DIVERSITY OF YEASTS IN VETIVER PHYLLOPLANE IN THAILAND AND THEIR CAPABILITY TO PRODUCE INDOLE-3-ACETIC ACID, A PLANT GROWTH PROMOTER

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Abstract

The phylloplane is a term for the aboveground surface of plants and usually refers to the external surface of plant leaves, a dominant aerial part of plant. It is known to be colonized by a large number of microorganisms including bacteria, yeasts and fungi. Yeasts in phylloplane belong to either phylum Ascomycota or phylum Basidiomycota. In this study diversity of yeasts in the external surface of vetiver grass leaf in Thailand was investigated by culture dependent method and yeast strains were investigated for their capability to produce indole-3-acetic acid (IAA), a plant growth promoter. Thirty-four samples of vetiver leaf were collected from eight provinces *viz.* Ratchaburi, Kanchanaburi, Uthai Thani, Chiang Mai, Phayao, Uttaradit, Trat and Udon Thani. Yeasts were isolated from surface of leaf by an enrichment technique using yeast extract-malt extract broth supplemented with 0.025% sodium propionate and 0.02% chloramphenicol. A total of 40 yeast strains were obtained from 29 leaf samples (85.3%). Yeasts were identified on the basis of molecular taxonomy by analysis of the D1/D2 region of the large subunit rRNA gene sequence similarity and phylogeny. Thirty-nine strains were identified to be 13 known species belong to Ascomycota *viz. Ambrosiozyma monospora*, *Candida carpophila*, *Candida jaroonii*, *Candida michaelii*, *Candida tropicalis*, *Hyphopichia burtonii*, *Kodamaea ohmeri*, *Meyerozyma caribbica* and *Pichia kudriavzevii*, and Basidiomycota *viz. Cryptococcus laurentii*, *Rhodosporidium paludigenum*, *Rhodotorula mucilaginosa* and *Trichosporon asahii*. One strain was found to represent a new yeast species in the genus *Rhodosporidium* closest to *R. toruloides*. The most prevalent yeast species in vetiver grass phylloplane was *M. caribbica* with a frequency of occurrence of 35% and followed by *C. tropicalis* that represented 25%, while only one to two strains were obtained from the other 10 species. Individual leaf sample was generally accommodated by only one yeast species (65.5%) and two yeast species were found in 31.0% of the samples. Determination of IAA production by the vetiver grass phylloplane yeasts revealed that only nine strains produced IAA in the range of 11.0-332.9 mg/L when cultivated in yeast extract peptone dextrose broth supplemented with 0.1% L-tryptophan. They consisted of two strains each of *C. michaelii*, *M. caribbica* and *R. paludigenum*, and one strain each of *C. tropicalis*, *P. kudriavzevii* and new *Rhodosporidium* species. The two strains of *R. paludigenum* produced high IAA including strain DMKU-LV61 produced the highest IAA of 332 mg/L and strain DMKU-LV56 produced 109.9 mg/L.

Keywords: Yeasts, phylloplane, biodiversity, indole-3-acetic acid, Thailand

1. Introduction

The phylloplane is usually referred to the external surface of plant leaves that has been recognized as an important habitat for microorganisms (Phaff and Starmer, 1987; Fonseca and Inacio, 2006). 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. While bacteria are the most abundant phylloplane microorganisms, yeasts and yeast-like fungi are also active phylloplane colonizers (Andrews and Harris, 2000; Lindow and Brandle, 2003). The phylloplanes of plants have been found to be colonized by both basidiomycetous and ascomycetous yeasts (Nakase et al., 2001; Inácio et al., 2005; Fonseca and Inacio, 2006; Slavikova et al., 2009; Glushakova and Chernov, 2010; Landell et al., 2010). In Thailand, yeasts that were previously reported to isolate from phylloplane of plants were both known and new species in the phylum Ascomycota and Basidiomycota such as *Candida chumphoensis*, *Candida jaroonii*, *C. mattraensis*, *C. michaelii*, *C. nivariensis*, *C. phyllophia*, *C. rugosa*, *C. sirachaensis*, *C. tropicalis*, *C. vitiphila*, *Cyberlindnera fabianii*, *Cy. rhodanensis*, *Debaryomyces hasenii*, *D. nepalensis*, *Hanseniaspora guilliermondii*, *H. opuntiae*, *Kazachstania siamensis*, *K. thailandica*, *Kodamaea ohmerii*, *Lachancea thermotolerans*, *Lodderomyces elongisporus*, *Metschnikowia koreensis*, *Meyerozyma caribbica*, *Millerozyma koratensis*, *Ogataea phyllophilla*, *O. kanchanaburiensis*, *O. wongdongensis*, *Pichia kudriavzevii*, *Torulaspora delbrueckii* and *Wickerhamomyces edaphicus* belong to the phylum Ascomycota and *Bensingtonia musae*, *Bullera peniseticola*, *Sporidiobolus ruineniae*, *Cryptococcus flavescens*, *Cr. laurentii*, *Cr. rajasthanensis*, *Kwoniella heveanensis*, *Rhodosporidium fluviale*, *R. paludigenum*, *Rhodotorula mucilaginosa*, *Rh. sesimbrana*, *Rh. taiwanensis*, *Sporidiobolus ruineniae*, *Sporobolomyces carnicolor*, *S. nylandii* and *Trichosporon asahii* belong to the phylum Basidiomycota (Nakase et al., 2001; Koowadjanakul et al., 2011; Limtong and Kaewwichian, 2013; Limtong et al., 2013; Limtong et al., 2014)

