

Yeasts from phylloplane and their capability to produce indole-3-acetic acid

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Abstract Yeasts were isolated from the phylloplane of various plant species collected from seven provinces in Thailand. A total of 114 yeast strains and 10 strains of a yeast-like fungus were obtained by enrichment isolation from 91 out of 97 leaf samples (93.8 %). On the basis of the D1/D2 domain of the large subunit rRNA gene sequence similarity, 98 strains were identified to be of 36 yeast species in 18 genera belonging to Ascomycota viz. *Candida*, *Clavispora*, *Cyberlindnera*, *Debaryomyces*, *Hanseniaspora*, *Hyphopichia*, *Kazachstania*, *Kluyveromyces*, *Kodamaea*, *Lachancea*, *Metschnikowia*, *Meyerozyma*, *Pichia*, *Starmerella*, *Torulasporea* and *Wickerhamomyces*, and to Basidiomycota viz. *Sporidiobolus* and *Trichosporon*. Three strains were found to represent two novelties *Candida* species which were previously described as *C. sirachaensis* and *C. sakaensis*. Ten strains of yeast-like fungus were identified as *Aureobasidium pullulans* of the phylum Ascomycota. Ascomycetous yeast species accounted altogether for 98.0 % of the 98 strains. The prevalent species was *Candida tropicalis* with a low frequency of isolation (14.3 %). Diversity of yeasts other than ballistoconidium-forming yeast in phylloplane in a tropical country in Asia has been reported for the first time. All strains obtained were accessed for the capability to produce IAA and result revealed that 39 strains in 20 species, one strain each of an undescribed and a novel species, and two unidentified strains showed the

capability of producing IAA when cultivated in yeast extract peptone dextrose broth supplemented with 0.1 % L-tryptophan. All five strains of *Candida maltosa* produced relatively high concentrations of IAA.

Keywords Phylloplane · Yeast · Indole-3-acetic acid · Thailand

Introduction

The external surface of plant leaves, which is usually referred to as the phylloplane or phyllosphere, has been recognized as an important habitat for epiphytic microorganisms (Fonseca and Inacio 2006; Phaff and Starmer 1987). In the phylloplane, the growth of microorganisms is dependent on nutrients from plant metabolites that are secreted to the phylloplane or on compounds in materials from external sources that drop on the plant surface. The plant metabolites are organic substances, mostly simple sugars e.g. glucose, fructose and sucrose, while the materials from external sources are inorganic nutrients (Xin et al. 2009). While bacteria are the most abundant phylloplane microorganisms, yeasts and yeast-like fungi such as *Aureobasidium pullulans* are also active phylloplane colonizers (Andrews and Harris 2000). The phylloplane of diverse temperate, tropical and Mediterranean plants have been found to be colonized by both basidiomycetous and ascomycetous yeasts (Fonseca and Inacio 2006; Glushakova and Chernov 2010; Glushakova et al. 2007; Inácio et al. 2005; Landell et al. 2010; Nakase et al. 2001; Slavikova et al. 2009). Although the common phylloplane yeasts were basidiomycetous species belonging to the genera *Bullera*, *Cryptococcus*, *Dioszegia*, *Rhodotorula*, *Sporobolomyces*, *Tilletiopsis* and

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Trichosporon such as *Cryptococcus laurentii*, *Rhodotorula mucilaginosa*, *Rhodotorula glutinis* and *Sporobolomyces roseus* (de Azeredo et al. 1998; Fonseca and Inacio 2006; Glushakova et al. 2007; Nakase et al. 2001; Sharma et al. 2009; Slavikova et al. 2009), some ascomycetous species have also been found such as *Debaryomyces hansenii*, *Hanseniaspora uvarum*, *Kazachstania barnettii*, *Metschnikowia pulcherima*, *Metschnikowia reukaufii*, *Pichia membranifaciens*, *Saccharomyces cerevisiae* and various species of *Candida* (Glushakova and Chernov 2010; Glushakova et al. 2007; Koowadjanakul et al. 2011; Landell et al. 2010; Sharma et al. 2009; Slavikova et al. 2009). Yeasts colonizing the phylloplane were studied intensively; however, limited investigation on diversity of yeasts in phylloplane in tropical area was conducted so far. For example Nakase et al. (2001) reported ballistoconidium-forming yeasts found in the phylloplane of Thailand.

Indole-3-acetic acid (IAA) is the major member of plant growth promoter in the auxin class that is known to stimulate both rapid and long-term responses in plants by regulation of various developmental and physiological processes (Cleland 1990). It is produced by plants and microorganisms including bacteria (Xinxian et al. 2011), actinomycetes (Khamna et al. 2010), yeasts (El-Tarabily 2004; Nakamura et al. 1991; Nassar et al. 2005), and filamentous fungi (Ruanpanun et al. 2010). IAA-producing microorganisms are receiving attention as good sources of biofertilizer (Sasikala and Ramana 1998). Applications of IAA producing yeasts, such as *S. roseus*, *Candida valida*, *R. glutinis* and *Trichosporon asahii*, *Lindera (Williopsis) saturnus* and *R. mucilaginosa*, to promoting growth of plants have been reported (El-Tarabily 2004; Nassar et al. 2005; Perondi et al. 1996; Xin et al. 2009).

There is little information on IAA-producing yeasts, especially those of the phylloplane. Therefore, the objectives of this study were isolation and identification of yeasts isolated from phylloplane in Thailand, followed by assessment of their capacity to produce indole-3-acetic acid in vitro.

Materials and methods

Sample collection

Green and undamaged plant leaves were collected and placed in plastic bags, sealed and transferred in ice-box to laboratory. The samples were kept at 8 °C until subjected for yeast isolation.

Yeast isolation

Yeast was isolated by an enrichment technique using yeast extract malt extract (YM) broth (3 g/L yeast extract, 3 g/L

malt extract, 5 g/L peptone and 10 g/L glucose) supplemented with 0.025 % sodium propionate and 0.02 % chloramphenicol (Limtong et al. 2007). Three grams of cut leaves, derived from cutting few leaves to the size that can be put into a 250 mL Erlenmeyer, was inoculated into 50 mL enrichment broth in the flask and incubated on a rotary shaker at 30 ± 3 °C for 2 days. A loopful of the enriched culture was streaked on YM agar supplemented with 25 mg/L sodium propionate and 20 mg/L chloramphenicol. Yeast colonies of different morphologies were picked and purified by cross streaking on YM agar. Purified yeast strains were suspended in YM broth supplemented with 10 % v/v glycerol and maintained at -80 °C.

