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Original Research

Molecular characterization and identification of marine yeasts from coastal and offshore regions in the southeastern Arabian Sea

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Abstract

This study investigates the diversity and distribution of marine yeasts in southeastern Arabian Sea, focusing on coastal and offshore regions. Yeast strains were identified through ITS and D1/D2 domain rRNA gene sequencing and phylogenetic analysis. Microscopic analysis was performed to examine budding patterns and cell morphology. A total of 45 yeast strains were isolated from water samples collected at various depths from locations, including Kochi, Kollam, Trivandrum, Rameswaram, and the coastal waters of the Lakshadweep Islands (Minicoy, Kalpeni, and Kavaratti). Morphologically, the yeast strains were predominantly oval to rod-shaped and clustered into six clades: *Debaryomyces*, *Kodamaea*, *Meyerozyma*, *Starmerella* (Ascomycota), *Rhodotorula* and *Sterigmatomyces* (Basidiomycota). Notably, *Starmerella* strains were the most abundant, particularly in coastal waters. Among them, *S. etchellsii* and *M. caribbica* exhibited significant dry weight and protein concentrations, ranging from 13.9% to 65.1%. This study expands the limited knowledge of marine yeast diversity in Indian waters and provides insights into their molecular identification and potential biotechnological applications.

Keywords: D1/D2 domain, ITS region, Morphology, Phylogenetic analysis, Protein yield

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1. Introduction

Coastal ecosystems are inherently dynamic, characterized by vast biological diversity and high primary production (Danovaro & Pusceddu, 2007). This productivity results in abundant organic matter, much of which persists as detritus even after herbivore grazing (Wiegner & Seitzinger, 2001). The decomposition of this detritus is facilitated by a diverse community of microorganisms (Manini et al., 2003; Pusceddu et al., 2003). Among them, chemoheterotrophic archaea and bacteria play a dominant role in detritus degradation (Moran & Miller, 2007; Mou et al., 2008). However, despite their ecological significance, the biodiversity, and functional roles of eukaryotic heterotrophs, including yeasts, remain relatively understudied (Fenchel, 2008; Giovannoni & Stingl, 2005; Strom, 2008).

1.1 Marine yeast diversity and distribution

Yeasts are unicellular, non-motile, budding eukaryotic microorganisms commonly found in diverse marine ecosystems. They are categorized into *obligate* marine yeasts, which depend strictly on marine salts for survival, and *facultative* marine yeasts, originating primarily from terrestrial and freshwater environments but capable of adapting to marine conditions (Kutty & Philip, 2008). Marine yeasts have been reported across a variety of habitats, including associations with copepods, fish, marine mammals, seabird excreta, and seaweeds (Yu et al., 2023).

Yeast abundance in marine environments varies significantly with depth and nutrient availability. Coastal waters typically exhibit high yeast cell counts (>1000 cells/L), while deeper, oligotrophic waters have substantially lower numbers (>10 cells/L). The predominant yeast genera influencing marine fungal distributions include *Candida*, *Cryptococcus*, *Debaryomyces*, and *Rhodotorula* (Kutty & Philip, 2008). These yeasts are versatile, thriving in both aerobic and anaerobic conditions, and actively participate in the decomposition of refractory organic matter, thereby playing a critical role in marine nutrient cycling (Jebaraj et al., 2010; Segal-Kischinevzky et al., 2022; Yu et al., 2023).

Studies investigating fungal diversity in deep-sea sediments (~5000 m depth) from the Indian Ocean have utilized both culture-dependent and culture-independent methodologies (Singh et al., 2010, 2011, 2012). Specifically, research by Kutty et al. (2013a) has highlighted the occurrence and ecological significance of black yeasts isolated from slope sediments of the Bay of Bengal. Another study by Kutty et al. (2013b) identified various yeast species from slope sediments of the Arabian Sea and the Bay of Bengal, utilizing cultural and biochemical characterizations. Additionally, marine oxygen-deficient habitats have been explored, further expanding the understanding of yeast communities under extreme conditions (Jebaraj et al., 2010, 2012).

Further research has expanded our understanding of yeast diversity in marine and estuarine environments. Nefla et al. (2023) investigated the impact of flood events on yeast diversity in mangrove ecosystems, highlighting environmental influences on microbial communities. Prasanna Kumar et al. (2020) examined yeast distribution in marine sediments, identifying distinct populations across different marine habitats. Studies by Coelho et al. (2010) and de Almeida (2005) analysed yeast community dynamics in Tagus River estuary, revealing fluctuations under varying environmental conditions. Manjusha et al. (2023) compared yeast diversity in mangrove-associated sediments and water, providing insights into habitat-specific variations. These studies contribute significantly to our understanding of marine yeast diversity and ecological functions, particularly in sediment-associated environments, where physiological and biochemical characterization methods have been widely used.

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1.2 Yeast identification and biotechnology

Yeast identification and biotechnology are closely interconnected, as precise species identification is fundamental to optimizing their industrial and biotechnological applications. Molecular techniques play a crucial role in yeast taxonomy, with the D1/D2 domain of 28S rRNA gene (~600–650 bp) widely used for species identification and phylogenetic analysis (Fell et al., 2000; Kurtzman & Robnett, 1998). Additionally, the internal transcribed spacer (ITS1 and ITS2) regions, along with the 5.8S rRNA gene, serve as standard markers for species identification and epidemiological studies in medical mycology (Korabecna, 2007).

Accurate yeast identification enables targeted applications in biotechnology. Yeasts play a pivotal role in food industry, particularly in ethanol production for baking, brewing, and wine distillation. They are also valuable as food supplements due to their high lipid, protein, and vitamin content. One of their most promising biotechnological applications is the bioconversion of organic matter into yeast biomass, known as single-cell protein (SCP) production. This high-nutritional-value biomass holds significant potential as an alternative protein source for animal and aquaculture feed (Kutty & Philip, 2008; Rhishipal & Philip, 1998; Yu et al., 2023).

Beyond conventional applications, marine yeasts have emerged as valuable bioresources with unique physiological traits. Yeasts isolated from seaweed genera have demonstrated beneficial properties and warrant further exploration (Francis et al., 2016). Compared to terrestrial yeasts, marine yeasts exhibit higher osmotic tolerance, enhanced production of specialized chemicals, and the ability to synthesize industrial enzymes, making them particularly promising for various industrial applications (Zaky et al., 2014). Additionally, marine yeasts have potential applications in medical and environmental fields and are rich sources of bioactive compounds. Their identification not only contributes to biodiversity conservation but also facilitates their use as bioresources (Sivakumar et al., 2020; Yu et al., 2023).

Recent advancements have also highlighted the potential of marine yeasts in brewing. Ordulj et al. (2024) demonstrated, for the first time, the experimental use of marine yeast isolates in beer fermentation, underscoring their potential as nonconventional yeasts in brewing. By integrating molecular identification techniques with biotechnological innovations, yeasts can be effectively harnessed for diverse applications in industry, medicine, and environmental sustainability.

