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THESIS

THE SPECIES DIVERSITY OF YEASTS IN SOME NATURAL HABITATS OF THAILAND

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Two hundred and eighty-three strains of yeast were isolated from insect frass (144 strains), flowers (24 strains), leaves (54 strains), mosses (27 strains), mushrooms (28 strains), and some other habitats (6 strains), which were collected from various places in Thailand. Among 283 strains taxonomically studied, 194 strains belonged to ascomycetous yeasts and 89 strains belonged to basidiomycetous yeasts. Based on the sequence analysis of D1/D2 domain of 26S rDNA, 139 strains (49%) were assigned to 56 known species because their D1/D2 domain sequences differed by 0-1 nucleotide substitution from known species. Among the strains were assigned to be known species, 97 strains belonged to 39 species of 17 genera of ascomycetous yeasts and 42 strains belonged to17 species of 9 genera of basidiomycetous yeasts. One hundred and sixteen strains (41%) were considered to represent 101 new species because their D1/D2 domain sequences differed by 4 nucleotide substitutions or more from closest species. Among the strains assigned to new species, 87 strains belonged to 76 species of 14 genera of ascomycetous yeasts and 29 strains belonged to 25 species of 7 genera of basidiomycetous yeasts. The remaining 28 strains (10%) are not yet identified because their D1/D2 domain sequences showed 2-3 nucleotide substitutions from known species.

Twenty-one new species were selected for precise taxonomic studies by considering phylogenetic clusters and nucleotide differences. These yeasts were studied by polyphasic approaches, including conventional taxonomy, chemotaxonomic study and molecular taxonomy and described as *Candida easanensis* sp. nov., *Candida flosculi* sp. nov., *Candida hasegawae* sp. nov., *Candida jaroonii* sp. nov., *Candida kazuoi* sp. nov., *Candida koratica* sp. nov., *Candida lignicola* sp. nov., *Candida nakhonratchasimensis* sp. nov., *Candida pattanina* sp. nov., *C. pattaniensis* sp. nov., *Candida songkhlaensis* sp. nov., *Candida thailandica* sp. nov., *Candida udonthanina* sp. nov., *Candida* sp. 1 (ST-331), *Hanseniaspora thailandica* sp. nov., *Kloeckera siamensis* sp. nov., *Kloeckera songkhlaensis* sp. nov., *Kloeckera tradensis* sp. nov., *Pichia koratensis* sp. nov., *Pichia nongratonensis* sp. nov., and *Trichosporon siamense* sp. nov.

The frequency of isolation of respective species showed that, *Candida tropicalis* (9 strains) and *Saccharomyces kluyveri* (8 strains) are the dominant species of ascomycetous yeasts and *Cryptococcus heveanensis* (8 strains) is the dominant species of basidiomycetous yeasts. Among 101 new species found in the present study, 93 species (92.1%) comprised one strain and the remaining 8 species (7.9%) comprised 2-5 strains. In contrast, 26 known species (44.8%) comprised one strain and 32 known species (55.2%) comprised 2-9 strains. This fact means that, in the natural environment, the number of yeast cells of known species is bigger than those of new species. Significantly, 14 species (14 strains) of undescribed yeasts differed in 60-110 nucleotide substitutions (10-18%) from closest known species. These species may represent new groups, probably new genera or families, of yeasts. It is concluded that hitherto unknown yeast are rich in the natural habitats of Thailand and many new groups, new genera or new families, will be found from these unknown yeasts.

Sasitorn Jindamorakot Savitree Limtong 19 / 05 / 2006

Student's signature Thesis Advisor's signature

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INTRODUCTION

It is well known that yeast is one of the most important microorganisms for human life. They have been used in many industrial processes such as production of alcoholic beverages and bread (Phaff *et al*., 1978), enzymes, vitamins, organic acids and animal feed (Domain *et al*., 1998; Spencer *et al*., 2002). However yeast species used for these industries are limited. On the other hand, it is estimated that a vast number of yeasts are living in the natural environment and many of them belong to not yet described species. In Thailand, taxonomic and ecological study of yeasts started in the late 1970's mainly on yeasts associated with fermented foods and related substrates (Davahuti, 1978; Tummarat, 1978). Yeast species found in these substrates are limited and most of them belong to known species such as *Saccharomyces cerevisiae, S. bayanus, S. kluyveri, S. pastorianus, Kloeckera apiculata, Pichia membranifaciens, Candida krusei, C. parapsilosis, Zygosaccharomyces rouxii* and etc. (Davahuti, 1978; Tummarat, 1978; Suzuki *et al*., 1987; Jindamorakot, 2000). However, in the study of yeasts from fermented foods and related substrates, 2 new species, *C. stellimalicola* and *Citeromyces siamensis* were described by Suzuki *et al*. (1994) and Nagasuka *et al*. (2002), respectively.

The study of yeasts living in the natural environment of Thailand was started in the late 1980's. The study of ballistoconidium-forming yeasts, a kind of basidiomycetous yeast producing ballistoconidium, started to elucidate the yeast flora in the phyllosphere in Thailand. In contrast to the result obtained from the study of yeasts in fermented foods and related substrates, ballistoconidium-forming yeasts in the phyllosphere were rich in biodiversity. Many undescribed species were found in these studies and 17 of them were described as new species so far (Nakase *et al*., 1991; Prillinger *et al*., 1997; Takashima *et al*., 1995; Takashima *et al*., 1998; Takashima and Nakase, 2000, 2001; Fungsin *et al*., 2001, 2002, 2003)

Recently, Limtong and coworkers studied on the thermotolerant methylotrophic yeasts and found that these kinds of yeast were widely distributed in the natural environment in Thailand. Four new species of genera *Pichia* and *Candida*, *P. siamensis*, *P. thermomethanolica*, *C. krabiensis* and *C. sithepensis* were described (Limtong *et al*., 2004, 2005). A new ascomycetous yeast from soils in Nam Nao national park, *Tetrapisispora namnaonensis*, was described (Sumpradit *et al*., 2005).

These finding suggests that yeasts found in the natural environment are rich in biodiversity and a vast number of undescribed yeasts are living in the natural environment of Thailand. However, a little is known about yeasts living in Thailand. It should be emphasized that the studies of yeasts in the natural environment in Thailand are still in the early stage and further extensive studies are required for the progress of yeast systematics, biodiversity and for the effective utilization of Thai yeasts for human welfare.

The aims of the present studies are:

1. To collect and identify yeasts from some natural habitats of Thailand such as flowers, insect frass, mosses and mushrooms.
2. To obtain interesting yeast species from the taxonomic and phylogenetic viewpoints.
3. To obtain taxonomic data of collected yeasts.

LITERATURE REVIEWS

1. Definition of Yeasts

Yeasts are ascomycetous or basidiomycetous fungi that reproduce vegetatively by budding or fission and that form sexual states, which are not enclosed in a fruiting body (Boekhout and Kurtzman, 1990). Yeast cells are usually spherical, ovoidal, ellipsoidal or cylindrical in form. The apiculate, ogival and elongate cells may be produced characteristically by certain yeasts, nevertheless, the shape of the active cell is not and exact means of species identification. The cells may vary from 1-5 μm or more in width and from 1-10 μm or more in length (Phaff *et al*., 1978). Yeast cells may vary considerably in dimensions, depending on the species, nutrition, age and other factors.

Yeasts are of benefit to mankind because they are widely used in many industrial processes, alcoholic beverages such as wine and beer, ethanol, baker's yeast, foods and feed, vitamins, lipid, polysaccharides and enzymes as shown in Table 1. Yeasts also cause spoilage of foods and beverages and are of medical importance.

2. Historical Survey on the Taxonomy of Yeasts

2.1 The increase of recognized species

Since Reess described the first yeast species in 1870, about 3,000 species have been described. However, many of them were later considered to be identical to previously described species and regarded as synonyms of previous species. Two recent monographs on yeasts accepted only approximately 700 species (Kurtzman and Robnett, 1998; Barnett *et al*., 2000). However, many new species have been described in a recent few years and the number of yeast is close to 1,000.

Table 1 Potential uses of yeast in the foods, beverages and fermentation industries.

Application	**Yeasts**
Ethanol fermentation	*Saccharomyces cerevisiae*
Ale fermentation	*Saccharomyces cerevisiae*
Lager beer fermentation	*Saccharomyces carlsbergensis* (= *Saccharomyces pastorianus*)
Wine fermentation	*Saccharomyces cerevisiae*
Lactose and milk fermentation	*Candida psuedotropicalis* (=*Candida kefyr*), *Kluyveromyces fragilis, Kluyveromyces marxianus, Kluyveromyces lactis*
Shoyu, Miso	*Zygosaccharomyces rouxii*
Bread and dough leavening	*Saccharomyces cerevisiae, Saccharomyces exiguus, Saccharomyces rosei* (=*Tolurspora delbrueckii*)
Fish and poultry feeds (astaxanthin)	*Phaffia rhodozyma*
Fodder and single cell protein	*Candida utilis*
Emulsifier	*Candida lipolytica*
D-Arabitol (sweetener)	*Candida diddensiae*
Mannitol (humectant)	*Torulopsis mannitofaciens*
Xylitol (sweetener)	*Torulopsis candida*
D-xylose fermentation	*Candida shehatae, Pachysolen tannophilus, Pichia stipitis, Pichia segobiensis*

Source: Domain *et al*. (1998)

A famous monograph entitled “The Yeasts, a Taxonomic Study” (Lodder and Kreger-van Rij) was published in 1952. This book has been used as a kind of standard book of yeast taxonomy and succeeding editions were published in 1970, 1983 and 1998. In the first edition of this book 164 species in 26 genera were included. In the 2nd edition edited by Lodder (1970), 394 species belonging to 39 genera were listed. The 3rd edition by Kreger-van Rij (1984), comprised 500 species in 50 genera. In the latest edition (Kurtzman and Fell, 1998), a total of 689 species in 94 genera were listed. In this edition, yeasts were classified into the Phylum Ascomycota and Phylum Basidiomycota. The ascomycetous yeasts (Phylum ascomycota) are distributed in three classes; Archiascomycetes, Euascomycetes and Hemiascomycetes. The classification system of ascomycetous yeasts is shown in Table 2. The basidiomycetous yeasts (Phylum basidiomycota) are distributed in tree classes: Hymenomycetes, Urediniomycetes and Ustilaginomycetes. The classification system of basidiomycetous yeasts is shown in Table 3.

Table 2 Classification of the ascomycetous yeasts.

Class **Order** **Family**[a] **Genus**	**Family**[a] **Genus**
Phylum: Ascomycota	Lipomycetaceae E.K. Novák & Zsolt
"Archiascomycetes"	*Babjevia*
Schizosaccharomycetales Prillinger, Dörfler, Laaser, Eckerlein & Lehle ex Kurtzman	*Dipodascopsis*
Schizosaccharomycetceae Beijerinck ex Klöcker	*Lipomyces*
Schizosaccharomyces	*Zygozyma*
Taphrinales Gäumann & C.W. Dodge	Metschnikowiaceae T. Kamienski
Taphrina	*Clavispora*
Lalaria (Anamorph of *Taphrina*)	*Metschnikowia*
Protomycetales Luttrell ex D.Hawksworth & O.E. Eriksson	Saccharomycetaceae G. Winter
Protomycetaceae Gray	? *Arxiozyma*
Protomyces	? *Citeromyces*
?*Saitoella* (Anamorphic genus)	? *Cyniclomyces*
Pneumocystidaceae O.E. Eriksson	? *Debaryomyces*
Pneumocystis	? *Dekkera*
Euascomycetes	? *Issatchenkia*
? *Endomyces*[b,c] (*E. scopularum*)	*Kluyveromyces*
Oosporidium	? *Lodderomyces*
Hemiascomycetes	? *Pachysolen*
Saccharomycetales Kudryavtsev	? *Pichia*
(synonym Endomycetales Gäumann)	*Saccharomyces*
Ascoideaceae J. Schröter	? *Saturnispora*
Ascoidea	*Torulaspora*
Cephaloascaceae L.R. Batra	? *Williopsis*
Cephaloascus	*Zygosaccharomyces*
Dipodascaceae Engler & E. Gilg	Saccharomycodaceae Kudryavtsev
Dipodascus	? *Hanseniaspora*
Galactomyces	? *Nadsonia*
? *Sporopachydermia*	*Saccharomycodes*
? *Stephanoascus*	? *Wickerhamia*
? *Wickerhamiella*	Saccharomycopsidaceae von Arx & van der Walt
? *Yarrowia*	? *Ambrosiozyma*
? *Zygoascus*	*Saccharomycopsis*
Endomycetaceae J. Schröter	Candidaceae Windisch ex van der Walt
? *Endomyces*[b,c] (*E. decipiens*)	*Aciculoconidium*
? *Helicogonium*[b]	*Arxula*
? *Myriogonium*	*Blastobotrys*
? *Phialoascus*	*Botryozyma*
? *Trichromonascus*	*Candida*
Eremotheciaceae Kurtzman	*Geotrichum*
Eremothecium	*Kloeckera*
? *Coccidiascus*	*Myxozyma*
	Schizoblastosporion
	Sympodiomyces
	Trigonopsis

[a] A question mark preceding the genus name indicates that family assignment is uncertain.

[b] Placement in the class *Hemiascomycetes* is uncertain.

[c] The genus *Endomyces* and the family Endomycetaaceae are uncertain

Source: Kurtzman and Fell (1998)

Table 3 Classification of the basidiomycetous yeasts.

Characteristic **Order** **Family** **Genus**	**Order** **Family** **Genus**
Teleomorphic taxa	Microstromaceae
I. With "simple" septal pores	Microstroma
A. Basidia cylindric, transversely septate	Exobasidiales
Ustilaginales	Exobasidiaceae
Ustilaginaceae	*Brachybasidium*
Microbotryum	*Dicellomyces*
Schizonella	*Exobasidiellum*
Sorosporium	*Exobasidium*
Sphacelotheca	*Llaurobasidium*
Sporisorium	II. With dolipore septa, parenthesomes cupulate
Ustilago	A. basidia "cruciate-septate"
Ustilentyloma and probably with *Ustilago*-type-basidia[a]	Tremellales
Spordidiales	Sirobasidiaceae
Sporidiobolaceae	*Fibulobasidium*
Leucosporidium	*Siobasidium*
Rhodosporidium	Tremellaceae
Sporidiobolus	*Bulleromyces*
? Erythrobasidium	*Itersonilia*[e]
? Kondoa	*Holtermannia*
? Sakaguchia	*Phyllogloea*
Platygloeales	*Sirotrema*
Cystobasidiaceae	*Tremella*
Colacogloea	*Trimorphomyces*
Cystobasidium	B. Basidia aseptate
Kriegeria[b]	Filobasidiales
Mycogloea	Filobasidiaceae
Occultifer	*Cystofilobasidium*[f]
Tijbodasia	*Filobasidiella*
Septobasidiales	*Filobasdium*
Septobasidiaceae	*Mrakia*
Auriculoscypha	*xanthophyllomyces*
Coccidiodictyon[c]	Syzygosporaceae
Ordonia[c]	*Christiansenia*
Septobasiium	*Syzygospora*
Atractiellales[d]	Anamorphic taxa
Chionosphaeraceae	Sporobolomycetaceae
Chionosphaera	*Bensingtonia* pro parte
Stilbum	*Kurtzmananomyces*
Atractogloeaceae	*Rhodotorula* pro parte
Atractogloea	*Sporobolomyces* pro parte
Agaricostilbales	*Sterigmatomyces*
Agaricostilbaceae	Cryptococcaeae
Agaricostilbum	*Bullera*
B. Basidia globose, nonseptate	*Cryptococcus*
Graphiolales	*Fellomyces*
Graphiolaceae	*Kockovaella*
Graphiola	*Phaffia*
C. basidia cylindric, nonseptate	*Trichosporon*
Cryptobasidiales	*Tsuchiyaea*
Cryptobasidiaceae	*Udeniomyces*
Conyodyctum	*? Hyalodendron*
Cryptobasidium	*? Moniliella*

Table 3 (continued)

Remark:

[a] Although economically important smuts have studied in detail, type of teliospore germination is unknown is many species. Direct conjugation of basidial cells occurs in some taxa with Ustilago-like basidia and this can result in the complete absence or infrequent occurrence of a yeast state.

[b] *Kriegeria* (*Xenogloea*) eriophori (monotypic) parasitizes monocots: its relationship to the mycoparasitic taxa placed in the Cystobasidiaceae and to most other Platygloeales, may be distant.

[c] Yeast states probably occur in these two genera

[d] Basidia can be cylindric, transeversely septate (e.g., in *Stilbum*), or clavate, holobasidia (e.g., as in *Chionosphaera*).

[e] *Itersonilia perplexans* appears to belong in this group (but see also under f), but basidia have not been found. Because of the known features, and the rather isolated position among anamorphic yeast groups, it is classified with the Tremellales here.

[f] *Cystofilobasidium* has thick-walled teliospores which germinate with holobasida. Because of biochemical traits such as cell wall composition, dolipore without parenthesomes, and apparently unique molecular characteristics, we tentativelyplace the genus here, Recent partial 26s rDNA Mrakia, sequences (Fell *et al*., 1992, 1995) suggest a more distant relationship between *Cystofilobasidium*, *Xanthophylomyces* and *Itersonilia* on one side with the Tremellales and the genera *Filobasidium* and *Filobasidiella* on the other.

Source: Kurtzman and Fell (1998)

As mentioned, the number of yeast species rapidly increased in the recent years, however, it is assumed that the species recognized only a small part of yeasts living on the earth. Scorzetti and Fell (2002) wrote that the discovery of basidiomycetous yeast species is in a primordial phase as possibly only 1% of the species in nature have been collected and described". The most of yeast species are still unknown.

2.2 Criteria for yeasts classification

The criteria used for yeast classification are conventional (Yarrow, 1998), chemotaxonomic (Phaff, 1998; Yamazaki *et al*., 1998) and molecular taxonomic characteristics (Kurtzman, 1998; Kurtzman and Blanz, 1998).

Conventional characteristics:

1. Morphological characteristics
   1.1. Characteristics of vegetative reproduction
      - Modes of vegetative reproduction
      - Characteristics of vegetative cells
   1.2. Characteristics of sexual reproduction
      - Characteristics of ascospore formation
      - Characteristics of basidiospore formation

2. Physiological and biochemical characteristics
   - Fermentation of carbohydrates
   - Assimilation of carbon compounds
   - Assimilation of nitrogen compounds
   - Growth in vitamin-free medium and vitamin requirements
   - Growth in media of high osmotic pressure
   - Growth at 37°C and at other temperatures
   - Acid formation from glucose
   - Formation of extracellular amyloid compounds (starch formation)
   - Hydrolysis of urea
   - Splitting of fat
   - Cycloheximide resistance
   - Tolerance of 1% of acetic acid
   - Diazonium Blue B color reaction
   - Canavanine-Glycine-Bromthymol blue (CGB) agar (for identifying the varieties of *Filobasidiella neoformans* (*Cryptococcus neoformans*)
   - Melanin synthesis on DOPA medium
   - Tetrazolium indicator medium (TTC medium)
   - Straining nuclei

Chemotaxonomic characteristics:

- Coenzyme Q (ubiquinone) system
- Carbohydrate composition of cell walls and extracellular carbohydrates
- Capsule polysaccharides
- Electrophoretic comparisons of enzymes

Molecular characteristics:

- Mol% G+C
- DNA sequencing
- DNA fingerprint
- DNA-DNA reassociation

The first period of yeast systematics (1838-1960) is characterized by a thorough study of morphology, comparative nutritional physiology and conventional genetics. Initially, the response on only a limited number of carbon and nitrogen compounds was used for taxonomic processes. Wickerham (1951) expanded the number of compound tested. Today approximately 60 tests are being performed routinely, including fermentation, assimilation of carbon and nitrogen compounds, vitamin requirements, resistance to cycloheximide, maximum growth temperature etc.

The second period of yeast systematics (from 1960 until present) is characterized by the introduction of new technologies. Morphological characteristics were studied by using the electron microscope in addition to the light microscope. Chemotaxonomic characteristics, such as carbohydrate composition of cell walls and capsules (Weijman and Rodrigues de Miranda, 1983; Suzuki and Nakase, 1998; Prillinger *et al*., 1993), proton magnetic resonance spectra of cell wall polysaccharides (Spencer and Gorin, 1969, 1970), ubiquinone system (Yamada and Kendo, 1973; Yamada *et al*., 1976, 1977), fatty acid composition (Cottrel *et al*., 1986; Viljoen *et al*., 1986) and isozyme patterns (Yamazaki *et al*., 1983), have been extensively used for taxonomic distinctions. The soluble proteins of the yeast cytoplasm can also be used for identification and classification. In another sense, these proteins are already used as criteria for yeast identification, as the proteins

comprise the enzymes, which are used by the organisms to metabolize the standard compounds for identification. However, this method is less powerful than those, which determine the similarities and differences in nucleic acid directly.

Prillinger *et al*., (1993) differentiated cell wall type of ascomycetous and basidiomycetous yeasts to 3 and 4 types, respectively.

Cell wall types occur within the ascomycetous yeast:

1) *Saccharomyces*-type: mannose and glucose present.
2) *Schizosaccharomyces*-type: galactose, glucose and mannose present.
3) *Protomyces*-type: glucose predominant, rhamnose, mannose present and galactose commonly present.

Cell wall types occur within the basidiomycetous yeast:

1) *Microbotryum*-type: mannose dominant, glucose present, fructose usually present and rhamnose sometimes present.
2) *Ustilago*-type: glucose dominant, mannose and galactose present.
3) *Dacrymyces*-type: xylose present, glucose and mannose present in equal amounts, traces of galactose may be present, but extracellular amyloid compounds are usually absent.
4) *Tremella*–type: glucose predominant, xylose, mannose, and galactose present, and extracellular amyloid compounds are often present.

Other methods in taxonomic investigations of yeasts based on determination of the structure of their macromolecules include determination of the NMR (PMR; proton magnetic resonance) spectra of the cell wall mannans and study of the soluble proteins of yeasts, particularly the spectra of isozymes (Kurtzman and Phaff, 1987). Gorin *et al*. (1969) suggested that the polysaccharides with similar proton magnetic resonance spectra have related chemical structures, and thus mannans from different species with similar spectra may indicate that these species have a close phylogenetic relationship. Gorin and Spencer (1970) took the PMR spectra of the mannans of most of known yeast species, and grouped the species according to similarities, and showed that isolates being compared were different strains or species. Similarities in the spectra did not indicate relationships. The type strain of

Candida parapsilosis was shown to be unrelated to proposed perfect stage, *Lodderomyced elongisporus*. Gorin and Spencer (1970) had shown that the cell wall mannans of these species were dissimilar, and Kurtzman and Phaff (1987) had confirmed the absence of relationship by determination of DNA homologies. NMR spectra can provide an independent fingerprint for placing a given yeast isolate in a small group of species, thus reducing the time and labor required for final identification.

Phenotypic characteristics have long been used as important taxonomic criteria for yeasts. However, these characteristics are often unstable because these characteristics varied by environment conditions and require time-consuming tests. It requires much training before we can get correct results. Furthermore, these characteristics often contradict the results of molecular studies. To overcome these problems, yeast-identification techniques have been directed to molecular methods. The principles and methods of molecular biology are increasingly important in the identification, classification, and elucidation of relationships among organisms.

In the last two decades the application of molecular techniques has made a great impact on the taxonomy of yeasts. The first methods investigated were the determination of the G+C content of both genomic and mitochondrial DNA and reassociation of RNA and DNA. The nucleic acid base composition (mol% G+C) can differentiate phenotypically similar strains of different species (Kurtzman and Phaff, 1987), while strains with the same base composition do not necessary represent a single species. The range of nucleic acid base compositions differs for ascomycetous and basidiomycetous yeasts. Most ascomycetous yeasts have a mol% G+C about 27-50%, while that of basidiomycetous yeasts is approximately 50-70% (Kurtzman, 1998) as shown in Table 4., except for the narrow range of 48-52%, where some overlap occurs. The taxonomic affinity of anamorphs can be reliably presumed from their base composition. The range of G+C contents among species within a genus is quite often 10% or less as found in *Debaryomyces, Hanseniaspora, Issatchenkia, Kluyveromyces, Metschnikowia* and several other genera. Genera showing a range of greater than 10% among species may be polyphyletic, but a narrower range does not ensure monophyletic (Kurtzman, 1998). The comparing strains, which were differed by more than 1.5-2.0 mol% G+C, do not closely related (Price *et al*., 1978). However,

determination of G+C content cannot distinguish the relations among yeasts with similar DNA base compositions.

DNA-DNA reassociation experiment is very laborious but most powerful for species definition because it gives a measure of overall DNA similarity, while DNA sequencing is a more limited measure. Price *et al*. (1978) examined yeast species in four genera and proposed that strains having 80% or greater than DNA relatedness were conspecific. The comparison of DNA-DNA relatedness of fertility among pairs of heterothallic yeast strains showed the strains having DNA relatedness at 70% or more are usually conspecific (Kurtzman, 1998). This suggestion same as the homothallic yeasts was reported by Kurtzman 1987 and 1991. The sequences of the ribosomal RNAs (rRNA) and ribosomal DNA (rDNA) have been investigated as taxonomic criteria and DNA sequencing can be used to infer phylogenetic relationships at virtually any level (Guého *et al*., 1990; Kurtzman and Robnett, 1991; Kurtzman, 1992; Molina *et al*., 1992, 1993; Mendonca-Hagler *et al*., 1993 and Kurtzman, 1994).

Table 4 Distribution of mol% G+C among ascomycetous and basidiomycetous Yeasts.

Mol% G+C	**Percentage of Ascomycetous Yeasts**	**Percentage of Basidiomycetous Yeasts**
25-29	0.75	-
30-34	14.5	-
35-39	28.0	1.0
40-44	32.5	1.0
45-49	16.0	10.0
50-54	5.5	35.0
55-59	2.0	31.0
60-64	0.75	18.0
65-69	-	4.0

Source: Barnett *et al*. (1990)

Ribosomal RNA/DNA sequence comparisons have been used extensively in recent years to assess both close and distant relationships among many kinds of organisms. The interest in rRNA/rDNA comes from two important properties: (1) ribosomes are present in all cellular organisms and appear to share a common evolutionary origin, thus providing a molecular history shared by all organisms, (2) some rRNA/rDNA sequences are sufficiently conserved that they are homologous for all organisms and serve as reference points that enable alignment of the less conserved areas used to measure evolutionary relationships (Kurtzman and Blanz, 1998). Complete sequences were not often determined because McCarroll *et al*. (1983) and Lane *et al*. (1985) demonstrated that partial sequences of small subunit rRNAs provided essentially the same phylogenies as complete sequences.

Ribosomal RNA/DNA has been preferred for molecular taxonomy over other molecules because of (1) its universality, which allows comparisons among virtually any organism, (2) the presence of multiple copies which evolve in concert, and (3) the belief that it is homologous, having originated only once in evolution times (Sogin *et al*., 1986), allowing comparison of different levels of relationship among yeast systematics is presented in Table 5. The PCR primers that are used to amplify and sequence respective rDNA regions are listed in Table 6.

The analysis of rDNA provided valuable information for the phylogeny and systematics of yeasts (Kurtzman, 1992). Yeast rDNA comprised the 26S, 18S and 5.8S rDNAs occur as tandem repeats with as 100-200 copies (Kurtzman and Blanz, 1998). The structure model of rDNA domain of yeast is shown in Figure 1. In addition, nuclear genes, mitochondria gene or regions other than rDNA have been used to solve relationships among microorganisms but not so extensively studied for yeast taxonomy.

There are many methods for characterize the genetic relationship such as the use of species-specific PCR primers (Fell, 1995; Haynes *et al*., 1995; Mannarelli and Kurtzman, 1998; Mitchell *et al*., 1994), analysis of RFLPs (Magee *et al*., 1987), PFGE, randomly amplified polymorphic DNA (Boekhout *et al*., 1997).

Table 5 Summary of commonly sequenced regions and their used ranges in yeast systematics.

Molecule	Region	Higher categories	Family	Genus	Species	Sister species	Subspecies/variety	Strains
5S rDNA	Total	+	+					
5.8S rDNA	Total			+				
18S rDNA	Total	+	++	+++	++			
18S rDNA	18S-1627 (1451-1618)	+[a]		+++	++			
25S rDNA	25S-635 (D2, V3, B or 463-622)	+[a]		+++	+++		+	
25S rDNA	25S-1841 (1611-1835)	+[a]		++				
25S rDNA	D1/D2			+	+++	++	+	
ITS	Total				+	+	+	
IGS	[b]				+		+	+
Mitochondrial	[b]				+	+		+

[a] Combined regions of 18S (18S-1627) and 25S rDNA (25S-1841 and 25S-635).

[b] Restriction analysis and total or partial sequencing.

+ Little used; ++ used; +++ much used.

Source: Valente *et al*. (1999)

Table 6 List of the primers used for amplification and/or sequencing of the rDNA regions most commonly used for yeast systematics.

Region	Primer (sequence)	Approximate	References [a]
5S rDNA total	[b]	120 bp	Walker and Doolittle (1982)
5.8S rDNA total	Sequenced with the entire ITS region	150 bp	Mitchell *et al*. (1992)
18S rDNA total	Direct sequencing 5' TGGAATTACCGCGGCTGCTGGCACC 3' 5' CCGTCAATTCCTTTAAGTTTCAGCC 3' 5' TCTGGGCCGCACGCGCGCTACACTG 3' 5' GACGGGCGGTGTGTACAAAGGGCAG 3'	1750 bp	Suh *et al*. (1996)
18S rDNA total	Sequencing of PCR product NS1-5' GTAGTCATATGCTTGTCTC 3' NS8-5' TCCGCAGGTTCACCTACGGA 3' Basid2-5' CTGTTAAGACTACAACGGAGCAGGC 3' Basid3-5' AGAGTGTTCAAAGCAGGA 3'	1800 bp	Swann and Taylor (1993)
18S rDNA 18S-1627 (1451-1618)	Direct sequencing 5' ACGGGCGGTGTGTAC 3'	167-172 bp	Guého *et al*. (1990)
25S rDNA 25S-635 (D2, B, V3 or 493-622)	Direct sequencing 5' GGTCCGTGTTTCAAGACGG 3'	200-300 bp	Guého *et al*. (1990)
25S rDNA 25S-1841 (1611-1835)	Direct sequencing 5' TTGGAGACCTGCTGCGG 3'	225 bp	Guého *et al*. (1990)
25S rDNA D1/D2	Sequencing of PCR product NL1-5' GCATATCAATAAGCGGAGGAAAAG 3' NL4-5' GGTCCGTGTTTCAAGACGG 3'	600 bp	Kurtzman and Robnett (1997)
ITS	Sequencing of PCR product ITS1-5' TCCGTAGGTGAACCTGCGG 3' or ITS5-5' GGAAGTAAAAGTCGTAACAAGG 3' ITS4-5' TCCTCCGCTTATTGATATGC 3'	Variable (<400->800 bp)	James *et al*. (1996)
IGS[c]	Sequencing of PCR product AC10-5' GAGGCTAGAGGTGCCAG 3' AC19-5' ATTCGAGAAGTTATTATG 3'	Variable (~2.4 kb)	Fan *et al*. (1995)

[a] References are examples of manuscripts based on the corresponding region, and were chosen because they include a description of the primers.

[b] Studies concerning this molecule are restricted to isolation and posterior analysis of the 5S rDNA, or analysis of the whole IGS region.

[c] IGS region was analyzed mostly by restriction.

Source: Valente *et al*. (1999)

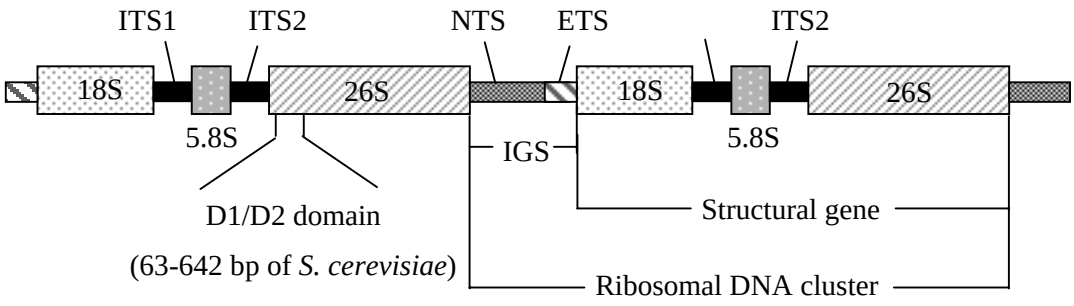

Figure 1 Structure of the ribosomal RNA gene cluster of yeasts. The cluster is split into coding (18S, 5.8S and 26S genes) and non-coding (Internal Transcribed Spacer or ITS and Inter-Genic Spacer or IGS. IGS consist of Non-Transcribed Spacer (NTS) and External Transcribed Spacer (ETS) regions.

Source: modification from:
http://departments.oxy.edu/biology/Stillman/bi221/110300/rna_polymerases.htm

Molnar *et al*. (1996) distinguished 17 *Kluyveromyces* species by Random Amplified Polymorphism DNA-PCR (RAPD-PCR) analysis, but their RAPD-PCR patterns appeared quite complex. Andrighetto *et al*. (2001) identified 42 yeast strains isolated from cheeses by using RAPD-PCR with primers M13 and RF2. This method was applied to the identification at species level. RAPD-PCR analysis of the type strains of different yeast species gave distinctive band profiles that allowed a clear differentiation of all the considered species.

Restriction Fragment Length Polymorphism (RFLP) is most suited to study at the intraspecific level or among closely related taxa. Presence and absence of fragments resulting from changes in recognition sites are used identifying species or populations. The coding (26S, 18S, 5.8S and ITS) and non-coding regions (IGS, ETS and NTS) of rDNA are used for analysis. Bellechetal (1998) have used PCR/RFLP of the Internal transcribe spacer (ITS)-5.8S region to identified *Kluyveromyces* species, but this region is not highly variable, and two restriction patterns were necessary for the differentiation of *K. lactic* and *K. marxianus*. Nguyen *et al*. (2000) proposed the rapid method based on PCR/RFLP of the non-

transcribed space (NTS) from the ribosomal DNA (rDNA) cluster to differentiate *K. lactic* and *K. marxianus* using only one restriction enzyme. This method can be used as rapid and reliable method for identification of the yeast populations (Cadez *et al*., 2002; Graf *et al*., 2004 and Romero *et al*., 2005). Estimates of evolutionary relationships from RFLP patterns have been reported for species assigned to *Candida* (Magee *et al*., 1987), to *Cryptococcus* (Vilgalys and Hester, 1990) and *Debaryomyces* (Romero *et al*., 2005). Such estimates derived from sequence comparisons because as evolutionary distances increase, the extent of patterns similarities less certain.

Molecular systematics of yeasts has emphasized either coding on D1/D2 variable domains of the 26S rDNA or the complete 18S rDNA or 5.8S rDNA. The 5.8S rDNA data will become available in near future as the ITS (Internal transcribed spaces ITS1 and ITS2) regions of the ribosomal are analyzed. These regions are considered to be chronometers because of their universal occurrence, functional constraints and presences of both variable and conserved regions (Worse, 1987). The length of the sequenced portion of D1/D2 region was about 600 nucleotides (63-642 bp of *Saccharomyces cerevisiae*). This region is useful for analysis at the species level (Kurtzman and Robnett, 1998) and a large database is now available. However, the D1/D2 sequence might not distinguish sister species or varieties. The most frequently used numerical methods for sequence comparison are parsimony, neighbor-joining (Saitou and Nei**,** 1987) and maximum likelihood methods (Felsenstein, 1988). The phylogenetic trees need to be statistically tested to set confidence limits for branching order by bootstrap analysis (Felsenstein, 1988).

Electrophoretic karyotype analysis has been proved to be useful for epidemiological studies of *Candida albicans* and *C. glabrata* because this method can distinguish not only the species identification but also the differences among the strains of the same species. (Doi *et al*., 1994). Boekhout *et al*. (1998) studied on seven species of *Malassezia*, all have different karyotypes which do not vary intraspecifically, except for *M. furfur*, which displayed two different karyotypes. In 2002, Cadez *et al*. studied on accurate identification of *Hanseniaspora* and *Kloeckera* species as well as for determining inter- and intraspecific relationships of 74 strains by using pulse-field gel electrophoresis (PFGE). Electrophoretic

karyotyping produced chromosomal profiles by which the seven species could be divided into four groups sharing similar karyotypes. Although most of the 60 strains examined exhibited a common species-specific pattern, a different degree of chromosomal-length polymorphism and a variable number of chromosomal DNA fragments were observed within species.

