BIODIVERSITAS
Volume 23, Number 3, March 2022
Pages: 1408-1419

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230327

First report of seaweed-associated yeast from Indonesia: Species composition and screening of their polysaccharides-degrading enzymes

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Manuscript received: 3 February 2022. Revision accepted: 21 February 2022.

Abstract. *Sibero MT, Frederick EH, Sabdono A, Wijayanti DP, Pringgenies D, Radjasa OK, Zilda DS, Murwani R. 2022. First report of seaweed-associated yeast from Indonesia: Species composition and screening of their polysaccharides-degrading enzymes. Biodiversitas 23: 1408-1419.* Yeast has been widely utilized in various industries due to its enzyme properties. Therefore, plenty of studies focus on exploring yeast biodiversity from diverse sources. However, there are limited reports regarding marine yeast biodiversity and its potential from Indonesia. This study aimed to isolate and identify the seaweed-associated yeast from Jepara in Central Java and Sepanjang Beach in Gunung Kidul, Yogyakarta, then examined their potential to produce extracellular polysaccharides-degrading enzymes (EPEs). Marine yeasts were isolated using standard marine agar (STD) and potato dextrose agar (PDA). All isolates were characterized by their salinity tolerance, morphology, and species confirmation using DNA barcoding. Three EPEs consisting of agarase, alginate-lyase, and carrageenase were screened using polysaccharides-enriched agar media. A specific agarase encoding gene was detected with specific primers. In total, 21 seaweed-associated yeast were successfully isolated from 6 seaweeds and noted as facultative marine yeast. The DNA barcoding study discovered that these yeasts belonged to 5 genera, consisted of *Aureobasidium, Candida, Debaryomyces, Hortaea,* and *Rhodotorula*. Moreover, *Candida* was noted as the most abundant genus (71.42%). It was noted that *Hortaea werneckii* MTJ.11 and MTJ.13 were positive for all enzymes; *Aureobasidium melanogenum* MTGK.31 gave a positive result for agarase and carrageenase; while [*Candida*] *zeylanoides* MTGK.23 only exhibited alginate-lyase activity. Unfortunately, none of the primers used to detect the presence of genes encoding polysaccharide-degrading enzymes were successfully amplified.

Keywords: Diversity, Indonesia, marine yeast, polysaccharides-degrading enzyme

INTRODUCTION

Marine oligosaccharides are the converted products of marine polysaccharides such as agar, carrageenan and alginate. It is highlighted that marine oligosaccharides contain hexoses sugar monomers (Stiger-Pouvreau et al. 2016; Jutur et al. 2016; Zhu et al. 2021). O'Sullivan et al. (2010) reported these substances to have various biological activities to support health. Marine oligosaccharides were noted to increase the metabolism of digestive organs, enhance the growth of beneficial microorganisms in the colon, inhibit the growth of pathogenic agents, and prevent intestinal inflammation (Ji et al. 2010; Kang et al. 2015; Wang et al. 2021). Therefore, they have been utilized as the central element of dietary supplements (Gurpilhares et al. 2019). Some studies have developed various methodologies to produce marine oligosaccharides (Ren et al. 2019). Simpson et al. (2012) reported that carbohydrase is frequently used in the food industries. This enzyme degrades polysaccharides into oligosaccharides through hydrolysis that beneficial in functional food production (Raveendran et al. 2018). These enzymes are classified as extracellular enzymes since they are secreted outside the cells (Tan and Zou 2001; Carrasco et al. 2012; Zhu and Ning 2015; Sibero et al. 2019; Lee et al. 2019).

Interestingly, some marine microorganisms are found producing suitable enzymes to produce the marine oligosaccharides naturally and effectively (Chand et al. 2016; Davani-Davari et al. 2019). They have been widely reported as carbohydrase producers, such as agarase, amylase, alginate-lyase, carrageenase, cellulase, chitinase, pectinase, phytase, and xylanase (Joseph and Raj 2007; Carrasco et al. 2012; Zaky et al. 2014; Zhu and Ning 2015; Zeng et al. 2016; Veliz et al. 2017; Zilda et al. 2019; Bruno et al. 2019). Based on the research of Yopi et al. (2017) it is known that *Bacillus tequilensis* can produce xylanase that is able to hydrolyze xylan to produce oligosaccharides with tri-hexasaccharide size as the main product. The production of a specific component with these enzymes offers many advantages, such as simple treatment, rapid multiplication

under controlled conditions, salt-tolerant, and high yield production (Zhang and Kim 2010; Bruno et al. 2019; Ruginescu et al. 2020). More interestingly, most studies in Indonesia only reported the potential of seaweed-associated bacteria and filamentous fungi as the source of polysaccharides degrading enzymes (Zilda et al. 2019; Hutapea et al. 2021; Wijaya et al. 2021). Yet, the study of polysaccharides degrading enzyme from Indonesia marine yeast has never been reported.