Plant growth promoters produced by plants and microorganisms play an important role in growth and development of plants through induction some important physiological responses at different stage of plant development at low concentration such as regulation of seed germination, root formation branching and tillering and fruit ripening (Tsavkelova et al., 2006; Ma et al., 2008). Indole-3-acetic acid (IAA) is the major member of plant growth promoter in the auxin group and is known to stimulate both rapid and long-term responses in plants by regulation of various developmental and physiological processes (Cleland 1990). Microorganisms which are capable of IAA production include bacteria (Sasirekha et al., 2012), actinomycetes (Khamna et al., 2010), yeasts (Nakamura et al., 1991; El-Tarabily, 2004; Nassar et al., 2005), and filamentous fungi (Ruanpanun et al., 2010). Applications of IAA producing yeasts, such as *Candida valida*, *Cyberlindnera saturnus*, *R. glutinis*, *R. mucilaginosa*, *Sporobolomyces roseus* and *Trichosporon asahii* to promoting the growth of plants have been reported (Perondi et al., 1996; El-Tarabily, 2004; Nassar et al., 2005; Xin et al., 2009).

Although yeasts inhabiting the phylloplane have been studied intensively but until present, no study on yeasts in the phylloplane of vetiver grass had been reported. Therefore, we carried out this work to investigate the diversity of yeasts in the phylloplane of vetiver grass in Thailand and determine the capacity of indole-3-acetic acid (IAA) production in vitro of the yeast strains obtained.

2. Materials and Methods

Sample collection

Green and healthy leaves of vetiver grass (*Vetiveria zizanioides*) 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 250 mg/L sodium propionate and 200 mg/L chloramphenicol (Limtong et al., 2007). Three g 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 250 mg/L sodium propionate and 200 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 region of the large subunit (LSU rRNA) gene sequences similarities (Kurtzman and Robnett, 1998 and Fell et al., 2000). The sequence of the D1/D2 region of the LSU rRNA gene was determined from PCR products amplified from genomic DNA. The D1/D2 region of the LSU rRNA gene was amplified and sequenced with primers, NL1 (50-GCATATCAATAA GCGGAGGAAAAG-30) and NL4 (50-GGTCCGTGTTTCAAGACGG-30) (Kurtzman and Robnett, 1998). Methods for DNA extraction and amplification of the D1/D2 region of the LSU rRNA gene and ITS region were as described previously (Limtong et al., 2007). The PCR products were checked by agarose gel electrophoresis and purified by using the QIAquick purification kit (Qiagen, Germany). The purified products were submitted to Macrogen Inc. (Korea) for sequencing. The sequences were compared pairwise using a BLAST 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 1.81 (Thompson et al., 1997). A phylogenetic tree was constructed from the evolutionary distance data with Kimura's two-parameter correction (Kimura, 1980), by the neighbor joining method (Saitou and Nei, 1987) using the MEGA software version 6.0 (Tamura et al., 2013). The confidence levels of the clades were estimated from bootstrap analysis (1,000 replicates) (Felsenstein, 1985). *Schizosaccharomyces pombe* NRRL Y-12796^T was used as the outgroup species in the analysis.