1.3 Scope of the present study

Given the limitations of previous studies, there is a need for more comprehensive research on marine yeasts, particularly concerning their diversity, phylogenetic relationships, and potential for SCP production for biotechnological applications (Kutty & Philip, 2008; Nimsi et al., 2023a, 2023b, 2024; Rekha et al., 2024; Shivaji & Prasad, 2008). In this context, the present study aims to characterize the taxonomic diversity of marine yeasts in southeastern Arabian Sea using morpho-molecular approaches and to assess their potential for SCP production.

2. Materials and methods

2.1 Study area

The study was conducted in southeastern Arabian Sea, a region influenced by seasonally reversing monsoonal currents and wind patterns. The area experiences significant variations in nutrient availability and plankton distribution due to coastal upwelling, particularly during the southwest monsoon (June–September). Previous studies have documented high phytoplankton biomass during this period (Jyothibabu et al., 2010). Seawater samples were collected during

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a 21-day research cruise aboard *ORV Sagar Sampada* from August to September 2011. Sampling was conducted at multiple depths in both coastal and offshore waters near Kochi, Kollam, and Trivandrum. Additionally, coastal water samples were obtained from three beaches in the Lakshadweep Islands (Kalpeni, Kavaratti, and Minicoy) and from Dhanushkodi, Rameswaram. The spatial distribution of sampling locations for the isolation of marine yeasts in southeastern Arabian Sea is shown in Fig. 1.

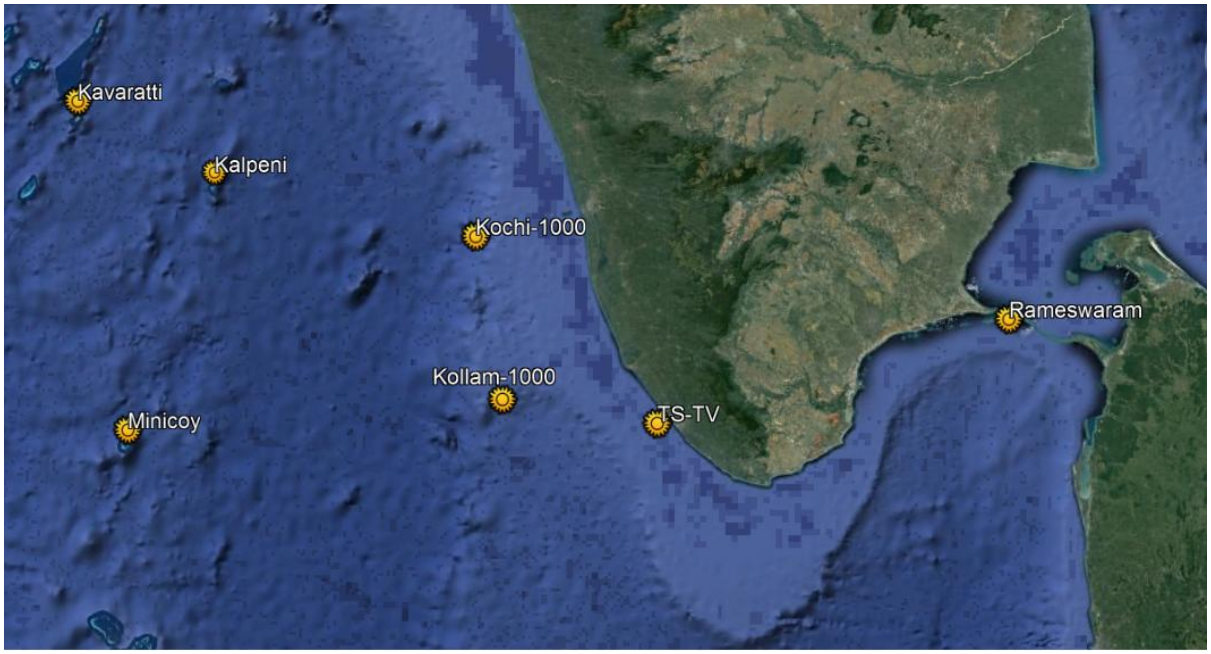

Fig. 1. The spatial distribution of sampling locations in southeastern Arabian Sea [Modified from Google Maps (2021)]

2.2 Sample collection and analysis

Seawater samples were collected from 18 stations, yielding a total of 120 samples. Sampling was conducted at multiple depths using a Rosette sampler equipped with Niskin bottles, targeting depths of surface, 5, 10, 20, 30, 50, 75, 100, 150, 200, 350, 500, 750, and 1000 m. Immediately after collection, samples were transferred into sterile, screw-capped plastic bottles and stored at 4°C to preserve microbial integrity. Samples designated for microbiological analysis were handled under aseptic conditions and processed upon arrival at the laboratory.

2.3 Microbiological analysis

Approximately 100 µL of each seawater sample was diluted in sterile 2% saline before being plated on Yeast Extract Peptone Dextrose (YPD) agar. The YPD agar medium was prepared with the following composition (g/L): Yeast Extract, 10; Peptone, 20; Dextrose, 20; NaCl, 20; Agar, 20. To inhibit bacterial growth, chloramphenicol (100 mg/L) was added to the medium. The inoculated plates were incubated at 25°C for one week to allow yeast colony development. Colonies that grew on the plates were purified and routinely maintained on YPD agar or broth for further studies. Cell morphology was examined using an upright fluorescent microscope (Eclipse 80i, Nikon, Japan). Differential staining with methylene blue, safranin, and lactophenol cotton blue was performed to enhance visualization of morphological features.

2.4 Genomic DNA isolation and phylogenetic analysis

Genomic DNA was extracted from 5 mL of broth culture, and specific gene regions, including ITS1, 5.8S rRNA gene, ITS2, and D1/D2 domain of 28S rRNA gene were amplified using

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primers ITS1 and NL4. Sequencing of the amplified products was carried out using primers NL1, NL2A, NL3A, ITS3, and ITS4 (Kurtzman & Robnett, 1998; Lin et al., 1995). Details of the amplification and sequencing protocols are provided in Prasad et al. (2005).

2.5 Phylogenetic analysis

Phylogenetic analyses were conducted using MEGA version 6 (Tamura et al., 2013). Separate phylogenetic trees were constructed for Ascomycota (29 sequences) and Basidiomycota (11 sequences). The evolutionary history was inferred using the Maximum Likelihood (ML) method based on the Tamura-Nei model (Tamura & Nei, 1993). The tree topology reliability was assessed using a bootstrap test with 1000 replicates, with bootstrap values displayed next to branches (Felsenstein, 1985) (Figs. 3 and 4). For heuristic searching, the initial trees were generated using the Neighbour-Joining (NJ) method based on pairwise distances estimated via the Maximum Composite Likelihood (MCL) approach. The phylogenetic trees were drawn to scale, with branch lengths representing the number of substitutions per site. Gaps and missing data were excluded from the analysis.