Despite its lack of studies, yeast is considered one of the pioneers of applied microorganisms for the food and beverage industries (Barnett 2003). This fungus has been utilized in fermentation for many centuries to produce bread, wine, and other liquors (Amoikon et al. 2019; Walker and Stewart 2016). Based on the geological distribution, it is classified into terrestrial yeast and marine yeast (Branda et al. 2010; Kandasamy et al. 2012; Buzzini et al. 2017; Aljohani et al. 2018). In fact, the terrestrial yeasts have been dominated the studies, while marine yeasts are rarely reported. On the other hand, Zaky et al. (2014) stated that marine yeast has a range of benefits in industrial applications, including enzyme producers. Also, this microorganism is widely distributed and isolated from various marine substrates, such as seawater, sediments, vegetation, and vertebrates (Kutty and Philip 2008; Kandasamy et al. 2012). In addition, the latest study about marine yeast from Indonesia was conducted by Sumerta and Kanti (2018). They successfully isolated 85 isolates from the coastal area in Karimun Besar Island, Riau. Thereafter, there is no other study that reports the diversity of marine yeast from Indonesia. Regarding the lack information about marine yeasts diversity and their polysaccharide degrading enzymes from Indonesia, this study was carried out to fill those information gaps.

MATERIALS AND METHODS

Seaweed collection

Sampling was conducted at Sepanjang Beach, Gunung Kidul District, Yogyakarta Province, Indonesia (8°08'14,21"S, 110°34'01,6"E); and two locations at Jepara District, Central Java Province, Indonesia, namely Teluk Awur (06°36'58,6"S, 110°38'18,8"E; 06°37'16,7"S; 110°38'07,5"E) and Panjang Island (06°34'20,4"S, 110°37'36,4"E) (Figure 1). In total ten seaweeds were collected in the zip lock bags then transported to the laboratory, and processed on the same day. Photographs of each sample were taken using Canon EOS 90D camera to assist in seaweed identification. The identification was carried out based on morphological and environment data (e.g., substrate, holdfast, thallus form, and air bladder) according to (Coppejans et al. 2017).

The sampling coordinate and environmental physical properties were recorded. At each sampling location, the dissolved oxygen and temperature were measured with a dissolved oxygen meter (Lutron DO-5510). At the same time, a refractometer was used for the salinity measurement, and a GPS (Garmin 60i) was used for marking the location coordinate.

Isolation, purification, and morphological characterization

Algal samples were washed with sterile natural seawater before proceed for fungal isolation. Standard marine agar (STD) [37.5 marine agar (Difco), 5 g glucose (Sigma Aldrich) 10 g yeast extract (HiMedia), 5 g beef extract (HiMedia), 5 g peptone (HiMedia) per 1 L distilled water] and potato dextrose agar (PDA, HiMedia) were used as isolation medium. Chloramphenicol (1 mg/mL) was added to the medium to eliminate the bacterial contamination.

The swab tap method was carried out to isolate the yeast. Algal samples were roughly ground aseptically then transferred and inoculated evenly on the agar media. As a control, environmental control plates were prepared. In total a 100 µL seawater from the sampling location were inoculated onto the agar media. Isolation was done with two replications. Agar plates were incubated at 27°C, then the colonies were checked each day after inoculation.

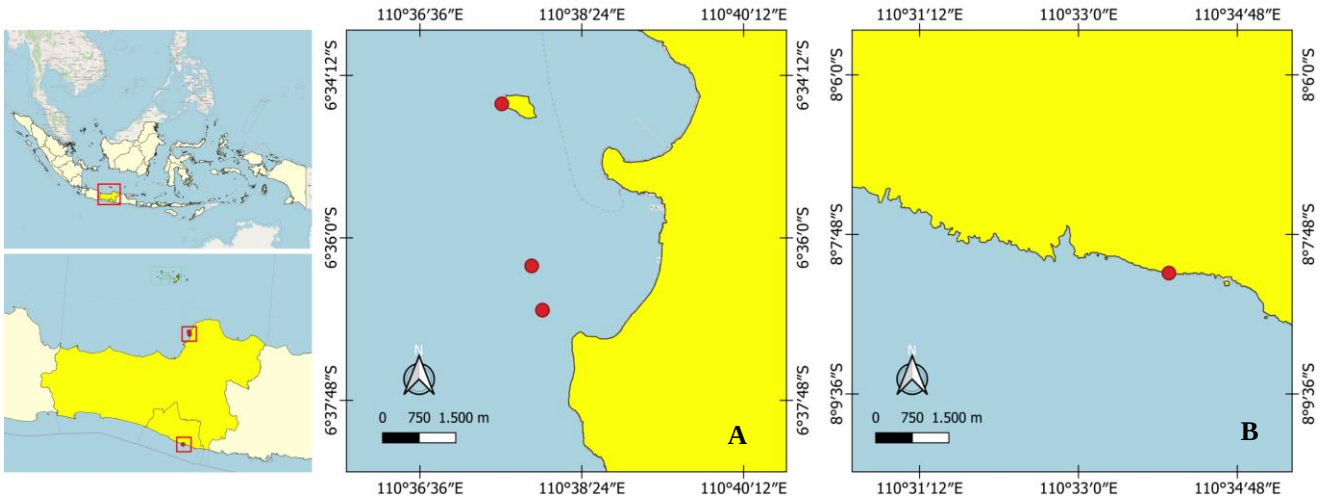

Figure 1. Sampling location in Central Java (A) and Yogyakarta (B), Indonesia

Each growing colony was purified and inoculated on PDA using the four-quadrant streak method. Colonies were distinguished based on colony size, margin, elevation, color, and texture. In order to confirm the cell and budding shape, microscopic observation was performed using lactophenol cotton blue (HiMedia). In order to obtain the seaweed-associated, hence the isolates that had same characteristics with the yeast from the seawater were discarded. A Canon EOS 90D camera took photographs of agar plates while the budding shape with a microscope camera.

