Group V (ST-515)

ST-515 had the mol %G+C of 38.4 similar with *H. vineae* (38.8-40.7%), the nearest species. In the phylogenetic tree, this strain was located at the cluster where the type strain of *H. vineae* was located. In the D1/D2 domain sequences, strain ST-515 differed in 3 nucleotides (including 1 gap) from the type strain of the closest species and 7 nucleotides or more from the type strains of related species (Table 29). In the DNA-DNA hybridization experiment, ST-515 showed similarities 89.5% with *H. vineae* (Table 30). The taxonomic criteria commonly employed of ST-515 is the same with *H. vineae*. Aparently this strain was identified as *Hanseniaspora vineae*.

4.1.1 Description of new species of *Hanseniaspora*

***Hanseniaspora thailandica* sp. nov. (ST-250 and ST-306)**

Growth in 2% glucose-yeast extract-peptone-broth: After 3 days at 25°C, the cells proliferate by bipolar budding. They are apiculate, ovoidal to elongate, 2-7 x 3-9 μm, single or in pairs (Fig. 8A). Sediment is present.

Growth in 2% glucose-yeast extract-peptone-agar: After one month at 25°C, the streak culture is pale cream colored, smooth, glossy, flat to slightly elevated at the center with slightly undulate margin.

Growth on the surface of assimilation media: Pellicles are not formed.

Ascospore formation: Asci containing 2-4 hat-shaped ascospore are observe af ter 2 weeks on potassium acetate agar at 25°C (Fig. 8B).

Slide culture on on potato dextrose agar: Pseudomycelium is formed (Fig. 8C).

Fermentation: Glucose is fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-
Sucrose	-	D-Mannitol	-
Maltose	-	D-Glucitol	-
Cellobiose	+	Xylitol	-

Trehalose	Weak	L-Arabinitol	-
Lactose	-	α-Methyl-D-glucoside	-
Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	+
Melezitose	-	D-Gluconic acid	+
Inulin	-	2-Ketogluconic acid	+
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	-	DL-Lactic acid	-
L-Arabinose	-	Succinic acid	-
D-Arabinose	-	Citric acid	-
D-Ribose	-	Saccharic acid	-
L-Rhamnose	-	D-Glucuronic acid	-
D-Glucosamine	-	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	Propane 1,2 diol	-
Ethanol	-	Butane 2,3 diol	-
Glycerol	-	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	+	Cadaverine	+
Ethylamine	-		

Additional tests:

Starch formation	-	Acid formation	-
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	nd	Lipase	-
0.1% Cycloheximide	nd	Maximum temperature	36-37°C
50% Glucose	nd	Ubiquinone system	Q-6
10% NaCl + 5% Glucose	nd	Mol% G+C (by HPLC)	34.8-34.9
Vitamins required	Pantothenate, inositol, niacin and pyridoxine		

Type strain: ST-250, isolated from insect frass collected in Hala-Bala, Narathiwat province, Thailand, Mar. 2001, is the type strain of this species. It was deposited at BIOTEC Culture Collection as BCC 11776.

Etymology: The specific epithet "*thailandica*" refers to "Thailand", the country of the strain isolated.

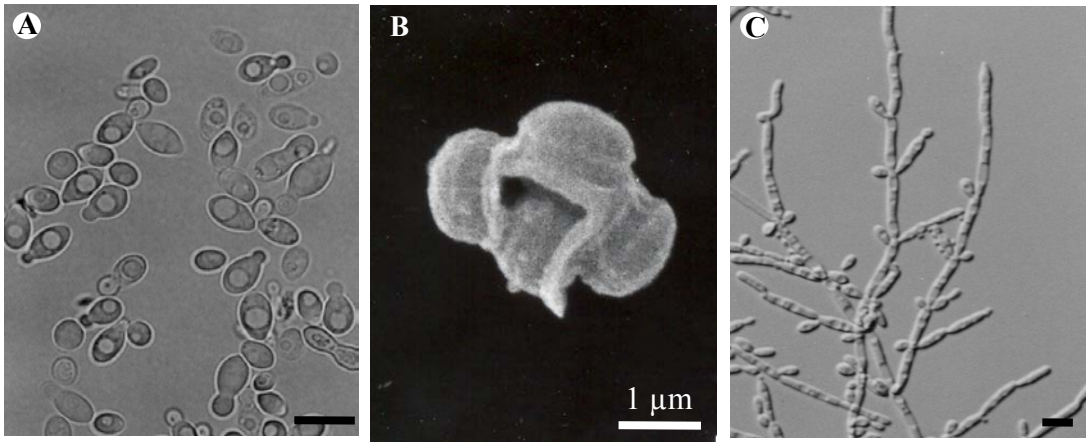

Figure 8 Morphological characteristics of *Hanseniaspora thailandica* (ST-250); (A) cells grown in YMB for 3 days at 25°C; (B) ascospore produced on potassium acetate agar after 7 days at 25°C; (C) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

.

***Kloeckera siamensis* sp. nov. (ST-464, ST-493 and ST-613)**

Growth in 2% glucose-yeast extract-peptone-broth: After 3 days at 25°C, the cells proliferated by bipolar budding. They are apiculate, ovoidal to elongate, 2-7 x 2-10 μm, single or in pairs (Fig. 9A). Sediment is present.

Growth in 2% glucose-yeast extract-peptone-agar: After one month at 25°C, the streak culture is pale cream colored, smooth, glossy, flat to slightly elevated at the center with slightly undulate margin.

Growth on the surface of assimilation media: Pellicles are not formed.

Slide culture on potato dextrose agar: A rudimentary pseudomycelium is formed (Fig. 9B).

Fermentation: Glucose is fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-
Sucrose	-	D-Mannitol	-
Maltose	-	D-Glucitol	-
Cellobiose	+	Xylitol	-
Trehalose	Weak	L-Arabinitol	-
Lactose	-	α-Methyl-D-glucoside	-
Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	+
Melezitose	-	D-Gluconic acid	+
Inulin	-	2-Ketogluconic acid	+
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	-	DL-Lactic acid	-
L-Arabinose	-	Succinic acid	-
D-Arabinose	-	Citric acid	-
D-Ribose	-	Saccharic acid	-
L-Rhamnose	-	D-Glucuronic acid	-
D-Glucosamine	-	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	Propane 1,2 diol	-
Ethanol	-	Butane 2,3 diol	-
Glycerol	-	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	+	Cadaverine	+
Ethylamine	-		

Additional tests:

Starch formation	-	Acid formation	-
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	nd	Lipase	-
0.1% Cycloheximide	nd	Maximum temperature	36-38°C
50% Glucose	nd	Ubiquinone system	Q-6
10% NaCl + 5% Glucose	nd	Mol% G+C (by HPLC)	34.9-35.3
Vitamins required	nd		

Type strain: ST-464, isolated from lichen collected in Tone Nga Chang Waterfall, Songkhla province, Thailand, Feb. 2003, is the type strain of this species. It was deposited at BIOTEC Culture Collection as BCC 14938.

Etymology: The specific epithet "*siamensis"* refered to "Siam", the old name of Thailand where this yeast was isolated.

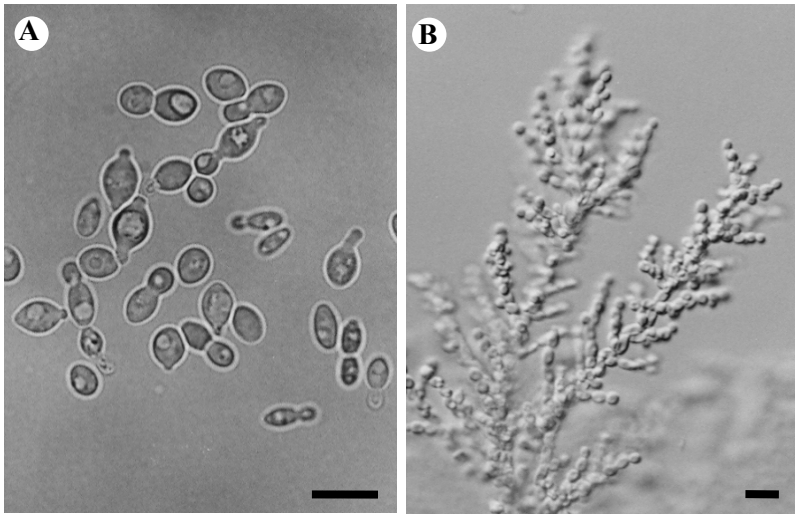

Figure 9 Morphological characteristics of *Kloeckera siamensis* (ST-464); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

***Kloeckera songkhlaensis* sp. nov. (ST-476)**

Growth in 2% glucose-yeast extract-peptone-broth: After 3 days at 25°C, the cells proliferated by bipolar budding. They are apiculate, ovoidal to elongate, 2-7 x 2-9 μm, single or in pairs (Fig. 10A). Sediment is present.

Growth in 2% glucose-yeast extract-peptone-agar: After one month at 25°C, the streak culture is pale cream colored, smooth, glossy, flat to slightly elevated at the center with slightly undulate margin.

Growth on the surface of assimilation media: Pellicles are not formed (Fig. 10B).

Slide culture on potato dextrose agar: Pseudomycelium is formed .

Fermentation: Glucose is fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-
Sucrose	-	D-Mannitol	-
Maltose	-	D-Glucitol	-
Cellobiose	+	Xylitol	-
Trehalose	-	L-Arabinitol	-
Lactose	-	α-Methyl-D-glucoside	-
Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	+
Melezitose	-	D-Gluconic acid	+
Inulin	-	2-Ketogluconic acid	+
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	-	DL-Lactic acid	-
L-Arabinose	-	Succinic acid	-
D-Arabinose	-	Citric acid	-
D-Ribose	-	Saccharic acid	-
L-Rhamnose	-	D-Glucuronic acid	-

D-Glucosamine	-	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	Propane 1,2 diol	-
Ethanol	-	Butane 2,3 diol	-
Glycerol	-	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	+	Cadaverine	+
Ethylamine	-		

Additional tests:

Starch formation	-	Acid formation	-
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	nd	Lipase	-
0.1% Cycloheximide	nd	Maximum temperature	33-34°C
50% Glucose	nd	Ubiquinone system	Q-6
10% NaCl + 5% Glucose	nd	Mol% G+C (by HPLC)	36.9
Vitamins required	Pantothenate, inositol, niacin, pyridoxine and thiamine stimulative		

Holotype: ST-476, isolated from mushroom (*Hygrophorus* sp.) collected in Tone Nga Chang Waterfall, Songkhla province, Thailand, Feb. 2003, is the holotype of this species. It was deposited at BIOTEC Culture Collection as BCC 14939.

Etymology: The specific epithet "*songkhlaensis*" was derived from the province, where this yeast was isolated.

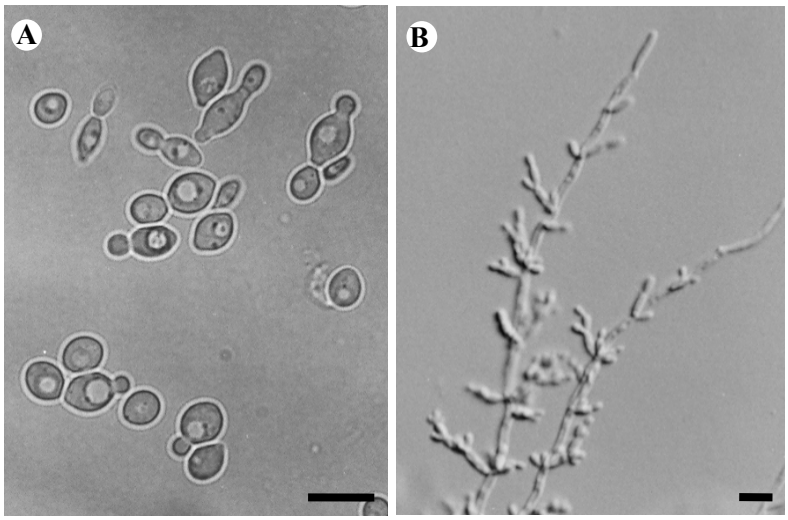

Figure 10 Morphological characteristics of *Kloeckera songkhlaensis* (ST-476); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

***Kloeckera tradensis* sp. nov. (ST-391)**

Growth in 2% glucose-yeast extract-peptone-broth: After 3 days at 25°C, the cells proliferated by bipolar budding. They are apiculate, ovoidal to elongate, 2-8 x 2-9 μm, single or in pairs (Fig. 11A). Sediment is present.

Growth in 2% glucose-yeast extract-peptone-agar: After one month at 25°C, the streak culture is pale cream colored, smooth, glossy, flat to slightly elevated at the center with slightly undulate margin.

Growth on the surface of assimilation media: Pellicles are not formed.

Slide culture on potato dextrose agar: A rudimentary pseudomycelium is formed (Fig. 11B).

Fermentation: Glucose is fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-
Sucrose	-	D-Mannitol	-

Maltose	-	D-Glucitol	-
Cellobiose	+	Xylitol	-
Trehalose	-	L-Arabinitol	-
Lactose	-	α-Methyl-D-glucoside	-
Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	-
Melezitose	-	D-Gluconic acid	-
Inulin	-	2-Ketogluconic acid	-
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	-	DL-Lactic acid	-
L-Arabinose	-	Succinic acid	-
D-Arabinose	-	Citric acid	-
D-Ribose	-	Saccharic acid	-
L-Rhamnose	-	D-Glucuronic acid	-
D-Glucosamine	-	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	Propane 1,2 diol	-
Ethanol	-	Butane 2,3 diol	-
Glycerol	-	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	+	Cadaverine	+
Ethylamine	-		

Additional tests:

Starch formation	-	Acid formation	-
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	+	Lipase	-
0.1% Cycloheximide	nd	Maximum temperature	34-35°C
50% Glucose	+	Ubiquinone system	Q-6

60% Glucose	nd	Mol% G+C (by HPLC)	34.9-35.3
Vitamin required	nd		

Holotype: ST-391, isolated from flowers collected in mangrove forest, Trad province, Thailand, Jan. 2002, is the holotype of this species. It was deposited at BIOTEC Culture Collection as BCC 14935.

Etymology: The specific epithet "*tradensis*" was derived from the name of province, where this yeast was found.

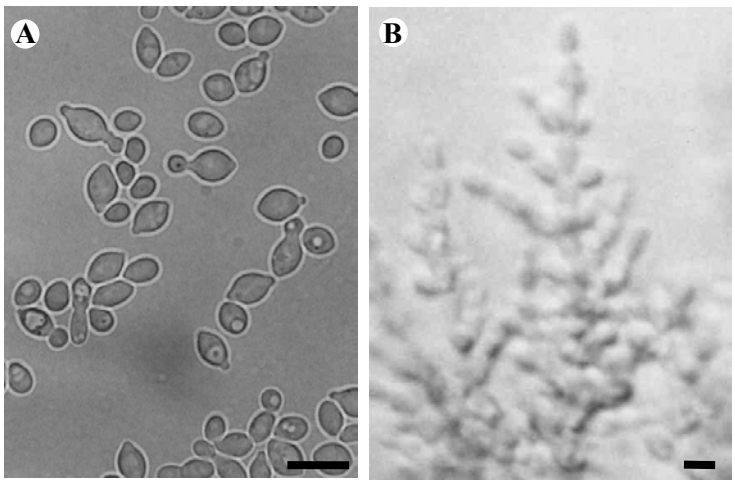

Figure 11 Morphological characteristics of *Kloeckera tradensis* (ST-391); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

4.1.2 Classification System of *Hanseniaspora/Kloeckera*

Since the description of *Saccharomyces apiculatus* (=*Kloeckera apiculata*, anamorph of *Hanseniaspora uvarum*) by Reess (1870), many new apiculate yeasts have been described in the genus *Hanseniaspora* and its anamorphic counterpert *Kloeckera*. Meyer *et al*. (1978) carried out detailed taxonomic studies on these yeasts and classified them into six species, *H. guilliermondii* (anamorph: *K. apis*), *H. occidentalis* (anamorph: *K. javanica*), *H. osmophila* (anamorph: *H. corticis*),

H. uvarum (anamorph: *K. apiculata*). *H. valbyensis* (anamorph: *K. japonica*) and *H. vineae* (anamorph: *K. africana*). This classification was adopted in the 3rd and 4th editions of The Yeasts, a Taxonomic Study (Smith, 1984, 1998). In addition to these six pairs of species, *Kloekera lindneri* is maintained as distinct species but its teleomorph has not been described.

Yamada *et al*. (1992) divided six species of *Hanseniaspora* into two clusters, one cluster consisted of *H. guilliermondii, H. uvarum* and *H. valbyensis*, the second cluster consisted of *H. occidentalis, H. osmophila* and *H. vineae*. They considered the two clusters as genera and reinstated the genus *Kloeckraspora* to accommodate the latter three species, which are characterized by spheroidal and warty ascospores. The genus *Kloeckeraspora* introduced by Niehaus (1932) was considered a synonym of *Hanseniaspora* by various authors (Lodder and Kreger-van Rij, 1952; Phaff, 1970; Meyer *et al*.,1978; Smith, 1984, 1998). Boekhout *et al*. (1994) demonstrated that *Hanseniapora* species could be divided into the same subgroups as Yamada *et al*. (1992), however, they maintained all of species in *Hanseniaspora* on the basis of both the heterogeneous distribution of phenetic properties among species as well as low statistical support in the 26S rDNA tree for the separation of the two subgroups.

