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ORIGINAL PAPER

Occurrence and Diversity of Yeasts in the Mangrove Ecosystems in Fujian, Guangdong and Hainan Provinces of China

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Abstract Mangrove wetland is a unique ecosystem and has rich bioresources. In this article, the roots, stems, branches, leaves, barks, fruits, and flowers from 12 species of the mangrove plants and six species of the accompanying mangrove plants, seawater and sediments in mangrove ecosystems in China were used as sources for isolation of yeasts. A total of 269 yeasts strains were obtained from the samples. The results of routine identification and phylogenetic analysis showed that they belonged to 22 genera and 45 species. Of all the 269 strains, *Candida* spp. was predominant with the proportion of 44.61%, followed by *Kluyveromyces* spp. (8.55%), *Pichia* spp. (7.44%), *Kodamaea ohmeri* (5.58%), *Issatchenkia* spp. (4.83%) and *Debaryomyces hansenii* (4.46%). We also found that strains N02-2.3 and ST3-1Y3 belonged to the undescribed species of *Pichia* sp. and *Trichosporon* sp. respectively while strain HN-12 was not related to any known yeast strains. This means that different yeast strains of *Candida* spp. especially *C. tropicalis* were widely distributed in the mangrove ecosystems and may have an important role in the mangrove ecosystems. The results also showed that some of them may have potential applications.

Keywords Yeast diversity · Mangrove ecosystems · Diversity · *Candida* spp. · Undescribed yeasts

Introduction

Mangrove ecosystems are typical intertidal wetland systems of the tropics and subtropics [1]. In recent years, microbial community in mangrove ecosystem has been intensively exploited in China and it has been found that it is mainly composed of bacteria, fungi and actinomyces [2]. At the same time, it has been found that yeasts are prevalent in salt marshes or mangrove ecosystems where the yeasts play an important role in the detrital food web [3], and they might be a food source for some marine invertebrates and zooplanktons. Yeasts also can be involved in marine habitats in decomposition and nutrient cycling, biodegradation of xenobiotics such as petroleum and its derivatives, and as parasites. Mangroves offer numerous microhabitats with the potential of harboring yeast communities [4, 5]. Some human-associated species and the prevalent species in polluted water were common in the mangrove ecosystem. Further extensive research could reveal the ecological roles of these yeasts and their interaction with the other organisms of the salt marsh. For example, the species *Kluyveromyces aestuarii* predominated the yeast communities of two detritus feeding crabs and *Candida* spp. were frequently isolated [6]. However, little has been known about yeast community in the mangrove ecosystems in China.

In this article, we dealt with all of the yeast strains isolated from the mangrove areas in South China, and tried to give a general understanding of the yeasts biodiversity of the Chinese mangrove.

Materials and Methods

Sampling

The samples (Table 1) which were collected from the mangrove ecosystem and used as the sources for yeast isolation in this study were described by Buzdar et al. [7].

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Table 1 The tested mangrove species, their typical accompanying plants and the sites where they lived

Tested mangrove species	DZG	DHI	NSI	HCD	LYB	SAB
Acanthus ilicifolius	+	+	+		+	+
Aegiceras corniculata	+		+	+	+	+
Avicennia mariana			+	+	+	
Bruguiera gymnorrhiza	+	+	+	+		
Bruguiera sexangula (Lour.) Poir.	+					
Clerodendrum inerme						+
Ceriops tagal	+		+			
Excoecaria agallocha	+			+	+	+
Heritiera littoralis						
Kadelia candel	+		+	+		
Rhizophora stylosa	+	+	+			+
Sonneratia apetala	+					
Acrostichum aureum[a]		+	+			
Thespesia populnea[a]	+	+				
Cassytha filiformis L.[a]			+			+
Phragmites communis Trin.[a]			+			
Pandanus tectorius[a]		+				+
Pluchea indica Less.[a]			+			+

"+" Means the corresponding mangrove species from where the samples were collected, *DZG* Dongzhai habour, Haikou, Hainan Province; *DHI* Donghai island, Zhanjiang, Guangdong Province; *NSI* Nansan island, Zhanjiang, Guangdong Province; *HCD* Haicang dock, Xiamen, Fujian Province; *LYB* Luoyang bridge, Quanzhou, Fujian Province; *SAB* Su'ai bay, Shantou, Guangdong Province

