

12-Month Report
(September 2009- August 2010)



**A Study on the diversity of yeast endosymbionts from mushroom-feeding
and wood-feeding Beetles**

R252124

By

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For

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National Center for Genetic Engineering and Biotechnology (BIOTEC)**

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Project Title **A Study on the Diversity of Yeast Endosymbionts from mushroom-feeding and wood-feeding Beetles**

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Method

Yeast isolation

Yeasts were isolated from gut of beetles by disinfecting the beetle surfaces with 95% ethanol for 1-2 min. The alcohol wash was followed by a 0.7% saline (NaCl) rinse, the rinse liquid was plated on acidified YM agar (Difco YM broth, 2% plain agar, adjusted to pH 3.5 with HCl). Forceps, dissecting needles, and minute insect pins are used to dissect the beetles on sterile microscope slides under a dissecting microscope. The beetle gut is removed aseptically, cut into pieces, and transferred to tubes containing 0.7% saline. Gut segments are crushed in the saline solution with a pipette tip and streaked with a loop onto the surface of acidified YM agar plates. Plates are incubated at 25°C. After incubation, yeast colonies were picked and purified by streaking on YM agar. Purified yeast strains were suspended in YM broth supplemented with 10% glycerol and maintained at -80 °C.

DNA sequencing and phylogenetic analysis

The sequences of the D1/D2 domain of the LSU rRNA gene were determined from PCR products amplified from genomic DNA extracted from yeast cells by using a slightly modified version of the method described by Lachance *et al.* (1999). The D1/D2 domain of the LSU rRNA gene was amplified by PCR with the forward primer NL-1 and the reverse primer NL-4 (O'Donnell, 1993). The PCR product was checked by agarose gel electrophoresis and purified by using the DNA purification kit NucleoSpin^R Extract (MARCHEREY-NAGEL). The purified PCR product was submitted to Macrogen Inc. (Korea) for sequencing with the external primers NL-1 and NL-4. The sequences were compared pairwise using a BLASTN search (Altschul *et al.*, 1997) and were aligned with the sequences of related species retrieved from GenBank using the multiple alignment program CLUSTAL_X version 2.0 (Larkin *et al.*, 2007). A phylogenetic tree was constructed from the evolutionary distance data with Kimura's two-parameter correction (Kimura, 1980), using the neighbor-joining method (Saitou & Nei, 1987). Confidence levels of the clades were estimated from bootstrap analysis (1000 replicates) (Felsenstein, 1985).

Phenotypic characterization

The strains of novel species were characterized morphologically, biochemically, and physiologically according to the standard methods described by Yarrow (1998). Assimilation of nitrogen compounds was examined on solid media with starved inocula following the method of Nakase & Suzuki (1986). Growth at various temperatures was determined by cultivation in YM

broth. Ubiquinones were extracted from cells cultivated in a 500 ml Erlenmeyer flask containing 200 ml of YM broth on a rotary shaker at 25 °C were harvested in the early stationary growth phase. The cells were washed with distilled water and freeze-dried. Extraction and purification of ubiquinones were carried out according to Nakase & Suzuki (1986). Ubiquinone isoprenologues were identified by HPLC.

Results

Two hundred and forty-three insect samples were collected from 3 provinces in Thailand (KhaoYai National Park; Nakhon Ratchassima, Khao Nan National Park; Nakhon Sri Thammarat and Nam Nao National Park; Phitsanulok). Ninety-five insect samples were collected from mushrooms, one hundred and ten samples were collected from wood, nine samples were collected from mushroom on the log and twenty-nine samples can't identified to the habitat as these insects were collected in the bungalows of the national park or picked from the ground or from plants. Insect samples were identified to be 21 families as Anthicidae, Brentidae, Carabidae, Cerylonidae, Chrysomelidae, Ciidae, Cleridae, Coccinellidae, Curculionidae, Elateridae, Endomychidae, Erotylidae, Histeridae, Hydrophilidae, Lucanidae, Nitidulidae, Passalidae, Passandridae, Scarabaeidae, Staphylinidae and Tenebrionidae. One hundred and fifty-three yeast strains were isolated from gut of 62 insect samples in 15 families as Carabidae, Cerylonidae, Chrysomelidae, Curculionidae, Elateridae, Endomychidae, Erotylidae, Histeridae, Lucanidae, Nitidulidae, Passalidae, Passandridae, Scarabaeidae, Staphylinidae and Tenebrionidae. Not found yeast from gut of Anthicidae, Brentidae, Ciidae, Cleridae, Coccinellidae and Hydrophilidae. Table 1 shows the list of the insect families and the number of insects belonging to these families as well as the number of yeasts that have been isolated from these families.

One hundred and forty-three yeast strains were identified by sequences analysis of the D1/D2 domain of the LSU rRNA gene (Table 2, 3). Among 143 strains taxonomically studied, a total of 38 strains (26.6 %) were identified to be described species or known species. Thirty-four strains were assigned to 8 described species in four genera of the phylum Ascomycota (*Candida*, *Debaryomyces*, *Pichia* and *Pseudozyma*) consisted of *Candida haemulonii*, *C. insectorum*, *C. oleophila*, *C. parapsilosis*, *Debaryomyces polymorphus*, *Pichia caribbica*, *P. stipitis* and *Pseudozyma aphidis*. Eight strains were assigned to two described species in two genera of the phylum Basidiomycota consisted of *Cryptococcus humicola* and *Rhodotorula mucilaginosa*. Thirty-three strains were similar to the undescribed species (23.1%) in 6 species as *Blastobotrys* sp. NRRL YB-2290, *Candida* sp. AST2009a, *Candida* sp. BG02-7-15-009-2-1, *Candida* sp. CBS 10852, *Candida* sp. GE13S02 and *Rhodotorula* sp. SJ15L05. Seventy-two strains (50.3%) were identified

to be seven 15 new species closest with *Blastobotrys* sp. NRRL YB-2290, *Candida kazuoi*, *Candida maxi*, *Candida panamericana*, *Candida* sp. AS2.4033, *Candida* sp. BG99-8-18-1-6 , *Candida* sp. SJ-1, *Candida* sp. ST-370, *Candida* sp. ST-431, *Candida* sp. UWO(PS)99-305.2, *Candida subhashii*, *Candida terraborum*, *Debaryomyces polymorphus*, *Endomyces scopularum*, *Pichia* sp. BET4 and *Trichosporon dulcitum*.

Table 1 Insect identifications and the number of yeasts found from gut of insects samples.

