing, a well-established technique, offers a powerful approach to characterize microbial diversity in environmental samples. This technique circumvents the limitations associated with culture bias and provides extensive data on microbial abundance and diversity in a specific sample. Its successful application in studying microbial diversity in marine samples from India and globally is well-documented (Bacosa et al. 2016, Fernandes et al. 2020). For this study, seawater samples were collected from one of Ramakrishna Beach's busiest locations on a high-traffic Sunday. The samples were subjected to amplicon sequencing of the ITS region to characterize the fungal diversity. Subsequently, the ecological functions of these fungi were predicted using the FUNGuild database (Nguyen et al. 2016).

Materials and methods

Study site and sample collection: Seawater samples were obtained from Ramakrishna Beach, Visakhapatnam, Andhra Pradesh, India (17.714 °N, 83.324 °E) on a notably crowded day, Sunday, 04.08.2019, during the peak of the southwest monsoon season (Figure 1). The samples were collected in three sets of 20 litres each and were promptly transported to the laboratory in pre-cleaned, acid-washed Nalgene containers with a 20-liter capacity.

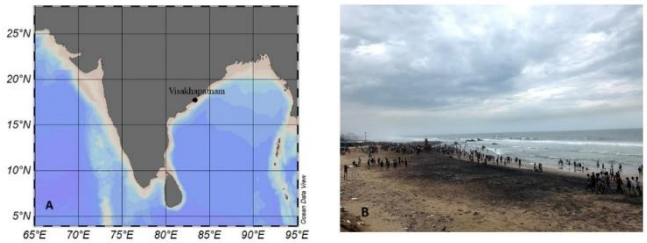

Figure 1. A) Geographic representation of Visakhapatnam in India. B). Sampling site at Ramakrishna Beach in Visakhapatnam

Sample processing: A low-pressure vacuum pump was used to filter 4.5 litres of the seawater through 0.22 µm Whatman filter paper for fungal amplicon sequencing. The filter paper, which had the filtered material, was preserved at -20 °C in absolute alcohol (EMSURE ACS, ISO, Merck Germany) until further processing.

Amplicon sequencing and analysis: The filter paper with the filtered material was sent to Eurofins Genomics India Pvt. Ltd., Bangalore, India (https://www.eurofins.com/) where further technical processing was carried out. This included the isolation of metagenomic DNA from the sample using a commercial Nucleospin kit. The Nextera XT Index Kit (Illumina Inc.) was utilized for the creation of amplicon libraries (Part # 15044223 Rev. B). Amplification of the fungal ITS region was performed using the primers ITS-F (5'-GCATCGATGAAGAAC GCAGC-3') and ITS-R (5'-TCCTCCGCTTATTGATATGC-3'). The Quantitative Insights into Microbial Ecology (QIIME) pipeline (http://qiime.org/1.4.0/) (Caporaso et al. 2010) was used to analyze the sequence data obtained from the sample. Clean reads were produced using Trimmomatic v0.38 (Bolger et al. 2014). The sequences received were grouped into operational taxonomic units (OTUs) at 97% sequence similarity rate using the UCLUST algorithm (Edgar 2010). Fungal representative sequences were obtained from the UNITE database (version 7.2; https://unite.ut.ee/) (Nilsson et al. 2018). Shannon alpha diversity index was calculated using QIIME. A rarefaction curve, depicting the distribution of the OTUs, was plotted to analyse the fungal species richness of the seawater sample.

New taxonomic records and ecological function predictions: The taxonomic information obtained from amplicon sequencing was compared with existing literature, including Shanti and Kondalarao (2019) and Maruthi et al. (2012). As a result, new taxonomic records at the genus level for the coastal waters of Visakhapatnam were identified. Insights into potential ecological functions of the OTUs discovered in this study were gained using the FUNGuild database (http://www.funguild.org/).

Results

Amplicon sequencing analysis of the seawater samples collected from Ramakrishna Beach led to the identification of a diverse range of fungal species/ OTUs. The comprehensive results of this analysis are detailed in Table 1. The most abundant fungal phylum identified was Ascomycota (78.01 %) (Figure 2). Within this classification, the genus *Candida* (42.7 %) was the most frequently identified, with *Candida tropicalis* (22.06 %) and *C. hyderabadensis* (9.4 %) being the most abundant species. The genus *Aspergillus* (14.78 %) was also prominent, with *A. penicillioides* (7.11 %) and *A. conicus* (1.13 %) among the top 10 most abundant species. Other significant species included *Teunomyces kruisii* (= *Candida kruisii*) (5.14 %), *Kluyveromyces lactis* (3.33 %), *Candida palmioleophi*

e-ISSN: 2582-7278
https://doi.org/10.59265/mycoasia.2024-01

(2.77 %), *C. glabrata* (1.76 %), and *Candolleomyces candolleanus* (*=Psathyrella candolleana*) (1.71 %).

