



BIODIVERSITY OF YEASTS ISOLATED FROM CON DAO ISLAND – BA RIA VUNG TAU

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Received: 30 October 2016; Accepted for publication: 30 May 2017

ABSTRACT

During the study on yeast diversity from natural samples in Viet Nam, we have collected 38 samples of flowers, fruits, leaves, litter and soils in Con Dao Island and isolated 54 yeast strains. They were identified based on morphology observation and sequences of D1/D2 domain of 26S rDNA. The results showed that they belonged to 13 genera, 28 species, of which 15 strains were suspected as 13 new species. Thirty-nine strains were identified as Ascomycetes belonging 7 genera, 11 species, and the fifteen remaining strains belonged to Basidiomycetes, 6 genera and 13 species. The results also showed that four Ascomycetous and nine Basidiomycetous isolated were suspected to be new species. The results also showed that *Candida* was the genus with highest number isolates- 26 strains, and half of these were *Candida rugosa*. The most diverse species in this study was found in *Cryptococcus* genus with 7 species from 8 isolates only, and all of them were suspected to be new species. This study also proved that the samples had differences in yeast diversity and species composition among them. Therefore, Con Dao Island is multi potential to discover and publish new yeast species in Vietnam.

Keywords: Con Dao, yeasts, biodiversity.

1. INTRODUCTION

In Viet Nam, like other South-east Asian countries, yeast research has been started in early stage of microbial studies of traditional fermented products, food, beverages and starters of fermentation [1-3]. In the past, some studies have been conducted to investigate yeast biodiversity from natural environment of Viet Nam such as bio-diversity of yeasts isolated from leaves at Cuc Phuong National Park and dioxin polluted area in Da Nang military airport [4][5] and leaf, litter and soil samples collected in Ma Da, Phong Nha-Ke Bang National Park, Cat Tien and Langbiang [6-8]. Based on these studies, yeast biodiversity in the natural environment of Viet Nam was predicted to be abundant. They found that 10-30 % of yeast isolates were undescribed species. These reports indicated that more novel yeast species living in natural environment of Viet Nam are considerably discovery potential.

The Con Dao islands are an archipelago of Ba Ria - Vung Tau Province, in the South region of Viet Nam. Con Dao is not only a place for tourism with ecotourism program but also a nature reserve for science researches. The biodiversity of plants and animals at the island are discovered to be rich. However, there is no study on microbial diversity in this location; the report "Yeast diversity in Con Dao Island" contributes to understanding microbial diversity, particularly yeast diversity in Viet Nam.

2. MATERIALS AND METHODS

2.1. Soil sampling and treatment

Samples: 38 samples (29 flower, leaf, fruit, 4 soil and 5 litter samples) were collected from Con Dao Island on 17 to 19 November 2012.

2.2. Isolation of yeasts

Leaf samples were processed following the method of Landell et al. (2006) [9] and isolated using the dilution method and cultured on YM agar plates (g / l: glucose-10; malt extract-3; yeast extract-3; peptone-5; chloramphenicol-0.4; agar-16; pH 6.0) [10]. Litter and soil samples are diluted and cultured directly on YM agar. The fruit samples were enriched in YM broth with 20 % glucose. After 3 days, enriched samples were diluted and spreaded on YM agar with 20 % glucose. Colonies were picked up based on morphological characteristics after 3-7 days incubated at 28 °C.

2.3. Identification of yeasts

- Morphological characteristics were carried out according to the methods of Kurtzman et al. [11].

- Sequencing and phylogenetic analysis. Genomic DNA was extracted following the method of Manitis et al. [12]. The partial sequence of D1/D2 domain of 26S rDNA, was determined after PCR amplification of the DNA. Both strands of the PCR products were directly sequenced [13]. Generated sequences were aligned with related species by using the CLUSTAL X computer program [14]. The phylogenetic tree was constructed from the evolutionary distance data according to Kimura [15] using the neighbor-joining method [16]. Sites where gaps existed in any sequences were excluded. Bootstrap analyses were performed from 1000 random repetitions only over 50 % of the value shown. Reference sequences used for the phylogenetic study were obtained from the database of GenBank/DDBJ, the access stands after the name of species.

3. RESULTS AND DISCUSSION

3.1. Sample collection

During the study on yeasts at Con Dao Islands, we have collected 38 samples (flower, fruit, leaf, litter and soil) from different sites (Con Dao market, So Ray, Ong Dung, Saigon-Con Dao hotel); 8°41'2 " – 8°43'55" North latitude and 106°35'20" – 106°37'18" East longitude, 1 to 151m altitude, temperature 27.3 – 30.2 °C, moisture from 68.2 to 92.4 %.