3. Phylogenetic Position of Yeasts

Molecular comparisons are leading to an understanding of phylogenetic relationships among yeasts (Barns *et al*., 1991; Hendriks *et al*., 1992; Kurtzman and Robnett, 1991; Wilmotte *et al*., 1993). Because of the phylogenetic-species concept, especially when based on cladistic analysis of molecular characteristics, offers constancy in the circumscription of species and gives a more realistic appraisal of biodiversity. Kurtzman (1994) examined the impact of molecular comparisons, notably rRNA/rDNA sequence divergence, on the current phenotypically defined classification of yeasts. Principal findings include: 1) budding ascomycetous yeasts are monophyletic and represent a sister group to the filamentous ascomycetes, 2) fission yeasts are ancestral to budding and filamentous ascomycetes, 3) the molecular phylogeny of basidiomycetous yeasts is generally congruent with type of hyphal septum, presence or absence of teliospores in the sexual state, and occurrence of cellular xylose. In order to better understand the evolutionary relationships among the yeasts and to construct more stable and robust phylogenetic frameworks, many more complete rRNA sequences must be determined.

3.1 The phylogenetic position of ascomycetous yeasts

The phylogeny of the ascomycetous yeasts has been vigorously debated since the time of Guilliermond (1912). The impact of rRNA/rDNA comparisons on the taxonomy of ascomycetous yeasts is just beginning. Additional work is required to evaluate the assignment of species to genera and the relationships among genera.

Examination of rRNA/rDNA sequence divergence from a limited number of taxa indicated the ascosporogenous yeasts, with the exception of *Schizosaccharomyces*, to from a monophyletic group (clade) distinct from the filamentous species (Barns *et al*., 1991; Burns *et al*., 1991; Hausner *et al*., 1992; Hendrinks *et al*., 1992; Kurtzman, 1993 and 1994; Nishida and Sugiyama 1993; Walker 1985 and Wilmotte *et al*., 1993). Kurtzman and Robnett (1998) analyzed rRNA sequence divergence from type species of all cultivatable ascomycetous yeasts and yeastlike taxa. This work demonstrated that yeasts, as well as yeastlike genera such as *Ascoidea* and *Cephaloascus* to comprise a clade sister to the "filamentous" ascomycetes (euascomycetes).

However, major findings to date include: (1) The majority of yeasts and yeastlike species are phylogenetically separate from the euascomyces and included in hemiascomycetes, (2) the fission yeast genus *Schizosaccharomyces* is phylogenetically distant from the "budding" yeast clade and the euascomycetes, resulting in the reassignment of the fission yeasts to a separate order, the *Schizosaccharomycetales* and (3) the demonstration that many phenotypic characters such as ascospore morphology are poor indicators of phylogeny (Kurtzman and Robnett, 1991; 1994). On the basis of rDNA sequence comparisons, Nishida and Sugiyama (1993) suggested the name Archiascomycetes for the clade comprising *Schizosaccharomyces, Saitoella, Taphrina, Protomyces* and *Pnumocystis*.

In 1998, 500 species of ascomycetous yeasts were studied and phylogenetically based on nuclear large-subunit (26S) ribosomal DNA partial sequences by Kurtzman and Robnett (1998). This study is the first one to include essentially all known ascomycetous yeasts in the same molecular dataset. Their results indicated that nearly all currently recognized ascomycetous yeasts could be identified from their unique D1/D2 sequences. They demonstrated for ascomycetous yeasts that strains differing by 1% nucleotide substitutions in D1/D2 domain represent separate species. Consequently the use of this database markedly increase the accuracy of yeast identifications.

3.2 Phylogenetic position of basidiomycetous yeasts

The basidiomycetous yeasts, as currently recognized, are distributed among the three classes of the Basidiomycota: Ustilaginomycetes, Urediniomycetes and Hymenomycetes. These yeasts have considerable economic, agricultural and medical importance but is estimated indicate that the number of known yeasts may represent about 1% of the species that exist in nature (Fell *et al*., 2002). There is an increased interest in discovering these species for economic exploitation and there is a need to understand their biodiversity and ecological roles. Identification and phylogenetic placement of the basidiomycetous yeasts are not always easy because of their polyphyletic nature.

The significant advances in basidiomycete systematics have been achieved by sequence analysis of the large and small subunits of rRNA/rDNA (Boekhout *et al*., 1995; Fell and Kurtzman, 1990; Fell *et al*., 1995; Guého *et al*., 1989, 1993; Nakase *et al*., 1993; Sugiyama and Suh, 1993; Suh and Sugiyama, 1993; Suh and Nakase, 1995; Swann and Taylor, 1995; and Van de Peer *et al*., 1992).

In 2000, Fell *et al*. studied the systematics of 337 basidiomycetous yeasts by large subunit rDNA D1/D2 domain sequence analysis. They suggested that strains that differed by two or more nucleotides in the D1/D2 region represented different taxa. The majority of the species can be identified using D1/D2 analyses, although the internal transcribed spacer region is required to distinguish closely related species. The intergenic spacer region is recommended for additional differentiation of species and strains.

Scorzetti *et al*. (2002) presented that the taxonomy of basidiomycetous yeasts can be clarified by ITS analysis. In addition to the ITS region, the IGS region is useful and may be required for strain separations as demonstrated with *Xanthophyllomyces, Phaffia* (Fell and Blatt, 1999) and *Markia* (Diaz and Fell, 2000).

4. Yeast Research in Thailand

It is easily estimated that microorganisms including yeasts are rich in Thailand because it belongs to the tropics with hot and wet climate. However, until recent years, a little has been known about the yeasts living in Thailand, except for yeasts associated with fermented foods and related materials. In the early stage of yeast research in Thailand, the study of yeast was focused on isolation and collection of alcoholic fermenting strains including fermentation process development. However, the study of yeasts living in the natural environment has just started.

4.1 Yeasts for the production of useful substances

Many strains of yeast were isolated for selected of yeast strains suitable for alcoholic fermentation in Thailand (Chaitiemwong, 1973; Chatisatienr, 1977; Tammarat, 1978; Punpeng, 1980; Pakdisupapol, 1980; Phoopat, 1983). Genetic improvements were used for improved yeast strains for high ethanol production (Seki *et al*., 1983; Khunajakr, 1987; Pirapatrungsuriya, 1991; Dejing, 1995; Chomtong, 1995).

Genetic improvements were used also for conduct yeast strains with lysine rich (Rupasut, 1993), methionine rich (Chanklan, 1996), lysine and methionine rich (Disyen, 1999), and increase free methionine and selenium accumulation (Sririporn, 2002).

Several strains of yeasts, which were isolated in Thailand, were reported to suitable to use as single cell protein. *Candida intermedia* and *Debaryomyces hansenii* had high protein content with high growth rate in soybeas waste (Siriroj, 1978). *Candida valida* isolated from soil in Kasetsart University and *Hansenula polymorpha* from sugarcane bagasses, were reported to be suitable to cultivated in slop waste for high protein content (Kunteeya, 1983; Sophonsathien, 1990).

Noppanakheepongse (1984) studied on protein production from xylose, xylan and sulfite spent liquor media by yeasts. Strains 4R-39.2 contained high protein content in sulfite-spent liquor hydrolyzed by crude xylanase enzyme. *Kloeckera japonica* and *Endomycopsis* sp., were able to hydrolyze starch at 45ºC and 40ºC (Naiyabootra, 1989).

A thermotolerant yeast strain, *Candida ishiwadae,* isolated from plant material in Thailand has ability to produce lipase and biosurfactant. *C. ishiwadae* could be a potential candidate for producing monoacylglycerols which are useful in industrial applications (Thanomsub, 2004).

4.2 Yeasts associated with fermented foods and related substrates

Saito *et al* (1983) isolated 386 strains of yeasts from 54 samples of fermented foods and related substrates and identified them as 21 species in 11 genera. All of them belong to known species. *Saccharomyces cerevisiae* was the dominant species and occupied 30.6% of the isolates and found in 39.4% of samples, followed by *Issatchenkia orientalis* (Anamorph: *Candida krusei*) (28.2% of isolates), *Hanseniaspora valbyensis* (9.8% of isolates), *Candida tropicalis* (5.4% of isolates), *Pichia membranifaciens* (4.7% of isolates), *Pichia ohmeri* (4.4% of isolates) and *Saccharomycopsis fibuligera* (3.9% of isolates). The remaining 14 species occupied 1.8-0.3% of the isolates, respectively.

Suzuki *et al*. (1987) studied 80 strains of yeasts, which were isolated from fermented foods and related substrates in 1984. They identified these yeasts as 17 species in 9 genera. *Issatchenkia orientalis* was the dominant species and occupied 42.5% of the isolates, followed by *Saccharomyces cerevisiae* (11.3%), *Issatchenkia occidentalis* (Anamorph: *Candida sorbosa*) (10.0%) and *Candida tropicalis* (6.3%). In this study, a strain was found to represent a new species and described as *Candida stellimalicola* (Suzuki *et al*. 1994). In these two studies on yeasts associated with fermented foods and related substrates, yeast species were common to those found in fermented foods in other countries, not only in Southeast Asian countries but also in European countries and in Japan. Further, more than 80% of the isolates belong to species that found in these two studies.

A total of 137 strains of halotolerant yeasts associated with fermented foods and related substrates, which were collected by Limtong (1986a, 1986b, and 1987), were identified by Jindamorakot (2000). They were assigned to 7 genera, *Candida*, *Citeromyces*, *Debaryomyces*, *Issatchenkia*, *Pichia*, *Saccharomyces* and *Zygosaccharomyces*. Among them, 123 isolates were identified as 33 known species. *Issatchenkia orientalis* was the dominant species and occupied 27.0% of the isolates followed by *Saccharomyces cerevisiae* (8.8%), *Candida parapsilosis* (7.3%) and *Candida glabrata* (5.8%). In this study, 57.6% of isolates belong to species commonly found in the studies of Saito *et al*. (1983) and/or Suzuki *et al*. (1987), in spite of the use of high salt content media for isolation. Two strains from fermented soybean and dried salted squid were identified as *Citeromyces matritensis* in this study. However, further detailed studies including 18S rDNA sequence analysis and phenotypic characteristics revealed that the two strains represented a new species. They were described as *Citeromyces siamensis* (Nagatsuka *et al*., 2002). Jindamorakot assigned the remaining 12 strains to the genera *Debaryomyces*, *Saccharomyces* and *Candida* but these strains could not identifed as any known species of these genera. Probably, these strains represent hitherto undescribed species.

Limtong *et al*. (2002) reported that most of the loog-pang, a traditional solid starter for alcoholic fermentation in Thailand, comprised *Saccharomycopsis fibuligera*, which were showed strong amylolytic activity.

4.3 Medically important yeasts

From 1999 to 2002, a total of 202 *Candida* isolates causing candidemia were recovered from 202 individual patients in the largest tertiary hospital in Bangkok, Thailand. *C. albicans* comprised 44.55% of all isolates. Non-albicans *Candida* spp. isolates accounted for 55.45% of all candidemia episodes and were primarily due to *C. tropicalis* (45%) followed by *C. parapsilosis* (6%), *C. glabrata* (4%), and *C. krusei* (0.5%) (Foongladda, 2004).

Imai (2000) studied on geographic grouping of *Cryptococcus neoformans* var. *gattii*, which were isolated from Thailand, by RAPD fingerprint patterns and ITS sequence divergence. The high discriminatory power of PFGE infers the benefit

of subtyping which lead to better understanding on the epidemiology and pathogenic potential of *C. neoformans* subtypes. Moreover, PCR fingerprinting and RAPD infer the feasibility of detail analysis between serotypes A and D for unencapsulated *C. neoformans* (Ngamwongsatit, 2005).

Sugita *et al*. (2003) isolated three *Pseudozyma* strains from the blood of patients in Thailand. While one isolate was identified as *P. antarctica* by rDNA sequence analysis, the other two were considered to be new species and were named *P. parantarctica* and *P. thailandica*. This is the first isolation of *Pseudozyma* strains from humans.

4.4 Yeast in natural environment

In the past decade, extensive studies have been carried out on the ballistoconidium-forming yeasts living in the Thai phyllosphere. In 1987, 42 samples of various plants were collected in the forests, fields, rice fields, roadsides in the western suburb of Bangkok and near Ayutthaya, and also in the urban areas of Bangkok including plant leaves collected in several markets. Yeasts were isolated from these samples at 23 and 30ºC by ballistoconidium-fall method (Nakase and Takashima, 1993). Sixty-three strains of ballistoconidium-forming yeasts were isolated from 20 samples (50%) examined. The frequency of isolation reached to 81.2% when samples collected in the suburbs of Bangkok and Ayutthaya but it was very low (15%) when samples collected in the urban areas of Bangkok including markets (Nakase *et al*., 2001).

Sixty-three strains were identified as 16 species in the genera *Bullera, Kockovaella, Bensingtonia, Sporidiobolus/Sporobolomyces* and *Tilletiopsis*. Two species, *Kockovaella imperatae* and *Kockovaella thailandica,* were described by Nakase *et al*. (1991). In addition to new species of *Kockovaella, Sporobolomyces nylandii* and *Sporobolomyces vermiculatus* were also described (Takashima and Nakase, 2000). Three strains out of six of yeast-like fungi were assigned to *Tilletiopsis*. They were found to represent three new species, *Tilletiopsis derxii, Tilletiopsis oryzicola* and *Tilletiopsis penniseti* (Takashima and Nakase, 2001).

In 1990, 73 strains of ballistoconidium-forming yeasts were isolated from 33 plant materials (82.5%) out of 40 collected in forests, grasslands and rice fields along the southeastern seacoast of Bangkok to Pattaya. The isolation of yeasts was carried out at 25°C. These yeasts were identified as 13 species in the genera *Bullera, Kockovaella, Bensingtonia and Sporidiobolus/Sporobolomyces. Bensingtonia musae* (Takashima *et al*., 1995), *Bullera penniseticola*, *Kockovaella sacchari* (Takashima and Nakase, 1998), *Sporobolomyces blumeae* and *Sporobolomyces poonsookiae* (Takashima and Nakase, 2000) were described as new species. In this isolation study, *Tilletiopsis* strains were not isolated though they are commonly found in the samples.

A total of 136 strains of ballistoconidium-forming yeasts isolated in 1987 and 1990 were identified as 21 species, 105 strains (77.2%) as 9 known species and 31 strains (22.8%) as 12 undescribed species (Nakase *et al*., 2001). Eight of them were commonly isolated in 1987 and 1990 but the remaining 13 species were isolated in either year. In the isolation study of 1987, *Sporobolomyces shibatanus* (Teleomorph: *Sporidiobolus pararoseus*) was the dominant species and found in 21.4% of plants examined, followed by *Bullera sinensis* (19.0%), *Bullera crocea* (16.7%) and *Sporobolomyces salmonicolor* (11.9%). In the isolation study in 1990, the most frequently isolated species was *Bullera sinensis* and found in 52.5% of plant samples examined, then followed by *Sporobolomyces shibatanus* (30.0%), *Sporidiobolus ruineniae* (17.5%) and *Sporobolomyces poonsookiae* (15.0%). *Sporobolomyces roseus*, the most frequently encountered ballistoconidium-forming species in the Temperate Zones, was not found in these studies. Among ballistoconidium-forming yeasts isolated in 1987 and 1990, a strain had Q-9 as the major component of ubiquinone and was described as *Bensingtonia musae* (Takashima *et al*., 1995). The remaining yeasts had Q-10 and were assigned to the genera *Bullera, Kockovaella, Sporidiobolus, Sporobolomyces* and *Tilletiopsis*. No yeast was found to have Q-10 (H_2) as the major ubiquinone (Nakase *et al*., 2001).

In 1996, Fungsin isolated 175 strains of yeasts from plants collected in a protected tropical rain forest in Sakaerat, Nakhon Ratchasima Province, northeastern Thailand, by ballistoconidium-fall method (Fungsin, 2003). After confirming the ballistoconidium-forming ability, he assigned 151 strains to the genera *Bullera* (51 strains), *Dioszegia* (2 strains), *Kockovaella* (4 strains),

Bensingtonia (10 strains), *Rhodotorula* (6 strains), *Sporidiobolus* (2 strains), *Sporobolomyces* (57 strains) and *Tilletiopsis* (18 strains). He identified 141 of them as 47 species and a variety, 14 known species, 33 undescribed species and an undescribed variety. Among 141 strains identified, 106 (75.2%) belong to undescribed species or an undescribed variety. This result clearly suggests that ballistoconidium-forming yeasts associated with plants in protected forests of Thailand are rich in biodiversity and a numerous number of unknown species are living in these substrates.

Among undescribed species of ballistoconidium-forming yeasts mentioned above, 6 strains were described as new species in the genera *Bensingtonia, Kockovaella* and *Bullera*, i. e., *Bensingtonia thailandica* (Fungsin *et al*., 2001), *Kockovaella barringtoniae* (Fungin *et al*., 2002a), *Bullera arundinariae* (Fungsin *et al*., 2002b), *Bullera siamensis, Bullera panici* (Fungsin *et al*., 2003a) and *Bullera sakaeratica* (Fungsin *et al*., 2003b). In this study, Fungsin found 19 Q-10(H_2) having yeast from 57.7% of plant samples examined. This suggested yeast diversity is very rich in the protected forest compared with Bangkok and its suburban regions. In addition to basidiomycetous yeasts mentioned above, Prillinger *et al*. (1997) found a new stalked conidium-forming yeast from a lichen in Thailand and described it as *Fellomyces thailandicus*.

Four new species of thermotolerant methylotrophic yeasts were described and namely *Candida krabiensis* sp. nov., *Candida sithepensis* sp. nov., *Pichia siamensis* sp. nov., and *Pichia thermomethanolica* sp. nov. by Limtong *et al*. (2004 and 2005). *C. krabiensis*, *C. sithepensis* and *Pichia thermomethanolica* were isolated from soils and three strains of *P. siamensis* were isolated from flowers and tree flux in Thailand.

Twenty-one strains of a novel ascomycetous yeast species, *Tetrapisispora namnaonensis* sp. nov., were isolated from soil collected in three kinds of natural forest, namely a dry dipterocarp forest, a mixed deciduous forest and a pine forest, in Nam Nao National Park, Phetchabun province, Thailand (Sumpradit *et al*., 2005).

A total of 33 new yeast species found in Thailand are showed in Table 7. It is suggested that species diversity of yeasts is richer in the natural environment than in fermented foods and related substrates.

Table 7 Lists of new yeast species found in Thailand.

Species	**Sources**	**References**
Ascomycetous yeasts		
Candida easanensis	Insect frass	Jindamorakot *et al*. (2004)
Candida krabiensis	Soil	Limtong *et al*. (2004)
Candida nakhonratchasimensis	Insect frass	Jindamorakot *et al*. (2004)
Candida pattaniensis	Insect frass	Jindamorakot *et al*. (2004)
Candida sithepensis	Soil	Limtong *et al*. (2004)
Candida stellimalicola	Star apple	Suzuki *et al*. (1994)
Citeromyces siamensis	Dried salted squid, Fermented soy bean	Nagatsuka *et al*. (2002)
Pichia siamensis	Flowers, tree flux	Limtong *et al*. (2004)
Pichia nongkratonensis	Insect frass	Nakase *et al*. (2005)
Tetrapisispora namnaonensis	Soil	Sumpradit *et al*. (2005)
Pichia thermomethanolica	Soil	Limtong *et al*. (2005)
Basidiomycetous yeasts		
Bensingtonia musae	Leaves	Takashima *et al*. (1995)
Bensingtonia thailandica	Leaves	Fungsin *et al*. (2001)
Bullera penniseticola	Leaves	Takashima *et al*. (1998)
Bullera arundinariae	Leaves	Fungsin *et al*. (2002b)
Bullera panici	Leaves	Fungsin *et al*. (2003a)
Bullera sakaeratica	Leaves	Fungsin *et al*. (2003b)
Bullera siamensis	Leaves	Fungsin *et al*. (2003a)
Fellomyces thailandicus	Lichen	Prillinger *et al*. (1997)
Kockovaella thailandica	Leaves	Nakase *et al*. (1991)
Kockovaella imperatae	Leaves	Nakase *et al*. (1991)
Kockovaella sacchari	Leaves	Takashima *et al*. (1998)
Kockovaella barringtoniae	Leaves	Fungsin *et al*. (2002a)

Table 7 (Continued)

Species	**Sources**	**References**
Pseudozyma parantarctica	Blood	Sigita *et al*. (2003)
Pseudozyma thailandica	Blood	Sigita *et al*. (2003)
*Sporobolomyces nylandi*i	Leaves	Takashima and Nakase (2000)
Sporobolomyces poonsookiae	Leaves	Takashima and Nakase (2000)
Sporobolomyces blumeae	Leaves	Takashima and Nakase (2000)
Sporobolomyces vermiculatus	Leaves	Takashima and Nakase (2000)
Tilletiopsis derxii	Leaves	Takashima and Nakase (2001)
Tilletiopsis oryzicola	Leaves	Takashima and Nakase (2001)
Tilletiopsis penniseti	Leaves	Takashima and Nakase (2001)
Trichosporon siamense	Insect frass	Nakase *et al*. (2006)

MATERIALS AND METHODS

1. Sample Collection

Samples were collected from various kinds of natural habitats such as flowers, insect frass, mosses, mushrooms and some others such as fruits and exudates, from several places of Thailand (Fig. 2).

Figure 2 Places where samples were collected (●).

2. Isolation of Yeasts

The yeasts were isolated by direct streaking of samples on YM agar plates supplemented with 100 μg/l chloramphenical and 0.2% sodium propionate (Appendix A). The enrichment technique was also used for isolation. The technique was carried out by addition of small amount of sample into YM broth supplemented with 100 μg/l chloramphenical and 0.2% sodium propionate (Appendix A) and incubated for several days until a film and / or sediment of yeasts are produced. The film of yeasts was streaked on YM agar supplemented with 100 μg/l chloramphenical and 0.2% sodium propionate. The sediment was also streaked on agar after removal of film and media by decantation. After incubation for several days, colonies appeared on agar media were picked up and purified by the conventional streaking technique on YM agar (Appendix A). The pure cultures were suspended in YM broth supplemented with 10% glycerol as a cryoprotectant and maintained in a deep freezer (-80°C).

3. Identification of Yeasts

Yeasts were identified by polyphasic taxonomic studies. The molecular, chemotaxonomic and conventional techniques were used for this purpose. The sequences of D1/D2 domain of 26S rDNA were compared with nucleotide database by BLASTn programs (http://www.ncbi.nlm.nih.gov/BLAST/ and http://www.ddbj.nig.ac.jp/E-mail/homology.html). The morphological, physiological, biochemical and chemotaxonomic characteristics were studied and compared with the standard descriptions in The Yeasts, A Taxonomic Study 4th ed. (Kurtzman and Robnett, 1998) and Yeasts: Characteristics and identification (Barnett et al., 2000). DNA-DNA reassociation experiment was used for confirmation of the correctness of identification if it was necessary.

3.1 Molecular techniques

3.1.1 Nucleotide sequencing

a. Isolation of DNA for Polymerase Chain Reaction (PCR). Isolation of DNA was carried out by boiling of cells with lysis buffer according to the methods of Manitis et al. (1982) with slight modification. A loopful of yeast cells was transferred to 1.5 ml Eppendorf tube. The 100 µl of lysis buffer was added. Cell suspensions were boiled in water bath or metal block bath for 15 min. After boiling, 100 µl of 2.5 M potassium acetate (pH 7.5) was added and placed on ice for 1 hr, and centrifuged at 14,000 rpm for 5 min. Supernatant was extracted twice with 100 µl of chloroform:isoamyl alcohol (24:1 v/v). DNA was precipitated with isopropanol, placed at 20°C for 10 min and centrifuged at 15,000 rpm for 15 min. DNA pellet was rinsed with 70% and 90% ethanol and then dried up (15-30 min at room temperature). The dried DNA was dissolved in 30 µl milli Q water.

b. Polymerase Chain Reaction (PCR) for D1/D2 domain of 26S RDNA. The divergent D1/D2 domain of 26S rDNA was amplified with primers NL-1 (5'- GCA TAT CAA TAA GCG GAG GAA AAG-3') and NL4 (5'-GGT CCG TGT TTC AAG ACG G-3') (Kurtzman and Robnett, 1998). Amplification was carried out in 100 µl reaction mixture conditioning 100 ng of genomic DNA, 2.5 U of Taq polymerase, 20 mM of each dNTP, 40 mM of each primer, 10 mM Tris-HCl and 1.5 mM $MgCl_2$. The reaction was pre-denatured at 94°C for 5 min, then repeated for 30 PCR cycles with denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2.5 min and then followed by the final extension at 72°C for 10 min. The amplified DNA was purified with QIAquick PCR Purification Kit according to the manufacturer's instruction. Visualization of the purified amplified DNA was performed by electrophoresis using 0.8% agarose gel in 1X TBE buffer and stained with ethidium bromide ($8x10^{-5}$ µg/ml) and observed under UV illuminator.

c. D1/D2 domain of 26S rDNA sequencing. The nucleotide sequences of D1/D2 domain of 26S rDNA were directly determined using PCR products according to Kurtzman and Robnett (1998) with slight modification. Cycle

sequencing of the D1/D2 domain was employed with forward primer NL1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG-3'), and reverse primer, NL4 (5'-GGT CCG TGT TTC AAG ACG G-3'), by ABI Prism™ BigDye™ Terminator Cycle Sequence Ready Reaction Kit (Applied Biosystems, Stafford, USA) according to the manufacturer's instruction.

3.1.2 BLAST analysis

The sequences of D1/D2 domain of 26S rDNA were compared by BLASTn Homology Search (http://www.ncbi.nlm.nih.gov/blast).

3.1.3 Phylogenetic analysis

Generated sequences were aligned with related species by using the CLUSTAL X ver. 1.8 computer programs (Thompson et al., 1997). The phylogenetic trees were constructed from the evolutionary distance data according to Kimura (1980) by the neighbor-joining method (Saitou and Nei, 1987). Sites where gaps existed in any sequences were excluded. Bootstrap analysis (Felsenstien, 1985) is performed from 1,000 random re-samplings.

3.1.4 DNA-DNA reassociation

DNA-DNA reassociation was carried out by photobiotin microplate hybridization method (Ezaki et al., 1989).

a. Isolation and purification of DNA. Two methods were used for isolation and purification of DNA.

Method I: This method was modified from Holm et al. (1986). A loopful of cells was inoculated into 50 ml of YPD in a 200 ml flask and incubated overnight at 25° C with shaking. Cells were harvested by centrifugation in a sterile 25 ml centrifuge tube at 3,000 rpm for 5 min. The cell pellet was resuspended in 20 ml of ice-cooled 50mM EDTA (pH 7.5). The pellets were collected and resuspended in 4 ml SCE (Appendix A) after centrifuge at 6,000 rpm for 5 min. Suspended cells were lysed by incubation with 0.1 ml Zymolyase solution (Appendix A) at 37°C for 1-1.5 hr. The supernatant was removed completely after centrifugation at 15,000 rpm for 3 min. Two volume of ice cooled of 99% ethanol was added and mixed gently after the

solution reached to room temperature. The supernatant was removed completely after centrifugation at 12,000 rpm for 3 min. The DNA pellet was dissolved completely in 1 ml of 10X TE (Appendix A) with a spatula. The DNA solution was incubated with 30 µl RNase solution (Appendix A) at 37°C for 1 hr. Then the DNA solution was incubated with 30 µl Proteinase K solution (Appendix A) at 65°C for 30 min. After the DNA solution reached to room temperature, 3 ml Tris saturated phenol was added and mixed. Aqueous layer was removed to a clean centrifuge tube after centrifugation at 15,000 rpm for 5 min. DNA was precipitated with 1/10 volume of sodium acetate and 2 volumes of ice cooled 99% ethanol with gently shaking to mix. Precipitated DNA was washed by mixed with 500 µl of 80% ethanol. Purified DNA was dried at 65°C for 15-30 min. Dried DNA was incubated with 500 µl TE at 50°C to dissolve. DNA solution was purified again by 300 µl Tris saturated phenol and mixed. Aqueous layer was transferred to a clean centrifuge tube after centrifugation at 12,000 rpm for 5 min. Aqueous layer was incubated with 10 µl RNase at 37°C for 1 hr. The solution was incubated with 10 µl Proteinase K solution at 65°C for 30 min. After the solution reached to room temperature, 300 µl Tris saturated phenol was added and mixed by vortex. Aqueous layer was transferred to a clean centrifuge tube after centrifugation at 12, 000 rpm for 5 min. DNA was precipitated with 1/10 volume of sodium acetate and 2 volumes of ice cooled 99% ethanol with gently shaking to mix. Precipitated DNA was transfered to a microtube containing 500 µl of 80% ethanol. Purified DNA was dried at 65°C for 15-30 min. Dried DNA was dissolved in 100-500 µl of sterilized distilled water. Protein and the DNA concentration were measured by spectrometer. Unpurified DNA was precipitated and purified again to clean up DNA further.

Method II (Modification of Mamar's Methods): Cells were grown in 500 ml Erlenmeyer flask containing 250 ml of YM broth on a rotary-shaker at 150 rpm at 25°C and were harvested in the logarithmic growth phase. The cells were washed twice with 1/15 M phosphate buffer with 0.1 M EDTA (pH 7.5). Cells (5-10 g) were suspended in 1.5 vol. of 1/15M phosphate buffer with 0.1 M EDTA and lysed by incubation with 5 mg of 100T Zymolyase and 0.2 ml of mercaptoethanol for 30 min under gentle shaking. The suspension was incubated with 0.5% of SDS at 35°C for

15min. The reaction mixture was vigorously shaken with equal volume of chloroform:isoamyl alcohol (24:1) and 5% of sodium perchlorate to denature proteins. The supernatant was removed after centrifugation. DNA was precipitated by addition of 2 vol. of chilled ethanol, and then spooled around a glass rod. Crude DNA was dissolved in 4.5 ml of 0.1 XSSC, then 0.5 ml of 10 XSSC was added. The remaining protein was digested with Proteinase K treatment at 37°C for 30 min. The solution was repeatedly by treated with 1 volume of chloroform:isoamyl alcohol (24:1) with vigorous shaking, and then centrifuged to get thin denatured protein layer. RNA was removed by incubation with 0.2 mg of RNase A at 37°C for 2 hr. DNA solution was treated with chloroform:isoamyl alcohol (24:1) to remove RNase. After centrifugation, DNA was precipitated with ethanol, and then spooled around a glass rod. DNA was dissolved in 4.5 ml 0.1X SSC and 0.5 ml of 10X SSC (Appendix A) was added. After addition of 0.5 ml of acetate-EDTA, 0.54 vol. of chilled isopropanol was added and DNA was spooled around a glass rod. Purified DNA was washed stepwise with 70%, 80%, 90% and 99.5% ethanol.

b. Immobilization of DNA. DNA solution was heated in boiling water bath for 5 min, and then quickly transferred to ice water bath. Heat denatured DNA solution was diluted to 4 ng/μl with PBSM1 (Appendix A). 100 μl of diluted DNA solution was dispensed to respective wells of microplate (Immunoplate MaxiSorp, Nunc), and then left at 28°C for 16 hr. The solution was discarded from wells, and washed twice with 200 μl of PBS, then the plate was dried at 60°C for 2 hr. Dried plate was stored at room temperature until use.

c. Labeling of DNA with photobiotin. Amount of 0.5-1 mg DNA was dissolved in 1 ml sterilized milli Q water. DNA solution was mixed with two volumes of photobiotin solution (Appendix A), with vigorous shaking in the dark room (the amount of solution should be 50 μl). Eppendorf tube (without cap) was stored on ice for 20 min under murcury lamp (distance ca. 10 cm). TE buffer, pH 9.0 was added in Eppendorf tube to get the final volume of 100 μl. After that 100 μl of isobutanol was added and mixed well by shaking. Upper layer (isobutanol layer) was

removed after centrifugation at 12,000 rpm for 10 sec. This step was repeated again. The pellet was dissolved in 200 μl of water, and then 5 μl of 3M sodium acetate solution and 100 μl of ethanol were added to the Eppendorf tube. The Eppendorf tube was stored in -20°C freezer over night and centrifuged at 12,000 rpm to obtain brown colored precipitate. The DNA pellet was washed with 70% ethanol and dried. DNA pellet was dissolved in approximate amount of TE and stored at -20°C until use.

d. Hybridization. Amount of 200 μl of pre-hybridization solution was added to respective wells with immobilized DNA, and then incubated at 37°C for 30 min. Microplate was turned upside down and liquid was removed carefully. Amount of 100 μl of hybridization solution containing 0.5 μg of heat-denatured photobiotin labeled DNA was added to respective wells and cover with plate seal, then left at 42°C for 24 hr. The solution was removed and wells were washed two times with 0.2X SSC.

e. Binding of streptavidin-β-galactosidase complex (SABG) to DNA. Amount of 100 μl of PBS-BSA-Triton solution (Appendix A) was added into wells and left at room temperature for 10 min. The solution was removed, and 100 μl of SABG solution (Appendix A) was added and left at 37°C for 30 min then wells were washed two times with 200 μl of PBS (Appendix A).

f. Measurement of β-galactosidase activity (measurement of 4-MUF). Amount of 200 μl of MUF-Gal solution (Appendix A) was added into wells. After that, 1 μl/ml MUF-Gal solution was added in A01 well for primary setting and checking of the microplate reader, and MUF-Gal solution was used as a blank by adding to A02 well, left at room temperature. The results were checked by measuring the released 4-methylumbelluferon by microplate reader, after 0 min, 10 min, 20 min and 30 min.

g. Calculation of% DNA similarity

% Similarity of DNA=100X(X-N) / (P-N)

X: measured value of DNA to be tested

P: measured value of DNA as probe

N: measured value of DNA that has no relationship to DNA to be tested

(P/N should be more than 8-10)

3.1.5 Determination of DNA base composition

DNA base composition was analyzed according to the method of Nakase and Suzuki (1986b). Amount of 20 µl (300-500 mg) of DNA solution (from 14.1) in 0.1XSSC was boiled for 10 min and rapidly cooled in ice water. Amount of 20 µl of 2 unit/ml Nuclease P1 was added and incubated at 50°C for 60 min. Amount of 20 µl of 2.4 unit/ml alkaline phosphatase was added and incubated at 37°C for 60 min. The DNA base composition was determined by HPLC using Cosmosil column (Waters, 5C18, 4.6 mm x 250 mm). The elution system was 0.02 M $NH_4H_2PO_4$: acetonitrile (20:1 v/v) at a flow rate of 1 ml/min. The nucleosides were detected at 270 nm. DNA-GC Kit (Yamasa Co., Tokyo) was used as the quantitative standard.

3.2. Morphological characteristics

Most of methods used to examine the morphological, physiological and biochemical characteristics are those described in The Yeasts, a Taxonomic Study, 4th ed. (Yarrow, 1998) except for several experiments mentioned later.

3.2.1 Morphology of vegetative cells

Yeast culture was inoculated in YM broth and incubated at 25°C for 3-5 days. The morphology of cells was examined under microscope. The shape, arrangement and the size of cells were recorded.

3.2.2 Cultural characteristics

Yeast culture was inoculated on YM agar and YM broth and incubated at 25°C until 4 weeks. The characteristics of colony on YM agar such as

color, size, surface, margin, and texture were recorded. The characteristics of culture observed in YM broth in the formation of sediment, coherent, flocculent or mucous, a ring, islets or a pellicle.

3.2.3 Pseudomycelium and true mycelium formation

The formation of pseudomycelium and true mycelium were examined using slide culture method. Glass slide in Petri dish with U-shaped glass rod (on a filter paper) and a tweezers wrapped with aluminium foil are sterilized at 160-170°C for 30-60 min. Sterilized glass slide was removed from Petri dish by sterilized tweeezer and was dipped into molten 50-55°C PDA (Difco), then placed on U-shaped glass rod in the Petri dish and allowed agar to solidify. After drying of agar surface, yeast was inoculated with a tiny loop (light inoculum), a sterilized cover slip was placed on the line and incubated at 25°C. The formation of pseudomycelium and true mycelium were examined under microscope at a few day's intervals until 2 weeks.

3.2.4 Morphology of ascospores

The active young culture grown on YM agar was streaked on YM agar, Acetate-GSH agar (Appendix A) and 5% malt extract agar (Appendix A). Mode of ascus formation and shape, surface structure, number per ascus and size of ascospores were examined under microscope after incubation for 7, 14, 21 and 28 days at 25°C.

3.2.5 Morphological characteristics of vegetative cells and ascospores under scanning electron microscope

Yeasts were grown in 1.5 ml YM broth for at 25°C 48 hr. The 0.5 ml of 1-2% glutaraldehide in 1/15 M phosphate buffer pH 7.0 was added to yeast culture. Yeast cells were harvested by centrifugation. Yeast cells were fixed by 0.5 ml 1-2% glutaraldehide in 1/15 M phosphate buffer pH 7.0 at room temperature for 1 hr 30 min, then centrifuged and washed with phosphate buffer for 3 times. The 0.5 ml of 2% osmium was added and left at the room temperature for 1-2 hr, then washed with phosphate buffer for 3 times. Fixed cells were washed stepwise with 0.5 ml of

increase alcohol concentration from 30%, 50%, 70%, 80%, 90%, 95% for 30 min and 100% for 30 min for 2 times. Fixed cells were dried with 0.5 ml of alcohol:acetone (1:1) for 30 min. After centifugation, fixed cells were dried twice with acetone for 30 min then centrifuged and 0.5 ml of isoamyl acetate:acetone (1:1) was added and left for 30 min, then centrifuged. Isoamyl acetate was added and left for 1 hr, then centrifuged. This step was repeated. Cells were moved to critical point dryer then coat by platinum. Cells were observed by scanning electron microscope.