No.	Family	Total Insect sample	Yeast found	
			No	Yes
1	Anthicidae	5	5	0 (0%)
2	Brentidae	1	1	0 (0%)
3	Carabidae	26	24	2 (7.6%)
4	Cerylonidae	2	1	1 (50%)
5	Chrysomelidae	3	2	1 (33.3%)
6	Ciidae	1	1	0 (0%)
7	Cleridae	1	1	0 (0%)
8	Coccinellidae	1	1	0 (0%)
9	Curculionidae	4	3	1 (25%)
10	Elateridae	3	2	1 (33.3%)
11	Endomychidae	10	9	1 (10%)
12	Erotylidae	8	3	5 (62.5%)
13	Histeridae	13	12	1 (7.7%)
14	Hydrophilidae	3	3	0 (0%)
15	Lucanidae	4	3	1 (25%)
16	Nitidulidae	7	6	1 (14.3%)
17	Passalidae	16	10	6 (37.5%)
18	Passandridae	2	1	1 (50%)
29	Scarabaeidae	11	3	8 (72.7%)
20	Staphylinidae	9	8	1 (11.1%)
21	Tenebrionidae	113	82	31 (27.4%)
		243	181	62 (25.5%)

Table 2 List of yeast species found from gut of beetles.

No.	Result of Identification	No. of Isolated
Known species		
1	<i>Candida haemulonii</i> type II (U44819)	16
2	<i>Candida insectorum</i> (U45791)	3
3	<i>Candida oleophila</i> (U45793)	1
4	<i>Candida parapsilosis</i> (U45754)	2
5	<i>Debaryomyces polymorphus</i> (U45836)	2
6	<i>Pichia caribbica</i> (EU348786)	5
7	<i>Pichia stipitis</i> (U45741)	3
8	<i>Pseudozyma aphidis</i> CBS 517.83 (AJ235303)	2
9	<i>Cryptococcus humicola</i> CBS571 (AF189836)	2
10	<i>Rhodotorula mucilaginosa</i> (AF070432)	2
Sub total		38
Undescribed species		
1	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	10
2	<i>Candida</i> sp. AST2009a (FJ008044.3)	9
3	<i>Candida</i> sp. BG02-7-15-009-2-1 (AY520324)	1
4	<i>Candida</i> sp. CBS 10852 (EU570106)	1
5	<i>Candida</i> sp. GE13S02 (FJ527140)	11
6	<i>Rhodotorula</i> sp. SJ15L05 (EU547814)	1
Sub total		33
New species		
1	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	1
2	<i>Candida maxii</i> BG01-7-21-006A-1-1 (AY242253)	3
3	<i>Candida panamericana</i> BG01-7-26-006B-2-1 (AY242273)	2
4	<i>Candida</i> sp. AS2.4033 (FJ613525)	5
5	<i>Candida</i> sp. BG99-8-18-1-6 (AY242348)	4
6	<i>Candida</i> sp. SJ-1 (EF653272)	1
7	<i>Candida</i> sp. ST-370 (DQ404513)	15
8	<i>Candida</i> sp. ST-431 (DQ404522)	28
9	<i>Candida</i> sp. UWO(PS)99-305.2	2
10	<i>Candida subhashii</i> (EU836708)	1
11	<i>Candida terraborum</i> BG02-7-15-019A-2-1 (AY309810)	1
12	<i>Debaryomyces polymorphus</i> (U45836)	1
13	<i>Endomyces scopularum</i> (U40092)	1
14	<i>Pichia</i> sp. BET4 (FM179640)	3
15	<i>Trichosporon dulcitum</i> (AF075517)	4
Sub total		72
Total		143

Table 3 Yeast identification by sequence analysis of the D1/D2 domain of the LSU rRNA gene

No.	Strain No.	Closest species	Identities		Gap		Substitutions		Result of identification	Query (letters)	Length of closest species
			No.	%	No.	%	No.	%			
1	MS01/1	<i>Pichia caribbica</i> (EU348786)	570/570	100.0	0	0	0	0.0	<i>Pichia caribbica</i>	570	570
2	MS01/1.1	<i>Pichia caribbica</i> (EU348786)	470/470	100.0	0	0	0	0.0	<i>Pichia caribbica</i>	470	570
3	MS01/1.2	<i>Pichia caribbica</i> (EU348786)	570/570	100.0	0	0	0	0.0	<i>Pichia caribbica</i>	570	570
4	MS01/1.3	<i>Pichia caribbica</i> (EU348786)	570/570	100.0	0	0	0	0.0	<i>Pichia caribbica</i>	570	570
5	MS03/01	<i>Candida oleophila</i> (U45793)	572/572	100.0	0	0	0	0.0	<i>Candida oleophila</i>	572	572
6	MS3/03	<i>Debaryomyces polymorphus</i> (U45836)	569/570	99.8	0	1	1	0.2	<i>Debaryomyces polymorphus</i>	570	570
7	MS3/08	<i>Pichia</i> sp. BET4 (FM179640)	534/562	95.0	2	26	26	4.6	New species	561	584
8	MS3/09	<i>Debaryomyces polymorphus</i> (U45836)	511/548	93.2	18	19	19	3.7	New species	530	570
9	MS04/05	<i>Pichia</i> sp. BET4 (FM179640)	534/563	95.0	3	26	26	4.6	New species	562	584
10	MS04/06	<i>Pichia</i> sp. BET4 (FM179640)	534/562	95.0	2	26	26	4.6	New species	561	584
11	MS20/3.1	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0	0	0.0	Undescribed species	569	544
12	MS20/3.2	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0	0	0.0	Undescribed species	569	544
13	MS20/4	<i>Candida</i> sp. ST-370 (DQ404513)	538/561	95.9	3	20	20	3.6	New species	560	578
14	MS20/6	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0	0	0.0	Undescribed species	560	544
15	MS20/8	<i>Candida terraborum</i> (AY309810)	525/547	96.0	1	26	26	4.8	New species	560	568
16	MS21/2.3	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0	0	0.0	Undescribed species	569	544
17	MS21/2.4	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0	0	0.0	Undescribed species	569	544
18	MS21/3.2	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0	0	0.0	Undescribed species	569	544
19	MS21/3.3	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0	0	0.0	Undescribed species	569	544
20	MS21/5.2.III	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0	0	0.0	Undescribed species	569	544
21	MS21/5.3	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0	0	0.0	Undescribed species	569	544
22	MS21/6.1	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	21	21	3.7	New species	561	578
23	MS21/6.2 I	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	21	21	3.7	New species	561	578
24	MS21/6.3 II	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	21	21	3.7	New species	561	578
25	MS21/6.3I	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	21	21	3.7	New species	561	578
26	MS21/7.1	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	21	21	3.7	New species	561	578
27	MS21/7.2I	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	21	21	3.7	New species	561	578
28	MS21/7.2II	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	21	21	3.7	New species	561	578
29	MS21/7.3II	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	21	21	3.7	New species	561	578
30	MS21/8.2	<i>Cryptococcus humicola</i> (AF189836)	576/578	98.1	1	1	1	0.2	<i>Cryptococcus humicola</i>	577	625