To further validate evolutionary relationships, additional phylogenetic methods were applied: 1) Neighbour-Joining (NJ) method (Saitou & Nei, 1987): The tree was drawn to scale, with branch lengths in the same units as the evolutionary distances used for inference. Evolutionary distances were calculated using the Maximum Composite Likelihood method (Tamura et al., 2004) and expressed as the number of base substitutions per site. 2) Maximum Parsimony (MP) method: The MP tree was constructed using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar, 2000) with search level 1, where initial trees were generated via random sequence addition (10 replicates). The tree was drawn to scale, maintaining branch lengths in the same units as those used for evolutionary distance computations. For rooting the phylogenetic trees, *Saccharomyces cerevisiae* CBA 1171^{T} (NR111007) was used as the outgroup for Ascomycota, while *Cryptococcus fonsecae* DSM 26992^{T} (LK023835) was used for Basidiomycota.

2.6 Estimation of the biomass and protein content

Yeast biomass was determined by culturing isolates in YPD broth supplemented with 100 mg/L chloramphenicol to prevent bacterial contamination. Cultures were inoculated into 100 mL of YPD broth in conical flasks using an initial inoculum of 1 mL (OD_{600} = 0.1). Incubation was carried out at room temperature for five days, maintaining pH 8.0 with shaking at 120 rpm to ensure aeration. After incubation, cultures were centrifuged, and the supernatant was discarded. The harvested yeast cells were washed twice with 1% sterile saline to remove residual media components. The resulting cell pellets were dried at 60°C for 12 hours, and biomass was quantified as dry weight (mg/100 mL). For protein estimation, yeast cells from the same cultures (after five days of growth) were processed. A standardized biomass of 0.025 mg was suspended in lysis buffer and incubated at 90°C for 10 minutes to extract proteins, following von der Haar (2007). The extracted protein content was quantified using Lowry's method, and the percentage of protein was calculated by correlating protein concentration (µg/mL) with dry biomass weight.

3. Results

3.1 Isolation of yeast strains

A total of 45 marine yeast strains were isolated from seawater samples collected at various depths and locations in southeastern Arabian Sea. Yeast isolation was performed based on colony morphology and microscopic examination. Most isolates (24 strains) were obtained from surface water samples, while the remaining strains were recovered from different depths

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throughout the water column (Table 1). Approximately 31% (14 strains) of the yeast isolates originated from coastal beach waters, including sites in the Lakshadweep Islands (Kalpeni, Kavaratti, and Minicoy) and Rameswaram (Dhanushkodi beaches). Table 1 provides detailed information on sampling locations, water depths (m), and collection times for the isolation of marine yeast strains from southeastern Arabian Sea.

Table 1. Sampling details of yeast strains isolated from surface and deep waters of southeastern Arabian Sea

S. No.	Strain no.	Sampling station	Water depth (m)
1	E1	Kollam-100M	100
2	E2	Minicoy beach	0
3	E3	Time Series-Trivendrum-27-06:00PM	0
4	E4	Kochi-1000M	1000
5	E5	Kollam-100M	0
6	E6	Kavratti beach	0
7	E7	Kavratti beach	0
8	E8	Kollam-30M	10
9	E9	Rameswaram beach-2	0
10	E10	Kollam-200M	100
11	E11	Kochi-50M	5
12	E12	Rameswaram beach-4A	0
13	E13	Rameswaram beach-3A	0
14	E14	Rameswaram beach-3B	0
15	E15	Kochi-50M	0
16	E16	Kalpeni beach	0
17	E17	Rameswaram beach-1B	0
18	E18	Kollam-100M	0
19	E19	Time Series-Trivendrum-27-12:00PM	0
20	E20	Kalpeni beach	0
21	E21	Kollam-100M	0
22	E22	Time Series-Trivendrum-27-06:00AM	0
23	E23	Minicoy beach	0
24	E24	Rameswaram beach-1A	0
25	E25	Kollam-200M	200
26	E26	Rameswaram beach-4B	0
27	E27	Kalpeni beach	0
28	E28	Kollam-1000M	1000
29	E29	Kochi-500M	150
30	E30	Kochi-50M	5
31	E31	Kollam-1000M	150
32	E32	Kollam-1000M	150
33	E33	Kollam-200M	75
34	E34	Kochi-50M	20
35	E35	Kochi-50M	20
36	E36	Time Series-Trivendrum-27-09:00AM	0
37	E37	Kollam-200M	0
38	E38	Kollam-200M	0
39	E39	Kollam-200M	50
40	E40	Kollam-350M	75
41	E41	Kollam-350M	75
42	E42	Kollam-350M	75
43	E43	Kollam-100M	100
44	E44	Kollam-350M	20
45	E45	Kochi-100M	75

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3.2 Colony and cell morphology of yeast strains

The study revealed a diverse range of colony morphotypes, ranging from non-pigmented (white to cream) to pigmented colonies displaying various colours, including brown, green, grey, pink, orange, and red. Notably, some yeast colonies initially appeared non-pigmented but developed pigmentation over time. Table 2 presents the colony characteristics—including size, shape, colour, and texture—of marine yeast strains isolated from southeastern Arabian Sea. Additionally, Fig. 2 illustrates the microscopic features, such as cell shape, size, and budding patterns.

Table 2. Colony characteristics of yeast strains isolated from southeastern Arabian Sea

S. No.	Strain No.	Colony morphology
1	E1	Highly raised, crateriform, early-stage white, later turned to grey
2	E2	Medium, convex, cream, entire margin
3	E3	White, raised, very rough, brittle later
4	E4	Small, convex, cream, entire margin
5	E5	Medium, convex, cream, entire margin
6	E6	Small, convex, cream, entire margin
7	E7	Medium, convex, cream, entire margin
8	E8	Light orange to red, glistening, soft to slimy, entire margin
9	E9	White, round, large colonies
10	E10	Brown, center raised, early-stage grey, later brown with a black backside
11	E11	White, flat, powdery-like with light brown backside
12	E12	White, round, large colonies
13	E13	White, round, medium, transparent
14	E14	Pink, irregular, medium colonies
15	E15	Round, convex at the center, wrinkled/rough, cream, entire margin
16	E16	Medium, round, convex, cream to white, entire margin
17	E17	Red, irregular, large colonies
18	E18	Small, round, convex, cream to white, entire margin
19	E19	Brown, raised, black backside
20	E20	Medium, round, convex, cream to white, entire margin
21	E21	Medium, round, convex, cream to white, entire margin
22	E22	Medium, round, convex, cream to white, entire margin
23	E23	Medium, round, convex, cream to white, entire margin
24	E24	Red, irregular, small colonies
25	E25	Medium, convex, cream, entire margin
26	E26	White, round, large colonies
27	E27	Medium, convex, cream, entire margin
28	E28	Small, convex, cream, entire margin
29	E29	Grey, large size, wrinkled
30	E30	White, raised, white backside
31	E31	Green, flat
32	E32	White, cotton-like early stages. The color turned to pink later
33	E33	Highly raised, early-stage white, later turned to green with a white backside
34	E34	Grey, large and spread completely later
35	E35	White early stage, later tuned to light pink, wrinkled, white backside
36	E36	Grey, cotton-like, white backside
37	E37	Grey early stage, turned to pink, wrinkled, very large and spread entire petri dish
38	E38	White, medium size, white backside
39	E39	Grey early stage later turned to pink, large colonies
40	E40	White, round, soft, raised
41	E41	White, cotton-like, very large size, light pink in middle
42	E42	White, cotton-like early stage, light green later stage
43	E43	Grey, large size, grey backside
44	E44	White, medium size, brown backside
45	E45	White, cotton-like, black center and white around the backside