Table 1. Summary of amplicon sequence analysis results

Item description	Results
Number of reads	195367
Total bases	103939568
Phylum level distribution	Ascomycota (78.01 %), Basidiomycota (8.1 %), Mucoromycota (0.72 %), Entomophthoromycota (0.19 %)
Class level distribution	Saccharomycetes (47.61 %), Eurotiomycetes (23.22 %), Agaricomycetes (7.67 %), Dothideomycetes (6.75 %), Mucoromycetes (0.7232 %), Cystobasidiomycetes (0.217 %), Basidiobolomycetes (0.1929 %), Sordariomycetes (0.0964 %), Leotiomycetes (0.0723 %), Microbotryomycetes (0.0723 %), Tremeliomycetes (0.0482 %), Ustilaginomycetes (0.0482 %)
Order level distribution	Saccharomycetales (47.61 %), Eurotiales (23 %), Agaricales (6.34 %), Pleosporales (3.81 %), Botryosphaeriales (1.45 %), Polyporales (1.11 %), Dothideales (0.7715 %), Mucorales (0.7232 %), Capnodiales (0.675 %), Cystobasidiales (0.217 %), Basidiobolales (0.1929 %), Onygenales (0.1205 %), Chaetothyriales (0.0964 %), Hypocreales (0.0964 %), Auriculariales (0.0723 %), Thelebolales (0.0723 %)
Family level distribution	Saccharomycetales fam. *incertae sedis* (42.7 %), Aspergillaceae (22.35 %), Saccharomycetaceae (3.59 %), Psathyrellaceae (2.36 %), Didymellaceae (1.49 %), Apiosporellaceae (1.04 %), Agaricaceae (0.9643 %), Pleosporaceae (0.8679 %), Schizophyllaceae (0.7956 %), Aureobasidiaceae (0.7715 %), Debaromycetaceae (0.675 %), Trichocomaceae (0.5304 %), Choanephoraceae (0.4822 %), Cladosporiaceae (0.4339 %), Botryosphaeriaceae (0.4098 %)
Genus level distribution	*Candida* (42.7 %), *Aspergillus* (14.78 %), *Kluyveromyces* (3.33 %), *Psathyrella* (1.88 %), *Phoma* (1.49 %), *Aplosporella* (1.04 %), *Schizophyllum* (0.7956 %), *Agaricus* (0.7232 %), *Curvularia* (0.6268 %), *Penicillium* (0.6027 %), *Debaryomyces* (0.5786 %), *Talaromyces* (0.5304 %), *Choanephora* (0.4822 %)
Species level distribution	*Candida tropicalis* (22.06 %), *C. hyderabadensis* (9.4 %), *Aspergillus penicillioides* (7.11 %), *Candida kruisii* (*=Teunomyces kruisii*, 5.14 %), *Kluyveromyces lactis* (3.33 %), *Candida palmioleophi* (2.77 %), *C. glabrata* (1.76 %), *Candolleomyces candolleanus* (*=Psathyrella candolleana*) (1.71 %), *Aspergillus conicus* (1.13 %), *A. ochraceopetaliformis* (1.01 %), *A. halophilicus* (0.9643 %), *Candida albicans* (0.9161 %)

The Shannon alpha-diversity index of 4.87 indicated high fungal diversity at Ramakrishna Beach. The following are the new fungal records at genus level for seawater of Ramakrishna beach, Visakhapatnam: *Agaricus*, *Agrocybe*, *Aplosporella*, *Arthrographis*, *Aureobasidium*, *Auricularia*, *Basidiobolus*, *Bipolaris*, *Candida*, *Candolleomyces*, *Chlorophyllum*, *Choanephora*, *Cladophialophora*, *Coprinellus*, *Coprinopsis*, *Curvularia*, *Cystobasidium*, *Debaryomyces*, *Diplodia*, *Exophiala*, *Fusarium*, *Ganoderma*, *Gymnopilus*, *Hanseniaspora*, *Hymenochaete*, *Kazachstania*, *Kluyveromyces*, *Lasiodiplodia*, *Lentinus*, *Meripilus*, *Naganishia*, *Panus*, *Perenniporia*, *Phanerochaete*, *Phoma*, *Pichia*, *Pisolithus*, *Podoscypha*, *Preussia*, *Pseudo-cercospora*, *Pyrenochaeta*, *Rhodosporidiobolus*, *Schizophyllum*, *Schwanniomyces*, *Septoria*, *Talaromyces*, *Toxicocladosporium*, *Trichoderma*, *Trichophyton*, *Ustilago* and *Westerdykella*.