[a] Typical accompanying plants

Table 2 The sampling sites and their Latitude and longitude

Sampling sites	Latitude and longitude
DZG, Haikou, Hainan Province	N19°53′ E110°19′
DHI, Zhanjiang, Guangdong Province	N21°00′ E110°23′
NSI, Zhanjiang, Guangdong Province	N21°08′ E110°30′
HCD, Xiamen, Fujian Province	N24°26′ E118°02′
LYB, Quanzhou, Fujian Province	N24°57′ E118°40′
SAB, Shantou, Guangdong Province	N23°19′ E116°43′

Latitude and longitude of the sampling sites are shown in Table 2.

Yeast Isolation

The yeast isolation from the samples was carried out by using the methods described by Buzdar et al. [7]. Colony and cell morphology observation and fermentation tests of the yeasts were performed using the methods described by Kurtzman and Robnett [8].

Metabolic Characterization of the Yeasts

The yeast strains were also identified by using BIOLOG system TM (Biolog MicroStation with Microlog System, Release 4.20, Biolog, Hayward, CA, USA), Biolog Universal Yeast Agar (Biolog) and Biolog Yeast microplate (Biolog) according to the procedures offered by the manufacturer [9].

DNA Extraction and PCR

The total genomic DNA of the yeast strains was isolated and purified by using the methods as described by Sambrook et al. [10]. Amplification and sequencing of D1/D2 26S rDNA sequences from the yeasts were performed according to the methods described by [11]. The common primers for amplification of D1/D2 26S rDNA in yeasts were used, the forward primer was NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and the reverse primer was NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′).

Phylogenetic Analysis and Identification of the Yeasts

The sequences obtained above were aligned by using BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST). The sequences which shared over 98% similarity with currently available sequences are considered to be the same species and multiple alignments was performed by using Clustal X 1.83 and phylogenetic trees were constructed by MEGA 4.0 [12].

Screening of Killer Toxin-Producing Yeasts

Screening of killer toxin-producing yeasts was performed according to the methods described by Wang et al. [14].

Screening of Single Cell Protein-Producing Yeasts

Screening of single cell protein-producing yeasts was conducted based on the methods described by Gao et al. [22].

Results and Discussion

Description of the Samples

The yeast strains were isolated from the roots, stems, branches, leaves, barks, fruits, and flowers from 12 species of the mangrove plants, six species of the typical accompanying plants, seawater and sediments in the mangrove ecosystems in Hainan Province, Guangdong Province and Fujian Province (Table 1). Therefore, the yeast strains isolated can represent the main yeast community in the mangrove ecosystems in China. From Table 2, it can be seen that all the trees involved in this study live in tropical and subtropical areas in China. Table 3 shows the chemical parameters at the sampling sites. The results in Table 3 indicated that the sampling sites had the characteristics of tropical and subtropical areas as well as marine environments, such as the high temperature, pH and salt.