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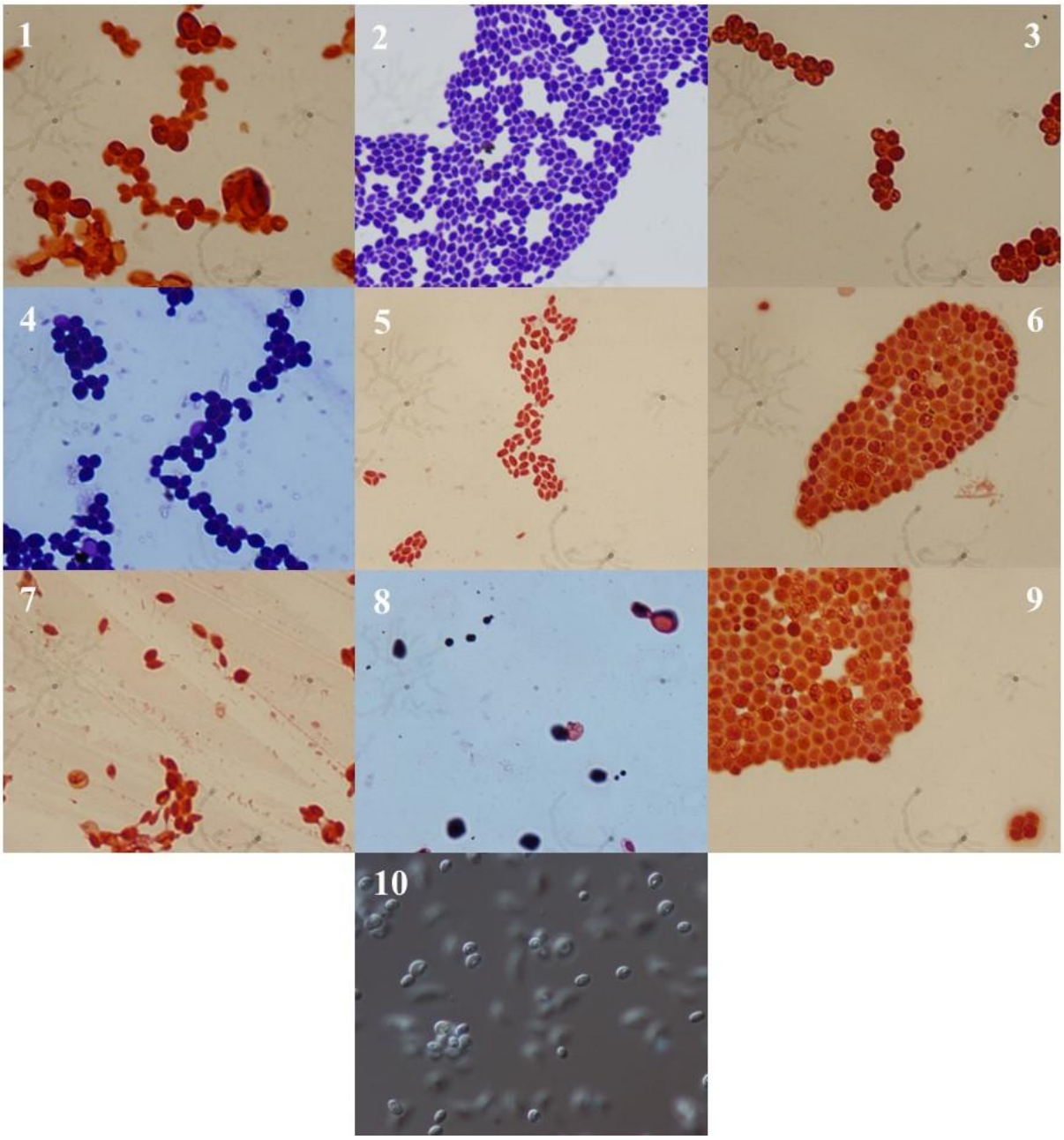

Fig. 2. Photomicrographs of marine yeasts grown in YPD broth. 1. (E3), 2. *Debaryomyces fabryi* (E4), 3. *Starmerella etchellsii* (E5), 4. *Rhodotorula mucilaginosa* (E8), 5. *Kodamaea ohmeri* (E12), 6. *R. mucilaginosa* (E17), 7. (E19), 8. (E22), 9. *R. mucilaginosa* (E24), 10. *S. etchellsii* (E25).

3.2.1 Identification using molecular analysis

Of the 45 yeast strains, 22 strains were selected for sequencing and phylogenetic analysis. The sequence lengths of these isolates ranged from 610 bp to 921 bp. Sequences were compared with GenBank using BLAST analysis and subsequently submitted to GenBank, receiving accession numbers PP355379 to PP355400. The sequence analysis results, along with accession numbers, are presented in Table 3. All strains, except strain E7, exhibited 98.9% to 100% sequence similarity (E-value: 0.0) with their closest type strains. However, strain E7 showed 96.26% sequence similarity (E-value: 0.0) with *Starmerella etchellsii* CBS 1750^{T}. Table 3 provides details on sequence length (bp), GenBank accession numbers, sequence

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coverage (ITS1 to 28S), closest relatives, and percentage identity (%) of marine yeast strains isolated from southeastern Arabian Sea.

3.3 Phylogenetic analysis

The phylogenetic analysis of yeast strains isolated in this study provides a comprehensive insight into their taxonomic relationships (Figs. 3 and 4). To ensure robust clustering patterns, phylogenetic trees were constructed using three different methods: Maximum Likelihood (ML), Neighbour-Joining (NJ), and Maximum Parsimony (MP). The resulting trees delineated relationships within Ascomycota and Basidiomycota, confirming the stability of clustering patterns across all methodologies.

