

Short Communication: *Candida* sp. isolated from mangrove soils for biological decolorization of textile reactive dyes

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Manuscript received: 1 May 2024. Revision accepted: 20 June 2024.

Abstract. Boontanom P, Chantarasiri A. 2024. Short communication: *Candida* sp. isolated from mangrove soils for biological decolorization of textile reactive dyes. *Biodiversitas* 25: 2595-2602. Effluent discharge from dye-related industries is a significant environmental pollution problem and a concern for human health. Dyes have harmful effects on aquatic organisms due to their toxic properties. One possible solution is using microorganisms for the biological decolorization of textile reactive dyes as an eco-friendly and potentially lucrative alternative to physicochemical processes. Therefore, this study isolated an effective dye-decolorizing yeast from mangrove soil samples in Thailand and investigated the decolorization efficiency of eight textile reactive dyes under experimental conditions. Yeasts have several advantages over other bacteria and fungi for the biological decolorization of textile reactive dyes, but the number of dye-decolorizing yeasts discovered in mangrove soils remains scarce. The most effective dye-decolorizing yeast, isolate RY21703, showed Remazol Brilliant Blue R (RBBR) decolorization of $37.50 \pm 0.07\%$ within 72 h of incubation. This yeast isolate was genetically and phylogenetically identified as *Candida* sp. strain RY21703 and produced 0.97 ± 0.08 U/mL of crude laccase activity, an essential enzyme in dye decolorization mechanisms. *Candida* sp. strain RY21703 showed strong decolorization efficiency after 72 h of incubation in all azo-reactive dyes, ranging from $27.32 \pm 3.34\%$ (CI Reactive Orange 122) to $61.45 \pm 1.43\%$ (CI Reactive Green 19). This strain also decolorized phthalocyanine reactive dye (CI Reactive Blue 21) by 60.71%. Findings suggested that this mangrove yeast can be used for the biological decolorization of dyeing wastewater.

Keywords: *Candida*, decolorization, dyeing wastewater treatment, mangrove soil, reactive dye

INTRODUCTION

Dyes have been used for thousands of years and are now widely utilized in several modern industries (Benkhaya et al. 2017), with applications in printing cellulosic fibers, cosmetics, food, leather, and pharmaceuticals. Dyes are categorized into different groups based on their chemical structure (anthraquinone, azo, phthalocyanine, sulfur, indigo, nitro, and nitroso dyes), application methods (dispersed, direct, acid, reactive, basic, and vat dyes), and color index (CI) number (Benkhaya et al. 2020). About 70 billion tons of synthetic dyes are produced annually for the global textile industry (Benkhaya et al. 2017; Benkhaya et al. 2020). The fixation efficiency of dyes on fibers typically ranges from 50 to 80% (Khatri et al. 2015), with 20 to 50% of the dye lost in the effluent water and discharged to aquatic and related environments (Khatri et al. 2015; Chantarasiri 2020). The effluent discharged from dye-related industries contains dyes, chemicals, metals, and pollutants (Kant 2012; Shindhal et al. 2021) characterized by high Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), pH, and color (Varadarajan and Venkatachalam 2016). Dyes negatively affect the environment and aquatic organisms due to their recalcitrant, bio-accumulative, toxic, mutagenic, and carcinogenic properties (Ardila-Leal et al. 2021). Colored wastewater also inhibits sunlight

penetration into water bodies and hampers the photosynthetic mechanisms of aquatic producers (Rahman and Bari 2011). Dye-laden wastewater is now a major environmental concern resulting from increased textile industry wastewater discharge in many parts of the world (Adane et al. 2021).

Decolorization presents a challenging issue in dyeing wastewater treatment processes (Chen et al. 2018). Many previous studies reported decolorization methods achieved through physical adsorption and electrochemical treatment (Chen et al. 2018). However, these methods are costly and not eco-friendly (Vantamuri and Kaliwal 2016; Bhamare et al. 2021). Biological methods for treating dye-containing wastewater are more promising than other techniques (Al-Tohamy et al. 2022) because they do not leave any harmful chemicals in the environment (Ravi et al. 2024). Biological decolorization has previously been achieved using various microbes such as algae, bacteria, fungi, and yeast (Chantarasiri 2020; Al-Tohamy et al. 2022; Ravi et al. 2024). Biological decolorization involves mechanisms of microbial enzyme-mediated degradation (Sarkar et al. 2017), such as azo-reductases, peroxidases, and laccases (Gupta et al. 2015; Imran et al. 2015; Chantarasiri 2020). In addition, microbial laccases degrade textile dyes in promising percentages (Sarkar et al. 2017). Laccase enzymes have been used in many biotechnological applications, such as the detoxification of industrial