Yeasts identification and phylogenetic analysis

Yeasts were identified by molecular taxonomy based on the analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene sequences similarities according to a guideline of Kurtzman and Robnett (1998) that yeast strains with 0–3 nucleotide differences are conspecific or sister species and yeast strains showing nucleotide substitutions greater than 6 are usually different species. Therefore, a strain showing nucleotide substitutions greater than six from the type strain of the closest species could be designed as the novel species. Undescribed species are the species that the D1/D2 domain of the LSU rRNA gene sequences was deposited in the GenBank without description.

Methods for DNA isolation and amplification of the D1/D2 domain of the LSU rRNA gene were as described previously by Limtong et al. (2007). The PCR product was checked by agarose gel electrophoresis and purified by using the QIA quick purification kit (Qiagen, Hilden, Germany). The purified product was sequenced commercially by Macrogen Inc. (Seoul, Korea) with primers, NL1 and NL4. The sequences were compared pairwise using a BLAST search (Altschul et al. 1997).

A phylogenetic tree was constructed from the evolutionary distance data with Kimura's two-parameter correction (Kimura 1980), using the neighbor-joining method (Saitou and Nei 1987). Confidence levels of the clades were estimated from bootstrap analysis (1,000 replicates; Felsenstein 1985).

Determination of indole-3-acetic acid production

Production of indole-3-acetic acid (IAA) by the phylloplane yeasts was investigated by the method of Xin et al. (2009). A yeast culture cultivated for 1–2 days on YM agar at 25 °C was inoculated in 5 mL of yeast extract peptone dextrose (YPD) broth (10 g/L yeast extract, 2 g/L peptone and 2 g/L dextrose) supplemented with 1 g/L L-tryptophan in a test tube and incubated on a shaker at 30 ± 2 °C and

150 rpm for 7 days. An aliquot of 1.5 mL of the culture broth was centrifuged at 8,000 rpm for 5 min and the supernatant was collected for determination of IAA concentration. One mL of supernatant was mixed with 1 mL of Salkowski reagent (12 g/l FeCl₃ and 7.9 M H₂SO₄; Glickmann and Dessaux 1994), and the intensity of pink color developing in the mixture after 30 min was quantified with a spectrophotometer (UV-1700, Shimadzu, Japan) at a wavelength of 530 nm. Calibration curve using pure IAA was established for calculation of IAA concentration. Growth was determined as dry weight by drying cells after centrifugation at 100 °C until constant weight was obtained.

Results

Sample collection and yeast isolation

Yeasts were isolated from the phylloplane of 76 leaf samples of 45 plant species and 21 samples of unknown plants which had been collected from 19 locations in seven provinces in the eastern, central, north–eastern and peninsular regions of Thailand during April and May 2009 (Table 1).

A total of 114 yeast strains and 10 strains of yeast-like fungus were obtained from 91 samples representing 93.8 % of the samples investigated (Table 1). Among these 91 samples three samples contained only the yeast-like fungus.

Yeast identification

On the basis of the D1/D2 domain of the LSU rRNA gene sequence similarity and the generally accepted criteria of Kurtzman and Robnett (1998), 96 yeast strains were identified to be 36 species in 18 genera (Tables 1 and 2). Thirty-four species were in 16 genera of Phylum Ascomycota (15 genera) viz. *Candida* (15 species), *Clavispora* (1 species), *Cyberlindnera* (1 species), *Debaryomyces* (1 species), *Hanseniaspora* (3 species), *Hyphopichia* (1 species), *Kazachstania* (1 species), *Kluyveromyces* (1 species), *Kodamaea* (1 species), *Lachancea* (1 species), *Metschnikowia* (1 species), *Meyerozyma* (1 species), *Pichia* (2 species), *Starmerella* (1 species), *Torulaspora* (2 species) and *Wickerhamomyces* (1 species), and two species were in Phylum Basidiomycota (2 genera) viz. *Sporidiobolus* (1 species), and *Trichosporon* (1 species). Four strains were similar to three undescribed species in Ascomycota and nine strains require further analysis for identification. Three strains were found to represent two novels *Candida* species which were previously proposed to be *C. sirachaensis* and *C. sakaeensis*. The 10 strains of yeast-like fungus were

identified to be *A. pullulans* (Table 1, 2). Phylogenetic positions of all yeast species obtained in this study were shown in Fig. 1. Most *Candida* species were distributed in five phylogenetically distinct clades including *Kodamaea*, *Lodderomyces-Spathospora*, *Nakaseozyma*, *Starmerella* and *Yamadazyma* clades while *Candida rugosa* was not placed in any clade.

The results indicated that 98.0 % of the strains isolated by the enrichment technique were ascomycetous yeasts, and only 2.0 % represented basidiomycetous species. The dominant species was *Candida tropicalis*, although only 14 strains of this species were isolated from 14 samples, represented a 14.3 % frequency of isolation (Table 2). Ten strains of *A. pullulans*, the yeast-like fungus, were isolated from 10 samples, giving a frequency of isolation of 7.8 %.

Iodole-3-acetic acid production

Among the 114 strains of yeast, 39 strains in 20 species, one strain of an undescribed species, one strain of a novel species, and two unidentified strains showed the capability of producing IAA when cultivated in YPD broth supplemented with 0.1 % L-tryptophan (Table 3). The other 71 strains grew in this medium, but no IAA was produced. All five strains of *C. maltosa* produced relatively high concentrations (121.4–234.1 mg/L) of IAA. This result indicated IAA production was strain-dependent; some strains of some species were able to produce IAA while others were not. All 10 strains of *A. pullulans* produced IAA; however, the concentrations were relatively low.