3.3 Physiological and biochemical characteristics

3.3.1 Assimilation of carbon compounds

Assimilation of carbon compounds was investigated in liquid media according to the method described by van der Walt and Yarrow (1984) and Yarrow (1998). In the present study 42 carbon compounds were employed in the description of each species (Table 8). Ten folds medium stock solution (Appendix A) was prepared by filter sterilization and stored in a freezer at -20°C until use. Amount of 0.2 ml of thawed stock solution was added to 1.8 ml of sterilized water in a cotton plugged test tube (13x100 mm). Media for inulin, soluble starch, ethanol, galactitol, 2-ketogluconic acid and 5-ketogluconic acid were prepared at every experiment. In the case of ethanol and 5-ketogluconic acid, 3% and 0.3% solution were employed, respectively. The young culture grown on YM agar was used as the inoculums. A very light suspension in Yeast Nitrogen Base (YNB) medium was prepared and incubated at 25°C for 1 week to exhaust the carbohydrate pool in the cells.

A drop of inoculums was inoculated to the carbon assimilation test medium by sterilized Pasteur pipette and incubated at 25°C for 4 weeks. Basal medium without carbon source was employed as a negative control. The growth was observed and recorded every week up to 4 weeks. After dispersed the cells by shaking, the degree of growth was assessed by eye by placing tubes against a white card on which lines of 0.75 mm thick were drown 5 mm apart with Indian Ink. The result was scored as +++ if the lines are completely obscured; as ++ if the lines appears as diffuse bands; as + if the lines are distinguishable as such but have blurred edges; as – if the lines were distinct and sharp edged.

The results are presented in the descriptions as follows:

+, positive, either a ++ or +++ reading after 1 week, or 2 weeks

l, delayed positive (latent), a ++ or +++ reading develops rapidly, but after 2 weeks or longer

s, slow positive, a ++ or +++ reading develops slowly over a period exceeding 2 weeks

w, weakly positive, a + reading

-, negative

v, variable: some strains are positive, others negative

+/w, positive or weak: all strains grow, but some of them grow slowly

w/-, weak or negative

Table 8 Carbon compounds used in the description of each species

Group	Carbon compounds
Hexoses	Glucose, galactose and sorbose
Disaccharides	Cellobiose, lactose, maltose, melibiose, sucrose and trehalose
Trisaccharides	Melizitose and raffinose
Polysaccharides	Inulin and soluble starch
Pentose	D-arabinose, L-arabinose, D-ribose, L-rhamnose and D-xylose
Alcohols	Galactitol, meso-erythritol, D-glucitol, glycerol, myo-inositol, D-mannitol, ribitol, ethanol and methanol
Organic acids	Citric acid, DL-lactic acid, succinic acid and D-gluconic acid
Glycosides	α-methyl-D-glucoside and salicin
Other compounds	D-glucosamine hydrochloride, *N*-acetyl-D-glucosamine and hexadecane
Additional compounds	2-keto-D-gluconate, 5-keto-D-gluconate, saccharate, xylitol, D-glucuronate and L-arabinitol

3.3.2 Assimialtion of nitrogen compounds

Assimilation of nitrogen compounds was investigated on solid media using starved inoculum according to the method of Nakase and Suzuki (1986a). Six kinds of nitrogen compounds including amonium sulfate ($(NH_4)_2SO_4$), potassium nitrate (KNO_3), sodium nitrite ($NaNO_2$), ethylamine, L-lysine and cadaverine were employed. The young culture grown on YM agar was used as the inoculums. A very light suspension in YCB medium (Appendix A) was prepared and incubated at 25°C for 1 week to exhaust the carbohydrate pool in the cell. A half-drop of starved culture was inoculated on nitrogen agar plate with sterilized Pasteur pipette and incubated at 25°C. The YCB agar without nitrogen source was used as a negative control. The growth of yeast was observed every 2-4 days up to 14 days.

3.3.3 Production of starch-like substances

The production of starch-like substance was determined according to the mothod described by Wicherham (1951). After assimilation test was finished, Lugol's solution (Appendix A) was added to glucose assimilation medium and anmonium sulfate assimilation medium. A positive result was indicated by the development in the culture of a color varying from dark blue to green.

3.3.4 Fermentation of sugar

Fermentation test was examined according to the method described by Wickerham (1951). A loop full of young culture grown on YM agar was inoculated into fermentation medium (Appendix A) and incubated at 25°C. Carbondioxide gas production was observed and recorded every day until 7 days and then at 14, 21 and 28 days of cultivation.

3.3.5 Vitamin requirements

Vitamin requirements were determined according to Komagata and Nakase (1967). Young culture grown on YM agar for 2-4 days was suspended in the basal medium (Appendix A) and incubated for 5-7 days at 25°C to deplete the vitamins carried from the pre-culture medium and vitamin pool of cells. A drop of

starved cell suspension was inoculated into the test medium then incubated at 25°C for 3 weeks. The growth of yeast was observed at 3, 5, 7, 10, 14 and 21 days.

3.3.6 Miscellaneous tests

a. Gelatin liquefaction

The ability to liquefy gelatin was examined according to the method described by Wickerham (1951). The yeast was inoculated by a needle into the gelatin medium (Appendix A) tube and incubated at 25°C. The cultures were examined regularly up to 3 weeks for signs of liquefaction.

b. Lipase test

Lipase activity was determined according to Eijkman (1901). Yeast cells was inoculated by streaking on the lipase test medium (Appendix A) and incubated at 25°C for 1 week. The result was recorded as positive if the strain produced an opaque zone.

c. Acid formation from glucose

The production of acid from glucose was determined according to van der Walt (1970). The yeast was streaked onto slants or plate of Custer's chalk medium (Appendix A) and incubated at 25°C for 2 weeks for clearing of the medium around the streaks. The result was recorded as positive if the chalk dissolved.

d. Cycloheximide resistant

Cycloheximide resistant was determined according to Whiffen (1948). This experiment was examined in liquid Bacto Yeast Nitrogen Base with D-glucose (basal medium), with cycloheximide added to give a final concentration of either 100 ppm or 1000 ppm. The inoculation and detection was done in the same way as for the carbon assimilation test.

e. Growth at 50% glucose medium

The ability to at high sugar concentration was examined according to the method described by Wickerham (1951). Young culture of yeasts was inoculated on a slant of 50% glucose agar medium (Appendix A). The growth of yeast was observed after 7 days.

f. Maximum growth temperature

The maximum growth temperature was examined according to the method described by Wickerham (1951). Young culture of yeasts was inoculated into YM broth medium. The growth of yeast was observed every week until 3 weeks. The result was detected as same as the assimilation of carbon compounds.

3.4 Chemotaxonomic Characteristics

3.4.1 Ubiquinone analysis

The analysis of biquinone system was carried out following the method of Nakase and Suzuki, 1986b). Cells grown in YPD medium (1% yeast extract, 2% peptone and 2% glucose) at 25°C for 16-30 hr with shaking were harvested by centrifugation at 6,000 rpm for 5 min. Harvested cells from 400 ml of medium were washed with distilled water and freeze-dried. Dried cells are suspended in 50 ml chloroform/methanol (2:1) and kept at room temperature for one night. Cells were removed by paper filtration. Filtrate was evaporated to dryness using a rotary evaporator and then residues were dissolved with 0.5 ml of acetone. Ubiquinone was purified by using the preparative thin-layer chromatography (0.5 mm silica gel, 60F254 layers on 20 x 20 cm glass plate, Merck, with hexane:diethyl ether (85:15) as developer). A band of ubiquinone detected under short wave UV light was scrapped off. Yeast having respective ubiquinones, Q-6 (*Saccharomyces cerevisiae,* NBRC 10515), Q-7 (*Pichia anomala* NBRC 10213), Q-8 (*Saccharomycopsis vini* NBRC 1749), Q-9 (*Debaryomyces hansenii* var. *hansenii* NBRC 1751) and Q-10 (*Schizosaccharomyces pombe* NBRC 1608*)* were used as references. Scrapped powder was transferred to a tube and extracted with 1 ml of acetone. The solution was filtered with a 0.2 μm membrane filter and concentrated by the blow of N_2 gas.

Ubiquinone homologues were identified by HPLC (Cosmosil column (Waters, 5C18, 4.6 mm x 250 mm), using methanol:isopropyl alcohol (2:1) as mobile phase at flow rate of 1 ml/min, detected at 275 nm, and identified by comparing with known ubiquinones as standards).

3.4.2 Characterization of cell wall polysaccharides

Yeasts were grown on YM agar plates supplemented with 2% glucose for 3 days at 25°C and harvested with by centrifugation after filtration of cell suspensions by defatted cotton cloth, then was washed twice with purified water. Mannose-containing polysaccharides were extracted and purified according to the methods of Gorin and Spencer (1970). The polysaccharides were extracted by boiling of ca. 5 g of packed cells with 25 ml of 2% potassium hydroxide solution for 2 hr. After cooling the suspension was neutralized with acetic acid to pH 6-7 and centrifuged at 15,000 rpm for 7 min. The supernatant was evaporated to approximately 10 ml, then 200 ml of methanol was added and mixed and placed overnight at 5°C. After centrifugation at 15,000 rpm for 7 min, the precipitate was washed twice with 40 ml of methanol and 3 times with 40 ml of acetone, then air-dried at room temperature. Fine powder of crude polysaccharide (0.2 g) was dissolved with 10 ml of water and boiled for 2 hr. The insoluble materials were removed by centrifugation at 15,000 rpm for 7 min, then 10 ml of Fehling solution was added to the supernatant and mixed, then left overnight at 5°C. The copper complex was washed twice with 20 ml of 2% potassium hydroxide and 3 times with 20 ml of methanol. The copper complex was decomposed by shaking in purified water with Amberite IR 120. The resins were washed twice with small amount purified water. The polysaccharide solution was evaporated to approximately 2 ml, then 20 ml of methanol and one drop of conc. hydrochloric acid were added. After centrifugation, the precipitated was washed 3 times with 2 ml of methanol and dried by washing 3 times with 2 ml of acetone.

The purified polysaccharide was hydrolyzed with 1N H_2SO_4 (10 mg/ml) at 100°C for 18 hr. The hydrolyzate was neutralized by adding barium hydroxide ($Ba(OH)_2$) to pH 6-7 and centrifuged at 15,000 rpm for 7 min. The supernatant was applied to TLC plate with ethyl acetate:isopropanol:water, 65:22:11 (v/v), as developer. A band of polysaccharide was detected by spraying with *p*-anisidine solution (Appendix A). D-glucose, D-mannose and D-galactose were used as standard sugar. The proton NMR spectra of purified polysaccharides were determined in D_2O at 70°C with tetramethylsilane as external standard by JEOL 500 MHz NMR spectrometer.

RESULTS AND DISCUSSION

1. Isolation of Yeasts from Natural Habitats

Two hundred and eighty-three strains of yeasts were isolated from insect frass (144 strains), flowers (24 strains), leaves (54 strains), mosses (27 strains), mushrooms (28 strains), and some other habitats such as coconut juice (1 strain), exudates (1 strain), fruit (1 strain), lichens (1 strain), and rotted wood (2 strains) as detailed in Table 9. These samples were collected from various places in the natural habitats of Thailand (Table 10). Places of sample collections are shown in Appendix B Table B1. The yeast strains were purified by conventional streaking technique and maintained at -80°C at BIOTEC Culture Collection.

Table 9 Number of yeasts isolated from various habitats.

Year	Flowers	Insect frass	Leaves	Mosses	Mushrooms	Others	Total
2000	12	26	0	6	9	0	53
2001	2	104	54	4	18	0	182
2002	7	6	0	8	0	1	22
2003	3	8	0	9	1	5	26
Total	**24**	**144**	**54**	**27**	**28**	**6**	**283**

Table 10 Number of yeasts isolated from four regions of Thailand.

Year	Regions				
	Northern	Eastern	Central	Southern	Total
2000	0	27	26	0	**53**
2001	0	114	3	65	**182**
2002	1	11	4	6	**22**
2003	0	0	20	6	**26**
Total	**1**	**152**	**53**	**77**	**283**

2. Diversity of Yeasts Estimated Based on Sequences of D1/D2 Domains of 26S rDNA

Tentative identification was carried out based on the sequences of D1/D2 (ca. 600 nucleotides) domain of 26S rDNA according to a guideline of Kurtzman and Robnett (1998), 0-1 nucleotide differences are conspecific species, 2-3 nucleotide differences are conspecific or sister species and 4 or more nucleotide differences are different species. Undescribed species, whose D1/D2 sequences were registerd at DNA databanks, were regarded as known species. Yeast diversity of Thai natural habitats was discussed based on this tentative identification. The detailed taxonomic studies were carried out on selective groups in which new species were presumed by D1/D2 sequences. They were studied from the view point of polyphasic taxonomy; morphology, physiology, biochemistry, chemotaxonomy and molecular taxonomy.

Among 283 strains of yeasts isolated from flowers (24 strains), insect frass (144 strains), leaves (54 strains), mosses (27 strains), mushrooms (28 strains) and others (6 strains) tentatively identified, 194 strains belong to ascomycetous yeasts and 89 strains belong to basidiomycetous yeasts. Based on the sequence analysis of D1/D2 domain of 26S rDNA, 139 strains (49%) differed in 0-1 nucleotides from known species and were assigned to 56 known species of 26 genera including 39 species (97 strains) of 17 genera of ascomycetous yeasts and 17 species (42 strains) of 9 genera of basidiomycetous yeasts (Table 11). Details of identification are shown in Table 12. *Candida tropicalis* (9 strains) is the dominant species of ascomycetous yeasts and followed by *Saccharomyces kluyveri* (8 strains), *Saccharomyces cerevisiae* (5 strains), *Hanseniaspora opuntiae* (5 strains), *Metschnikowia koreensis* (5 strains) and *Debaryomyces nepalensis* (4 strains). In the case of basidiomycetous yeasts, *Cryptococcus heveanensis* (8 strains) is the dominant species and followed by *Exobasidium vexans* (4 strains), *Sporobolomyces* sp. (4 strains), and *Trichosporon asahii* (4 strains).

Figure 3 Places where new species were found.

- Places where sample were collected.
- Places where new species were found.

Table 11 Known species of ascomycetous and basidiomycetous yeasts isolated from some natural habitats of Thailand.

Species	No. of Strains	Species	No. of Strains
Ascomycetous yeasts			
Ambrosiozyma monospora	2	*Saccharomyces cerevisiae*	5
Aureobasidium pullulans	2	*S. kluyveri*	8
Blastobotrys catitulata	1	*S. unisporus*	2
Candida diversa	3	*Stephanoascus smithiae*	3
C. fukuyamaensis	3	*Torulaspora delbrueckii*	1
C. gotoi	2	*Torulaspora* sp. IFO 11061	1
C. leandrae	1	*Wililopsis saturnus* var. *mrakii*	1
C. natalensis	1	*W. saturnus* var. *subsufficiens*	1
C. palmae	1	*Zygosaccharomyces* sp. IFO 11070	1
C. parapsilosis	3	**Total 17 genera, 39 species, 2 varities**	**97**
C. rancensis	1		
C. sithepensis	1	**Basidiomycetous yeasts**	
C. tropicalis	9	*Bullera dendrophila*	1
Candida sp. NRRL Y-17456	4	*Bullera sinensis*	2
Candida sp. UWO(PS)00-147.3	1	*Cryptococcus heveanensis*	8
Debaryomyces nepalensis	4	*C. humicola*	2
D. polymorphus	3	*C. laurentii*	3
D. polymorphus var. *africanus*	1	*Cryptococcus* sp. CBS 8372	1
D. vanrijiae var. *yarrowii*	3	*Exobasidium vexans*	4
Debaryomyces sp. NRRL-7804	1	*Rhodosporidium paludigenum*	1
Geotrichum fragrans	2	*R. toruloides*	1
Hanseniaspora opuntiae	5	*Rhodotorula nothofagi*	1
H. vineae	1	*Sporidiobolus ruineniae*	4
Hanseniaspora sp. CBS 8772	1	*Sporobolomyces bannaensis*	1
Kloeckera lindneri	1	*S. odoratus*	3
Kluyveromyces lactis	2	*S. poonsookiae*	1
Kodamaea (Pichia) ohmeri	3	*Sporobolomyces* sp. TY-241 / TY-253	4
Metschnikowia koreensis	5	*Tilletiopsis* sp. TY-2352	1
Pichia nakazawae var. *akitaensis*	2	*Trichosporon asahii*	4
P. stipitis	2	**Total 9 genera, 17 species**	**42**
P. sydowiorum	2		
Pichia sp. UWO(PS)99-305.1	1		

Table 12 List of known species found in this study.

Strain	Nearest Species with Accession Number of DNA DataBank	Nucleotide Identity	No. of Nucleotide Difference
	Ascomycetous yeasts		
ST-232	*Ambrosiozyma monospora* U40106	566/566 (100)	0
ST-241	*Ambrosiozyma monospora* U40106	565/565 (100)	0
ST-21	*Aureobasidium pullulans* AF050239	571/571 (100)	0
ST-74	*Aureobasidium pullulans* AF050239	571/571 (100)	0
ST-62	*Blastobotrys capitulata* U40104	574/575 (99.8)	1
ST-35	*Candida diversa* U71064	533/534 (99.8)	1
ST-85	*Candida diversa* U71064	533/533 (100)	0
ST-568	*Candida diversa* U71064	532/533 (99.8)	1
ST-64	*Candida fukuyamaensis* U62311	570/570 (100)	0
ST-80	*Candida fukuyamaensis* U62311	570/570 (100)	0
ST-419	*Candida fukuyamaensis* U62311	556/556 (100)	0
ST-24	*Candida gotoi* AY489112	528/529 (99.8)	1
ST-252	*Candida gotoi* AY489112	527/528 (99.8)	1
ST-27	*Candida leandrae* AY449659	499/500 (99.8)	1
ST-362	*Candida natalensis* U45818	566/566 (100)	0
ST-267	*Candida palmae* AY197725	472/473 (99.8)	1
ST-20	*Candida parapsilosis* U45754	570/570 (100)	0
ST-125	*Candida parapsilosis* AJ508576	569/570 (99.8)	1

Table 12 (Continued)

Strain	Nearest Species with Accession Number of DNA DataBank	Nucleotide Identity	No. of Nucleotide Difference
ST-364	*Candida parapsilosis* AF374609	564/564 (100)	0
ST-40	*Candida rancensis* AJ508580	508/509 (99.8)	1
ST-194	*Candida sithepensis* AB120220	566/567 (99.8)	1
ST-136	*Candida* sp. NRRL Y-17456 U45775	569/570 (99.8)	1
ST-161	*Candida* sp. NRRL Y-17456 U45775	568/569 (99.8)	1
ST-403	*Candida* sp. NRRL Y-17456 U45775	555/556 (99.8)	1
ST-368	*Candida* sp. NRRL Y-17456 U45775	528/529 (99.8)	1
ST-15	*Candida* sp. UWO(PS)00-147.3 AF530612	586/586 (100)	0
ST-48	*Candida tropicalis* U45749	564/564 (100)	0
ST-55	*Candida tropicalis* U45749	570/570 (100)	0
ST-61	*Candida tropicalis* U45749	570/570 (100)	0
ST-68	*Candida tropicalis* U45749	570/570 (100)	0
ST-83	*Candida tropicalis* U45749	570/570 (100)	0
ST-165	*Candida tropicalis* U45749	570/570 (100)	0
ST-251	*Candida tropicalis* U45749	570/570 (100)	0
ST-298	*Candida tropicalis* U45749	566/566 (100)	0
ST-308	*Candida tropicalis* U45749	564/564 (100)	0
ST-117	*Debaryomyces polymorphus* U45836	569/570 (99.8)	1
ST-120	*Debaryomyces polymorphus* U45836	569/570 (99.8)	1

Table 12 (Continued)

Strain	Nearest Species with Accession Number of DNA DataBank	Nucleotide Identity	No. of Nucleotide Difference
ST-404	*Debaryomyces polymorphus* U45836	569/570 (99.8)	1
ST-126	*Debaryomyces polymorphus* var. *africanus* AB054994	511/512 (99.8)	1
ST-124	*Debaryomyces nepalensis* U45839	570/570 (100)	0
ST-327	*Debaryomyces nepalensis* U45839	564/564 (100)	0
ST-332	*Debaryomyces nepalensis* U45839	564/564 (100)	0
ST-336	*Debaryomyces nepalensis* U45839	570/570 (100)	0
ST-313	*Debaryomyces vanrijiae* var. *yarrowii* U45843	569/570 (99.8)	1
ST-319	*Debaryomyces vanrijiae* var. *yarrowii* U45843	569/570 (99.8)	1
ST-321	*Debaryomyces vanrijiae* var. *yarrowii* U45843	569/570 (99.8)	1
ST-46	*Debaryomyces* sp. NRRL Y-7804 U45771	540/540 (100)	0
ST-53	*Geotrichum fragrans* U40119	403/404 (99.8)	1
ST-54	*Geotrichum fragrans* U40119	403/404 (99.8)	1
ST-5	*Hanseniaspora opuntiae* AY267820	571/572 (99.8)	1
ST-8	*Hanseniaspora opuntiae* AY267820	561/562 (99.6)	1
ST-9	*Hanseniaspora opuntiae* AY267820	559/560 (99.8)	1
ST-448	*Hanseniaspora opuntiae* AY267820	572/572 (99.5)	0
ST-481	*Hanseniaspora opuntiae* AY267820	571/572 (99.8)	1
ST-515	*Hanseniaspora vineae* U84224	518/519 (99.8)	1
ST-398	*Hanseniaspora* sp. CBS 8772 AJ512455	570/570 (100)	0

Table 12 (Continued)

Strain	Nearest Species with Accession Number of DNA DataBank	Nucleotide Identity	No. of Nucleotide Difference
ST-484	*Kloeckera lindneri* U84226	571/572 (99.8)	1
ST-214	*Kluyveromyces lactis* U94922	538/538 (100)	0
ST-340	*Kluyveromyces lactis* U94922	545/545 (100)	0
ST-13	*Kodamaea (Pichia) ohmeri* AF335976	493/493 (100)	0
ST-326	*Kodamaea (Pichia) ohmeri* AY267821	492/492 (100)	0
ST-356	*Kodamaea (Pichia) ohmeri* AF335976	509/509 (100)	0
ST-28	*Metschnikowia koreensis* AF296438	495/495 (100)	0
ST-258	*Metschnikowia koreensis* AF296438	517/517 (100)	0
ST-265	*Metschnikowia koreensis* AF296438	516/517 (99.8)	1
ST-389	*Metschnikowia koreensis* AF296438	516/517 (99.8)	1
ST-410	*Metschnikowia koreensis* AF296438	493/493 (100)	0
ST-361	*Pichia nakazawae* var. *akitaensis* U45766	529/530 (99.8)	1
ST-363	*Pichia nakazawae* var. *akitaensis* U45766	529/530 (99.8)	1
ST-312	*Pichia stipitis* U45741	567/568 (99.8)	1
ST-317	*Pichia stipitis* U45741	567/568 (99.8)	1
ST-41	*Pichia sydowiorum* AJ508573	569/570 (99.8)	1
ST-247	*Pichia sydowiorum* AJ508573	536/536 (100)	0
ST-23	*Pichia* sp. UWO(PS)99-305.1 AF322059	557/557 (100)	0
ST-541	*Saccharomyces cerevisiae* U53879	547/547 (100)	0

Table 12 (Continued)

Strain	Nearest Species with Accession Number of DNA DataBank	Nucleotide Identity	No. of Nucleotide Difference
ST-543	*Saccharomyces cerevisiae* U53879	573/573 (100)	0
ST-555	*Saccharomyces cerevisiae* U53879	557/557 (100)	0
ST-570	*Saccharomyces cerevisiae* U53879	581/581 (100)	0
ST-586	*Saccharomyces cerevisiae* U53879	539/539 (100)	0
ST-453	*Saccharomyces kluyveri* U68552	566/567 (99.8)	1
ST-457	*Saccharomyces kluyveri* U68552	536/537 (99.8)	1
ST-516	*Saccharomyces kluyveri* U68552	570/571 (99.8)	1
ST-529	*Saccharomyces kluyveri* U68552	570/571 (99.8)	1
ST-551	*Saccharomyces kluyveri* U68552	570/571 (99.8)	1
ST-552	*Saccharomyces kluyveri* U68552	570/571 (99.8)	1
ST-554	*Saccharomyces kluyveri* U68552	570/571 (99.8)	1
ST-578	*Saccharomyces kluyveri* U68552	570/571 (99.8)	1
ST-82	*Saccharomyces unisporus* AB087392	565/565 (100)	0
ST-495	*Saccharomyces unisporus* AB087392	570/570 (100)	0
ST-34	*Stephanoascus smithiae* U76531	566/566 (100)	0
ST-231	*Stephanoascus smithiae* U76531	566/566 (100)	0
ST-303	*Stephanoascus smithiae* U76531	566/566 (100)	0
ST-375	*Torulaspora delbrueckii* U72156	572/573 (99.8)	1
ST-266	*Torulaspora* sp. IFO 11061 AB087935	572/572 (100)	0

Table 12 (Continued)

Strain	Nearest Species with Accession Number of DNA DataBank	Nucleotide Identity	No. of Nucleotide Difference
ST-31	*Williopsis saturnus* var. *markii* U94929	574/574 (100)	0
ST-316	*Williopsis saturnus* var. *markii* U94929	573/574 (99.8)	1
ST-583	*Zygosaccharomyces* sp. IFO 11070 AB087401	580/581 (99.8)	1
	Basidiomycetous yeasts		
ST-75	*Bullera dendrophila* AF189870	599/600 (99.8)	1 (1)
ST-150	*Bullera sinensis* AF189884	595/595 (100)	0
ST-176	*Bullera sinensis* AF189884	595/595 (100)	0
ST-69	*Cryptococcus heveanensis* AF075467	598/599 (99.8)	1
ST-77	*Cryptococcus heveanensis* AF075467	598/599 (99.8)	1
ST-146	*Cryptococcus heveanensis* AF075467	598/599 (99.8)	1
ST-148	*Cryptococcus heveanensis* AF075467	593/594 (100)	1
ST-160	*Cryptococcus heveanensis* AF075467	598/599 (99.8)	1
ST-162	*Cryptococcus heveanensis* AF075467	598/599 (99.8)	1
ST-210	*Cryptococcus heveanensis* AF075467	598/599 (99.8)	1
ST-223	*Cryptococcus heveanensis* AF075467	598/599 (99.8)	1
ST-1	*Cryptococcus humicola* AF189836	593/594 (99.8)	1
ST-7	*Cryptococcus humicola* AF189836	591/591 (100)	0
ST-147	*Cryptococcus laurentii* AF459662	592/592 (100)	0
ST-149	*Cryptococcus laurentii* AF459662	592/592 (100)	0

Table 12 (Continued)

Strain	Nearest Species with Accession Number of DNA DataBank	Nucleotide Identity	No. of Nucleotide Difference
ST-305	*Cryptococcus laurentii* AF459662	597/597 (100)	0
ST-322	*Cryptococcus* sp. CBS 8372 AF444707	596/596 (100)	0
ST-140	*Exobasidium vexans* AJ235288	580/580 (100)	0
ST-166	*Exobasidium vexans* AJ235288	568/568 (100)	0
ST-182	*Exobasidium vexans* AJ235288	552/552 (100)	0
ST-217	*Exobasidium vexans* AJ235288	581/582 (99.8)	1
ST-296	*Rhodosporidium paludigenum* AF363640	575/575 (100)	0
ST-209	*Rhodosporidium toruloides* AF444764	568/568 (100)	0
ST-70	*Rhodotorula nothofagi* AF444736	588/588 (100)	0
ST-11	*Sporidiobolus ruineniae* var. *ruineniae* AF387128	574/574 (100)	0
ST-12	*Sporidiobolus ruineniae* var. *ruineniae* AF387129	573/574 (99.8)	1
ST-307	*Sporidiobolus ruineniae* AF387129	554/555 (99.8)	1
ST-185	*Sporobolomyces bannaensis* AY274823	582/582 (100)	0
ST-174	*Sporobolomyces odoratus* AF387125	568/569 (99.8)	1
ST-204	*Sporobolomyces odoratus* AF387125	566/567 (99.8)	1
ST-208	*Sporobolomyces odoratus* AF387125	570/571 (99.8)	1
ST-344	*Sporobolomyces poonsookiae* AF387124	569/570 (99.8)	1
ST-107	*Sporobolomyces* sp. TY-241 AY313053	573/574 (99.8)	1
ST-93	*Sporobolomyces* sp. TY-257 AY313054	568/568 (100)	0
ST-132	*Sporobolomyces* sp. TY-257 AY313054	568/568 (100)	0

Table 12 (Continued)

Strain	Nearest Species with Accession Number of DNA DataBank	Nucleotide Identity	No. of Nucleotide Difference
ST-187	*Sporobolomyces* sp. TY-257 AY313054	574/574 (100)	0
ST-101	*Telletiopsis* sp. TY 235 AY313021	562/563 (99.8)	1
ST-72	*Trichosporon asahii* AF105393	597/597 (100)	0
ST-63	*Trichosporon asahii* AF105393	519/519 (100)	0
ST-81	*Trichosporon asahii* AF105393	592/592 (100)	0
ST-407	*Trichosporon asahii* AF105393	612/612 (100)	0

One hundred and sixteen strains (41%) demonstrated 4 or more nucleotide substitutions from respective nearest species in the D1/D2 domain of 26S rDNA sequences (Table 13). These strains are considered to represent new species as discussed by Kurtzman and Robnett (1998). They were classified into 101 species belonged to 76 species (87 strains) of ascomycetous yeasts and 25 species (29 strains) of basidiomycetous yeasts. The frequencies of isolateion of new species are very high in respective habitats collected in various places in Thailand; flowers (11 strains, 46%), insect frass (56 strains, 41%), leaves (26 strains, 48%), mosses (6 strains, 26%), and mushrooms (14 strains, 50%). The places where new species were found are shown in Fig. 3. The remaining 28 strains (10%) out of 283 strains may be known or new species because 2-3 nucleotides were different from respective nearest species (Table 14). As mentioned above, these species are conspecific or sister species from each other. DNA-DNA reassociation experiment is required to identify these strains.

In the present study, 283 strains isolated from various kinds of natural habitat collected in Thailand were identified as 56 known species of 26 genera, 101 species of hitherto undescribed species and 28 unidentified species, based on the D1/D2 sequences. Namely, 41.0% of isolates belong to undescribed species. This frequency is much higher than that of fermented foods and related substrates, which were reported by Saito *et al*. (1983), Suzuki *et al*. (1987), Suzuki *et al*. (1994), Jindamorakot (2000) and ballistoconidium-forming yeasts from Bangkok and southern seacoast from Bangkok to Pattaya (Nakase *et al*., 2001) and resembles the case of ballistoconidium-forming yeasts from Sakaerat (Fungsin, 2003). It is considered that yeasts in the natural habitats of Thailand, especially in protected forests, are very rich in biodivesity, not only in ballistoconidium-forming yeasts previous reported but also in ascomycetous yeasts and basidiomycetous yeasts other than ballistoconidium-forming yeasts.

Table 13 List of new species found in this study.