A further analysis conducted using the FUNGuild database to infer the trophism and ecological guilds of the identified fungal species revealed interesting insights into the ecological functions and potential contributions of these fungi to the coastal ecosystem of Ramakrishna Beach. The findings from this analysis are presented in Table 2.

Discussion

In this research, we investigated the fungal diversity and their ecological roles in the coastal waters of Ramakrishna Beach, Visakhapatnam, India, using amplicon sequencing of environmental DNA. This research resulted in interesting insights into the taxonomy and ecological significance of fungi in the chosen marine environment and their contributions to the dynamics of the coastal ecosystem (Tables 1 and 2).

e-ISSN: 2582-7278
https://doi.org/10.59265/mycoasia.2024-01

Table 2: Taxonomic classification, trophic modes, and guilds of fungal taxa identified in the study, alongside their absolute counts

Sl. No.	Taxon Name	Trophic Mode	Trophic Guild	Absolute Count
1	***Candida tropicalis*** (Castell.) Berkhout	Pathotroph, Saprotroph, Symbiotroph	Animal Pathogen-Endophyte-Undefined Saprotroph	915
2	***Candida hyderabadensis*** R. Sreen. Rao, Bhadra, N.N. Kumar & Shivaji	No info.	No info.	390
3	***Aspergillus penicillioides*** Speg.	Pathotroph	Animal Pathogen	295
4	***Teunomyces kruisii*** (Kock.-Krat. & Ondrush.) Kurtzman & M. Blackw. (=*Candida kruisii* (Kock.-Krat. & Ondrush.) S.A. Mey. & Yarrow)	No info.	No info.	213
5	***Aspergillus*** sp.	No info.	No info.	167
6	***Kluyveromyces lactis*** (Stell.-Dekk.) Van der Walt	Saprotroph	Undefined Saprotroph	138
7	***Candida palmioleophila*** Nakase & Itoh	No info.	No info.	115
8	***Candida glabrata*** (H.W. Anderson) S.A. Mey. & Yarrow	Pathotroph, Saprotroph	Animal Pathogen-Undefined Saprotroph	73
9	***Candolleomyces candolleanus*** (Fr.) D. Wächt. & A. Melzer	No info.	No info.	71
10	***Phoma* sp.**	Pathotroph, Saprotroph, Symbiotroph	Endophyte, Dung Saprotroph, Lichen Parasite, Litter Saprotroph, Plant Pathogen, Soil Saprotroph, Wood Saprotroph	62
11	***Aspergillus conicus*** Blochwitz	No info.	No info.	47
12	***Aplosporella* sp.**	Pathotroph	Plant Pathogen	43
13	***Aspergillus ochraceopetaliformis*** Bat. & Maia	Pathotroph	Animal Pathogen	42
14	***Aspergillus halophilicus*** M. Chr., Papav. & C.R. Benj.	No info.	No info.	40
15	***Candida albicans*** (C.P. Robin) Berkhout	Pathotroph	Animal Pathogen	38
16	***Schizophyllum commune*** Fr.	Pathotroph, Saprotroph	Animal Pathogen, Endophyte, Wood Saprotroph	33
17	***Debaryomyces* sp.**	Saprotroph	Undefined Saprotroph	24
18	***Penicillium euchlorocarpium*** Yaguchi, Someya & Udagawa (=***Talaromyces euchlorocarpius*** Yaguchi, Someya & Udagawa)	No info.	No info.	22
19	***Agaricus* sp.**	No info.	No info.	20
20	***Choanephora cucurbitarum*** (Berk. & Ravenel) Thaxt.	Pathotroph	Plant Pathogen	20
21	***Toxicocladosporium irritans*** Crous & U. Braun	No info.	No info.	18
22	***Curvularia hawaiiensis*** (Bugnic. ex M.B. Ellis) Manamgoda, L. Cai & K.D. Hyde	No info.	No info.	14
23	***Penicillium citrinum*** Thom	No info.	No info.	14
24	***Aureobasidium pullulans*** (de Bary & Löwenthal) G. Arnaud	Pathotroph, Symbiotroph	Animal Pathogen, Endophyte-Epiphyte, Plant Pathogen	13