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 into 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 one ml of Salkowski reagent (12 g/L $FeCl_3$ and 7.9 M H_2SO_4) (Glickmann and Dessaux, 1994), and the intensity of pink color developing in the mixture after 30 min was quantified with a

spectrophotometer (Shimizu, Japan) at a wavelength of 530 nm. A calibration curve using pure IAA was established for calculation of IAA concentration.

3. Results and Discussion

Sample collection and yeast isolation

Thirty-four samples of vetiver grass leaf were collected from 8 provinces, consisting of three provinces in the central part of Thailand *viz.* Ratchaburi (14 samples), Kanchanaburi (7 samples) and Uthai Thani (1 sample); three provinces in the northern part *viz.* Chiang Mai (1 sample), Phayao (1 sample) and Uttaradit (4 samples); one province in the eastern part *viz.* Trat (1 sample); and one province in the north-eastern part *viz.* Udon Thani (6 samples) (Table 1). Therefore, most of the samples were collected from the central part of Thailand (22 samples)

A total of 40 yeast strains were isolated from 29 leaf samples (85.3%) while yeast could not be isolated from 4 samples collected from Kanchanaburi Province.

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 similarity together with the generally accepted regulation that ascomycetous yeast strains with 0 to 3 nucleotide differences in the D1/D2 region are either conspecific or sister species and yeast strains showing nucleotide substitutions greater than six are usually different species (Kurtzman and Robnett, 1998), and strains of the same basidiomycetous yeast species showing identical or one nucleotide substitution but strains that differed by two or more nucleotides represent different taxa (Fell et al., 2000). Thirty-nine strains were identified to be 13 known species that belong to Ascomycota *viz. Ambrosiozyma monospora*, *Candida fukuyamaensis*, *Candida jaroonii*, *Candida michaelii*, *Candida tropicalis*, *Hyphopichia burtonii*, *Kodamaea ohmeri*, *Meyerozyma caribbica* and *Pichia kudriavzevii*, and Basidiomycota *viz. Cryptococcus laurentii*, *Rhodosporidium paludigenum*, *Rhodotorula mucilaginosa*, and *Trichosporon asahii* (Tables 1, 2). One strain was found to represent a new yeast species closest to *Rhodosporidium toruloides*. The similarity analysis of the D1/D2 domain of the large subunit (LSU rRNA) gene sequences was also supported by the phylogenetic tree based on the sequences of the D1/D2 region of the LSU rRNA gene (Fig. 1). The identified strains were at the same positions as the type strains of the relative known yeast species in the tree.

The prevalent species of vetiver grass phylloplane yeasts was *M. caribbica* with a frequency of occurrence of 35% followed by *C. tropicalis* that represented 25%, while only one to two isolates were obtained from the other 10 species (Table 3). Individual leaf sample was generally accommodated by only one yeast species (65.5%) and two yeast species were found in 31.0% of the samples, while one sample contained three yeast species (Table 1).

Most yeast species found in vetiver grass phylloplane in this study have been reported to detect in phylloplane of various plants in Thailand such as sugarcane and rice (Limtong and Koowadjanakul, 2012; Limtong and Kaewwichian, 2014; Limtong et al., 2014) except *Ambrosiozyma monospora* and *Candida fukuyamaensis* that have never been reported. However, the two species detected in vetiver grass phylloplane were detected only one strain for each species, so it might be presented by accident. *M. caribbica* that detected in vetiver phylloplane at high frequency of occurrence (35%) was also reported to present in sugarcane phylloplane at high frequency of 23% (Limtong et al., 2014). Also, *C. tropicalis* found at high frequency of occurrence (25%) in vetiver phylloplane were also found in phylloplane sugarcane and rice at high frequency of occurrence, 10 and 16%, respectively (Limtong and Kaewwichian, 2014; Limtong et al., 2014).