Isolation and Routine Identification of the Yeast Strains

After isolation and purification, a total of 269 yeasts strains were obtained from the samples. According to the results of their colony and cell morphology, fermentation tests and metabolic characteristics, it was found that they belonged to 22 genera and 45 species. One hundreds and twenty isolates (44.61%) were identified as 14 species in the genera *Candida* spp. (Table 4). It was worthy to notice that 74 isolates (27.51%) were identified as *Candida tropicalis* and it could be found in seawater and sediments in all the mangrove ecosystems, and on fruits, root, barks, leaves and branches of most of the mangrove trees. This meant that *Candida* spp. especially *C. tropicalis* were widely distributed in the mangrove ecosystems in China. This may be related to high temperature and salts in the sampling sites (Table 3). However, *C. phangngensis* (0.37%), *C. aaseri* (0.74%), *C. boidinii* (0.38%), *C. Hollandica* (0.37%) and *C. maltosa* (0.37%) could not be isolated from any samples from the mangrove trees. It also has been reported that *Candida* spp. are the most frequently isolated genus in Brazilian mangrove ecosystem [6] and at Sepetiba Bay [13]. Among the *Candida* spp., *C. parapsilosis*, *C. tropicalis*, *C. valida-like*, *C. krusei*, *C. sorbosa*, *C. colliculosa-like*, *C. famata-like*, *C. guilliermondii*, *C. albicans*, *C. silvae* and *C. boidinii* have been found to occur in other mangrove ecosystems [1, 3, 4, 6]. However, *C. valida*-like, *C. krusei*, *C. sorbosa*, *C. colliculosa*-like, *C. famata*-like, *C. guilliermondii*, *C. albicans* were not found in this study (Table 4). In contrast, it can be seen from Table 4 that *C. maltosa*, *C. orthopsilosis*, *C. tenuis*, *C. asseri*, *C. butyric*, *C. thaimueangensis* were obtained in the mangrove ecosystems in China. The results in Table 4 showed that *C. tropicalis* outnumbered other *Candida* species greatly and was considered to be a highly dominant species. It has been well documented that *C. tropicalis* could degrade different kinds of pollutants [14, 16]. Other *Candida* spp. such as *C. parapsilosis* [15] and *C. intermedia* [16] also have ability to degrade oil pollutants.

Table 3 Chemical parameters of the sampling sites

Sampling sites	pH	Temperature (°C)	Salt (%)
DZG	8.1	35	2.02
DHD	8.0	35	1.70
NSD	7.9	35	1.71
HCD	8.1	35	2.30
LYB	7.8	34	1.69
SAB	7.9	36	1.57

Table 4 Yeasts of *Candida* spp. and the amount, proportion of every species and sources

Yeasts species	Amount of isolates	Proportion (%)	Sources
C. aaseri	2	0.74	Sediments
C. boidinii	1	0.37	Sediments
C. butyric	9	3.35	Branches, barks, flowers, fruits, roots
C. catenulata	6	2.24	Branches, flowers, fruits, sediments
C. hollandica	1	0.37	Seawater
C. intermedia	11	4.09	Leaves, waters, barks, fruits, flowers, branches, roots
C. maltosa	1	0.37	Sediments
C. parapsilosis	6	2.23	Leaves, fruits, barks, branches
C. orthopsilosis	1	0.37	Fruits
C. phangngensis	1	0.37	Sediments
C. silvae	3	1.12	Branches
C. tenuis	1	0.37	Branches
C. thaimueangensis	3	1.12	Roots, branches, leaves
C. tropicalis	74	27.51	Sediments, fruits, root, barks, leaves, branches, water
Total	120	44.61	

Other yeasts (146 strains) obtained from the mangrove ecosystems included *Aureobasidium pullulans* (1.12%), *Clavispora lusitaniae* (2.6%), *Debaryomyces hansenii* (4.46%), *Galactomyces geotrichum* (1.49%), *Geotrichum* sp. (3.35%), *Issatchenkia occidentalis* (0.37%), *I. orientalis* (2.97%), *I. siamensis* (1.49%), *Kazachstania exigua* (2.6%), *K. aestuarii* (4.46%), *K. nonfermentans* (0.37%), *K. siamensis* (3.72%), *Metschnikowia koreensis* (0.74%), *Kodamaea ohmeri* (5.58%), *Pichia anomala* (3.35%), *P. guilliermondii* (1.12%), *P. mexicana* (2.23%), *P. spartinae* (0.74%), *Rhodotorula mucilaginosa* (0.74%), *Rhodosporidium paludigenum* (0.37%), *Saccharomyces exiguous* (0.37%), *Saccharomycete* sp. (1.47%), *Saturnispora mendoncae* (1.47%), *Trichosporon asahii* (2.6%), *Williopsis saturnus* (0.74%), *Yarrowi lipolytica* (2.24%) and *Zygoascus steatolyticus* (0.74%) (Table 5). Among *Kluyveromyces* spp., *K. nonfermentans* have been obtained from deep-sea samples [1]. However, we found that it also appeared in the mangrove ecosystems. It has been reported that *K. aestuarii* was frequently isolated from marine environments and could become the feed for invertebrates in mangrove ecosystem [6]. Consistent with the finding, 4.46% of the yeast strains isolated from the mangrove ecosystems in this study were *K. aestuarii.* This meant that the mangrove ecosystems indeed had some characteristics of marine environments (Table 3).