wastewater and synthetic dye decolorization (Theerachai et al. 2017). Yeast is considered a viable biological candidate for dye wastewater treatment. Yeast species have several advantages over other microbes due to their active growth rates and ability to tolerate varied environmental conditions (Al-Tohamy et al. 2022). Several ascomycetous yeast species have been reported as dye-containing wastewater degraders, such as *Candida*, *Debaryomyces*, *Kluyveromyces*, and *Saccharomyces* (Al-Tohamy et al. 2022; Ravi et al. 2024), but the isolation of effective dye-decolorizing yeasts from various environments are still challenged.

Mangrove forests are coastal intertidal wetland ecosystems in tropical and sub-tropical regions (Friess 2016a; Thomas et al. 2017). They provide multiple services that increase local communities' economic or food security and many cultural services to coastal populations (Friess 2016b). Mangrove ecosystems are hotspots for microbial diversity, and they are extremely rich in yeast communities and interesting habitat sampling sites for novel yeast species (Kunthiphun et al. 2018; Hoondee et al. 2019); therefore, mangrove forests are also preferred for isolating and screening novel and effective yeast species that can decolorize textile reactive dyes.

Southeast Asia contains 33.8% of global mangrove forests (Thomas et al. 2017). Thailand is one of the 10 member countries of the Association of Southeast Asian Nations (ASEAN). The coastal zone of Thailand is covered by rich mangrove forests (Pumijumnong 2014). Many recent studies have investigated the diversity and applications of mangrove yeast in Thailand. However, only some studies have reported on the dye-decolorizing yeast species isolated from mangrove soils in Thailand and the Southeast Asia region.

Consequently, this study isolated and genetically identified a dye-decolorizing yeast from mangrove soil samples in Rayong Province, Thailand. Under experimental conditions, the active yeast was tested for biological decolorization of eight textile reactive dyes. This isolated

dye-decolorizing yeast showed promise as a candidate for future dyeing wastewater treatment and related applications.

MATERIALS AND METHODS

Study area

The sampling area in this study was the Phra Chedi Klang Nam Mangrove Forest (12°39'55.5" N, 101°14'50.1" E) in Rayong Province, Thailand. This riverine mangrove forest is part of the Rayong River, with its estuary connecting to the Gulf of Thailand, as Bamrunpanichtavorn et al. (2023) described its characteristics. The scenery in the study area is shown in Figure 1.

Procedures

Sampling mangrove soils and isolation of mangrove yeasts

Moreover, 30 soil samples were collected at a depth of 0 to 10 cm from the Phra Chedi Klang Nam Mangrove Forest. The temperatures of the mangrove soil samples were measured by a needle probe thermometer (Extech Instruments, USA) at the sampling sites. All mangrove soil samples were kept at 4°C in sterilized plastic bags and processed for yeast isolation within 24 h of collection.

The isolation of mangrove yeasts followed the method of Wongchamrearn et al. (2023). Briefly, the mangrove soil samples were diluted with sterilized deionized water to obtain 1:1,000,000 dilutions. Then, 100 µL of each diluted sample was spread-plated on Dichloran Rose Bengal Chloramphenicol (DRBC) agar (HiMedia, India). All cultures were incubated at 27.8°C (average soil temperature of the sampling sites) in an incubator cabinet for 72 h. The yeast isolates were selected based on the distinct colony morphology and then streak-plated on Yeast Malt (YM) agar (HiMedia, India) for colony purification.