Table 3 (Continued)

No.	Strain No.	Closest species	Identities		Gap		Substitutions		Result of identification	Query (letters)	Length of closest species
			No.	%	No.	%	No.	%			
31	MS21/8.3	<i>Cryptococcus humicola</i> (AF189836)	593/594	99.8	0	0.2	1	0.2	<i>Cryptococcus humicola</i>	594	625
32	MS21/9.1	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	3.7	21	3.7	New species	561	578
33	MS21/9.11	<i>Candida</i> sp. ST-370 (DQ404513)	461/482	95.6	2	3.9	19	3.9	New species	482	578
34	MS21/9.2	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	3.7	21	3.7	New species	561	578
35	MS21/9.21	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	3.7	21	3.7	New species	561	578
36	MS21/9.31	<i>Candida</i> sp. ST-370 (DQ404513)	537/562	95.6	4	3.7	21	3.7	New species	561	578
37	MS21/9.3II	<i>Candida</i> sp. ST-370 (DQ404513)	514/539	95.4	4	3.9	21	3.9	New species	538	578
38	MS22/03	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0.0	0	0.0	Undescribed species	569	544
39	MS22/6	<i>Candida</i> sp. GE13S02 (FJ527140)	542/542	100.0	0	0.0	0	0.0	Undescribed species	569	544
40	MS26/1.1	<i>Trichosporon dulcitum</i> (AF075517)	592/606	97.7	0	2.3	14	2.3	New species	608	625
41	MS26/1.2	<i>Trichosporon dulcitum</i> (AF075517)	581/595	97.6	0	2.3	14	2.3	New species	595	625
42	MS26/1.3	<i>Trichosporon dulcitum</i> (AF075517)	551/567	97.2	1	2.6	15	2.6	New species	566	625
43	MS26/4	<i>Trichosporon dulcitum</i> (AF075517)	581/595	97.6	0	2.3	14	2.3	New species	595	625
44	MS28/4.1	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	514	514
45	MS46/2.2	<i>Pseudozyma aphidis</i> (AJ235303)	586/590	99.3	2	0.3	2	0.3	<i>Pseudozyma aphidis</i>	619	588
46	MS50.2/1	<i>Candida</i> sp. CBS 10852 (EU570106)	556/557	99.8	0	0.2	1	0.2	Undescribed species	557	557
47	MS56/1.1	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	553	514
48	MS56/1.2	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	553	514
49	MS56/1.3	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	514	514
50	MS56/3.1	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	514	514
51	MS56/3.5	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	514	514
52	MS58/3.1	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	514	514
53	MS58/3.4	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	514	514
54	MS58/3.5	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	514	514
55	MS79/1.1	<i>Candida parapsilosis</i> (U45754)	570/570	100.0	0	0.0	0	0.0	<i>Candida parapsilosis</i>	570	570
56	MS79/1.3	<i>Candida parapsilosis</i> (U45754)	569/570	99.8	0	0.0	0	0.0	<i>Candida parapsilosis</i>	570	570
57	MS87/1.1	<i>Candida insectorum</i> (U45791)	513/515	99.6	1	0.2	1	0.2	<i>Candida insectorum</i>	514	530
58	MS87/3.1	<i>Candida insectorum</i> (U45791)	529/530	99.8	0	0.0	0	0.0	<i>Candida insectorum</i>	536	530
59	MS87/3.3	<i>Candida insectorum</i> (U45791)	528/530	99.6	0	0.4	2	0.4	<i>Candida insectorum</i>	595	530
60	MS91/7.1	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	553	514

Table 3 (Continued)

No.	Strain No.	Closest species	Identities		Gap		Substitutions		Result of identification	Query (letters)	Length of closest species
			No.	%	No.	%	No.	%			
61	MS100/3.1	<i>Pichia stipitis</i> (U45741)	567/568	99.8	0	0.0	0	0.0	<i>Pichia stipitis</i>	568	568
62	MS100/3.3	<i>Pichia stipitis</i> (U45741)	567/568	99.8	0	0.0	0	0.0	<i>Pichia stipitis</i>	568	568
63	MS100/3.4	<i>Pichia stipitis</i> (U45741)	567/568	99.8	0	0.0	0	0.0	<i>Pichia stipitis</i>	568	568
64	MS102/1.4	<i>Candida</i> sp. AS2.4033 (FJ613525)	429/488	87.9	6	10.9	53	10.9	New species	487	515
65	MS102/1.5	<i>Candida</i> sp. AS2.4033 (FJ613525)	429/488	87.9	6	10.9	53	10.9	New species	487	515
66	MS102/3.1	<i>Candida</i> sp. AS2.4033 (FJ613525)	436/495	88.1	6	10.7	53	10.7	New species	494	515
67	MS102/3.2	<i>Candida</i> sp. AS2.4033 (FJ613525)	429/488	87.9	6	10.9	53	10.9	New species	487	515
68	MS102/3.3	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	559/568	98.4	1	1.4	8	1.4	New species	568	3244
69	MS102/3.4	<i>Candida</i> sp. AS2.4033 (FJ613525)	429/488	87.9	6	10.9	53	10.9	New species	487	515
70	MS106/1.2	<i>Candida panamericana</i> (AY242273)	531/547	97.1	1	2.7	15	2.7	New species	558	569
71	MS106/1.3	<i>Candida panamericana</i> (AY242273)	531/547	97.1	1	2.7	15	2.7	New species	558	569
72	MS108/1.12	<i>Candida</i> sp. UWO(PS)99-305.2	475/503	94.4	10	3.6	18	3.6	New species	502	513
73	MS108/1.3	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	1.1	6	1.1	New species	569	556
74	MS108/1.4	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	1.1	6	1.1	New species	569	556
75	MS108/1.7	<i>Candida</i> sp. UWO(PS)99-305.2	475/503	94.4	10	3.6	18	3.6	New species	502	513
76	MS108/1.9	<i>Rhodotorula</i> sp. SJ15L05 (EU547814)	574/574	100.0	0	0.0	0	0.0	Undescribed species	609	576
77	MS109/3.1	<i>Candida</i> sp. BG02-7-15-009-2-1 (AY520324)	565/565	100.0	0	0.0	0	0.0	Undescribed species	582	587
78	MS115/2.2	<i>Rhodotorula mucilaginosa</i> (AF070432)	583/583	100.0	0	0.0	0	0.0	<i>Rhodotorula mucilaginosa</i>	586	600
79	MS133/3.1	<i>Debaryomyces polymorphus</i> (U45836)	569/570	99.8	0	0.2	1	0.2	<i>Debaryomyces polymorphus</i>	570	570
80	MS136/1.3	<i>Candida subhashii</i> (EU836708)	542/565	95.9	1	3.9	22	3.9	New species	569	656
81	MS150/3.1	<i>Candida</i> sp. AST2009a (FJ008044.3)	530/530	100.0	0	0.0	0	0.0	Undescribed species	530	560
82	MS150/3.4	<i>Candida</i> sp. AST2009a (FJ008044.3)	530/530	100.0	0	0.0	0	0.0	Undescribed species	530	560
83	MS151/1.1	<i>Candida</i> sp. AST2009a (FJ008044.3)	530/530	100.0	0	0.0	0	0.0	Undescribed species	530	560
84	MS151/1.2	<i>Candida</i> sp. AST2009a (FJ008044.3)	529/530	99.8	0	0.0	0	0.0	Undescribed species	530	560
85	MS157/1.1	<i>Candida</i> sp. AST2009a (FJ008044.3)	529/530	99.8	0	0.0	0	0.0	Undescribed species	530	560
86	MS157/3.1	<i>Candida</i> sp. AST2009a (FJ008044.3)	530/530	100.0	0	0.0	0	0.0	Undescribed species	530	560
87	MS157/3.5	<i>Rhodotorula mucilaginosa</i> (AF070432)	581/581	100.0	0	0.0	0	0.0	<i>Rhodotorula mucilaginosa</i>	583	600
88	MS162/1.1	<i>Candida</i> sp. AST2009a (FJ008044.3)	530/530	100.0	0	0.0	0	0.0	Undescribed species	530	560
89	MS162/3.2	<i>Candida</i> sp. AST2009a (FJ008044.3)	530/531	99.8	1	0.0	0	0.0	Undescribed species	530	560
90	MS165/1.12	<i>Candida</i> sp. AST2009a (FJ008044.3)	530/530	100.0	0	0.0	0	0.0	Undescribed species	530	560