Salinity experiment

All isolates were cultivated onto PDA medium in freshwater (0 ppm) and natural seawater (35 ppm). Isolate that grew only on natural seawater PDA was considered as marine obligate yeast, while isolate that grew on both media indicated was considered as facultative marine yeast (Wijaya et al. 2020).

DNA extraction

All isolates were cultivated for 3-Each yeast DNA was extracted using a Quick-DNA^{TM} Fungal/Bacterial Miniprep Kit (D6005, Zymo Research). After extraction, each DNA template was stored at -20°C until it was required.

Polymerase Chain Reaction (PCR) and sequencing

Internal Transcribed Spacer (ITS) region was used for yeast identification with ITS1 and ITS4 primers (Schoch et al. 2012) (Table 1). PCR mix composition and protocol were carried out according to (Sibero et al. 2016). Each reaction mixture had a total volume of 25 µL containing 12.5 µL GoTaq Green Master Mix DNA polymerase (Promega), 1 µL ITS1 primer, 1 µL ITS4 primer, 1.75 µL nuclease-free water (Thermo Scientific), and 8.75 µL DNA template. The amplification was carried out using a thermal cycler machine (Bio-Rad T100^{TM}) following this condition: 35 cycles of denaturation at 95°C for 1 min, annealing at 50-55°C for 1 min, and extension at 72°C for 1 min while the final extension was done at 72°C for 7 min. All samples were continued to electrophoresis (Submarine electrophoresis) to check the amplification quality. Then, the products were sent and sequenced by 1st Base Laboratories Sdn Bhd, Malaysia. The sequences were deposited as GenBank Accession Numbers. Then, all sequences were accumulated, edited, and aligned with MEGA X and identified by the Basic Local Alignment Search Tool (BLAST) in NCBI. The phylogenetic tree was reconstructed using maximum likelihood with bootstrap number 1000.

Screening of EPEs

The screening was performed according to (Hutapea et al. 2021). The media were prepared to contain different amounts of enzyme substances, as described below: (i) Agarase: contained 2% agar, 0.1% yeast extract, and 0.5% peptone. (ii) Alginate-lyase: contained 2% agar, 0.1% yeast extract, 0.5% peptone, and 0.5% alginate. (iii) Carrageenase: contained 2% carrageenan, 0.1% yeast extract, and 0.5% peptone.

All isolates were cultivated on the medium then incubated at room temperature for seven days. After incubation, all plates were flooded with 1 mL of 2.5% iodine solution, and the positive result was defined as a clear halo around the colony. The enzymatic index value was determined using a formula based on (Ayuningtyas et al. 2021).

Screening of EPEs was also carried out using specific primers to detect the encoding genes, as mentioned in Table 1. The PCR conditions were adjusted following the condition for the DNA barcoding.

RESULTS AND DISCUSSION

Seaweed collections and yeast isolation

Ten seaweeds were obtained from 3 different sampling sites. They have consisted of six seaweeds from Gunung Kidul, three seaweeds from Teluk Awur, and one seaweed from Panjang Island. The water quality of each sampling site had a different value of salinity, temperature, and dissolved oxygen (Table 2). Based on morphological identification, five seaweeds belonged to Phaeophyta, three Rhodophyta, and two Chlorophyta (Table 2 and Figure 2). Twenty-one yeasts have been successfully isolated from these samples, which was consisted of 16 isolates on PDA and 5 others on STD.

Table 1. Primers that used in this study

Primer's Name	Oligonucleotides	Target	References
ITS1	5′-TCC GTA GGT GAA CCT GCG G-3′	Fungal Barcoding	Sibero et al. (2016)
ITS4	5′ -TCC TCC GCT TAT TGA TAT GC-3′		
Aga4383F	5′-TCA TCA TAT GCA AGA TTG GGC ACA AAT TCC-3′	β-Agarase encoding gene	Hou et al. (2015)
Aga4383F	5′- CGA CAA GCT TTT ATT CTT TGA TAA TCC TCT G-3′		
agaM1F	5′-AGT AAG GAT CCC AAT ACG ACT GGG ATA ACA TTG CAA TTC C-3′	β-Agarase encoding gene	Li et al. (2018)
agaM1R	5′-AGT AAG ACG TCT AGC TTT GTT AGT TTG CGA GTG ACC C-3′		
ZH0-1F	5′-GGA TCC CAC CCC TTC GAC CAG GCC GTC GTG-3′	Alginate-lyase encoding gene	He et al. (2018)
ZH0-1R	5′-GCG GCC GCT CAG CTC GAG TGC TTT ACG TGG AG-3′		
CgkF	5′-ATG AAA ATA AAT AAA CAG G-3′	κ-carrageenase encoding gene	Kobayashi et al. (2012)
CgkR	5′-ATT TAC CGT GAT CAT AAC CGT G-3′		