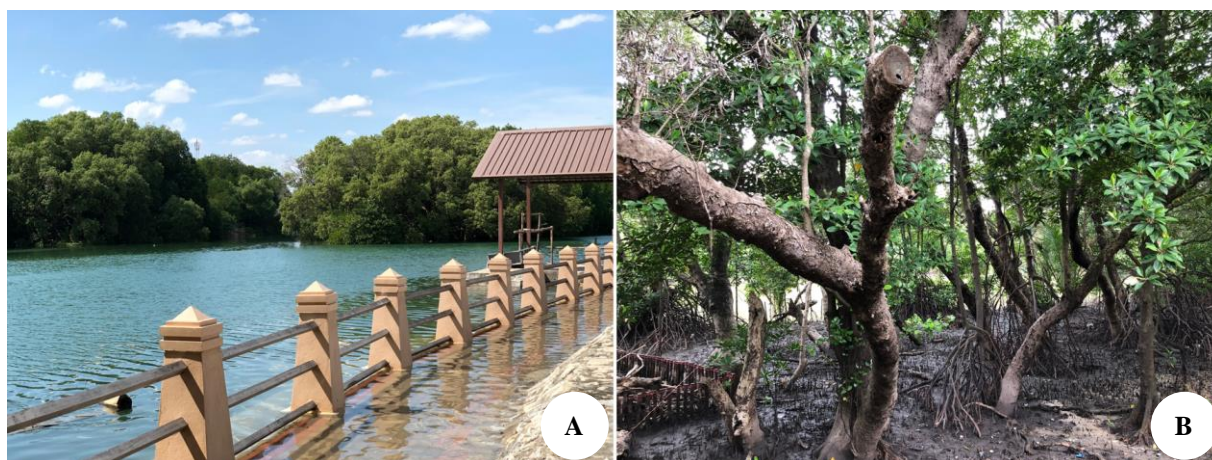


Figure 1. Scenery in the study area: A. Rayong River and B. The Phra Chedi Klang Nam Mangrove Forest in Rayong Province, Thailand

Screening of dye-decolorizing yeasts by the rapid RBBR method

The rapid Remazol Brilliant Blue R (RBBR) method for the determination of laccase-producing bacteria, previously described by Chang et al. (2014), was modified for the screening of dye-decolorizing yeasts in this study following Chantarasiri (2020). Each isolated mangrove yeast was cultured in 5 mL of BSGYP medium supplemented with 0.01% (w/v) RBBR reactive dye or CI Reactive Blue 19 dye (Sigma-Aldrich, Germany) at 27.8°C for 72 h in a shaking incubator at 120 rpm (LSI-3016R incubator, Daihan Labtech, Korea). The formula of the BSGYP culture medium was described by Chantarasiri (2020). All cultures were incubated in the dark to avoid unexpected visible light decolorization of the RBBR reactive dye.

Yeast cells were centrifuged at 10,400 rpm for 15 min (Digicen 21 R centrifugation, Ortoalresa, Spain) and discarded from the culture medium. The leftover RBBR in the culture medium was determined in the cell-free supernatant by spectrometric analysis at 592 nm, the λ_{\max} of the RBBR reactive dye. The uninoculated BSGYP medium supplemented with 0.01% (w/v) RBBR reactive dye was considered the reference. The decolorization percentage of the reactive dye was calculated using the formula presented by Chantarasiri and Boontanom (2017) as decolorization percentage (%) = $((\text{Abs}_i - \text{Abs}_f) / \text{Abs}_i) \times 100$, where Abs_i is the absorbance of cell-free supernatant at the initial incubation, and Abs_f is the final absorbance of cell-free supernatant after 72 h of incubation. All experiments were assayed in triplicate.

Genetic and phylogenetic identification of the dye-decolorizing yeast

A genomic DNA isolation kit extracted the genomic DNA of the dye-decolorizing yeast according to the protocol described by Bio-Helix (Taiwan). The extracted genomic DNA was used as the polymerase chain reaction (PCR) template. The amplification process was performed by a OnePCR reaction mixture (Bio-Helix, Taiwan) using the universal ITS1 and ITS4 primers to amplify the Internal Transcribed Spacer (ITS) regions. PCR cycling was conducted in an Eppendorf Mastercycler nexus gradient (Eppendorf, Germany) for 35 amplification cycles, with PCR cycling conditions following Wongchamrearn et al. (2023) as an initialization step at 95°C for 5 min, a denaturation step at 95°C for 1 min 30 sec, an annealing step at 55°C for 2 min, an extension step at 72°C for 3 min, and a final extension step at 72°C for 5 min. The resulting PCR products were electrophoresed on 1.5 (%w/v) GenedireX agarose gel (Bio-Helix, Taiwan) and then visualized by Novel Juice DNA staining reagent (Bio-Helix, Taiwan).