e-ISSN: 2582-7278
https://doi.org/10.59265/mycoasia.2024-01

25	***Candida zeylanoides*** (Castell.) Langeron & Guerra	Pathotroph	Animal Pathogen	12
26	***Kazachstania* sp.**	No info.	No info.	11
27	***Phanerodontia chrysosporium*** (Burds.) Hjortstam & Ryvarden	Saprotroph	Wood Saprotroph	11
28	***Aspergillus carbonarius*** (Bainier) Thom	No info.	No info.	10
29	***Curvularia lunata*** (Wakker) Boedijn	Pathotroph, Symbiotroph	Endophyte, Plant Pathogen	10
30	***Gymnopilus* sp.**	Saprotroph	Wood Saprotroph	10
31	***Lentinus squarrosulus*** Mont.	No info.	No info.	10
32	***Preussia* sp.**	Saprotroph	Undefined Saprotroph	10
33	***Rhizopus stolonifer*** (Ehrenb.) Vuill. 1902 (=*Rhizopus arrhizus sensu* Cunningham)	Pathotroph, Symbiotroph	Endophyte, Plant Pathogen	10
34	***Cystobasidium* sp.**	Pathotroph	Fungal Parasite	9
35	***Basidiobolus ranarum*** Eidam	Pathotroph, Saprotroph, Symbiotroph	Animal Endosymbiont, Animal Pathogen, Undefined Saprotroph	8
36	***Candida digboiensis*** G.S. Prasad, Mayilraj, Sood & Ban. Lal	No info.	No info.	8
37	***Chlorophyllum globosum*** (Mossebo) Vellinga	No info.	No info.	8
38	***Coprinellus aureogranulatus*** (Uljé & Aptroot) Redhead, Vilgalys & Moncalvo	No info.	No info.	8
39	***Coprinellus* sp.**	No info.	No info.	8
40	***Psathyrella* sp.**	Saprotroph	Wood Saprotroph	7
41	***Agaricus rotalis*** K.R. Peterson, Desjardin & Hemmes	No info.	No info.	6
42	***Agrocybe* sp.**	Saprotroph	Dung Saprotroph, Soil Saprotroph, Undefined Saprotroph	6
43	***Penicillium multicolor*** Grig.-Man. & Porad.	No info.	No info.	6
44	***Aspergillus templicola*** Visagie, Hirooka & Samson	No info.	No info.	5
45	***Diplodia alatafructa*** Mehl & Slippers 2011	No info.	No info.	5
46	***Penicillium capsulatum*** Raper & Fennell 1948	No info.	No info.	5
47	***Phanerochaete* sp.**	Saprotroph	Wood Saprotroph	5
48	***Trichophyton* sp.**	Saprotroph	Undefined Saprotroph	5
49	***Aspergillus sydowii*** (Bainier & Sartory) Thom & Church	Pathotroph	Animal Pathogen	4
50	***Candida boleticola*** Nakase	Pathotroph	Animal Pathogen	4
51	***Ganoderma*** sp.	Pathotroph, Saprotroph	Plant Pathogen, Wood Saprotroph	4
52	***Meripilus giganteus*** (Pers.) P. Karst.	No info.	No info.	4
53	***Pichia* sp.**	Pathotroph, Saprotroph, Symbiotroph	Animal Endosymbiont, Animal Pathogen, Plant Pathogen, Undefined Saprotroph	4
54	***Pyrenochaeta* sp.**	No info.	No info.	4
55	***Schwanniomyces etchellsii*** (Kreger-van Rij) M. Suzuki & Kurtzman	No info.	No info.	4
56	***Aspergillus flavus*** Link	No info.	No info.	3
57	***Auricularia nigricans*** (Sw.) Birkebak, Looney & Sánchez-García	No info.	No info.	3