3.3.1 Ascomycota clustering

Among Ascomycota, ten strains (E2, E5, E7, E10, E16, E20, E21, E23, E25, and E27) clustered closely with *Starmerella etchellsii*, indicating a strong representation of this species among the isolates. Three strains (E4, E18, and E28) grouped within *Debaryomyces*, specifically aligning with *D. fabryi*, *D. hansenii*, and *D. coudertii*, suggesting a close evolutionary relationship within this genus. Another distinct cluster comprised strains E12, E13, and E26, which were affiliated with *Kodamaea ohmeri*, a yeast species known for its presence in diverse ecological niches. Two isolates (E11 and E15) clustered with *Meyerozyma caribbica*, further expanding the fungal diversity identified in this study. Notably, strain E7, while clustering within *Starmerella*, formed a distinct clade, suggesting genetic divergence and a potential novel taxonomic placement (Fig. 3, Supplementary Figs. 1 and 3).

3.3.2 Basidiomycota clustering

Within Basidiomycota, three strains (E8, E17, and E24) clustered with *Rhodotorula mucilaginosa* and *R. dairenensis*, both species known for their ecological versatility and biotechnological potential. Additionally, one strain (E1) clustered closely with *Sterigmatomyces elviae*, a lesser-known but ecologically significant basidiomycete (Fig. 4, Supplementary Figs. 2 and 4). The consistency across multiple phylogenetic algorithms reinforces the robust taxonomic placement of the identified strains.

3.4 Overall distribution and industrial significance

The distribution analysis of the identified yeast strains showed that *Starmerella etchellsii* was the dominant species, comprising 45.5% of the isolates. The remaining strains were distributed as follows: *Kodamaea ohmeri* (13.6%), *Debaryomyces fabryi* (13.6%), *Meyerozyma caribbica* (13.6%), *Rhodotorula mucilaginosa* (9.1%), and *Sterigmatomyces elviae* (4.5%) (Fig. 5). The dominance of *Starmerella etchellsii* is particularly significant, as species within this genus are known for their biotechnological applications, including lipid production and biofuel synthesis.

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Table 3. Molecular characterization of yeast strains from the southeastern Arabian Sea, including strain details, sequence data, BLAST results, and percent identity

Strain No.	Sequence length (bp)	Sequence accession number	Sequence Coverage (ITS1 to 28S)	First BLAST result	Strain and Sequence accession numbers	Per cent identity (%)
E1	853	PP355379	ITS2, 28S	*Sterigmatomyces elviae*	CBS 5922^{T}, KY109786	100
E2	772	PP355380	5.8S, ITS2, 28S	*Starmerella etchellsii*	CBS 1750^{T}, KY102077	99.22
E4	772	PP355381	ITS2, 28S	*Debaryomyces fabryi*	CBS 789^{T}, MK394103	100
E5	749	PP355382	5.8S, ITS2, 28S	*Starmerella etchellsii*	CBS 1750^{T}, KY102077	99.60
E7	852	PP355383	ITS1, 5.8S, ITS2, 28S	*Starmerella etchellsii*	CBS 1750^{T}, KY102077	96.26
E8	904	PP355384	5.8S, ITS2, 28S	*Rhodotorula mucilaginosa*	CBS 316^{T}, KY109056	99.47
E10	866	PP355385	ITS1, 5.8S, ITS2, 28S	*Starmerella etchellsii*	CBS 1750^{T}, KY102077	99.65
E11	740	PP355386	ITS2, 28S	*Meyerozyma caribbica*	CBS 9966^{T}, MH545919	98.92
E12	757	PP355387	5.8S, ITS2, 28S	*Kodamaea ohmeri*	CBS 5367^{T}, MK394144	99.87
E13	610	PP355388	ITS2, 28S	*Kodamaea ohmeri*	CBS 5367^{T}, MK394144	99.51
E15	850	PP355389	5.8S, ITS2, 28S	*Meyerozyma caribbica*	CBS 9966^{T}, MH545919	100
E16	790	PP355390	ITS1, 5.8S, ITS2, 28S	*Starmerella etchellsii*	CBS 1750^{T}, KY102077	99.62
E17	844	PP355391	5.8S, ITS2, 28S	*Rhodotorula mucilaginosa*	CBS 316^{T}, KY109056	100
E18	921	PP355392	5.8S, ITS2, 28S	*Debaryomyces fabryi*	CBS 789^{T}, MK394103	99.78
E20	771	PP355393	5.8S, ITS2, 28S	*Starmerella etchellsii*	CBS 1750^{T}, KY102077	99.61
E21	767	PP355394	5.8S, ITS2, 28S	*Starmerella etchellsii*	CBS 1750^{T}, KY102077	99.21
E23	769	PP355395	5.8S, ITS2, 28S	*Starmerella etchellsii*	CBS 1750^{T}, KY102077	99.21
E24	718	PP355396	ITS2, 28S	*Rhodotorula mucilaginosa*	CBS 316^{T}, KY109056	100
E25	768	PP355397	5.8S, ITS2, 28S	*Starmerella etchellsii*	CBS 1750^{T}, KY102077	99.48
E26	783	PP355398	ITS1, 5.8S, ITS2, 28S	*Kodamaea ohmeri*	CBS 5367^{T}, MK394144	99.74
E27	795	PP355399	ITS1, 5.8S, ITS2, 28S	*Starmerella etchellsii*	CBS 1750^{T}, KY102077	99.37
E28	802	PP355400	ITS2, 28S	*Debaryomyces fabryi*	CBS 789^{T}, MK394103	99.88