Discussion

The two findings of the present study were, first, that phylloplane yeasts are present on diverse plant species in Thailand and *C. tropicalis* was frequently found and second, that approximately 37.7 % of the phylloplane yeasts found were capable of in vitro IAA biosynthesis. The study found that by enrichment isolation at 30 ± 3 °C most yeasts obtained from phylloplane in Thailand were in the phylum Ascomycota (98.0 %); this is in contrast with the other investigations, which report the dominance of basidiomycetous yeasts on the phylloplane in the other regions (de Azeredo et al. 1998; Fonseca and Inacio 2006; Glushakova et al. 2007; Nakase et al. 2001; Sharma et al. 2009). This difference may have resulted from the different technique and incubation temperature employed for isolation. While in most investigations leaf washing followed by dilution plating technique was used, Nakase et al. (2001) used the ballistoconidium fall method with YM agar without any antibacterial and antifungal agents, and they found more yeast species when the incubation temperature

Table 1 Leaf from diverse plant species and yeasts isolated from phylloplane with their accession numbers of the D1/D2 domain of the large subunit rRNA gene

Sample collection		Identification of yeast and yeast like fungus				Identification result	
Location (date)	Plant	Strain	Closest species (GenBank accession no.)	Nucleotide substitutions/total nt	Species	Accession no. (D1/D2)	
Wachirawat Boy Scout Camp, Si Racha district, Chonburi province (26 April 2009)	Bermese Ebony (<i>Pterocarpus macrocarpus</i> Kurz.)	LM001	<i>Candida glabrata</i> (U44808)	1/581	<i>C. glabrata</i>	AB617902	
	Teak (<i>Tectona grandis</i> Linn.)	LM002	<i>Kazachstania siamensis</i> (AB258462)	0/574	<i>K. siamensis</i>	AB617903	
	Eucalyptus (<i>Eucalyptus camaldulensis</i> Dehnh.)	LM003	<i>Candida stigmatidis</i> (GQ184144)	0/423	<i>C. stigmatidis</i>	AB617904	
	Mango (<i>Mangifera indica</i> Linn.)	–	–	–	–	–	
	Black Wattle (<i>Acacia auriculaeformis</i> Cunn.)	LM004	<i>Candida etchellsii</i> (U45723)	0/482	<i>C. etchellsii</i>	AB617905	
	Canna (<i>Canna indica</i> Linn.)	LM005	<i>Candida apicola</i> (U45703)	2/480	<i>C. apicola</i>	AB617906	
	Cashew Nut (<i>Anacardium occidentale</i> Linn.)	–	–	–	–	–	
	Jack Fruit (<i>Artocarpus eterophyllus</i> Lam.)	LM006	<i>Pichia kudriavzevii</i> (U76347)	0/564	<i>P. kudriavzevii</i>	AB617907	
	–	LM007	<i>Hanseniaspora opuntiae</i> (AJ512453)	0/572	<i>H. opuntiae</i>	AB617908	
	Bengal Almond (<i>Terminalia catappa</i> Linn.)	–	–	–	–	–	
	Copper pod (<i>Peltophorum pterocarpum</i> (DC.) Backer ex K. Heyne.)	LM008	<i>Candida bombi</i> (AF406929)	50/485	<i>Candida sirachaensis</i> sp. nov. ^a	AB617909	
	Flame Tree (<i>Delonix regia</i> (Bojer) Raf.)	LM009	<i>K. siamensis</i> (AB258462)	0/574	<i>K. siamensis</i>	AB617910	
	Rain Tree (<i>Samanea saman</i> Merr.)	–	–	–	–	–	
	Siamese Rough Bush (<i>Streblus asper</i> Lour.)	LM010	<i>C. glabrata</i> (U44808)	1/581	<i>C. glabrata</i>	AB617911	
	Kurchi (<i>Wrightia religiosa</i> Benth.)	–	–	–	–	–	
	Chinese Rose (<i>Hibiscus rosa-sinensis</i> Linn.)	LM011	<i>C. glabrata</i> (U44808)	1/581	<i>C. glabrata</i>	AB617912	
	Tamarind (<i>Tamarindus indica</i> Linn.)	LM012	<i>C. glabrata</i> (U44808)	1/581	<i>C. glabrata</i>	AB617913	
	Devil Tree (<i>Alstonia scholaris</i> (L.) R. Br.)	LM013	<i>Candida potacharoentiae</i> (AB537430)	0/450	<i>C. potacharoentiae</i>	AB617914	
	She Oak (<i>Casuarina junghuhntiana</i> Miq.)	–	–	–	–	–	
	Golden Shower Tree (<i>Cassia fistula</i> Linn.)	LM014	<i>C. etchellsii</i> (U45723)	0/482	<i>C. etchellsii</i>	AB617915	
	Coconut (<i>Cocos nucifera</i> Linn.)	LM015	<i>Sporidiobolus ruineniae</i> (AF387128)	1/574	<i>S. ruineniae</i>	AB617916	
	–	LM016	–	–	–	–	
	–	LM017	<i>A. pullulans</i> (FJ515219)	0/571	<i>A. pullulans</i>	AB617918	
	Bougainvillea (<i>Bougainvillea</i> sp.)	LM018	<i>A. pullulans</i> (FJ515219)	1/571	<i>A. pullulans</i>	AB617919	
Unknown	LM019	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617920		
West Indian Jasmine (<i>Jaxora chinensis</i> Lamk.)	LM020	<i>A. pullulans</i> (FJ515219)	0/571	<i>A. pullulans</i>	AB617921		
Papaya (<i>Carica papaya</i> Linn.)	LM021	<i>A. pullulans</i> (FJ515219)	0/577	<i>A. pullulans</i>	AB617922		
Bamboo (<i>Bambusa</i> sp.)	LM022	<i>Hyphopichia burtonii</i> (U45712)	0/524	<i>H. burtonii</i>	AB617923		
–	LM023	<i>Pichia</i> sp. ST-335 (DQ404505)	0/530	Similar to <i>Pichia</i> sp. ST-335	AB617924		
Jujube (<i>Zizyphus mauritiana</i> Lamk.)	LM024	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617925		