e-ISSN: 2582-7278
https://doi.org/10.59265/mycoasia.2024-01

58	***Candida diddensiae*** (Phaff, Mrak & O.B. Williams) Fell & S.A. Mey.	Pathotroph	Animal Pathogen	3
59	***Geomyces* sp.**	Saprotroph	Soil Saprotroph	3
60	***Hanseniaspora uvarum*** (Niehaus) Shehata, Mrak & Phaff ex M.T. Sm.	Pathotroph	Animal Pathogen	3
61	***Panus similis*** (Berk. & Broome) T.W. May & A.E. Wood	No info.	No info.	3
62	***Perenniporia* sp.**	Saprotroph	Wood Saprotroph	3
63	***Rhodosporidiobolus nylandii*** (M. Takash. & Nakase) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout	No info.	No info.	3
64	***Septoria* sp.**	Pathotroph	Plant Pathogen	3
65	***Agaricus chartaceus*** T. Lebel, in Lebel & Syme	No info.	No info.	2
66	***Agaricus bisporus*** (J.E. Lange) Imbach	No info.	No info.	2
67	***Arthrographis arxii*** Guarro, A. Giraldo, Gené & Cano	No info.	No info.	2
68	***Bipolaris* sp.**	Pathotroph	Plant Pathogen	2
69	***Cladophialophora* sp.**	Saprotroph	Undefined Saprotroph	2
70	***Coprinellus* sp. 1**	Saprotroph	Undefined Saprotroph	2
71	***Coprinellus* sp. 2**	Saprotroph	Undefined Saprotroph	2
72	***Curvularia pseudorobusta*** Meng Zhang & T.Y. Zhang	No info.	No info.	2
73	***Exophiala oligosperma*** Calandron ex de Hoog & Tintelnot 2003	No info.	No info.	2
74	***Bisifusarium penzigii*** (Schroers, Summerb. & O'Donnell) L. Lombard & Crous	No info.	No info.	2
75	***Ganoderma orbiforme*** (Fr.) Ryvarden	No info.	No info.	2
76	***Hymenochaete cana*** S.H. He & Hai J. Li	No info.	No info.	2
77	***Lasiodiplodia* sp.**	Pathotroph	Plant Pathogen	2
78	***Naganishia albida*** (Saito) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout	No info.	No info.	2
79	***Pisolithus albus*** (Cooke & Massee) Priest	No info.	No info.	2
80	***Podoscypha* sp.**	Saprotroph	Undefined Saprotroph	2
81	***Pseudocercospora* sp.**	Pathotroph	Plant Pathogen	2
82	***Trichoderma reesei*** E.G. Simmons, in Bigelow & Simmons	No info.	No info.	2
83	***Ustilago* sp.**	Pathotroph	Plant Pathogen	2
84	***Westerdykella dispersa*** (Clum) Cejp & Milko	No info.	No info.	2

e-ISSN: 2582-7278
https://doi.org/10.59265/mycoasia.2024-01

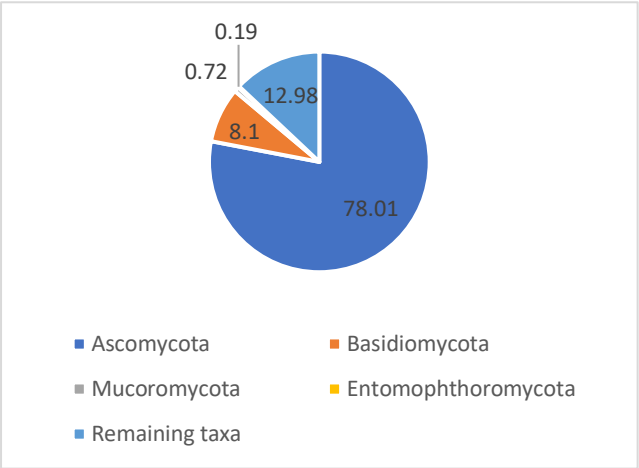

Figure 2: Distribution (%) of fungal communities at the phylum level in coastal waters of Ramakrishna Beach

Diversity index: Our study revealed a high fungal diversity in the seawaters off Ramakrishna Beach, with Shannon alpha-diversity index of 4.87. However, this is lower than the bacterial diversity observed in the same sample, which has Shannon alpha-diversity index of 7.0 (Unpublished data). There are few studies focusing on fungal diversity in the coastal waters of India that employ amplicon sequencing of the ITS region. Further studies are needed to fill this research gap.

Taxonomic profiling: The predominant phylum identified in this study was Ascomycota, accounting for 78.01 % of the total fungal diversity observed (Table 1). Notably, a significant portion of the fungal community belonged to an unidentified family within the Saccharomycetales, labelled as 'Saccharomycetales fam. *incertae sedis*,' representing 47.61 % of the identified taxa. This finding highlights the potential presence of previously unrecorded fungal taxa within the region, underscoring the rich biodiversity and the possibility of discovering novel species.

Among the fungal species identified, *Candida tropicalis* emerged as the most prevalent, constituting 22.06 % of the fungal community. Additionally, the study uncovered the presence of *Candida hyderabadensis* and *Aspergillus penicillioides*, with relative abundance of 9.4 % and 7.11 %, respectively. These findings suggest the existence of potential pathogenic species within the coastal waters, possibly indicative of anthropogenic influences on the composition of the fungal community. There is a need for further studies on this issue, particularly involving experts in medical mycology.

Dominance of the Ascomycota: The dominance of phylum Ascomycota within marine fungal communities is a well-documented phenomenon that underscores their adaptability and ecological versatility across various habitats (Tedersoo et al. 2014). The predominance of Ascomycota is attributed to their wide array of life strategies, including saprophytism, pathogenicity, and symbiosis, enabling them to exploit diverse ecological niches effectively. These fungi exhibit a remarkable capacity for ecological diversity and adaptability, playing pivotal roles in aquatic ecosystems ranging from coastal waters (Barnes et al. 2018) to the depths of marine sediments (Damare and Raghukumar 2008).