Since the description of *H. guilliermondii* by Pijper (1928), practically no new species have been described in the genus *Hanseniaspora*. New species proposed after 1928 were found to be synonyms of formerly described species (Smith, 1998). Recently, however, four new species, *H. meyeri, H. opuntiae, H. clermontiae* and *H. lachancei*, were described (Cadez *et al*., 2003). In addition, as mentioned above, 4 new species were isolated in the present study from Thailand. The finding of these new species resulted in the increase of diversity of *Hanseniaspora*. So, detailed taxonomic studies are urgently required for the construction of rational classification system of *Hanseniaspora* and *Kloeckera*. The characterization of cell wall polysaccharides were carried out by NMR spectrum to make clear the intrageneric structure of the genus.

Twenty-four samples of polysaccharides of 24 strains of *Haniasporaspora/ Kloeckera* purified via copper complexes are mannans because the monosaccharide that was detected in the hydrolysates are only mannose (Fig. 12). The H-1 proton of NMR spectra of mannans of *Haniasporaspora/Kloeckera* were classified into 2 types (Fig.13). Type I comprised *H. clermontiae, H. guilliermondii, H. lachancei, H. myeri, H. opuntiae, H. pseudoguilliermondii, H. uvarum, K. lindneri*, and 4 new species of *Hanseniaspora* (1)*/Kloeckera* (3) isolated in Thailand. Type II comprised *H. osmophilla, H. vineae* and *H. occidentalis* (with slightly different). These results coincide with the serological characteristics (Tsuchiya et al., 1966), NMR spectra of mannans (Spencer and Gorin, 1968) and phylogenetic analysis of the sequences of Ribosomal DNA (Yamada *et al*., 1992).

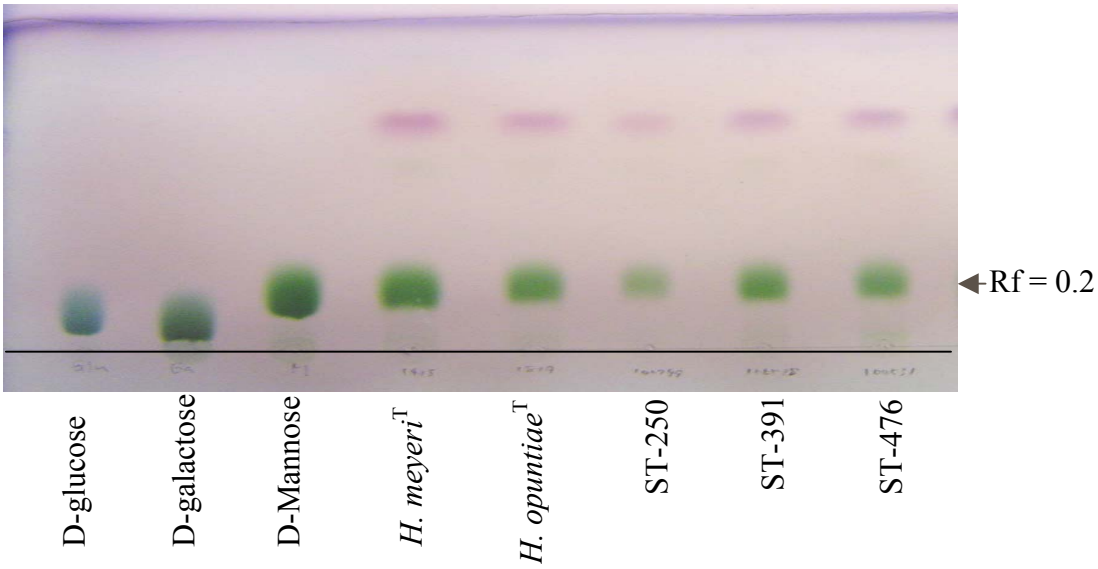

Figure 12 Thin layer chromatography of acid hydrolyzates of polysaccharides from *Hanseniaspora/Kloeckera*.

The data of the sequences of Ribosomal DNA, NMR spectrum of cell wall mannans and serological characteristics supported to divided strains of *Hanseniaspora* and *Kloeckera* into 2 groups and should be distinguished from each other at the generic level. Group I contained 9 species namely *H. clermontiae, H. guilliermondii, H. lachancei, H. myeri, H. opuntiae, H. pseudoguilliermondii* (in press)*, H. uvarum* and *K. lindneri*. As proposed by Yamada and coworkers (1992), *H. occidentalis, H.*

osmophilla and *H. vineae* should be transferred to the genus *Kloeckerispora*. However, the genus *Hanseniaspora/Kloeckera* had heterogeneous morphological, serological and chemotaxonomic characteristics. The further study such as ascospore morphology, sequences of other regions; 18S rDNA, ITS1/ITS2 and IGS, enzymes and amino acids patterns, are necessary to completed generic divergence classification of the genus *Hanseniaspora/Kloeckera*.

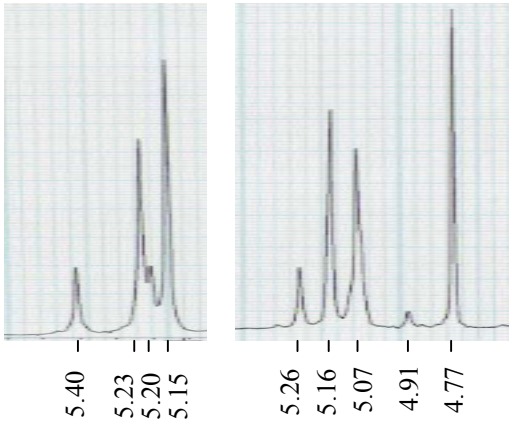

Figure 13 The NMR spectra of H-1 region of the mannans of *Hanseniaspora*/ *Kloeckera*. Type I; *H. clermontiae, H. guilliermondii, H. lachancei, H. myeri, H. opuntiae, H. pseudoguilliermondii, H. uvarum, K. lindneri*, and *H. thailandica*, *K. siamensis*, *K. tradensis* and *K. songkhlaensis.* Type II; *H. osmophilla, H. vineae* and *H. occidentalis*. Numerials indicate chemical shift of samples in ppm.

4.2 Taxonomic study on new species of the genus *Candida*

4.2.2 Strain related to *Candida coipomoensis* (ST-33)

Strain ST-33 was isolated from insect frass collected from Khao Yai National Park, Nakhon Ratchasima, Thailand. This strain proliferated by multilateral budding, showed negative DBB and urease reactions and did not produced ascospores. These characteristics coincided with the genus *Candida*.

In the phylogenetic tree based on D1/D2 domain of 26S rDNA sequences constructed by neighbor-joining method, ST-33 constituted a cluster with *Candida coipomoensis* that was connected with *C. ergastensis* (Fig. 14). A pairwise comparison of the D1/D2 domain of 26S rDNA sequence showed that this strain differed in 9 nucleotides (1.6%) from *C. coipomoensis*, the nearest species. In the morphological, physiological and chemotaxonomic properties, ST-33 resembles *C. coipomoensis* but differed by the assimilation of L-arabinose, L-rhamnose, glucono-δ-lactone, the lack of assimilation of lactose and growth at 35°C. These facts mentioned above clearly suggested that ST-33 represented a hitherto undescribed species of anamorphic ascomycetous yeasts and was named *Candida lignicola* sp. nov.

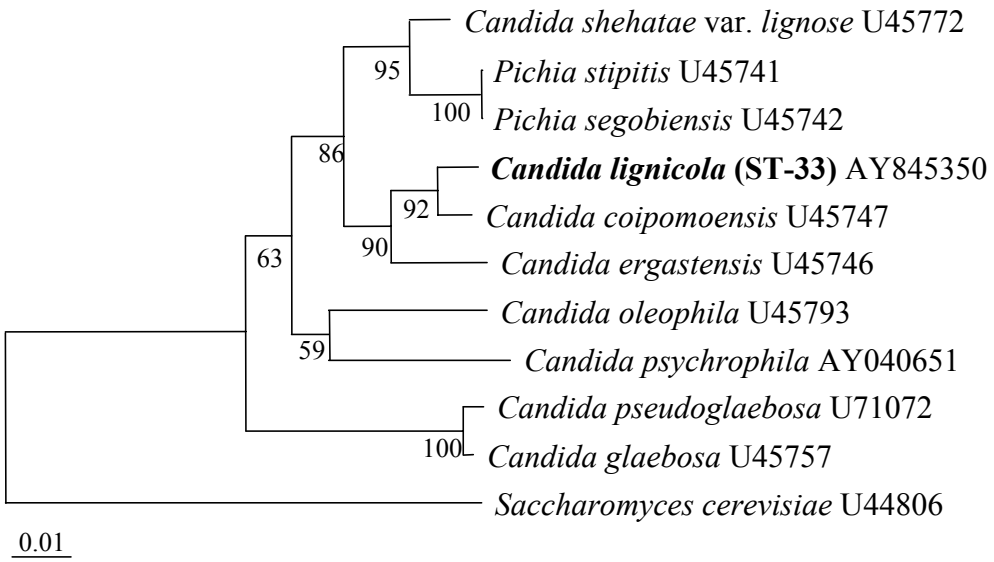

Figure 14 Phylogenetic tree showing the position of *Candida lignicola* (ST-33) based on the sequences of the D1/D2 domain of 26S rDNA with bootstrap values by 1000 re-sampling (< 50% is not shown).

***Candida lignicola* sp. nov. (ST-33)**

Growth in 2*%* glucose-yeast extract-peptone-broth: After 3 days at 25°C, the cells proliferate by multilateral budding. They are round, ovoidal and ellipsoidal, 1.9-3.8 x 2.0-6.4 mm, single or in pairs (Fig. 15A).

Growth in 2% glucose-yeast extract-peptone-agar*:* After one month at 25°C, the streak culture is tannish-yellow, smooth, flat, shiny, soft and the margin is entire.

Growth on the surface of assimilation media*:* Pellicles are not formed.

Slide culture on potato dextrose agar*:* After 14 days at 25^{o}C, pseudomycelia are formed and true mycelia are formed (Fig. 15B).

Fermentation: Glucose and galactose are fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	+
Galactose	+	Ribitol	+
L-Sorbose	+	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	nd
Trehalose	+	L-Arabinitol	nd
Lactose	-	α-Methyl-D-glucoside	+
Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	+
Melezitose	+	D-Gluconic acid	nd
Inulin	-	2-Ketogluconic acid	-
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	+	DL-Lactic acid	-
L-Arabinose	+	Succinic acid	+
D-Arabinose	+	Citric acid	+
D-Ribose	+	Saccharic acid	nd
L-Rhamnose	Weak	D-Glucuronic acid	-
Ethanol	-	D-Galacturonic acid	-
Glycerol	+	Inositol	-

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	+
Ethylamine	+		

Additional tests:

Starch formation	-	Acid formation	+
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	+	Lipase	-
0.1% Cycloheximide	+	Maximum temperature	nd
50% Glucose	-	Ubiquinone system	Q-9
10% NaCl + 5% Glucose	nd	Mol% G+C (by HPLC)	44.8
Vitamin free medium	+		

Holotype: The strain ST-33 isolated from insect frass collected in Khao Yai National Park of Thailand in 2001 is the holotype of this species. This strain is deposited at the BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Thailand, as BCC7733.

Etymology: The specific epithet "*lignicola"* means "dweller on wood" that is related with a source of this strain.

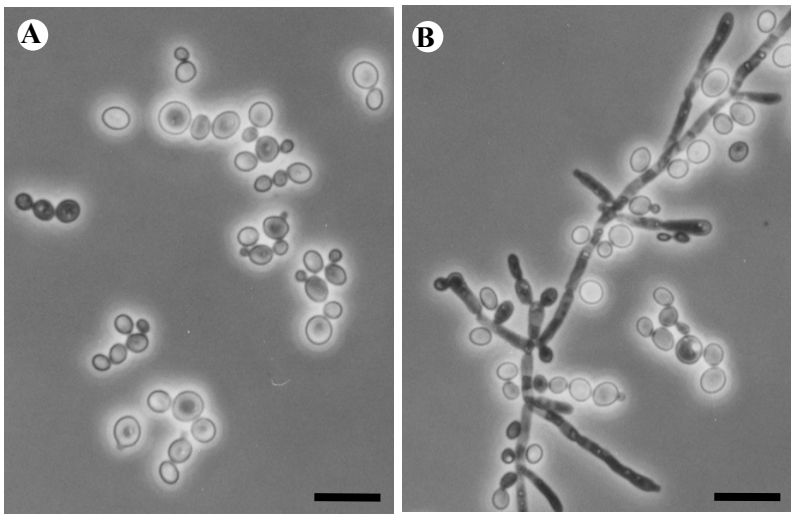

Figure 15 Morphological characteristics of *Candida lignicola*; (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

4.2.3 Strain related to *Candida etchellsii* (ST-22)

The strain ST-22 was isolated from a flower in Khao Yai National Park, Nakhon Ratchasima, Thailand. This strain proliferated by multilateral budding, lacked sexual reproduction, showed negative urease reactions and had Q-9 as a major ubiquinone. These characteristics coincided with the genus *Candida*.

By BLAST search of the D1/D2 sequences of 26S rDNA, this strain was closest with *Candida etchellsii*. In the phylogenetic tree based on D1/D2 sequences constructed by neighbor-joining method, ST-22 constituted a cluster where *C. etchellsii* was located with high statistical support (Fig. 16).

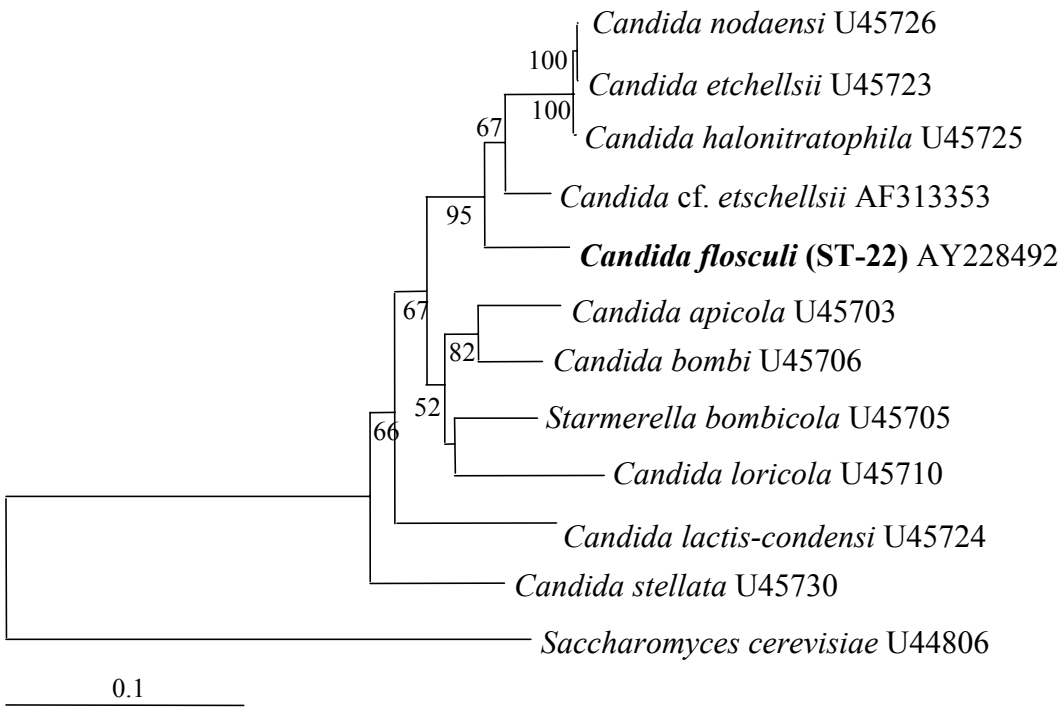

Figure 16 Phylogenetic tree showing the position of *Candida flosculi* based on the sequences of the D1/D2 domain of 26S rDNA with bootstrap values by 1000 re-sampling (< 50% is not shown).

ST-22 differed in 44 nucleotides (9.1%) from *C. etschellsii*, the most closely related species. The great nucleotide difference in the sequence clearly indicated that ST-22 is different species from *C. etchellsii*. In addition, ST-22 is distinguished from *C. etchellsii* by it's ability to assimilate sucrose, raffinose and salicin, resistance to 0.1 % (w/v) cycloheximide, growth in vitamin free medium and inability to grow on 50% (w/v) glucose agar medium. Eventhough the G+C contents of the strain ST-22 and the type strain of *C. etchellsii* showed a little difference (52.0 and 52.4 mol%, respectively), the sequence analysis of D1/D2 and taxonomic characteristics clearly suggested that ST-22 represented a new ascomycetous anamorphic yeast. ST-22 was proposed to name *Candida flosculi*.