Yeast identification

The morphological characteristics dominated by medium colony size, circular shape, entire margin, convex elevation, smooth texture, and white cream color. All isolates were dominated with ellipsoidal cell shape and formed a monopolar type of budding (Table 3). The DNA barcoding approach discovered that the 21 isolates consisted of four isolates as *Candida sake*, ten isolates as [*Candida*] *zeylanoides*, one isolate as [*Candida*] *santamariae* var. *membranifaciens*, one isolate as *Debaryomyces prosopidis*, one isolate as *Rhodotorula mucilaginosa*, two isolates as *Aureobasidium melanogenum*, and two isolates as *Hortaea werneckii* (Table 4). The photograph of the seaweed-associated yeast is shown in Figure 3, while Figure 4 shows the percentage of yeast species. Based on the phylogenetic tree, four clades were reconstructed (Figure 5). The Debaryomycetaceae clade contained the isolates with genera *Candida* and *Debaryomyces*, *Saccotheciaceae* included genus *Aureobasidium*, Teratosphaeriaceae for genus *Hortaea*, while genus *Rhodotorula* was separated in a group of Sporidiobolaceae clade. Further, all isolates were identified as facultative marine yeast from its ability to grow both on freshwater and seawater medium.

Enzyme activity

A screening of EPEs was conducted to evaluate their ability to produce four extracellular enzymes. The enzyme activity is shown in Table 7, which is presented as enzymatic index (EI) value. Four out of 21 isolates have shown enzyme activity, with 2.55 as the highest EI value produced by MTJ.13 on alginate-lyase assay, whereas MTJ.11 showed the lowest EI with a value of 0.53. After performing a molecular study to detect the encoding genes, unfortunately the primers that we used could not amplified any targeted genes.

Figure 2. Seaweed collections; A to F seaweeds from Gunung Kidul, Yogyakarta; G to I seaweeds from Teluk Awur, and J seaweed from Panjang Island, Jepara, Central Java, Indonesia

Table 2. Sampling location, its physical property of seawater, seaweed samples, and seaweed-associated yeast

Sampling location	Physical Properties of Seawater			Number of samples	Sample code	Suggested seaweed identity	Number of isolates
	Temp. (°C)	Salinity (ppm)	Dissolved Oxygen (mg/L)				
Sampling site 1							
Sepanjang Beach, Gunung Kidul	26.9	28	5.6	6	GK.1	*Padina* sp.1	12
					GK.2	*Codium repens*	1
					GK.3	*Asparagopsis* sp.	1
					GK.4	*Chondrophycus* sp.	2
					GK.5	*Acanthophora spicifera*	-
					GK.6	*Chaetomorpha* sp.	3
Sampling site 2							
Teluk Awur, Jepara	27.1	35	7	3	JPR.1	*Sargassum crassifolium*	2
					JPR.2	*Padina* sp.2	-
					JPR.3	*Sargassum* sp.	-
Sampling site 3							
Panjang Island, Jepara	30.5	33	5.3	1	PP.1	*Turbinaria decurrens*	-