Strain	GenBank Accession No.	Nearest Species with Accession No. of DNA DataBank	Nucleotide Identity (%)	No. of Nucleotide Different (No. of Gap)	Remark
		Ascomycetous yeast			
ST-370	DQ404513	*Candida ambrosiae* AY013716	530/559 (94.8)	29 (1)	*C. ambrosiae* ≠ *C. tanzawaensis*
		Candida tanzawaensis U44811	530/559 (94.8)	29 (1)	(Eight nucleotides are different between 2 species)
ST-249	DQ404488	*Candida asparagi* AY450920	453/532 (85.2)	79 (22)	-
ST-19	DQ400365	*Candida azyma* U62312	549/553 (99.3)	4	-
ST-239	DQ404486	*Candida berthetii* U62298	567/574 (98.9)	7 (4)	-
ST-29	DQ400367	*Candida* cf.. *etchellsii* AY257048	451/482 (93.6)	31 (1)	-
ST-449	DQ404525	*Candida* cf.. *glabrata* UWO(PS)98-110.4 AF313362	581/594 (97.8)	13 (1)	-
ST-33	AY845350	*Candida coipomoensis* U45747	553/562 (98.4)	9	-
ST-22	AY228492	*Candida etchellsii* UWO(PS)00-226.2 AF313353	472/501 (94.2)	29 (1)	-
ST-60	DQ400376	*Candida fermentati* AY187283	553/557 (99.3)	4 (1)	-
ST-253	DQ404490	*Candida fermentati* AY187283	552/556 (99.3)	4 (4)	-
ST-377	DQ404514	*Candida floricola* U45710	481/486 (98%)	5 (3)	-
ST-390	DQ404518	*Candida floricola* U45710	481/485 (99.2)	4 (2)	-
ST-331	DQ404502	*Candida friedrichii* U45781	534/548 (97.4)	14 (1)	-
ST-43	DQ400371	*Candida friedrichii* U45781	504/547 (92.1)	43 (12)	-

Table 13 (Continued)

Strain	GenBank Accession No.	Nearest Species with Accession No. of DNA DataBank	Nucleotide Identity (%)	No. of Nucleotide Different (No. of Gap)	Remark
ST-300	DQ404493	*Candida friedrichii* U45781	541/547 (98.9)	6	ST-300 = ST-365 = ST-366
ST-365	DQ404511	*Candida friedrichii* U45781	542/548 (98.9)	6	
ST-366	DQ404512	*Candida friedrichii* U45781	541/547 (98.9)	6	
ST-328	DQ404499	*Candida friedrichii* U45781	539/547 (98.5)	8	ST-328 = ST-329 = ST-333
ST-329	DQ404500	*Candida friedrichii* U45781	539/547 (98.5)	8	
ST-333	DQ404503	*Candida friedrichii* U45781	539/547 (98.5)	8	
ST-451	DQ404526	*Candida musae* U44814	496/534 (92.9)	38 (11)	-
ST-238	DQ404485	*Candida insectalens* U62304	331/338 (97.9)	7 (1)	-
ST-211	DQ404478	*Candida intermedia* AJ508588	451/516 (80.4)	65 (8)	-
ST-246	DQ404487	*Candida llanquihuensis* U70190	534/554 (96.4)	20	-
ST-164	DQ404469	*Candida membranifaciens* U45792	529/542 (97.6)	13	-
ST-79	DQ400380	*Candida mokoenaii* AY559042	570/574 (99.3)	4	-
ST-337	DQ404506	*Candida musae* U44814	453/532 (85.2)	79 (22)	-
ST-380	DQ404515	*Candida natalensis* U45818	563/567 (99.3)	4 (1)	-
ST-95	DQ404454	*Candida palmae* AY197725	414/488 (84.8)	74 (9)	-
ST-18	DQ400364	*Candida parapsilosis* U45754	531/571 (93.0)	40 (3)	-
ST-234	DQ404482	*Candida pseudointermedia* AF533069	514/518 (99.2)	4 (1)	-
ST-387	DQ404516	*Candida rancensis* AJ508580	481/510 (94.3)	29 (1)	-

Table 13 (Continued)

Strain	GenBank Accession No.	Nearest Species with Accession No. of DNA DataBank	Nucleotide Identity (%)	No. of Nucleotide Different (No. of Gap)	Remark
ST-330	DQ404501	*Candida savonica* U62307	451/478 (94.4)	27	-
ST-224	DQ404479	*Candida silvanorum* U71068	439/522 (84.1)	83 (11)	-
ST-490	DQ404529	*Candida sorbosivorans* AY008842	489/502 (97.4)	13	-
ST-533	DQ404531	*Candida sorbosivorans* AY008842	451/462 (97.6)	11	ST-533 = ST-536
ST-536	DQ404532	*Candida sorbosivorans* AY008842	436/447 (97.5)	11	
ST-78	DQ400379	*Candida tammaniensis* AF017243	519/543 (95.6)	21 (3)	-
ST-358	DQ404510	*Candida trypodendroni* AF017240	525/532 (98.7)	7 (1)	-
ST-388	DQ404517	*Candida tammaniensis* AF017243	476/515 (92.4)	39 (4)	-
ST-17	AY228491	*Candida tsuchiyae* U49064	448/514 (87.2)	66 (13)	-
ST-49	DQ400372	*Candida tsuchiyae* U49064	400/515 (77.7)	105 (8)	-
ST-50	DQ400373	*Candida tsuchiyae* U49064	403/513 (78.6)	110 (22)	-
ST-431	DQ404522	*Candida bolitotheri* AY309867	534/556 (96.0)	22 (1)	-
ST-297	DQ404492	*Candida* sp. BG99-8-18-1-6 AY242348	502/567 (88.5)	65 (9)	-
ST-315	DQ404498	*Candida* sp. BG99-8-18-1-6 AY242348	517/552 (93.7)	35 (3)	-
ST-32	DQ400369	*Candida* sp. NRRL Y-17678 AF017237	553/562 (98.4)	11 (3)	-
ST-309	DQ404495	*Debaryomyces vanrijiae* var. *yarrowii* U45843	566/570 (99.3)	4	ST-309 = ST-310
ST-310	DQ404496	*Debaryomyces vanrijiae* var. *yarrowii* U45843	566/570 (99.3)	4	
ST-338	DQ404507	*Dipodascus ovetensis* U40116	399/405 (98.5)	6	-

Table 13 (Continued)

Strain	GenBank Accession No.	Nearest Species with Accession No. of DNA DataBank	Nucleotide Identity (%)	No. of Nucleotide Different (No. of Gap)	Remark
ST-2	DQ400361	*Endomyces scopularum* AF267231	448/495 (90.5)	47 (1)	-
ST-36	DQ400370	*Galactomyces geotrichum* U40118	537/548 (98.0)	11 (2)	-
ST-391	DQ404519	*Hanseniaspora meyeri* AJ 512461	545/569 (94.1)	24 (2)	-
ST-493	DQ404530	*Hanseniaspora meyeri* AJ 512461	553/577 (96.0)	24 (3)	-
ST-464	DQ404527	*Hanseniaspora meyeri* AJ 512461	552/572 (96.8)	19	} ST-464 = ST-613
ST-613	DQ414467	*Hanseniaspora meyeri* AJ 512461	479/498 (96.2)	19 (1)	
ST-476	DQ404528	*Hanseniaspora clermontiae* AJ512456	568/578 (98.3)	10 (2)	-
ST-250	DQ404489	*Hanseniaspora meyeri* AJ 512461	552/571 (96.7)	19	ST-250 may be same ST-306
ST-306	DQ404494	*Hanseniaspora meyeri* AJ 512461	560/569 (98.4)	19 (1)	(One nucleotide is different between 2 species)
ST-314	DQ404497	*Issatchenkia* sp. NRRL Y-12824 AF017398	513/559 (91.8)	46 (4)	-
ST-343	DQ404509	*Kluyveromyces* sp. IFO 1884 AB041001	496/516 (96.1)	20 (3)	-
ST-233	DQ404481	*Metschnikowia agaveae* U84243	520/549 (94.7)	29 (3)	-
ST-57	DQ400374	*Metschnikowia drosophilae* AF279303	503/537 (93.7)	34 (5)	-
ST-96	DQ404455	*Metschnikowia lunata* U45733	431/454 (94.9)	23	-
ST-237	DQ404484	*Pichia acaciae* U45767	563/569 (98.9)	6	-
ST-37	AY634567	*Pichia americana* U73575	563/569 (98.9)	6	-
ST-236	DQ404483	*Pichia anomala* AF330115	567/573 (99.0)	4	-

Table 13 (Continued)

Strain	GenBank Accession No.	Nearest Species with Accession No. of DNA DataBank	Nucleotide Identity (%)	No. of Nucleotide Different (No. of Gap)	Remark
ST-84	DQ404448	*Candida* sp. BG02-7-15-015A-2-1 AY520326	512/521 (86.2)	8	-
ST-240	AB191049	*Pichia dryadoides* U75422	464/570 (81.4)	106 (13)	-
ST-335	DQ404505	*Pichia mexicana* U45797	522/531 (98.3)	9 (1)	-
ST-433	DQ404523	*Pichia mississippiensis* U74597	522/531 (98.3)	9	-
ST-311	AY634568	*Pichia fabianii* AF335971	561/570 (98.4)	9	ST-311 = ST-320 *P. fabianii* = *P. veronae*
		Pichia veronae U73576	561/570 (98.4)	9	
ST-320	AY634569	*Pichia fabianii* AF335971	561/570 (98.4)	9	
		Pichia veronae U73576	561/570 (98.4)	9	
ST-225	AY634570	*Pichia japonica* U73579	564/570 (98.9)	6	ST-225 = ST-228 = ST-229
ST-228	AY634571	*Pichia japonica* U73579	564/570 (98.9)	6	
ST-229	AY634572	*Pichia japonica* U73579	564/570 (98.9)	6	
ST-3	DQ400362	*Pichia pijperi* U75418	561/568 (98.8)	7	ST-3 = ST-4
ST-4	DQ400363	*Pichia pijperi* U75418	561/568 (98.8)	7	
ST-30	DQ400368	*Pichia pijperi* U75418	561/568 (98.8)	7 (1)	-
ST-334	DQ404504	*Pichia pijperi* U75418	562/568 (98.9)	6	-
ST-445	DQ404524	*Pichia pijperi* U75418	542/551 (98.4)	9	-
ST-339	DQ404500	*Pichia* sp. UWO(PS)85-301.3 AF530614	547/558 (98.0)	11	-
ST-422	DQ404521	*Saccharomyces cerevisiae* AY048154	588/595 (98.8)	7 (5)	-

Table 13 (Continued)

Strain	GenBank Accession No.	Nearest Species with Accession No. of DNA DataBank	Nucleotide Identity (%)	No. of Nucleotide Different (No. of Gap)	Remark
ST-394	DQ404520	*Saccharomyces servazzii* AF406921	575/591 (97.3)	16 (1)	-
ST-269	DQ404491	*Schizoblastosporion chiloense* U84347	269/347 (77.5)	78 (40)	-
ST-26	DQ400366	*Trichosporonoides spathulata* AF335526	289/309 (93.5)	30	-
ST-122	DQ404462	*Wickerhamia fluorescens* U45719	454/545 (83.3)	91 (26)	-
		Basidiomycetous yeast			
ST-144	DQ404464	*Bullera pseudovariabilis* AF544247	567/610 (92.9)	43 (11)	-
ST-186	DQ404472	*Bullera* sp. TY-145 AY312997	529/586 (90.3)	57 (1)	-
ST-201	DQ404475	*Cryptococcus luteolus* AF075482	581/588 (98.8)	7 (1)	-
ST-111	DQ404459	*Cryptococcus luteolus* AF075482	600/606 (99.0)	6	-
ST-71	DQ400377	*Cryptococcus* sp. CBS 8507 AF444742	592/600 (98.7)	8	-
ST-73	DQ400378	*Cryptococcus* sp. CBS 8363 AF444699	582/597 (97.5)	15	-
ST-145	DQ404465	*Cryptotrichosporon anacardii* AY550003	503/515 (97.7)	12	-
ST-98	DQ404456	*Sporidiobolus pararoseus* AF189978	582/586 (99.3)	4	ST-98 = ST-115= ST-128 = ST-195 = ST-206
ST-115	DQ404460	*Sporidiobolus pararoseus* AF189978	528/532 (99.2)	4	
ST-128	DQ404463	*Sporidiobolus pararoseus* AF189978	582/586 (99.3)	4	
ST-195	DQ404473	*Sporidiobolus pararoseus* AF189978	573/577 (99.3)	4	
ST-206	DQ404477	*Sporidiobolus pararoseus* AF189978	582/586 (99.3)	4	
ST-87	DQ404449	*Sporidiobolus pararoseus* AF189978	538/544 (98.9)	6 (1)	-

Table 13 (Continued)

Strain	GenBank Accession No.	Nearest Species with Accession No. of DNA DataBank	Nucleotide Identity (%)	No. of Nucleotide Different (No. of Gap)	Remark
ST-91	DQ404452	*Sporidiobolus pararoseus* AF189978	558/563 (99.1)	5	-
ST-92	DQ404453	*Sporidiobolus pararoseus* AF189978	574/580 (98.9)	6	-
ST-102	DQ404457	*Sporidiobolus pararoseus* AF189978	579/586 (98.8)	4	-
ST-88	DQ404450	*Sporidiobolus ruineniae* var. *ruinenie* AF070438	572/588 (97.3)	16	Two nucleotide is different between 2 strains
ST-90	DQ404451	*Sporidiobolus ruineniae* var. *ruinenie* AF070438	559/575 (97.2)	16	
ST-202	DQ404476	*Sporobolomyces bannaensis* AY274823	538/560 (96.1)	28 (17)	-
ST-119	DQ404461	*Sporobolomyces carnicolor* AY070008	549/555 (98.9)	9 (1)	-
ST-151	DQ404466	*Sporobolomyces japonicus* AY070009	559/564 (91.1)	5	-
ST-156	DQ404468	*Sporobolomyces japonicus* AY070009	566/572 (99.0)	6 (2)	-
ST-184	DQ404471	*Sporobolomyces odoratus* AF387125	558/566 (98.6)	8	-
ST-153	DQ404467	*Sporobolomyces* sp. TY-215 AY313049	555/592 (93.8)	37 (2)	-
ST-198	DQ404474	*Sporobolomyces odoratus* AF387125	546/552 (98.9)	6	-
ST-226	DQ404480	*Sporobolomyces odoratus* AF387125	514/518 (99.2)	4	-
ST-173	DQ404470	*Tilletiopsis albescens* AJ235289	519/584 (88.9)	65 (6)	-
ST-59	DQ400375	*Trichosporon faecale* AF105395	573/577 (99.3)	4 (2)	-
ST-318	AB164370	*Trichosporon loubieri* AY101608	710/731 (97.1)	21 (6)	-

Table 14 List of not yet identified species found in this study.

Strain	Nearest Species with Accession No. of DNA DataBank	Nucleotide Identity (%)	No. of Nucleotide Different (No. of Gap)	Remark
	Ascomycetous yeast			
ST-446	*Candida albicans* AF156536	585/587 (99.7)	2	-
ST-108	*Candida dendronema* U45751	528/530 (99.6)	2 (1)	-
ST-385	*Candida floricola* U45710	481/484 (99.6)	3 (1)	-
ST-127	*Candida maltosa* U45745	570/571 (99.8)	2 (1)	-
ST-441	*Candida quercitrusa* U45831	563/565 (99.8)	2	-
ST-39	*Candida rancensis* AJ508580	507/510 (99.4)	3 (1)	-
ST-248	*Candida* sp. BG00-7-5-1-2-1 AY242247	557/560 (99.5)	3	-
ST-116	*Debaryomyces* sp. NRRL Y-7804 U45771	553/556 (99.1)	3	-
ST-235	*Pichia acaciae* U45767	562/564 (99.6)	2	-
ST-38	*Pichia stipitis* U45741	566/568 (99.8)	2	-
ST-14	*Torulaspora* sp. IFO 11061 AB087395	571/574 (99.5)	3 (1)	-
	Basidiomycetous yeast			
ST-172	*Bullera sinensis* AF189884	571/574 (99.5)	3	ST-172 # ST-181 (Three nucleotide is different between 2 strains)
	Bullera derxii AF189857	571/574 (99.5)	3	
ST-181	*Bullera sinensis* AF189884	557/560 (99.5)	3	
	Bullera derxii AF189857	557/560 (99.5)	3	
ST-52	*Cryptococcus heveanensis* AFO75467	605/608 (99.5)	3 (1)	-

Table 14 (Continued)

Strain	Nearest Species with Accession No. of DNA DataBank	Nucleotide Identity (%)	No. of Nucleotide Different (No. of Gap)	Remark
ST-323	*Cryptococcus humicola* AF189836	603/606 (99.5)	3 (1)	-
ST-213	*Exobasidium vexans* AJ235288	576/578 (99.6)	2 (2)	-
ST-170	*Sporobolomyces japonicus* AY070009	583/586 (99.5)	3	ST-170 = ST-175 = ST-178 = ST-183
ST-175	*Sporobolomyces japonicus* AY070009	569/572 (99.5)	3	
ST-178	*Sporobolomyces japonicus* AY070009	583/586 (99.5)	3	
ST-183	*Sporobolomyces japonicus* AY070009	536/539 (99.4)	3	
ST-94	*Sporobolomyces odoratus* AF387125	488/490 (99.6)	2 (1)	-
ST-100	*Sporobolomyces odoratus* AF387125	540/543 (99.4)	3	ST 100 ≠ ST-123 (4)
ST-123	*Sporobolomyces odoratus* AF387125	568/571 (99.5)	3	ST-100 ≠ ST-192 (3)
ST-192	*Sporobolomyces odoratus* AF387125	552/555 (99.5)	3	ST-123 ≠ ST-192 (7/4)
ST-159	*Sporobolomyces* sp. TY-257 AY313054	571/574 (99.5)	3	-
ST-197	*Tilletiopsis* sp. TY-363 AY313023	593/595 (99.7)	2	-
ST-121	*Trichosporon multisporum* AF139984	593/595 (99.7)	2	-
ST-299	*Trichosporon mycotoxinivorans* AJ601388	600/602 (99.7)	2 (2)	-

Table 15 Nucleotides difference from nearest species of 283 strains of Thai yeast.

No. of Nucleotide Difference	No. of Strains	No. of Species (No. of Genera)	No. of Strains/No. of Species
0-1	139 (49%)	56 (26)	2.3
2-3	28 (10%)	21 (10)	1.3
≥4	116 (41%)	101 (21)	1.1
Nucleotides difference of 116 strains that differed from nearest species by 4 or more nucleotides showed as below.			
4-6 (0.7-1%)	35 (30%)	26	1.3
7-12 (>1-2%)	28 (24%)	25	1.1
13-24 (>2-4%)	19 (17%)	15	1.3
25-60 (>4-10%)	20 (17%)	19	1
61-110 (>10-18%)	14 (12%)	14	1

The number of nucleotide differences of 283 strains of Thai yeasts from their respective nearest species are shown in Table 15. One hundred and sixteen strains of unknown yeasts, which were classified into 101 species, showed nucleotide substitutions of 4 or more (0.7% or more) in the D1/D2 domains of 26S rDNA from their respective nearest known species including 4-6 nucleotides (0.7-1%) in 35 strains of 26 species, 7-12 nucleotides (>1-2%) in 28 strains of 24 species, 13-24 nucleotides (>2-4%) in 20 strains of 17 species, 25-60 nucleotides (>4-10%) in 19 strains of 19 species and 60-110 nuceotides (>10-18%) in 14 strains of 14 species. It is very interesting that 12.1% (14 species) of new species demonstrated great nucleotide substitutions in D1/D2 domain from respective nearest known yeasts. Apparently, these 14 strains that had nucleotide substitutions of 10% or more belong to new groups of yeasts and are considered to differ at the level of higher taxonomic ranks such as genus and family. At present, the numbers of strains belong to these groups are not enough to define the concept of genus or family. However, further extensive isolation studies will provide data to define these new yeast groups.

As clearly shown in Table 15, the number of isolated strains per species is different in respective categories of species. In the known species, the average of 2.3 strains were isolated. On the other hand, the average of 1.1 strains were recovered in new species and the average of 1.3 strains, the intermediate number between known species and new species, were isolated in not identified species (species considered conspecific or sister species with respective known species). Generally speaking, among new species, the average number of isolated strains decreased accompanied by the increase of nucleotide differences from 1.3 strains in species with 4-6 nucleotide differences to 1 strain in species with 61-110 nucleotide differences. The number of strains in species showing 4-6 nucleotide substitution is the same as not yet identified species which showed 2-3 nucleotide substitutions (0.3-0.6%) and only 1 strain was isolated in the species showing more than 4% substitutions.

The above mentioned tendency is clearer when we look at the number of strains of respective species. The isolation data for respective species showed that, among 101 new species found in the present study, 93 species (92.1%) comprised one strain and the remaining 8 species (7.9%) comprised 2-5 strains (Table 13). In 28 not yet identified species, 24 species (85.7%) comprised one strain and the remaining 1 species (14.3%) comprised 4 strains (Table 14). In contrast, 26 known species (42.9%) comprised one strain and 32 known species (57.1%) comprised 2-9 strains (Table 11). This is quite different from yeasts associated with fermented foods where 2 or more strains were isolated in the most of species (Saito *et al*., 1983; Suzuki *et al*., 1987; Jindamorakot, 2000). Based on the fact mentioned above, it is assumed that, in the natural environment, the number of yeast cells of known species is bigger than those of new species (unknown species) so that yeast researchers could find and described these species in early stages of yeast taxonomy. Meanwhile, species with small cell numbers in the environment still remain “unknown” to mankind.

2.1 Diversity of yeasts from flowers

Twenty-four yeast strains isolated from flowers were identified as 17 species comprised of 8 known species (13 strains; 52%) and 9 new species (11 strains; 48%). Species found from flowers are shown in Table 16. Among new species, 9 strains (8 species) related to *Candida* and the remaining 2 strains related to *Hanseniaspora* and *Trichosporonoides*.

The frequency of yeasts isolated from flowers were shown that 6 species contained only 1 strain, 1 species contained 2 strains and 1 species contained 5 strains. *Metchnikowia koreensis* is the dominant species (5 strains) and occupied 20.8% of the strains and followed by *Sporidiobolus ruineniae* var. *ruineniae* (2 strains; 8.3%).

Table 16 List of species found from flowers collected in Thailand.

Species	**No. of Strains**	**Species**	**No. of Strains**
Ascomycetous yeasts		**Basidiomycetous yeasts**	
Known species		**Known species**	
Aureobasidium pullulans	1	*Sporidiobolus ruineniae*	2
Candida leandrae	1	var. *ruineniae*	
Candida parapsilosis	1	**Total 1 species**	**2**
Debaryomyces sp. NRRL Y-7804	1		
Hanseniaspora sp. CBS 8772	1		
Kodamaea ohmeri	1		
Metschnikowia koreensis	5		
Total 7 species	**11**		
New species		**New species**	
ST-22, ST-26, ST-29, ST-32, ST-387, ST-388, ST-390, ST-391, ST-490, ST-533 and ST-536		-	
Total 9 species	**11**	**Total 0 species**	**0**
Not yet identified species		**Not yet identified species**	
-		**-**	
Total 0 species	**0**	**Total 0 species**	**0**

2.2 Diversity of yeasts from insect frass

Based on the sequences of D1/D2 of 26S rDNA, 134 strains out of 144 from insect frass were identified as 84 species comprised of 36 known species (78 strains; 58.2%) of 12 genera of ascomycetous yeasts (26 species, 57 strains) and 7 genera of basidiomycetous yeasts (10 spececies, 21 strains), 48 new species (56 strains; 41.8%) of ascomycetous (44 strains) and basidiomycetous yeasts (4 strains). Species found from insect frass are shown in Table 17. The remaining 10 strains (8%) are not yet identified, they were differed in 2-3 nucleotides from nearest species. These strains were considered to belong to known species or sister species of nearest

known species. Probably, some of them represent new species closely related to respective known species.

Table 17 List of species found from insect frass collected in Thailand.

Species	No. of Strains	Species	No. of Strains
Ascomycetous yeasts		**Basidiomycetous yeasts**	
Known species		**Known species**	
Ambrosiozyma monospora	2	*Bullera dendrophila*	1
Aureobasidium pullulans	1	*Cryptococcus heveanensis*	8
Blastobotrys capitulata	1	*Cryptococcus laurentii*	3
Candida diversa	1	*Cryptococcus* sp. CBS 8372	1
C. fukuyamaensis	3	*Rhodosporidium paludigenum*	1
C. gotoi	2	*Rhodosporidium toruloides*	1
C. parapsilosis	1	*Rhodotorula nothofagi*	1
C. rancensis	1	*Sporidiobolus ruineniae*	1
C. sithepensis	1	*Sporobolomyces poonsookiae*	1
C. tropicalis	8	*Trichosporon asahii*	3
Candida sp. NRRL Y-17456	3	**Total 10 species**	**21**
Candida sp. UWO(PS)00-147.3	1		
Debaryomyces nepalensis	4		
D. polymorphus	3		
D. polymorphus var. *africanus*	1		
D. vanrijiae var. *yarrowii*	3		
Geotrichum fragrans	2		
Kluyveromyces lactis	2		
Kodamaea (Pichia) ohmeri	2		
P. stipitis	2		
P. sydowiorum	1		
Pichia sp. UWO(PS)99-305.1	1		
Saccharomyces cerevisiae	2		
S. kluyveri	5		
S. unisporus	1		
Stephanoascus smithiae	2		
Williopsis saturnus var. *subsufficiens*	1		
Total 26 species	**57**		

Table 17 (Continued)

Species	No. of Strains	Species	No. of Strains
Ascomycetous yeasts		**Basidiomycetous yeasts**	
New species		**New species**	
ST-17, ST-33, ST-37, ST-43, ST-49,ST-50, ST-57, ST-60, ST-78, ST-79,ST-84, ST-95, ST-96, ST-112, ST-164, ST-211, ST-224, ST-225, ST-228, ST-229, ST-233, ST-234, ST-236, ST-237, ST-238, ST-239, ST-240, ST-249, ST-250, ST-297, ST-300, ST-306, ST-309, ST-310, ST-311, ST-314, ST-315, ST-320, ST-328, ST-329, ST-330, ST-331, ST-333, ST-334, ST-335, ST-337, ST-338, ST-339, ST-394, ST-431,ST-433 and ST-493		ST-59, ST-71, ST-73 and ST-318	
Total 44 species	**52**	**Total 4 species**	**4**
Not yet identified species		**Not yet identified species**	
ST-38, ST-39, ST-108, ST-116, ST-127, ST-235 and ST-248		ST-52, ST-121 and ST-299	
Total	**7**	**Total**	**3**

Based on the sequences of D1/D2 domain of new species, 52 strains of them belong to ascomycetous yeasts and were classified into 44 species. They are related to species of *Candida* (24 species; 26 strains), *Debaryomyces* (1 species; 2 strains), *Dipodascus* (1 species; 1 strain), *Hanseniaspora* (2 species; 3 strains), *Issatchenkia* (1 species; 1 strain), *Metschnikowia* (3 species; 3 strains), *Pichia* (11 species; 14 strains), *Saccharomyces* (1species; 1 strain) and *Wickerhamia* (1 species; 1 strain). The remaining 4 strains belong to basidiomycetous genera *Cryptococcus* (2 species; 2 strains) and *Trichosporon* (2 species; 2 strains). Therefore, it is considered that about a half of yeasts isolated from insect frass represent new species.

The results of D1/D2 sequences showed the diversity of yeasts from insect frass in natural environment of Thailand. *Candida tropicalis* and *Cryptococcus heveanensis* are the dominant species (8 strains of each species) and occupied 5.8% of the strains from insect frass followed by *Saccharomyces kluyveri* (5 strains; 3.6%) and *Debaryomyces nepalensis* (4 strains; 2.9%). Thirty-nine percent (56 strains) of yeasts isolated from insect frass were found to represent new species. Twenty-three species contained only 1 strain, 8 species contained 2 strains, 1-2 species contained 3-5 strains, and only 2 species contained 8 strains.

2.3 Diversity of yeasts from mosses

Twenty-two strains out of 27 isolated from mosses belonged to 18 species; 12 known species (17 strains; 63.0%) of 7 genera of ascomycetous yeasts (11 species, 16 strains) and a genus of basidiomycetous yeasts (1 species, 1 strain) and 6 new species (6 strains; 22.2%). The remaining 4 strains (14.8%) are not yet identified. Species found from mosses are shown in Table 18. Eighteen species contained only 1 strain, 2 species contained 2 strains and only 1 species contained 3 strains. *Saccharomyces cerevisiae* (3 strains; 13.6%) is the dominant species and followed by *Candida diversa* (2 strains; 9.1%) and *Saccharomyces kluyveri* (2 strains; 9.1%). Six strains of new species were related to *Candida* (2 strains), *Pichia* (2 strains), *Galactomyces* (1 strain) and *Schizoblastosporion* (1 strain).

2.4 Diversity of yeasts from mushrooms

Twenty- six strains out of 28 isolated from mushrooms belonged to 22 species; 10 known species (12 strains; 43%) of 5 genera of ascomycetous yeasts (9 species, 10 strains) and a genus of basidiomycetous yeasts (1 species, 1 strain), 12 new species (13 strains; 46.4%) of ascomycetous yeasts. The species are related to genus *Candida* (8 species; 9 strains), *Endomyces* (1 species; 1 strain), *Hanseniaspora* (1 species; 1 strain), *Kluyveromyces* (1 species; 1 strain), and *Pichia* (1 species; 2 strains). The remaining 2 strains (7%) are not yet identified. The most of species

found from mushrooms contained only 1 strain and 3 species contained 2 strains. Species found from mosses are shown in Table 19.

Table 18 List of species found from mosses collected in Thailand.

Species	**No. of Strains**	**Species**	**No. of Strains**
Ascomycetous yeasts		**Basidiomycetous yeasts**	
Known species		**Known species**	
Candida diversa	2	*Trichosporon asahii*	1
Candida palmae	1	**Total 1 species**	**1**
Hanseniaspora opuntiae	2		
Hanseniaspora vineae	1		
Pichia sydowiorum	1		
Saccharomyces cerevisiae	3		
Saccharomyces kluyveri	2		
Saccharomyces unisporus	1		
Torulaspora sp. IFO 11061	1		
Williopsis saturnus var. *markii*	1		
Zygosaccharomyces sp. IFO 11070	1		
Total 11 species	**16**		
New species		**New species**	
ST-30, ST-36, ST-269, ST-445, ST-449 and ST-451		-	
Total 6 species	**6**	**Total 0 species**	**0**
Not yet identified species		**Not yet identified species**	
ST-14, ST- 385 and ST-441and ST-446		-	
Total	**4**	**Total**	**0**

Table 19 List of species found from mushrooms collected in Thailand.

Species	**No. of Strains**	**Species**	**No. of Strains**
Ascomycetous yeasts		**Basidiomycetous yeasts**	
Known species		**Known species**	
Candida natalensis	1	*Cryptococcus humicola*	2
Candida parapsilosis	1	**Total 1 species**	**2**
Candida sp. NRRL Y-17456	1		
Candida tropicalis	1		
Hanseniaspora opuntiae	2		
Pichia nakazawae var. *akitaensis*	2		
Stephanoascus smithiae	1		
Torulaspora delbrueckii	1		
Total 9 species	**10**		
New species		**New species**	
ST-2, ST-3, ST-4, ST-18, ST-19, ST-246, ST-253, ST-343, ST-358, ST-365**,** ST-366, ST-370 and ST-377		-	
Total 12 species	**14**	**Total 0 species**	**0**
Not yet identified species			
ST-380		ST-323	
Total	**1**	**Total**	**1**

2.5 Diversity of yeasts from leaves

Thirty strains out of 53 from leaves belonged to 7 known species (15 strains; 28.3%) of 4 genera of basidiomycetous yeasts, 18 new species (26 strains; 47.2%) and 13 (24.5%) not yet identified strains. Species found from leaves are shown in Table 20. New species are related with the genera *Bullera* (2 species; 2 strains), *Cryptococcus* (1 species; 2 strains), *Cryptotrichosporon* (1 species; 1 strains), *Sporidiobolus* (3 or 4 species; 11 strains), *Sporobolomyces* (7 species; 8 strains) and *Tilletiopsis* (1 species; 1 strains). The remaining 13 strains (24.5%) are not yet

identified. The dominant genus of yeast isolated form leave is *Sporobolomyces* (17 species, 21 strains; 67.7%). From the phylogenetic analysis based on the D1/D2 domain sequences of 26S rDNA, 18 species contained only 1 strain, 5 species contained 2 strains, 5 species contained 4 strains and only 1 species contained 5 strains.

Table 20 List of species found from leaves collected in Thailand.

Species	**No. of Strains**
Ascomycetous yeasts	0
Basidiomycetous yeasts	
Known species	
Bullera sinensis	2
Exobasidium vexans	4
Sporobolomyces bannaensis	1
Sporobolomyces odoratus	3
Sporobolomyces sp. TY-241	1
Sporobolomyces sp. TY-257	3
Telletiopsis sp. TY 235	1
Total 7 species	**15**
New species	
ST-87, ST-88, ST-90, ST-91, ST-92, ST-98, ST-102, ST-105, ST-111,ST-115, ST-119, ST-128, ST-144, ST-145, ST-151, ST-153, ST-156, ST-173, ST-184, ST-186, ST-195, ST-198, ST-201, ST-202, ST-206 and ST-226	
Total	**26**
Not yet identified species	
ST-94, ST-100, ST-123, ST-159, ST-170, ST-172, ST-175, ST-178, ST-181, ST-183, ST-192, ST-197 and ST-213	
Total	**13**

3. Taxonomic and Phylogenetic Positions of New Yeasts from Thailand

The phylogenetic trees for new species isolated in the natural environment of Thailand were constructed based on the D1/D2 sequences together with their closest known species. A tree for new ascomycetous yeasts comprised 86 strains of 75 species and their closest known species (Fig. 4) and that for new basidiomycetous yeasts comprised 30 strains (26 species) and their closest known species (Fig.5). The new yeasts isolated from natural habitat of Thailand were widely distributed in the phylogenetic trees but not concentrated in certain groups of yeasts.

Eighty-six strains of new ascomycetous yeasts were classified into 14 genera of 6 families (Order Saccharomycetales, Class Hemiascomycetes) and represented 76 species (Table 21). Thirty strains of new basidiomycetous yeasts were distributed in 7 genera of 3 classes, Uredinomycetes, Hymenomycetes and Ustilaginomycetes (Table 21). Urediniomycetes included 19 strains which were classified into the genera *Sporidiobolus* and *Sporobolomyces* representing 11 or 12 species. Hymenomycetes comprised 9 strains of the genera *Bullera, Cryptococcus, Trichosporon* and *Cryptotrichosporon*. Ustilaginomycetes comprised 2 strains of the genus *Tilletiopsis*.

As already mentioned, most of new ascomycetous species contained only 1 strain. Some species have more than 1 strains but less than 4. In the case of new basidiomycetous yeasts, most species have 1 strain same as ascomycetous yeasts. Although 9 strains (ST-87,ST-91,ST-92,ST-98,ST-102,ST-115,ST-128,ST-195 and ST-206) were very close to *Sporidiobolus pararoseus* (Fig. 5), they may be separated into 2 or 3 species because 0-4 nucleotides (including gaps) differences were observed among them (Table 22).

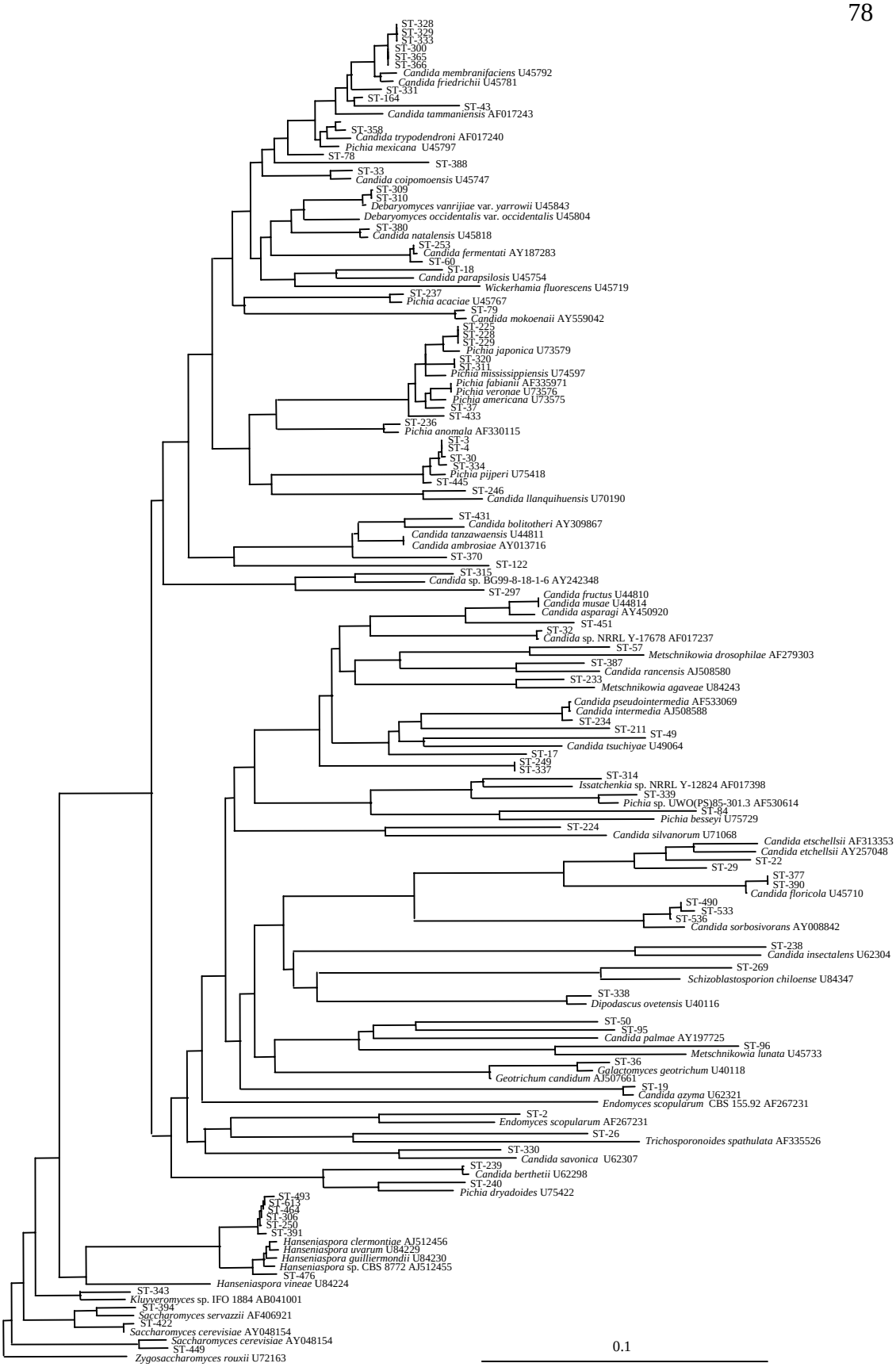

Figure 4 The phylogenetics tree of the new species of ascomycetous yeasts and their closest species based on the D1/D2 domain sequences of 26S rDNA.

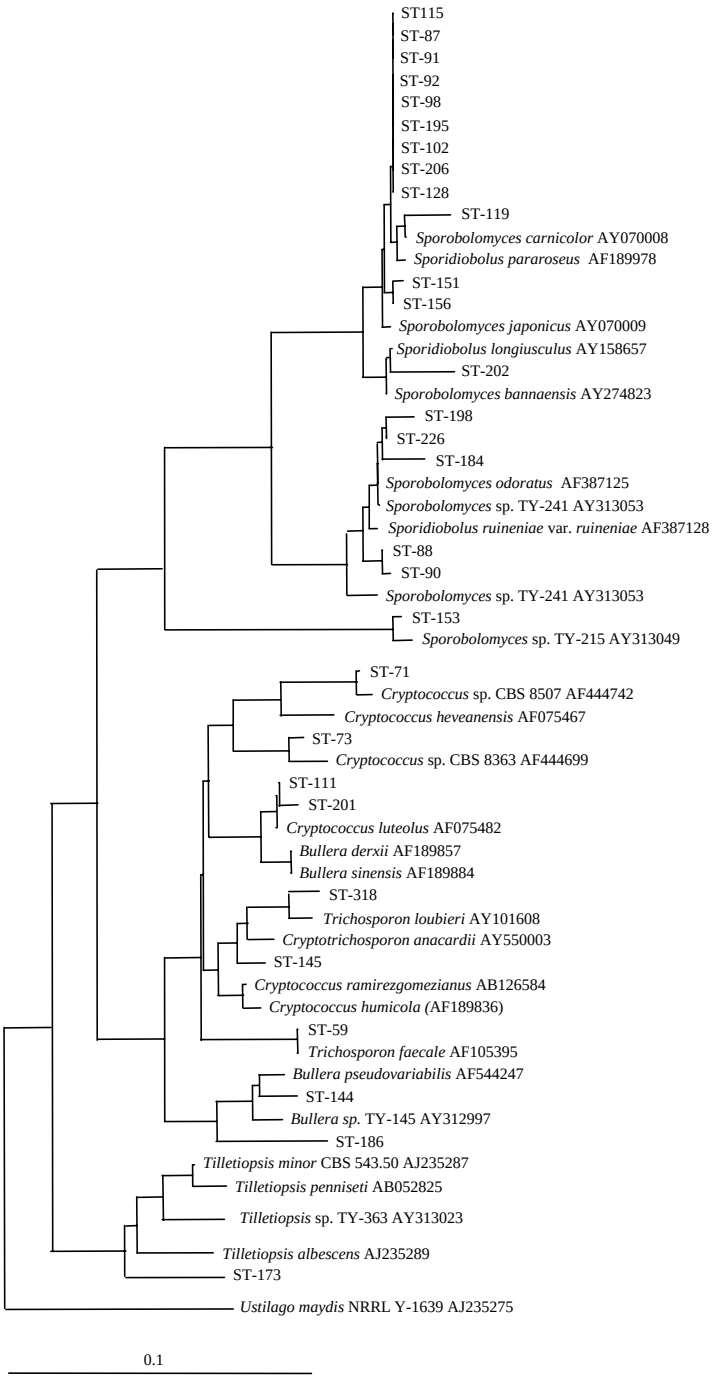

Figure 5 The phylogenetics tree of the new species of basidiomycetous yeasts and their closest species based on the D1/D2 domain sequences of 26S rDNA.