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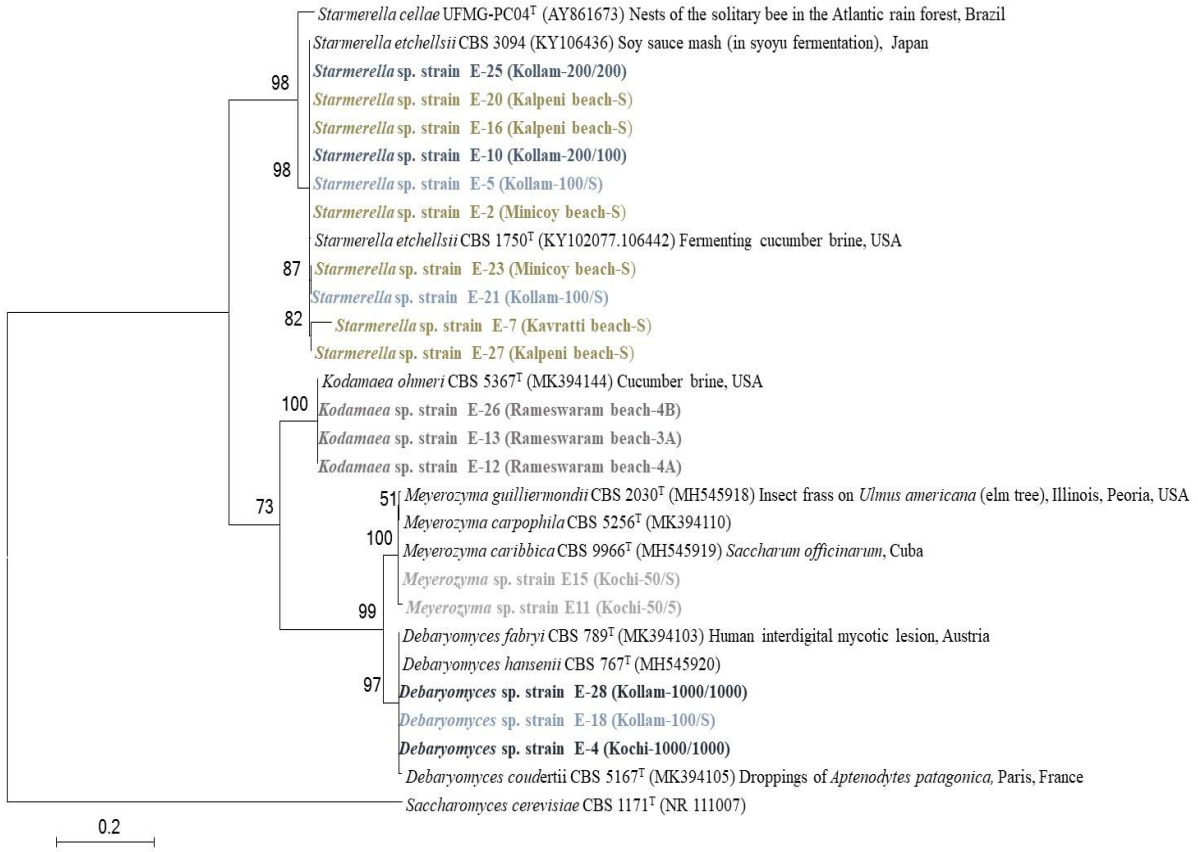

Fig. 3. Maximum likelihood tree depicting phylogenetic relationships of yeast strains from southeastern Arabian Sea (Ascomycota) with related type strains. Strains are color-coded by sampling points: Beach (grey), Coastal (green), Offshore surface (light blue), and Offshore bottom (dark blue). Bootstrap values >50% are shown at nodes. *Saccharomyces cerevisiae* CBA 1171^{T} (NR111007) serves as the outgroup. Scale bar = 0.05 substitutions per nucleotide position.

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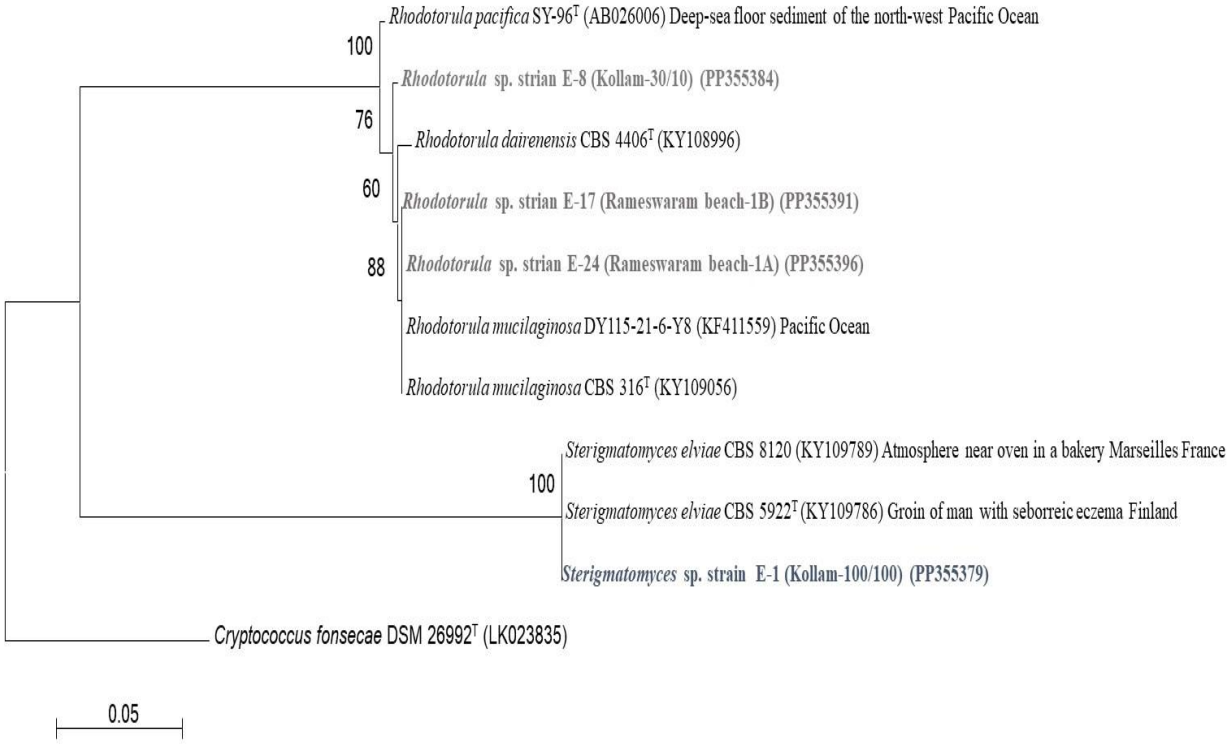

Fig. 4. Maximum likelihood tree depicting phylogenetic relationships of yeast strains from southeastern Arabian Sea (Basidiomycota) with related type strains. Strains are color-coded by sampling points: Beach (grey), Coastal (green), Offshore surface (light blue), and Offshore bottom (dark blue). Bootstrap values >50% are shown at nodes. *Cryptococcus fonsecae* DSM 26992^{T} (LK023835) serves as the outgroup. Scale bar = 0.2 substitutions per nucleotide position.