***Candida flosculi* sp. nov. (ST-22)**

Growth in 2% glucose-yeast extract-peptone-broth: After 3 days at 25°C, the cells proliferate by multilateral budding. They are round, ovoidal and ellipsoidal, 1.7-4.2 x 2.0-6.5 μm, single or in pairs (Fig. 17).

Growth in 2% glucose-yeast extract-peptone-agar: After one month at 25°C, the streak culture is tannish-yellow, smooth, flat, shiny, soft and the margin is entire.

Growth on the surface of assimilation media: Pellicles are not formed.

Slide culture on potato dextrose agar: After 14 days at 25°C, pseudomycelia and true mycelia are not produced.

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	+	Ribitol	-
L-Sorbose	+	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	-	D-Glucitol	+
Cellobiose	-	Xylitol	nd
Trehalose	-	L-Arabinitol	nd
Lactose	-	α-Methyl-D-glucoside	-
Melibiose	-	Salicin	+

Raffinose	+	Glucono-δ-lactone	+
Melezitose	-	D-Gluconic acid	nd
Inulin	-	2-Ketogluconic acid	+
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	-	DL-Lactic acid	-
L-Arabinose	-	Succinic acid	+
D-Arabinose	-	Citric acid	+
D-Ribose	+	Saccharic acid	nd
L-Rhamnose	-	D-Glucuronic acid	-
D-Glucosamine	nd	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	nd	Inositol	-
Methanol	nd	Propane 1,2 diol	nd
Ethanol	-	Butane 2,3 diol	nd
Glycerol	-	Hexadecane	nd

Assimilation of nitrogen compounds:

Potassium nitrate	+	L-Lysine	+
Sodium nitrite	+	Cadaverine	+
Ethylamine	+		+

Additional tests:

Starch formation	-	Acid formation	+
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	+	Lipase	-
0.1% Cycloheximide	+	Maximum temperature	30°C
50% Glucose	-	Ubiquinone system:	Q-9
10% NaCl + 5% Glucose	nd	Mol% G+C (by HPLC)	52.0
Vitamin free medium	+		

Holotype: The strain ST-22 isolated from a flower collected in Khao Yai National Park of Thailand in 2000 is the holotype of this species. This strain is deposited at the BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Thailand, as BCC7722.

Etymology: The specific epithet "*flosculi"* refers to a little flower that is a source of this strain.

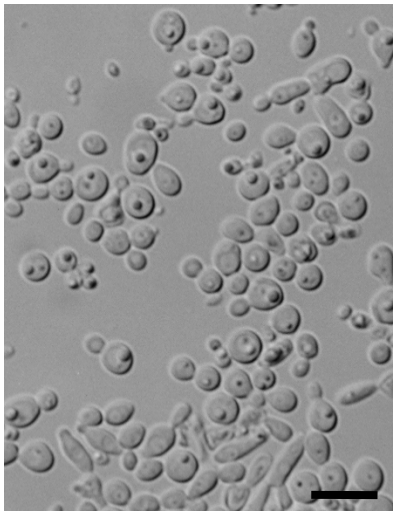

Figure 17 Morphological characteristics of *Candida flosculi* grown in YMB for 3 days at 25°C. Scale = 10 μm.

4.2.3 Strains related to *Candida friedrichii* (Group I; ST-328, ST-329 and ST-333, Group II; ST-300, ST-365 and ST- 366, Group III; ST-331, and Group IV; ST-43)

In the phylogenetic tree constructed based on the sequences of D1/D2 domain of 26S rDNA, eight strains were most closely related with *Candida friedrichii* (Fig. 18). They were separated to 4 groups, Group I (ST- 328, ST-329 and ST-333), Group II (ST-300, ST-365 and ST- 366), Group III (ST-331) and Group IV (ST-43).

Group I contained 3 strains, ST- 328, ST-329 and ST-333. They were isolated from insect frass that was collected from Tone Nga Chang Waterfall, Songkhla, Thailand. These 3 strains had the same sequences in D1/D2 domain so that they are located at the same position in a cluster with *C. friedrichii*, their closest species (Fig. 18). They differed in 8 nucleotides from *C. friedrichii* and were assigned to a new species and named *Candida songkhlaensis* sp. nov. The DNA-DNA homology of these 3 strains showed similarities of 54.6% or more between them, 21.1% or less with *C. friedrichii*, 54.2% or less with other strains of Group II, Group III and Group IV as shown in Table 29. However, DNA-DNA homology should be repeated again to completed identification of these strains.

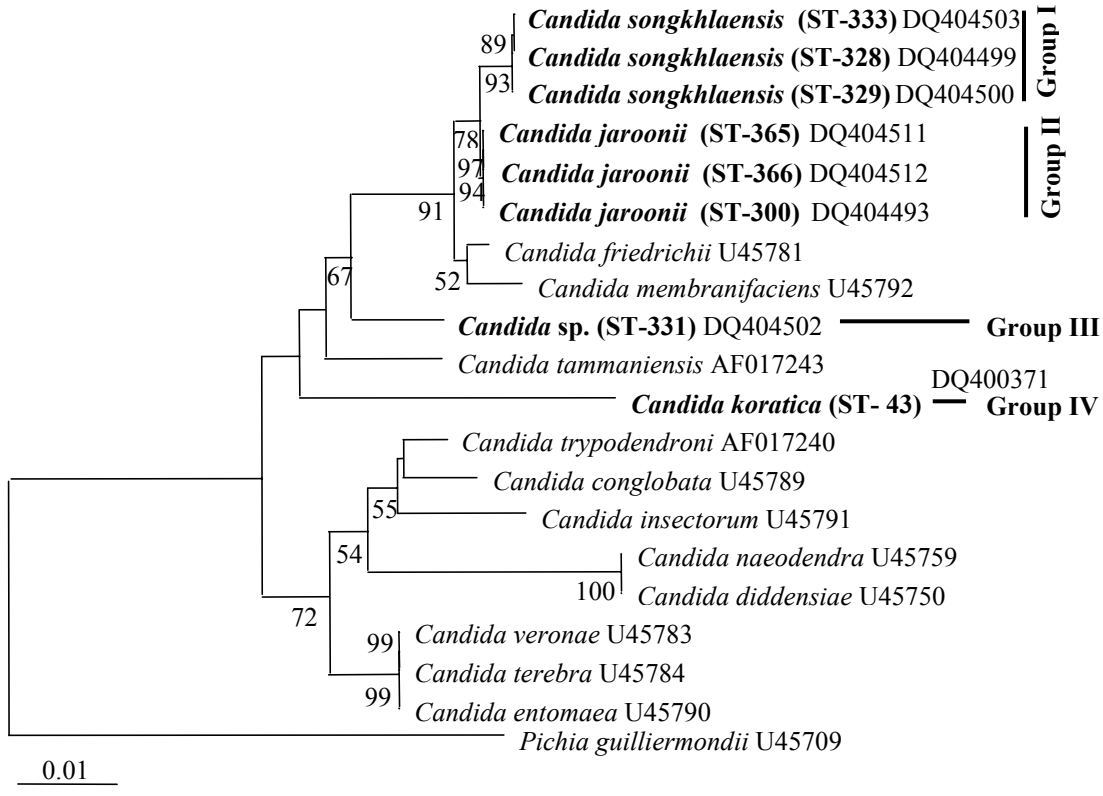

Figure 18 Phylogenetic tree showing the positions of strains related to *Candida friedrichii* based on the sequences of the D1/D2 domain of 26S rDNA with bootstrap values by 1000 resampling (< 50% is not shown).

Table 31 DNA-DNA hybridization of strains related to *C. friedrichii*.

Group/Strains		ST-328	ST-329	ST-333	ST-300	ST-365	ST-366	ST-331	ST-43
	ST-328	100	74.2	184.6	45.1	37.6	32.5	18.1	26.7
Group I	ST-329	164.9	100	198.7	27.8	18.6	25.7	21.9	27.7
	ST-333	135.3	54.6	100	12.9	2.4	9.7	10.2	20.1
	ST-300	21.4	12.3	54.2	100	126.1	95.9	69.9	50.5
Group II	ST-365	20.9	47.2	34.7	98.4	100	106.3	60.7	42.7
	ST-366	40.1	17.3	39.3	98.3	108.7	100	47.6	60.2
Group III	ST-331	16.3	0.2	6.7	17.1	2.4	2.3	100	21.6
Group IV	ST-43	24.3	0	39.5	19.7	7.1	18.6	66.6	100
C. friedrichii NBRC 10277		5.5	0	21.1	24.1	0.9	0	20.1	8.4

Group II contained 3 strains, ST-300, ST-365 and ST- 366. Strain ST-300 was isolated from insect frass collected in Ko Yao, Pattani, Thailand and 2 strains, ST-365 and ST-366, were isolated from Tone-Nga-Chang Waterfall, Songkhla province, Thailand. These 3 strains had the same sequences in D1/D2 domain and differed in 6 nucleotides from *C. friedrichii*, their closest species. In the phylogenetic tree, they are located at the same position in a cluster with *C. friedrichii* (Fig. 18) and close to Group I. Group II was separated from Group I by 2 nucleotide differences and located in the different position in phylogenetic tree. In the conventional taxonomic characteristics, Group II differed from Group I in the assimilation of L-sorbose and L-rhamnose. The DNA-DNA homology of these 3 strains showed similarities of 98.3 % or more between them, 24.1% or less with *C. friedrichii* and 54.2% or less from strains of Group I, Group III and Group IV as shown in Table 29. Apparently, Group II represents a new species. The name *Candida jaroonii* sp. nov. was given for this species.

Group III contained a strain, ST-331, which was isolated from insect frass that was collected from Tone Nga Chang Waterfall, Songkhla province, Thailand. This strain wass located at the position distant from the most closely related species, *C. friedrichii*, by 14 nucleotides difference (Fig. 18) and is considered to represent a new species. The DNA-DNA homology of this strain showed similarity of 20.1% with *C. friedrichii,* and differed from other strains of Group I, Group II and Group IV as shown in Table 29. Recently, however, a new species *Candida amphixiae* was reported (Suh *et al*., 2005). This species differed from ST-331 in 2 nucleotides in D1/D2 region. So, ST-331 is conspecific with this species or sister species. Tentatively, ST-331 is dealt as *Candida* sp.1.

Group IV contained a strain, ST-43, isolated from insect frass that was collected from Khao Yai National Park, Nakhon Ratchasima, Thailand. In the phylogenetic tree, this strain was located at the position distant from the cluster in which nearest species is located. ST-43 differed in 43 nucleotides (including 12 gaps) from *C. friedrichii* (Fig. 18). The DNA-DNA homology of this strain showed similarities of 8.4% with *C. friedrichii,* and 60.2% or less from other strains of Group I, Group II and Group IV as shown in Table 29. This strain was assigned to a new species and named *Candida koratica* sp. nov.

Description of new species related to *Candida friedrichii* showed as below.

***Candida jaroonii* sp. nov. (ST-300, ST-365 and ST-366)**

Growth in 2% glucose-yeast extract-peptone-broth: After 3 days at 25^{o}C, the cells proliferate by multilateral budding. The cells of pellicle are ellipsoidal to cylindrical, elongate, 2.5-6 x 6-14 μm, single, in pairs and in pseudomycelia (Fig. 19A). The cells of sediment are round to short ovoidal, single or in pairs, pseudomycelial cells elongate, 3-10 x 4-12 μm (Fig. 19B).

Growth in 2% glucose-yeast extract-peptone-agar: After one month at 25^{o}C, the streak culture are grayish white, smooth, dull-shining, soft and has entire margin.

Growth on the surface of assimilation media: After one month at 25^{o}C, thin, creeping pellicle and sediment are present.

Fermentation: Glucose and galactose are fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	+
Galactose	+	Ribitol	+
L-Sorbose	+	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	+
Trehalose	+	L-Arabinitol	+
Lactose	-	α-Methyl-D-glucoside	+
Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	+
Melezitose	+	D-Gluconic acid	Latent
Inulin	-	2-Ketogluconic acid	+
Soluble starch	Weak	5-Ketogluconic acid	-
D-Xylose	+	DL-Lactic acid	Latent
L-Arabinose	+	Succinic acid	+
D-Arabinose	+	Citric acid	+
D-Ribose	+	Saccharic acid	-
L-Rhamnose	+	D-Glucuronic acid	-
D-Glucosamine	Latent	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	+	Inositol	-
Methanol	-	Propane 1,2 diol	-
Ethanol	+ or latent	Butane 2,3 diol	-
Glycerol	+	Hexadecane	+

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	+
Ethylamine	-		

Additional tests:

Starch formation	-	Acid formation	weak
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	-	Lipase	-
0.1% Cycloheximide	-	Maximum temperature	37-38°C
50% Glucose	nd	Ubiquinone system	Q-9
10% NaCl + 5% Glucose	+	Mol% G+C (by HPLC)	33.0-33.94
Vitamin required	Thiamine		

Type strain: ST-300, isolated from insect frass collected in Ko Yao, Pattani province, Thailand, Mar. 2001, is the type strain of this species. It was deposited at BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Thailand, as BCC 11783.

Etymology: The specific epithet was chosen in honor of Dr. Jaroon Kumnuanta for his contribution of yeast researches in Thailand.

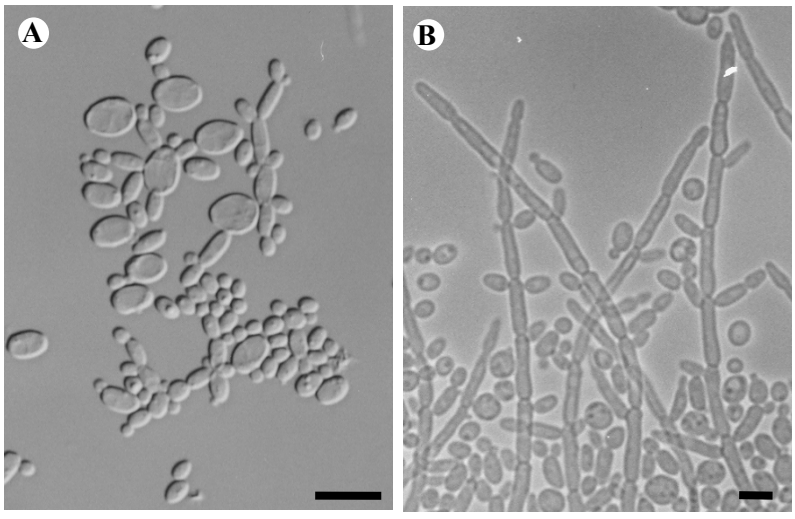

Figure 19 Morphological characteristics of *Candida jaroonii* (ST-300); (A) cells of pellicle grown in YMB for 3 days at 25°C; (B) cells of sediment grown in YMB for 3 days at 25°C. Scales = 10 μm.

***Candida koratica* sp. nov. (ST-43)**

Growth in 2% glucose-yeast extract-peptone-broth: After 3 days at 25°C, the cells proliferate by multilateral budding. They are round to ovoid, sometimes elongate, 3.5-10 x 4-12 μm, single, in pairs, in short chains (Fig. 20).

Growth in 2% glucose-yeast extract-peptone-agar: After one month at 25°C, the streak culture are grayish white, smooth, shining, soft and has entire margin.

Growth on the surface of assimilation media: After one month at 25°C, trace of a ring and sediment are present.

Fermentation: Glucose and galactose are fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	+	Ribitol	+
L-Sorbose	Latent	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	Latent
Trehalose	+	L-Arabinitol	-
Lactose	-	α-Methyl-D-glucoside	+
Melibiose	-	Salicin	Latent
Raffinose	-	Glucono-δ-lactone	+
Melezitose	+	D-Gluconic acid	Latent
Inulin	-	2-Ketogluconic acid	-
Soluble starch	Latent & weak	5-Ketogluconic acid	-
D-Xylose	+	DL-Lactic acid	-
L-Arabinose	Latent	Succinic acid	+
D-Arabinose	+	Citric acid	+
D-Ribose	+	Saccharic acid	-
L-Rhamnose	+	D-Glucuronic acid	-
D-Glucosamine	Latent	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	+	Inositol	-

Methanol	-	Propane 1,2 diol	-
Ethanol	-	Butane 2,3 diol	-
Glycerol	+	Hexadecane	+

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	+
Ethylamine	-		

Additional tests:

Starch formation	-	Acid formation	-
Gelatin liquefaction	Latent & weak	Urease	-
0.01% Cycloheximide	-	Lipase	-
0.1% Cycloheximide	-	Maximum temperature	35-36°C
50% Glucose	-	Ubiquinone system	Q-9
10%Nacl+5%Glucose	-	Mol% G+C (by HPLC)	37.94
Vitamins required	Pyridoxine and thiamine		

Holotype: ST-43, isolated from insect frass collected in Khao Yai National Park, Nakhon Ratchasima province, Thailand, Jan. 2001, is the holotype of this species. It was deposited at BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Thailand, as BCC 7743.

Etymology: The specific epithet "*koratica*" was derived from "Korat" the common name of procvince where this yeast was found.