The resulting PCR products were nucleotide sequenced using the Sanger sequencing services of Macrogen Inc. (South Korea), and the nucleotide sequences were aligned for genetic identification by the BlastN program based on the Eukaryota nt (nt_euk) databases of the National Center for Biotechnology Information (NCBI). A phylogenetic tree was generated for species identification of the dye-decolorizing yeast using Seaview software version

5.0.1 (Gouy et al. 2010) and FigTree software version 1.4.4 (<http://tree.bio.ed.ac.uk>), while the BIONJ algorithm generated 1,000 bootstraps. The resulting ITS region of the dye-decolorizing yeast was deposited in the GenBank database of the NCBI under accession number KY582845.

Determination of laccase activity

Crude laccase was prepared following the method of Chantarasiri (2020) with some modifications. The isolated dye-decolorizing yeast was cultured in BSGYP medium supplemented with 0.01% (w/v) Kraft lignin (Sigma-Aldrich, USA) at 27.8°C for 72 h in a shaking incubator at 120 rpm. The culture was centrifuged at 10,400 rpm for 15 min to obtain cell-free supernatant as the crude laccase solution. The crude laccase was then partially purified and concentrated by 10 kDa Amicon ultra centrifugal filter units (Millipore, Ireland), with all crude laccase solutions carefully stored at 4°C.

The activity of crude laccase was determined following the method of Chang et al. (2014) and measured by incubating 0.2 mL of crude laccase with 0.8 mL of reaction mixture containing 75 mM catechol (Sigma-Aldrich, USA) in 50 mM sodium phosphate buffer at pH 7.0. The laccase reaction was spectrophotometrically monitored at 440 nm for 10 min. Therefore, one unit (U) of laccase activity was defined as the amount of laccase catalyzing 1 μmol of catechol oxidized per min of reaction. All experiments were conducted in triplicate.

Dye decolorization efficiency

The determination of dye decolorization efficiency was modified from Chantarasiri (2020). The isolated dye-decolorizing yeast with 1% (v/v) inoculum was inoculated in BSGYP medium supplemented with 0.01% (w/v) reactive dye and cultured at 27.8°C for 72 h in a shaking incubator at 120 rpm. The textile reactive dyes used in this experiment were CI Reactive Blue 19 or RBBR (λ_{\max} = 592 nm) (Sigma-Aldrich, Germany), CI Reactive Black 1 (λ_{\max} = 606 nm), CI Reactive Brown 1 (λ_{\max} = 489 nm), CI Reactive Blue 194 (λ_{\max} = 594 nm), CI Reactive Blue 21 (λ_{\max} = 670 nm), CI Reactive Green 19 (λ_{\max} = 636 nm), CI Reactive Orange 122 (λ_{\max} = 489 nm) and CI Reactive Yellow 167 (λ_{\max} = 408 nm) (INDAFIX, Thailand). All λ_{\max} values of the dyes were obtained from Chantarasiri (2020).

Furthermore, 500 μL of medium culture was collected aseptically at regular 12 h intervals to evaluate dye decolorization efficiency. The cell-free supernatant was used to observe the decolorization of textile reactive dyes using a spectrophotometer at the λ_{\max} of each dye. The decolorization percentage was calculated and conducted according to the previously mentioned formula. Therefore, to ensure the reliability of the results, all experiments were conducted in triplicate. The control consisted of BSGYP medium supplemented with 0.01% (w/v) textile reactive dye inoculated with a non-dye decolorizing oleaginous yeast, *Kodamaea ohmeri* strain YB8, following Planonth and Chantarasiri (2022).

Data analysis

The statistical analyses in this study were performed using R software version 4.3.3 (R Foundation for Statistical Computing, Austria). Multiple comparisons were determined by one-way ANOVA and Tukey's test at a 95% confidence interval ($p < 0.05$).

RESULTS AND DISCUSSION

Characteristics of mangrove soil samples and isolation of mangrove yeasts

Moreover, 30 soil samples were collected as muddy black sediments from the Phra Chedi Klang Nam Mangrove Forest in Rayong Province, Thailand. The average temperature of the mangrove soils at the sampling sites was $27.85 \pm 0.15^\circ\text{C}$. Therefore, all microbial cultures analyzed in this study were incubated at 27.8°C .