Pichia spp. is also frequently obtained from marine environments. For example, *P. guilliermondii*, *P. ohmeri*, *P. fermentans*, *P. burtonii* and *P.chia anomala* were obtained from surface of *Sargassum pallidum*, gut of *Hexagrammos otakii*, gut of *yellow-fin tuna*, gut of

Table 5 Other yeasts, the numbers of the strains, proportion of every species and sources

Yeasts species	Numbers of strains	Proportion (%)	Sources
Aureobasidium pullulans	3	1.12	Roots, fruits, leaves
Clavispora lusitaniae	7	2.60	Barks, leaves, branches, sediments
Debaryomyces hansenii	12	4.46	Leaves, branches, roots, barks, sediments
Galactomyces geotrichum	4	1.49	Branches, leaves
Geotrichum sp.	9	3.35	Waters, roots, fruits, sediments
Issatchenkia occidentalis	1	0.37	Branches of *Amcerana marma*
Issatchenkia orientalis	8	2.97	Sediments, branches, fruits,
Issatchenkia siamensis	4	1.49	Sediments, leaves, fruits
Kazachstania exigua	7	2.60	Sediments, branches, fruits, leaves
Kluyveromyces aestuarii	12	4.46	Branches, sediments, leaves, fruits
Kluyveromyces nonfermentans	1	0.37	Leaves
Kluyveromyces siamensis	10	3.72	Branches, flowers, fruits, barks, roots
Metschnikowia koreensis	2	0.74	Leaves
Kodamaea ohmeri	15	5.58	Roots, leaves, fruits, flowers, barks
Pichia anomala	9	3.35	Flowers, sediments, leaves, roots
Pichia guilliermondii	3	1.12	Roots, branches, fruits
Pichia mexicana	6	2.23	Leaves, fruits, roots, sediments
Pichia spartinae	2	0.74	Flowers, sediments
Rhodotorula mucilaginosa	2	0.74	Leaves
Rhodosporidium paludigenum	2	0.74	Fruits and leaves of *Aegiceras corniculatum*
Rhodosporidium sphaerocarpum	1	0.37	Fruits of *Ceriops tagal*
Saccharomyces exiguous	1	0.37	Leaves of *Aegiceras corniculatum*
Saccharomycete sp.	4	1.47	Sediments
Saturnispora mendoncae	4	1.47	Leaves and roots
Trichosporon asahii	7	2.60	Barks, fruits, branches, sediments
Williopsis saturnus	2	0.74	Sediments
Yarrowi lipolytica	6	2.24	Roots, fruits, leaves, branches
Zygoascus steatolyticus	2	0.74	Leaves, fruits
Pichia sp. N02-2.3 (undescribed species)	1	0.37	Barks of *Bruguiera gymnorrhiza*
Trichosporon sp. ST3-1Y3 (undescribed species)	1	0.37	Leaves of *Pluchea indica*
Strain HN1-2 (undescribed genus)	1	0.37	Leaves of *Kadelia candel*
Total	149	55.3	

Lepidorigla microptera and gut of *sea squirt* [17]. In this study, 7.44% of the yeast strains obtained from the mangrove ecosystems were *Pichia* spp. (Table 4). *P. anomala* and *P. membranaefaciens*, also have been reported to occur in mangrove ecosystems [1, 4–6].

In our previous study, we found that *K. ohmerii* is widely distributed in different marine environments and the marine-derived yeast *K. ohmerii* BG3 can produce a large amount extracellular phytase [18]. In this study, 5.58% of the yeast strains isolated from the mangrove ecosystems were found to be *K. ohmerii* (Table 4). This revealed that the mangrove ecosystems are also the natural habitat for growth of *K. ohmerii.*

Debaryomyces hansenii has been found to be the common member in marine environment as it can tolerate high concentration of NaCl [19]. In this study, 4.46% of the yeast strains obtained from the mangrove ecosystems were *D. hansenii.* This again demonstrated that the mangrove ecosystems had some characteristics of marine environments (Table 3).