Table 3 (Continued)

No.	Strain No.	Closest species	Identities		Gap		Substitutions		Result of identification	Query (letters)	Length of closest species
			No.	%	No.	%	No.	%			
91	MS183/1.1	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0	0	0.0	<i>Candida haemulonii</i> type II	514	514
92	MS183/1.3	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0	0	0.0	<i>Candida haemulonii</i> type II	514	514
93	MS184/3.1	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	567/567	100.0	0	0	0	0.0	Undescribed species	567	3244
94	MS184/3.6	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0	0	0.0	<i>Candida haemulonii</i> type II	514	514
95	MS186/1.1	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	567/567	100.0	0	0	0	0.0	Undescribed species	567	3244
96	MS186/1.2	<i>Candida haemulonii</i> type II (U44819)	514/514	100	0	0	0	0	<i>Candida haemulonii</i>	514	514
97	MS205/1.3	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	569	556
98	MS207/1.31	<i>Pichia caribbica</i> (EU348786)	570/570	100.0	0	0	0	0.0	<i>Pichia caribbica</i>	570	570
99	MS207/1.4	<i>Candida</i> sp. SJ-1 (EF653272)	538/566	95.1	8	20	3.5	3.5	New species	566	578
100	MS220/3.12	<i>Pseudozyma aphidis</i> (AJ235303)	586/588	99.7	0	2	0.3	0.3	<i>Pseudozyma aphidis</i>	605	588
101	MS260/1.1	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	6	1.1	1.1	New species	569	556
102	MS261/1.2	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	567/567	100.0	0	0	0	0.0	Undescribed species	567	3244
103	MS261/1.4	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	569	556
104	MS261/1.5	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	569	556
105	MS261/3.62	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	569	556
106	MS262/1.1	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	6	1.1	1.1	New species	565	556
107	MS262/1.5	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	6	1.1	1.1	New species	569	556
108	MS264/3.1	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	569	556
109	MS265/1.1	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	569	556
110	MS265/3.21	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	569	556
111	MS266/1.32	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	6	1.1	1.1	New species	569	556
112	MS266/3.11	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	569	556
113	MS266/3.3	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	565	556
114	MS267/1.1	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	567/567	100.0	0	0	0	0.0	Undescribed species	567	3244
115	MS267/1.3	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	6	1.1	1.1	New species	569	556
116	MS267/1.5	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	565	556
117	MS267/3.21	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	565	556
118	MS267/3.221	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	565	556
119	MS268/1.1	<i>Candida</i> sp. BG99-8-18-1-6 (AY242348)	497/532	93.5	2	33	6.2	6.2	New species	547	554
120	MS268/1.2	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.8	1	6	1.1	1.1	New species	569	556

Table 3 (Continued)

No.	Strain No.	Closest species	Identities		Gap		Substitutions		Result of identification	Query (letters)	Length of closest species
			No.	%	No.	%	No.	%			
121	MS268/1.3	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	567/567	100.0	0	0.0	0	0.0	Undescribed species	567	3244
122	MS268/3.4	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	1.1	6	1.1	New species	569	556
123	MS269/1.1	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	514	514
124	MS269/3.4	<i>Candida haemulonii</i> type II (U44819)	514/514	100.0	0	0.0	0	0.0	<i>Candida haemulonii</i> type II	514	514
125	MS271/1.1	<i>Endomyces scoullarum</i> (U40092)	550/563	97.7	2	2.0	11	2.0	New species	563	561
126	MS271/1.3	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	567/567	100.0	0	0.0	0	0.0	Undescribed species	567	3244
127	MS271/3.4	<i>Candida maxii</i> (AY242253)	523/547	95.6	2	4.0	22	4.0	New species	560	571
128	MS272/1.1	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	567/567	100.0	0	0.0	0	0.0	Undescribed species	567	3244
129	MS272/1.5	<i>Candida</i> sp. BG99-8-18-1-6 (AY242348)	496/532	93.5	2	6.2	33	6.2	New species	547	554
130	MS273/1.1	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	1.1	6	1.1	New species	569	556
131	MS273/1.5	<i>Candida</i> sp. BG99-8-18-1-6 (AY242348)	497/532	93.5	2	6.2	33	6.2	New species	547	554
132	MS273/3.11	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	1.1	6	1.1	New species	569	556
133	MS273/3.3	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	1.1	6	1.1	New species	569	556
134	MS273/3.4	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	567/567	100.0	0	0.0	0	0.0	Undescribed species	567	3244
135	MS276/1.1	<i>Candida maxii</i> (AY242253)	530/554	95.7	2	4.0	22	4.0	New species	567	574
136	MS276/1.2	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	1.1	6	1.1	New species	569	556
137	MS276/1.3	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	1.1	6	1.1	New species	569	556
138	MS276/1.5	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	1.1	6	1.1	New species	569	556
139	MS276/1.7	<i>Candida</i> sp. BG99-8-18-1-6 (AY242348)	497/532	93.5	2	6.2	33	6.2	New species	547	554
140	MS276/1.8	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	567/567	100.0	0	0.0	0	0.0	Undescribed species	567	3244
141	MS284/1.4	<i>Candida maxii</i> (AY242253)	530/554	95.7	2	4.0	22	4.0	New species	567	574
142	MS284/1.5	<i>Candida</i> sp. ST-431 (DQ404522)	550/557	98.7	1	1.1	6	1.1	New species	569	556
143	MS285/1.2	<i>Blastobotrys</i> sp. NRRL YB-2290 (DQ442700)	567/567	100.0	0	0.0	0	0.0	Undescribed species	567	3244