Table 1 continued

Sample collection		Identification of yeast and yeast like fungus				Identification result	
Location (date)	Plant	Strain	Closest species (GenBank accession no.)	Nucleotide substitutions/total nt	Species	Accession no. (D1/D2)	
Khlong Bod reservoir, Mueang Nakhon Nayok district, Nakhon Nayok province (10 May 2009)	Governor's plum (<i>Flacourtia cataphracta</i> Roxb.)	LM025	<i>A. pullulans</i> (FJ515219)	0/571	<i>A. pullulans</i>	AB617926	
	Bengal Almond (<i>T. catappa</i> Linn.)	LM026	<i>Candida trypodendronii</i> (AF017240)	2/532	<i>C. trypodendronii</i>	AB617927	
	Bitter Bush (<i>Eupatorium odoratum</i> Linn.)	LM028	<i>Wickerhamomyces edaphicus</i> (AB436763)	0/567	<i>W. edaphicus</i>	AB617929	
		LM029	<i>Metschnikowia korensis</i> (AF296438)	0/512	<i>M. korensis</i>	AB617930	
		LM030	<i>P. kudriavzevii</i> (U76347)	0/564	<i>P. kudriavzevii</i>	AB617931	
		LM031	<i>A. pullulans</i> (FJ515219)	0/578	<i>A. pullulans</i>	AB617932	
		LM032	<i>Starmerella meliponinorum</i> (AF313354)	1/478	<i>S. meliponinorum</i>	AB617933	
Khundanprakanchon dam, Mueang Nakhon Nayok district, Nakhon Nayok province (10 May 2009)	Black Wattle (<i>A. auriculaeformis</i> Cunn.)	LM034	<i>C. amphixiae</i> (FJ614671)	2/542	<i>C. amphixiae</i>	AB617935	
	Frangipan (temple tree) (<i>Plumeria</i> sp.)	LM035	<i>S. meliponinorum</i> (AF313354)	1/478	<i>S. meliponinorum</i>	AB617936	
		LM036	<i>A. pullulans</i> (FJ515219)	0/577	<i>A. pullulans</i>	AB617937	
	Tamarind (<i>T. indica</i> Linn.)	LM037	<i>Kodamaea ohmeri</i> (U45702)	0/486	<i>K. ohmeri</i>	AB617938	
	Golden Shower Tree (<i>C. fistula</i> Linn.)	LM038	<i>A. pullulans</i> (FJ515219)	0/557	<i>A. pullulans</i>	AB617939	
		LM039	<i>C. jaroonii</i> (DQ404493)	0/541	<i>C. jaroonii</i>	AB617940	
	Flame Tree (<i>D. regia</i> (Bojer) Raf.)	LM040	<i>P. kudriavzevii</i> (U76347)	0/562	<i>P. kudriavzevii</i>	AB617941	
Chak Pong reservoir, Mueang district, Prachin Buri province (10 May 2009)	Bougainvillea (<i>Bougainvillea</i> sp.)	LM041	<i>Torulasporea delbrueckii</i> (U72156)	0/573	<i>T. delbrueckii</i>	AB617943	
		LM042	<i>A. pullulans</i> (FJ515219)	0/571	<i>A. pullulans</i>	AB617944	
	Mango (<i>M. indica</i> Linn.)	LM043	<i>H. opuntiae</i> (AJ512453)	0/572	<i>H. opuntiae</i>	AB617945	
	Black Wattle (<i>A. auriculaeformis</i> Cunn.)	LM044	<i>Pichia manshurica</i> (EF550223)	0/566	<i>P. manshurica</i>	AB617946	
	Wild Tamarind (<i>Leucaena leucocephala</i> (Lam) de Wit.)	LM045	<i>Torulasporea pretoriensis</i> (U72157)	0/574	<i>T. pretoriensis</i>	AB617947	
		LM046	<i>H. burtonii</i> (U45712)	0/524	<i>H. burtonii</i>	AB617948	
		LM047	<i>Lachancea thermotolerans</i> (U69581)	0/571	<i>L. thermotolerans</i>	AB617949	
Ito mountain, Mueang district, Prachin Buri province (10 May 2009)	Bitter Bush (<i>E. odoratum</i> Linn.)	LM048	<i>A. pullulans</i> (FJ515219)	0/571	<i>A. pullulans</i>	AB617950	
	Sacred Fig Tree (<i>Ficus religiosa</i> Linn.)	LM049	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617951	
		LM050	<i>C. rugosa</i> (U45727)	1/485	<i>C. rugosa</i>	AB617952	
	Devil Tree (<i>A. scholaris</i> (L.) R. Br.)	LM051	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617953	
	Tamarind (<i>T. indica</i> Linn.)	LM052	<i>K. ohmeri</i> (U45702)	2/493	<i>K. ohmeri</i>	AB617954	
		LM053	<i>C. rugosa</i> (U45727)	2/484	<i>C. rugosa</i>	AB617955	
	Burma Padauk (<i>Pterocarpus indicus</i> Willd.)	LM054	<i>P. kudriavzevii</i> (U76347)	0/564	<i>P. kudriavzevii</i>	AB617956	
Frangipan (temple tree) (<i>Plumeria</i> sp.)		LM055	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617957	
		LM057	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617958	