Table 21 Taxonomic position of new yeast species found in this study.

Clasification	No. of species (No. of strains)
Phylum Ascomycota	
Class Hemiascomycetes	
Order Saccharomycetales	
Family Dipodascaceae	
Genus *Dipodascus*	1 (1)
Galactomyces	1 (1)
Trichosporonoides	1 (1)
Family Endomycetaceae	
Genus *Endomyces*	1 (1)
Family Metschnikowiaceae	
Genus *Metschnikowia*	3 (3)
Family Saccharomycetaceae	
Genus *Debaryomyces*	1 (2)
Issatchenkia	1 (1)
Kluyveromyces	1 (1)
Pichia	14 (18)
Saccharomyces	2 (2)
Family Saccharomycodaceae	
Genus *Hanseniaspora/Kloeckera*	6 (7)
Wickerhamia	1 (1)
Family Candidaceae	
Genus *Candida*	41 (47)
Schizoblastosporion	1 (1)
Total 14 genera	**76 (87)**
Phylum Basidiomycota	
Class Urediniomycetes	
Order Sporidiobolales	
Family Sporidiobolaceae	
Genus *Sporidiobolus*	7 (11)
Family Sporobolomycetaceae	
Genus *Sporobolomyces*	8 (8)
Class Hymenomycetes	
Genus "*Cryptotrichosporon*"	1 (1)
Family Cryptococcaceae	
Genus *Bullera*	2 (2)
Cryptococcus	4 (4)
Trichosporon	2 (2)
Class Ustilaginomycetes	
Order Entylomatales	
Family Entylomataceae	
Genus *Telletiopsis*	1 (1)
Total 7 genera	**25 (29)**

Table 22 Number of nucleotide differences in new species of genus *Sporidiobolus* found in this study.

	ST-87	ST-91	ST-92	ST-98	ST-115	ST-128	ST-195	ST-206	ST-102
ST-87	0								
ST-91	4(2)	0							
ST-92	4(1)	4(1)	0						
ST-98	2(1)	3(1)	2	0					
ST-115	2(1)	3(1)	2	0	0				
ST-128	2(1)	2(1)	2	0	0	0			
ST-195	2(1)	2(1)	2	0	0	0	0		
ST-206	2(1)	2(1)	2	0	0	0	0	0	
ST-102	3(2)	2(1)	2	1(1)	1(1)	1(1)	1(1)	1(1)	0

Remark: Numerals in parentheses indicate the number of gaps.

4. Taxonomic Study on Selected Groups

Twenty-one new species were selected for precise taxonomic studies by considering phylogenetic clusters and nucleotide diffences. These yeasts were studied by polyphasic approaches, conventional taxonomy, chemotaxonomic study and molecular taxonomy. These strains belong to the genus *Candida* (14 species), *Hanseniaspora/Kloeckera* (4 species), *Pichia* (2 species), and *Trichosporon* (1 species). Their conventional taxonomic characteristics are shown in Appendix B (Appendix Table B2 and B3). Their taxonomy and describtions are shown below.

4.1 Taxonomic studies on *Hanseniaspora*/*Kloeckera* (anamorph)

Fifteen strains isolated in the present study proliferated by bipolar budding, buds are produced repeatedly from the same position (anneallation) only at the pole of the cell in both sides (Fig. 6). In the phylogenetic tree based on the D1/D2 domain sequences, they were located in the cluster where species of the genus *Hanseniaspora* and its anamorph counterpart *Kloeckera* were located. They were separated into 5 groups, Group I, Group II, Group III, Group IV and Group V as shown in Fig. 7.

Nucleotide similarities and number of nucleotide differences from closest species were shown in Table 23.

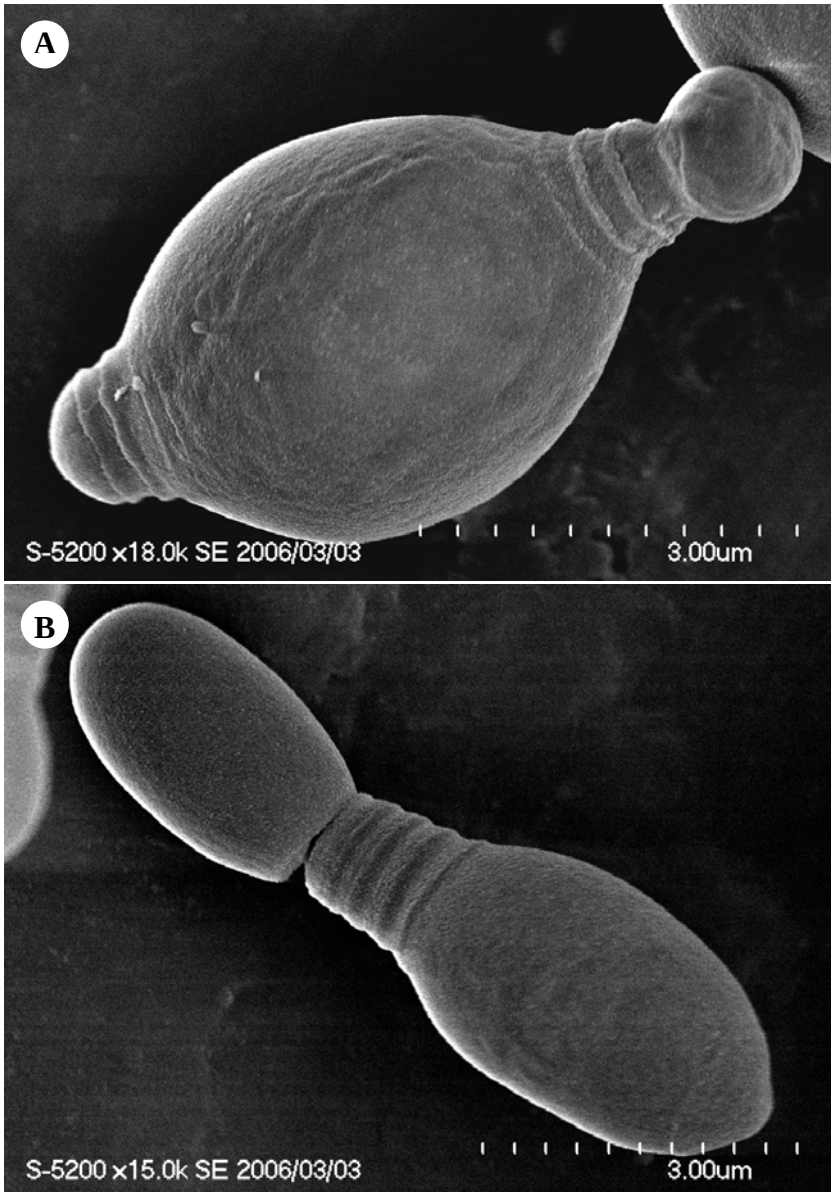

Figure 6 Cell morphology of *Hanseniaspora thailandica* (strain ST-250), after 2 days at 25°C in YPD broth, (A) trypical apiculate cell (B) matured new bud releasing from mother cell.

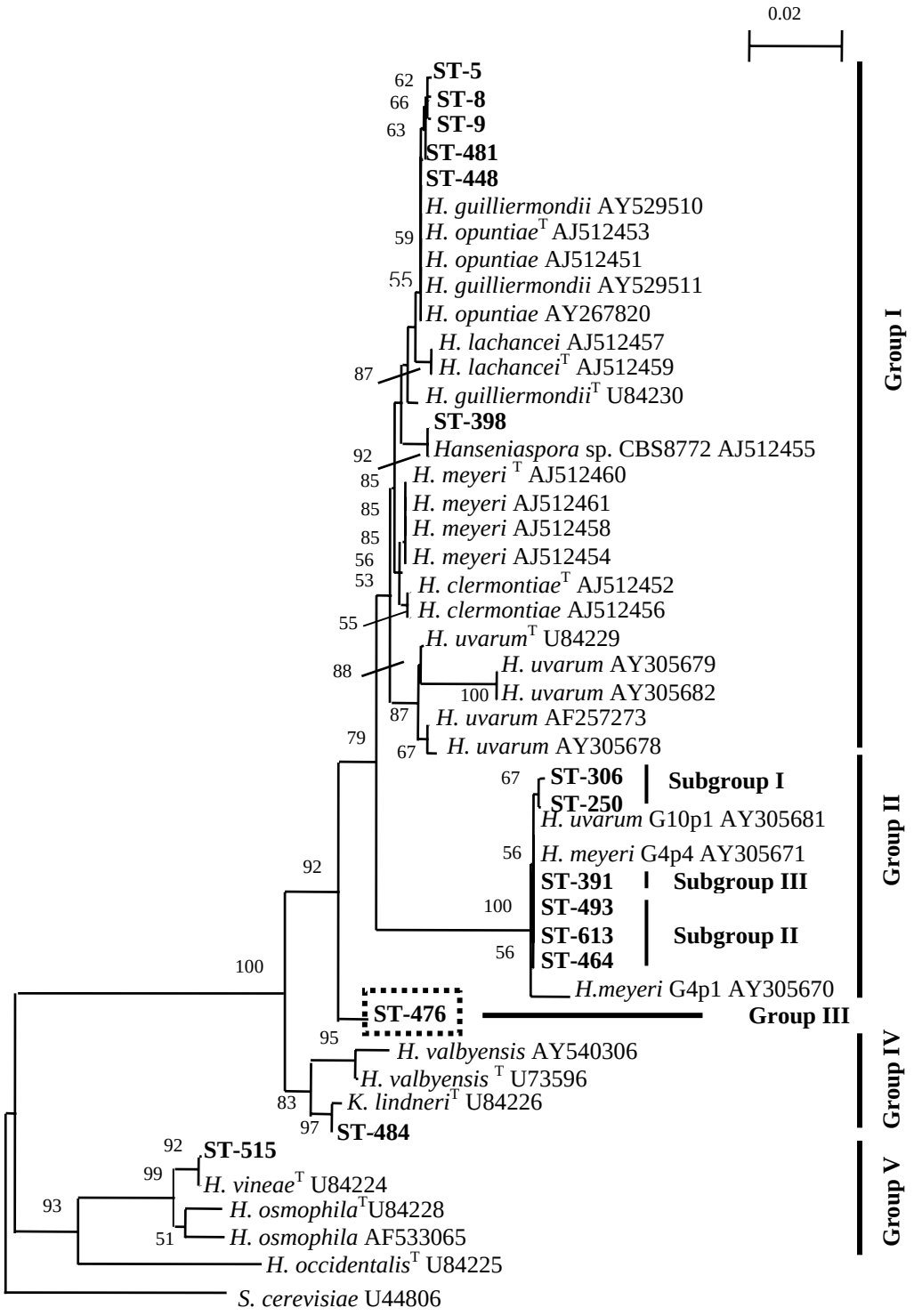

Figure 7 Phylogenetic tree showing the positions of *Hanseniaspora/Kloeckera* isolated in Thailand and their related species based on the sequences of the D1/D2 domain of 26S rDNA with bootstrap values by 1000 re-samplings (< 50% is not shown).

Table 23 Nucleotide similarity in D1/D2 domain sequence of 26S rDNA of Thai strains and their closest species (Type strains).

Group	Strains	Closest Species with GeneBank Accession No.	Nucleotide Identity (%)	No. of Nucleotide Difference	Remark
I	ST-5	*H. opuntiae* AY267820	571/571 (99.8)	1	Known species
	ST-8	*H. opuntiae* AY267820	560/561 (98.9)	1	Known species
	ST-9	*H. opuntiae* AY267820	559/560 (99.8)	1	Known species
	ST-448	*H. opuntiae* AY267820	572/572 (100)	0	Known species
	ST-481	*H. opuntiae* AY267820	571/572 (99.8)	1	Known species
	ST-398	*H. pseudoguilliermondii* AJ512455 (in press)	570/570 (100)	0	Known species
II	ST-250	*H. meyeri* AJ 512460	548/568 (96.3)	20	New species
	ST-306	*H. meyeri* AJ 512460	548/569 (96.3)	21	New species
	ST-493	*H. meyeri* AJ 512460	558/577 (96.5)	19	New species
	ST-613	*H. meyeri* AJ 512460	479/498 (96.2)	19	New species
	ST-464	*H. meyeri* AJ 512460	563/582 (96.6)	19	New species
	ST-391	*H. meyeri* AJ 512460	542/566 (95.9)	24	New species
III	ST-476	*H. clermontiae* AJ512456	568/578 (98.6)	10	New species
IV	ST-484	*Kloeckera lindneri* U84226	571/572 (99.8)	1	Known species
V	ST-515	*H. vineae* U84224	518/519 (99.8)	1	Known species

Group I (ST-5, ST-8, ST-9, ST-398, ST-448 and ST-481)

Six strains of group I had similar mol% G+C of 34.1-36.1 with those (33.6-35.3) of the nearest species (Table 24). They constituted a cluster with known species of *Hanseniaspora*, *H. guilliermondii*, *H. opunitae*, *H. lachancei*, *H. meyeri*, *H. clermontiae* and *H. uvarum* (Fig. 7). Among 6 strains, 5 strains, ST-5, ST-8, ST-9, ST-448 and ST-481, were identified as *Hanseniaspora opuntiae*. ST-448 showed the identical sequence with the type strain of *H. opuntiae* and 4 strains, ST-5, ST-8, ST-9, ST-448, differed in 1 nucleotide from the type strain (Table 23). In the DNA-DNA hybridization experiment among these 5 strains showed the similarities more than 95% with the type strain of *H. opuntiae* (Table 24). In the conventional taxonomy, these strains had the same morphological and physiological characteristics with *H.*

opuntiae. Apparently, these 5 strains are conspecific with *H. opuntae*. In the D1/D2 domain, ST-398 showed the identical sequence with *Hanseniaspora* sp. CBS 8772 and constituted a cluster with this strain. These two strains differed in 4-5 nucleotides from other strains of Group I (Table 25). In the DNA-DNA hybridization experiment, ST-398 was differentiated from other strains of Group I though the value to *H. opuntiae* was fairly high, 55.0-74.2% (Table 24). ST-398 and CBS 8772 are considered to represent a single new species different from *H. opuntiae*. According to Smith, CBS 8722 will be described soon as a new species *Hanseniaspora pseudoguilliermondii* (personal communicatetion to Y. Imanishi, NBRC). So, ST-398 is the second strain of *H. pseudoguilliermondii*.

Group II (ST-250, ST-306, ST-391, ST-464, ST-493 and ST-613)

Six strains of Group II constituted a cluster with known species of *Hanseniaspora, H. guilliermondii, H. opuntiae, H. lachancei, H. meyeri, H. clermontiae* and *H. uvarum* (Fig. 7). They had mol%G+C lower than *H. meyeri*, the nearest species but the differences are not significant (Table 26). In the D1/D2 domain sequence, they differed in 19-22 nucleotides from the type strain of nearest species (Table 27). Based on the DNA-DNA hybridization and nucleotide differences in D1/D2 domain, 6 strains of Group II were suggested to be separated to 3 subgroups, Subgroup I, Subgroup II and Subgroup III (Table 26 and Table 27). In the phylogenetic tree of *Hanseniaspora*, strains of Group II constituted a cluster with *H. uvarum* G10p1, *H. meyeri* G4p4 and *H. meyeri* G4p1, which were registed to DNA databank however they do not belong *to H. uvarum* and *H. meyeri* because their D1/D2 sequence much differ from respective type strains. (Fig. 7). *H. uvarum* G10p1 and *H. meyeri* G4p4 differed from Group II of Thai strains in 3 and 4 nucleotides, respectively. So that these strains are conspecific or different species of Group II. *H. meyeri* G4p1 differed from Group II in 20 nucleotides. In the taxonomic criteria commonly employed, strains of subgroup I are differentiated from Group II by ability to assimilate trehalose and requirement of thiamine. Subgroup III differed from subgroup I and subgroup II by weak assimilation of 2-ketogluconic acid, inability to assimilate trehalose, glucono-δ-lactone, and no growth at 36°C.

Table 24 Similarity and mol% G+C of DNA of *Hanseniaspora/Kloeckera* Group I and related strains.

Group I	Mol % G+C	% Similarity										
		1	2	3	4	5	6	7	8	9	10	11
1 ST-5	34.8	100										
2 ST-8	36.1	96.5	nd									
3 ST-9	35.4	95.8	nd	nd								
4 ST-448	35.3	97.8	nd	nd	nd							
5 ST-481	35.3	108.1	nd	nd	nd	nd						
6 *H. opuniae* CBS 8733	33.6-35.3	118.1	140.2	153.4	159.4	109.7	100					
7 *H. lachancei* CBS 8818	34.8-35.6	57.9	57.3	54.1	56.0	55.9	50.2	100				
8 *H. guiliermondii* BCC 4316	32.9-34.2	27.8	30.4	27.5	37.2	57.7	48.0	28.0	100			
9 *H. clermontiae* CBS 8821	35.7-37.2	25.4	28.9	22.0	27.3	39.3	35.5	25.9	33.7	100		
10 *H. meyeri* CBS 8734	36.6-37.4	19.6	24.3	20.1	28.4	31.2	39.2	20.2	30.3	58.0	100	
11 ST-398	34.9	55.0	nd	nd	nd	nd	74.2	47.7	51.4	43.9	9.8	ND

Remark: nd, not determined

Table 25 Number of nucleotide differences in D1/D2 domain among *Hanseniaspora/Kloeckera* Group I and type strains of related species.

Group I	ST-5	ST-8	ST-9	ST-448	ST-481	*H.opu.*	*H.gui.*	*H.mey.*	*H.lac.*	*H.cle.*	CBS 8772	ST-398	*H. uva.*
ST-5		1	1	1	1	1	3	4	4	5	5	5	6
ST-8	0.2		0	1	1	1	3	4	4	5	5	5	6
ST-9	0.2	0		1	1	1	3	4	4	5	5	4	6
ST-448	0.2	0.2	0.2		1	0	2	3	3	4	4	4	5
ST-481	0.2	0.2	0.2	0.2		1	3	4	4	5	5	5	6
H. opuntiae	0.2	0.2	0.2	0.2	0.2		2	3	3	4	4	4	5
H. guilliermondii	0.6	0.6	0.6	0.4	0.6	0.4		3	5	4	4	4	5
H. meyeri	0.8	0.8	0.8	0.6	0.7	0.6	0.6		6	1	5	5	4
H. lachancei	0.8	0.8	0.8	0.6	0.7	0.6	0.9	1.1		5	5	5	6
H. clermontiae	0.9	0.9	0.9	0.7	0.9	0.7	0.7	0.2	0.9		4	4	3
Hanseniaspora sp.CBS 8772	0.9	0.9	0.9	0.7	0.9	0.7	0.7	0.9	0.9	0.7		0	5
ST-398	1.1	1.1	1.0	0.8	0.9	0.8	0.8	0.9	0.9	0.7	0		5
H. uvarum	1.1	1.1	1.1	1.3	1.1	0.9	0.9	0.8	1.1	0.6	0.6	0.9	

Remark: A upper right triangle refers to nucleotide differences in D1/D2 including gaps and lower triangle refers to % nucleotide differences.

Table 26 Similarity and mol% G+C of DNA of *Hanseniaspora/Kloeckera* Group II and type strain of related species. Hybridization was performed at 40°C.

Group II	Strains	Mol% G+C	% Similarity						
			ST-250	ST-306	ST-464	ST-493	ST-613	ST-391	CBS 8734
Sub Group I	ST-250	34.9	100						
	ST-306	34.8	91.6	nd					
Sub Group II	ST-464	35.3	63.0	nd	nd				
	ST-493	35.2	67.4	nd	nd	nd			
	ST-613	34.9	72.5	nd	nd	nd	nd		
SubGroup III	ST-391	35.0	58.4	nd	nd	nd	nd	nd	
	H. meyeri CBS 8734	36.6-37.4	23.6	20.8	27.7	29.0	28.7	18.1	100

Remark: nd, not determined

Table 27 Number of nucleotide differences in D1/D2 domain among *Hanseniaspora/Kloeckera* Group II and related species.

Group II	Strains	ST-250	ST-306	ST-464	ST-493	ST-613	ST-391	G10p1	G4p4	G4p1	*H. mey.*	*H. uva.*
Subgroup I	ST-250		2(1)	1	1	1	4(2)	3(1)	5(4)	20(6)	20	22
	ST-306	0.2		1	1	1	3(1)	4(2)	6(5)	21(7)	21(1)	23(1)
Subgroup II	ST-464	0.2	0.2		0	0	5(2)	2(1)	4(4)	20(7)	19	21
	ST-493	0.2	0.2	0		0	5(2)	2(1)	4(4)	20(7)	19	21
	ST-613	0.2	0.2	0	0		5(2)	2(1)	4(4)	20(7)	19	21
Subgroup III	ST-391	0.7	0.5	1.2	1.2	1.2		7(3)	5(4)	24(8)	22(2)	25(2)
	H. uvarum G10p1	0.5	0.7	0.4	0.4	0.4	1.3		5(4)	20(7)	22(1)	23(1)
	H. meyeri G4p4	0.9	1.1	0.8	0.8	0.8	1.1	1.0		23(10)	23(4)	25(4)
	H. meyeri G4p1	3.8	4.0	4.8	4.8	4.8	4.4	3.6	4.3		38(7)	39(6)
	H. meyeri	3.5	3.6	3.2	3.2	3.2	3.9	3.8	4.0	6.8		4(0)
	H. uvarum	3.8	4.0	3.7	3.7	4.3	4.3	4.2	4.7	4.7	0.7	

Remark: A upper right triangle refers to nucleotide differences in D1/D2 including gaps. (Numerals in parenthes indicate the number of gaps) and lower triangle refers to % nucleotide differences.

Subgroup I contained 2 strains, ST-250 and ST-306. These strains had 34.8-34.9 mol% G+C. The sequence of D1/D2 domain of these two strains differed from type strain of *H. meyeri*, their closest species, by 20 (3.5%) and 22 (3.6%) nucleotides, respectively. ST-250 and ST-306 differed in 2 nucleotides including 1 gap between them. However, these two strains close to 3 undescribed yeasts. ST-250 and ST-306 differed from *H. uvarum* G10p1 and *H. meyeri* G4p4 in 3 and 6 nucleotides (including 1-5 gaps), respectively (Table 27). So that these strains are conspecific or sister species. Further, ST-250 and ST-306 are different species with *H. meyeri* G4p1 by 20-21 nucleotides including 6-7 gaps (Table 27). In the DNA-DNA hybridization, ST-250 and ST-306 showed the similarities of 20.8-23.6 % with the type strain of *H. meyeri* and 91.6% between them (Table 26). Subgroup I showed the similarities of 63.0-72.5 % and 58.4% with strains of subgroup II and subgroup III, respectively (Table 26). In the taxonomic criteria commonly employed, strains of subgroup I are differentiated from *H. meyeri* by ability to assimilate trehalose, sodium nitrate, growth at 36-37°C, inability to assimilate potassium nitrate and ethylamine and growth in 0.1% cycloheximide is negative. These data clearly suggested that ST-250 and ST-306 belonged to the same species and represented a new species of *Hanseniaspora*.

Subgroup II contained 3 strains, ST-464, ST-493 and ST-613. These strains had 34.9-35.3 mol% G+C (Table 26). They had identical sequences of D1/D2 domain and differed in 19 nucleotides from the type strain of *H. meyeri*, the nearest species (Table 27). However, subgroup II differed from undescribed yeasts of *H. uvarum* G10p1 and *H. meyeri* G4p4 in 2 and 4 nucleotides (including 1 and 2 gaps), respectively (Table 27). So that these strains are conspecific or sister species of subgroup II. Further, subgroup II is different species with *H. meyeri* G4p1 because 20 nucleotides including 7 gaps are different (Table 27). In the DNA-DNA hybridization, ST-464, ST-493 and ST-613 showed the similarities of 27.7-29.0% with type strain of *H. meyeri* (Table 26). In the taxonomic criteria commonly employed, subgroup II is differentiated from *H. meyeri* by ability to assimilate sodium nitrite, growth at 36-38°C, inability to assimilate potassium nitrate and ethylamine and growth in 0.1% cycloheximide is negative. These data clearly suggested that ST-464, ST-493 and ST-

613, are different species from known species and subgroup I, However, ascospore has not been found in this study so that strains in subgroup II will be described as new species of the genus *Kloeckera*.

Subgroup III contained a strain, ST-391. This strain had 35 mol %G+C. In the D1/D2 domain, ST-391 differed in 3-5 nucleotides from Subgroup I and Subgroup II (Table 27). This strain differed in 22 (3.9%) nucleotides including 2 gaps from the type strain of *H. meyeri*, the nearest species. ST-391 close to 3 undescribed yeasts by differed from *H. uvarum* G10p1 and *H. meyeri* G4p4 in 7 and 5 nucleotides (including 3 and 4 gaps), respectively (Table 27). Further, subgroup III are different from *H. meyeri* G4p1 by 24 nucleotides including 8 gaps (Table 27). In the DNA-DNA hybridization experiment, ST-391 showed similarity of 18.1% with the type strain of *H. meyeri* (Table 26). In the taxonomic criteria commonly employed, strains of ST-391 is differentiated from *H. meyeri* by ability to assimilate sodium nitrite, growth at 36-38°C, inability to assimilate glucono-δ-lactone, potassium nitrate and ethylamine and no growth in 0.1% cycloheximide. However, ascospore has not been found in this study so that strains in subgroup II will be described as new species of the genus *Kloeckera*.

Group III (ST-476)

ST-476 had a mol% G+C of 36.9 similar with the type strain of *H. clermontiae* (35.7-37.2%), the closest species. In the phylogenetic tree, this strain is distant from any known species of *Hanseniaspora* (Fig. 7). In D1/D1 domain, ST-476 differed in 10 nucleotides (1.7%) including 2 gaps from the type strain of *H. clermontiae*, the closest species, and more than 10 nucleotides from the type strains of related species (Table 28). In the taxonomic criteria commonly employed, strains of ST-476 is differentiated from *H. clermontiae* by ability to assimilate sodium nitrite, growth at 33-34°C, inability to assimilate potassium nitrate, ethylamine and no growth in 0.1% cycloheximide. Apparently, this strain represents a new species of *Kloeckera* because ascospore has not been found in this study.

Table 28 Number of nucleotide differences in D1/D2 domain among *Hanseniaspora/ Kloeckera* Group III and type strains of related species.

Group III	ST-476	*H. cle.*	*H. uva.*	*H. opu.*	CBS 8772	*H. mey.*	*H. lac.*
ST-476		10(2)	11	12(2)	12(2)	12(3)	14(3)
H. clermontiae	1.4		3	4	4	1	5
H. uvarum	1.9	0.6		5	5	4	6
H. opuntae	2.1	0.7	0.9		4	3	3
Hanseniaspora sp.CBS 8772	2.1	0.7	0.6	0.7		5	5
H. meyeri	2.1	0.2	0.8	0.6	0.9		6
H.lachancei	2.4	0.9	1.1	0.6	1.1	1.1	

Remark: A upper right triangle refers to nucleotide differences in D1/D2 including gaps. (Numerals in parenthes indicate the number of gaps) and lower triangle refers to % nucleotide differences.

Group IV (ST-484)

ST-484 had a mol% G+C of 36.1. In the phylogenetic tree, this strain was located at the cluster where the type strain of the closest species, *Kloeckera lindneri*, was located (Fig. 7). In the D1/D2 domain, ST-484 differed in 1 nucleotide from the type strain of *K. lindneri* and 7 nucleotides from the type strain of *H. valbyensis* (Table 29). The taxonomic criteria commonly employed of ST-484 is the same with *K. lindneri*. These data clearly suggests that ST-484 belongs to the species *Kloeckera lindneri*.

Table 29 Number of nucleotide differences in D1/D2 domain among *Hanseniaspora/Kloeckera* Group IV and Group V and type strains of related species.

Group IV	ST-484	*K. lindneri*	*H. valbyensis*	**Group V**	ST-515	*H. vineae*	*H. osmophila*	*H. occidentalis*
ST-484		1	7 (1)	ST-515		3 (1)	7 (1)	34 (1)
K. lindneri	0.2		8 (1)	*H. vineae*	0.6		8	36 (1)
H. valbyensis	1.2	1.4		*H. osmophila*	1.3	1.4		34 (1)
				H. occidentalis	6.4	6.7	6.0	

Remark: A upper right triangle refers to nucleotide differences in D1/D2 including gaps. (Numerals in parenthes indicate the number of gaps) and lower triangle refers to % nucleotide differences.

Table 30 Similarity and mol% G+C of DNA of *Hanseniaspora* Group IV, Group V and related strains.

Strains	% Mol G+C	% Similarity				
		ST-515	ST-484	*H. vineae*	*H. osmophila*	*K. lindneri*
ST-515 (Group V)	38.4	nd				
ST-484 (Group IV)	36.1	nd	nd			
H. vineae IFO 1415	38.8-40.7	89.5	6.1	100		
H. osmophila IFO 10834	39.8-40.5	21.7	20.1	nd	100	
K. lindneri	nd	nd	nd	nd	nd	nd

Remark: nd, not determined

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.

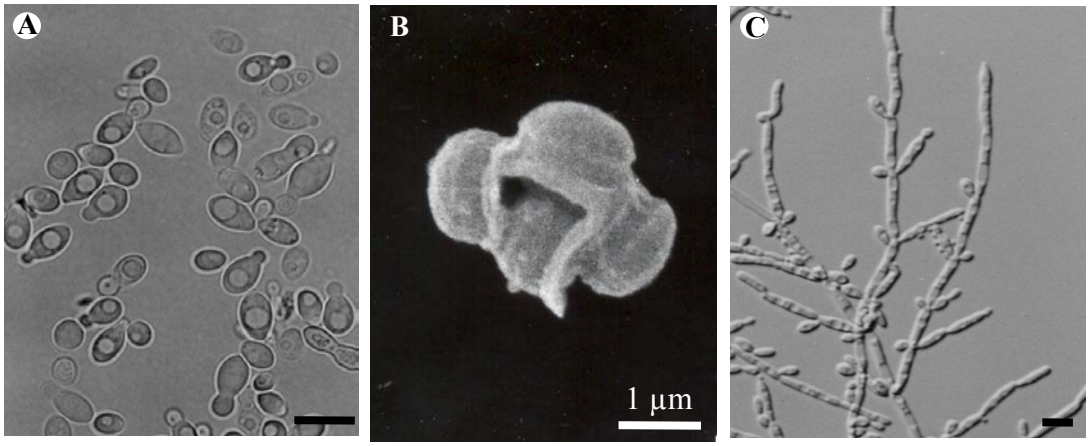

Figure 8 Morphological characteristics of *Hanseniaspora thailandica* (ST-250); (A) cells grown in YMB for 3 days at 25°C; (B) ascospore produced on potassium acetate agar after 7 days at 25°C; (C) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

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***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.

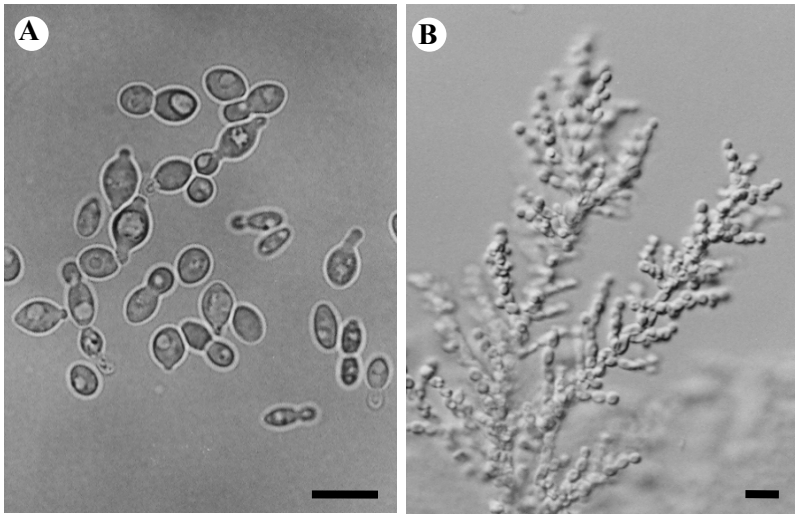

Figure 9 Morphological characteristics of *Kloeckera siamensis* (ST-464); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

***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.

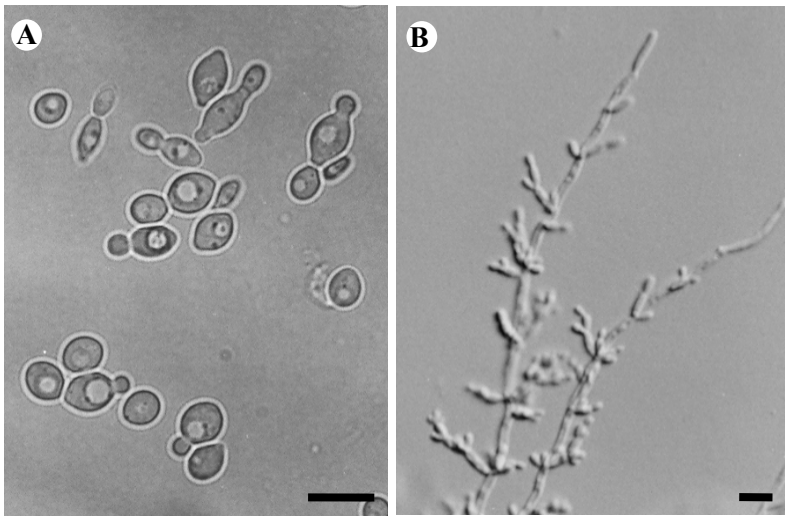

Figure 10 Morphological characteristics of *Kloeckera songkhlaensis* (ST-476); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

***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.

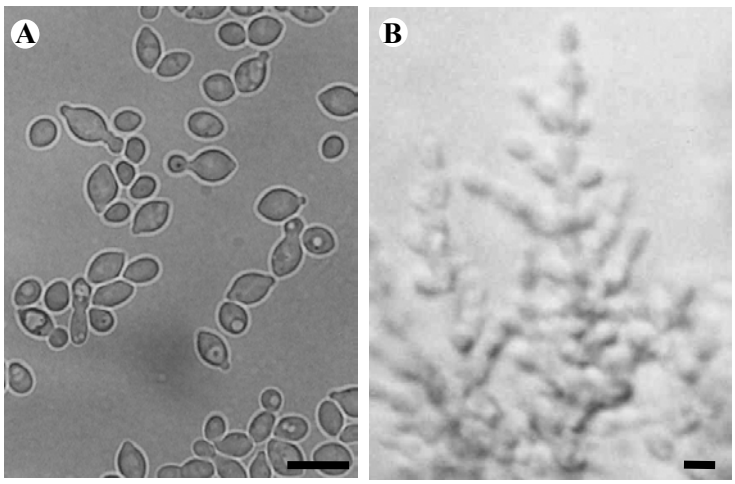

Figure 11 Morphological characteristics of *Kloeckera tradensis* (ST-391); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

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).

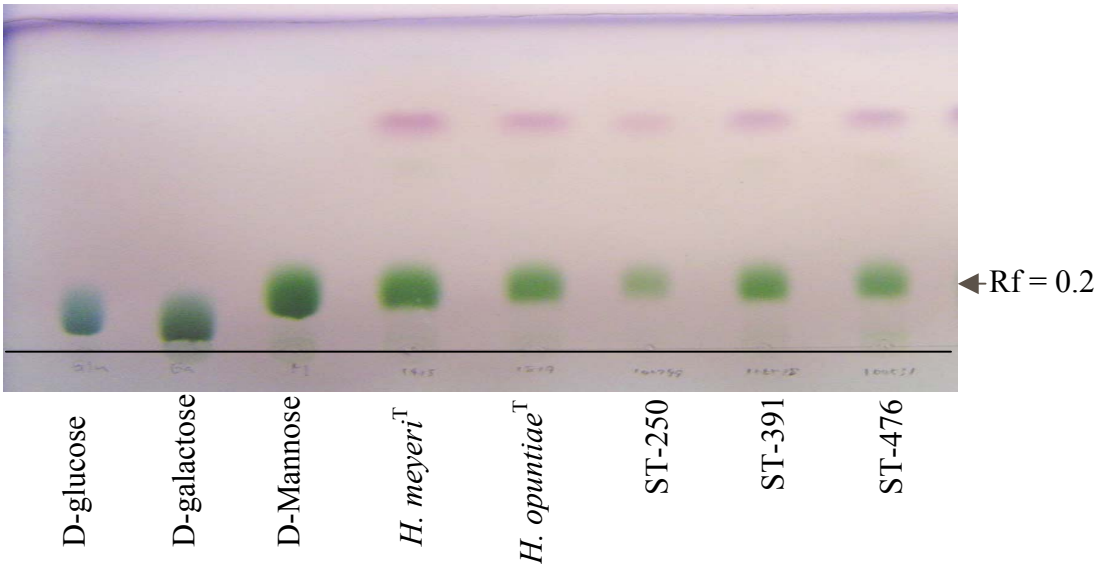

Figure 12 Thin layer chromatography of acid hydrolyzates of polysaccharides from *Hanseniaspora/Kloeckera*.