Many new yeasts species were found in this work closest with a previous study of wood- and basidiocarp-feeding beetles in North America (*Candida maxii* BG01-7-21-006A-1-1, *Candida panamericana* BG01-7-26-006B-2-1, *Candida* sp. BG99-8-18-1-6 and *Candida terraborum* BG02-7-15-019A-2-1). The results revealed that yeasts from beetle guts maybe have evolved closely. One of 15 new species were selected for polyphasic approaches, including conventional taxonomy, chemotaxonomic study and molecular taxonomy and described as *Candida guttulata* sp. nov. The characteristics of this new species is presented in the draft manuscript (see Appendix) to be submitted for publication in International Journal of Systematic and Evolutionary Microbiology; IJSEM (IF = 2.12) in Title: *Candida guttulata* sp. nov., a new ascomycetous yeast species isolated from gut of beetles in Thailand.

From the results we found that 26.6% of newly isolated from gut of beetles were identified to be only 10 known species and found 3 strains of the xylose-fermenting yeast, *Pichia stipitis*. However, more than 70% were undescribed and new species that the ability to ferment xylose is still not known. The ability to ferment xylose must be a goal for future work and will be an added scope during the extension of this project as only a few yeast species have been reported to ferment D-xylose to ethanol. These include *Brettanomyces naardenensis*, *Candida lyxosophila*, *C. intermedia*, *C. shehatae*, *C. tenuis*, *Pachysolen tannophilus*, *Pichia segobiensis*, *Scheffersomyces* (*Pichia*) *stipitis* (Barnett *et al.*, 2000; Kurtzman & Fell, 1998), *Candida jeffriesii*, *Spathaspora passalidarum* (Nguyen *et al.*, 2006), and *Spathaspora arborariae* (Cadete *et al.*, 2009). At present, lignocelluloses in plant biomass with xylose as a major sugar component is of interest as a substrate for production of many microbial products, such as xylitol, acetic acid, and ethanol.

Recommendations for follow-on work:

Given the high number of new species discovered in this project, the gut of the beetles have provided us a new avenue where to look for hidden taxa and species description should be one of the main aims of the second phase of the project. Sampling was done only in a limited period of time and only at a few national parks in Thailand. For the next phase, more beetle collection should be done in the North or in the Northeastern part of the country and compare the diversity of the beetles and the yeasts in each of the geographical location. It would also be of immense importance in the future to be able to study the relationships of the yeasts to the mushrooms where the beetles feed on and if these yeasts in the beetle guts are the same population as those found in the mushrooms. For the utilization of beetle gut yeasts, native xylose fermenting yeasts are important

aspects and the screening of these xylose fermenters will be done during the next phase of this project.

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Running word: *Candida guttulata* sp. nov.; gut; beetle; yeast

The GenBank, EMBL/DBJ accession number for the sequence of the D1/D2 domain of the LSU rRNA of 'MS21/6.1' are XXXXXXXX.

1 Full paper

2 ***Candida guttulata* sp. nov., a new ascomycetous yeast species**
3 **isolated from gut of beetles in Thailand**

4
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19
20 **Running word:** *Candida guttulata* sp nov.; gut; beetle; yeast

21
22 The GenBank/EMBL/DDBJ accession number for the sequence of the D1/D2 domain of the
23 LSU rRNA gene of MS21/6.1^T are XXXXXXXXX.

Summary

Three novel yeast strains (MS21/6.1^T, MS21/7.1 and MS21/9.1) were isolated from gut of beetles, family Scarabaeidae, collected in Khao-Yai National Park, Nakhonrachasima, Thailand. Analysis of the D1/D2 domains of the large subunit rRNA gene sequences revealed that the sequence of D1/D2 domains of the LSU rDNA of three were identical. The closest recognized species of strains MS21/6.1^T, MS21/7.1 and MS21/9.1 in terms of pairwise sequence similarity were *Candida* sp. ST-370 and *Candida panamericana*, but with 3.7% and 4.2% nucleotide substitution respectively. Base on the morphological, biochemical, physiological and chemotaxonomic characteristics, and the sequence analysis of the D1/D2 domain of the large subunit rRNA gene, the three strains were found to represent a novel *Candida* species in the *Candida tanzawaensis* clade. Three strains (MS21/6.1^T, MS21/7.1 and MS21/9.1) were assigned as a single novel species, which was named *Candida guttulata* sp. nov. The type strain is MS21/6.1^T (= CBS XXXXX^T = NBRC XXXXX^T = BCC XXXXX^T).

1 Introduction

Associations between yeasts and mushroom- and wood-feeding beetles have largely gone unnoticed until now. In recent years, the diversity and novel species of yeast from natural habitat in Thailand were constantly reported. Many new yeast species were isolated from insect frass such as *Candida easanensis*, *C. hasegawae*, *C. kazuoi*, *C. nakhonratchasimensis* and *C. pattaniensis* (Jindamorakot *et al.*, 2004; Nakase *et al.*, 2007) but yeasts from the digestive tract of insects had still not been reported in Thailand. A previous study of basidiocarp-feeding beetles in North America, 650 yeasts were isolated from beetles in 26 families and almost 30% of yeast isolates formed a clade with *Candida tanzawaensis* (Nakase *et al.*, 1988; Suh *et al.*, 2004). At present *Candida tanzawaensis* clade consists of 23 recognized species (Kurtzman, 2001; Nakase *et al.*, 1988; Suh *et al.*, 2004). In this clade 16 species (*Candida guaymorum*, *C. bokatorum*, *C. kunorum*, *C. terraborum*, *C. emberorum*, *C. wounanorum*, *C. yuchorum*, *C. chickasaworum*, *C. choctaworum*, *C. bolitotheri*, *C. atakaporum*, *C. panamericana*, *C. bribrorum*, *C. maxi*, *C. anneliseae* and *C. taliae*) were isolated from digestive tract of basidiocarp-feeding beetles and 2 species (*C. ambrosiae* and *C. pyralidae*) were isolated from insect frass.