Table 1 continued

Sample collection		Identification of yeast and yeast like fungus			Identification result	
Location (date)	Plant	Strain	Closest species (GenBank accession no.)	Nucleotide substitutions/total nt	Species	Accession no. (D1/D2)
Mueang Sa Kaeo district, Sakoeo province (11 May 2009)	Manaila Tamarind (<i>Pithecellobium dulce</i> (Roxb.) Benth.)	LM059	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617959
		LM060	<i>C. rugosa</i> (U45727)	1/485	<i>C. rugosa</i>	AB617960
Wathana Nakhon district, Sakoeo province (11 May 2009)	Crown Flower (<i>Calotropis gigantea</i> (Linn.) R.Br.ex Ait.)	LM061	<i>K. ohmeri</i> (U45702)	0/493	<i>K. ohmeri</i>	AB617961
	Jujube (<i>Z. mauritiana</i> Lamk.)	LM062	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617962
	Sugarcane (<i>Saccharum officinarum</i> Linn.)	LM063	<i>P. kudrivazevii</i> (U76347)	0/564	<i>P. kudrivazevii</i>	AB617963
		LM064	<i>H. opuntiae</i> (AJ512453)	0/572	<i>H. opuntiae</i>	AB617964
		LM065	–	–	Unidentified	–
Phra Prong reservoir, Wathana Nakhon district, Sakoeo province (11 May 2009)	Devil Tree (<i>A. scholaris</i> (L.) R. Br.)	LM066	<i>C. rugosa</i> (U45727)	1/485	<i>C. rugosa</i>	AB617966
	Golden Shower Tree (<i>C. fistula</i> Linn.)	LM067	<i>H. opuntiae</i> (AJ512453)	1/572	<i>H. opuntiae</i>	AB617967
	Frangipan (temple tree) (<i>Plumeria</i> sp.)	LM068	<i>Candida morakotiae</i> (DQ400364)	14/574	<i>Candida sakaeensis</i> sp. nov. ^a	AB617968
	Lotus (<i>Nelumbo nucifera</i> Gaertn.)	LM069	<i>C. mallosa</i> (U45745)	0/572	<i>C. mallosa</i>	AB617969
	Teak (<i>T. grandis</i> Linn.)	LM070	<i>C. glabrata</i> (U44808)	1/581	<i>C. glabrata</i>	AB617970
	Dasheen (<i>Colocasia esculenta</i> (L.) Schott.)	LM071	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617971
	Crown Flower (<i>C. gigantea</i> (Linn.) R.Br.ex Ait.)	LM072	<i>C. rugosa</i> (U45727)	1/485	<i>C. rugosa</i>	AB617972
Huai Chan reservoir, Ta Phraya district, Sakoeo province (11 May 2009)	Crown Flower (<i>C. gigantea</i> (Linn.) R.Br.ex Ait.)	LM073	<i>P. manshurica</i> (U75738)	0/560	<i>P. manshurica</i>	AB617973
	Unknown	LM074	<i>C. rugosa</i> (U45727)	0/484	<i>C. rugosa</i>	AB617974
	Coconut (<i>C. nucifera</i> Linn.)	LM075	<i>Cyberlindnera rhodanensis</i> (U73571)	3/578	<i>C. a rhodanensis</i>	AB617975
	Burma Padauk (<i>P. indicus</i> Willd.)	LM076	<i>H. burtonii</i> (U45712)	0/524	<i>H. burtonii</i>	AB617976
	Sacred Fig Tree (<i>F. religiosa</i> Linn.)	LM077	<i>Candida metapsilosis</i> (FJ746055)	0/556	<i>C. metapsilosis</i>	AB617977
Water reservoir, Mueang district, Buri Ran province (11 May 2009)	Weeping fig (<i>Ficus benjamina</i> Linn.)	LM078	<i>C. morakotiae</i> (DQ400364)	14/574	<i>C. sakaeensis</i> sp. nov. ^a	AB617978
	West Indian Jasmine (<i>J. chinensis</i> Lamk.)	LM079	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617979
	Star Gooseberry (<i>Phyllanthus acidus</i> (Linn.) Skeels.)	LM080	<i>H. opuntiae</i> (AJ512453)	0/582	<i>H. opuntiae</i>	AB617980
	Pineapple (<i>Ananas comosus</i> (L.) Merr.)	LM081	<i>Kluyveromyces marxianus</i> (U94924)	1/545	<i>K. marxianus</i>	AB617981
	Sugarcane (dry leaves) (<i>S. officinarum</i> Linn.)	LM082	<i>C. glabrata</i> (U44808)	1/581	<i>C. glabrata</i>	AB617982
		LM083	<i>C. lusitanae</i> (GQ396270)	0/517	<i>C. lusitanae</i>	AB617983
		LM084	<i>Candida sorboxylosa</i> (U62314)	4/339	<i>C. sorboxylosa</i>	AB617984
		LM085	<i>Trichosporon asahii</i> (AF105393)	0/606	<i>T. asahii</i>	AB617985
		LM086	–	–	Unidentified	–

Table 1 continued

Sample collection		Identification of yeast and yeast like fungus			Identification result	
Location (date)	Plant	Strain	Closest species (GenBank accession no.)	Nucleotide substitutions/total nt	Species	Accession no. (D1/D2)
Hua Hin district, Prachuap Khiri Khan province (18 May 2009)	Sugarcane (<i>S. officinarum</i> Linn.)	LM087	–	–	Unidentified	–
	Sugarcane (dry leaves) (<i>S. officinarum</i> Linn.)	LM088	–	–	Unidentified	–
Mueang Chumphon district, Chumphon province (18 May 2009)	Jujube (<i>Z. mauritiana</i> Lamk.)	LM089	<i>P. kudriavzevii</i> (U76347)	0/564	<i>P. kudriavzevii</i>	AB617989
Paknam Chumphon, Chumphon province (18 May 2009)	Palm (<i>Palmae</i> sp.)	LM090	<i>K. ohmeri</i> (U45702)	0/493	<i>K. ohmeri</i>	AB617990
		LM091	<i>Debaryomyces nepalensis</i> (U45839)	1/570	<i>D. nepalensis</i>	AB617991
	Palm (dry leaves) (<i>Palmae</i> sp.)	LM092	<i>Geotrichum</i> sp. SKK15 (AB500211)	0/545	Similar to <i>Geotrichum</i> sp. SKK15	AB617992
		LM093	–	–	Unidentified	–
	LM094	–	–	–	Unidentified	–
	LM095	<i>D. nepalensis</i> (U45839)	1/570	<i>D. nepalensis</i>	AB617995	
	Para rubber (<i>Hevea brasiliensis</i> Müll. Arg.)	LM125	<i>Candida</i> sp. 05-7-186T (FJ537069)	0/495	Similar to <i>Candida</i> sp. 05-7-186T	AB618025
	Pumelo (<i>Citrus maxima</i> (Burm.) Merr.)	LM126	<i>Candida</i> sp. 05-7-186T (FJ537069)	0/495	Similar to <i>Candida</i> sp. 05-7-186T	AB618026
	Unknown	LM127	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB618027
	Mangosteen (<i>Garcinia mangostana</i> Linn.)	LM128	<i>P. kudriavzevii</i> (U76347)	0/564	<i>P. kudriavzevii</i>	AB618028
	Banana (<i>Musa sapientum</i> Linn.)	LM129	<i>H. guilliermondii</i> (U84230)	0/572	<i>H. guilliermondii</i>	AB618029
Forest on Mattra island, Chumphon Islands National Park, Chumphon province (19 May 2009)	Banana (<i>M. sapientum</i> Linn.)	LM096	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB617996
		LM097	<i>Hanseniaspora thailandica</i> (DQ404527)	0/572	<i>H. thailandica</i>	AB617997
	Unknown	LM098	<i>H. thailandica</i> (DQ404527)	0/579	<i>H. thailandica</i>	AB617998
	Unknown	LM099	<i>Candida parapsilosis</i> (U45754)	0/570	<i>C. parapsilosis</i>	AB617999
	Unknown	LM100	<i>C. maltosa</i> (U45745)	2/572	<i>C. maltosa</i>	AB618000
	Unknown	LM101	<i>D. nepalensis</i> (U45839)	1/570	<i>D. nepalensis</i>	AB618001
	Unknown	LM102	<i>Candida nivariensis</i> (AY627305)	1/549	<i>C. nivariensis</i>	AB618002
	Unknown	LM103	<i>Candida maltosa</i> (U45745)	2/572	<i>C. maltosa</i>	AB618003
	Unknown	LM104	<i>D. nepalensis</i> (U45839)	1/570	<i>D. nepalensis</i>	AB618004
	Unknown	LM105	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB618005
	Unknown	LM106	–	–	Unidentified	–
Unknown	LM107	<i>D. nepalensis</i> (U45839)	1/570	<i>D. nepalensis</i>	AB618007	
Unknown	LM109	<i>C. nivariensis</i> (AY627305)	1/549	<i>C. nivariensis</i>	AB618009	
Unknown	LM110	<i>C. maltosa</i> (U45745)	2/572	<i>C. maltosa</i>	AB618010	
Unknown	LM111	<i>K. ohmeri</i> (U45702)	0/493	<i>K. ohmeri</i>	AB618011	