The mangrove yeasts were isolated from the soil samples using DRBC agar and then purified using YM agar. The resulting 40 yeast isolates were grouped into five distinct colony morphologies and purified. Each purified isolate was labeled with a unique code ranging from RY21700 to RY21705. All the isolated yeasts had an opaque, circular shape with an entire margin and convex elevation, with their morphology differing in colony pigmentation and diameter.

Screening of dye-decolorizing yeasts by the rapid RBBR method

The yeast isolates were tested for their ability to decolorize the RBBR reactive dye (CI Reactive Blue 19) using the modified rapid RBBR method. The yeasts were grown in BSGYP medium supplemented with 0.01% (w/v) of the dye. All the isolated yeasts showed robust growth in this medium, but only one isolate, RY21703, could decolorize the dye at more than 5% decolorization percentage. This isolate exhibited the maximum RBBR decolorization of $37.50 \pm 0.07\%$ within 72 h of incubation. Interestingly, the colony color of isolate RY21703, originally opaque white, remained unchanged after incubation in the BSGYP medium supplemented with RBBR dye. This suggested that the decolorization was due to enzymatic activity rather than dye molecule cell adsorptions. Therefore, isolate RY21703 was selected for further experiments. The result of RBBR decolorization by isolate RY21703 is shown in Figure 2.

Genetic and phylogenetic identification of the isolated dye-decolorizing yeast

The genomic DNA of the yeast isolates RY21703 was extracted and amplified using PCR to target the ITS regions. The resulting PCR products were purified, nucleotide sequenced and aligned for genetic identification

using the BlastN program against the Eukaryota NT databases of the NCBI. Results showed that isolate RY21703 was similar to *Candida* sp. isolate CtBYITSBCKV12 (accession number MN327101.1) with 94.94% identity and zero E value. To confirm the identification of the yeast isolate, a circular phylogenetic tree was generated by Seaview software using the BIONJ algorithm with 1,000 bootstrap replications (Figure 3). The phylogenetic tree revealed that isolate RY21703 belonged to the clade of *C. tropicalis* with a bootstrap value of 70. Bootstrap values among representative *C. tropicalis* strains obtained from GenBank in the clade ranged from 40 to 100.

Based on the nucleotide alignment and phylogenetic tree results, the isolate RY21703 was identified as *Candida* sp. strain RY21703. The ITS region of *Candida* sp. strain RY21703 was deposited in the GenBank database of the NCBI under accession number KY582845.

Laccase activity of dye-decolorizing *Candida* sp. strain RY21703

Laccase-producing microbes have been utilized as a potentially cost-effective and alternative method for the bioremediation of various textile dyes. The isolated *Candida* sp. strain RY21703 was cultured and prepared for the crude laccase solution. The enzymatic activity results showed that *Candida* sp. strain RY21703 produced 0.97 ± 0.08 U/mL of laccase activity in 50 mM sodium phosphate buffer at pH 7.0. Therefore, it was designated as a laccase-producing microorganism.

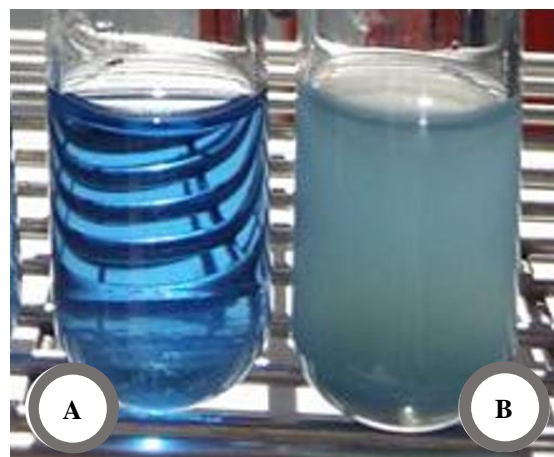


Figure 2. Decolorization of RBBR reactive dye in BSGYP medium: A. Uninoculated BSGYP medium (control) and B. Inoculated BSGYP medium with the dye-decolorizing yeast isolate RY21703 after 72 h of incubation