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*.

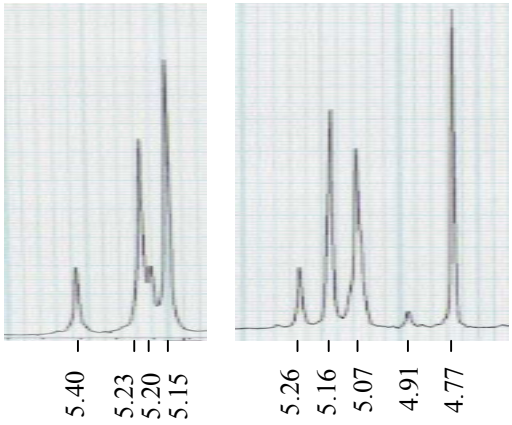

Figure 13 The NMR spectra of H-1 region of the mannans of *Hanseniaspora*/ *Kloeckera*. Type I; *H. clermontiae, H. guilliermondii, H. lachancei, H. myeri, H. opuntiae, H. pseudoguilliermondii, H. uvarum, K. lindneri*, and *H. thailandica*, *K. siamensis*, *K. tradensis* and *K. songkhlaensis.* Type 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.

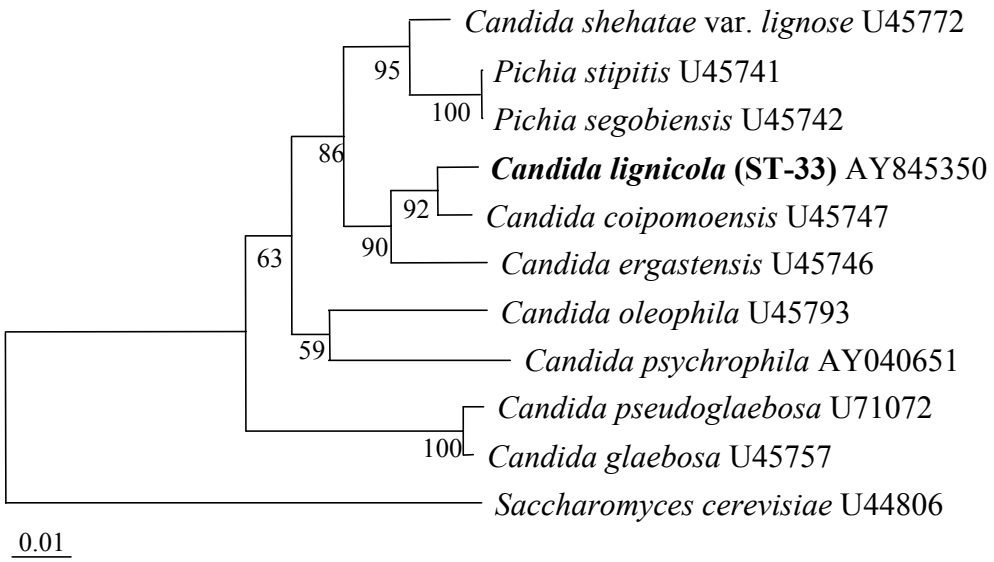

Figure 14 Phylogenetic tree showing the position of *Candida lignicola* (ST-33) based on the sequences of the D1/D2 domain of 26S rDNA with bootstrap values by 1000 re-sampling (< 50% is not shown).

***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.

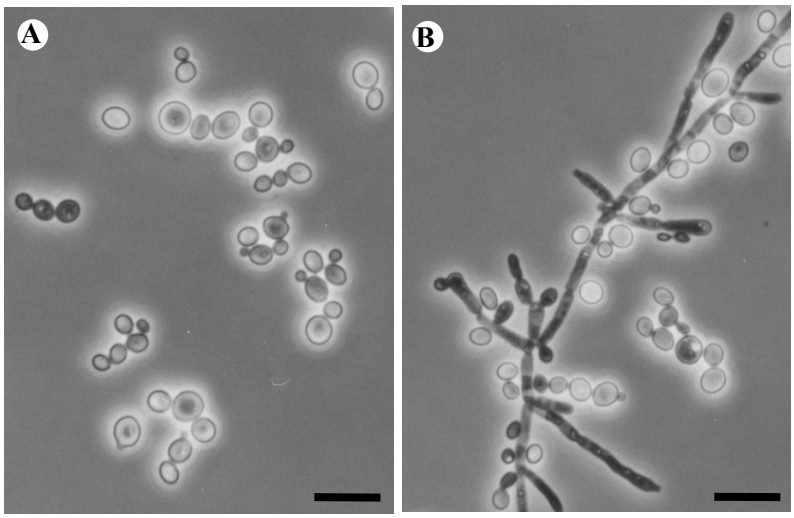

Figure 15 Morphological characteristics of *Candida lignicola*; (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

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).

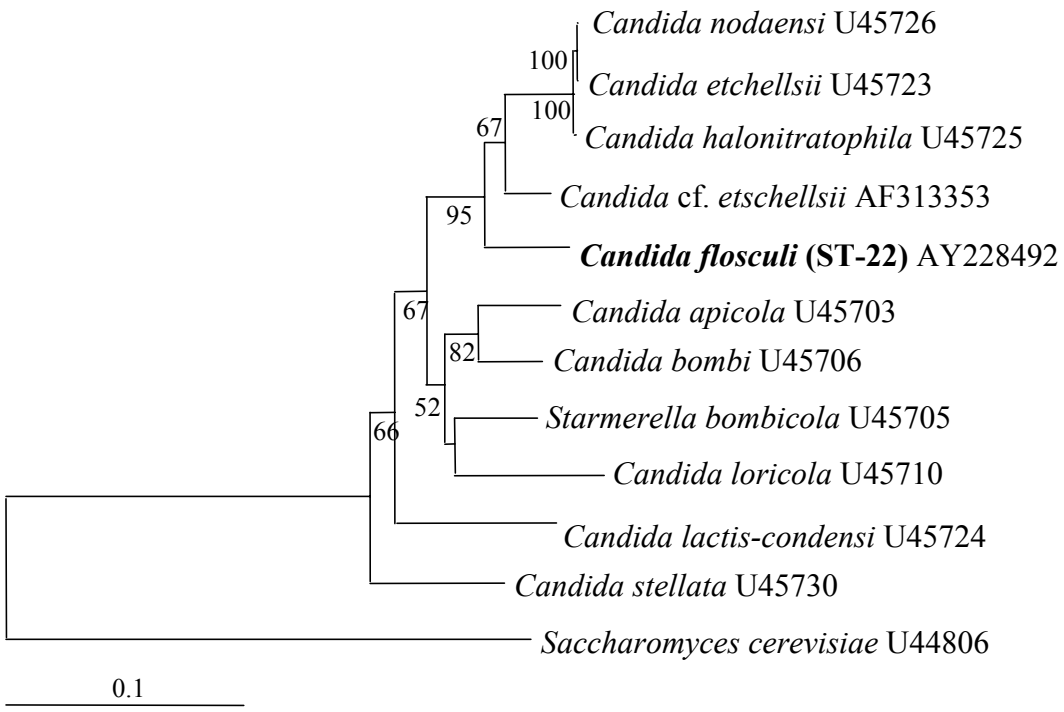

Figure 16 Phylogenetic tree showing the position of *Candida flosculi* based on the sequences of the D1/D2 domain of 26S rDNA with bootstrap values by 1000 re-sampling (< 50% is not shown).

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.

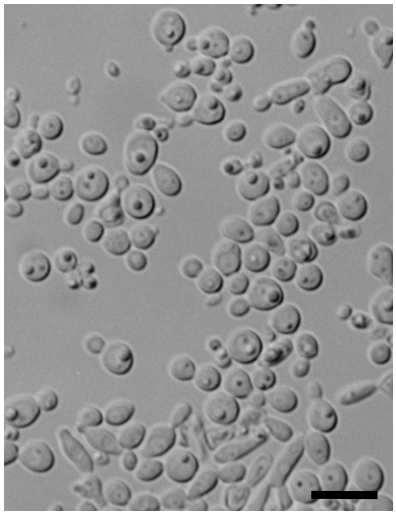

Figure 17 Morphological characteristics of *Candida flosculi* grown in YMB for 3 days at 25°C. Scale = 10 μm.

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.

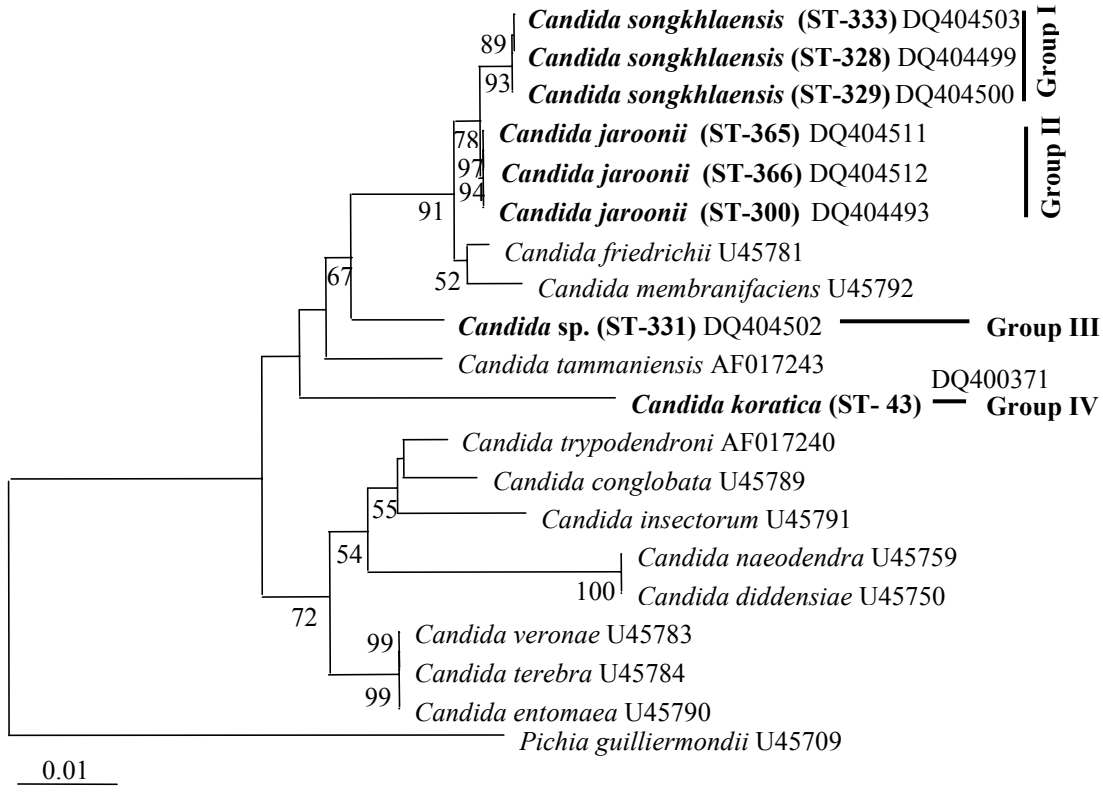

Figure 18 Phylogenetic tree showing the positions of strains related to *Candida friedrichii* based on the sequences of the D1/D2 domain of 26S rDNA with bootstrap values by 1000 resampling (< 50% is not shown).

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.

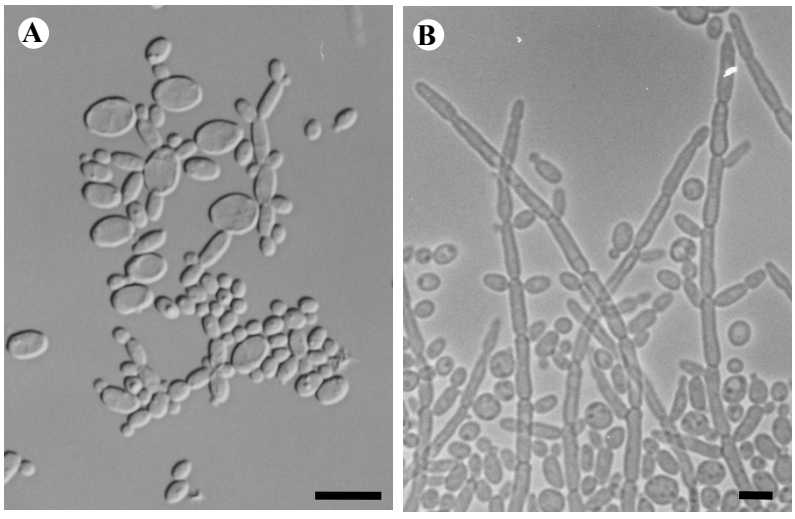

Figure 19 Morphological characteristics of *Candida jaroonii* (ST-300); (A) cells of pellicle grown in YMB for 3 days at 25°C; (B) cells of sediment grown in YMB for 3 days at 25°C. Scales = 10 μm.

***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.

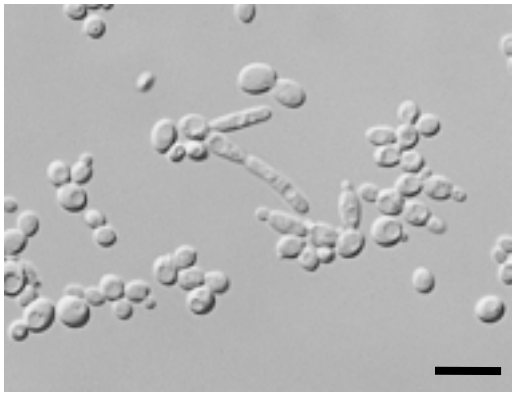

Figure 20 Morphological characteristics of *Candida koratica* (ST-43) grown in YMB for 3 days at 25°C. Scale = 10 μm.

***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.

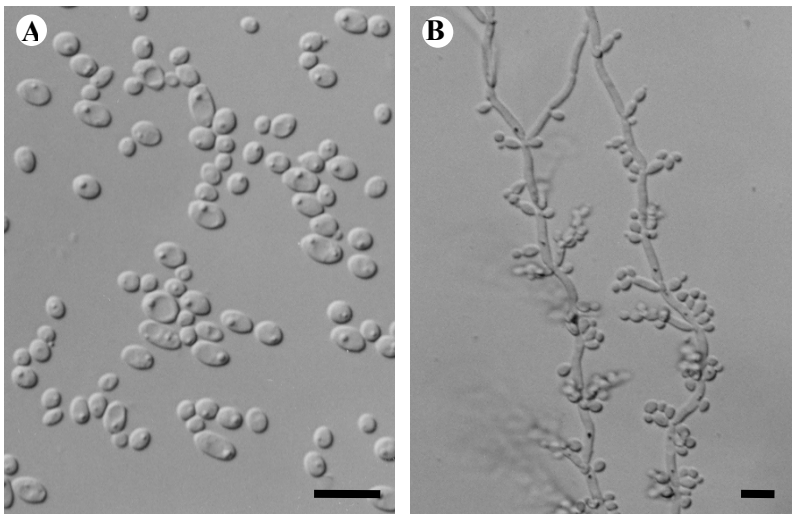

Figure 21 Morphological characteristics of *Candida songkhlaensis* (ST-328); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

***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.

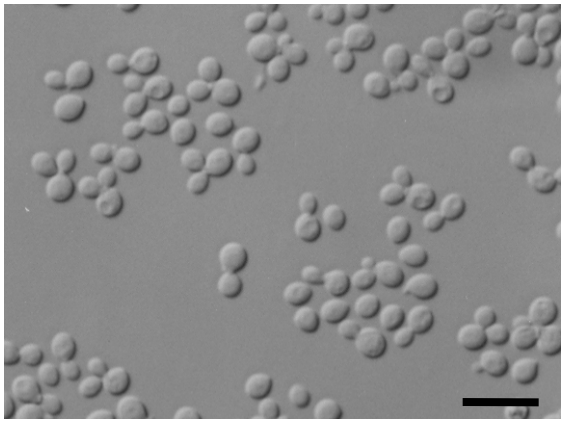

Figure 22 Morphological characteristics of *Candida* sp. (ST-311) grown in YMB for 3 days at 25°C. Scale = 10 μm.

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).

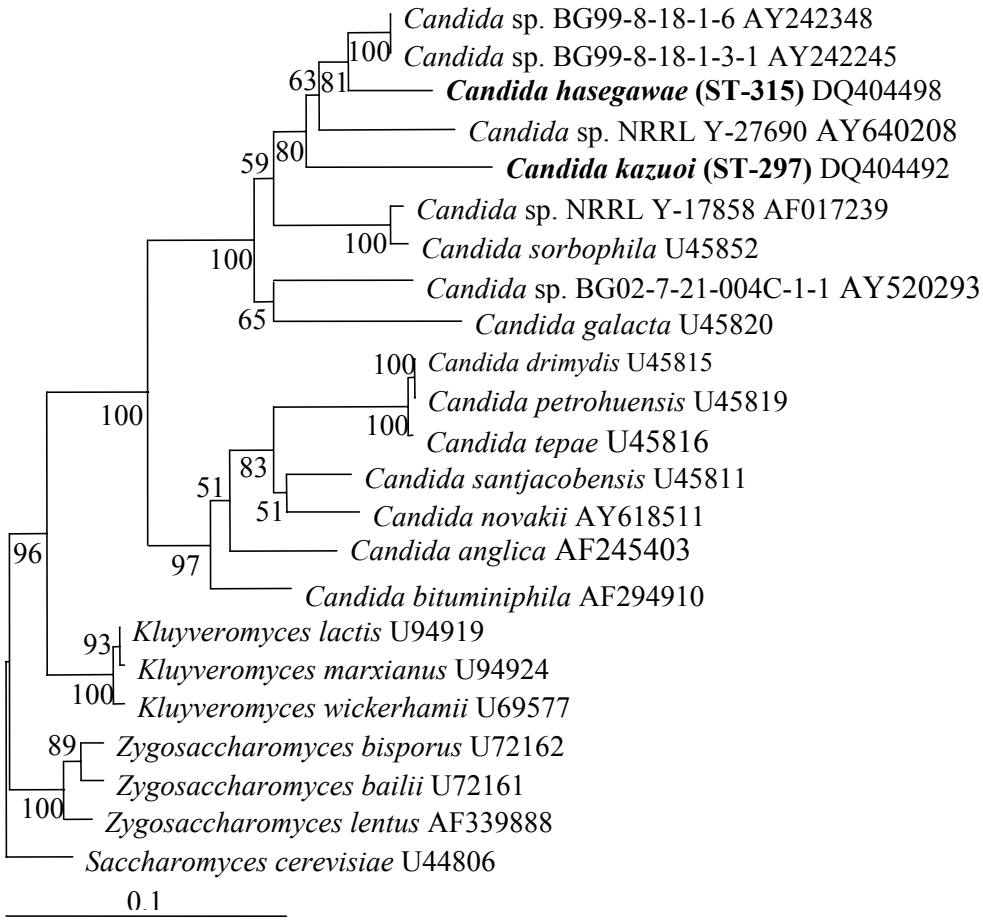

Figure 23 Phylogenetic tree for two new species, *Candida kazuoi* and *Candida hasegawae,* constructed by the neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the bootstrap values of 1,000 resamplings.

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.

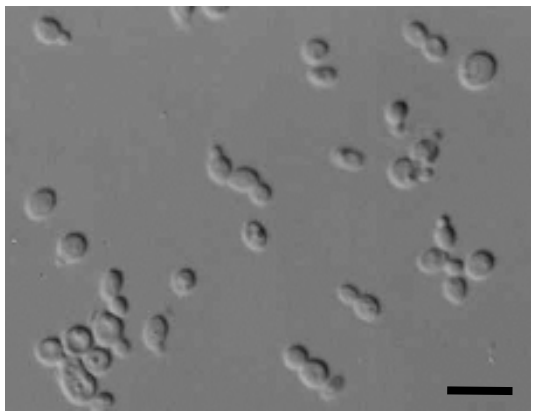

Figure 24 Morphological characteristics of *Candida kazuoi* (ST-297) grown in YMB for 3 days at 25°C. Scale = 10μm.

***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.

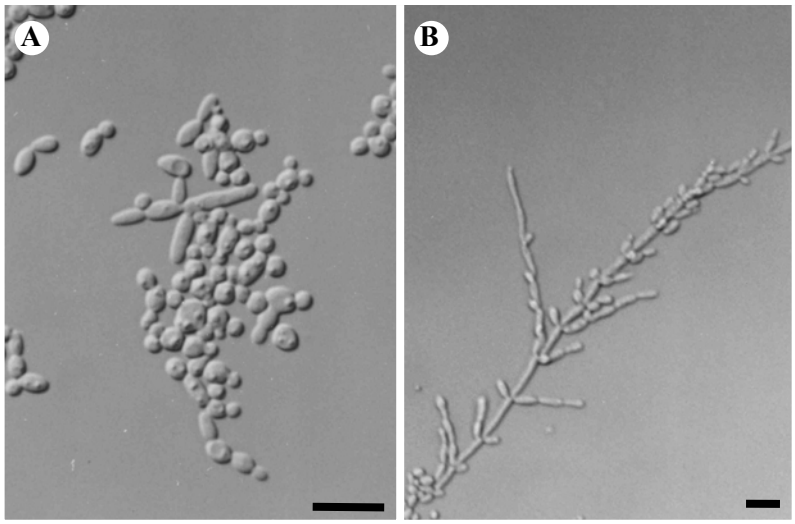

Figure 25 Morphological characteristics of *Candida hasegawae* (ST-315); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

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.

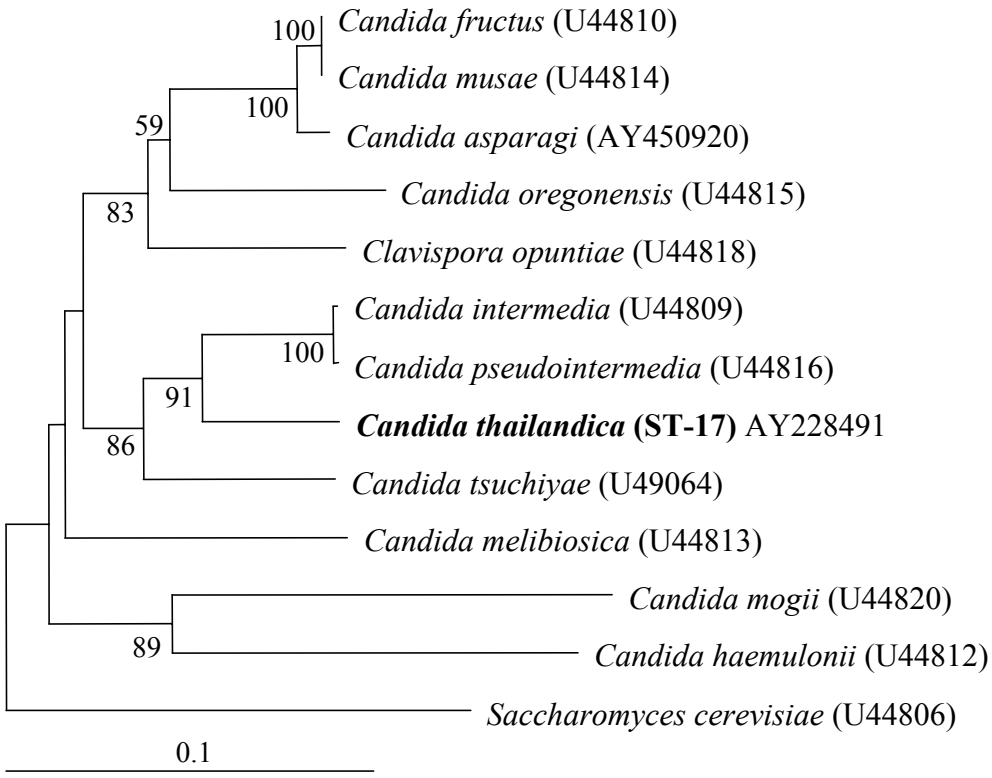

Figure 26 Phylogenetic tree showing the position of *Candida thailandica* (ST-17) based on the sequences of the D1/D2 domain of 26S rDNA with bootstrap values by 1000 re-sampling (< 50% is not shown).

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.

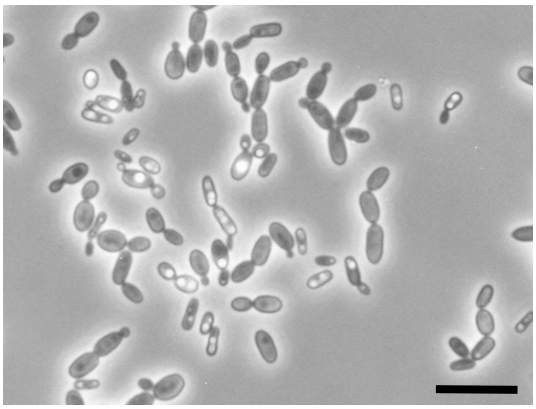

Figure 27 Morphological characteristics of *Candida thailandica* (ST-17) grown in YMB for 3 days at 25°C. Scale = 10 μm.

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).

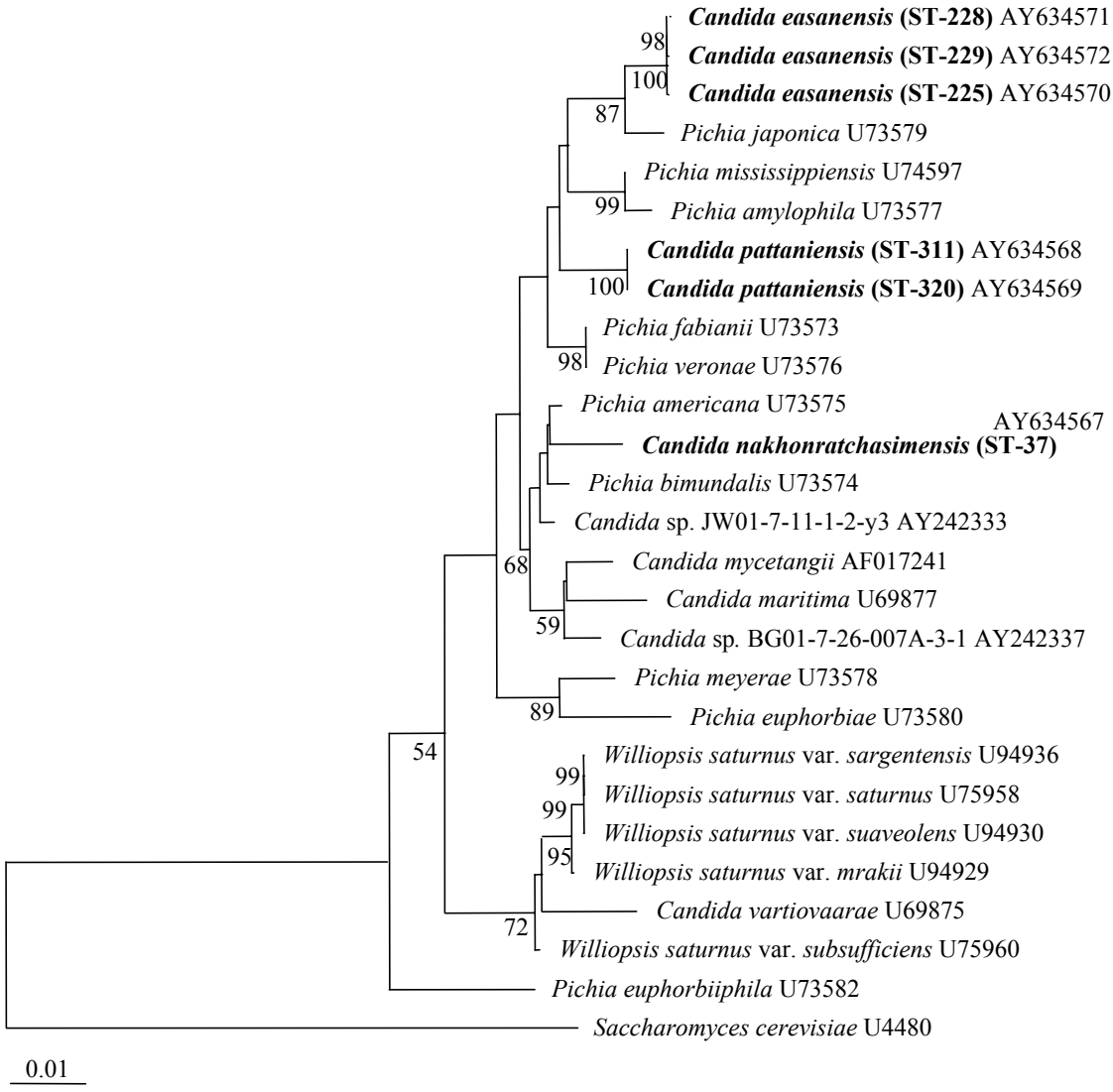

Figure 28 Phylogenetic tree for *Candida easanensis*, *Candida pattaniensis* and *Candida nakhonratchasimensis* isolated in Thailand constructed by the neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the bootstrap values of 1,000 trials.

***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.

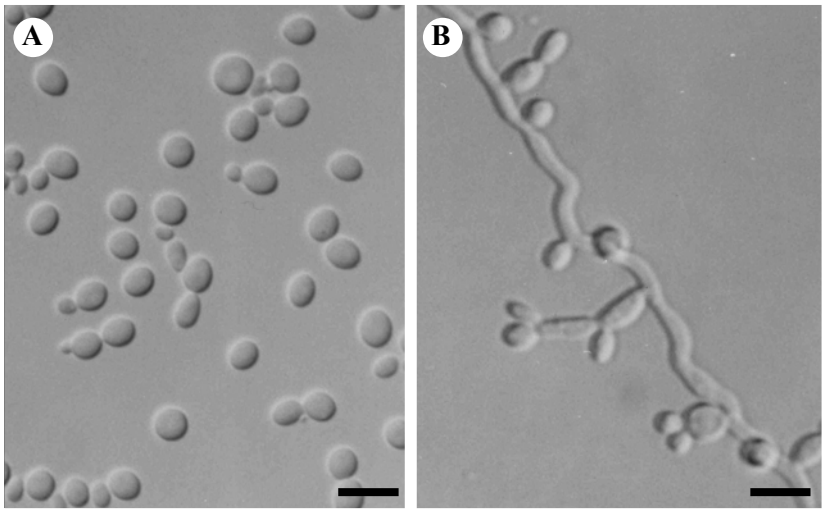

Figure 29 Morphological characteristics of *Candida nakhonratchasimensis* (ST-37); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

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.

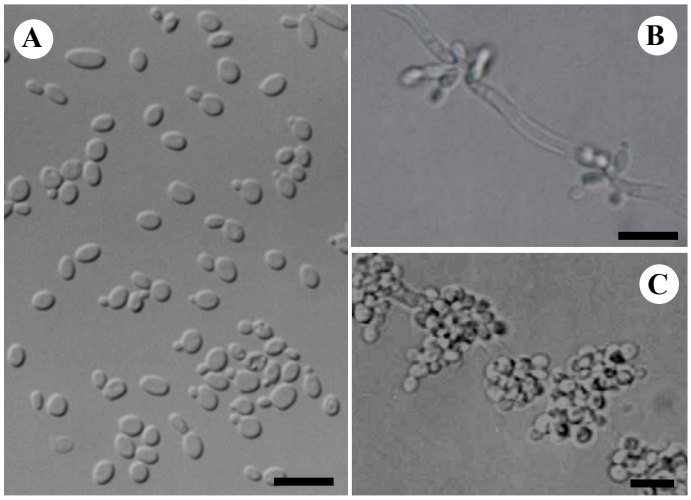

Figure 30 Morphological characteristics of *Candida easanensis* (ST-225); (A) cells grown in YMB for 3 days at 25°C; (B, C) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

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.

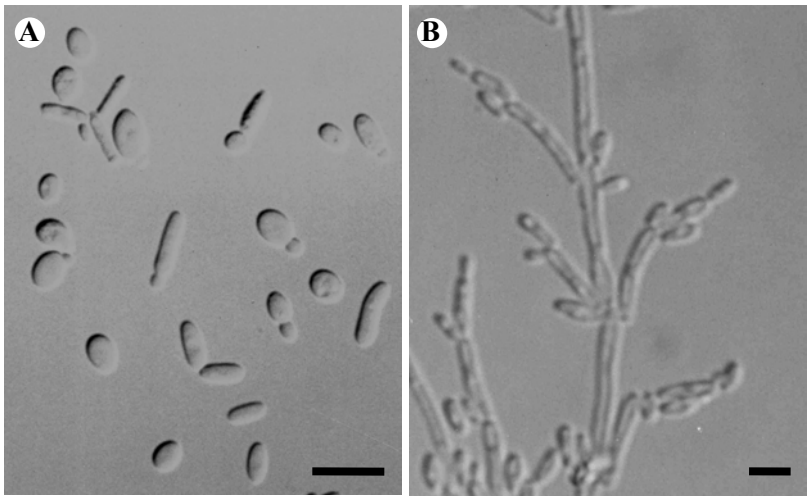

Figure 31 Morphological characteristics of *Candida pattaniensis* (ST-311); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

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.

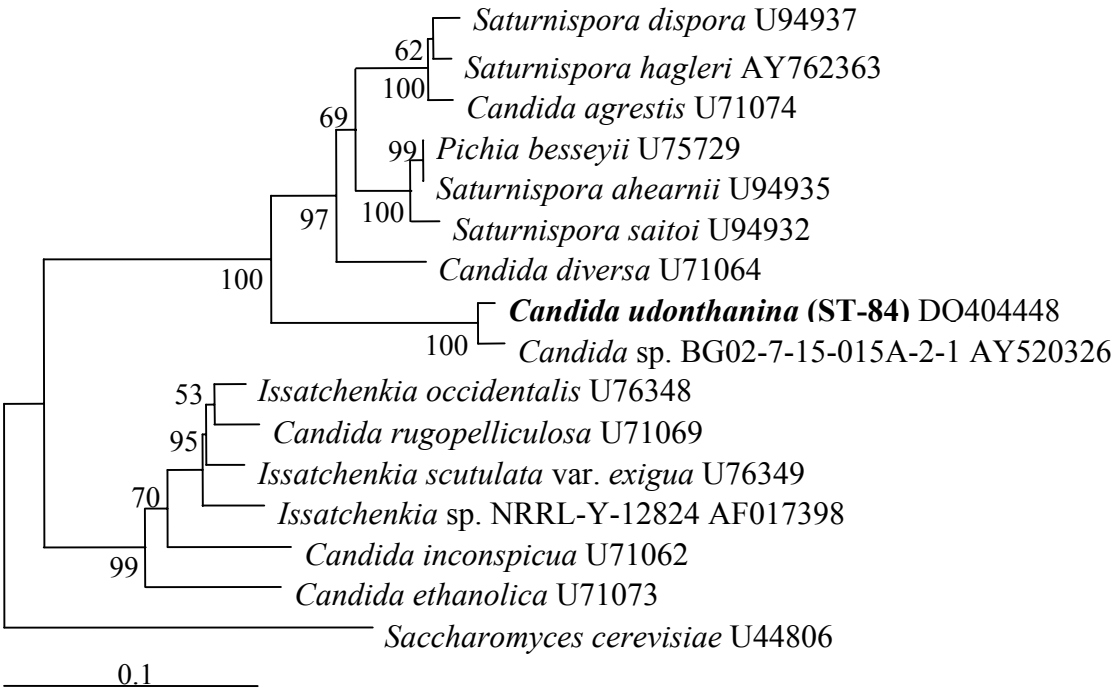

Figure 32 Phylogenetic tree for a new species, *Candida udonthanina,* constructed by the neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the bootstrap values of 1,000 resamplings.

***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.

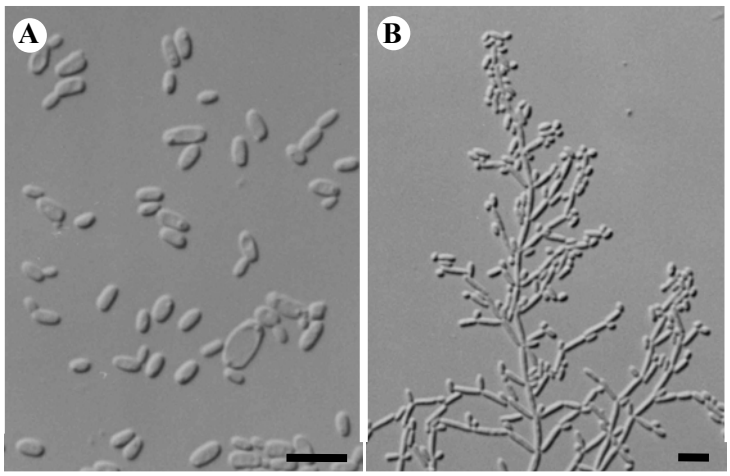

Figure 33 Morphological characteristics of *Candida udonthanina* (ST-84); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

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.