Table 1. Isolation and identification of yeast strains isolated from surface of vetiver grass leaves

Sample code	Location (province)	Date of sample collection	Strain No.	Accession number	Closest species with GenBank accession number	Nucleotide in D1/D2 region		Result of identification
						substitution / total nt	% identity	
R-1	Ratchaburi	1/7/2009	DMKU-LV34	AB831045	*Meyerozyma caribbica* CBS 9966^T (EU348786)	0/570	100	*Meyerozyma caribbica* (A)
R-2	Ratchaburi	1/7/2009	DMKU-LV36	AB831046	*Meyerozyma caribbica* CBS 9966^T (EU348786)	0/525	100	*Meyerozyma caribbica* (A)
		1/7/2009	DMKU-LV37	AB831047	*Candida tropicalis* CBS 94^T (U45749)	0/570	100	*Candida tropicalis* (A)
R-3	Ratchaburi	1/7/2009	DMKU-LV38	AB831048	*Kodamaea ohmeri* CBS 5367^T (U45702)	0/493	100	*Kodamaea ohmeri* (A)
R-4	Ratchaburi	1/7/2009	DMKU-LV39	AB831049	*Candida tropicalis* CBS 94^T (U45749)	0/570	100	*Candida tropicalis* (A)
R-5	Ratchaburi	1/7/2009	DMKU-LV40	AB831050	*Candida tropicalis* CBS 94^T (U45749)	0/570	100	*Candida tropicalis* (A)
R-6	Ratchaburi	1/7/2009	DMKU-LV41	AB831051	*Candida tropicalis* CBS 94^T (U45749)	0/570	100	*Candida tropicalis* (A)
	Ratchaburi	1/7/2009	DMKU-LV42	AB831052	*Meyerozyma caribbica* CBS 9966^T (EU348786)	0/570	100	*Meyerozyma caribbica* (A)
R-7	Ratchaburi	1/7/2009	DMKU-LV43	AB831053	*Candida tropicalis* CBS 94^T (U45749)	0/570	100	*Candida tropicalis* (A)
R-8	Ratchaburi	1/7/2009	DMKU-LV44	AB831054	*Meyerozyma caribbica* CBS 9966^T (EU348786)	0/570	100	*Meyerozyma caribbica* (A)
R-9	Ratchaburi	1/7/2009	DMKU-LV45	AB831055	*Candida tropicalis* CBS 94^T (U45749)	0/570	100	*Candida tropicalis* (A)
R-10	Ratchaburi	1/7/2009	DMKU-LV46	AB831056	*Candida tropicalis* CBS 94^T (U45749)	0/570	100	*Candida tropicalis* (A)
R-11	Ratchaburi	1/7/2009	DMKU-LV47	AB831057	*Rhodotorula mucilaginosa* CBS 316^T (AF070432)	0/573	99.8	*Rhodotorula mucilaginosa* (B)
	Ratchaburi	1/7/2009	DMKU-LV48	AB831058	*Trichosporon asahii* CBS 2479^T (AF105393)	0/600	100	*Trichosporon asahii* (B)
R-12	Ratchaburi	1/7/2009	DMKU-LV49	AB831059	*Candida tropicalis* CBS 94^T (U45749)	0/570	100	*Candida tropicalis* (A)
R-13	Ratchaburi	1/7/2009	DMKU-LV50	AB831060	*Meyerozyma caribbica* CBS 9966^T (EU348786)	1/570	99.8	*Meyerozyma caribbica* (A)
R-14	Ratchaburi	1/7/2009	DMKU-LV51	AB831061	*Meyerozyma caribbica* CBS 9966^T (EU348786)	0/570	100	*Meyerozyma caribbica* (A)
			DMKU-LV52	AB831062	*Pichia kudriavzevii* CBS 5147^T (U76347)	0/564	100	*Pichia kudriavzevii* (A)
K-1	Kanchanaburi	15/8/2010	-	-	-	-	-	-
K-2	Kanchanaburi	15/8/2010	-	-	-	-	-	-
K-3	Kanchanaburi	15/8/2010	-	-	-	-	-	-
K-4	Kanchanaburi	15/8/2010	-	-	-	-	-	-
K-5	Kanchanaburi	21/8/2010	-	-	-	-	-	-
K-6	Kanchanaburi	21/8/2010	DMKU-LV54	AB831063	*Candida michaelii* NRRL Y-27705^T (AY520329)	1/530	99.8	*Candida michaelii* (A)
K-7	Kanchanaburi	21/8/2010	DMKU-LV55	AB831064	*Candida michaelii* NRRL Y-27705^T (AY520329)	0/530	100	*Candida michaelii* (A)
			DMKU-LV56	AB831065	*Ambrosiozyma monospora* CBS 2554^T (EU011590)	0/566	100	*Ambrosiozyma monospora* (A)
P-1	Phayao	6/4/2011	DMKU-LV57	AB831066	*Trichosporon asahii* CBS 2479^T (AF105393)	0/597	100	*Trichosporon asahii* (B)
			DMKU-LV58	AB831067	*Rhodosporidium paludigenum* CBS 6566^T (AF363640)	0/574	100	*Rhodosporidium paludigenum* (B)
			DMKU-LV59	AB831068	*Cryptococcus laurentii* PYCC 3966^T (AF075469)	1/597	99.8	*Cryptococcus laurentii* (B)
Ut-1	Uttaradit	7/4/2011	DMKU-LV60	AB831069	*Candida jaroonii* CBS 10930^T (DQ404493)	0/548	100	*Candida jaroonii* (A)