Aureobasidium pullulans is popularly known as black yeast and widely distributed in the phyllosphere of many crop plants, on various tropical fruits, in fresh water, estuarine, marine sediments, hypersaline habitats, seawater and deep sea [20]. In this study, the mangrove swamp was proven to be another natural habitat that was suitable for *A. pullulans* though it was not the major species.

The results in Table 4 indicated that *Saccharomycete* sp. could not be isolated from any samples of the mangrove trees, either. We found that strains N02-2.3 and ST3-1Y3 were identified as the undescribed species of *Pichia* sp. and *Trichosporon* sp. respectively while strain HN-12 was assigned to the undescribed genus (Table 4). This suggested that there were still unknown yeast species or genera in the mangrove ecosystems in China and more new genera or species can be found if further studies are carried on.

The results in Tables 4 and 5 also showed that the diversity of the yeast species was very rich and they belonged to 22 genera and 45 species.

Phylogenetic Analysis of Partial Sequences of the D1/D2 26S rDNA

It has been confirmed that most yeast species can be easily identified at species level from sequence

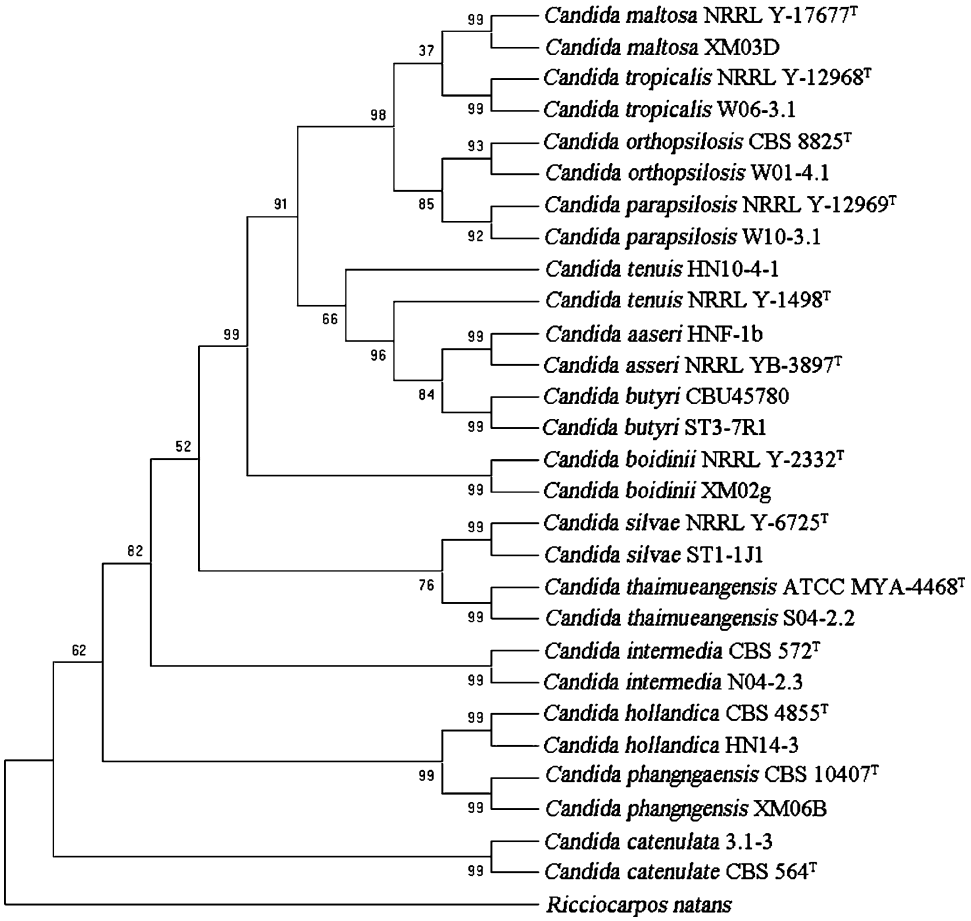

Fig. 1 Consensus tree of 120 isolates based on 26S rRNA gene sequences obtained in this study. The type strains were selected from CBS database, and the previously published sequences of the type strains were obtained from GenBank. The outgroup we used was *R. natans*. The *numbers* above the branches are bootstrap. *T* stands for type strains