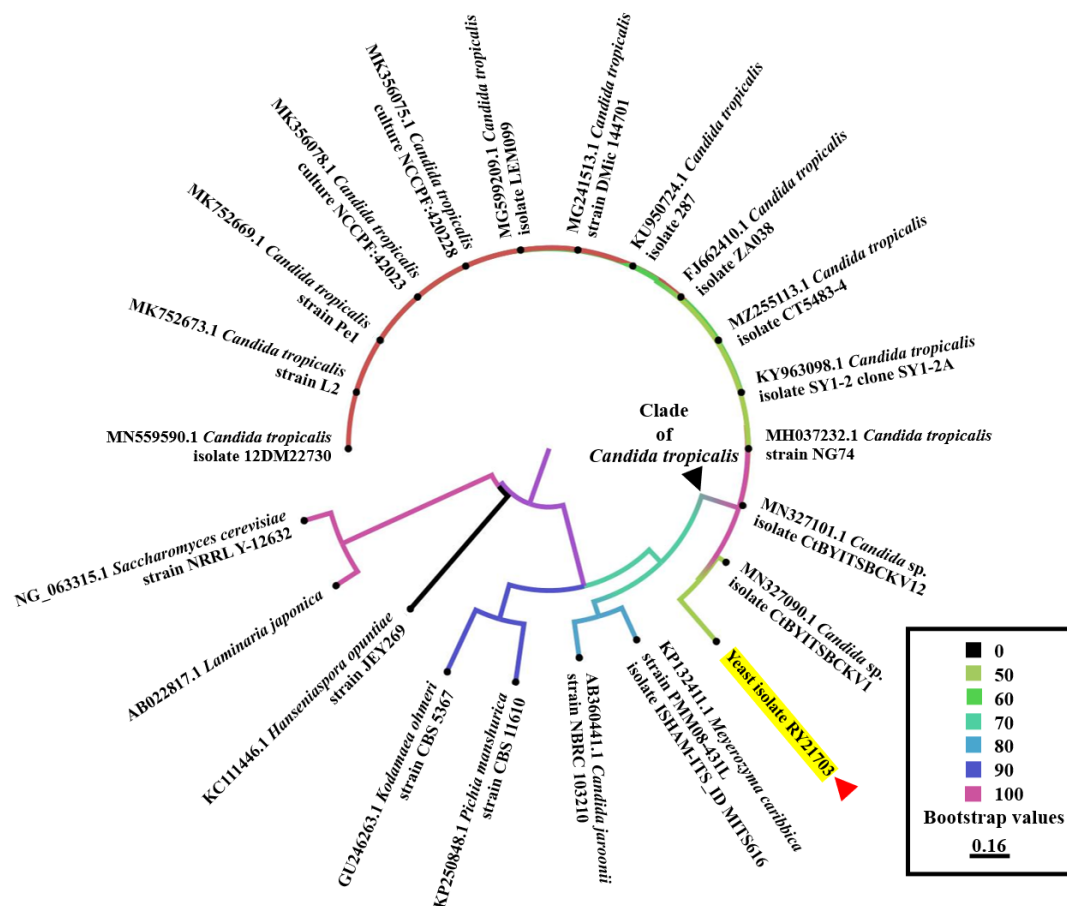


Figure 3. A circular phylogenetic tree of yeast isolates RY21703 was analyzed by the BIONJ algorithm with 1,000 bootstraps and generated using Seaview and FigTree software

The efficiency of dye-decolorizing *Candida* sp. strain RY21703

The dye decolorization efficiency of *Candida* sp. strain RY21703 was determined in BSGYP medium supplemented with textile reactive dye. The decolorization shows that this strain satisfactorily decolorized several textile reactive dyes within 12 h of incubation, including CI Reactive Blue 21 (phthalocyanine dye) by $48.67 \pm 1.49\%$ and CI Reactive Green 19 (azo dye) by $47.17 \pm 3.48\%$. In comparison, other textile-reactive dyes were decolorized within 24 to 60 hours of incubation. The highest decolorized dye in this study was CI Reactive Blue 194 (azo dye) at $60.92 \pm 2.25\%$ after 72 h of incubation. The second most decolorized dyes were CI Reactive Green 19 and CI Reactive Blue 21 by $61.45 \pm 1.43\%$ and $60.71 \pm 5.34\%$ after 72 h of incubation, respectively. However, results also showed that CI Reactive Orange 122 (azo dye) and CI Reactive Yellow 167 (azo dye) were persistent in decolorization by *Candida* sp. strain RY21703, being decolorized at only $27.32 \pm 3.34\%$ and $33.02 \pm 3.28\%$ after 72 h of incubation, respectively. CI Reactive Blue 19, the only anthraquinone dye in this study, was decolorized by $37.50 \pm 0.07\%$ at 72 h of incubation. The control yeast, *K. ohmeri* strain YB8, exhibited less than 3% decolorization for each reactive dye. Results in Figure 4 and Table 1 illustrate the decolorization efficiency of

various textile reactive dyes by *Candida* sp. strain RY21703 under experimental conditions.