Table 1 continued

Sample collection		Identification of yeast and yeast like fungus			Identification result	
Location (date)	Plant	Strain	Closest species (GenBank accession no.)	Nucleotide substitutions/total nt	Species	Accession no. (D1/D2)
Bang Saphan district, Prachuap Khiri Khan province (20 May 2009)	Unknown	LM112	<i>H. thailandica</i> (DQ404527)	0/572	<i>H. thailandica</i>	AB618012
	Unknown	LM113	–	–	Unidentified	–
	Unknown	LM114	<i>C. maltosa</i> (U45745)	2/572	<i>C. maltosa</i>	AB618014
	Unknown	LM115	<i>C. nivariensis</i> (AY627305)	1/549	<i>C. nivariensis</i>	AB618015
	Unknown	LM116	<i>C. nivariensis</i> (AY627305)	1/549	<i>C. nivariensis</i>	AB618016
	Unknown	LM118	<i>C. tropicalis</i> (U45749)	0/570	<i>C. tropicalis</i>	AB618018
	Unknown	LM119	<i>H. burtonii</i> (U45712)	0/524	<i>H. burtonii</i>	AB618019
	Unknown	LM120	<i>M. guilliermondii</i> (U45709)	2/570	<i>M. guilliermondii</i>	AB618020
	Sugarcane (<i>S. officinarum</i> Linn.)	LM121	<i>C. glabrata</i> (U44808)	1/581	<i>C. glabrata</i>	AB618021
	Fig (<i>Ficus racemosa</i> Linn.)	LM122	<i>C. amphixiae</i> (AY520327)	2/533	<i>C. amphixiae</i>	AB618022
	Coconut (<i>C. nucifera</i> Linn.)	LM123	<i>C. nivariensis</i> (AY627305)	1/543	<i>C. nivariensis</i>	AB618023
	Unknown	LM124	<i>D. nepalensis</i> (U45839)	3/570	<i>D. nepalensis</i>	AB618024
	Frangipan (temple tree) (<i>Plumeria</i> sp.)	LM130	<i>K. ohmeri</i> (U45702)	1/493	<i>K. ohmeri</i>	AB618030

^a Limtong et al. (2012)

was at 23 °C than at 30 °C. This may due to the fact that most of basidiomycete yeasts grow rather slow and they have fairly low maximum growth temperature in comparison with ascomycete yeasts and the enrichment isolation at high temperature (30 ± 3 °C) selects rapid growing yeasts. Therefore, more ascomycete yeasts were obtained.

Among yeast species found on phylloplane in this study only *S. ruineniae* has been stated that its primary habitat is possible to be the phylloplane (Sampaio 2011). Strains of many yeast species found in this study were reported to isolate from insects viz. *Candida amphixiae*, *C. apicola*, *C. etchellsii*, *C. glabrata*, *C. trypodendroni*, *Debaryomyces nepalensis*, *Hanseniaspora guilliermondii*, *Hyphopichia burtonii*, *Kluyveromyces marxianus*, *Kodamae ohmeri*, *Lachances thermotolerans* and *Starmerella meliponinorum* (Cadez and Smith 2011; Kurtzman 2011a, d; Lachance 2011b; Lachance and Kurtzman 2011a, b; Rosa et al. 2003) and from plants including flowers, fruits and tree parks viz. *Candida jaroonii*, *C. nivariensis*, *C. potacharoeniae*, *C. stigmatis*, *Clavispora lusitaniae*, *Hanseniaspora opuntiae*, *H. thailandica*, *Metschnikowia koreensis*, *Meyerozyma guilliermondii* and *Pichia kudriavzevii* (Cadez and Smith 2011; Imanishi et al. 2008; Kurtzman 2011b, c; Lachance 2011a, c; Lachance et al. 2011; Nakase et al. 2010; Jindamorakot et al. 2009; Sipiczki 2010). The present of these yeast species on the phylloplanes in this study may resulted from visiting of insects that carried these yeasts to the phylloplane. Strains of some species obtained in this study were previously reported to be the novel species discovered in Thailand isolated from the other sources viz. *C. jaroonii* (Imanishi et al. 2008), *C. potacharoeniae* (Nakase et al. 2010), *H. thailandica* (Jindamorakot et al. 2009), *Kazachstania siamensis* (Limtong et al. 2007) and *Wickerhamomyces edaphicus* (Limtong et al. 2009). Moreover, *A. pullulans*, the yeast-like fungus, which was found to occur regularly on the leaves of fruit trees in the Czech Republic (Slavikova et al. 2009), was also frequently found in the present study.

Investigation of the IAA production capability of the phylloplane yeasts in this study revealed that about 37.7 % of investigated yeast strains possessed this ability. It was found that among the species of one genus, both IAA producing species and non-producing species were detected. Moreover, not all strains within the same species had the ability to produce IAA. It therefore seems that IAA production capability is strain-dependent in these phylloplane yeasts. Variation in IAA biosynthesis among strains within the same species in the other microorganisms has also been reported by the other investigators (Ruanpanun et al. 2010; Tsavkelova et al. 2006).