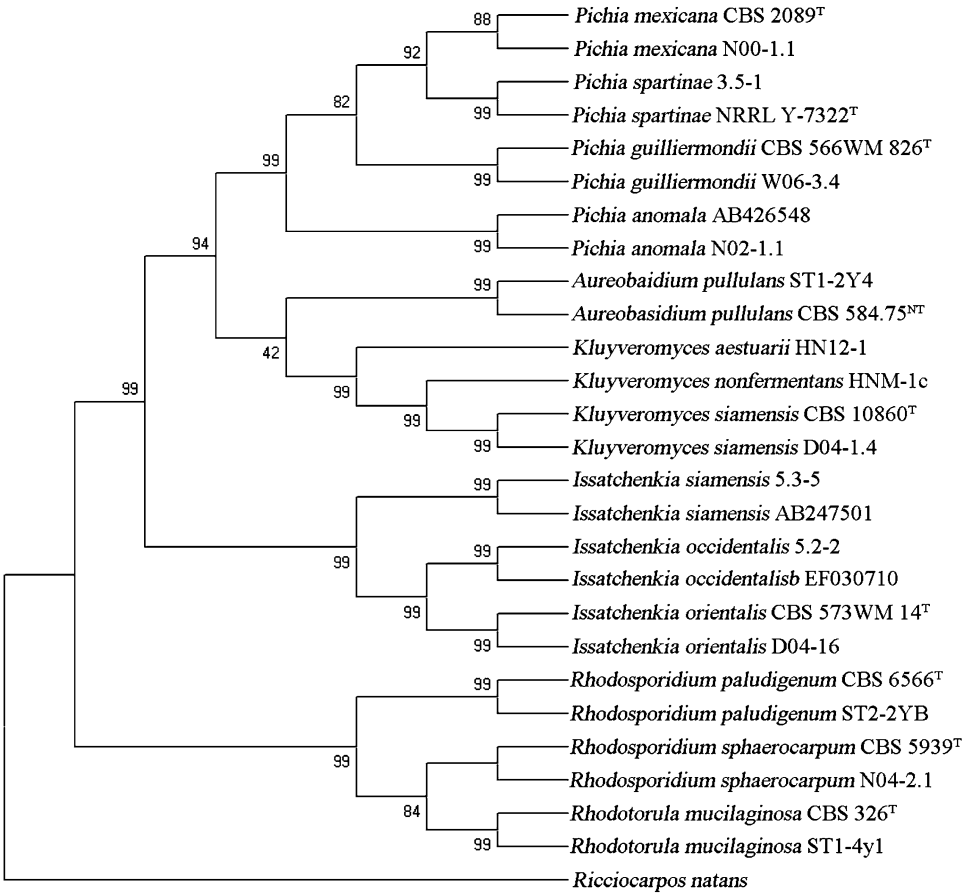

Fig. 2 Consensus tree of 64 isolates based on 26S rRNA gene sequences obtained in this study. The type strains were selected from CBS database and the previously published 26S rRNA gene sequences of the type strains were obtained from GenBank. The outgroup we used was *R. natan*. The *numbers* above the branches are bootstrap. *NT* means neotype and *T* stands for type strains

divergence in the D1/D2 domain [21]. In this study, in order to certify the taxonomic status of the isolates and the inter- and intra-specific relationships, 26S domain D1/D2 of each isolate obtained was sequenced, the sequences obtained were aligned. The phylogenetic trees were constructed when *Cafeteria roenbergensis* and *Ricciocarpos natans* were used as out-groups, respectively. The topology of the phylogram in Figs. 1, 2 and 3 demonstrated that the 266 isolates obtained in this study were closely related to the known different type strains, respectively. However, the results in Fig. 4 indicated that strains N02-2.3 and ST3-1Y3 belonged to the undescribed species of *Pichia* sp. and *Trichosporon* sp. respectively while strain HN-12 was not related to any known yeast strains. This meant that bioresource and diversity of yeasts in the mangrove ecosystems needed to be further exploited.