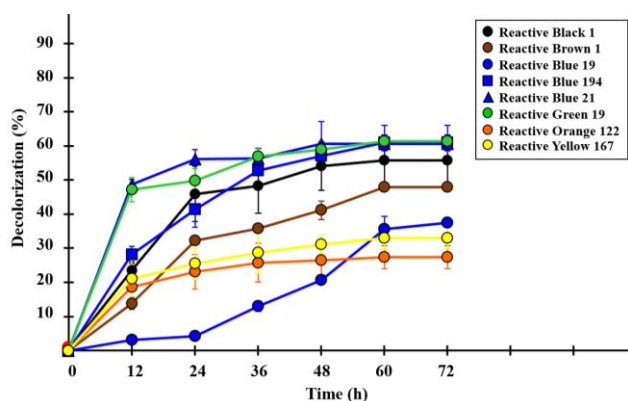
Discussion

Microbial decolorization and degradation is an environmentally friendly and cost-competitive alternative to chemical decomposition processes (Jafari et al. 2013). Various microbial strains are capable of decolorizing raw textile wastewater (Imran et al. 2015). Among these, yeast species are particularly attractive for biological decolorization of textile reactive dyes because they have advantages over bacteria and filamentous fungi (Imran et al. 2015). Mangrove swamps contain many yeasts crucial for the ecosystem's functioning (Nimsi et al. 2023). Yeasts in mangrove sediments have recently gained attention due to their potential characteristics (Vidya and Sebastian 2022). However, only a few mangrove yeasts have been isolated and studied for their efficiency in decolorizing textile reactive dyes. This study reports on the isolation, screening, and genetic identification of effective dye-decolorizing yeasts from mangrove soil in Eastern Thailand. The dye decolorization shows that *Candida* sp. strain RY21703 was the most effective, with satisfactory decolorization efficiency of various textile reactive dyes. This strain showed promise for further bioremediation applications.

Table 1. Decolorization efficiency of textile reactive dyes by *Candida* sp. strain RY21703 under experimental conditions after 72 h of incubation

Incubation time (h)	Decolorization (%)							
	CI Reactive Black 1 (azo dye)	CI Reactive Brown 1 (azo dye)	CI Reactive Blue 19 (RBBR) (anthraquinone dye)	CI Reactive Blue 194 (azo dye)	CI Reactive Blue 21 (phthalocyanine dye)	CI Reactive Green 19 (azo dye)	CI Reactive Orange 122 (azo dye)	CI Reactive Yellow 167 (azo dye)
12	23.38±2.61 ^a	13.73±1.58 ^a	3.18±1.15 ^a	28.07±2.51 ^a	48.67±1.49 ^a	47.17±3.48 ^a	18.59±3.02 ^a	20.97±5.54 ^a
24	45.95±8.07 ^b	32.19±1.32 ^b	4.17±0.72 ^a	41.30±5.15 ^b	56.07±2.81 ^a	49.79±3.53 ^a	23.03±5.02 ^a	25.46±4.56 ^a
36	48.25±7.92 ^b	35.76±0.61 ^b	13.07±1.48 ^b	52.77±1.27 ^c	56.38±2.90 ^a	56.98±2.22 ^b	25.78±5.70 ^a	28.78±6.04 ^a
48	54.06±7.06 ^b	41.14±2.68 ^c	20.75±0.30 ^c	56.99±3.59 ^c	60.60±6.60 ^a	58.91±1.44 ^b	26.54±5.05 ^a	31.14±2.57 ^a
60	55.82±6.52 ^b	47.89±1.05 ^d	35.66±3.78 ^d	60.90±2.13 ^c	60.69±5.30 ^a	61.42±1.42 ^b	27.30±3.15 ^a	32.98±3.31 ^a
72	55.84±6.51 ^b	47.90±1.06 ^d	37.50±0.07 ^d	60.92±2.25 ^c	60.71±5.34 ^a	61.45±1.43 ^b	27.32±3.34 ^a	33.02±3.28 ^a