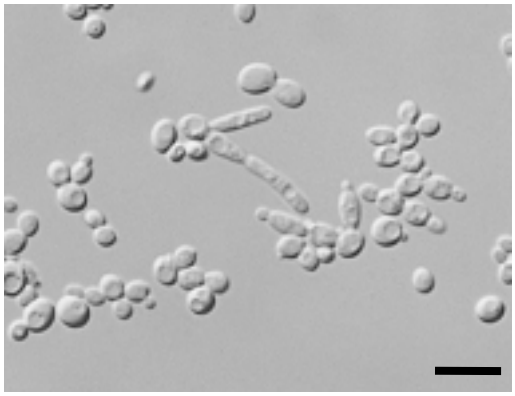

Figure 20 Morphological characteristics of *Candida koratica* (ST-43) grown in YMB for 3 days at 25°C. Scale = 10 μm.

***Candida songkhlaensis* sp. nov. (ST-328, ST-329 and ST-333)**

Growth in 2% glucose-yeast extract-peptone-broth: After 3 days at 25°C, the cells proliferate by multilateral budding. They are round to short oval, elongate, pseudomycelia observed, 3.5-10 x 4-14 μm, single or in pairs (Fig. 21A).

Growth in 2% glucose-yeast extract-peptone-agar: After one month at 25°C, the streak culture are grayish white, smooth, shining, soft and has entire margin.

Slide culture on on potato dextrose agar***:*** After 7 days at 25°C, pseudomycelia is formed (Fig. 21B).

Growth on the surface of assimilation media: After one month at 25°C, trace of a ring and sediment are present.

Fermentation: Glucose and galactose are fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	+
Galactose	+	Ribitol	+
L-Sorbose	- / latent & weak	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	+
Trehalose	+	L-Arabinitol	+
Lactose	-	α-Methyl-D-glucoside	+

Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	+
Melezitose	+	D-Gluconic acid	+
Inulin	-	2-Ketogluconic acid	+
Soluble starch	- or weak	5-Ketogluconic acid	-
D-Xylose	+	DL-Lactic acid	Latent / latent & weak
L-Arabinose	+	Succinic acid	+
D-Arabinose	+ / latent	Citric acid	+
D-Ribose	+	Saccharic acid	-
L-Rhamnose	-	D-Glucuronic acid	-
D-Glucosamine	+	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	+	Inositol	-
Methanol	-	Propane 1,2 diol	-
Ethanol	Latent	Butane 2,3 diol	-
Glycerol	+	Hexadecane	+

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	+
Ethylamine	Weak		

Additional tests:

Starch formation	-	Acid formation	-
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	-	Lipase	-
0.1% Cycloheximide	-	Maximum temperature	38-39°C
50% Glucose	nd	Ubiquinone system	Q-9
10%NaCl+5% Glucose	+	Mol% G+C (by HPLC)	33.79-34.80
Vitamins required	Pyridoxine and thiamine		

Type strain: ST-328, isolated from insect frass collected at Tone Nga Chang Waterfall, Songkhla province, Thailand, Mar. 2001, is the type strain of this species. It was deposited at BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Thailand, as BCC 11804.

Etymology: The specific epithet was derived from "Songkhla" the name of procvince where this yeast was found.

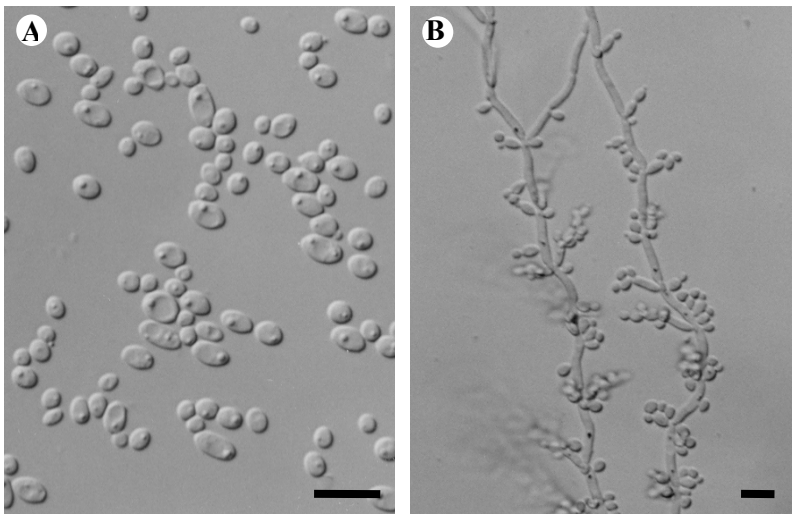

Figure 21 Morphological characteristics of *Candida songkhlaensis* (ST-328); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

***Candida* sp.1 (ST-331)**

Growth in 2% glucose-yeast extract-peptone-broth: After 3 days at 25°C, the cells proliferate by multilateral budding. They are short oval to oval, ellipsoidal, single or in pairs, 3-8.5 x 4.5-10 μm (Fig. 22).

Growth in 2% glucose-yeast extract-peptone-agar: After one month at 25°C, the streak culture are grayish white, smooth, shining, soft and has entire margin.

Growth on the surface of assimilation media: After one month at 25°C, Trace of a ring and sediment are present.

Fermentation: Glucose and galactose are ferment.

Assimilation of carbon compounds:

Glucose	+	Erythritol	+
Galactose	+	Ribitol	+
L-Sorbose	Latent	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	+
Trehalose	+	L-Arabinitol	+
Lactose	Latent	α-Methyl-D-glucoside	+
Melibiose	Latent	Salicin	+
Raffinose	-	Glucono-δ-lactone	+
Melezitose	+	D-Gluconic acid	+
Inulin	-	2-Ketogluconic acid	+
Soluble starch	Weak	5-Ketogluconic acid	-
D-Xylose	+	DL-Lactic acid	-
L-Arabinose	+	Succinic acid	Latent
D-Arabinose	+	Citric acid	+
D-Ribose	+	Saccharic acid	+
L-Rhamnose	+	D-Glucuronic acid	-
D-Glucosamine	+	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	+	Inositol	-
Methanol	-	Propane 1,2 diol	-
Ethanol	+	Butane 2,3 diol	-
Glycerol	+	Hexadecane	+

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	+
Ethylamine	-		

Additional tests:

Starch formation	-	Acid formation	Weak
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	-	Lipase	-
0.1% Cycloheximide	-	Maximum temperature	38-39°C
50% Glucose	nd	Ubiquinone system	Q-9
10% NaCl+5% Glucose	+	Mol% G+C (by HPLC)	32.95%
Vitamins required	Pyridoxine and thiamine		

Holotype: ST-331, isolated from insect frass collected in Tone Nga Chang Waterfall, Songkhla province, Thailand, Mar. 2001, is the holotype of this species. It was deposited at BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Thailand, as BCC 11807.

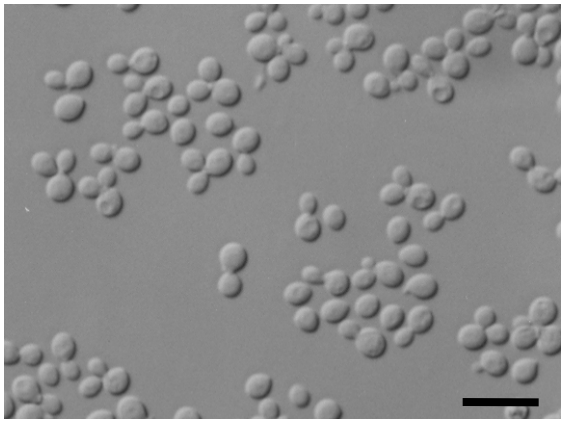

Figure 22 Morphological characteristics of *Candida* sp. (ST-311) grown in YMB for 3 days at 25°C. Scale = 10 μm.

4.2.4 Strains related to *Candida sorbophila* (ST-297 and ST-315)

ST-297 and ST-315 were isolated from insect frass collected in Ko Yao, Pattani, Thailand. In the phylogenetic tree based on the D1/D2 domain sequences of 26S rDNA, strain ST-315 constituted a cluster with two strains of undescribed *Candida* species, BG99-8-18-1-6, BG99-8-18-1-3-1 and connected with *Candida* sp. NRRL Y-27690 then with ST-297 (Fig. 23). The cluster comprising these 5 yeasts connected with a cluster including *C. sorbophila*. ST-315 differed from two undescribed *Candida* in 35 nucleotides (6.6%) in D1/D2 domain and ST-297 differed in 63 nucleotides (12.0%) from these *Candida* strains in this region. Strains ST-297 and ST-315 differed from each other in 74 nucleotides (13.7%) in D1/D2 domain. Furthermore, ST-297 differed from *C. sorbophila*, the nearest known species in D1/D2 sequences, in 79 nucleotides (14.8%) and ST-315 differed from *C. sorbophila*, the nearest known species in D1/D2 sequences, in 84 nucleotides (15.2%). It is concluded that ST-297 and ST-315 represent different undescribed species that were phylogenetically distant from the known species (Fig. 23).

In the phenotypic characteristics, ST-297 and ST-315 resemble from each other but are distinguished by the assimilability of ribitol as a carbon source and ethylamine and cadaverine as nitrogen sources, and alcoholic fermentative ability, negative for ST-297 and positive for ST-315. The two strains showed some resemblances to several *Candida* species such as *C. antillancae*, *C. apis*, *C. geochares* and *C. gropengiesseri*. However, they are distinguished from these four species by the good assimilation of hexadecane and the requirement of pyridoxine. ST-297 and ST-315 are described below as respective new species, *Candida kazuoi* sp. nov. (ST-297) and *Candida hasegawae* sp. nov. (ST-315).

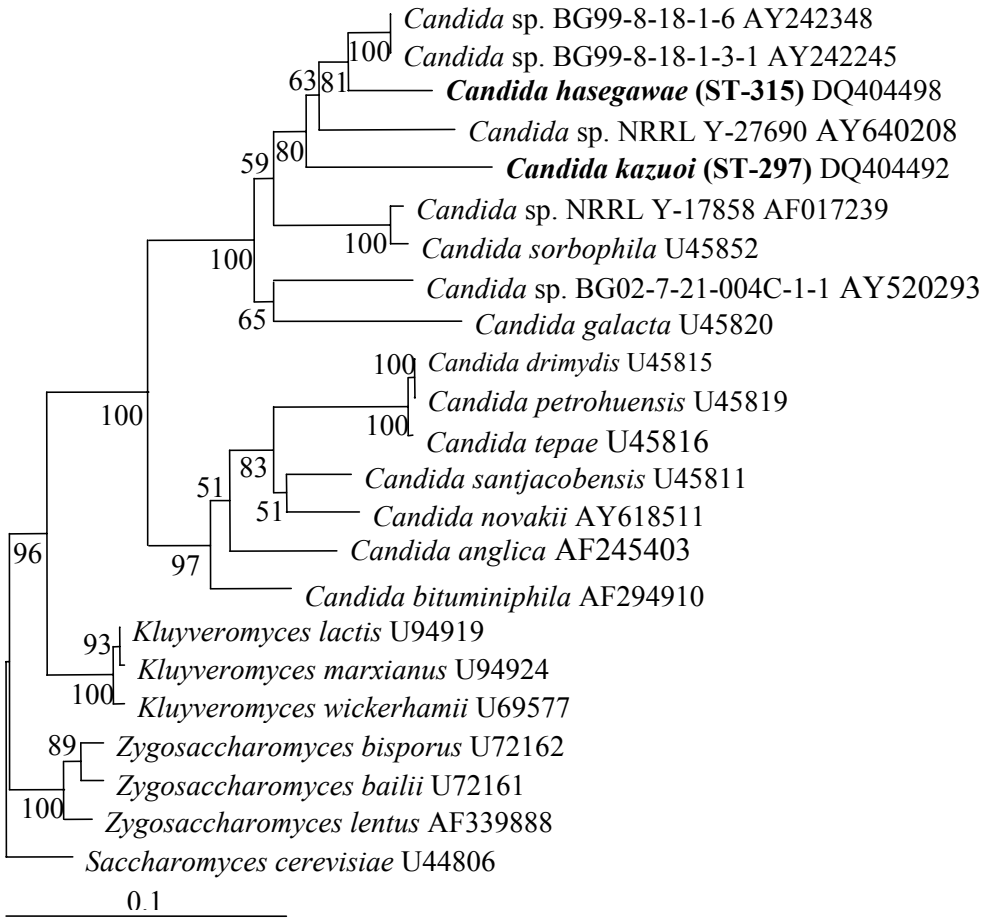

Figure 23 Phylogenetic tree for two new species, *Candida kazuoi* and *Candida hasegawae,* constructed by the neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the bootstrap values of 1,000 resamplings.

Description of strains related to *Candida sorbophila* showed as below.

***Candida kazuoi* sp. nov. (ST-297)**

Growth in YM broth: After 3 days at 25°C, cells are globose, short-ovoidal to ovoidal, 2.0-4.5 x 2.0-5.0 μm, single, in pairs or in short chains (Fig. 24). Thin pellicle and a sediment are formed. After 1 month at 20°C, fragile islets, a ring and a sediment are present.

Growth on YM agar: After 1 month at 20°C, the streak culture is grayish white to pale brown, smooth, semi-shining, soft and has an entire to erose margin.

Slide culture on potato dextrose agar: Pseudomycelium is not produced.

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-
Sucrose	-	D-Mannitol	+
Maltose	-	D-Glucitol	+
Cellobiose	-	Xylitol	+
Trehalose	-	L-Arabinitol	-
Lactose	-	α-Methyl-D-glucoside	-
Melibiose	-	Salicin	-
Raffinose	-	Glucono-δ-lactone	+
Melezitose	-	D-Gluconic acid	Latent
Inulin	-	2-Ketogluconic acid	-
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	-	DL-Lactic acid	-
L-Arabinose	-	Succinic acid	+
D-Arabinose	-	Citric acid	+
D-Ribose	-	Saccharic acid	-
L-Rhamnose	+	D-Glucuronic acid	-
D-Glucosamine	Latent	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	Propane-1,2-diol	-
Ethanol	-	Butane-2,3-diol	-
Glycerol	-	Hexadecane	+

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	-
Ethylamine	-		

Additional tests:

Starch formation	-	Urease	-
Gelatin liquefaction	Slow	Lipase	-
0.01% Cycloheximide	-	Maximum temperature	35-36°C
0.1% Cycloheximide	-	Ubiquinone system:	Q-9
Acid formation	-	Mol% G+C (by HPLC)	56.3 %
10%NaCl + 5% glucose	Weak		
Vitamins required	Biotin, pyridoxine and thiamine		

Holotype: ST-297, isolated from insect frass collected in Ko Yao, Pattani province, Mar. 2001, is the type strain of this species. It was deposited at BIOTEC Culture Collection as BCC 11780. This strain was also deposited at Japan Collection of Microorganisms (JCM), RIKEN, as JCM 12558. The strain is maintained by freezing and/or lyophilization in these culture collections.

Etymology: The specific epithet was chosen in honor of Dr. Kazuo Komagata for his many contributions to microbial diversity including yeasts in Asian countries.

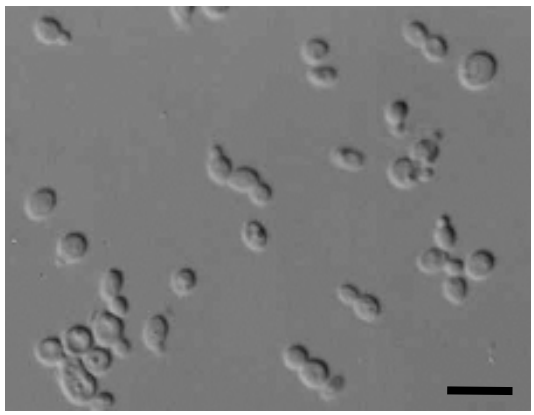

Figure 24 Morphological characteristics of *Candida kazuoi* (ST-297) grown in YMB for 3 days at 25°C. Scale = 10μm.

***Candida hasegawae* sp. nov. (ST-315)**

Growth in YM broth: After 3 days at 25°C, cells are globose, ovoidal to long ovoidal or ellipsoidal, 2.5-4.5 x 2.5-6 μm, single, in pairs or in short-chains, sometimes pseudomycelia are present (Fig. 25A). Pseudomycelial cells are elongate measuring up to 13 μm in length. An incomplete ring and a sediment are formed. After 1 month at 20°C, fragile pellicle and a sediment are present.

Growth on YM agar: After 1 month at 20°C, the streak culture is grayish white, smooth, dull, soft and has an entire margin.

Slide culture on potato dextrose agar: Pseudomycelia are well developed (Fig. 25B).