We also found that the 26S rRNA gene sequence of each species isolated from different niches in the mangrove ecosystems was the same (Figs. 1, 2, 3, 4).

All the results in Figs. 1, 2, 3 and 4 were in agreement with those of fermentation texts and metabolic identification by Biolog (Tables 4, 5).

Potential Applications of the Yeasts Isolated from the Mangrove Systems

The dried cells of some of the 269 yeasts strains obtained above were found to contain more than 30% (w/w) of crude proteins, especially the dried cells of *P. anomala* No2-1.1 had more than 44.8% (w/w) protein (Table 6). It have been confirmed that single-cell proteins have many uses in food and feed industries as they have high content of protein, high percentage of essential amino acids and other nutrients [22]. In addition, after the single cell proteins were hydrolyzed by alkaline protease, the hydrolysates obtained have angiotensin converting enzyme inhibitory activity and antioxidant activity [23]. In general, the protein content in the single cells for protein production should be between 29 and 73 g per 100 g of cell dry weight [24]. This meant that many yeast strains obtained from the mangrove systems have many potential uses.

It was also found that *K. siamensis* D04-1.4 among them could actively produce killer toxin against *M. bicuspidate* WCY, a pathogenic yeast in crab (Fig. 5). The killer toxins produced by marine yeasts can be applied to biocontrol, fermentation and pharmaceutical industries [25].