3.2. Isolation of yeasts

From 38 samples collected, 54 yeast strains were isolated (Table 1). Using enrichment method in YM broth with 20 % glucose, there were 12 isolates from 9 of 12 fruit samples. Using the dilution method, 31 yeast strains were isolated from 14 of 17 leaf samples; 7 strains from 5 of 5 litter samples and 4 strains from 4 of 4 soil samples. The results in table 1 shows that the number of yeast strains isolated in the leaf samples is maximum (2.3 isolates/samples), followed by litter samples with average of 1.4 isolates/sample, fruit samples with average of 1.2 isolates/sample and soil samples had the lowest (1.0 isolates/sample). Previous study on leaf samples collected in dioxin polluted area in Da Nang military airport had higher number of strains per sample than this study, with average from 3.4 to 4.4 strains/sample. In the other studies, yeasts isolated from leaf samples at Cuc Phuong National Park; Phong Nha-Ke Bang and Cat Tien were rate of 7; 7.5 and 6.9 strains/sample, respectively [8]. In the study on yeast in Phu Quoc Island, 171 yeast strains were isolated from 110 collected samples. The number of yeasts was on average 1.0 isolates/soil sample; 3.4 isolates/litter and leaf sample; 1.5 isolates/fruit sample and 1.7 isolates/fermented food sample [17].

Table 1. Number of yeast isolated at Con Dao Island.

Sources	No. of samples	Isolated method	No. of samples found yeasts	No. of isolates
Flowers, fruits and leaves	29	Enrichment	9/12	12
		Dilution plate	14/17	31
Litter	5	Dilution plate	5/5	7
Soil	4	Dilution plate	4/4	4
Total	38		32/38	54

3.3. Identification of yeast strains isolated from Con Dao Island

The morphology and sequences of D1/D2 domain of 26 rDNA of 54 yeast strains were determined. These yeasts were tentatively identified on the basis of the D1/D2 sequences by consulting a guideline of Kurzman and Robnett [13] and were revealed that they belong to 13 genera, 28 species, in particular, 39 strains of 15 described species (10 genera) and 15 strains of 13 undescribed species (6 genera).

The results presented in Table 2 indicated that Ascomycetous yeasts have 39 strains of 7 genera with 11 described species and four undescribed species of 2 genera. Among these genera, *Candida* accounted for the highest number of isolates, species and undescribed species (23 strains, 5 described species and 3 undescribed species); *Candida rugosa* has the most number of isolates (13 strains). Next was *Meyerozyma* (6 strains of *Meyerozyma guilliermondii*), *Pichia* genus (4 strains of *Pichia kudriavzevii*), *Aureobasidium* (3 strains, 1 described species and 1 undescribed species). *Kazachstania*, *Kodamaea* and *Saccharomyces* genera has only 1 strain of described species. In the other studies, *Candida* genus was dominant isolated from samples in Ma Da Reserve, Phong Nha-Ke Bang National Park [6, 8] and isolated from bromeliads in Brazil [9].

Table 2. The diversity of yeasts in Con Dao Island.

No.	Genus	Species	No. strain of species ^a	% ADN homology of nearest species	No. species of genus ^b
Ascomycetous yeasts					
1	<i>Aureobasidium</i>	<i>A. pullulans</i>	1	100	1
		<i>Aureobasidium</i> sp.	2(2)	99.0	1(1)
		<i>Candida albicans</i>	2	99.8-100	
		<i>Candida</i> cf. <i>azyma</i>	1	99.9	
2	<i>Candida</i>	<i>Candida diversa</i>	1	99.8	6(3)
		<i>Candida rugosa</i>	13	100	
		<i>Candida</i> sp.	3(3)	94.4-96.6	
		<i>Candida tropicalis</i>	3	99.8-100	1
3	<i>Kazachstania</i>	<i>Kazachstania africana</i>	1	100	1
4	<i>Kodamaea</i>	<i>Kodamaea ohmeri</i>	1	100	1
5	<i>Meyerozyma</i>	<i>Meyerozyma guilliermondii</i>	6	100	1
6	<i>Pichia</i>	<i>Pichia kudriavzevii</i>	4	99.8-100	1
7	<i>Saccharomyces</i>	<i>S. cerevisiae</i>	1	100	1
Basidiomycetous yeasts					
8	<i>Bullera</i>	<i>Bullera dendrophila</i>	2	99.8	1
9	<i>Cryptococcus</i>	<i>Cryptococcus</i> sp.	8(8)	96.1-99	7(7)
		<i>Pseudozyma</i>	<i>P. hubeiensis</i>	1	100
10		<i>P. parantarctica</i>	1	100	
11	<i>Sympodiomyopsis</i>	<i>Sympodiomyopsis</i> sp.	1(1)	97.7	1(1)
12	<i>Tremella</i>	<i>Tremella</i> sp.	1(1)	97.7	1(1)
13	<i>Ustilago</i>	<i>Ustilago esculenta</i>	1	100	1
Total			54 (15 ^a)		28 (13 ^b)

^aThe number of strains of undescribed species.