Fermentation: Glucose is latently and slowly fermented. Galactose, sucrose, maltose, lactose and raffinose are not fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	+	Ribitol	+
L-Sorbose	+ / latent	Galactitol	Latent / latent & weak
Sucrose	-	D-Mannitol	+
Maltose	-	D-Glucitol	+
Cellobiose	Latent	Xylitol	Latent
Trehalose	-	L-Arabinitol	Latent
Lactose	-	α-Methyl-D-glucoside	-
Melibiose	-	Salicin	-
Raffinose	-	Glucono-δ-lactone	Latent
Melezitose	-	D-Gluconic acid	-
Inulin	-	2-Ketogluconic acid	-
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	Latent	DL-Lactic acid	-
L-Arabinose	Latent	Succinic acid	+
D-Arabinose	+	Citric acid	+
D-Ribose	-	Saccharic acid	-

L-Rhamnose	-	D-Glucuronic acid	-
D-Glucosamine	-	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	Propane-1,2-diol	-
Ethanol	-	Butane-2,3-diol	-
Glycerol	+ or latent	Hexadecane	+

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	+
Ethylamine	+		

Additional tests:

Starch formation	-	Acid formation	-
Gelatin liquefaction	Slow	Urease	-
0.01% Cycloheximide	-	Lipase	-
0.1% Cycloheximide	-	Maximum temperature	35-36°C
50% glucose	-	Ubiquinone system:	Q-9
10% NaCl + 5% Glucose	-	Mol% G+C (by HPLC)	49.2
Vitamins required	Biotin, pyridoxine and thiamine		

Holotype: ST-315, isolated from insect frass collected in Ko Yao, Pattani province, Thailand, in Mar. 2001, is the type strain of this species. It was deposited at BIOTEC Culture Collection as BCC 11794. This strain is also maintained at Japan Collection of Microorganisms (JCM), RIKEN, as JCM 12559. The strain is maintained by freezing and/or lyophilization in these culture collections. .

Etymology: The specific epithet was chosen in honor of Dr. Takeji Hasegawa for his contribution to yeast systematics including early introduction of chemical method to define the genera of yeasts.

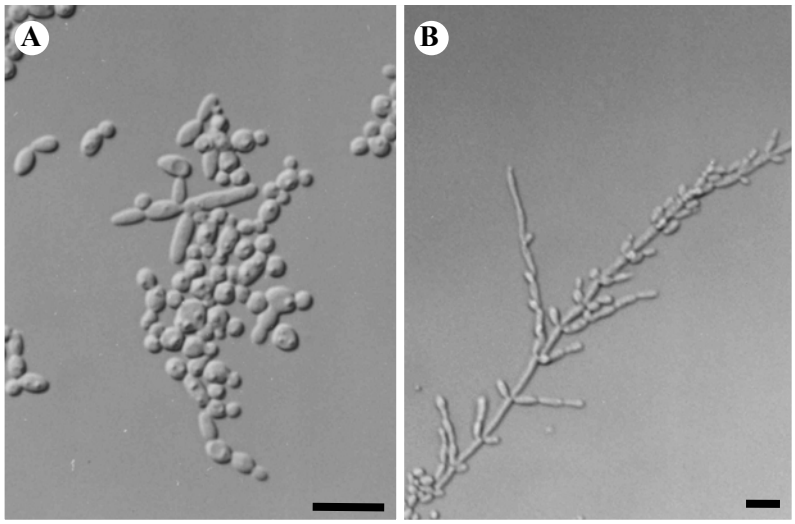

Figure 25 Morphological characteristics of *Candida hasegawae* (ST-315); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

4.2.5 Strain related to *Candida tsuchiyae* (ST-17)

ST-17 was isolated from insect frass that was collected from Khao Yai National Park of Thailand. This yeast poliferated by multilateral budding, showed negative DBB and urease reactions, did not produce ascospores, and had Q-9 as a major ubiquinone. These characteristics coincided with the genus *Candida*. This strain differed in 66 nucleotides (10.0%) from *C. tsuchiyae*, the most closely related species of this strain. In the phylogenetic tree based on the sequences of D1/D2 domain of 26S rDNA, ST-17 is located in a cluster with *C. intermedia, C. pseudointermedia* and *C. tsuchiyae*. (Fig. 26). The big nucleotide differences of 10% or more from closely related known species clearly indicated that ST-17 represented hitherto undescribed species.

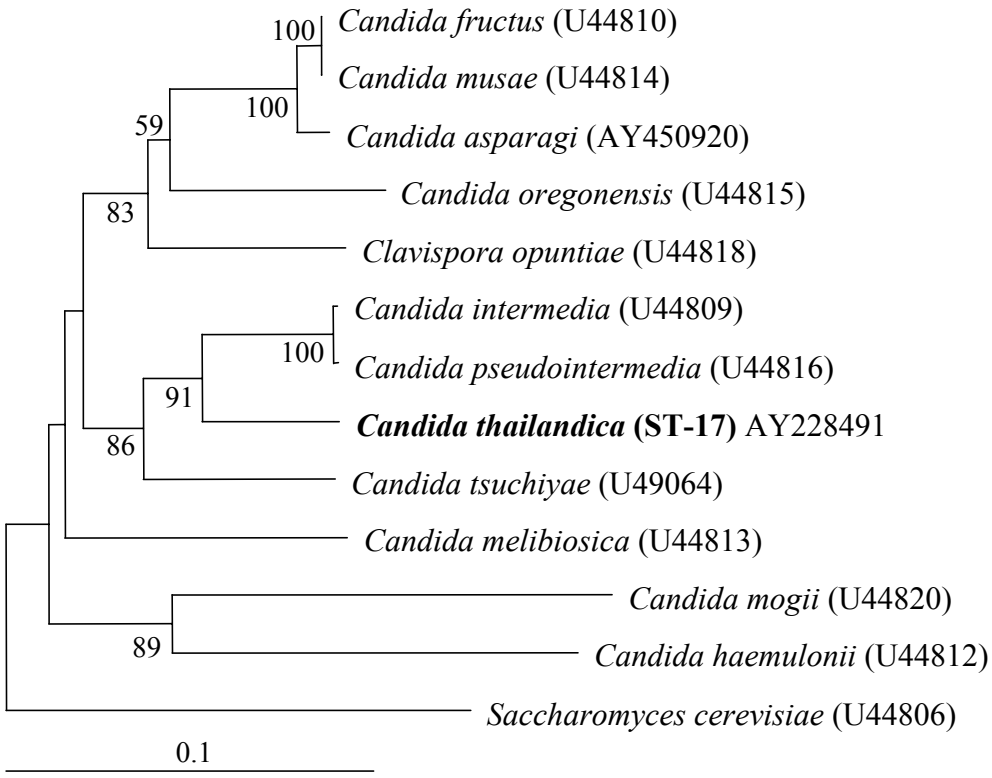

Figure 26 Phylogenetic tree showing the position of *Candida thailandica* (ST-17) based on the sequences of the D1/D2 domain of 26S rDNA with bootstrap values by 1000 re-sampling (< 50% is not shown).

In the phenotypic characteristics, this strain is distinguished from *C. tsuchiyae* based on fermentation of galactose, sucrose and raffinose, assimilation of galactose, cellobiose, lactose, raffinose, soluble starch, D-xylose, D-arabinose, ethanol, galactitol and salicin, growth on 50% glucose and growth at 30°C. The difference of 2.7 mol% G+C was found in the chromosomal DNA between this strain (44.8%) and *C. tsuchiyae* (47.5%). This strain was named *Candida thailandica.* It will be validly described in near future.

***Candida thailandica* sp. nov. (ST-17)**

Growth in 2% glucose-yeast extract-peptone-broth: After 3 days at 25°C, the cells proliferate by maltilateral budding. They are ovoidal to cylindrical, 2.0-7.0 x 2.0-7.0 μm, single or in pairs (Fig. 27).

Growth in 2% glucose-yeast extract-peptone-agar: After one month at 25°C, the streak culture is white, glossy, flat, striated and the margin is entire.

Growth on the surface of assimilation media: Pellicles are not formed.

Slide culture on potato dextrose agar: After 5 days at 25°C, pseudomycelia is produced.

Fermentation: Glucose and galactose are fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	+	Ribitol	+
L-Sorbose	+	Galactitol	+
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	nd
Trehalose	+	L-Arabinitol	nd
Lactose	+	α-Methyl-D-glucoside	+
Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	+
Melezitose	+	D-Gluconic acid	nd
Inulin	-	2-Ketogluconic acid	+
Soluble starch	Weak	5-Ketogluconic acid	-
D-Xylose	+	DL-Lactic acid	Weak
L-Arabinose	-	Succinic acid	+
D-Arabinose	+	Citric acid	+
D-Ribose	-	Saccharic acid	nd
L-Rhamnose	-	D-Glucuronic acid	-
D-Glucosamine	nd	D-Galacturonic acid	-

N-Acetyl-D-glucosamine	nd	Inositol	-
Methanol	nd	Propane 1,2 diol	nd
Ethanol	-	Butane 2,3 diol	nd
Glycerol	-	Hexadecane	nd

Assimilation of nitrogen compounds:

Potassium nitrate	+	L-Lysine	+
Sodium nitrite	+	Cadaverine	+
Ethylamine	+		

Additional tests:

Starch formation	-	Acid formation	+
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	-	Lipase	-
0.1% Cycloheximide	-	Maximum temperature	30°C
50% Glucose	-	Ubiquinone system	Q-9
60% Glucose	-	Mol% G+C (by HPLC)	44.7
Vitamin free medium	-		

Holotype: The strain ST-17 was isolated from insect frass collected from Khao Yai National Park of Thailand in 2000 is the holotype of this species. This strain is deposited at the BIOTEC Culture Collection, National Center for Genetics Engineering and Biotechnology, Thailand as BCC7717.

Etymology: The specific epithet "*thailandica"* refers to "Thailand", the country of the strain isolated.

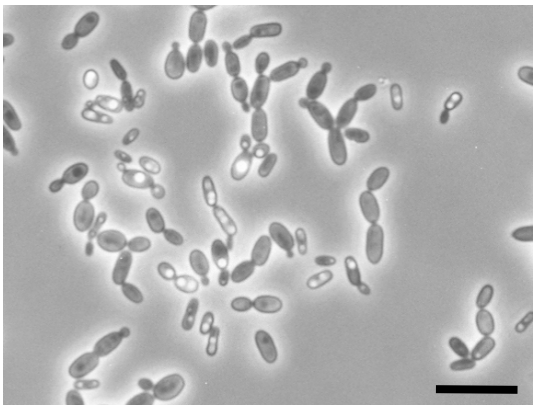

Figure 27 Morphological characteristics of *Candida thailandica* (ST-17) grown in YMB for 3 days at 25°C. Scale = 10 μm.

4.4.6 Strain related to *Pichia americana* (ST-37)

Strain ST-37 was isolated from insect frass collected in Ko Yao, Pattani, Thailand. This strain had Q-7 as the major component of ubiquinones. In the phylogenetic tree based on the D1/D2 sequences of 26S rDNA, strain ST-37 constituted a cluster with *Pichia americana* and *Pichia bimundalis* (Fig. 28). This strain differed in 6 (1.1%) and 7 nucleotides (1.2%) from the latter two species, respectively.

In the taxonomic criteria commonly employed, this strain is differentiated from these two species in the good fermentation of glucose and sucrose, and inability to assimilate raffinose. However, this strain was assigned to the genus *Candida* because it did not produce ascospores. Apparently, this strain represents a new species. It was named *Candida nakhonratchasimensis* sp. nov. (Jindamorakot *et al*., 2004).

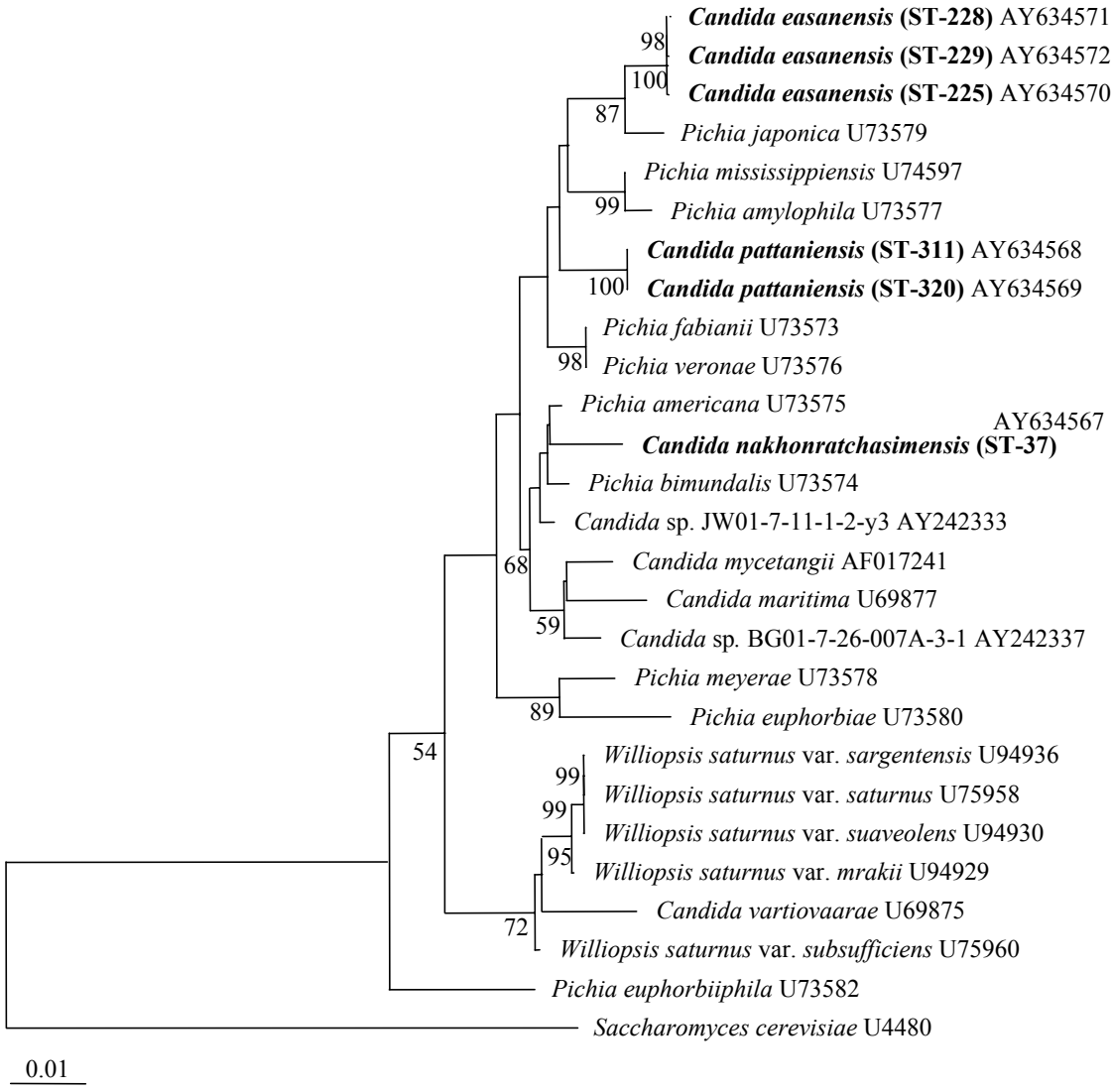

Figure 28 Phylogenetic tree for *Candida easanensis*, *Candida pattaniensis* and *Candida nakhonratchasimensis* isolated in Thailand constructed by the neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the bootstrap values of 1,000 trials.

***Candida nakhonratchasimensis* sp. nov. (ST-37)**

Growth in YM broth: After 3 days at 25°C, the cells are globose to short voildal, oval, 3.5-7 x 4-7 μm, single or in pair (Fig. 29A). A fragile ring and a sediment are produced. After one month at 20°C, trace of a ring and a sediment are present.

Growth on YM agar: After one month at 20°C, the streak culture is grayish-white, smooth, shining, soft and has an entire to ciliate margin.

Slide culture on potato dextrose agar: Well-developed pseudomycelia are abundantly produced (Fig. 29B).

Fermentation of sugars: Glucose and sucrose are fermented. Galactose, maltose, lactose, melibiose and raffinose are not fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	+
Trehalose	Latent	L-Arabinitol	-
Lactose	-	α-Methyl-D-glucoside	+
Melibiose	-	Salicin	+
Raffinose	+	Glucono-δ-lactone	+
Melezitose	+	D-Gluconic acid	+
Inulin	-	2-Ketogluconic acid	-
Soluble starch	Latent & weak	5-Ketogluconic acid	-
D-Xylose	+ or latent	DL-Lactic acid	+
L-Arabinose	-	Succinic acid	+
D-Arabinose	-	Citric acid	+
D-Ribose	-	Saccharic acid	-
L-Rhamnose	+	D-Glucuronic acid	-

D-Glucosamine	-	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	Propane 1,2 diol	+
Ethanol	+	Butane 2,3 diol	-
Glycerol	+	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	+	L-Lysine	+
Sodium nitrite	+	Cadaverine	+
Ethylamine	+		

Additional tests:

Starch formation	-	Urease	-
Gelatin liquefaction	-	Lipase	-
0.01% Cycloheximide	-	Maximum temperature	33-34°C
0.1% Cycloheximide	-	Ubiquinone system:	Q-7
10% NaCl + 5% glucose	-	Mol% G+C (by HPLC)	39.2%
Acid formation	-		
Vitamins required	Pyridoxine and thiamine		

Holotype: ST-37, isolated from insect frass collected in Jan 2001. , Khao Yai, Nakhon Ratchasima province, Thailand, is the type strain of this species. It was deposited at BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), as BCC 7737, Thailand Institute of Scientific and Technological Research as TISTR 5826 and at the Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, as JCM 12474.