During investigations of the diversity of yeasts from mushroom-feeding and wood-feeding beetles in Thailand, three yeast strains (MS21/6.1^T, MS21/7.1 and MS21/9.1) were found to represent a novel *Candida* species in *Candida tanzawaensis* clade based on the analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene sequence and to be described as *Candida guttulata* sp. nov.

1 **METHOD**

2 **Yeast isolation**

3 Yeasts were isolated from gut of beetles by disinfecting the beetle surfaces in 95%
4 ethanol for 1-2 min. The alcohol wash was followed by a 0.7% saline (NaCl) rinse, the rinse
5 liquid was plated on acidified YM agar (Difco YM broth, 2% plain agar, adjusted to pH 3.5
6 with HCl). Forceps, dissecting needles, and minute insect pins are used to dissect the beetles
7 on sterile microscope slides under a dissecting microscope. The beetle gut is removed
8 aseptically, cut into pieces, and transferred into tubes containing 0.7% saline. Gut segments
9 are crushed in the saline solution with a pipette tip and streaked with a loop onto the surface
10 of acidified YM agar plates. Plates are incubated at 25°C. After incubation, yeast colonies
11 were picked and purified by streaking on YM agar. Purified yeast strains were suspended in
12 YM broth supplemented with 10% glycerol and maintained at -80 °C.

13 **DNA sequencing and phylogenetic analysis**

14 The sequences of the D1/D2 domain of the LSU rRNA gene were determined from
15 PCR products amplified from genomic DNA extracted from yeast cells by using a slightly
16 modified version of the method described by Lachance *et al.* (1999). The D1/D2 domain of
17 the LSU rRNA gene was amplified by PCR with the forward primer NL-1 and the reverse
18 primer NL-4 (O'Donnell, 1993). The PCR product was checked by agarose gel
19 electrophoresis and purified by using the DNA purification kit NucleoSpin^R Extract
20 (MARCHERY-NAGEL). The purified PCR product was submitted to Macrogen Inc.
21 (Korea) for sequencing with the external primers NL-1 and NL-4. The sequences were
22 compared pairwise using a BLASTN search (Altschul *et al.*, 1997) and were aligned with the
23 sequences of related species retrieved from GenBank using the multiple alignment program

CLUSTAL_X version 2.0 (Larkin *et al.*, 2007). A phylogenetic tree was constructed from the evolutionary distance data with Kimura's two-parameter correction (Kimura, 1980), using the neighbor-joining method (Saitou & Nei, 1987). Confidence levels of the clades were estimated from bootstrap analysis (1000 replicates) (Felsenstein, 1985).

Phenotypic characterization

The strains of novel species were characterized morphologically, biochemically, and physiologically according to the standard methods described by Yarrow (1998). Assimilation of nitrogen compounds was examined on solid media with starved inocula following the method of Nakase & Suzuki (1986). Growth at various temperatures was determined by cultivation in YM broth. Ubiquinones were extracted from cells cultivated in a 500 ml Erlenmeyer flask containing 200 ml of YM broth on a rotary shaker at 25 °C were harvested in the early stationary growth phase. The cells were washed with distilled water and freeze-dried. Extraction and purification of ubiquinones were carried out according to Nakase & Suzuki (1986). Ubiquinone isoprenologues were identified by HPLC.

RESULTS AND DISCUSSION

Species delineation, classification and ecology

Three novel yeast strains (MS21/6.1^T, MS21/7.1 and MS21/9.1) were isolated from gut of beetles collected in Khao Yai National Park, Nakhon Ratchasima, Thailand. Analysis of the D1/D2 domains of the large subunit rRNA gene sequences revealed that the sequence of D1/D2 domains of the LSU rDNA of three strains were identical. With respect to their closest species in term of pairwise sequences similarity the three strains differed by 3.7%

nucleotide substitution (21 nucleotide substitutions and four gaps out of 562 bases) from undescribed species *Candida* sp. ST-370, 4.2% nucleotide substitutions (23 nucleotide substitutions and four gaps out of 550 nt) from the type strain of *Candida panamericana* and 5.2% nucleotide substitutions (29 nucleotide substitutions and three gaps out of 561 bases) from the type strain of *Candida tanzawaensis*. *Candida panamericana* and *Candida tanzawaensis* belong to the *Candida tanzawaensis* clade. According to Kurtzman & Robnett (1998), yeast strains showing nucleotide substitutions of greater than 1% in the D1/D2 domain of the LSU rRNA gene are usually representative of different species. The phylogenetic tree based on the sequences of the D1/D2 domain of the LSU rRNA gene further demonstrated that the three strains (MS21/6.1^T, MS21/7.1 and MS21/9.1) was placed in the *Candida tanzawaensis* clade and distinct from *Candida* sp. ST-370, *Candida panamericana*, *Candida tanzawaensis* and other related species in the this clade (Fig. 1).

Cells of the novel species proliferated by multilateral budding, and were negative for urease reactions. Starch-like compounds were not produced as do the other members of *Candida tanzawaensis* clade. Ascospores were not produced from individual strains or strains paired on 5% malt extract agar, Fowell's acetate agar and YM agar after 5 weeks at 25 °C. The characteristics fit well with those of the genus *Candida*. Therefore, we concluded that the three strains represent a novel species for which the name *Candida guttulata* sp. nov. is proposed to accommodate the three strains MS21/6.1^T, MS21/7.1 and MS21/9.1

In practice, *Candida guttulata* sp. nov. can be distinguished from *C. panamericana*, *C. atakporum* and *Candida tanzawaensis* by many characteristics as shown in Table 1.

The *Candida tanzawaensis* clade consists of 23 recognized species (Kurtzman, 2001; Nakase *et al.*, 1998; Suh and Blackwell, 2004). 16 species from this clade (*Candida*

guaymorum, *C. bokatorum*, *C. kunorum*, *C. terraborum*, *C. emberorum*, *C. wounanorum*, *C. yuchorum*, *C. chickasaworum*, *C. choctaworum*, *C. bolitotheri*, *C. atakaporum*, *C. panamericana*, *C. bribrorum*, *C. maxi*, *C. anneliseae* and *C. taliae*) were isolated from digestive tract of basidiocarp-feeding beetles similar with a novel *Candida* species in this work, *Candida guttulata* sp. nov. Two species (*C. ambrosiae* and *C. pyralidae*) were isolated from insect frass. The result of our work and from other studies revealed that the yeast taxa in *Candida tanzawaensis* clade are highly associated with the digestive tract of beetles. The digestive tract of beetles may be a major habitat of the yeasts in this clade.