Table 1 (continued)

Sample code	Location (province)	Date of sample collection	Strain No.	Accession number	Closest species with GenBank accession number	Nucleotide in D1/D2 region		Result of identification
						substitution / total nt	% identity	
Ut-2	Uttaradit	7/4/2011	DMKU-LV61	AB831070	*Rhodosporidium paludigenum* CBS 6566^{T} (AF363640)	0/574	100	*Rhodosporidium paludigenum* (B)
			DMKU-LV62	AB831071	*Meyerozyma caribbica* CBS 9966^{T} (EU348786)	0/570	100	*Meyerozyma caribbica* (A)
Ut-3	Uttaradit	7/4/2011	DMKU-LV64	AB831072	*Meyerozyma caribbica* CBS 9966^{T} (EU348786)	0/570	100	*Meyerozyma caribbica* (A)
			DMKU-LV65	AB831073	*Candida tropicalis* CBS 94^{T} (U45749)	0/570	100	*Candida tropicalis* (A)
C-1	Chiang Mai	11/4/2011	DMKU-LV66	AB831074	*Meyerozyma caribbica* CBS 9966^{T} (EU348786)	0/570	100	*Meyerozyma caribbica* (A)
			DMKU-LV67	AB831075	*Candida tropicalis* CBS 94^{T} (U45749)	0/570	100	*Candida tropicalis* (A)
Ud-1	Udon Thani	19/4/2011	DMKU-LV69	AB831076	*Hyphopichia burtonii* CBS 2352^{T} (U45712)	0/489	100	*Hyphopichia burtonii* (A)
			DMKU-LV70	AB831077	*Meyerozyma caribbica* CBS 9966^{T} (EU348786)	0/570	100	*Meyerozyma caribbica* (A)
Ud-2	Udon Thani	19/4/2011	DMKU-LV71	AB831078	*Meyerozyma caribbica* CBS 9966^{T} (EU348786)	0/570	100	*Meyerozyma caribbica* (A)
Ud-3	Udon Thani	19/4/2011	DMKU-LV72	AB831079	*Rhodosporidium toruloides* CBS 349^{T}(AF070426)	5/573	96.5	new species
Ud-4	Udon Thani	19/4/2011	DMKU-LV73	AB831080	*Cryptococcus laurentii* PYCC 3966^{T} (AF075469)	1/597	99.8	*Cryptococcus laurentii* (B)
Ud-5	Udon Thani	19/4/2011	DMKU-LV74	AB831081	*Meyerozyma caribbica* CBS 9966^{T} (EU348786)	0/570	100	*Meyerozyma caribbica* (A)
Ud-6	Udon Thani	19/4/2011	DMKU-LV77	AB831082	*Meyerozyma caribbica* CBS 9966^{T}(EU348786)	0/570	100	*Meyerozyma caribbica* (A)
Ud-7	Uthai Thani	27/4/2011	DMKU-LV78	AB831083	*Meyerozyma caribbica* CBS 9966^{T}(EU348786)	1/570	99.8	*Meyerozyma caribbica* (A)
T-1	Trat	27/4/2011	DMKU-LV81	AB831084	*Candida fukuyamaensis* CBS 7921^{T}(U62311)	0/570	100	*Candida carpophila* (A)