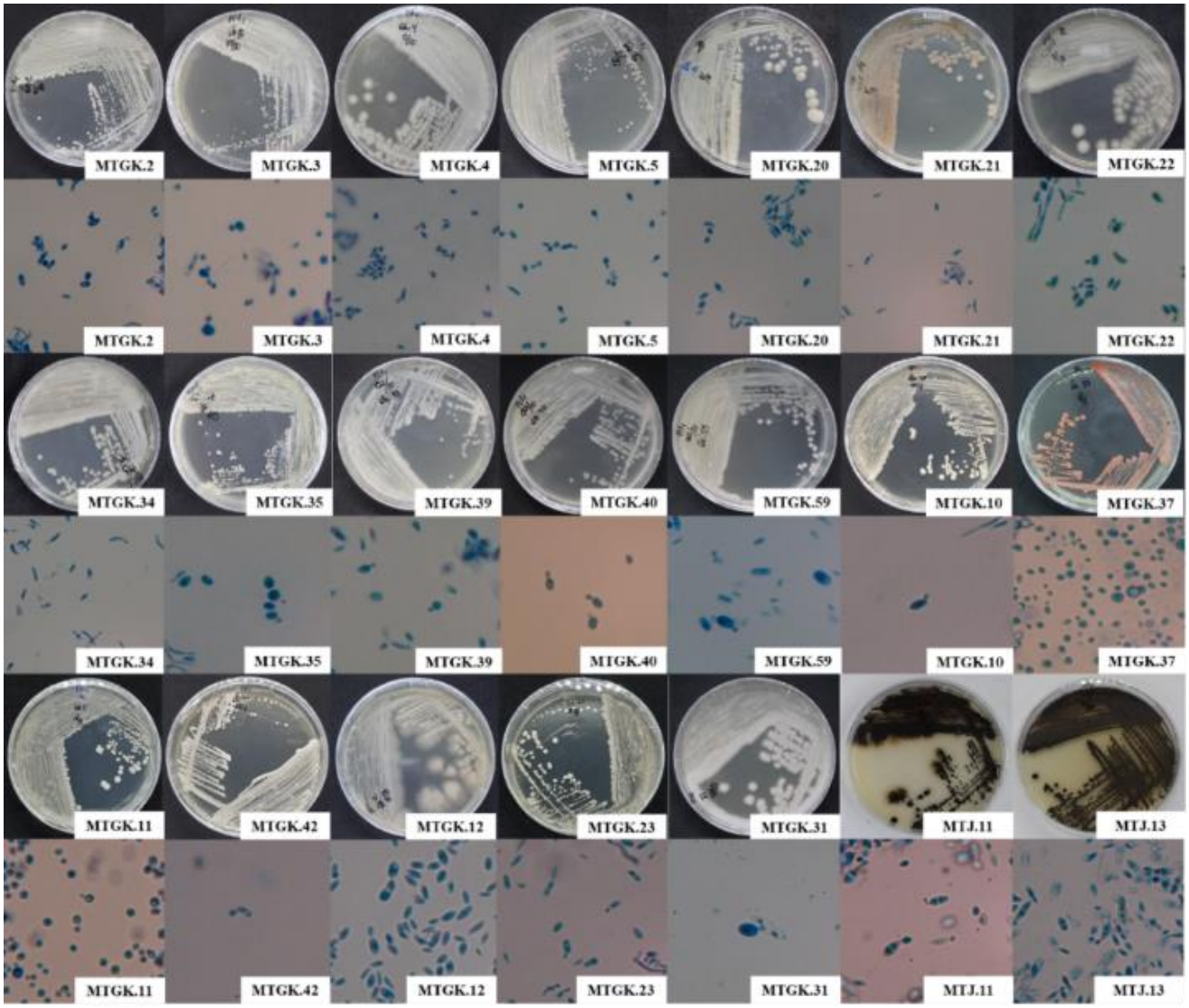

Figure 3. Morphological characteristics of seaweed-associated yeast

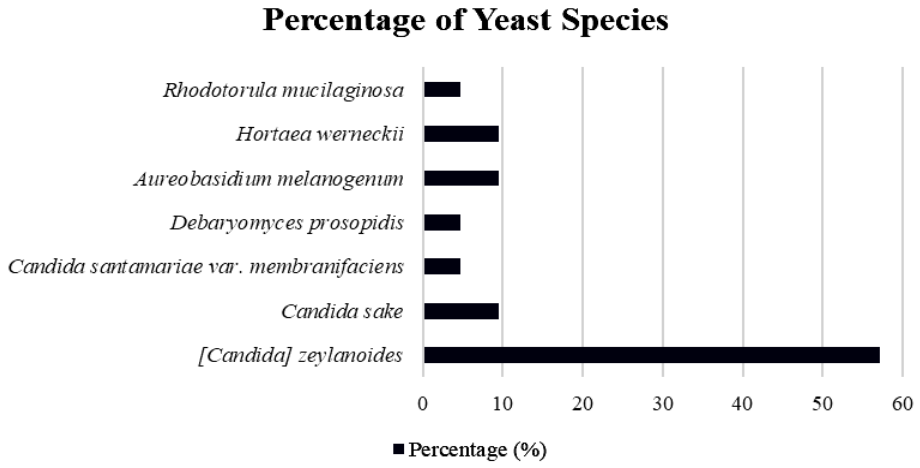

Figure 4. Relative abundance of seaweed-associated yeast

Figure 4. Relative abundance of seaweed-associated yeast

Isolate code	Macroscopic Identification						Microscopic Identification		
	Colony size	**Shape**	**Margin**	**Elevation**	**Texture**	**Color**	**Cell Shape**	**Budding**	**Type of Budding**
MTGK.2	Medium	Circular	Entire	Pulvinate	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.3	Medium	Circular	Entire	Pulvinate	Smooth	White Cream	Sub globose	Yes	Monopolar
MTGK.4	Medium	Circular	Undulate	Flat	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.5	Medium	Circular	Entire	Pulvinate	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.20	Large	Circular	Entire	Convex	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.21	Medium	Circular	Entire	Convex	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.22	Large	Circular	Undulate	Convex	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.34	Medium	Circular	Erose	Convex	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.35	Large	Circular	Filamentous	Flat	Smooth	White Cream	Sub globose	Yes	Monopolar
MTGK.39	Medium	Circular	Filamentous	Pulvinate	Smooth	White Cream	Sub globose	Yes	Monopolar
MTGK.40	Medium	Circular	Filamentous	Umbonate	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.59	Large	Circular	Filamentous	Convex	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.10	Large	Circular	Entire	Umbonate	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.37	Medium	Circular	Entire	Umbonate	Smooth	Pink Cream	Globose	Yes	Multilateral
MTGK.11	Large	Circular	Entire	Umbonate	Smooth	White Cream	Globose	Yes	Multilateral
MTGK.42	Medium	Circular	Entire	Pulvinate	Smooth	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.12	Medium	Circular	Entire	Convex	Smooth	White Cream	Ellipsoidal	Yes	Polar
MTGK.23	Medium	Irregular	Undulate	Convex	Wrinkled	White Cream	Ellipsoidal	Yes	Monopolar
MTGK.31	Large	Filamentous	Filamentous	Convex	Smooth	White Cream	Ellipsoidal	Yes	Polar
MTJ.11	Large	Circular	Rhizoid	Convex	Smooth	Black	Ellipsoidal	Yes	Bipolar
MTJ.13	Large	Circular	Filamentous	Convex	Smooth	Black	Ellipsoidal	Yes	Bipolar