Etymology: The specific epithet is derived from “Nakhonratchasima”, the name of a province in Thailand where this species was isolated.

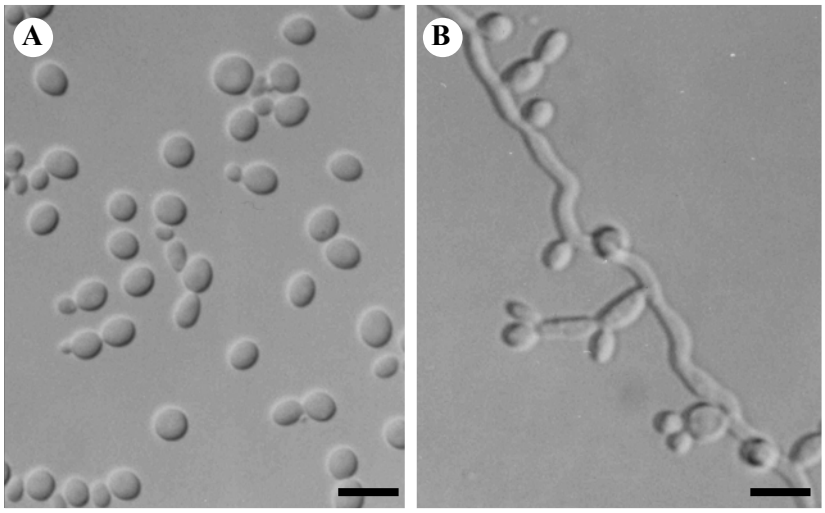

Figure 29 Morphological characteristics of *Candida nakhonratchasimensis* (ST-37); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

4.2.5 Strains related to *Pichia japonica* (ST-225, ST-228 and ST-229)

Three strains, ST-225, ST-228 and ST-229, had Q-7 as the major component of ubiquinones. In the phylogenetic tree based on the D1/D2 sequences of 26S rDNA, they were located in clusters with several *Pichia* species (Fig. 28). These facts suggest that the three strains have affinity to the genus *Pichia*. However, these strains were assigned to the genus *Candida* because they did not produce ascospores.

These three strains showed identical nucleotide sequences in the D1/D2 domain and showed taxonomic characteristics similar to each other, and were considered to represent a single species. The three strains were closely related to *Pichia japonica* but 6 nucleotides (1.1%) were different from the latter species. In the taxonomic criteria commonly employed, they are discriminated from *P. japonica* by the assimilation of soluble starch and L-arabinose. The name *Candida easanensis* is proposed for this new species (Jindamorakot *et al*., 2004).

***Candida easanensis* sp. nov. (ST-225, ST-228 and ST-229)**

Growth in YM broth: After 3 days at 25°C, the cells are globose, short ovoidal to ovoidal, ellipsoidal, cylindrical, 2-4.5 x 3-7.5 μm single or in pairs (Fig. 30A). Pseudomycelia are observed. Trace of a ring and a sediment are produced. After 1 month at 20°C, trace of a ring and a sediment are present.

Growth on YM agar: After 1 month at 20°C, the streak culture is grayish white, smooth, shining, soft and has an entire to ciliate margin.

Slide culture on potato dextrose agar: Well-developed pseudomycelia bearing many blastoconidia are abundantly produced (Fig. 30B, C).

Fermentation of sugars: Glucose is fermented. Galactose, sucrose, maltose, lactose, melibiose and raffinose are not fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	+ / latent
Trehalose	+	L-Arabinitol	+ / -
Lactose	-	α-Methyl-D-glucoside	+
Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	+
Melezitose	+	D-Gluconic acid	+ / latent
Inulin	-	2-Ketogluconic acid	-
Soluble starch	+	5-Ketogluconic acid	-
D-Xylose	+	DL-Lactic acid	+
L-Arabinose	Latent	Succinic acid	+
D-Arabinose	Latent / latent & weak	Citric acid	+
D-Ribose	-	Saccharic acid	-
L-Rhamnose	+	D-Glucuronic acid	-

D-Glucosamine	-	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	Propane 1,2 diol	+
Ethanol	+	Butane 2,3 diol	-
Glycerol	+	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	+
Ethylamine	+		

Additional tests:

Starch formation	-	Urease	-
Gelatin liquefaction	-	Lipase	-
0.01% Cycloheximide	-	Maximum temperature	41-42°C
0.1% Cycloheximide	-	Ubiquinone system	Q-7
10% NaCl + 5% glucose	-	Mol% G+C (by HPLC)	43.7-45.2
Acid formation	+		
Vitamins required	Pyridoxine and thiamine		

Type strain: ST-225, isolated from insect frass collected in Jan. 2001, in Nong Laung, Amnat Charoen, Northeastern Thailand, is the type strain of this species. It was deposited at BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), as BCC 11759, TISTR Culture Collection, Thailand Institute of Scientific and Technological Research (TISTR), as TISTR 5824, and at the Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, as JCM 12476. Other strains are maintained under the following numbers: ST-228 as BCC 11760=TISTR 5825=JCM 12477, ST-229 as BCC 11761.

Etymology: The specific epithet "*easanensis*" was chosen since all of strains of this species were isolated in several places of "Easan", a northeastern region of Thailand.

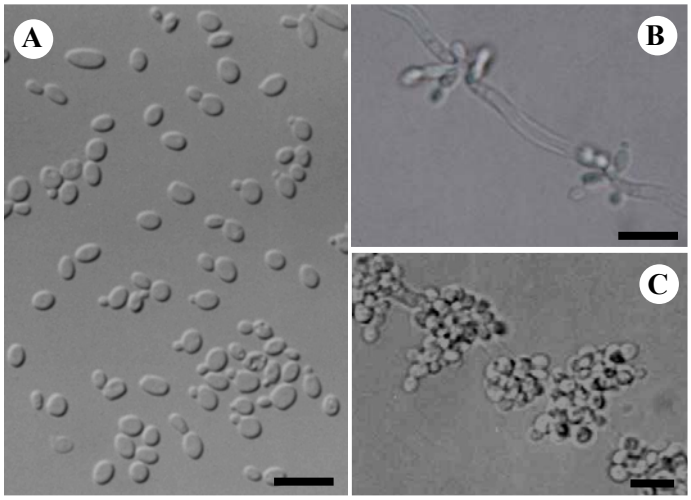

Figure 30 Morphological characteristics of *Candida easanensis* (ST-225); (A) cells grown in YMB for 3 days at 25°C; (B, C) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

4.2.6 Strains related to *Pichia veronae* and *Pichia fabianii* (ST-311 and ST-320)

Strains ST-311 and ST-320 were isolated from insect frass collected in Ko Yao, Pattani, Thailand. These strains had Q-7 as the major component of ubiquinones. In the phylogenetic tree based on the D1/D2 sequences of 26S rDNA, they were located in clusters with several *Pichia* species (Fig. 28). These two strains had identical nucleotide sequences in the D1/D2 domain and showed essentially the same taxonomic characteristics, and were considered to represent a single species. They are closely related to *Pichia veronae* and *Pichia fabianii* but 9 nucleotides (1.6%) are different from these two species.

In the taxonomic criteria commonly employed, they closely resemble *P. veronae* but differ in the mol% G+C, 43.2 to 43.9 mol% for the two strains and 46.5 or 49.5 mol% for *P. veronae* (Kurtzman, 1998). The two strains also resemble *P. fabianii* but differ in the assimilation of raffinose, lack of the assimilation of L-

arabinose and L-rhamnose, and absence of growth in 10% NaCl + 5% glucose medium and at 37°C. These two strains were assigned to the genus *Candida* because they did not produce ascospores. These strains were assigned to a new species and named *Candida pattaniensis* sp. nov. (Jindamorakot *et al*., 2004).

***Candida pattaniensis* sp. nov. (ST-311 and ST-320)**

Growth in the YM broth: After 3 days at 25°C the cells are short ovoidal to ovoildal to long ellipsoidal, sausage-shape, 1.5-4.5 x 3-10 μm, single or in pair (Fig. 31A). Trace of ring and a sediment are produced. After one month at 20°C, trace of a ring and a sediment are present.

Growth on YM agar: After one month at 20°C, the streak culture is grayish-white, smooth, shining, soft and has an entire to ciliate margin.

Slide culture on potato dextrose agar: Well developed pseudomycelia are abundantly produced (Fig. 31B). Usually they are tree-like.

Fermentation: Glucose and sucrose are fermented. Galactose, maltose, lactose, melibiose and raffinose are not fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	+
Trehalose	+	L-Arabinitol	Latent & weak
Lactose	-	α-Methyl-D-glucoside	+
Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	+
Melezitose	+	D-Gluconic acid	+ or latent
Inulin	-	2-Ketogluconic acid	-
Soluble starch	+	5-Ketogluconic acid	-
D-Xylose	+	DL-Lactic acid	+

L-Arabinose	Latent	Succinic acid	+
D-Arabinose	-	Citric acid	+
D-Ribose	-	Saccharic acid	-
L-Rhamnose	+	D-Glucuronic acid	-
D-Glucosamine	-	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	Propane 1,2 diol	+ or latent
Ethanol	+	Butane 2,3 diol	-
Glycerol	+	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	+
Ethylamine	+		

Additional tests:

Starch formation	-	Urease	-
Gelatin liquefaction	-	Lipase	-
0.01% Cycloheximide	-	Maximum temperature	34-35°C
10% NaCl + 5% glucose	-	Ubiquinone system:	Q-7
Acid formation	-	Mol% G+C (by HPLC)	43.2-43.9
Vitamins required	Biotin, pyridoxine and thiamine		

Type strain: ST-311, isolated from insect frass collected in Mar. 2001, Ko Yao, Pattani province, southern Thailand, is the type strain of this species. It was deposited at BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), as BCC 11790, TISTR Culture Collection, Thailand Institute of Scientific and Technological Research (TISTR), as TISTR 5827, and the Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, as JCM 12475. The other strain ST-320 is maintained at BCC as BCC 11799.

Etymology: The specific epithet is derived from “Pattani”, the name of a province in southern Thailand where this species was isolated.

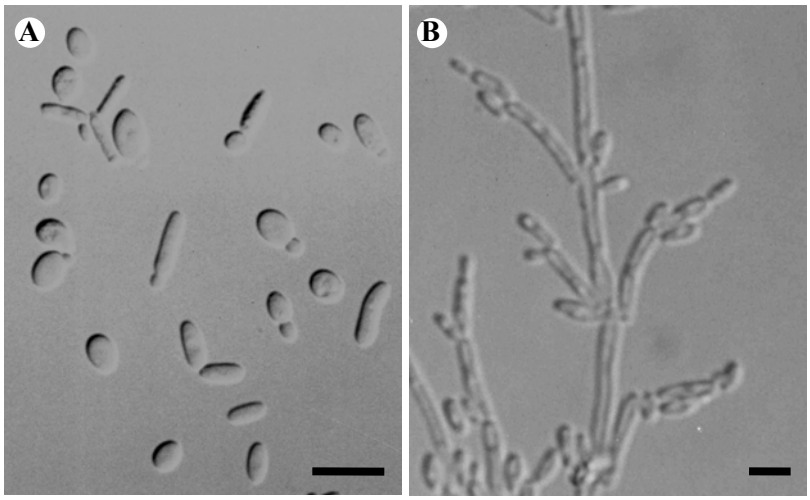

Figure 31 Morphological characteristics of *Candida pattaniensis* (ST-311); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

4.2.7 Strain related to *Pichia besseyii* (ST-84)

Strain ST-84 was isolated from insect frass in Haui Laung Dam, Udon Thani, Thailand. This strain was located in a cluster including *Pichia besseyii, Saturnispora ahearnii* and *Candida diversa* but was distant from these species in the phylogenetic tree based on the D1/D2 domain sequences of 26S rDNA (Fig. 32). Recently, D1/D2 sequence of *Candida* sp. BG02-7-15-015A-2-1 AY520326 was reported (Suh *et al*., 2005). This sequence was close to ST-84 but differed in 9 nucleotides in D1/D2 region from ST-84. This strains was assigned to the genus *Candida* because it did not produce ascospores. ST-84 represented a new species and was named *Candida udonthanina*. In the phenotypic characteristics, *C. udonthanina* showed some resemblances to *Pichia nakasei* and *Pichia deserticola* but distinguished from the former species in the assimilation of citric acid, growth in osmotic pressure medium (10% NaCl + 5% glucose), and pyridoxine requirement, and from the latter species in fermentative ability, assimilation of citric acid and the lack of growth at 37°C. Furthermore, mol% G+C of *C. udonthanina* is much higher than those of the two species.

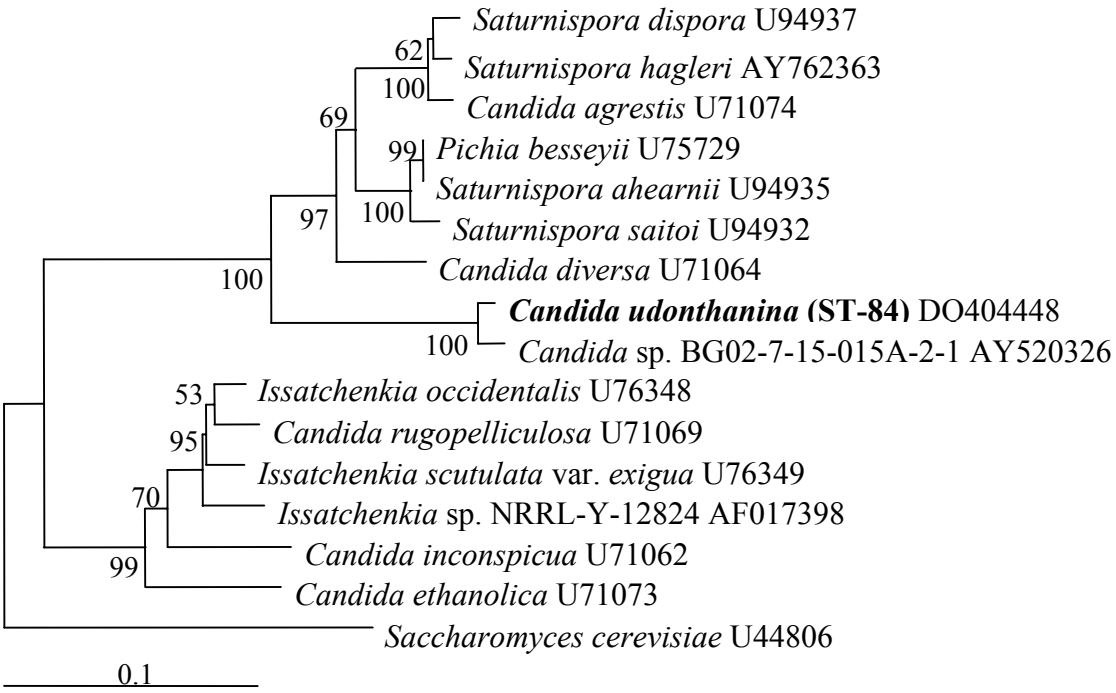

Figure 32 Phylogenetic tree for a new species, *Candida udonthanina,* constructed by the neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the bootstrap values of 1,000 resamplings.

***Candida udonthanina* sp. nov. (ST-84)**

Growth in YM broth: After 3 days at 25, cells are ovoidal, ellipsoidal to long ellipsoidal,cylindrical or elongate, 2-4.5 x 2.5-8.5 μm, single or in pairs (Fig. 33A).

Growth on YM agar: After one month at 25°C, grayish white, smooth, shining, soft and has entire margin.

Slide culture on potato dextrose agar: Pseudomycelia are produced (Fig. 33B).

Fermentation: Glucose is fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-

Sucrose	-	D-Mannitol	-
Maltose	-	D-Glucitol	-
Cellobiose	-	Xylitol	-
Trehalose	-	L-Arabinitol	-
Lactose	-	α-Methyl-D-glucoside	-
Melibiose	-	Salicin	-
Raffinose	-	Glucono-δ-lactone	-
Melezitose	-	D-Gluconic acid	-
Inulin	-	2-Ketogluconic acid	-
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	-	DL-Lactic acid	Latent & weak
L-Arabinose	-	Succinic acid	+
D-Arabinose	-	Citric acid	+
D-Ribose	-	Saccharic acid	-
L-Rhamnose	-	D-Glucuronic acid	-
D-Glucosamine	-	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	Propane-1,2-diol	-
Ethanol	+	Butane-2,3-diol	-
Glycerol	+	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	+
Ethylamine	+		

Additional tests:

Starch formation	-	Urease	-
Gelatin liquefaction	-	Lipase	-
0.01% Cycloheximide	-	Maximum temperature	35-36°C

0.1% Cycloheximide	-	Ubiquinone system	Q-7
10% NaCl + 5% glucose	-	Mol% G+C (by HPLC)	42.8
Acid formation	latent & weak		
Vitamin required	Biotin (stimulative), pyridoxine and thiamine		

Holotype: ST-84, isolated from insect frass collected in Khuan Haui-Laung, Udon Thani province, Feb. 2001, is the type strain of this species. This strain was deposited at BIOTEC Culture Collection as BCC 8320. It is also maintained at Japan Collection of Microorganisms (JCM), RIKEN, as JCM 12549. The strain is maintained by freezing and/or lyophilization in these culture collections. .