Note: Mean values of decolorization efficiency in the same column followed by the same superscript were not significantly different according to Tukey's test ($p < 0.05$) during the incubation time. All experiments were performed in triplicate

**Figure 4.** Under experimental conditions, the decolorization of eight textile reactive dyes by *Candida* sp. strain RY21703. All experiments were performed in triplicate

Candida species are commonly found in mangrove ecosystems, with *C. tropicalis* being the most regularly isolated yeast species (Chi et al. 2012; Vidya and Sebastian 2022). Two previous studies identified *C. tropicalis* in mangrove soil samples obtained from several provinces in Thailand (Hoondie et al. 2019; Wongchamrearn et al. 2023). *Candida* sp. strain RY21703 was identified as a manglicolous yeast belonging to the clade of *C. tropicalis* based on both genetic and phylogenetic analyses. Advanced molecular genetic and bioinformatic analyses are required to identify this yeast species accurately. *Candida* sp. strain RY21703 was also found to produce laccases, as enzymes from the family of multicopper oxidases have been proven effective in breaking down synthetic dyes (De Paula et al. 2022). Laccases have been detected in yeast species such as *Candida* and *Saccharomyces* (Akinyemi et al. 2022), and the advantages of using laccases for dye decolorization are that they produce low amounts of sludge at low cost (Ardila-Leal et al. 2021). Therefore, *Candida* sp. strain RY21703 was believed to decolorize the textile reactive dyes through the catalytic mechanisms of laccases.

This study revealed that *Candida* sp. strain RY21703 effectively decolorized eight textile reactive dyes under experimental conditions, with decolorization percentages

ranging from 27.32% to 61.45% within 72 h. Azo dyes, commonly used in textile production, can bind to suspended organic matter and harm aquatic life. At the same time, the toxic compounds present in colored water can transfer through the food chain to humans, causing health disorders (Ardila-Leal et al. 2021). *Candida* sp. strain RY21703 showed effective decolorization on six different types of azo dyes, including CI Reactive Black 1, CI Reactive Brown 1, CI Reactive Blue 194, CI Reactive Green 19, CI Reactive Orange 122, and CI Reactive Yellow 167. Previous studies also reported the high ability of *Candida* species to decolorize different azo dyes. For example, *C. palmiophila* strain JKS4 exhibited 85.7% decolorization of Reactive Black 5 (azo) (Jafari et al. 2013), while *C. albicans* showed 73 to 87% decolorization of Direct Violet 51 (azo) (Das et al. 2023). Anthraquinone dyes are highly toxic despite their widespread use (Ardila-Leal et al. 2021) and also resistant to chemical oxidation due to their stability. In this study, *Candida* sp. strain RY21703 decolorized CI Reactive Blue 19 (RBBR) by 37.50%, an improvement compared to an earlier report that *Candida* sp. strain S1 decolorized RBBR by approximately 30% (Khudhair et al. 2015). Phthalocyanine dyes are metallic complexes that persist in biological degradation. *Candida* sp. strain RY21703 decolorized CI Reactive Blue 21 by 60.71%; however, knowledge about the decolorization of phthalocyanine dye is very limited. One previous study reported that *C. krusei* strain G-1 decolorized Reactive Turquoise Blue KN-G (phthalocyanine dye) by 62% (Yu and Wen 2005).

Mangrove forests in Eastern Thailand have the potential to provide effective dye-decolorizing yeasts. Furthermore, 5 mangrove yeast isolates were identified, each with a distinct colony morphology. *Candida* sp. strain RY21703 was determined as a dye-decolorizing yeast using the rapid RBBR method. This strain also produces laccases, essential enzymes in the dye decolorization process. *Candida* sp. strain RY21703 decolorized six azo dyes, one anthraquinone dye, and one phthalocyanine dye (Figure 4 and Table 1). Therefore, this yeast strain can be considered a promising candidate for biological decolorization of reactive dyes. Further studies are recommended to identify

the species more accurately, examine the metabolites produced after decolorization, and conduct decolorization experiments in a natural environment.

ACKNOWLEDGEMENTS

This research was funded by the Faculty of Science, Energy and Environment, King Mongkut's University of Technology North Bangkok, Thailand with contract no. SCIEE-KMUTNB-66-001.

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