Table 4. BLAST result of seaweed-associated according to ITS rDNA sequence

Code	Sequence Length	Top BLAST Result	Query Cover (%)	Similarity (%)	Accession no.	Submitted Accession no.
MTGK.2	381	*Candida sake*	100	99.21	NR_151807.1	MZ891642
MTGK.3	382	*Candida sake*	100	98.95	NR_151807.1	MZ891700
MTGK.4	551	[*Candida*] *zeylanoides*	96	99.62	NR_131278.1	MZ891699
MTGK.5	561	[*Candida*] *zeylanoides*	94	99.63	NR_131278.1	MZ891701
MTGK.20	561	[*Candida*] *zeylanoides*	94	99.81	NR_131278.1	MZ891702
MTGK.21	557	[*Candida*] *zeylanoides*	94	99.81	NR_155224.1	MZ891990
MTGK.22	560	[*Candida*] *zeylanoides*	94	99.81	NR_131278.1	MZ891703
MTGK.34	377	*Candida sake*	100	99.20	NR_151807.1	MZ891991
MTGK.35	375	*Candida sake*	100	99.47	NR_151807.1	MZ891992
MTGK.39	561	[*Candida*] *zeylanoides*	94	99.81	NR_131278.1	OK078615
MTGK.40	554	[*Candida*] *zeylanoides*	95	99.81	NR_131278.1	MZ892384
MTGK.59	591	[*Candida*] *santamariae var. membranifaciens*	99	99.83	NR_111300.1	MZ892389
MTGK.10	562	[*Candida*] *zeylanoides*	94	99.43	NR_155224.1	MZ892543
MTGK.37	679	*Rhodotorula mucilaginosa*	98	98.96	NR_073296.1	MZ892603
MTGK.11	715	*Debaryomyces prosopidis*	98	99.34	NR_077067.1	OK078616
MTGK.42	556	[*Candida*] *zeylanoides*	94	99.05	NR_155224.1	MZ892598
MTGK.12	560	*Aureobasidium melanogenum*	97	99.27	NR_159598.1	MZ905358
MTGK.23	570	[*Candida*] *zeylanoides*	93	99.81	NR_155224.1	MZ905369
MTGK.31	555	*Aureobasidium melanogenum*	98	99.27	NR_159598.1	MZ905360
MTJ.11	525	*Hortaea werneckii*	90	97.90	NR_145338.1	MZ892609
MTJ.13	521	*Hortaea werneckii*	90	98.52	NR_145338.1	MZ892612