Etymology: The specific epithet was derived from "Udon Thani" the name of province where a sample of insect frass, from which this yeast was isolated, was collected.

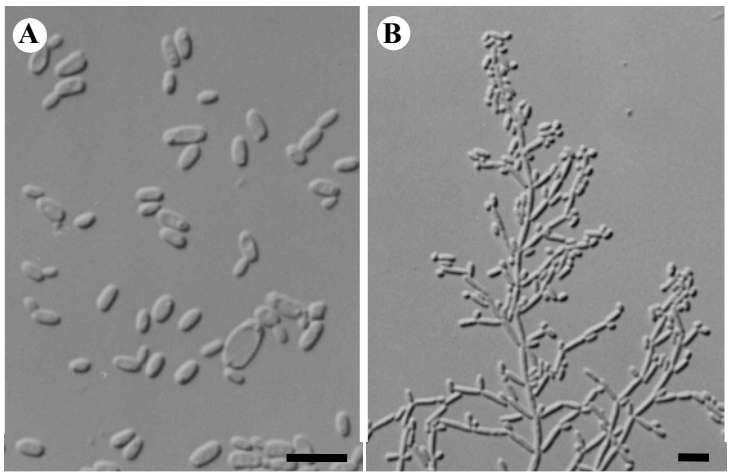

Figure 33 Morphological characteristics of *Candida udonthanina* (ST-84); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

4.2.8 Strain related to *Pichia norvegensis* (ST-314)

ST-314 was isolated from insect frass collected in Ko Yao, Pattani, Thailand. This strain was assigned to the genus *Candida* based on the conventional taxonomic criteria used for yeast classification. This strain has Q-7 as the major ubiquinone and is suggested to has close relationships to the genus *Pichia*. However, this strain was assigned to the genus *Candida* because it did not produce ascospores. In the phylogenetic tree based on the sequence of D1/D2 domain, ST-314 clustered with *Pichia norvegensis* (Fig. 34) but differed in 46 nucleotides (8.2%) in this domain. In the phenotypic characteristics, *C. pattanina* resembles *Pichia pseudocactophila* but differs in the lack of growth at 37°C. This strain was assigned to new species and named *Candida pattanina* sp. nov.

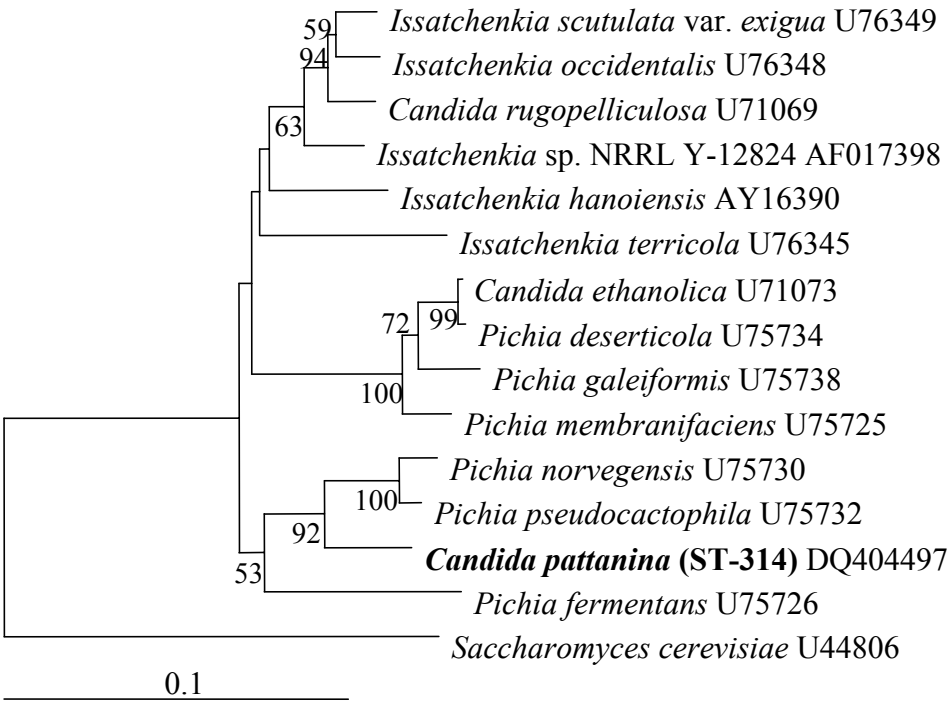

Figure 34 Phylogenetic tree for a new species, *Candida pattanina,* constructed by the neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the bootstrap values of 1,000 resamplings.

***Candida pattanina* sp. nov. (ST-314)**

Growth in YM broth: After 3 days at 25°C, the cells are globose, subovoidal, oval, 2.0-4.4 x 2.0-5.0 μm, single or in pairs (Fig. 35A). Pellicicle and a sediment are produced. After one month at 20°C, trace of a ring and a sediment are present.

Growth on YM agar: After one month at 20°C, the streak culture are grayish white, smooth, shining, soft and has entire margin.

Slide culture on potato dextrose agar: Pseudomycelia are produced (Fig. 35B).

Fermentation: Absent. Sometimes, a bubble is produced from glucose after 3 to 4 weeks.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-
Sucrose	-	D-Mannitol	-
Maltose	-	D-Glucitol	-
Cellobiose	-	Xylitol	-
Trehalose	-	L-Arabinitol	-
Lactose	-	α-Methyl-D-glucoside	-
Melibiose	-	Salicin	-
Raffinose	-	Glucono-δ-lactone	-
Melezitose	-	D-Gluconic acid	-
Inulin	-	2-Ketogluconic acid	-
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	-	DL-Lactic acid	+
L-Arabinose	-	Succinic acid	+
D-Arabinose	-	Citric acid	+
D-Ribose	-	Saccharic acid	-
L-Rhamnose	-	D-Glucuronic acid	-
D-Glucosamine	+	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-

Methanol	-	Propane-1,2-diol	-
Ethanol	+	Butane-2,3-diol	-
Glycerol	- / latent & weak	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	-	Cadaverine	+
Ethylamine	+		

Additional tests:

Starch formation	-	Acid formation	-
Gelatin liquefaction	-	Urease	-
0.01% Cycloheximide	-	Lipase	-
0.1% Cycloheximide	-	Maximum temperature	32-33°C
50% Glucose	-	Ubiquinone system:	Q-7
10% NaCl + 5% Glucose	-	Mol% G+C (by HPLC)	37.7-38.1
Vitamins required	Biotin and pyridoxin		

Holoytype: ST-314, isolated from insect frass collected in Ko Yao, Pattani province, Mar. 2001, is the type strain of this species. It was deposited at BIOTEC Culture Collection as BCC 11793. This strain was also deposited at Japan Collection of Microorganisms (JCM), RIKEN, as JCM 12548. The strain is maintained by freezing and/or lyophilization in these culture collections.

Etymology: The specific epithet was derived from “Pattani” the name of procvince where a sample of insect frass, from which this yeast was isolated, was collected.

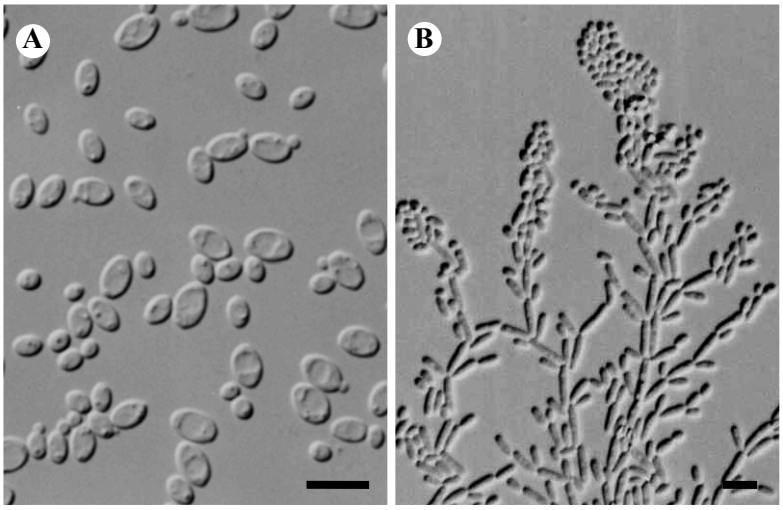

Figure 35 Morphological characteristics of *Candida pattanina* (ST-314); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

4.3 Taxonomic studies on new species of the genus *Pichia* (ST-237 and ST-240)

Strain ST-237, isolated from insect frass collected in Nong Kratone, Nakhon Ratchasima, Thailand, was found to represent a new species of the genus *Pichia*. Strain ST-237 produced hat-shaped ascospores and possessed Q-9 as the major component of ubiquinones. Billon-Grand (1989) divided species of the genus *Pichia* into three groups based on the type of coenzyme Q (ubiquinone) produced and transferred all Q-9 having species that produce hat-shaped ascospores to the newly described genus *Yamadazyma.* However, Kurtzman and Robnett (1998) found that species assigned to *Yamadazyma* characterized by coenzyme Q-9 were placed in several different clades. Based on this finding, Kurtzman and Robnett (1998) did not accept the genus *Yamadazyma* and retained all of species of this genus in the genus *Pichia*. The ubiquinone type was suggested to the one of the important taxonomic criteria to define the genus and Q-9 having species should be excluded from the genus *Pichia* because *Pichia membranifaciens*, type species of *Pichia*, has Q-7. However, at present, the genus *Yamadazyma* is not well-defined and the reclassification of *Pichia*-complex is required. Therefore, we decided to place ST-237 in the genus *Pichia*.

In the phylogenetic tree based on the D1/D2 domain sequences of 26S rDNA, strain ST-237 constituted a cluster with *Pichia (Yamadazyma) acaciae* with high bootstrap value (Fig. 36). The cluster connected with a cluster comprising *Pichia* (*Yamadazyma) farinosa* and *Candida cacaoi*. ST-237 differed in 6 nucleotides (1.1%) from *P. acaciae*, the nearest species in D1/D2 sequences, and could be regarded as distinct species from the latter species according to a guideline of Kurtzman and Robnett (1998).

ST-237 resembles *P. acaciae* also in the phenotypic characteristics but clearly differentiated from this species by the assimilation and fermentation of sucrose and the growth in vitamin-deficient medium. ST-237 is described bellow as a new species of *Pichia, Pichia koratensis*.

Strain ST-240, isolated from insect frass collected in Nong Kratone, Nakhon Ratchasima, Thailand, was found to represent a new species of the genus *Pichia.* Strain ST-240 proliferated by multilateral budding forming globose to ellipsoidal cells and produced hat-shaped ascospores without conjugation, and fitted to those of the genus *Pichia* (Kurtzman, 1998). It has Q-7 as the major component of ubiquinones and assimilated limited number of carbon compounds as found in many typical species of the genus.

In a phylogenetic tree constructed by neighbor-joining method based on the D1/D2 domain sequences of 26S rDNA, ST-240 constituted a cluster with *Pichia dryadoides* with high bootstrap confidence level (Fig. 36). However, ST-240 differed in 27 nucleotides (5.6%) from the latter species so that it is not so closely related to *P. dryadoides*. Undoubtedly, ST-240 is a different species from *P. dryadoides* and represents a new species of the genus *Pichia*.

ST-240 resembles *P. dryadoides* also in the phenotypic characteristics but is distinguished from the latter species in the ability to assimilate salicin (latent) and saccharic acid (latent) and in the inability to assimilate D-glucitol, 1,2-propanediol and 2,3-butanediol as carbon sources. In addition, ST-240 does not assimilate

cadaverine as a nitrogen source. This strain described as *Pichia nongkratonensis* sp. nov. (Nakase *et al*., 2005).

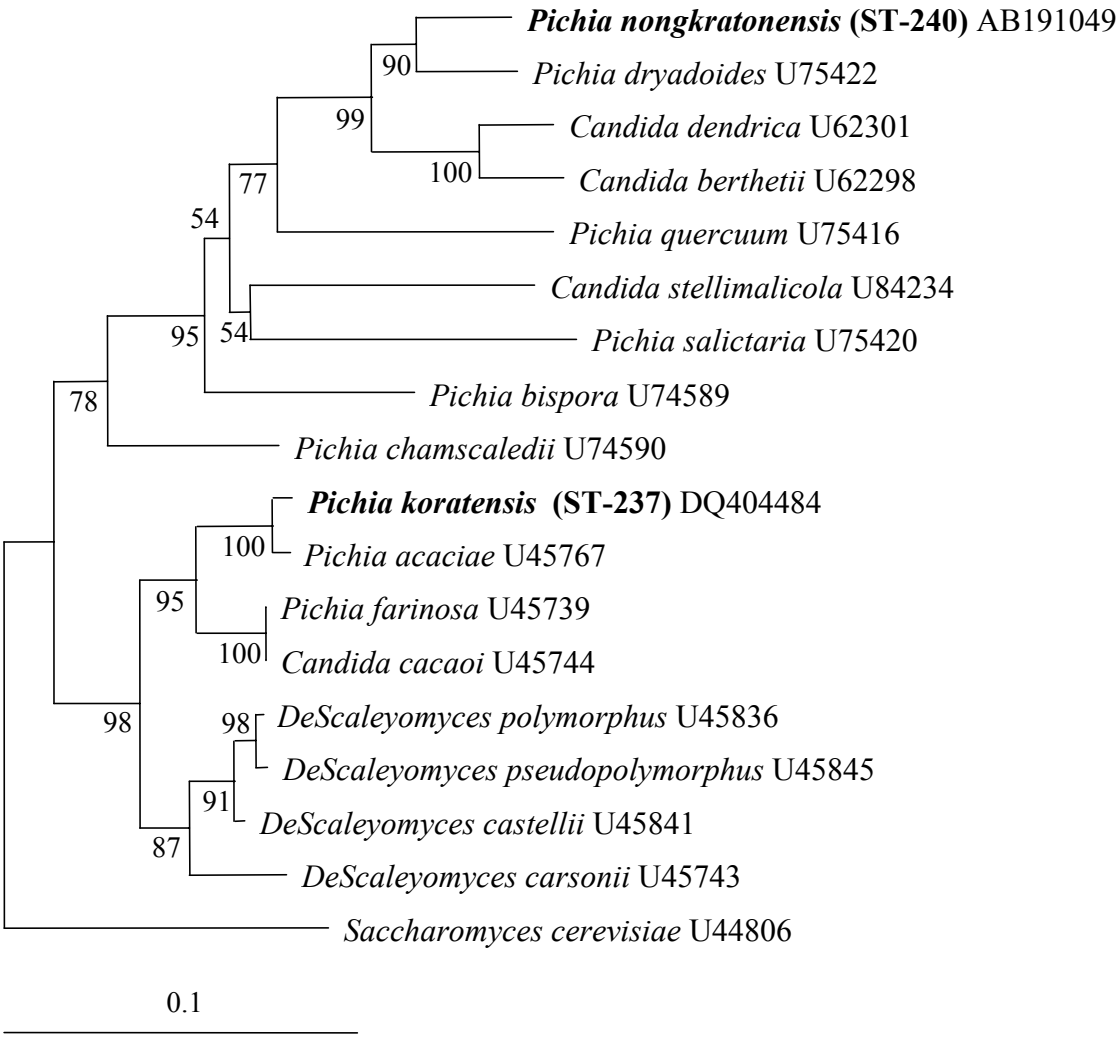

Figure 36 Phylogenetic tree for *Pichia koratensis* and *Pichia nongkratonensis* constructed by neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the values from 1,000 replicate bootstrap resamplings.

Description of new species of the genus *Pichia* showed as below.

***Pichia koratensis* sp. nov. (ST-237)**

Growth in YM broth: After 3 days at 25°C, cells are globose to short-ovoidal, 2.0-4.5 x 2.0-5.5 μm, single or in pairs (Fig. 37A).

Growth on YM agar: After one month at 20°C, the streak culture is grayish white, smooth, shining, soft and has entire margin.

Slide culture on potato dextrose agar: Not produce.

Ascospores: Ascospores are hat shape (Fig. 37B).

Fermentation: Glucose, sucrose and maltose are fermented. Galactose and lactose are not fermented.