IAA production by *C. maltosa* LM114 (314.3 mg/L) seemed to be higher than that reported for the other yeasts such as *Lindera (Williopsis) saturnus* (22.5 mg/L; Nassar

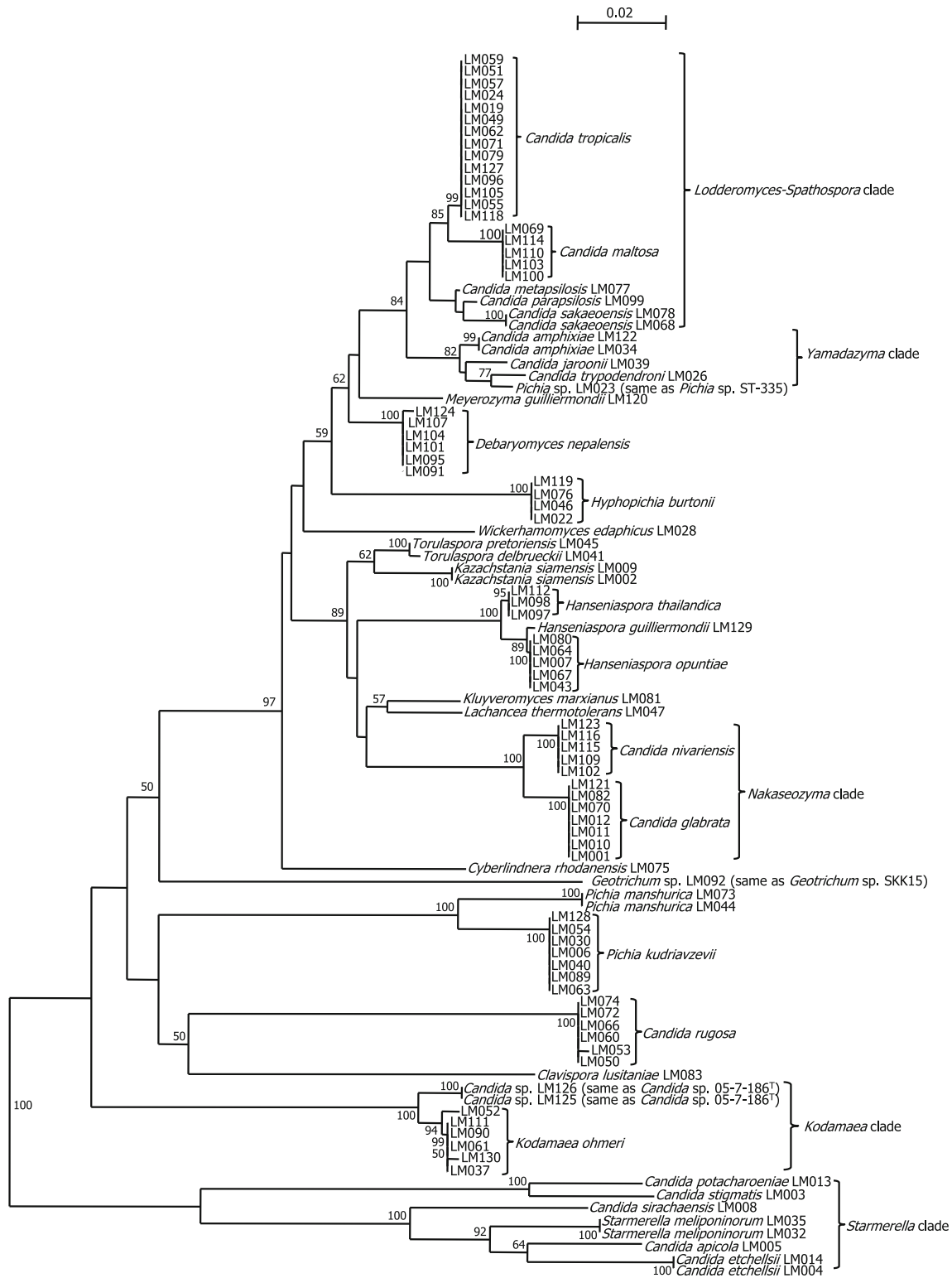


Fig. 1 Phylogeny of yeasts isolated from phylloplanes based on the D1/D2 domains of the LSU rRNA gene

et al. 2005); fungi such as *Aspergillus niger* (132.7 µg/mL; Bilkay et al. 2010); some actinomycetes such as *Streptomyces* sp. CMU-H009 (143.95 µg/mL; Khamna et al.

2010); and bacteria such as *Rubrivivax benzoatilyticus* JA2 (58.1 mg/L; Mujahid et al. 2001) and *Klebsiellas* sp. SN1 (291 mg/L; Xinxian et al. 2011). However, the

Table 2 Frequency of isolation of phylloplane yeasts obtained from diverse plant species in Thailand

Species and strain	No. of plant with this species	Frequency of isolation (%) ^a
Phylum Ascomycota		
<i>C. amphixiae</i>	2	2.04
<i>C. apicola</i>	1	1.02
<i>C. etchellsii</i>	2	2.04
<i>C. glabrata</i>	7	7.14
<i>C. jaroonii</i>	1	1.02
<i>C. maltosa</i>	5	5.10
<i>C. metapsilosis</i>	1	1.02
<i>C. nivariensis</i>	5	5.10
<i>C. parapsilosis</i>	1	1.02
<i>C. potacharoeniae</i>	1	1.02
<i>C. rugosa</i>	6	6.12
<i>C. sorboxylosa</i>	1	1.02
<i>C. stigmatis</i>	1	1.02
<i>C. tropicalis</i>	14	14.29
<i>C. trypodendroni</i>	1	1.02
<i>C. lusitaniae</i>	1	1.02
<i>C. rhodanensis</i>	1	1.02
<i>D. nepalensis</i>	6	6.12
<i>H. guilliermondii</i>	1	1.02
<i>H. opuntiae</i>	5	5.10
<i>H. thailandica</i>	3	3.06
<i>H. burtonii</i>	4	4.08
<i>K. siamensis</i>	2	2.04
<i>K. marxianus</i>	1	1.02
<i>K. ohmeri</i>	6	6.12
<i>L. thermotolerans</i>	1	1.02
<i>M. koreensis</i>	1	1.02
<i>M. guilliermondii</i>	1	1.02
<i>P. manshurica</i>	2	2.04
<i>P. kudriavzevii</i>	7	7.14
<i>S. meliponinorum</i>	2	2.04
<i>T. delbrueckii</i>	2	2.04
<i>W. edaphicus</i>	1	1.02
Subtotal of Ascomycota	96	97.96
Phylum Basidiomycota		
<i>S. ruineniae</i>	1	1.02
<i>T. asahii</i>	1	1.02
Subtotal of Basidiomycota	2	2.04
Total	98	100

^a Number of plant leaf sample from which that species was isolated/ number of plant leaf sample examined \times 100

concentration of IAA produced by *C. maltosa* LM114 needs to be confirmed with more specific method for IAA determination such as high-performance liquid chromatography or gas chromatography-mass spectrometry.