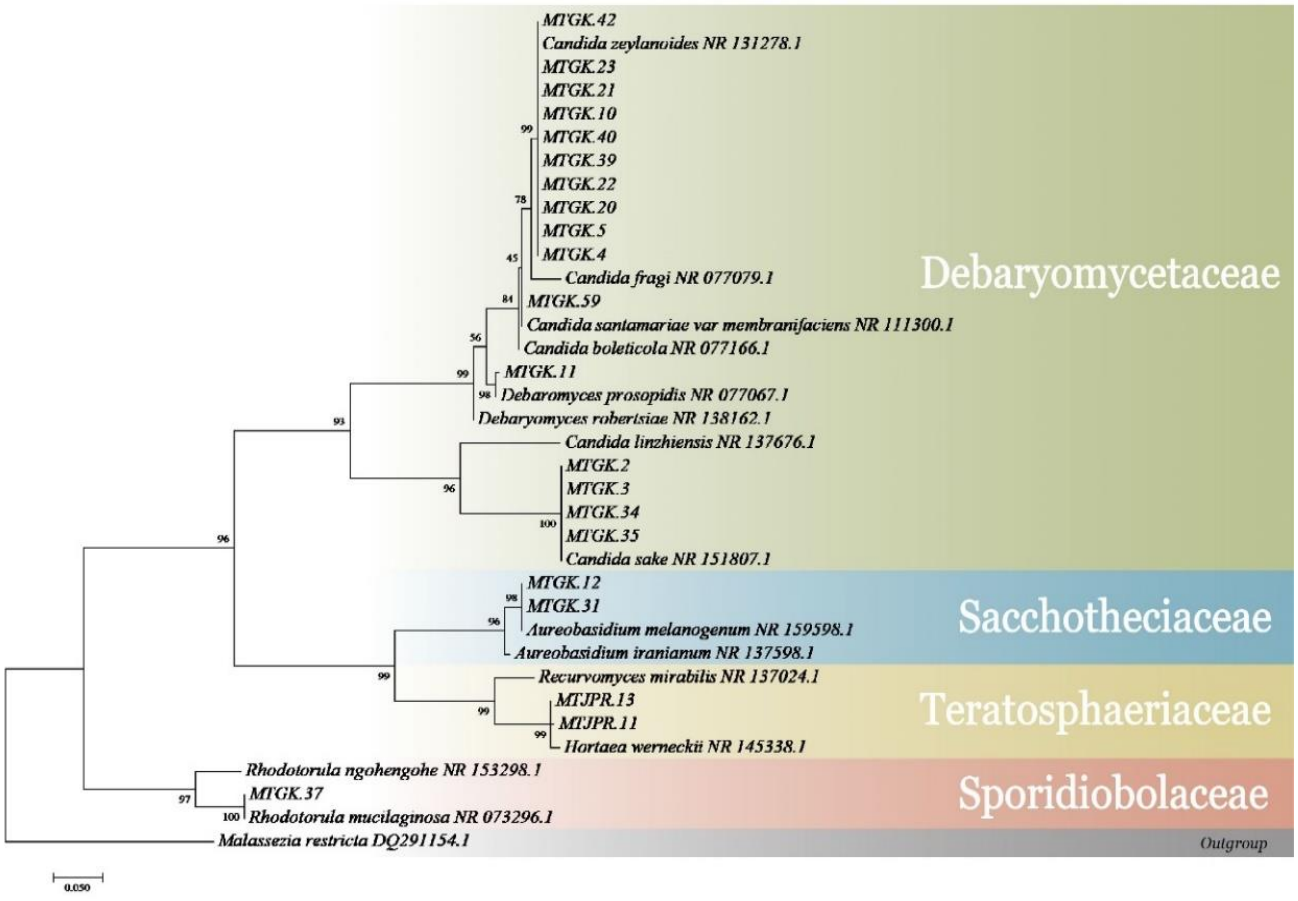

Figure 5. Phylogenetic tree based on ITS rDNA sequence of the seaweed-associated yeast

Table 5. Enzymatic Index (EI) value of prospective isolates

Code	Enzyme activity index		
	A	**B**	**C**
[*C.*] *zeylanoides* MTGK.23	-	1.66	-
A. melanogenum MTGK.31	2.07	-	1.30
H. werneckii MTJ.11	0.53	2.30	1.10
H. werneckii MTJ.13	1.79	2.55	1.46

Note: A. Agarase, B. Alginate-Lyase, C. Carrageenase

Discussion

Gunung Kidul and Jepara regions are well known for their ecotourism site due to their bioresources. Geographically, Gunung Kidul faces the South Java Sea, while Jepara faces the North Java Sea. Sudaryatno (2016) stated that the water characteristics in North and South Java Sea have different characters. Waters in the South Java Sea have more dynamic condition rather than in the North Java Sea, including the pH, salinity, and dissolved oxygen. This condition certainly affected the diversity and morphology of the seaweed (Erlania and Radiarta 2017; Widyartini et al. 2017; Romdoni et al. 2018). In addition, this condition certainly affected the flora and fauna diversity in both places (Putri et al. 2017; Utami et al. 2017; Rahmawati 2021; Sabdono et al. 2021). Previous studies have reported that seaweed from Chlorophyta and Rhodophyta are diverse in the Gunung Kidul region, while in Jepara is dominated by the Phaeophyta division (Wulandari et al. 2015; Chasani and Suyono 2020).

In this study, we collected six seaweeds from Gunung Kidul and four from Jepara (Figure 2). Based on the morphological identification five seaweeds belong to Phaeophyta, 3 Rhodophyta, and 2 Chlorophyta (Table 1). Generally, all samples were found attached on dead corals, pebbles, and sandy substrates. Our Phaeophyta were found in dense population with horizontally spread, brown thallus, and different shapes of blades (undulates, fan shape, dentate, and fleshy). For Rhodophyta, they have greenish to purplish red thallus, irregular branch, forming up to 11 cm high. Moreover, Chlorophyta have light green thallus, unbranched, and gregarious filaments. After performed the isolation, we obtained 21 seaweed-associated yeast. It was noted that the number of isolates from each seaweed was varied.

Morphological description is very essential to support yeast identifications (Nagahama et al. 2001; Singh et al. 2010; Francis et al. 2016). Therefore, morphological characteristics such as colony size, shape, margin, elevation, texture, color, cell shape, and type of budding were performed (Table 3). Based on the directions where it occurs, budding is classified as monopolar, polar, bipolar, and multilateral or multipolar. Monopolar is budding that is restricted to one pole of the cell, while budding that occurs at both poles is called bipolar. Polar budding is a bud that occurs on a narrow base and multilateral is budding from various sites on the cell (Kurtzman et al. 2011). In addition, we also conducted salinity experiment to determine their obligatory. We found that all isolates could grow on fresh and saline mediums. Therefore, all isolates were categorized as facultative marine fungi (Gal-Hemed et al. 2011; Sibero et al. 2017). In addition, Buzzini et al. (2017) reported their marine yeasts were classified as facultative marine fungi as well. Marine fungi are suggested to be carried from the terrestrial to the ocean through the biological vector, rain, wind or other natural phenomenon (Sibero et al. 2021). They are also equipped with special mechanism to tolerate the environmental conditions, such as morphology regulation, cell anatomy, cation transport entity modulation, and secondary metabolite production (Hohmann 2002; Ariño et al. 2010; Nagano et al. 2010; Sharma and Sharma 2017). To adapt with the environment conditions, several genera of yeasts (e.g., *Candida* spp., *Aureobasidium* spp., *Hortaea* spp., and *Exophiala* spp.) could convert their budding shape into filamentous-like shape called pseudohyphae (Branda et al. 2010; Novak Babič et al. 2016; Mukaremera et al. 2017). Therefore, those yeasts are commonly called yeast-like fungi due to their filamentous appearance (Kurtzman et al. 2011). This understanding can explain that although the isolates MTGK.12 and MTGK.31 were identified as *A. melanogenum*, they had different macroscopic characteristics.