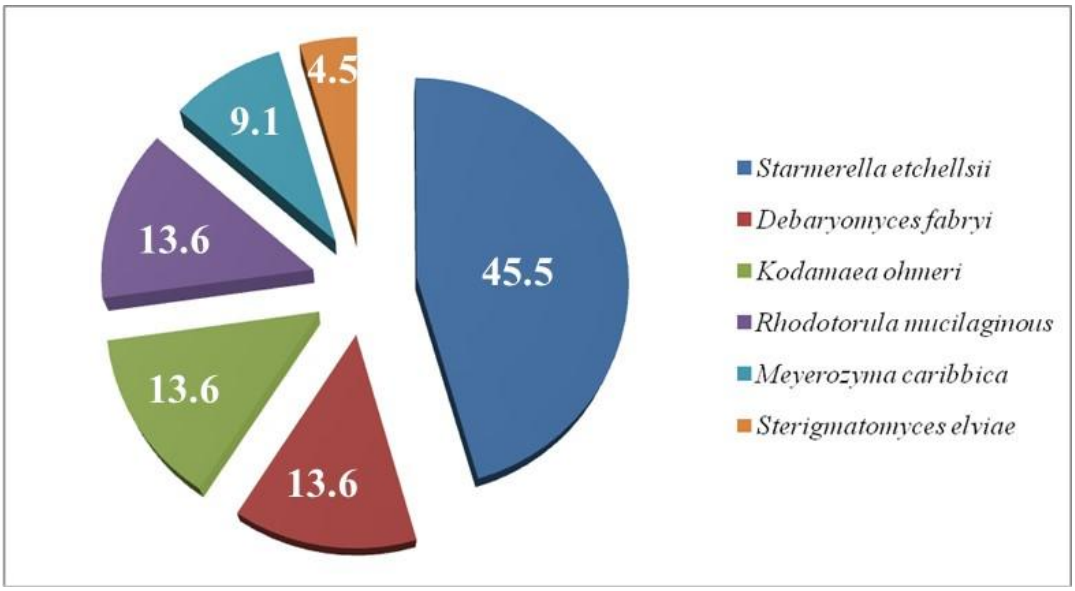

Fig. 5. Generic composition of the number of yeast isolates from each genus

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3.5 Bioprospecting of isolated yeast strains

Bioprospecting of marine yeast strains from southeastern Arabian Sea revealed significant variation in biomass production, protein concentration, and enzymatic activities (Table 4).

3.5.1 Biomass production

The dry weight of yeast strains ranged from 0.0573 to 0.1122 mg/100 mL, indicating variability in growth rates among the isolates (Table 4). Among them, *Starmerella etchellsii* (E27) exhibited the highest biomass production (0.1122 mg/100 mL), suggesting superior growth potential under the given conditions.

3.5.2 Protein concentration

The protein concentration among yeast strains ranged from 0.10 to 0.71 µg/mL (Table 4). Notably, *Starmerella etchellsii* (E27) exhibited the highest protein concentration (0.71 µg/mL), indicating a strong capacity for protein biosynthesis. When expressed as a percentage of biomass, *Starmerella etchellsii* (E25) had the highest protein content (65.1%), followed closely by E27 (63.3%). These strains could be valuable for industrial applications requiring high protein yields, such as single-cell protein (SCP) production for animal feed and biotechnological processes.

Table 4. Physiological characteristics of yeast strains isolated from southeastern Arabian Sea: Dry weight, Protein content, and Protein concentration (%)

Strain No.	Sampling station	Identified nearest taxon	Dry wt. (mg/100 ml)	Protein content (µg/ml)	Protein Conc. (%)
E1	KOL-100M/BOT 2	*Sterigmatomyces elviae*	0.0776	0.36	46.4
E2	S-5	*Starmerella etchellsii*	0.0710	0.25	35.2
E4	K0-1000M/BOT	*Debaryomyces fabryi*	0.0776	0.35	45.1
E5	KOL-100/SUR 1	*Starmerella etchellsii*	0.0693	0.10	14.4
E7	S-1	*Starmerella etchellsii*	0.0710	0.15	21.1
E10	KOL-200M/100M	*Starmerella etchellsii*	0.0668	0.30	44.9
E11	KO-50M/5HR 1	*Meyerozyma caribbica*	0.0726	0.42	57.9
E12	RAM-52-4A	*Kodamaea ohmeri*	0.0874	0.35	40.0
E13	RAM-53-3A	*Kodamaea ohmeri*	0.0894	0.30	33.6
E15	KOCHI-50M/SURFACE	*Meyerozyma caribbica*	0.1069	0.59	55.2
E16	S3-2A	*Starmerella etchellsii*	0.0717	0.10	13.9
E17	RAM-52-RED 1	*Rhodotorula mucilaginosa*	0.0573	0.18	31.4
E18	KOL-100M/SWIF 2	*Debaryomyces fabryi*	0.0693	0.29	41.8
E20	S3-2	*Starmerella etchellsii*	0.0637	0.25	39.2
E21	KOL-100M/SWIF 2	*Starmerella etchellsii*	0.0614	0.29	47.2
E23	S-9	*Starmerella etchellsii*	0.0823	0.40	48.6
E24	RAM-52-RED	*Rhodotorula mucilaginosa*	0.0712	0.18	25.3
E25	KOL-100M/200M	*Starmerella etchellsii*	0.0614	0.40	65.1
E26	RAM-52-4B	*Kodamaea ohmeri*	0.0712	0.27	37.9
E27	S3-1	*Starmerella etchellsii*	0.1122	0.71	63.3
E28	KOL-1000M/BOT-3	*Debaryomyces fabryi*	0.0626	0.26	41.5

4. Discussion

Previous studies on marine yeast biodiversity in Indian waters have utilized various identification methods, including colony morphology, microscopic examination, growth characteristics, and biochemical profiling. Marine yeasts have been isolated from diverse ecosystems, such as the Arabian Sea coast (Kutty et al., 2013a, 2013b), deep-sea sediments

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(~5000 m) of the Indian Ocean (Singh et al., 2010, 2011, 2012), marine oxygen-deficient environments (Jebaraj et al., 2010, 2012), coastal and offshore waters of Cochin (Rhishipal & Philip, 1998), hydrocarbon-degrading yeast from Mumbai waters (Oswal et al., 2002), and sediments from the Arabian Sea and Bay of Bengal slope (Kutty et al., 2013a, 2013b). Additional reports include marine yeast diversity from Vizhinjam and Rajakkamangalam coasts (Anusha et al., 2014), Muthupet mangrove environment (Jayalakshmi & Umamaheshwari, 2016), and the coastal waters of Konkan (Anuradha et al., 2016).

Yeast species identified in these studies include Black yeasts, *Bullera* sp., *Candida albicans*, *C. atmospherica*, *C. parapsilosis*, *C. tropicalis*, *Cryptococcus* sp., *Debaryomyces hansenii*, *Dekkera* sp., *Filobasidium* sp., *Leucosporidium* sp., *Lipomyces* sp., *Oosporidium* sp., *Pichia guilliermondii*, *Rhodotorula crocea*, *R. rubra*, *Saccharomyces italicus*, *S. chevalieri*, *S. rosei*, *Sporobolomyces hispanicus*, *S. odonus*, *Torulopsis candida*, *T. glabrata*, *Trichosporon* sp., *Yarrowia lipolytica*, and *Wingea* sp., with *Candida* being the predominant genus in several samples.