^bThe number of undescribed species (less than 99 % homology in their D1/D2 rDNA to the nearest species in BLAST search).

Fifteen strains of Basidiomycetous yeast were found to belong to 6 genera, 13 species (4 described species and 9 undescribed species), in which *Cryptococcus* genus was the dominant (8 strains of 7 species), followed by *Pseudozyma* (2 strains of 2 described species), *Bullera* (2 strains of a described species), *Sympodiomyopsis* (1 species of an undescribed species), *Tremella* (1 species of an undescribed species), *Ustilago* (1 species of an undescribed species). Con

Dao is different from the other studied areas that it has not any red yeasts isolated such as *Rhodotorula*, *Rhodospiridium*, *Sporidiobolus*, *Sporisorium* and *Sporobolomyces* (Table 2). In this study, *Cryptococcus* genus contains a large number of strains with high level of species diversity and high number of undescribed species. This is similar to the results found in Cat Tien National Park, Da Nang and Ma Da [5, 7, 8].

3.4. Yeast diversity of collected samples

Habitat and the interactions among the microbial communities will determine the population density and diversity of yeast living in the environment. In this study, we have found 13 genera, 28 species of yeasts from 32 out of 38 collected samples. Of these, 31 species (belonging to 9 genera, 20 species) were isolated from 14 leaf samples; 12 strains (4 genera, 6 species) isolated from 9 fruit samples; 7 strains (2 genera, 2 species) were isolated from 5 litter samples and 4 strains (2 genera, 3 species) were isolated from 4 soil samples.

The diversity of yeasts is different on each substrate: 4 species of 2 genera were found in 4/4 soil samples: *Candida* and *Meyerozyma*. 7 strains of these 2 genera were also isolated from 5 litter samples. In particular, *Candida rugosa* and *Meyerozyma guilliermondii* occupied the majority in soil and litter samples in Con Dao Island. From 9/12 fruit samples, 12 strains of 4 genera were isolated, including *Candida*, *Kodamaea*, *Pichia* and *Saccharomyces*. *Candida* genus has the highest number of species and strains (6 strains, 3 species), followed by 4 strains of *Pichia kudriavzevii*.

Leaf surface is regarded as an important habitat of yeasts [18]. Jager et al. [19] reported that the saprophytic yeast found on the surface of leaves belonged to the common genera such as *Candida*, *Cryptococcus*, *Pichia*, *Rhodotorula* and *Trichosporon*. In this study, leaves are the source with highest number of isolated yeasts including 31 strains of 9 genera, 20 species. In particular, *Candida* genus accounted for the highest number of isolated strains (12 strains of 4 species); followed by *Cryptococcus* with 8 strains of 7 species, *Aureobasidium* with 3 strains of 2 species. *Pseudozyma*, *Bullera*, *Kazachstania*, *Symptodiomyopsis*, *Tremella* and *Ustilago* genera has 1 to 2 strains each. This result is similar to the reports obtained in other parts of Viet Nam [5-8]. However, this result is opposite to the study by Sjamsuridzal et al. [20] in which yeasts were isolated from the leaves in the Cibodas (22 strains/12 leaf samples) and no yeast was found from leaf samples in Cibinong.

3.5. Discovery of novel yeast species

During our research program on the microbial diversity in Viet Nam, we isolated yeasts from different sources in Con Dao Island. The yeast strains were classified based on the homology of D1/D2 region of the LSU rDNA [13].