The molecular study was conducted to gather more accurate identification through a DNA barcoding approach. ITS rDNA region was reported as a conservative and recommended region to identify the fungi (Schoch et al. 2012). In addition, several previous studies also conducted the molecular study using several regions and primers to get appropriate result, such as nuclear ribosomal DNA, the 18s rRNA, and the 28s rRNA (Francis et al. 2016; Sumerta and Kanti 2018; Kaewkrajay et al. 2020). This study has successfully found that phylum Ascomycota dominated the number of yeast strains, with the total of 20 ascomycetous and 1 basidiomycetous yeast. In Table 4, it showed that several isolates had similarity under 99%, which could be expected there was a present of novel species and required further analysis (Schlaberg et al. 2012; Raja et al. 2017).

Although the selection of isolates by morphology characterization has been performed, [*Candida*] zeylanoides was noted as the most dominant species with 12 number of isolates. According to Thompson et al. (2011), *Candida* genera is equipped with a special mechanism to modify their morphology. This mechanism allows them to construct a special type of buds called pseudohyphae. Besides functioning as a tool for taking nutrition, this organ could increase its pathogenicity since *Candida* genera are widely known as a pathogen. Based on homology sequences with NCBI, we found that there was a

bracket "[]" mark on [*Candida*] *zeylanoides*. This symbol means that the name is waiting for appropriate action by the taxonomist (Schoch et al. 2020). Hence, we reconstructed the phylogenetic tree to determine the genetic relationship among all isolates (Figure 5).

All yeast genera in this study have been previously reported to have been isolated from various hosts both in terrestrial and marine environment (Russo et al. 2008; Romo-Sánchez et al. 2010; Carrasco et al. 2012; Francis et al. 2016; Jamili et al. 2016; Yurkov et al. 2016; Kaewkrajay et al. 2020). Previous studies stated that there are a wide variety of well-known marine yeasts genera such as *Candida*, *Cryptococcus*, *Debaryomyces*, *Pichia*, *Hansenula*, *Rhodotorula*, *Saccharomyces*, *Trichosporon*, and *Torulopsis*. In addition, ascomycetous yeasts are dominantly found in shallow waters, whereas basidiomycetous yeasts in deep waters (Kutty and Philip, 2008). Further, Francis et al. (2016) who studied the seaweed-associated yeast reported total yeasts species in their study were dominated by phylum Ascomycota. The results of this study are clearly different from previous studies, especially the total yeast isolates. We noted that most of genera were previously reported as human pathogens such as *Aureobasidium*, *Candida* and *Hortea*. This result is presumably because the sampling locations are impacted by anthropogenic activity such as tourism and wastewater (Monapathi et al. 2020). It was noted that the environmental conditions affected the yeast communities **(**Jun et al. 2012; Hou et al. 2017; Stanaszek-Tomal 2020).

We also evaluated the biotechnological application of the isolates as carbohydrase producer. Among all isolates, only several yeasts showed their ability to secrete the extracellular carbohydrase. It was noted that [*C.*] *zeylanoides* MTGK.23, *A. melanogenum* MTGK.31, *H. werneckii* MTJ.11, and *H. werneckii* MTJ.13. It was highlighted that *H. werneckii* MTJ.13 showed the highest enzymatic index with a value of 2.55 on the screening of alginate-lyase, while MTJ.11 had the lowest enzymatic index with a value of 0.53 on the agarase screening plate. Moreover, the plate-based assay is a preliminary assay for extracellular enzyme screening from microorganisms. While for confirmation, other method using spectrophotometry is strongly suggested (Bhagobaty and Joshi 2012). The difference in the results was affected by various factors, such as pH, salinity, temperature, humidity, and incubation period (Shahat 2017; Gomez et al. 2020; Becker et al. 2021). Abdel-Raheem and Shearer (2002) also stated that there are several explanations of the absence of a positive result. It could mean that either the enzyme is not produced; or it was not secreted from the cells into the agar medium, or that it is produced and secreted, but the medium inhibits the activity. Therefore, previous studies frequently performed the production of extracellular enzymes with the liquid medium (Bhagobaty and Joshi 2012; Lee et al. 2019; Zilda et al. 2019). Besides confirmation with spectrophotometry, detection of the encoding genes is another strategy to validate the activity (Olempska-Beer et al. 2006; Harakava 2005; Kim et al. 2008; Sibero et al. 2019). However, after performing the detection, all isolates showed no positive activity. The lack of positive result in the detection of the encoding genes comes from the unknown mechanism replaces the encoding genes (Söderholm and Jaakkola 2013) or the absence of the encoding genes (Nakazawa and Honda 2015) or the unspecific attachment of the primers (Kumar et al. 2017). In this case, designing the specific primer is needed to obtain the appropriate result (Xu et al. 2011).

In conclusion, this study is the first report of seaweed-associated yeast from Indonesia's waters. We successfully isolated 21 seaweed-associated yeast and performed the diversity study with *Candida* genera dominated the result. Various ITS sequence similarity was observed during this investigation. It is recommended to use more than one locus to identify the yeasts. Although this study has successfully examined the potential of seaweed-associated yeast as carbohydrase producer, further studies are required to support this result and produce the enzymes. More comprehensive studies of marine yeasts from Indonesia may provide further information regarding their biodiversity, ecological role, and biotechnological applications.

ACKNOWLEDGEMENTS

The authors thank to Universits Diponegoro, Semarang, Indonesia for funding this research through *Riset Publikasi Internasional* (RPI) scheme with contract number 233-18/UN7.6.1/2020.

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