Although some of these studies incorporated molecular techniques by sequencing ITS1, 5.8S rRNA gene, ITS2, or 28S rRNA gene, relatively few have extensively applied phylogenetic analysis for accurate species identification. For instance, molecular studies on Konkan coast samples (Anuradha et al., 2016) identified yeast isolates belonging to six genera—*Pichia*, *Cryptococcus*, *Kodamaea*, *Zygozyma*, *Candida*, and *Rhodotorula*. Similarly, black yeast isolates from Bay of Bengal slope sediments (Kutty et al., 2013a) were identified as *Hortaea werneckii*.

4.1 Yeast biodiversity in southeastern Arabian Sea

In this study, 45 yeast strains were isolated from southeastern Arabian Sea, with 53% obtained from surface waters, consistent with previous reports (Chen et al., 2009; Kutty & Philip, 2008). Additionally, beach water samples exhibited higher colony-forming units (CFUs), whereas fewer isolates were recovered from deeper waters (5, 10, 100, 200, and 1000 m), highlighting greater yeast diversity in surface waters.

Among the 45 isolates, 22 strains were identified using molecular methods, including sequencing of ITS1, 5.8S rRNA gene, ITS2, and the D1/D2 domain of 28S rRNA gene, followed by phylogenetic analysis. These isolates belonged to two major phyla—Ascomycota and Basidiomycota—comprising *Starmerella*, *Debaryomyces*, *Kodamaea*, *Meyerozyma*, *Rhodotorula*, and *Sterigmatomyces*.

Typically, Ascomycota yeasts—including *Candida*, *Debaryomyces*, *Kluyveromyces*, *Pichia*, and *Saccharomyces*—are dominant in upper water layers, whereas Basidiomycota yeasts, such as *Cryptococcus*, *Rhodosporidium*, *Rhodotorula*, and *Sporobolomyces*, are more common in deeper waters (Kutty et al., 2013a, 2013b). However, our findings deviated from this pattern. For instance, *Debaryomyces fabryi* was isolated from both surface and 1000 m depths, *Starmerella etchellsii* was found across various depths, and *Rhodotorula mucilaginosa* was isolated from surface waters.

The dominance of *Starmerella etchellsii* in this study is consistent with previous research in coastal and deep-sea environments (Rishipal & Philip, 1998; Kutty & Philip, 2008). Understanding marine yeast biodiversity has broader ecological implications, particularly in monitoring seawater pollution (Chopra et al., 2024; Hagler, 2006; Monapathi et al., 2020).

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4.2 Discovery of a potential novel yeast species

A noteworthy finding in this study was strain E7, which exhibited 96.26% sequence similarity with *Starmerella etchellsii*, suggesting it may represent a novel species within *Starmerella*. Phylogenetic analysis revealed that E7 clusters within *Starmerella* but forms a distinct clade (Fig. 3), indicating genetic divergence (>3% sequence divergence from the nearest type strain).

To confirm strain E7 as a novel species, further studies should assess its phenotypic characteristics, including morphology, growth conditions, and metabolic profiles, in comparison to closely related taxa. Additionally, whole-genome sequencing may be necessary to resolve its phylogenetic placement and identify unique genetic traits. The potential discovery of a novel yeast species holds important implications for microbial diversity, ecology, and evolutionary studies.

4.3 Biotechnological potential of marine yeasts

While marine yeast biodiversity has been widely studied, bioprospecting of marine yeasts from Indian waters remains poorly explored. Previous research has demonstrated their potential in various applications. *Saccharomyces cerevisiae* has been explored for bioethanol production (Saravanakumar et al., 2013), while strain improvement for thermostable α-amylase production has been reported (Sidhu et al., 1997). *Yarrowia lipolytica* has been investigated for single-cell oil production (Katre et al., 2012). The immunostimulant properties of marine yeasts have been evaluated in *Fenneropenaeus indicus* (Sarlin & Philip, 2011).

Additionally, *Yarrowia lipolytica* and *Candida* sp. have been studied for the biosynthesis of gold and silver nanoparticles (Agnihotri et al., 2009; Apte et al., 2013; Dinesh Kumar et al., 2011). Marine yeasts have also been experimentally used in beer fermentation, demonstrating their potential as nonconventional brewing yeasts (Ordulj et al., 2024). Beyond these applications, marine yeasts play a crucial role in pharmaceuticals, producing valuable compounds such as astaxanthin, riboflavin, β-glucans, and vaccines. They are also explored for biofuels and environmental biotechnology (Sivakumar et al., 2020).

4.4 Single-Cell Protein (SCP) potential

Single-cell protein (SCP) refers to microbial proteins derived from algae, bacteria, and yeasts, serving as a protein-rich alternative for human and animal nutrition (Chi et al., 2006). SCP is rich in essential amino acids and can replace conventional protein sources, such as soymeal, in addressing global protein shortages. Among microbial sources, yeasts are ideal candidates for SCP production. Previous studies have shown that marine yeast isolates used as SCP increased protein concentration from 38.5% to 70.4% (Rishipal & Philip, 1998), with *Candida* sp. exhibiting the highest activity. Similarly, *Cryptococcus aureus* G7a was identified as a high-protein yeast (Gao et al., 2007).

In this study, protein concentration among yeast isolates ranged from 13.9% to 65.1%, with the highest levels found in *Starmerella etchellsii*, followed by *Meyerozyma caribbica*, *Sterigmatomyces elviae*, *Debaryomyces fabryi*, *Kodamaea ohmeri*, and *Rhodotorula mucilaginosa*. Notably, no scientific reports have documented the use of *Starmerella*, *Meyerozyma*, *Sterigmatomyces*, or *Kodamaea* in SCP production, highlighting their untapped biotechnological potential.

5. Conclusion

This study highlights the diversity of marine yeasts, with higher species richness observed in surface and coastal waters. Traditional identification methods, including microscopic

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examination, colony morphology, and biochemical assays, remain useful for genus-level identification. However, the integration of morphological and molecular characterization enables precise species identification and facilitates the discovery of potentially novel strains. Notably, one genetically distinct strain (E7) was identified, suggesting the possibility of a novel species. The continued exploration of marine yeast diversity holds promise for discovering microorganisms with significant industrial and environmental applications. Marine yeasts not only offer biotechnological potential but also serve as bio-indicators of seawater pollution.

In this study, six yeast species were identified: *Debaryomyces fabryi*, *Kodamaea ohmeri*, *Meyerozyma caribbica*, *Rhodotorula mucilaginosa*, *Starmerella etchellsii* (the predominant species), and *Sterigmatomyces elviae*. Among these, *S. etchellsii* and *M. caribbica* exhibited high biomass production and protein content, indicating their potential use as single-cell protein (SCP) sources. These strains could be valuable as nutritional feed supplements in marine aquaculture. Future research should focus on optimizing the cultivation and industrial utilization of these yeasts, particularly in SCP production, biotechnology, and environmental monitoring.

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Conflict of Interest
There is no conflict of interest.

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