Remark: (A), Phylum Ascomycota**;** (B)**,** Phylum Basidiomycota

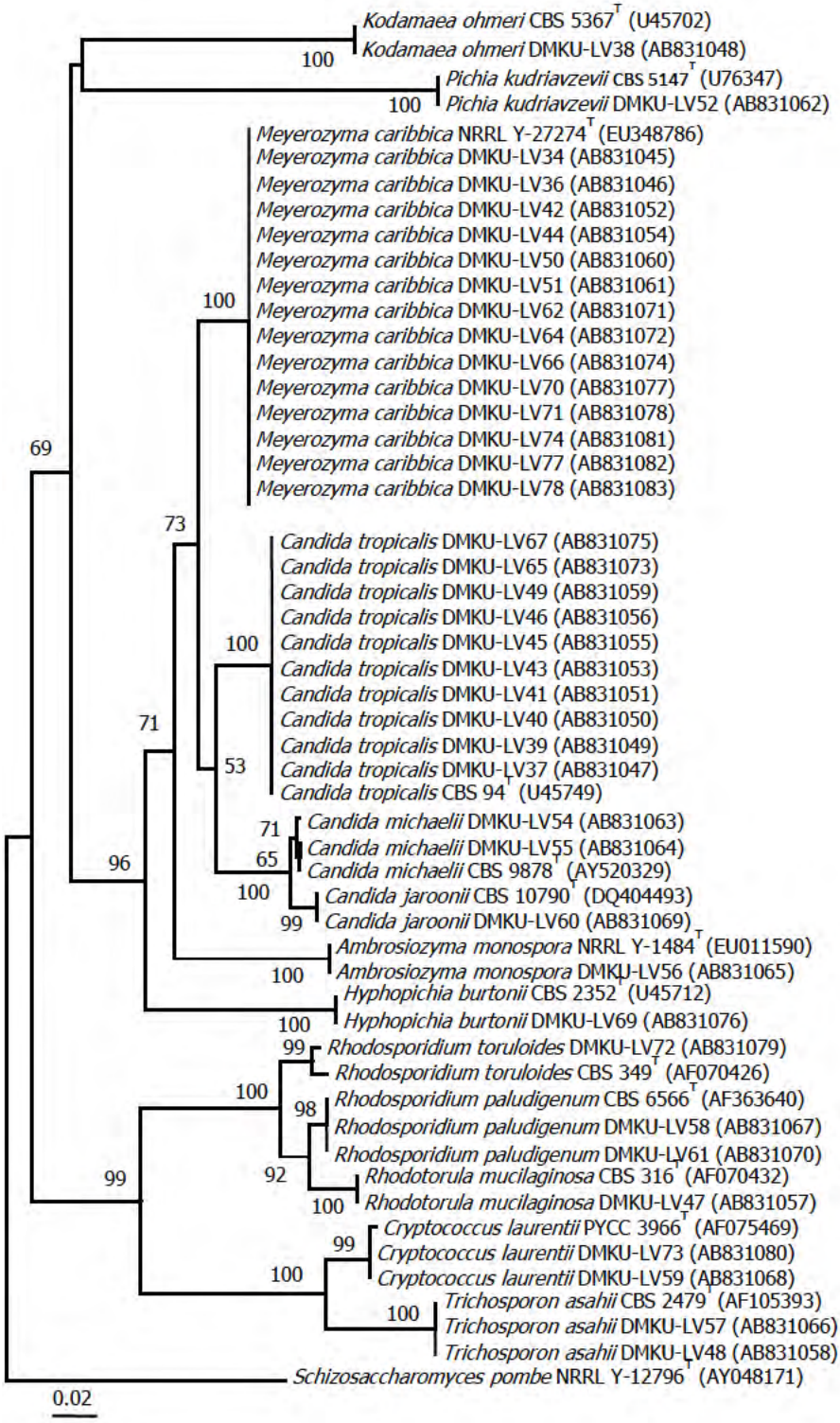

Figure 1. Phylogenetic tree based on the sequences of the D1/D2 region of the LSU rRNA gene, showing positions of strains isolated from vetiver grass phylloplane with respect to the type strains of known yeast species

Table 2. Yeasts from vetiver grass phylloplane and their frequency of occurrence

Species	No. of leaves sample with this yeast species	Frequency of occurrence (%)*
Phylum Ascomycota		
Ambrosiozyma monospora	1	2.5
Candida fukuyamaensis	1	2.5
Candida jaroonii	1	2.5
Candida michaelii	2	5.0
Candida tropicalis	10	25.0
Hyphopichia burtonii	1	2.5
Kodamaea ohmeri	1	2.5
Meyerozyma caribbica	14	35.0
Pichia kudriavzevii	1	2.5
Phylum Basidiomycota		
Cryptococcus laurentii	2	5.0
Rhodosporidium paludigenum	2	5.0
Rhodotorula mucilaginosa	1	2.5
Trichosporon asahii	2	5.0
New species closest to *Rhodosporidium toruloides*	1	2.5