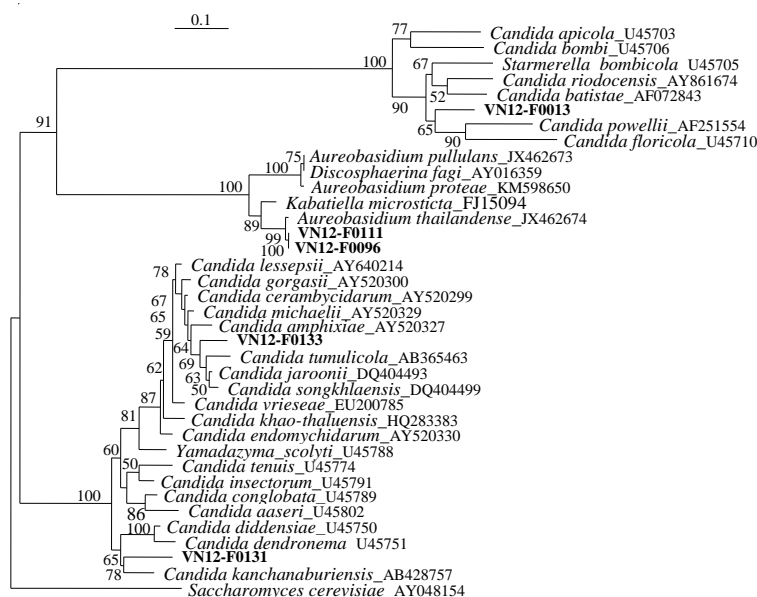


Figure. 1. Phylogenetic tree of suspected new species belong to Ascomycetous yeasts with type strains of related species based on D1 / D2 region of the 26S rDNA.

The classification was performed for 54 yeast strains. The sequence results of the D1/D2 region of the 26S rDNA showed that 15 strains had less than 99 % homology and were separated from the described species on the phylogenetic tree (Figures 1 and 2). The analysis of the sequence of D1/D2 region demonstrated that 15/54 isolates (27.8 %) representing 13 suspected new species. The suspected new species mainly belonged to *Cryptococcus* (7 species), followed by *Candida* (3 species), *Aureobasidium*, *Sympodiomyces* and *Tremella* (1 species). Further studies on the morphological characteristics, chemical classification, physiological and biochemical characteristics are needed in order to publish novel species. The study of yeast diversity in Con Dao Islands has also contributed knowledge on the microbial diversity of Viet Nam.

4. CONCLUSION

Fifty four yeast strains were isolated from 38 samples of fresh flowers, fruits, leaves, litter and soil collected in Con Dao Island. The highest number of yeast strains was found in fresh leaf samples and the lowest number was found in soil samples.

Classification of 54 yeast strains based on colony and cell morphology and D1 / D2 sequence analysis has been performed. They belong to 13 genera, 28 species. The diversity and distribution of yeasts were different in different samples.

Fifteen strains had the D1/D2 region sequence with less than 99 % homology and were separated from the described species on the phylogenetic tree, representing 13 suspected new species. *Cryptococcus* genus had the highest number of suspected new species found in this study.

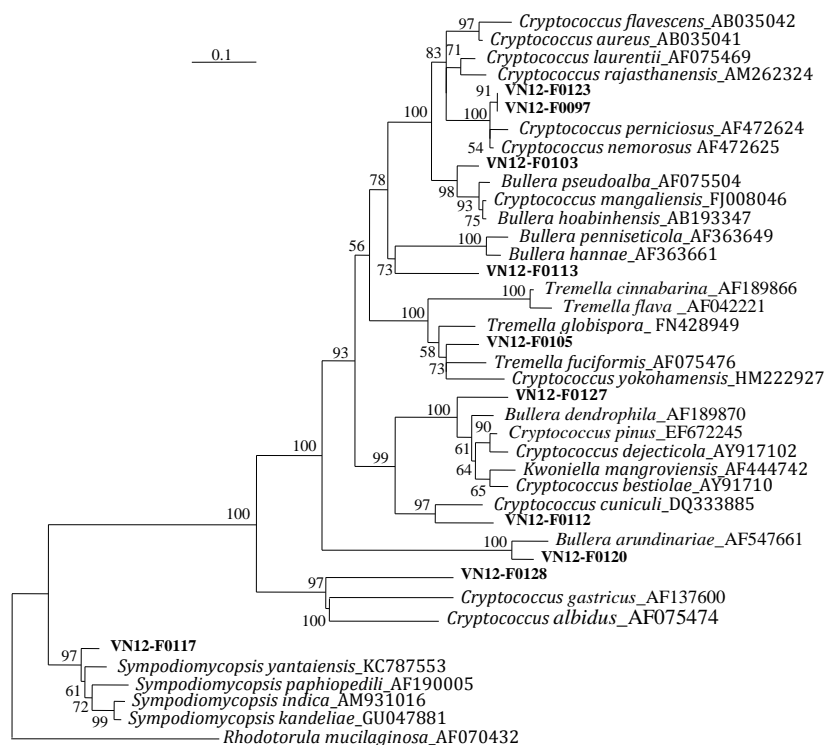


Figure. 2. Phylogenetic tree of suspected new species belong to Basidiomycetous yeasts with type strains of related species based on D1 / D2 region of the 26S rDNA.

Acknowledgement. This study was supported the funds of “Conservation and maintaining microbial bioresources” - the independent project of Viet Nam National University Hanoi and Ministry of Science and Technology.

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