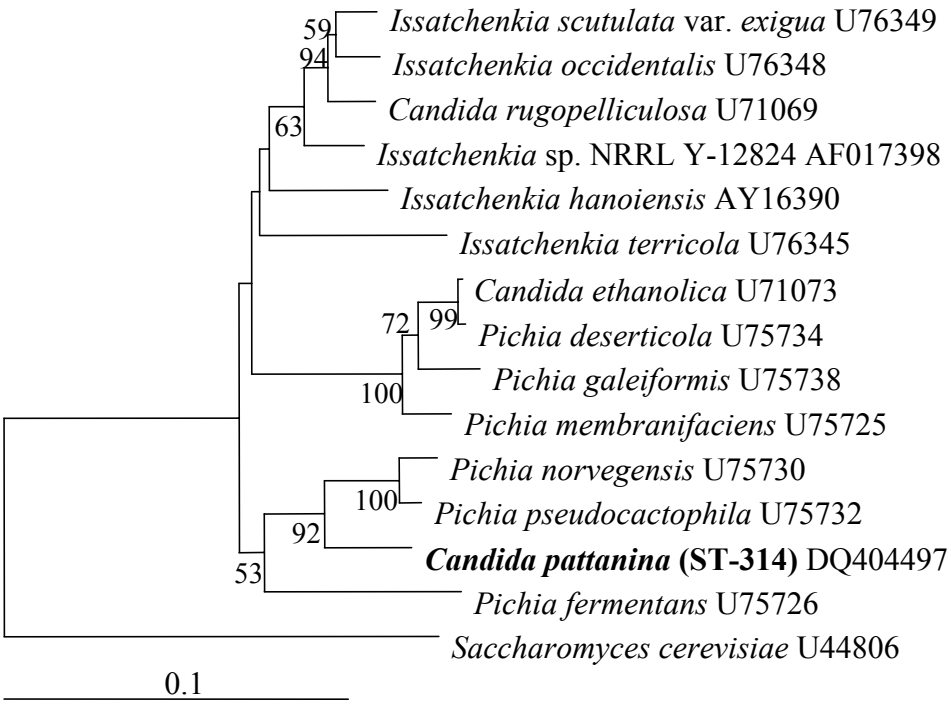

Figure 34 Phylogenetic tree for a new species, *Candida pattanina,* constructed by the neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the bootstrap values of 1,000 resamplings.

***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.

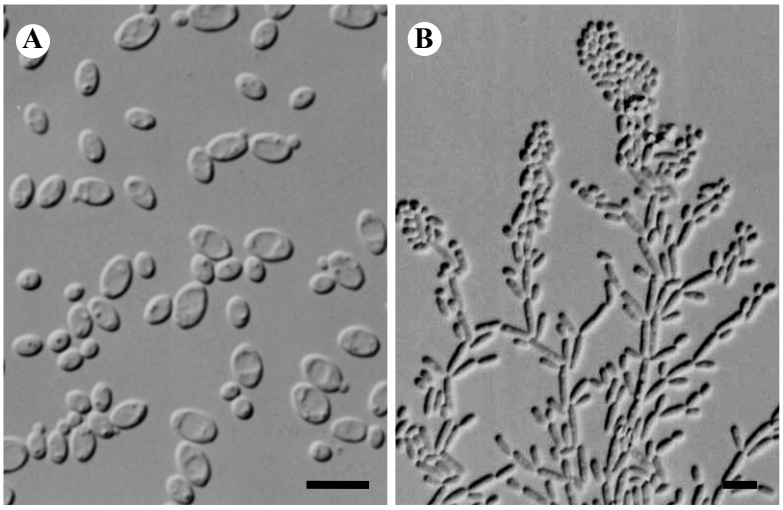

Figure 35 Morphological characteristics of *Candida pattanina* (ST-314); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

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).

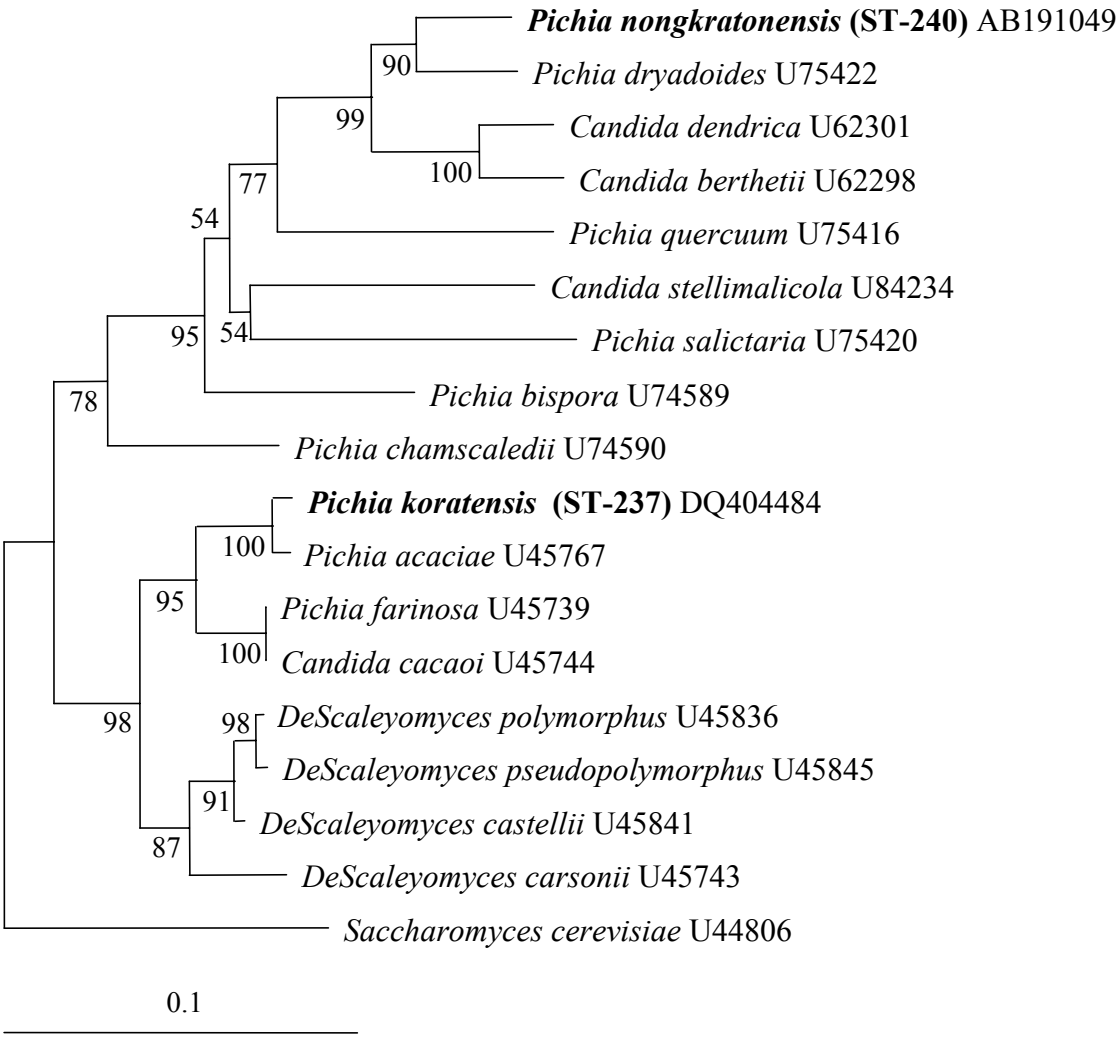

Figure 36 Phylogenetic tree for *Pichia koratensis* and *Pichia nongkratonensis* constructed by neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the values from 1,000 replicate bootstrap resamplings.

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.

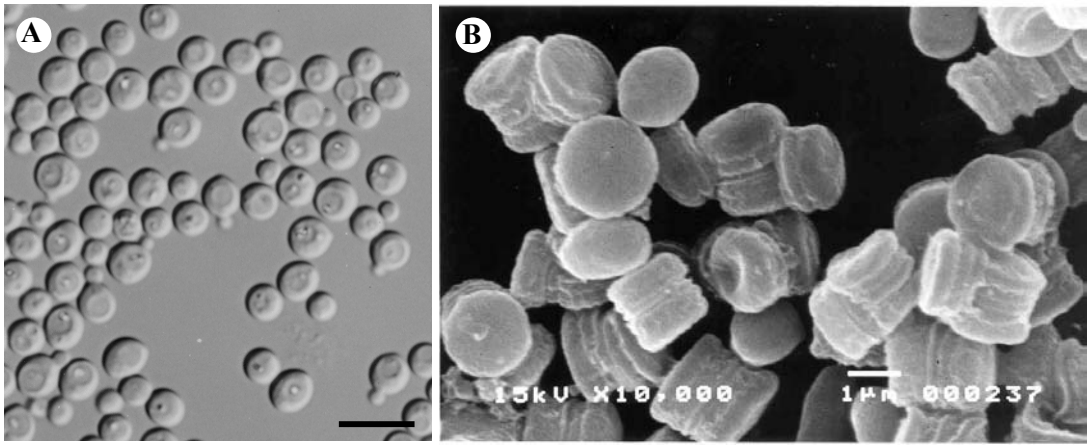

Figure 37 Morphological characteristics of *Pichia koratensis* (ST-237); (A) cells grown in YMB for 3 days at 25°C, Scale = 10 μm; (B) SEM picture of ascospore produced on YM agar after 14 days at 25°C.

***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.

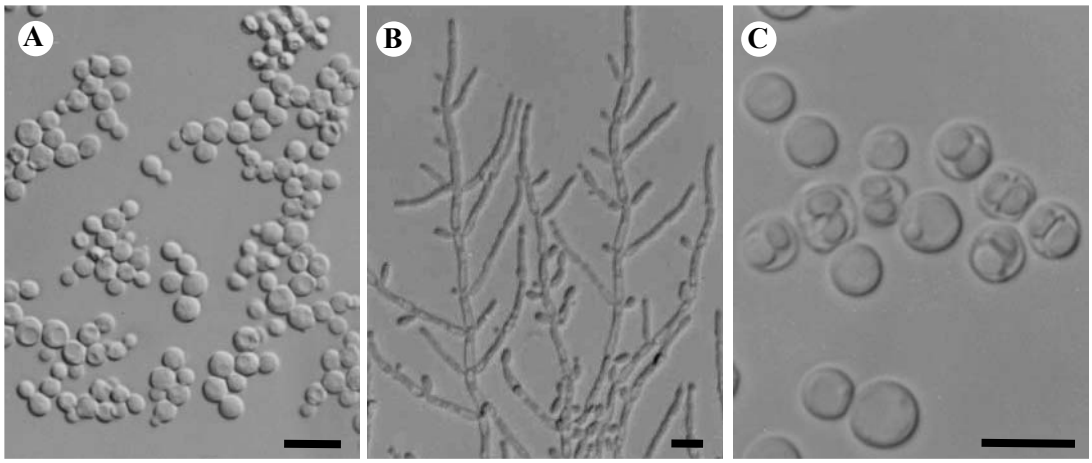

Figure 38 Morphological characteristics of *Pichia nongkratonensis* (ST-240); (A) cells grown in YMB for 3 days at 25°C; (B) pseudomycelium produced on YM agar after 7 days at 25°C; (C) ascospore produced on YM agar after 14 days at 25°C. Scales = 10 μm.

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).

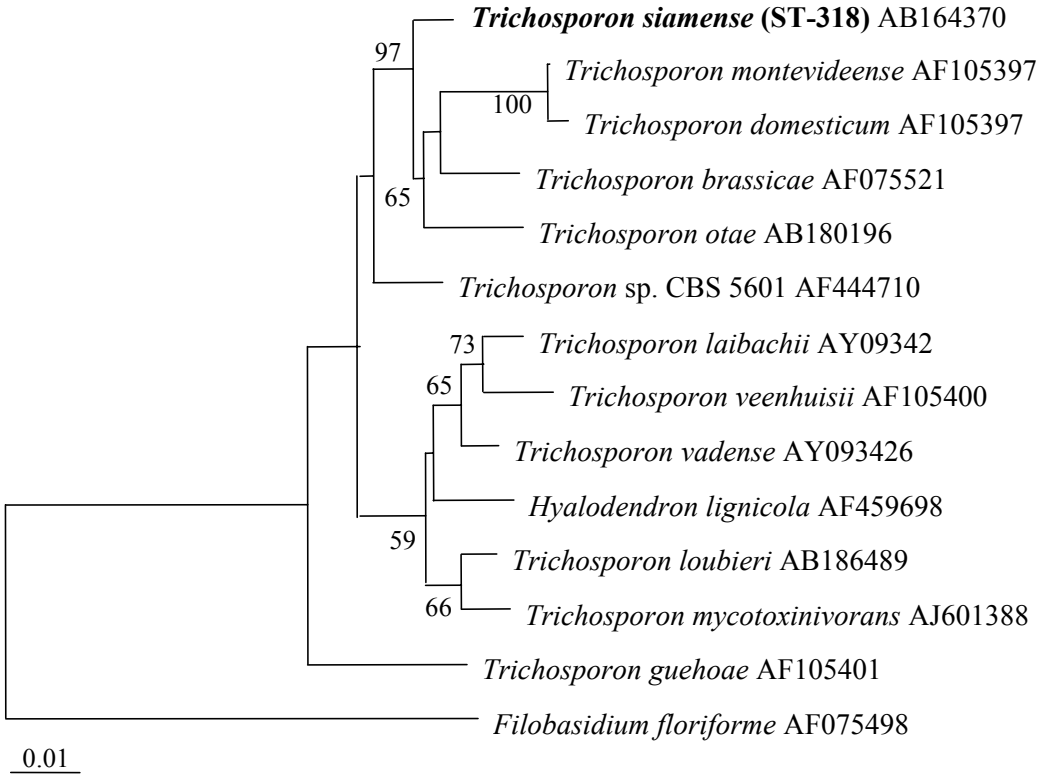

Figure 39 Phylogenetic tree for a new basidiomycetous yeast, *Trichosporon siamense,* constructed by neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The numerals indicate the values from 1,000 replicate bootstrap resamplings. Sequences were retrieved from the NCBI databases under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

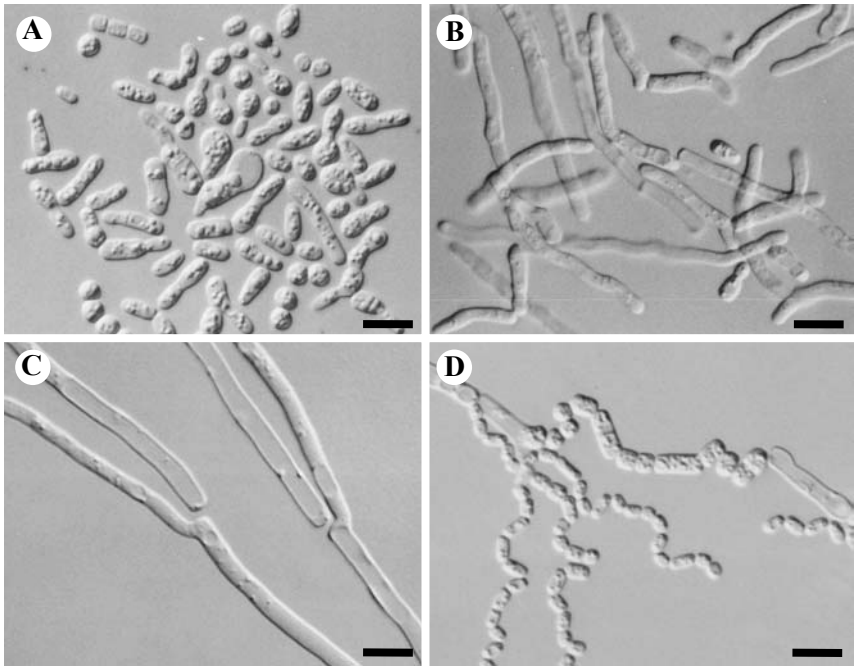

Figure 40 Morphological characteristics of *Trichosporon siamense* (ST-318); (A) cells grown in YMB for 3 days at 25°C; (B, C and D) mycelium produced on YM agar after 7 days at 25°C. Scales = 10 μm.

CONCLUSION

As already discussed, 116 strains (41.0%) out of 283 isolated from some natural habitats of Thailand were predicted to represent hitherto undescribed species of yeasts based on the D1/D2 sequences of 26S rDNA. This is quite different from yeasts found in fermented foods and related materials and resemble ballistoconidium forming yeasts found in the phyllosphere of Thailand.

In the present study, 283 strains isolated from various kinds of natural habitats collected in Thailand were identified as 58 known species of 26 genera, 76 species of 21 genera of hitherto undescribed species, based on the D1/D2 sequences. The result showed that 41.0% (116 strains out of 283 strains) of isolates belong to undescribed species. This frequency is much higher than that of fermented foods and related substrates and ballistoconidium-forming yeasts from Bangkok and southern seacoast from Bangkok to Pattaya and resembles the case of ballistoconidium-forming yeasts from Sakaerat. It is considered that yeasts in the natural environment of Thailand, especially in protected forests, are very rich in biodivesity, not only in ballistoconidium-forming yeasts previous reported but also in ascomycetous yeasts and basidiomycetous yeasts other than ballistoconidium-forming yeasts.

Almost all the undescribed species predicted based on D1/D2 sequences are considered to represent new species with high possibilities though detailed taxonomic studies are required before the description of new species. As discussed in the text, the detailed taxonomic studies were carried out on 21 species. They were described as *Candida easanensis* sp. nov., *Candida flosculi* sp. nov., *Candida hasegawae* sp. nov., *Candida jaroonii* sp. nov., *Candida kazuoi* sp. nov., *Candida koratica* sp. nov., *Candida lignicola* sp. nov., *Candida nakhonratchasimensis* sp. nov., *Candida pattanina* sp. nov., *C. pattaniensis* sp. nov., *Candida songkhlaensis* sp. nov., *Candida thailandica* sp. nov., *Candida udonthanina* sp. nov., *Candida* sp. (ST-331), *Hanseniaspora thailandica* sp. nov., *Kloeckera siamensis* sp. nov., *Kloeckera songkhlaensis* sp. nov., *Kloeckera tradensis* sp. nov., *Pichia koratensis* sp. nov., *Pichia nongratonensis* sp. nov., and *Trichosporon siamense* sp. nov.

It is interesting that the number of strains isolated per species is low in undescribed species in the present study and in 92.1% of species only 1 strain was isolated. It is assumed that the cell populations of these species are small in the natural habitats. In contrast, two or more strains were often isolated in the case of known species. The number of strains is high in popular known species such as *Candida tropicalis, Saccharomyces cerevisiae* and *Cryptococcus heveanensis*. It is considered that these popular species were described in the early history of yeast taxonomy since many cells are living in the natural environment in these species and researchers have many chances to find these species.

In D1/D2 domain sequences, 14 species of undescribed yeasts showed nucleotide substitutions more than 60 (10 %) from closest known species. These species may represent new groups, probably new genera or families, of yeasts. It is concluded that so many hitherto unknown yeast species are living in the natural environment of Thailand and many new groups, new genera or new families, will be found from these unknown yeasts. The study of these yeasts will much contribute to the progress of yeast science and technology, not only to the yeast systematics but also to the yeast technologies such as the production of useful substances and treatment of wastes because various kinds of new genes will be found.

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APPENDIX

APPENDIX A

MEDIA

Most of media used in this study are described by Yarrow (1998)

1. Yeast extract malt extract (YM) agar

Yeast extract	3.0	g
Malt extract	3.0	g
Peptone	5.0	g
Glucose	10.0	g
Agar	15.0	g
Distilled water	1000	ml
Sterilized at 121°C for 15 min		

2. Yeast extract malt extract (YM) broth

Yeast extract	3.0	g
Malt extract	3.0	g
Peptone	5.0	g
Glucose	10.0	g
Distilled water	1000	ml
Sterilized at 121°C for 15 min		

3. Yeast extract malt extract (YM) agar plates supplemented with chloramphinical (100 μg/l) and sodium propionate (0.2%).

Yeast extract	3.0	g
Malt extract	3.0	g
Peptone	5.0	g
Glucose	10.0	g
Chloramphenical	100	μg
Sodium propionate	2.0	g
Agar	15	g
Distilled water	1000	ml
Sterilized at 121°C for 15 min		

4. Yeast extract malt extract (YM) broth supplemented with chloramphinical (100 μg/l) and sodium propionate (0.2%).

Yeast extract	3.0	g
Malt extract	3.0	g
Peptone	5.0	g
Glucose	10.0	g
Chloramphenical	100	μg
Sodium propionate	2.0	g
Distilled water	1000	ml
Sterilized at 121°C for 15 min		

5. Yeast extract malt extract (YM) supplemented with 10% glycerol

Yeast extract	3.0	g
Malt extract	3.0	g
Peptone	5.0	g
Glucose	10.0	g
Glycerol	100	ml
Distilled water	1000	ml
Sterilized at 121°C for 15 min		

6. Yeast Peptone Dextrose (YPD)

Yeast extract	10.0	g
Peptone	10.0	g
Glucose	20.0	g
Glycerol	100.0	ml
Distilled water	1000	ml
Sterilized at 121°C for 15 min		

7. Acetate–GSH agar

Potassium acetate	15.0	g
Glucose	0.2	g
Yeast extract	0.1	g
Glutathione	10.0	mM
Agar	15.0	g
Distilled water	1000	ml

Sterilized at 121°C for 15 min

8. 5% malt extract agar

Malt extract	50	g
Agar	15	g
Distilled water	1000	ml

Sterilized at 115°C for 15 min

9. Stock carbon solution (10X)

Yeast Nitrogen Base (Difco)	6.7	g
Carbon compound	5.0	g
Distilled water	100	ml

Sterilized by filtration and stored in a freezer at -20°C until use

10. Stock nitrogen solution (10X)

Bacto Yeast Carbon Base (Difco)	11.7	g
Nitrogen compound*	x	g
Distilled water	100	ml

Sterilized by filtration and stored in a freezer at -20°C until use

* Nitrogen compound: $(NH_4)_2SO_4$ (0.5 g) or KNO_3 (0.78 g) or $NaNO_2$ (0.26 g) or ethylamine-HCl (0.64 g) or L-lysine-HCl (0.56 g) or cadaverine (0.68 g)

11. Yeast Carbon Base (YCB) medium:

1) Stock YCB solution (10X)

Bacto Yeast Carbon Base (Difco)	11.7	g
Distilled water	100	ml

Sterilized by filtration and stored in a freezer at -20°C until use

2) Ten fold of stock YCB solution (0.2 ml) was added to 13x100 mm test tube containing 1.8 ml sterilized distilled water

12. Yeast Nitrogen Base (YNB) medium

1) Stock YNB solution (10X)

Bacto Yeast Nitrogen Base (Difco)	6.7	g
Distilled water	100	ml

Sterilized by filtration and stored in a freezer at -20°C until use

2) Ten fold of stock YNB solution (0.2 ml) was added into 13 x 100 mm test tube containing 1.8 ml sterilized distilled water.

13. Carbon assimilation medium

Ten fold of stock carbon solution (0.2 ml) was added to 13 x 100 mm test tube containing 1.8 ml sterilized distilled water.

14. Nitrogen agar plate:

Ninety ml of 1.67 % agar solution was sterilized at 121°C for 15 min and left for cool down to 50-55°C, then 10 ml of stock nitrogen solution (10X) was added, mixed well and poured into the petri dishes.

15. Fermentation test medium

1) Basal medium

Yeast extract	4.5	g
Peptone	7.5	g
Bromothymol blue	small amount	
Distilled water	1000	ml

Fermentation basal medium (2 ml) was distributed into the cotton plugged test tubes (13x100 mm), insert Durham tube and sterilize at 121°C for 15 min. After cool down, add concentrate sugar, which was sterilized by membrane filtration to make the final concentration at 2% sugar (except 4% raffinose).

16. Gelatin liquefaction

Gelatin	100	g
Glucose	5.0	g
Bacto Yeast Nitrogen Base	6.7	g
Distilled water	1000	ml

Sterilized at 121°C for 15min

The medium in tubes was allowed to gel with the tubes in a vertical position

17. Vitamin requirement test medium

1) Vitamin requirement basal medium

Glucose	10.0	g
Vitamin-free cassamino acids (Difco)	5.0	g
$KH_2PO_4.7H_2O$	1	g
$MgSO_4$	0.5	g
NaCl	0.1	g
$CaCl_2$	0.1	g
Distilled water	1000	ml

Adjust pH = 5.5

Sterilized at 121°C for 15min

2) Vitamin stock solutions (100X) are prepared as follows:

- Biotin: 0.02 mg Biotin was dissolved in 100 ml distilled water, to make the final concentration at 2 μg/ml.
- Ca-Pantothenate: 4 mg Ca-Pantothenate was dissolved in 100 ml distilled water, to make the final concentration at 400 μg/ml.
- Folic acid: 0.02 mg Folic acid was dissolved in 100 ml distilled water, to make the final concentration at 2 μg/ml.
- Inositol: 20 mg Inositol was dissolved in 100 ml distilled water, to make the final concentration at 2,000 μg/ml.
- Niacin: 4 mg Niacin was dissolved in 100 ml distilled water, to make the final concentration at 400 μg/ml.
- ρ-Aminobenzoic acid (PABA): 2 mg PABA was dissolved in 100 ml distilled water, to make the final concentration at 200 μg/ml.
- Pyridoxine-HCl: 4 mg Pyridoxine-HCl was dissolved in 100 ml distilled water, to make the final concentration at 400 μg/ml.
- Riboflavin: 2 mg Riboflavin was dissolved in 100 ml distilled water, to make the final concentration at 200 μg/ml.
- Thimine-HCL: 4 mg Thimine-HCl was dissolved in 100 ml distilled water, to make the final concentration at 400 μg/ml.

3) Active test medium

One hundred folds of vitamin solution were diluted with 9 volumes of the basal medium and sterilized by membrane filtration. The composition of vitamins in each medium is shown in Table 32. A mount of 0.2 ml of sterilized 10X vitamin mixture was added aseptically into cotton plugged tube containing 1.8 ml of the sterilized basal medium then incubated at 25°C for 3 days at room temperature to confirm the sterility.

Appendix Table A1 Vitamin requirement test

Vitamin	Medium No.											Final concentration (μg/ml)
	1	2	3	4	5	6	7	8	9	10	11	
Biotin	X	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	2
Pantothenate	X	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	400
Folic acid	X	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	2
Inositol	X	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	2000
Niacin	X	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	400
PABA	X	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	200
Pyridoxine	X	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	400
Riboflavin	X	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	200
Thiamine	X	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	400

X: No add 10X vitamin solution.

✓: Add 10X vitamin solution.

Remark: Medium No.1: Negative control (with out vitamin); No.2: Biotin requirement; No.3: Pantothenate requirement; No.4: Folic acid requirement; No.5: Inositol requirement; No.6: Niacin requirement; No.7: PABA requirement; No.8: Pyridoxine requirement; No.9: Riboflavin requirement; No.10: Thiamine requirement and No.11: Positive control.

18. Lipase test medium

Fresh beef suet was melted and filtrated, then sterilized at 121°C for 15 min. Molten fat (0.5 ml) was pored into a slightly warmed sterilized petri dish and spread over the bottom and surplus fat was removed. Preti dish was moved into a refrigerator and left for 1-2 hr. Molten YM agar was cooled to about 40°C, and then poured over the fat.

19. Acid formation from glucose

Glucose	50.0	g
Calcium carbonate	5.0	g
Yeast extract	5.0	g
Agar	20.0	g
Distilled water	1000	ml

Sterilized at 121°C for 15min

Tubes was cooled to around 45°C and agitated gently to resuspend the chalk and then slanted or pored to plate.

20. Cycloheximide resistant

1) Basal medium

Cycloheximide 1 g in acetone	2.5	ml
Bacto Yeast Nitrogen Base (Difco)	6.7	g
Distilled water	100	ml

Sterilized by filtration

2) Active medium

Amount of 0.2 ml of 10 fold basal medium was added to 13 x 100 mm test tube containing 1.8 ml sterilized distilled water.

3) 50% glucose medium

This medium prepared by dissolving 13 g of agar in 1% solution of yeast extract, and then 500 g of glucose was added and sterilized at 110°C, for 10 min.

REAGENTS

1. Lugol's solution

Iodine	1	g
Potassium iodide	2	g
Demineralized water	300	ml

2. Lysis buffer

Tris (pH 8.0)	100	mM
EDTA (pH 8.0)	30	mM
Sodium dodecyl sulfate (SDS)	0.5	%

3. 10X SSC

NaCl	1.5	M
Trisodium citrate	0.15	M
Adjust pH = 7.0		

4. 1/15 M phosphate buffer with 0.1 M EDTA (pH 7.5)

K_2HPO_4 (1/15 M)	86.6	ml
KH_2PO_4 (1/15 M)	13.4	ml
Mix and adjust pH =7.5		
Add EDTA	0.1	M

5. Acetate-EDTA

Sodium acetate (pH 8.0)	3.0	M
EDTA (pH 8.0)	0.5	M

6. TBE buffer

Tris-borate	90.0	mM
EDTA (pH 8.0)	1.0	mM

7. SCE

Sorbitol	1.0	M
Sodium citrate	0.1	M
EDTA (pH 7.0)	0.006	M

Sterilized at 121°C for 15 min

8. Zymolyase solution (1 ml)

Zymolyase 100T	3	mg
2-mercaptoethanol	0.1	ml
SCE	0.9	ml

9. 10X TE buffer

Tris-HCl	0.1	M
EDTA (pH 8.0)	0.01	M

Sterilized at 121°C for 15 min

10. RNase solution

RNase	5	mg/ml
Tris-HCl (pH 7.5)	10	mM
NaCl	15	mM

Boil for 15 min and cooled solution was stored at -20°C

11. Proteinase K solution

Proteinase K	20	mg
Demineralized water	1	ml

Stored at –20°C

12. Tris-saturated phenol

Phenol	250	ml
Chloroform	240	ml
Isoamyl alcohol	10	ml

Equilibrated with Tris-HCl (pH 8.0)

13. PBS

$Na_2HPO_4.12H_2O$	8	mM
KH_2PO_4	1.5	mM
NaCl	137	mM
KCl	2.7	mM
pH 7.2		
Sterilized at 121°C for 15 min		

14. PBSM1, 2

$Na_2HPO_4.12H_2O$	8	mM
KH_2PO_4	1.5	mM
NaCl	137	mM
KCl	2.7	mM
$MgCl_2$	0.1	M
pH 7.2		
Prepare before use and do not autoclave		

15. Photobiotin solution

Photobiotin acetate	1	mg
Purified water	1	ml
(Prepared in 1.5ml Eppendorf tube, and vigorous shaking)		
Tris-HCl	0.1	M
EDTA (pH 9.0)	1	mM

16. Pre-hybridization solution (10 ml)

Deionized formamide	5.0	ml
20X SSC	1.0	ml
50X Denhardt solution	1.0	ml
10 mg/ml Salmon DNA (Type III, Sigma)	0.1	ml
Sterilized distilled water	2.9	ml

17. 20X SSC

NaCl	3.0	M
Trisodium citrate	0.3	M
Adjust pH = 7.0		

18. 0.1 mg/ml salmon DNA

Salmon DNA	10	mg
TE	1	ml

Denature by heating at 100°C for 5 min, then rapidly cooling in ice water

19. Hybridization solution (10 ml)

Deionized formamide	5.0	ml
20X SSC	1.0	ml
50X Denhardt solution	1.0	ml
10 mg/ml Salmon DNA (Type III, Sigma)	0.1	ml
50% Dextran sulphate sodium salt	1.0	ml
Denatured photobiotin labeled DNA	5.0	ml
Sterilized distilled water	2.9	ml

20. PBS-BSA-Triton solution

Triton X-100	0.1	ml
PBS	100	ml
Autoclave at 121°C for 15 min		
Add bovine serum albumin (Fraction V) (after autoclave)	0.5	g

21. SABG solution

Streptavidin-β-galactosidase	1	μl
PBS-BSA-Triton solution	1	ml

22. MUF-Gal solution (0.1 mg/ml)

4-methylumbelliferyl-β-galactopyranoside	0.1	mg
DMSO	25	μl
PBSM2	975	μl

23. TE buffer (pH 9.0)

Tris-HCl (pH 9.0)	0.1	M
EDTA (pH 8.0)	1.0	mM
Adjust pH = 9.0		

APPENDIX B

Appendix Table B1 Places where sample were collected.