Remark: * $\frac{\text{Number of plant leaf sample from which that species was isolated}}{\text{Number of plant leaf sample examined}} \times 100$

Indole-3-acetic acid production

Determination of IAA production by the phylloplane yeasts revealed that only nine strains produced IAA in the range of 11.0-332.9 mg/L when cultivated in yeast extract peptone dextrose broth supplemented with 0.1% L-tryptophan (Table 3). They consisted of two strains each of *C. michaelii*, *M. caribbica* and *R. paludigenum*, and one strain each of *C. tropicalis*, *P. kudriavzevii* and new *Rhodosporidium* species. The two strains of *R. paludigenum* produced high IAA including strain DMKU-LV61 that produced the highest IAA of 332 mg/L and strain DMKU-LV56 that produced 109.9 mg/L.

Though the IAA production by *R. paludigenum* DMKU-LV61 was relatively high when compared with fungi such as *Aspergillus niger* (132.7 mg/L**)** (Bilkay et al., 2010); some actinomycetes such as *Streptomyces* sp. CMU-H009 (143.95 mg/L) (Khamna et al., 2010); and bacteria such *Klebsiellas* sp. SN1 (291 mg/L) (Xinxian et al., 2011) and *Pseudomonas aeruginosa* (132 mg/L) (Sasirekha et al., 2012). However, it produced not much different IAA concentration comparing with some yeast isolates isolated from plant phyllophane in the other reports of our group such as *R. fluviale* DMKU-RK253 that produced 417.8 mg/L (Limtong et al., 2014), and *C. maltosa* LM114 (314.3 mg/L) (Limtong and Koowadjanakul, 2012)

Table 3. Indole-3-acetic acid production by yeast strains obtained from vetiver grass phylloplane in L-tryptophan-YPD broth in test tube

Species	Strain	Cell dry weight (g/L)	IAA (mg/L)
Ascomycota			
Ambrosiozyma monospora	DMKU-LV56	14.6	0
Candida jaroonii	DMKU-LV60	9.1	0
Candida michaelii	DMKU-LV54	6.6	12.2
	DMKU-LV55	9.2	75.2
Candida tropicalis	DMKU-LV37	8.5	0
	DMKU-LV39	9.0	0
	DMKU-LV40	10.9	0
	DMKU-LV41	11.9	0
	DMKU-LV43	11.0	0
	DMKU-LV45	12.6	0
	DMKU-LV46	10.6	0
	DMKU-LV49	8.7	0
	DMKU-LV65	11.8	0
	DMKU-LV67	9.0	11.0
Hyphopichia burtonii	DMKU-LV69	9.9	0
Kodamaea ohmeri	DMKU-LV38	8.6	0
Meyerozyma caribbica	DMKU-LV34	9.4	0
	DMKU-LV36	8.9	0
	DMKU-LV42	10.6	0
	DMKU-LV44	10.9	0
	DMKU-LV50	10.5	47.6
	DMKU-LV51	10.3	75.5
	DMKU-LV62	10.2	0
	DMKU-LV64	10.6	0
	DMKU-LV66	6.6	0
	DMKU-LV70	9.7	0
	DMKU-LV71	9.9	0
Pichia kudriavzevii	DMKU-LV52	12.0	16.5
Basidiomycota			
Cryptococcus laurentii	DMKU-LV59	8.9	0
	DMKU-LV73	10.7	0
Rhodosporidium paludigenum	DMKU-LV58	8.8	109.9
	DMKU-LV61	9.1	332.9
Rhodotorula mucilaginosa	DMKU-LV47	10.1	0
Trichosporon asahii	DMKU-LV48	10.1	0
	DMKU-LV57	14.0	0
New species closest to *Rhodosporidium toruloides*	DMKU-LV72	11.9	65.1

4. Conclusions

The result of this study revealed that yeast species in the vetiver grass phylloplane were similar to that present in the phylloplane of the other plant species. *M. caribbica* and *C. tropicalis*, frequently found in the vetiver grass phylloplane, were also present in the phylloplane of sugarcane and rice at high frequency of occurrence. Some yeast strains could produce IAA which may possibly contribute to promoting growth of vetiver grass in the field.

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