Latin diagnosis of *Candida guttulata* Nitiyon, Sudhadham, Khonsanit, Jindamorakot et Luangsa-ard sp. nov.

In agaro YM post dies 3 ad 25 °C cellulae ovoidae aut cylindricae, (1-4 × 2-7 µm), singulae, binae, aut pseudohyphae fiunt, per germinationem multipolarem reproducentes.

Cultura cremea, glabra, nitidae, margine glabra. In agaro

farinae Zea mays post dies 3 ad 25 °C pseudohyphae formantur, hyphae non formantur.

Ascosporae non formantur. D-Glucosum, D-galactosum et α,α-trehalosum fermentantur

at non sucrosum, maltosum, lactosum, melibiosum, nec raffinose. D-Glucosum, D-

galactosum, sucrosum, maltosum, cellobiosum, α,α-trehalosum, melezitose, amylum

solubile (infirme), D-xylosum, D-arabiosum (infirme), D-ribosum, ethanolum, glycerolum,

ribitolum, D-mannitolum, D-glucitolum, α-methyl-D-glucosidum, salicinum, α-glucono-

lactonum, acidum 2-ketogluconicum, acidum succinicum, acidum citricum, D-glucosaminum,

N-acetyl-D-glucosaminum, ethylaminum, L-lysinum et cadaverinum assimilantur. L-

Sorbose, lactosum, melibiosum, raffinose, inulinum, L-arabiosum, L-rhamnosum,

1 *erythritolum, galactitolum, acidum 5-ketogluconicum, acidum DL-lacticum, inositolum,*
2 *acidum D-glucuronicum, acidum D-galacturonicum, xylitolum, L-arabinitolum, acidum D-*
3 *gluconicum, propanum-1,2-diolum, butanum-1,2-diolum, methanolum, kalium nitricum nec*
4 *natrum nitrosum non assimilantur. Vitamina externa crescentiae non necessaria sunt. Crescit*
5 *postest in temperatura 35 °C at non in 37 °C. Non crescit in 0.01% cycloheximido nec 0.1%*
6 *cycloheximido. Crescit in 10% natrii chloridum/5% glucosum. Non crescit in 50% glucosum,*
7 *60% glucosum, 10% natrii chloridum/5% glucosum nec 15% natrii chloridum/5% glucosum.*
8 *Amylum non formatur. Ureum non hydrolysatur. Ubiquinonum majus: Q-9*

9 *Typus stirpis, MS21/6.1^T (= CBS XXXXX^T = NBRC XXXXX^T = BCC XXXXX^T)*
10 *isolatus designat stirpem typicum, Nakhonrachasima provincia, Thailandica, conservatur in*
11 *collectionibus culturarum quas Centraalbureau voor Schimmelcultures (Utrecht, The*
12 *Netherlands), NITE Biological Resource Center (Chiba, Japan) et BIOTEC Culture*
13 *Collection, Nationnal Central for Genetic Engineering and Biotechnology, Thailand*
14 *(Pathumthani, Thailand) deposita est.*

15
16 **Description of *Candida guttulata* Nitiyon, Sudhadham, Khonsanit, Mongkolsamrit,**
17 **Jindamorakot & Luangsa-ard sp. nov.**

18 After growth on YM agar for 3 days at 25 °C, cells are ovoid to cylindrical (1-4 × 2-7
19 µm) and occur singly, in pairs or in short chain (Fig. 2). Budding is multilateral. Colony is
20 cream-colored, smooth, glossy and has an entire margin. Pseudohyphae are formed, but true
21 hyphae are not formed in slide culture on cornmeal agar after 3 days at 25 °C. Ascospores are
22 not produced from individual strain or strains paired on 5% malt extract agar, Fowell's
23 acetate agar and YM agar after 5 weeks at 25 °C. The major ubiquinone is Q-9. Fermentation

of D-glucose, D-galactose and α,α -trehalose are positive but negative for sucrose, maltose, lactose, melibiose and raffinose. D-Glucose, D-galactose, sucrose, maltose, cellobiose, α,α -trehalose, melezitose, soluble starch (weak), D-xylose, D-arabinose (weak), D-ribose, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, α -methyl-D-glucoside, salicin, α -gluconolactone, 2-ketogluconic acid, succinic acid, citric acid, D-glucosamine, N-acetyl-D-glucosamine, ethylamine-HCl, L-lysine-HCl and cadaverine are assimilated but L-sorbose, lactose, melibiose, raffinose, inulin, L-arabinose, L-rhamnose, erythritol, galactitol, 5-ketogluconic acid, DL-lactic acid, inositol, D-glucuronic acid, D-galacturonic acid, xylitol, L-arabinitol, D-gluconic acid, propane-1,2-diol, butane-2,3-diol, methanol, potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin free medium is weak positive. Growth at 35 °C is positive, but at 37 °C is negative. Growth with 0.01% cycloheximide and 0.1% cycloheximide are negative. Growth on medium containing 10% (w/v) sodium chloride/5% (w/v) glucose but negative on 50% (w/v) glucose, 60% (w/v) glucose and 15% (w/v) sodium chloride/5% (w/v) glucose. Starch-like compounds are not produced and urease reactions are negative.

The type strain MS21/6.1^T (= CBS XXXXX^T = NBRC XXXXX^T = BCC XXXXX^T) was isolated from gut of beetle collected in Khao-Yai National Park, Nakhonrachasima, Thailand.