Assimilation of carbon compounds:

Glucose	+	Erythritol	+
Galactose	+	Ribitol	+
L-Sorbose	+ or latent	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	+
Trehalose	+	L-Arabinitol	+
Lactose	Latent	α-Methyl-D-glucoside	+
Melibiose	-	Salicin	+
Raffinose	-	Glucono-δ-lactone	Latent
Melezitose	-	D-Gluconic acid	+
Inulin	-	2-Ketogluconic acid	-
Soluble starch	+	5-Ketogluconic acid	-
D-Xylose	+	DL-Lactic acid	-
L-Arabinose	-	Succinic acid	+
D-Arabinose	-	Citric acid	+
D-Ribose	+	Saccharic acid	-
L-Rhamnose	-	D-Glucuronic acid	-

D-Glucosamine	+	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	+	Inositol	-
Methanol	-	Propane-1,2-diol	Latent
Ethanol	+	Butane-2,3-diol	+
Glycerol	+	Hexadecane	Latent and weak

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	-
Sodium nitrite	-	Cadaverine	-
Ethylamine	+		

Additional tests:

Starch formation	-	Diazonium Blue B	-
Gelatin liquefaction	Latent	Urease	-
0.01% Cycloheximide	nd	Lipase	-
0.1% Cycloheximide	nd	Maximum temperature	43-44°C
10% NaCl/5% glucose	+	Ubiquinone system:	Q-9
Acid formation	+	Mol% G+C (by HPLC)	43.0
Vitamin free medium	+		

Holotype: ST-237, isolated from insect frass collected in Nong Kratone, Nakhon Ratchasima province, Feb. 2001, is the type strain of this species. It was deposited at BIOTEC Culture Collection as BCC 11769. This strain was also deposited at Japan Collection of Microorganisms (JCM), RIKEN, as JCM 12576. The strain is maintained by freezing and/or lyophilization in these culture collections.

Etymology: The specific epithet was derived from “Korat”, a common name of Nakhonratchasima, where *Pichia koratensis* was isolated.

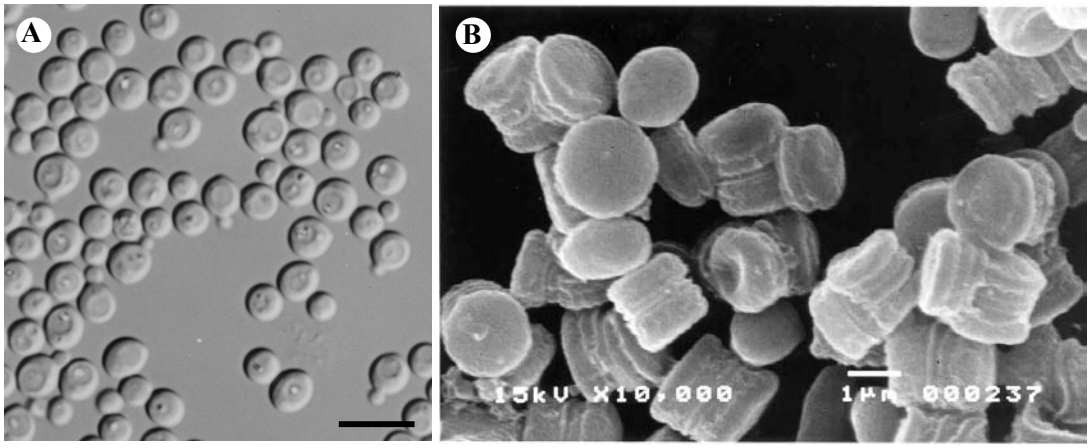

Figure 37 Morphological characteristics of *Pichia koratensis* (ST-237); (A) cells grown in YMB for 3 days at 25°C, Scale = 10 μm; (B) SEM picture of ascospore produced on YM agar after 14 days at 25°C.

***Pichia nongkratonensis* sp. nov. (ST-240)**

Growth in YM broth: After 3 days at 25°C, cells are globose to short-ovoidal, 2.5-4.5 x 2.5-5 μm, single, in pairs or in short chains (Fig. 38A). Sometimes pseudomycelia are observed. Pseudomycelial cells are elongate. An incomplete ring and a sediment are formed. After 1 month at 20°C, an incomplete ring and a sediment are present.

Growth on YM agar: After one month at 20°C, the streak culture is grayish white to grayish brown, smooth, semi-shining, soft and has an entire margin.

Slide culture: Pseudomycelia are produced on YM agar (Fig. 38B) but not produce on potato dextrose agar.

Ascospores: Diploid vegetative cells directly transform to asci and each ascus contains one to three, usually two ascospores. Ascospores are hat-shaped with prominent brims, 1.5-2.3 x 2.0-3.1 μm (Fig. 38C).

Fermentation: Glucose is slowly fermented after 3 weeks.

Assimilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	-	Ribitol	-
L-Sorbose	-	Galactitol	-
Sucrose	-	D-Mannitol	+
Maltose	-	D-Glucitol	-
Cellobiose	+ or latent	Xylitol	-
Trehalose	-	L-Arabinitol	-
Lactose	-	α-Methyl-D-glucoside	-
Melibiose	-	Salicin	+ or latent
Raffinose	-	Glucono-δ-lactone	+
Melezitose	-	D-Gluconic acid	Latent
Inulin	-	2-Ketogluconic acid	-
Soluble starch	-	5-Ketogluconic acid	-
D-Xylose	-	DL-Lactic acid	+ or latent
L-Arabinose	-	Succinic acid	+
D-Arabinose	-	Citric acid	+
D-Ribose	-	Saccharic acid	- or latent
L-Rhamnose	-	D-Glucuronic acid	-
D-Glucosamine	-	D-Galacturonic acid	-
N-Acetyl-D-glucosamine	-	Inositol	-
Methanol	-	1,2-Propanediol	-
Ethanol	+	2,3-Butanediol	-
Glycerol	+	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	+	L-Lysine	+
Sodium nitrite	+	Cadaverine	-
Ethylamine	+		

Additional tests:

Starch formation	-	Urease	-
Gelatin liquefaction	-	Lipase	-
0.01% Cycloheximide	+	Maximum temperature	39-40°C
0.1% Cycloheximide	Weak	Ubiquinone system:	Q-7
Acid formation	Weak	Mol% G+C (by HPLC)	33.4
Vitamin free medium	+ (stimulated by thiamine)		

Holotype: ST-240, isolated from insect frass collected in a tropical rain forest, Nong Kratone, Nakhon Ratchasima province, Thailand, in Feb., 2001, is the type strain of this species. The living culture from the type was deposited at BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology, as BCC 11772. Isotype was deposited at Japan Collection of Microorganisms (JCM), RIKEN, as JCM 12550, respectively. These cultures are maintained by freezing and/or lyophilization.

Etymology: The specific epithet "*nongkratonensis*" was derived from the place where this yeast was found.

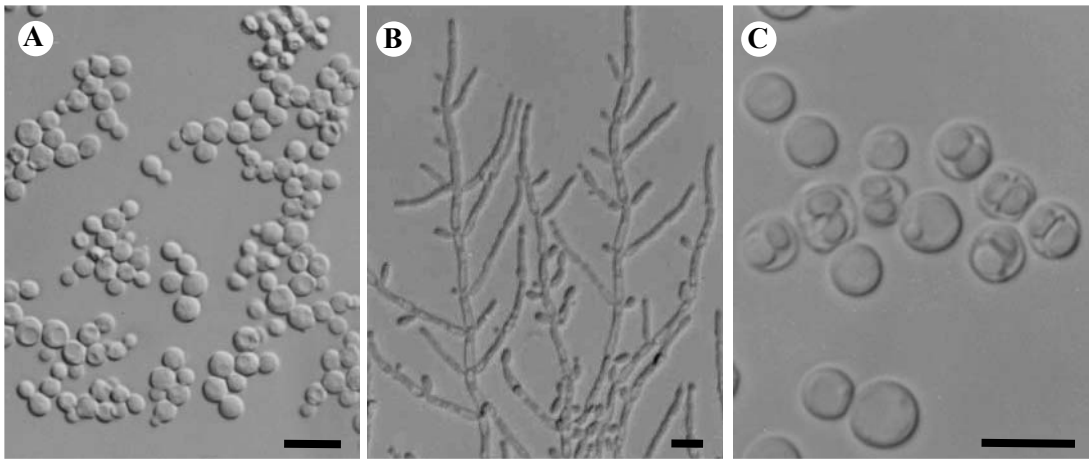

Figure 38 Morphological characteristics of *Pichia nongkratonensis* (ST-240); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C; (C) ascospore produced on YM agar after 14 days at 25°C. Scales = 10 μm.

4.4 Taxonomic study on new species of the genus *Trichosporon*

A yeast strain designated ST-318 was isolated from insect frass collected in Ko Yao, Pattani province, a southern region of Thailand. In the phylogenetic tree based on D1/D2 domain sequences, strain ST-318 was located in the *Trichosporonales* lineage and constituted a cluster with *Trichosporon brassicae*, *Trichosporon montevideense* and *Trichosporon domesticum* though the bootstrap confidence level was not high (Fig. 39). In the D1/D2 nucleotide sequences, ST-318 was close to *T. brassicae* and *Trichosporon* sp. CBS 5601, however, 12 nucleotides (1.8%) were substituted from the two species. These sequence differences clearly indicates the difference of ST-318 from the two yeasts at species level. It is described as *Trichosporon siamense* sp. nov. (Nakase *et al*., 2006).

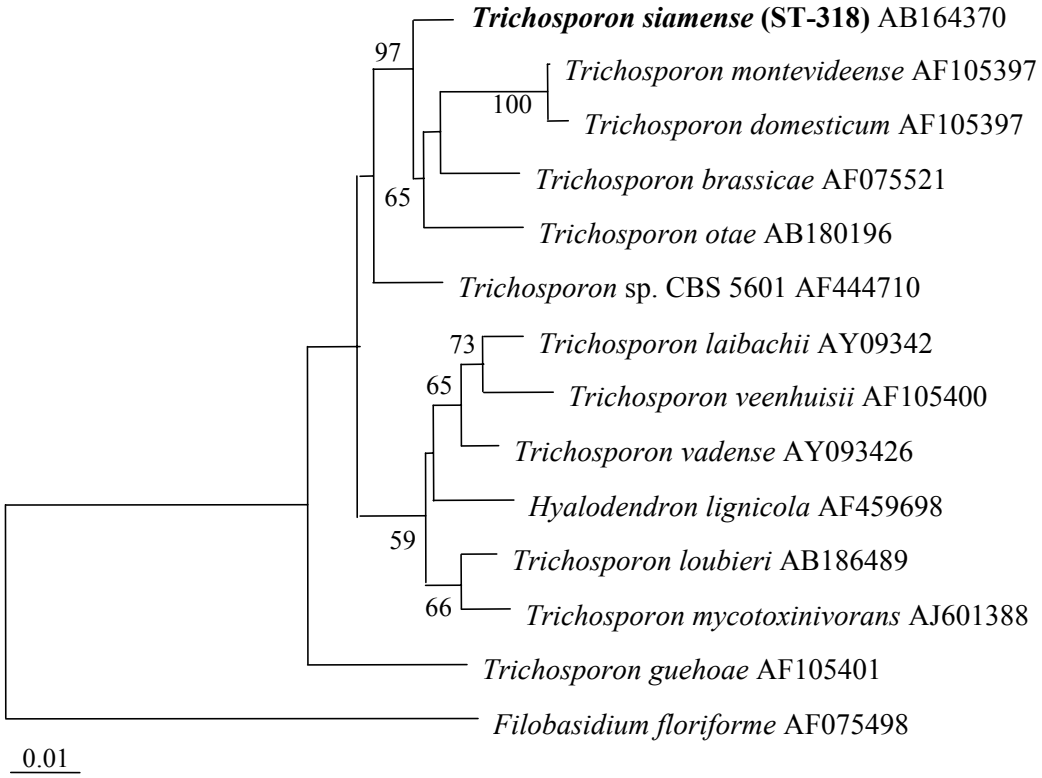

Figure 39 Phylogenetic tree for a new basidiomycetous yeast, *Trichosporon siamense,* constructed by neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the values from 1,000 replicate bootstrap resamplings. Sequences were retrieved from the NCBI databases under the accession numbers indicated.

In the phenotypic characteristics, *T. siamense* resembles species in the same cluster, especially *T. montevideense*. However, *T. siamense* is clearly discriminated from the latter species by its ability to assimilate L-sorbose, L-rhamnose, and sodium nitrite, its inability to assimilate galactitol and salicin, and the lack of growth on 50% glucose yeast extract agar.

4.4.1 Description of new species of the genus *Trichosporon*

***Trichosporon siamense* sp. nov. (ST-318)**

Growth in YM broth: After 3 days at 25°C, cell are globose, rectangular, cylindrical or elongate, single, in pairs or in chains, 2.5-7.5 x 2.5-15.0 μm, many septate mycelia and arthroconidia are present (Fig. 40A). A wrinkled creeping pellicle and a sediment are formed.

Growth on YM agar: After one month at 20°C, the streak culture is grayish yellow, fine hairs on the surface, dull, soft to butyrous and has a ciliate margin.

Slide culture on Potato Dextrose agar: Septate and branched mycelia are abundantly produced (Fig. 40B, C and D). They break up into arthroconidia that are often arranged in zigzag. Arthroconidia are rectangular, cylindrical, or close to subglobose, 2.5-4.5 x 3.5-10.0 μm

Assmilation of carbon compounds:

Glucose	+	Erythritol	-
Galactose	+	Ribitol	Latent
L-Sorbose	+	Galactitol	-
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	+
Trehalose	+	L-Arabinitol	-
Lactose	+	α-Methyl-D-glucoside	+
Melibiose	-	Salicin	-
Raffinose	-	Glucono-δ-lactone	+

Melezitose	+ or lateent	D-Gluconic acid	+
Inulin	-	2-Ketogluconic acid	+
Soluble starch	+	5-Ketogluconic acid	+
D-Xylose	+	DL-Lactic acid	+
L-Arabinose	-	Succinic acid	+
D-Arabinose	-	Citric acid	+
D-Ribose	+	Saccharic acid	+
L-Rhamnose	+	D-Glucuronic acid	+
D-Glucosamine	+	D-Galacturonic acid	+
N-Acetyl-D-glucosamine	+	Inositol	+
Methanol	-	Propane-1,2-diol	+
Ethanol	+	Butane-2,3-diol	+
Glycerol	+	Hexadecane	-

Assimilation of nitrogen compounds:

Potassium nitrate	-	L-Lysine	+
Sodium nitrite	+	Cadaverine	+
Ethylamine	+		

Additional tests:

Starch formation	-	Diazonium Blue B	+
Gelatin liquefaction	-	Urease	+
0.1% Cycloheximide	-	Lipase	-
50% glucose	-	Maximum temperature	35-36°C
Vitamins required	Thiamine	Ubiquinone system:	Q-9
Xylose in the whole cell hydrolysates		+	

Holotype: The strain ST-318 isolated from insect frass collected in Ko Yao, Pattani province, Thailandia, in March 2001, is the type strain of this species. Living culture from the holotype was deposited at BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), National

Science and Technology Development Agency (NSTDA), Pathumthani, Thailand, as BCC 11797, TISTR Culture Collection, Thailand Institute of Scientific and Technological Research (TISTR), Pathumthani, Thailand, as TISTR 5823, and Japan Collection of Microorganisms (JCM), RIKEN', Wako, Saitama 351-0198, Japan, as JCM 12478. These cultures are maintained by lyophilization and/or freezing.

Etymology: The specific epithet of this species was derived from "Siam", the old name of Thailand where this yeast was isolated.

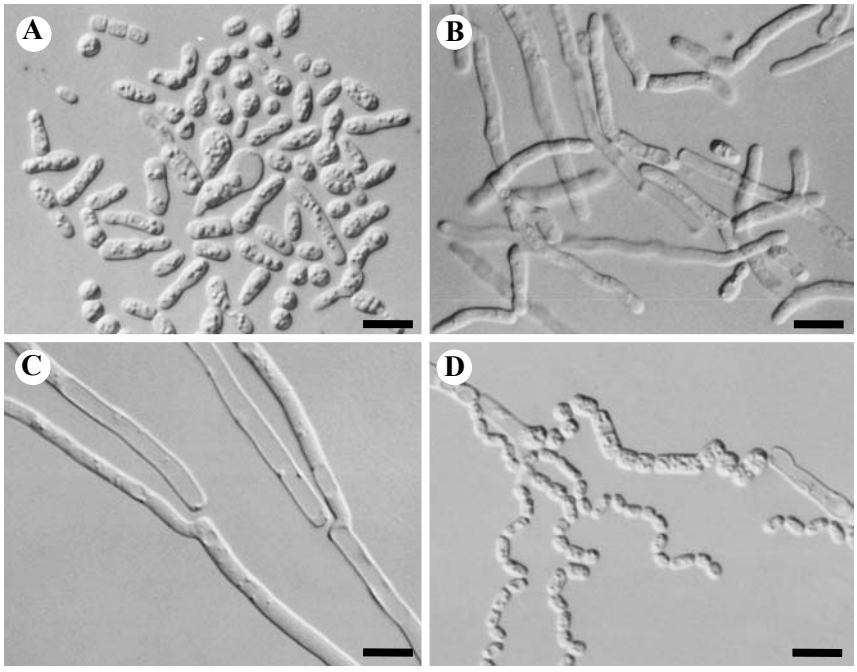

Figure 40 Morphological characteristics of *Trichosporon siamense* (ST-318); (A) cells grown in YMB for 3 days at 25°C; (B, C and D) mycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.