Table 3 Indole-3-acetic acid productions in YPD broth supplemented with 1 mg/L L-tryptophan in a test tube after 7 days by shake cultivation at 150 rpm and 30 \pm 3 °C

Species and strain	IAA (mg/L)
Phylum Ascomycota	
<i>C. amphixiae</i> LM034	–
<i>C. amphixiae</i> LM122	85.7
<i>C. apicola</i> LM005	–
<i>C. etchellsii</i> LM004, LM014	–
<i>C. glabrata</i> LM001	50.5
<i>C. glabrata</i> LM121	25.7
<i>C. glabrata</i> LM010, LM011, LM012, LM070, LM082	–
<i>C. jaroonii</i> LM039	–
<i>C. maltosa</i> LM069	121.4
<i>C. maltosa</i> LM100	130.5
<i>C. maltosa</i> LM103	237.1
<i>C. maltosa</i> LM110	159.5
<i>C. maltosa</i> LM114	314.3
<i>C. metapsilosis</i> LM077	81.0
<i>C. nivariensis</i> LM102	–
<i>C. nivariensis</i> LM109	39.0
<i>C. nivariensis</i> LM115	27.1
<i>C. nivariensis</i> LM116	37.6
<i>C. nivariensis</i> LM123	51.4
<i>C. parapsilosis</i> LM099	71.4
<i>C. potacharoeniae</i> LM013	–
<i>C. rugosa</i> LM050	–
<i>C. rugosa</i> LM060	98.6
<i>C. rugosa</i> LM066	101.9
<i>C. rugosa</i> LM074	66.9
<i>C. rugosa</i> LM053, LM072	–
<i>C. sorboxylosa</i> LM084	–
<i>C. stigmatis</i> LM003	–
<i>C. tropicalis</i> LM049	32.9
<i>C. tropicalis</i> LM051	54.3
<i>C. tropicalis</i> LM057	33.8
<i>C. tropicalis</i> LM062	31.9
<i>C. tropicalis</i> LM071	31.4
<i>C. tropicalis</i> LM105	43.8
<i>C. tropicalis</i> LM118	53.3
<i>C. tropicalis</i> LM127	37.6
<i>C. tropicalis</i> LM019, LM024, LM055, LM059, LM079, LM096	–
<i>C. trypodendroni</i> LM026	–
<i>C. lusitaniae</i> LM083	–
<i>C. rhodanensis</i> LM075	–
<i>D. nepalensis</i> LM107	32.4
<i>D. nepalensis</i> LM108	36.2
<i>D. nepalensis</i> LM091, LM124, LM095, LM101, LM104	–

Table 3 continued

Species and strain	IAA (mg/L)
<i>H. guilliermondii</i> LM129	–
<i>H. opuntiae</i> LM043	44.8
<i>H. opuntiae</i> LM007, LM064, LM067, LM080	–
<i>H. thailandica</i> LM097, LM112	–
<i>K. siamensis</i> LM002	43.3
<i>K. siamensis</i> LM009	–
<i>K. marxianus</i> LM081	–
<i>K. ohmeri</i> LM052	25.7
<i>K. ohmeri</i> LM111	24.3
<i>K. ohmeri</i> LM037, LM061, LM090, LM130	–
<i>L. thermotolerans</i> LM047	35.7
<i>M. koreensis</i> LM029	–
<i>M. guilliermondii</i> LM120	68.1
<i>H. burtonii</i> LM046	38.6
<i>H. burtonii</i> LM022, LM076, LM119	–
<i>P. manshurica</i> LM044, LM073	–
<i>P. kudriavzevii</i> LM128	27.1
<i>P. kudriavzevii</i> LM006, LM030, LM040, LM054, LM063, LM089	–
<i>S. meliponinorum</i> LM032, LM035	–
<i>T. delbrueckii</i> LM041	27.1
<i>T. pretoriensis</i> LM045	36.2
<i>W. edaphicus</i> LM028	–
Phylum Basidiomycota	–
<i>Sporidiobolus euineniae</i> LM015	84.3
<i>T. asahii</i> LM085	–
Novel species*	
<i>C. sirachaensis</i> LM068	31.9
<i>C. sakaeoensis</i> LM078	–
<i>C. sakaeoensis</i> LM008	–
Undescribed species	
Strain LM092 (similar to <i>Geotrichum</i> sp. SKK15)	–
Strain LM023 (similar to <i>Pichia</i> sp. ST-335)	–
Strain LM117 (similar to <i>Wickerhamia</i> sp. ST-122)	41.9
Strains LM125, LM126 (similar to <i>Candida</i> sp. 05-7-186T)	–
Unidentified strains	
Strain LM113 Unidentified	–
Strain LM093	62.9
Strain LM065	31.9
Strains LM016, LM086, LM087, LM088, LM094, LM106	–
Yeast-like fungus	
<i>A. pullulans</i> LM017	52.9
<i>A. pullulans</i> LM018	57.1
<i>A. pullulans</i> LM020	33.3
<i>A. pullulans</i> LM025	34.8
<i>A. pullulans</i> LM027	29.5

Table 3 continued

Species and strain	IAA (mg/L)
<i>A. pullulans</i> LM038	58.6
<i>A. pullulans</i> LM042	43.8
<i>A. pullulans</i> LM048	35.7
<i>A. pullulans</i> LM021, LM031, LM033, LM036	–

Remark: The results are means of two replications per strain

– = no IAA production

^a Limtong et al. (2012)

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