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Table 1. Differential phenotypic characteristics of *Candida guttulata* sp. nov., *C. panamericana*, *C. atakaporum* and *C. tanzawaensis*

Species: 1, *Candida guttulata*; 2, *C. panamericana*; 3, *C. atakaporum*; 4, *C. tanzawaensis*

+, Positive; w, weak; -, negative; nd. Data for *C. tanzawaensis* were taken from Meyer *et al.* (1998) and data for *C. atakaporum*, *C. atakaporum* were taken from Suh *et al.* (2004)

Characteristics	1	2	3	4
Fermentation				
Galactose	+	d	-	-
Melibiose	-	-	-	nd
Trehalose	+	+	d	w
Cabon assimilation				
Soluble starch	w	-	w	-
D-Xylose	+	+	+	-
D-Arabinose	w	-	w	-
D-Ribose	+	-	-	-
Ethanol	+	-	+	+
α -Glucono-lactone	+	nd	nd	nd
5-ketogluconic acid	-	nd	nd	-
DL-Lactic acid	-	-	d	-
D-Galacturonic acid	-	nd	nd	nd
D-gluconic acid	-	+	w	-
D-Glucosamine	+	d/+	d	-
N-Acetyl-D-glucosamine	+	nd	nd	+
Propane-1,2-diol	-	-	w	-
Other				
Growth w/o requirment	w	v	-	-
50% Glucose	-	+	w	-
60% Glucose	-	+	-	nd
15% NaCl	w	w/-	w	nd
35°C	+	d/+	+	-
Ubiquinone	Q9	nd	nd	Q9

1 **Figure legends**

2 **Fig. 1.** Phylogenetic tree based on the sequences of the D1/D2 domain of the LSU rRNA
3 gene, showing positions of *Candida guttulata* sp. nov. (MS21/6.1^T, MS21/7.1 and MS21/9.1)
4 with respect to closely related species. The phylogenetic tree was constructed from
5 evolutionary distance data with correction Kimura's two-parameter (Kimura, 1980), using the
6 neighbor-joining method. Numbers indicate percentages of bootstrap sampling, derived from
7 1000 samples.

8 **Fig. 2.** *Candida guttulata* sp. nov. MS21/6.1^T. (a) Cells grown on YM agar after 3 days at 25
9 °C and (b) pseudohyphae produced on cornmeal agar after 5 days at 25 °C; Bar, 10 µm.

	-	-	d	+
Fermenter	-	-	-	-
Galactose	-	-	-	-
Melibiose	-	-	-	-
Trehalose	-	-	-	-
Sucrose	-	-	-	-
Soluble Starch	-	-	-	-
D-Xylose	-	-	-	-
D-Arabinose	-	-	-	-
D-Ribose	-	-	-	-
Ethanol	-	-	-	-
α-Glucose-1-phosphate	-	-	-	-
β-Ketoglutarate	-	-	-	-
D,L-Lactate	-	-	-	-
D-Galactate	-	-	-	-
D-Glucose-6-phosphate	-	-	-	-
D-Glucose-1-phosphate	-	-	-	-
D-Glucose-2-phosphate	-	-	-	-
N-Acetyl-D-glucosamine	-	-	-	-
Propanol-1,2-diol	-	-	-	-
Other	-	-	-	-
Growth at 4 °C	-	-	-	-
50% Glycerol	-	-	-	-
60% Glycerol	-	-	-	-
15% NaCl	-	-	-	-
25 °C	-	-	-	-
Ubiquinone	-	-	-	-

Fig. 1.

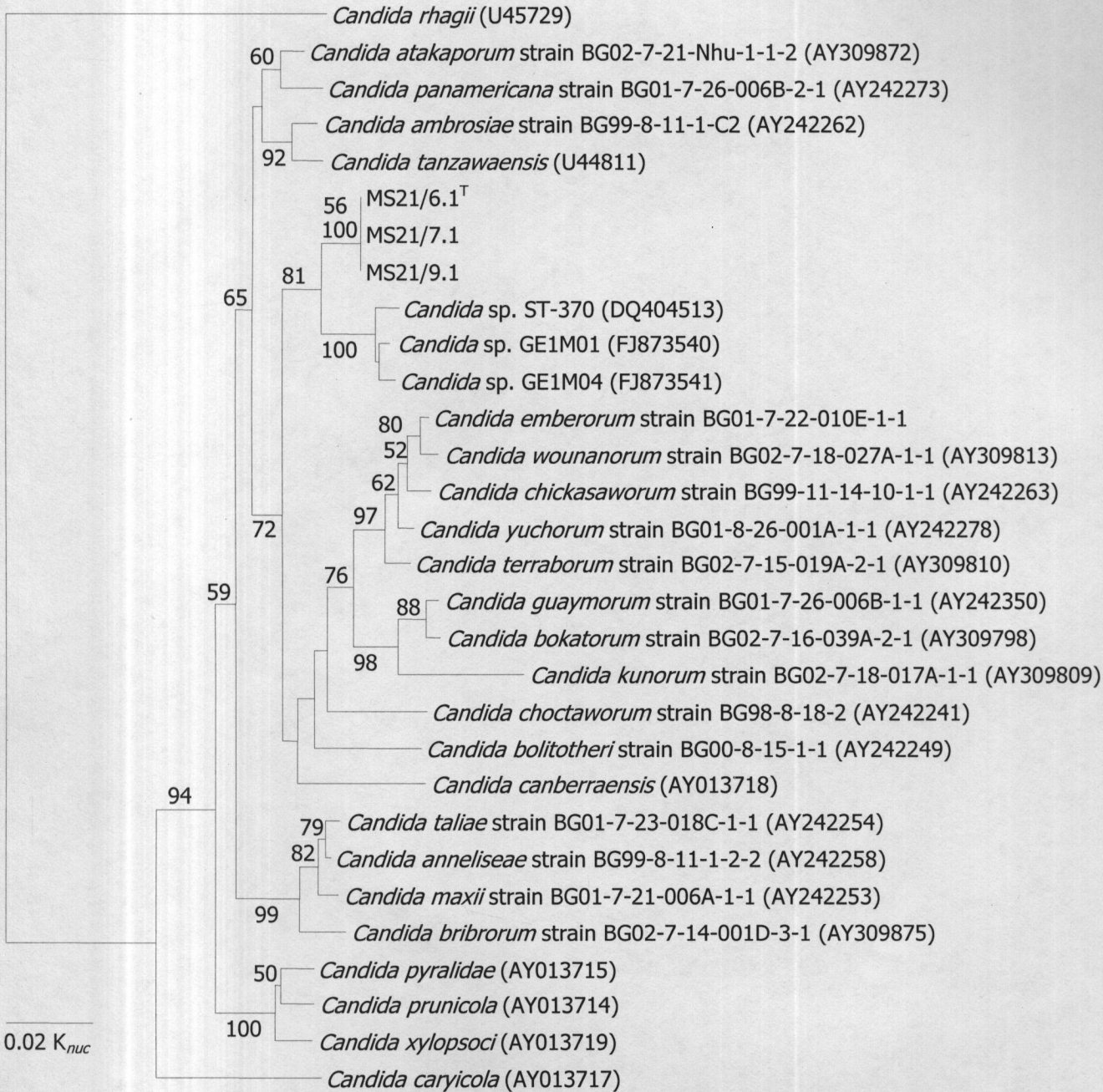


Fig. 2.