Dominance of the Saccharomycetes: The unexpected dominance of Saccharomycetes at Ramakrishna Beach highlights the adaptability and ecological versatility of these yeast species. The unicellular nature of Saccharomycetes facilitates their rapid colonization and exploitation of nutrient-rich niches, especially those impacted by organic pollution and terrestrial runoff, which are commonplace in coastal areas subjected to human activities (Gadanho and Sampaio 2005). Their capability to flourish in varied salinity levels and utilise diverse carbon sources positions Saccharomycetes as pivotal contributors in nutrient cycling and organic matter decomposition, potentially altering the functions of marine ecosystems (Kaewkrajay et al. 2020).

The presence of Mucoromycota and Entomophthoromycota: The detection of Mucoromycota (0.72 %) and Entomophthoromycota (0.19 %), traditionally associated with terrestrial and freshwater habitats, in Visakhapatnam's marine waters reveals new aspects of fungal diversity. The adaptation of Mucoromycota to saline conditions suggests possible involvement in organic matter decomposition and nutrient cycling (Dzurendova et al., 2022), while the presence of Entomophthoromycota, known for terrestrial pathogenicity, indicates potential interactions with marine invertebrates (Gryganskyi et al., 2012). However, their low abundance raises questions about their ecological impact, underscoring the need for further study to elucidate their roles in marine ecosystems.

Potential markers for water pollution: Genera such as *Candida*, *Aspergillus*, and *Aureobasidium* could serve as key markers for environmental health assessment (Hagler 2006, Pfliegler et al. 2020, Gostinčar et al. 2014). For instance, the presence of *Candida* species may indicate organic pollution (Hagler 2006), while *Aspergillus* suggests capabilities in contaminant degradation and mycotoxin production (Pfliegler et al. 2020). Additionally, the efficiency of *Aureobasidium pullulans* in degrading substances underlines its importance as a water quality marker (Gostinčar et al. 2014). However, our findings (Table 1 and Table 2),

e-ISSN: 2582-7278
https://doi.org/10.59265/mycoasia.2024-01

derived from a limited dataset, highlight the need for a comprehensive, polyphasic approach in environmental evaluations.

The role of *Candida* species: The presence of *Candida* species, including *C. tropicalis*, *C. glabrata* and *C. albicans* at Ramakrishna Beach, indicates a notable overlap between environmental microbiology and public health (Rao et al. 2007, Turner and Butler 2014). These species, particularly pathogenic to humans, underscore the potential health risks associated with marine environments. The detection of plant-pathogenic *C. hyderabadensis* (Rao et al. 2007), along with other *Candida* species, highlights the need for environmental monitoring to trace the pathways through which these pathogens can spread.

Why mostly terrestrial species? The detection of terrestrial fungal taxa such as *Agaricus*, *Ganoderma*, and *Trichoderma* in the marine waters of Ramakrishna Beach, Visakhapatnam, based on a single sample, hints at an intriguing overlap between terrestrial and marine ecosystems. This preliminary observation suggests that human activities, including pollution, agricultural runoff, urban discharge, and tourism, might contribute to introducing terrestrial fungi into marine environments (Bashir et al. 2020). While these findings elicit questions about the impacts of environmental and recreational activities on marine fungal diversity, they warrant cautious interpretation.

Why not that many obligate marine fungal species? The absence of obligate marine fungi in our study, utilizing amplicon sequencing of the ITS region, is noteworthy. Factors such as pollution, altered salinity, and nutrient influx from human activities may unfavorably affect obligate marine fungi, tilting the balance towards terrestrial and facultative marine species (Bonugli-Santos et al. 2015). Future studies, employing diverse methodologies and polyphasic data are required accurately capture marine fungal diversity.

Conclusion

Our investigation into the fungal diversity at Ramakrishna Beach, Visakhapatnam, highlighted a rich variety, predominantly of Ascomycota, using environmental DNA amplicon sequencing. We discovered notable species like *Candida tropicalis* and the presence of terrestrial fungi, Mucoromycota and Entomophthoromycota, in marine waters, suggesting a blend of terrestrial and marine ecosystems. Despite these insights, the absence of obligate marine fungi and reliance on a limited dataset call for comprehensive future studies. This research underscores the ecological versatility of fungi, the potential public health implications, and the need for advanced methodologies (and traditional culture-based studies) to fully understand the impact of fungal communities on marine ecosystems and biodiversity.