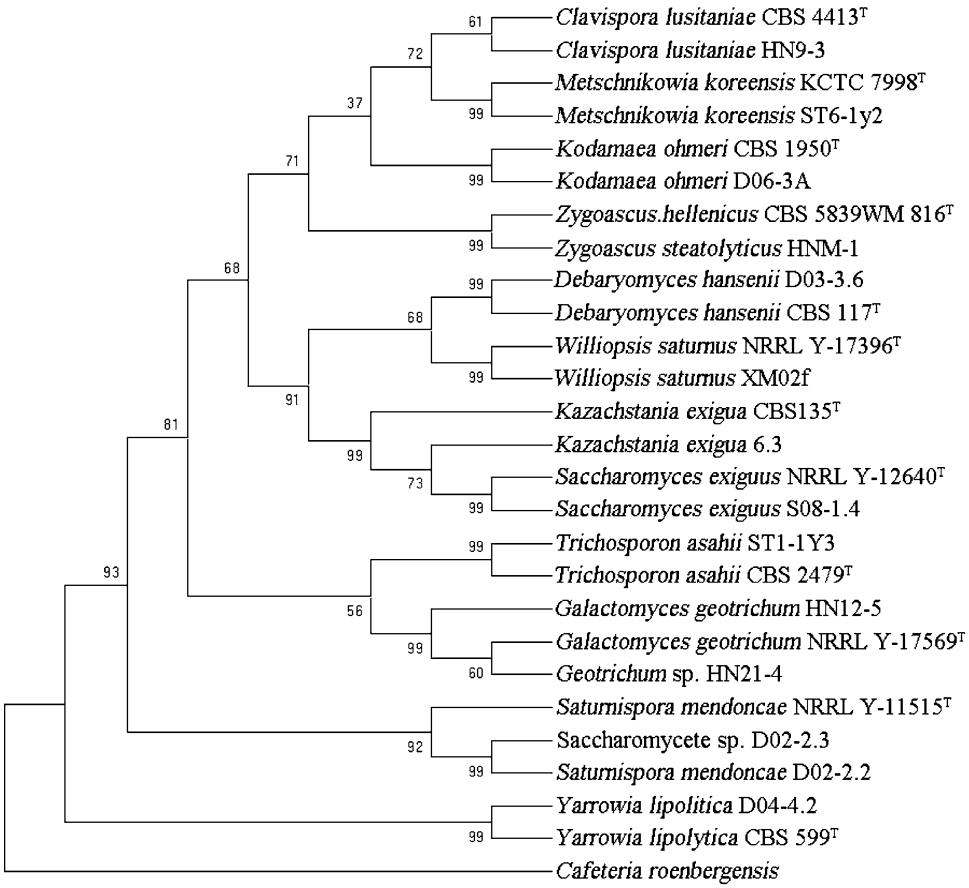

Fig. 3 Consensus tree of 82 isolates based on 26S rRNA gene sequences obtained in this study. The type strains were selected from CBS database while *M. koreensis* KCTC 7998T was from GenBank taxonomy database and the previously published 26S rRNA gene sequences of the type strains were obtained from GenBank. The outgroup we used was *C. roenbergensis*. The *numbers* above the branches are bootstrap. *T* stands for type strains

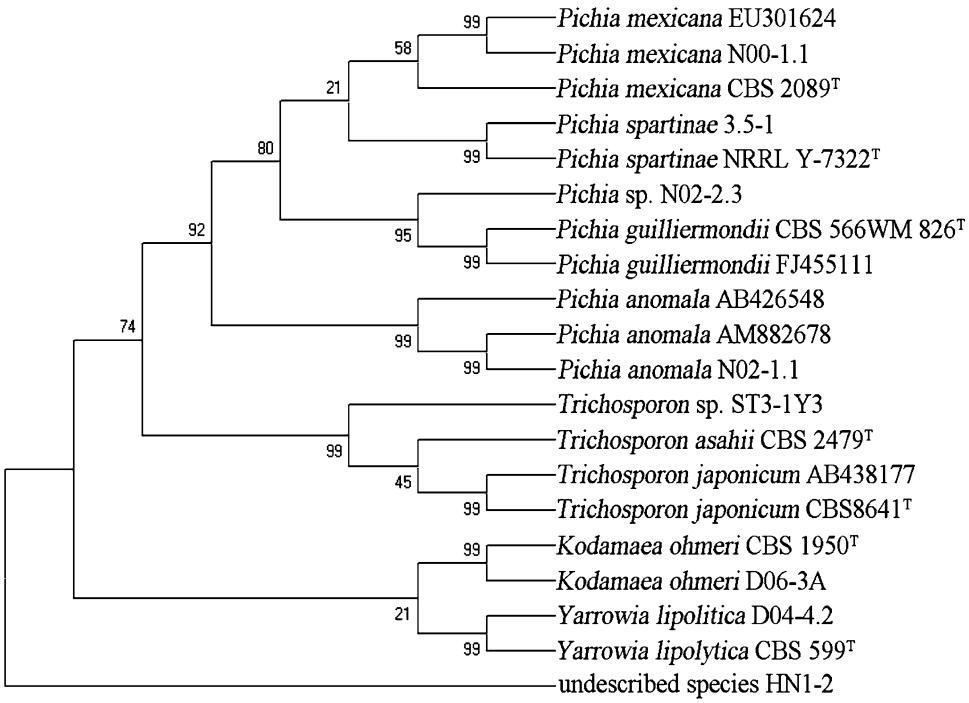

Fig. 4 Consensus tree of 3 isolates based on 26S rRNA gene sequences obtained in this study. The type strains were selected from CBS database and the previously published 26S rRNA gene sequences of the type strains were obtained from GenBank. The *numbers* above the branches are bootstrap. *T* stands for type strains

Table 6 Protein contents of some yeast strains isolated from the mangrove systems

Species	Protein content (%)
Pichia anomala No2-1.1	44.8 ± 1.4
Candida silvae ST3-1R1	35.9 ± 1.1
Candida butyri ST2-4R2	37.6 ± 0.4
Kluyveromyces siamensis HN12-1	30.0 ± 1.8
Trichosporon asahii ST3-1Y1	30.6 ± 0.9
Metschnikowia koreensis ST6-1y2	33.3 ± 1.3
Rhodosporidium paludigenum ST2-7G	35.0 ± 1.1
Rhodosporidium sphaerocarpum n04-2.1	34.1 ± 0.7

Data are given as means ± SD, $n = 3$

Fig. 5 The clear zone formed by the yeast *K. siamensis* D04-1.4 grown in the plate seeded with the pathogenic yeast *M. bicuspidata* WCY at 28°C for 4 days

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