Acknowledgement

We gratefully acknowledge the support and funding provided by the Seawater Quality Monitoring Program (SWQM) of the Ministry of Earth Sciences, Government of India (GAP 3195). We also extend our appreciation to Prof. Sunil Kumar Singh (Director, CSIR-National Institute of Oceanography), Dr. V. V. S. S. Sarma (Scientist In-Charge of CSIR-NIO Regional Centre Visakhapatnam) and our colleagues Dr. Moturi Sriramakrishna and Dr. T. N. R. Srinivas for their encouragement and support. We would like to express our sincere gratitude to Dr. Sreedevi for her invaluable assistance in providing guidance and support with the Visakhapatnam map. We would like to acknowledge the expert reviewers and editors whose insightful comments and suggestions significantly contributed to improving the quality of this manuscript. Their expertise and feedback were invaluable in shaping the final version of this work.

Conflict of interest

The authors declare no conflict of interest.

References

Bacosa HP, Thyng KM, Plunkett S, Erdner DL, Liu Z (2016) The tarballs on Texas beaches following the 2014 Texas City “Y” Spill: Modeling, chemical, and microbiological studies. Marine Pollution Bulletin 109:236–244. doi:10.1016/j.marpolbul.2016.05.076

Babu KR, Sailaja VH, Reddy KVS, Anand KG, Pratap GV, Raju MR (2014) Studies on microbial status and characteristic features from polluted coastal habitats at Visakhapatnam, India. International Journal of Multidisciplinary and Current Research 2:113–117.

Barnes NM, Khodse VB, Lotlikar NP, Meena RM (2018) Bioremediation potential of hydrocarbon-utilizing fungi from select marine niches of India. 3Biotech 8:21. https://doi.org/10.1007/s13205-017-1043-8

Bashir I, Lone FA, Bhat RA, Mir SA, Dar ZA, Dar SA (2020) Concerns and threats of contamination on aquatic ecosystems. Bioremediation and Biotechnology 27:1–26. doi: 10.1007/978-3-030-35691-0_1

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina Sequence Data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

e-ISSN: 2582-7278
https://doi.org/10.59265/mycoasia.2024-01

Bonugli-Santos RC, dos Santos Vasconcelos MR, Passarini MRZ, Vieira GAL, Lopes VCP, Mainardi PH, dos Santos JA, de Azevedo Duarte L, Otero IVR, da Silva Yoshida AM, Feitosa VA, Pessoa A Jr and Sette LD (2015) Marine-derived fungi: diversity of enzymes and biotechnological applications. Frontiers in Microbiology 6:269. doi: 10.3389/fmicb.2015.00269

Chakravarty MS, Ganesh PRC, Amarnath D, Santhi Sudha B, Samantha MH (2015) Bacteria of the recreational beach waters of Visakhapatnam, India. International Journal of Recent Scientific Research 6.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley Ga, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336. doi: 10.1038/nme th.f.303

Clark A, Torrey T, Dorothy KP, Goutham J, Kalavati C, Rajanna B (2003) Health hazards due to pollution of waters along the coast of Visakhapatnam, east coast of India. Ecotoxicology and Environmental Safety 56: 390–397.

Damare S, Raghukumar C (2008) Fungi and macroaggregation in deep-sea sediments. Microbial Ecology 56:168–177. doi: 10.1007/s00248-007-9334-y

Dzurendova S, Losada CB, Dupuy-Galet BX et al. (2022) Mucoromycota fungi as powerful cell factories for modern biorefinery. Applied Microbiology and Biotechnology 106:101–115. https://doi .org/10.1007/s00253-021-11720-1

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Fernandes GL, Shenoy BD, Damare SR (2020) Diversity of bacterial community in the oxygen minimum zones of Arabian Sea and Bay of Bengal as deduced by Illumina sequencing. Frontiers in Microbiology 10:3153. doi: 10.3389/fmicb. 2019.03153

Gadanho M, Sampaio JP (2005) Occurrence and diversity of yeasts in the mid-atlantic ridge hydrothermal fields near the Azores Archipelago. Microbial Ecology 50:408–417. doi: 10.1007/s00248-005-0195-y.

Gostinčar C, Ohm RA, Kogej T, Sonjak S, Turk M, Zajc J, Zalar P, Grube M, Sun H, Han J, Sharma A, Chiniquy J, Ngan CY, Lipzen A, Barry K, Grigoriev IV, Gunde-Cimerman N (2014) Genome sequencing of four *Aureobasidium pullulans* varieties: biotechnological potential, stress tolerance, and description of new species. BMC Genomics 15:549. doi: 10.1186/1471-2164-15-549

Gryganskyi AP, Humber RA, Smith ME, Miadlikowska J, Wu S, Voigt K, Walther G, Anishchenko IM, Vilgalys R (2012) Molecular phylogeny of the Entomophthoromycota. Molecular Phylogene tics and Evolution 65:682–694. doi: 10.1016/j.ympev.2012.07.026

Hagler AN (2006) Yeasts as indicators of environmental quality. In: Péter, G., Rosa, C. (eds) Biodiversity and Ecophysiology of Yeasts. The Yeast Handbook. Springer, Berlin, Heidelberg. pp. 515–532. https://doi.org/10.1007/3-540-30985-3_21

Kaewkrajay C, Thanongsak C, Savitree L (2020) Assessment of diversity of culturable marine yeasts associated with corals and Zoanthids in the Gulf of Thailand, South China Sea. Microorganisms 8,4:474. https://doi.org/10.3390/microorganisms8040474

Khandeparker L, Desai DV, Sawant SS, Krishnamurthy V, Anil AC (2020) Spatio-temporal variations in bacterial abundance with an emphasis on fecal indicator bacteria and *Vibrio* spp. in and around Visakhapatnam Port, East Coast of India. Asean Journal on Science and Technology for Development 37:91–99. https://doi.org/10.29037/ajstd.619

Maruthi YA, Hossain K, Priya DH, Tejaswi B (2012) Prevalence of keratinophilic fungi from sewage sludge at some wastewater outlets along the coast of Visakhapatnam: A case study. Advances in Applied Science Research 3:605–610.

Nguyen NH, Song Z, Bates ST, Branco, S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20:241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nilsson RH, Taylor AFS, Adams RI, Baschien C, Johan Bengtsson-Palme, Cangren P, Coleine C, Daniel H, Glassman SI, Hirooka Y, Irinyi L, Iršėnaitė R, Martin-Sanchez PM, Meyer W, Seung-Yoon Oh, Sampaio JP, Seifert KA, Sklenář F, Stubbe D, Suh SO, Summerbell R, Svantesson S, Unterseher M, Visagie CM, Weiss M, Woudenberg JH, Wurzbacher C, den

e-ISSN: 2582-7278
https://doi.org/10.59265/mycoasia.2024-01

Wyngaert SV, Yilmaz N, Andrey Yurkov, Kõljalg U, Abarenkov K (2018) Taxonomic annotation of public fungal ITS sequences from the built environment - a report from an April 10–11, 2017 workshop (Aberdeen, UK). MycoKeys 8:65–82. doi: 10.3897/mycokeys.28.20887

Pfliegler WP, Pócsi I, Győri Z and Pusztahelyi T (2020) The Aspergilli and their mycotoxins: Metabolic interactions with plants and the soil biota. Frontiers in Microbiology 10:2921. doi: 10.3389/fmicb.2019.02921

Rao RS, Bhadra B, Kumar NN, Shivaji S (2007) *Candida hyderabadensis* sp. nov., a novel ascomycetous yeast isolated from wine grapes. FEMS Yeast Research 7:489–493. doi: 10.1111/j.1567-1364.2006.00206.x.

Sailaja VH, Archana A, Babu KR (2013) Assessment of faecal indicator bacteria in the coastal waters of Visakhapatnam, India. Advances in Applied Science Research, 4.

Shanti IV, Kondalarao B (2009) Density distribution of fungi in the surface waters of Visakhapatnam fishing harbour, east coast of India. Journal of The Marine Biological Association of India 51:238–241.

Sudha Rani P, Kumar GS, Mukherjee J, Srinivas TNR, Sarma VVSS (2018) Perennial occurrence of heterotrophic, indicator and pathogenic bacteria in the coastal Bay of Bengal (off Visakhapatnam) - Impact of physical and atmospheric processes. Marine Pollution Bulletin 127:412–423. doi: 10.1016/j.marpolbul.2017.12.023

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Villarreal Ruiz L, Vasco-Palacios AM, Thu PQ, Suija A, Smith ME, Sharp C, Saluveer E, Saitta A, Rosas M, Riit T, Ratkowsky D, Pritsch K, Põldmaa K, Piepenbring M, Phosri C, Peterson M, Parts K, Pärtel K, Otsing E, Nouhra E, Njouonkou AL, Nilsson RH, Morgado LN, Mayor J, May TW, Majuakim L, Lodge DJ, Lee SS, Larsson KH, Kohout P, Hosaka K, Hiiesalu I, Henkel TW, Harend H, Guo LD, Greslebin A, Grelet G, Geml J, Gates G, Dunstan W, Dunk C, Drenkhan R, Dearnaley J, De Kesel A, Dang T, Chen X, Buegger F, Brearley FQ, Bonito G, Anslan S, Abell S, Abarenkov K (2014) Fungal biogeography. Global diversity and geography of soil fungi. Science 346:1256688. doi: 10.1126/science.1256688

Turner SA, Butler G (2014) The *Candida* pathogenic species complex. Cold Spring Harbor Perspectives in Medicine 4(9):a019778. doi: 10.1101/cshperspect.a019778