Strains	Source	Place	Date of Collected
ST-1	Mushroom	Sakaerat (Chachoengsao)	17-Oct-00
ST-2	Mushroom	Sakaerat (Chachoengsao)	17-Oct-00
ST-3, ST-4	Mushroom	Sakaerat (Chachoengsao)	17-Oct-00
ST-5	Mushroom	Sakaerat (Chachoengsao)	17-Oct-00
ST-7	Mushroom	Sakaerat (Chachoengsao)	17-Oct-00
ST-8	Mushroom	Sakaerat (Chachoengsao)	17-Oct-00
ST-9	Moss	Sakaerat (Chachoengsao)	17-Oct-00
ST-11	Flower (*Ixora robbii* Loud)	Chatuchak (Bangkok)	30-Oct-00
ST-12	Flower (*Cassia suratensis*)	Chatuchak (Bangkok)	30-Oct-00
ST-13	Flower of Coconut (*Cocos nucifera*)	Chatuchak (Bangkok)	30-Oct-00
ST-14	Moss	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-15	Insect frass	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-17	Insect frass	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-18, ST-19	Mushroom	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-20, ST-21	Flower	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-22	Flower	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-23, ST-24	Insect frass	Khao Yai (Nakhon Ratchasima)	04-Nov-00
ST-26	Flower	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-27, ST-28, ST-29	Flower	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-30, ST-31	Moss	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-32	Flower	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-33	Insect frass	Khao Yai (Nakhon Ratchasima)	03-Nov-00

Appendix Table B1 (Continued)

Strains	Source	Place	Date of Collected
ST-34	Insect frass	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-35, ST-36	Moss	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-37	Insect frass	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-38	Insect frass	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-39	Insect frass	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-40	Insect frass	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-41	Insect frass	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-43	Insect frass	Khao Yai (Nakhon Ratchasima)	03-Nov-00
ST-46	Flower of Coconut (*Cocos nucifera*)	Chatuchak (Bangkok)	12-Nov-00
ST-48	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-49	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-50	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-53	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-54	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-55	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-52	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-57	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-59, ST-60	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-61	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-62, ST-63	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-64	Insect frass	Putthamontol (Nakhon Pathom)	07-Dec-00
ST-68	Mushroom	Chatuchak (Bangkok)	16-Jan-01

Appendix Table B1 (Continued)

Strains	Source	Place	Date of Collected
ST-69	Insect frass	Khuan U-Bolratana (Khon Kaen)	01-Feb-01
ST-70, ST-71, ST-72	Insect frass	Khuan U-Bolratana (Khon Kaen)	01-Feb-01
ST-73, ST-74	Insect frass	Khuan U-Bolratana (Khon Kaen)	01-Feb-01
ST-75	Insect frass	Khuan U-Bolratana (Khon Kaen)	01-Feb-01
ST-77, ST-78	Insect frass	Khuan Haui Laung (Udon Thani)	02-Feb-01
ST-79, ST-80	Insect frass	Khuan Haui Laung (Udon Thani)	02-Feb-01
ST-81, ST-82	Insect frass	Khuan Haui Laung (Udon Thani)	02-Feb-01
ST-83, ST-84, ST-85	Insect frass	Khuan Haui Laung (Udon Thani)	02-Feb-01
ST-87, ST-88, ST-90, ST-91	Leave (*Eugenia* sp.)	Than Tong Waterfall (Nong Khai)	02-Feb-01
ST-92, ST-93, ST-94	Leave (*Triumfetta* sp.)	Than Tong Waterfall (Nong Khai)	02-Feb-01
ST-95, ST-96	Insect frass	Than Tong Waterfall (Nong Khai)	02-Feb-01
ST-98	Leave (*Bambusa* sp.)	Than Tong Waterfall (Nong Khai)	02-Feb-01
ST-100, ST-101, ST-102, ST-105	Leave (*Pharagmites* sp.)	Hin Mak Peang Temple (Nong Khai)	02-Feb-01
ST-106, ST-107	Leave	Hin Mak Peang Temple (Nong Khai)	02-Feb-01
ST-108	Insect frass	Hin Mak Peang Temple (Nong Khai)	02-Feb-01
ST-111, ST-115	Leave (*Merremia* sp.)	Than Tong Waterfall (Nong Khai)	02-Feb-01
ST-116	Insect frass	Than Tong Waterfall (Nong Khai)	02-Feb-01
ST-117	Insect frass	Nong Bo Kok (Nong Khai)	03-Feb-01
ST-119	Leave (*Eucaliptus* sp.)	Nong Bo Kok (Nong Khai)	03-Feb-01
ST-120, ST-121, ST-122	Insect frass	Pu Wao (Nong Khai)	03-Feb-01
ST-123	Leave	Pu Wao (Nong Khai)	03-Feb-01
ST-124, ST-125	Insect frass	Pu Wao (Nong Khai)	03-Feb-01

Appendix Table B1 (Continued)

Strains	Source	Place	Date of Collected
ST-126, ST-127	Insect frass	Pu Wao (Nong Khai)	03-Feb-01
ST-128	Leave (*Stepblus* sp.).	Pu Wao (Nong Khai)	03-Feb-01
ST-132	Leave (*Memycylon* sp.)	Pu Wao (Nong Khai)	03-Feb-01
ST-136	Insect frass	Pu Wao (Nong Khai)	03-Feb-01
ST-140	Leave (*Hymenoperamis* sp.)	Pu Wao (Nong Khai)	03-Feb-01
ST-144, ST-145	Leave (*Thelypteris parasitica*)	Pu Wao (Nong Khai)	03-Feb-01
ST-146, ST-147	Insect frass	Ban Paeng Nakhon Phanom)	03-Feb-01
ST-148	Insect frass	Ban Paeng (Nakhon Phanom)	03-Feb-01
ST-149	Insect frass	Ban Paeng (Nakhon Phanom)	03-Feb-01
ST-150, ST-151, ST-153	Leave	Ban Paeng (Nakhon Phanom)	03-Feb-01
ST-156, ST-159	Leave (*Thelypteris interrupta*)	Ban Paeng (Nakhon Phanom)	03-Feb-01
ST-160	Insect frass	Nong Han (Nakhon Phanom)	03-Feb-01
ST-161	Insect frass	Nong Han (Nakhon Phanom)	03-Feb-01
ST-162	Insect frass	Nong Han (Nakhon Phanom)	03-Feb-01
ST-164	Insect frass	Huai Deag (Sakon Nakhon)	04-Feb-01
ST-165	Insect frass	Huai Deag (Sakon Nakhon)	04-Feb-01
ST-166	Leave (*Artocarpus* sp.)	Phuphan (Sakon Nakhon)	04-Feb-01
ST-170, ST-172, ST-173	Leave (*Imperata cylindrica*)	Num Pung Dam (Sakon Nakhon)	04-Feb-01
ST-174, ST-175, ST-176	Leave (*Eragrostis* sp.)	Num Pung Dam (Sakon Nakhon)	04-Feb-01
ST-178, ST-181	Leave (*Pennisetum* sp.)	Num Pung Dam (Sakon Nakhon)	04-Feb-01
ST-182, ST-183, ST-184, ST-185, ST-186, ST-187	Leave (*Ageratum* sp.)	Num Pung Dam (Sakon Nakhon)	04-Feb-01

Appendix Table B1 (Continued)

Strains	Source	Place	Date of Collected
ST-192	Leave (*Commelina* sp.)	Song Dao (Sakon Nakhon)	04-Feb-01
ST-194	Insect frass	Kut Bak (Sakon Nakhon)	04-Feb-01
ST-195, ST-197, ST-198, ST-201, ST-202	Leave (*Manihot esculenta*)	Ban Tai (Sakon Nakhon)	04-Feb-01
ST-204	Leave (*Pederia* sp.)	Ban Tai (Sakon Nakhon)	04-Feb-01
ST-206, ST-208	Leave (*Erechtites* sp.)	Ban Tai (Sakon Nakhon)	04-Feb-01
ST-209	Insect frass	Waritchaphum (Sakhon Nakhon)	04-Feb-01
ST-211	Insect frass	Ban Chiong (Udon Thani)	04-Feb-01
ST-213	Leave (*Musa sapientum*)	Nong Meg (Udon Thani)	04-Feb-01
ST-214	Insect frass	Lum Pao Dam (Kalasin)	05-Feb-01
ST-217	Leave (*Bauhinia* sp.)	Huai Prong (Kalasin)	05-Feb-01
ST-223	Insect frass	Nong Laung (Amnat Charoen)	05-Feb-01
ST-224, ST-225	Insect frass	Nong Laung (Amnat Charoen)	05-Feb-01
ST-226	Leave	Nong Bo Mhu (Yasothon)	05-Feb-01
ST-228	Insect frass	Nong Bo Mhu (Yasothon)	05-Feb-01
ST-229	Insect frass	Nong Bo Mhu (Yasothon)	05-Feb-01
ST-231	Insect frass	Nong Kratone (Nakhon Ratchasima)	06-Feb-01
ST-232, ST-233	Insect frass	Nong Kratone (Nakhon Ratchasima)	06-Feb-01
ST-234	Insect frass	Nong Kratone (Nakhon Ratchasima)	06-Feb-01
ST-235, ST-236, ST-237	Insect frass	Nong Kratone (Nakhon Ratchasima)	06-Feb-01
ST-238, ST-239, ST-240, ST-241	Insect frass	Nong Kratone (Nakhon Ratchasima)	06-Feb-01
ST-246	Mushroom	Hala Bala (Narathiwat)	10-Mar-01

Appendix Table B1 (Continued)

Strains	Source	Place	Date of Collected
ST-247	Moss	Hala Bala (Narathiwat)	10-Mar-01
ST-248	Insect frass	Hala Bala (Narathiwat)	10-Mar-01
ST-249	Insect frass	Hala Bala (Narathiwat)	10-Mar-01
ST-250	Insect frass	Hala Bala (Narathiwat)	10-Mar-01
ST-251	Insect frass	Hala Bala (Narathiwat)	10-Mar-01
ST-252	Insect frass	Hala Bala (Narathiwat)	10-Mar-01
ST-253	Mushroom	Hala Bala (Narathiwat)	10-Mar-01
ST-258	Flower (*Plumeria acuminata*)	Tak Bi (Narathiwat)	10-Mar-01
ST-265	Flower (*Ixora* sp.)	Hala Bala (Narathiwat)	10-Mar-01
ST-266	Moss	Hala Bala (Narathiwat)	10-Mar-01
ST-267	Moss	Hala Bala (Narathiwat)	11-Mar-01
ST-269	Moss	Hala Bala (Narathiwat)	11-Mar-01
ST-296, ST-297	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-298	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-299	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-300	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-303	Mushroom	Ko Yao (Pattani)	12-Mar-01
ST-305, ST-306, ST-307	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-308	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-309	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-312, ST-313, ST-314	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-315, ST-316	Insect frass	Ko Yao (Pattani)	12-Mar-01

Appendix Table B1 (Continued)

Strains	Source	Place	Date of Collected
ST-317, ST-318, ST-319	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-310, ST-311	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-320	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-321	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-322	Insect frass	Ko Yao (Pattani)	12-Mar-01
ST-323	Mushroom	Hala Bala (Narathiwat)	13-Mar-01
ST-326, ST-327	Insect frass	Tone Nga Chang Waterfall (Songkhla)	13-Mar-01
ST-328, ST-329	Insect frass	Tone Nga Chang Waterfall (Songkhla)	13-Mar-01
ST-330	Insect frass	Tone Nga Chang Waterfall (Songkhla)	13-Mar-01
ST-331	Insect frass	Tone Nga Chang Waterfall (Songkhla)	13-Mar-01
ST-332, ST-333	Insect frass	Tone Nga Chang Waterfall (Songkhla)	13-Mar-01
ST-334	Insect frass	Tone Nga Chang Waterfall (Songkhla)	13-Mar-01
ST-335	Insect frass	Tone Nga Chang Waterfall (Songkhla)	13-Mar-01
ST-336	Insect frass	Tone Nga Chang Waterfall (Songkhla)	13-Mar-01
ST-337, ST-338, ST-339	Insect frass	Tone Nga Chang Waterfall (Songkhla)	13-Mar-01
ST-340	Insect frass	Tone Nga Chang Waterfall (Songkhla)	13-Mar-01
ST-343	Mushroom	Prom Lok Waterfall (Surat Thani)	14-Mar-01
ST-344	Insect frass	Prom Lok Waterfall (Surat Thani)	14-Mar-01
ST-356	Insect frass	Bangkhen (Bangkok)	30-May-01
ST-358	Mushroom	Tone Nga Chang Waterfall (Songkhla)	29-Sep-01
ST-361	Mushroom	Tone Nga Chang Waterfall (Songkhla)	30-Sep-01
ST-362, ST-363	Mushroom	Tone Nga Chang Waterfall (Songkhla)	30-Sep-01

Appendix Table B1 (Continued)

Strains	Source	Place	Date of Collected
ST-364	Mushroom	Krung Ching Waterfall (Nakhon Si Thammarat)	30-Sep-01
ST-365, ST-366	Mushroom (*Maraumius* sp.)	Krung Ching Waterfall (Nakhon Si Thammarat)	30-Sep-01
ST-368	Mushroom	Krung Ching Waterfall (Nakhon Si Thammarat)	30-Sep-01
ST-370	Mushroom	Krung Ching Waterfall (Nakhon Si Thammarat)	30-Sep-01
ST-375	Mushroom	Krung Ching Waterfall (Nakhon Si Thammarat)	30-Sep-01
ST-377	Mushroom	Nam Nao National Park (Petchabun)	05-Oct-01
ST-380	Mushroom	Sai Yok Waterfall (Kanchanaburi)	16-Oct-01
ST-385	Moss	Mae Hong Son	07-Jan-02
ST-387	Flower (*Bruguiera gymnorrhiza*)	Waeru Mangrove Forest (Trad)	13-Jan-02
ST-388	Flower (*Bruguiera gymnorrhiza*)	Waeru Mangrove Forest (Trad)	13-Jan-02
ST-389	Flower (*Rhizophora apiculata*)	Waeru Mangrove Forest (Trad)	13-Jan-02
ST-390	Flower (*Rhizophora apiculata*)	Waeru Mangrove Forest (Trad)	13-Jan-02
ST-391	Flower (*Sonneratia caseolaris*)	Waeru Mangrove Forest (Trad)	13-Jan-02
ST-394	Insect frass	Pan Pu (Trad)	13-Jan-02
ST-398	Flower (*Durio zibethinus*)	Ban pred Nai (Trad)	14-Jan-02
ST-403	Insect frass	Houy Num Kaow Mangrove Forest (Trad)	14-Jan-02
ST-404	Insect frass	Houy Num Kaow Mangrove Forest (Trad)	14-Jan-02
ST-407	Moss	Houy Num Kaow Mangrove Forest (Trad)	14-Jan-02
ST-410	Flower (*Lumnitzera recemosa*)	Houy Num Kaow Mangrove Forest (Trad)	14-Jan-02
ST-419	Insect frass	Prai Prong Prang (Samut Songkhram)	07-Feb-02
ST-422	Coconut juice (*Cocos nucifera*)	Prai Prong Prang (Samut Songkhram)	07-Feb-02
ST-431	Insect frass	Sakaerat (Chachoengsao)	10-Apr-02

Appendix Table B1 (Continued)

Strains	Source	Place	Date of Collected
ST-433	Insect frass	Sakaerat (Chachoengsao)	10-Apr-02
ST-441	Moss	Ranong Mangrove Biosphere (Ranong)	20-May-02
ST-445	Moss	Ngaow Waterfall (Ranong)	20-May-02
ST-446	Moss	Ngaow Waterfall (Ranong)	20-May-02
ST-448	Moss	Boonyaban Waterfall (Ranong)	20-May-02
ST-449	Moss	Ngaow Waterfall (Ranong)	20-May-02
ST-451	Moss	Ngaow Waterfall (Ranong)	20-May-02
ST-453	Moss	Tone Nga Chang Waterfall (Songkhla)	05-Feb-03
ST-457	Moss	Tone Nga Chang Waterfall (Songkhla)	05-Feb-03
ST-464	Lichen	Tone Nga Chang Waterfall (Songkhla)	05-Feb-03
ST-476	Rotted wood	Tone Nga Chang Waterfall (Songkhla)	05-Feb-03
ST-481	Mushroom	Tone Nga Chang Waterfall (Songkhla)	05-Feb-03
ST-484	Exudate of *Ficus* sp.	Tone Nga Chang Waterfall (Songkhla)	05-Feb-03
ST-490	Flower	Bangkhen (Bangkok)	05-Feb-03
ST-493	Insect frass	Tong Pha Phum (Kanchanaburi)	19-Feb-03
ST-495	Moss	Tong Pha Phum (Kanchanaburi)	19-Feb-03
ST-515	Moss	Tong Pha Phum (Kanchanaburi)	19-Feb-03
ST-516	Insect frass	Tong Pha Phum (Kanchanaburi)	19-Feb-03
ST-529	Insect frass	Tong Pha Phum (Kanchanaburi)	19-Feb-03
ST-533	Flower	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-536	Flower	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-541	Insect frass	Tong Pha Phum (Kanchanaburi)	20-Feb-03

Appendix Table B1 (Continued)

Strains	Source	Place	Date of Collected
ST-543	Moss	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-551	Insect frass	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-552	Insect frass	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-554	Insect frass	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-555	Insect frass	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-568	Moss	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-570	Moss	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-578	Rotted wood	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-583	Moss	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-586	Moss	Tong Pha Phum (Kanchanaburi)	20-Feb-03
ST-613	Rotted fruit (*Psidium guajava*)	Tong Pha Phum (Kanchanaburi)	04-Nov-03

Appendix Table B2 Assimilation of carbon and nitrogen compounds of yeast strains assigned to new species.

Strains	Carbon Assimilation																																							Nitrogen Assimilation					
	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Sol.starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol	a-Methyl-D-glucoside	Salicin	Glucono-δ-lactone	2-Ketogluconic acid	5-Ketogluconic acid	DL-lactic acid	Succinic acid	Citric acid	Inositol	Glucuronic acid	Galacturonic acid	Nitrate	Nitrite	Ethylamine	L-Lysine	Cadaverine
ST-2	+	-	-	+	+	+	+	-	-	-	+	-	+	w	-	-	+	-	-	+	-	+	-	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	+	+
ST-3	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+
ST-4	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+
ST-17	+	+	+	+	+	+	+	+	-	-	+	-	w	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	-	w	+	+	-	-	-	-	-	+	+	+
ST-18	+	+	+	+	+	+	+	-	-	-	+	-	w	+	+	-	+	-	-	+	-	+	-	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	+	+	+
ST-19	+	+	+	+	+	-	+	-	-	-	+	-	w	+	+	+	-	-	-	+	-	+	+	+	+	+	-	+	+	-	-	+	-	-	-	-	-	-	+	+	+
ST-22	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	+	+	+	w	+
ST-26	+	w	-	+	-	+	-	-	-	+	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-	-	-	+	w	-	-	-	+	+	-	-	+
ST-29	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	w	-	-	+	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	+	+	+	-	+
ST-30	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+
ST-32	+	-	+	+	+	-	+	-	-	-	+	-	-	+	-	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	+	+	-	-	-	-	-	+	+	+
ST-33	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	+	+	w	-	+	+	+	-	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	+	+
ST-36	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+	+
ST-37	+	-	-	+	+	+	+	-	-	+	+	w	w	+	-	-	-	+	-	+	-	-	-	+	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	+
ST-43	+	+	+	+	+	+	+	-	-	-	+	-	w	+	+	+	+	+	-	+	-	+	-	+	+	+	-	+	-	w	+	+	-	-	-	-	-	-	w	+	+
ST-49	+	+	+	+	+	-	+	-	-	+	+	+	+	+	-	-	+	-	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+

Appendix Table B2 (Continued)

Strains	Carbon Assimilation																																								Nitrogen Assimilation					
	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Sol.starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol	a-Methyl-D-glucoside	Salicin	Glucono-δ-lactone	2-Ketogluconic acid	5-Ketogluconic acid	DL-lactic acid	Succinic acid	Citric acid	Inositol	Glucuronic acid	Galacturonic acid	Nitrate	Nitrite	Ethylamine	L-Lysine	Cadaverine
ST-50	+	+	+	+	+	-	+	-	-	-	+	+	w	+	+	+	+	-	+	+	-	+	-	+	+	+	-	+	+	-	-	+	+	-	-	+	-	-	+	+	+
ST-57	+	+	+	-	-	-	-	-	-	-	-	+	w	+	-	-	-	-	+	+	-	+	-	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-	+	+	+
ST-59	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	w	+	-	+	+	+	+	+	+	w	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
ST-61	+	+	+	+	+	+	+	-	-	-	-	w	+	+	-	w	w	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+
ST-71	+	+	l	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	s	s	+	+	-	+	-	+	+	+	-	+	+	+	+
ST-73	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	-	l/-	l/-	-	l	-	l	+	+	+	+	+	+	+	+	l	+	+	+	+	+	-	-	+	+	+
ST-78	+	+	l	+	+	+	+	-	-	-	+	-	w	+	+	+	w	+	+	+	+	+	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	+	+	+
ST-79	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	l	+	-	-	+	+	+
ST-84	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+
ST-87	+	+	+	+	+	+	+	-	-	+	+	s	+	+	w/-	s	s	-	+	+	-	+	-	+	+	+	+	+	-	-	s	+	+	-	-	-	+	+	l	s	w
ST-88	s	-	-	-	-	-	+	-	-	-	-	-	-	s	-	-	-	-	-	-	-	-	-	-	-	-	-	w/-	-	-	-	w	-	-	-	-	-	-	+	+	+
ST-90	+	+	+	w/-	-	-	+	-	-	-	-	-	-	+	w/-	w/-	w/-	-	+	+	-	+	+	+	+	-	-	w	-	-	-	w	w	-	-	-	-	-	+	+	+
ST-91	+	-	+	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-
ST-92	+	+	+	+	+	+	+	-	-	+	+	w	+	s	-	+	+	-	+	+	-	+	-	+	+	+	+	+	-	-	w	+	+	-	-	-	+	+	+	-	-
ST-95	+	+	+	+	+	-	+	-	-	-	+	-	-	+	-	-	-	-	+	+	-	+	-	+	+	+	-	+	+	-	+	+	+	-	-	-	-	-	+	+	+
ST-98	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	-	w	+	+	+	+	+	-	+	+	s	+	-	-	-	-	+	+	-	-	-	-	-	+	+	+

Appendix Table B2 (Continued)

Strains	Carbon Assimilation																																					Nitrogen Assimilation				
	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Sol.starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol	a-Methyl-D-glucoside	Salicin	Glucono-δ-lactone	2-Ketogluconic acid	5-Ketogluconic acid	DL-lactic acid	Succinic acid	Citric acid	Inositol	Glucuronic acid	Galacturonic acid	Nitrate	Nitrite	Ethylamine	L-Lysine	Cadaverine
ST-102	+	+	+	+	+	+	+	-	-	+	+	w	+	+	-	+	+	-	+	+	-	+	-	+	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	-
ST-105	+	-	-	+	+	+	+	-	-	+	+	-	+	+	+	+	-	-	-	+	-	l	-	+	+	+	-	l	-	-	-	+	+	-	w	-	+	+	-	-	-
ST-111	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	s	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+
ST-115	+	+	+	+	+	w	+	-	-	+	+	l	-	l	-	w	-	-	+	+	-	+	-	+	+	-	+	+	-	-	w	+	+	-	-	-	+	+	+	w	-
ST-119	+	+	+	+	+	+	+	-	-	+	+	+	+	l	-	l	+	-	+	l	-	l	+	+	+	+	+	+	-	-	l	+	w	-	-	-	w	-	+	+	w
ST-122	+	+	+	+	+	+	+	-	-	+	-	-	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	+	+
ST-128	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	l	+	-	+	+	-	+	-	+	+	+	+	+	-	-	w	+	+	-	-	-	+	+	+	-	-
ST-144	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	l	+	-	-	-	-	+	+	-	+	w	l	+	+	l	+	-	+	+	+	+	w	w	w	w
ST-145	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	+	-	-	-	+	l	+	+	+	+	+	l	+	-	+	l	+	+	+	+	-	+	w	+
ST-151	+	l	l	+	+	+	-	-	-	+	+	l	+	l	-	+	-	-	+	+	-	l	-	+	+	l	+	+	-	-	-	+	+	-	-	-	+	w	-	+	+
ST-153	+	-	-	+	l	+	+	-	+	+	+	l	-	w	w	-	-	-	-	-	-	+	-	+	+	-	-	w	-	-	-	l	-	-	-	-	+	-	-	-	-
ST-156	+	l	+	+	+	+	+	-	-	+	+	w	+	-	-	+	w	-	+	+	-	+	-	+	+	+	+	+	-	-	-	+	+	-	-	-	+	-	w	+	w
ST-164	+	+	-	+	+	+	+	-	-	-	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	l	-	-	l	+	+	-	-	-	-	-	+	+	+
ST-184	+	-	+	+	-	-	-	-	-	l	-	-	-	l	-	-	-	-	l	+	-	+	-	+	+	-	-	l	-	-	-	w	-	-	-	-	+	+	-	w	-
ST-195	+	l	+	+	+	w	+	-	-	+	+	-	+	+	-	w	w	-	+	+	-	+	-	+	+	-	w	+	-	-	w	+	+	-	-	-	+	+	+	+	+
ST-198	+	-	+	+	-	-	l	-	-	w	-	-	-	+	-	-	-	-	+	+	-	+	-	-	+	-	w	-	-	-	-	w	-	-	-	-	+	+	-	w	-

Appendix Table B2 (Continued)

Strains	Carbon Assimilation																																						Nitrogen Assimilation				
	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Sol.starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol	a-Methyl-D-glucoside	Salicin	Glucono-δ-lactone	2-Ketogluconic acid	5-Ketogluconic acid	DL-lactic acid	Succinic acid	Citric acid	Inositol	Glucuronic acid	Galacturonic acid	Nitrate	Nitrite	Ethylamine	L-Lysine	Cadaverine
ST-201	+	+	+	+	+	+	+	-	+	+	+	-	w	+	+	+	w	+	+	+	+	w	+	+	l	+	+	+	+	+	-	+	l	+	+	+	-	+	+	+	+
ST-202	+	+	+	+	+	+	+	-	-	+	+	w	+	-	-	l	l	-	+	+	-	l	-	+	+	+	+	+	-	-	-	+	w	-	-	-	+	+	+	+	w
ST-206	+	w	+	+	+	l	+	-	-	+	+	w	+	l	-	w	+	-	+	+	-	l	-	+	+	l	+	+	-	-	w	+	+	-	-	-	+	+	+	w	-
ST-211	+	+	l	+	+	+	+	-	-	+	+	l	-	+	-	-	-	-	+	+	-	+	-	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+
ST-224	+	+	l	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	l	+	-	-	-	-	-	+	+	+
ST-225	+	-	-	+	+	+	+	-	-	-	+	-	+	+	+	+	-	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+
ST-226	+	-	+	+	-	-	+	-	-	+	-	-	-	+	-	w	-	-	+	+	-	+	-	+	+	-	-	w	-	-	-	w	-	-	-	-	+	+	-	w	-
ST-228	+	-	-	+	+	+	+	-	-	-	+	-	+	+	l	w	-	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+
ST-229	+	-	-	+	+	+	+	-	-	-	+	-	+	+	l	l	-	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+
ST-236	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	+
ST-237	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	+	-	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	+	+
ST-238	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	+	-	+	+	-	-	+	+	s	-	-	-	-	-	+	+	+
ST-239	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	s	-	+	+	-	-	+	+	+	-	-	-	+	+	+	+	+
ST-240	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-	-	-	+	+	-	-	-	+	+	+	+	-
ST-246	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	+	+	+	-	+	+	-	-	+	-	-	w/-	+	+	-	-	-	+	+	+	+	+
ST-249	+	-	+	+	+	+	+	-	-	-	+	-	+	+	-	-	-	-	+	-	-	+	-	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+

Appendix Table B2 (Continued)

Strains	Carbon Assimilation																																					Nitrogen Assimilation				
	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Sol.starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol	a-Methyl-D-glucoside	Salicin	Glucono-δ-lactone	2-Ketogluconic acid	5-Ketogluconic acid	DL-lactic acid	Succinic acid	Citric acid	Inositol	Glucuronic acid	Galacturonic acid	Nitrate	Nitrite	Ethylamine	L-Lysine	Cadaverine
ST-250	+	-	-	-	-	+	w	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	-	+	+
ST-253	+	+	+	+	+	+	+	-	+	+	+	+	w	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+
ST-269	+	+	+	+	+	+	+	-	-	-	+	-	w/-	+	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+
ST-297	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	+	-
ST-300	+	+	+	+	+	+	+	-	-	-	+	-	w	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	+
ST-306	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+
ST-311	+	-	-	+	+	+	+	-	-	w/-	+	-	+	+	l	-	-	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+
ST-314	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+
ST-315	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	+	s	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	+	+	+
ST-318	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-	-	+	+	+	+	-	l	-	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+
ST-320	+	-	-	+	+	+	+	-	-	-	+	-	+	+	s/-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	-	+	+	+	+
ST-328	+	+	-	+	+	+	+	-	-	-	+	-	w	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-	s	+	+	-	-	-	-	-	+	+
ST-329	+	+	-	+	+	+	+	-	-	-	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	+
ST-330	+	+	+	-	-	+	+	-	-	-	-	-	-	s	-	+	-	-	+	+	-	+	-	+	+	-	+	+	+	-	-	-	+	+	-	-	-	-	-	+	+
ST-331	+	+	+	+	+	+	+	+	-	-	+	-	w	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+
ST-333	+	+	l	+	+	+	+	-	-	-	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+

Appendix Table B2 (Continued)

Strains	Carbon Assimilation																																					Nitrogen Assimilation				
	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Sol.starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol	a-Methyl-D-glucoside	Salicin	Glucono-δ-lactone	2-Ketogluconic acid	5-Ketogluconic acid	DL-lactic acid	Succinic acid	Citric acid	Inositol	Glucuronic acid	Galacturonic acid	Nitrate	Nitrite	Ethylamine	L-Lysine	Cadaverine
ST-334	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	+	-	+	+	-	-	+	+	+	-	-	-	-	+	+	+	+
ST-335	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	l	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+
ST-337	+	-	+	+	+	+	+	-	-	-	+	-	+	l	-	-	-	+	+	-	-	s	-	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+
ST-338	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+	+
ST-339	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+
ST-343	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
ST-358	+	+	s	+	+	+	+	-	-	-	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+
ST-365	+	+	+	+	+	+	+	-	-	-	+	-	w/-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	w/-	+	+	-	-	-	-	-	+	+	+
ST-366	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+
ST-370	+	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-	s	-	+	+	-	+	-	+	+	-	+	+	+	w/-	-	+	+	-	-	-	-	-	+	+	+
ST-377	+	+	-	-	-	+	+	-	-	-	-	-	-	+	s	-	+	-	+	+	-	+	-	+	+	-	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+
ST-380	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+
ST-388	+	+	+	+	+	-	-	-	-	+	-	-	w/-	w/-	-	-	w	-	-	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-	-	-	-	+	+	+
ST-390	+	+	+	+	+	-	-	-	-	+	-	-	w	w/-	w/-	w/-	w	-	-	+	-	-	-	+	+	-	-	+	-	-	-	+	w/-	-	-	-	-	-	+	+	+
ST-391	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	w	-	-	-	-	-	-	-	+	-	+	+	+
ST-394	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix Table B2 (Continued)

Strains	Carbon Assimilation																																						Nitrogen Assimilation				
	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Sol.starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	D-Mannitol	D-Glucitol	a-Methyl-D-glucoside	Salicin	Glucono-δ-lactone	2-Ketogluconic acid	5-Ketogluconic acid	DL-lactic acid	Succinic acid	Citric acid	Inositol	Glucuronic acid	Galacturonic acid	Nitrate	Nitrite	Ethylamine	L-Lysine	Cadaverine
ST-422	+	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
ST-431	+	+	-	-	-	+	+	-	-	-	-	-	-	+	-	w	+	-	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-	-	-	-	-	+	+	+
ST-433	+	-	-	+	+	+	+	-	-	-	+	-	+	+	+	+	-	-	+	+	-	l	-	+	+	+	+	l	-	-	+	+	+	-	-	-	+	+	+	+	+
ST-445	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	+	+	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+
ST-449	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-
ST-451	+	+	+	+	+	+	+	-	-	-	+	-	w	+	-	w	+	-	-	+	-	+	-	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+
ST-464	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	+	+
ST-476	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	+	+
ST-490	+	+	+	+	-	+	-	-	-	+	-	-	-	s/-	-	-	+	-	-	+	-	+	-	+	+	-	+	+	+	-	-	+	+	-	-	-	+	+	+	+	+
ST-493	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	+	+
ST-533	+	+	+	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	+	-	+	+	+	-	-	+	+	-	-	-	+	+	+	+	+
ST-536	+	+	+	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	+	-	+	+	+	-	-	+	+	-	-	-	+	+	+	+	+
ST-613	+	-	-	-	-	+	w/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	+	+

Appendix Table B3 Fermentation and other characteristics of yeast strains assigned to new species.

Strain	Fermentation							Other Tests									
	Glucose	Galactose	Sucrose	Maltose	Lactose	Rafinose	Melibiose	Vitamin free medium	50% Glucose	Starch formation	Gelatin liquefaction	0.1% Cycloheximide	Acid formation	Lipase	Urease	Maximum growth temperature (°C)	Vitamin Requirement
ST-2	-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	35-36	nd
ST-3	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	35-36	nd
ST-4	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	35-36	nd
ST-17	s	w	-	-	-	-	-	-	-	-	-	-	-	-	-	35-36	nd
ST-18	+	+	-	+	-	-	-	-	+	-	-	-	+	-	-	33-34	nd
ST-19	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	33-34	nd
ST-22	vw	-	-	-	-	-	-	+	-	-	-	+	+	-	-	32-33	nd
ST-26	-	-	-	-	-	-	-	+	+	-	+	-	+	-	-	nd	nd
ST-29	l	-	-	-	-	-	-	-	-	-	-	+	+	-	-	30-31	nd
ST-30	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	35-36	nd
ST-32	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	35-36	nd
ST-33	+	+	-	-	-	-	-	+	-	-	-	+	+	-	-	36-37	nd
ST-36	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	35-36	nd
ST-37	+	-	+	-	-	+	-	w	-	-	-	-	+	-	-	33-34	Pyridoxine and thiamine
ST-43	w	-	-	-	-	-	-	-	-	-	lw	-	-	-	-	35-36	nd
ST-49	+	-	+	-	-	+	-	+	-	-	-	+	-	-	-	34-35	nd

Appendix Table B3 (Continued)

Strain	Fermentation							Other Tests									
	Glucose	Galactose	Sucrose	Maltose	Lactose	Rafinose	Melibiose	Vitamin free medium	50% Glucose	Starch formation	Gelatin liquefaction	0.1% Cycloheximide	Acid formation	Lipase	Urease	Maximum growth temperature (°C)	Vitamin Requirement
ST-50	+	l	w	-	-	-	-	+	-	-	-	-	w	-	-	35-36	nd
ST-57	+	-	-	-	-	-	-	-	+	-	-	-	w	-	-	nd	nd
ST-59	-	-	-	-	-	-	-	-	-	-	+	+	-	-	w	nd	nd
ST-61	+	w	+	s	-	-	-	+	+	-	-	nd	-	-	-	nd	nd
ST-71	-	-	-	-	-	-	-	+	+	-	+	-	+	-	+	35-36	Thiamin
ST-73	-	-	-	-	-	-	-	-	-	-	-	-	w	-	+	32-33	Thiamine
ST-78	+	vw	-	-	-	-	-	+	+	-	-	nd	+	+	-	36-37	nd
ST-79	w	-	-	-	-	-	-	+	-	-	-	nd	-	-	-	40-41	-
ST-84	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	35-36	Pyridoxin and thiamine
ST-87	-	-	-	-	-	-	-	-	nd	-	-	-	lw	-	+	nd	Folic acid
ST-88	-	-	-	-	-	-	-	nd	nd	-	-	-	nd	-	+	nd	nd
ST-90	-	-	-	-	-	-	-	nd	nd	-	-	-	nd	-	+	nd	nd
ST-91	-	-	-	-	-	-	-	+	nd	-	-	-	nd	-	+	nd	-
ST-92	-	-	-	-	-	-	-	+	nd	-	-	-	nd	-	+	nd	-
ST-95	+	vw	+	s	-	-	-	-	nd	-	-	-	nd	-	-	36-37	Thiamine
ST-98	-	-	-	-	-	-	-	-	nd	-	-	-	nd	-	+	nd	Biotin and thiamine

Appendix Table B3 (Continued)

Strain	Fermentation							Other Tests									
	Glucose	Galactose	Sucrose	Maltose	Lactose	Rafinose	Melibiose	Vitamin free medium	50% Glucose	Starch formation	Gelatin liquefaction	0.1% Cycloheximide	Acid formation	Lipase	Urease	Maximum growth temperature (°C)	Vitamin Requirement
ST-102	-	-	-	-	-	-	-	+	nd	-	-	-	nd	-	+	nd	-
ST-105	-	-	-	-	-	-	-	-	nd	nd	-	-	nd	-	+	nd	Thiamine
ST-111	-	-	-	-	-	-	-	-	nd	+	-	-	nd	-	+	nd	Thiamine
ST-115	-	-	-	-	-	-	-	nd	nd	nd	-	-	nd	-	+	nd	nd
ST-119	-	-	-	-	-	-	-	nd	nd	nd	-	-	nd	-	+	nd	nd
ST-122	+	+	+	+	-	-	-	nd	nd	nd	+	-	nd	-	-	39-40	nd
ST-128	-	-	-	-	-	-	-	nd	nd	nd	-	-	nd	-	+	nd	nd
ST-144	-	-	-	-	-	-	-	nd	-	nd	-	-	-	-	+	28-29	nd
ST-145	-	-	-	-	-	-	-	nd	-	nd	-	-	-	-	+	32-33	nd
ST-151	-	-	-	-	-	-	-	nd	-	nd	-	-	-	-	+	nd	nd
ST-153	-	-	-	-	-	-	-	nd	-	nd	-	-	-	-	+	nd	nd
ST-156	-	-	-	-	-	-	-	nd	-	nd	-	-	-	-	+	nd	nd
ST-164	+	+	-	-	-	-	-	-	-	nd	-	-	-	-	-	39-40	Biotin
ST-184	-	-	-	-	-	-	-	nd	lw	nd	-	-	-	-	+	29-30	nd
ST-195	-	-	-	-	-	-	-	nd	-	nd	-	-	-	-	+	nd	nd
ST-198	-	-	-	-	-	-	-	nd	w	nd	-	-	-	-	+	28-29	nd

Appendix Table B3 (Continued)

Strain	Fermentation							Other Tests									
	Glucose	Galactose	Sucrose	Maltose	Lactose	Rafinose	Melibiose	Vitamin free medium	50% Glucose	Starch formation	Gelatin liquefaction	0.1% Cycloheximide	Acid formation	Lipase	Urease	Maximum growth temperature (°C)	Vitamin Requirement
ST-201	-	-	-	-	-	-	-	nd	nd	nd	-	-	-	-	+	nd	nd
ST-202	-	-	-	-	-	-	-	nd	nd	nd	-	-	-	-	+	nd	nd
ST-206	-	-	-	-	-	-	-	nd	nd	nd	-	-	-	-	+	nd	nd
ST-211	+	+	+	-	-	+	-	nd	nd	-	-	-	-	-	-	37-38	-
ST-224	s	-	-	-	-	-	-	nd	nd	-	-	-	-	-	-	39-40	-
ST-225	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	41-42	Pyridoxine and thiamine
ST-226	-	-	-	-	-	-	-	nd	-	nd	-	-	-	-	+	nd	nd
ST-228	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	41-42	Pyridoxine and thiamine
ST-229	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	41-42	Pyridoxine and thiamine
ST-236	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	38-39	-
ST-237	+	s	+	+	-	-	-	+	nd	-	lw	-	w	-	-	43-44	-
ST-238	-	-	-	-	-	-	-	+	nd	-	-	-	-	-	-	39-40	-
ST-239	s	-	-	-	-	-	-	+	nd	-	-	+	w	-	-	39-40	-
ST-240	s	-	-	-	-	-	-	+	+	-	-	+	w	-	-	40-41	-
ST-246	+	-	-	-	-	-	-	-	nd	-	-	+	-	-	-	35-36	Thiamine
ST-249	vw	-	-	-	-	-	-	+	nd	-	-	-	-	-	-	33-34	-

Appendix Table B3 (Continued)

Strain	Fermentation							Other Tests									Vitamin Requirement
	Glucose	Galactose	Sucrose	Maltose	Lactose	Rafinose	Melibiose	Vitamin free medium	50% Glucose	Starch formation	Gelatin liquefaction	0.1% Cycloheximide	Acid formation	Lipase	Urease	Maximum growth temperature (°C)	
ST-250	+	-	-	-	-	-	-	-	nd	nd	-	-	-	-	-	nd	Inositol, niacin, pantothenate and pyridoxine
ST-253	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	nd	nd
ST-269	-	-	-	-	-	-	-	+	+	-	-	nd	-	-	-	37-38	-
ST-297	-	-	-	-	-	-	-	-	nd	-	lw	+	w	-	-	34-35	Biotin, pyridoxine and thiamine
ST-300	+	+	-	-	-	-	-	-	nd	-	-	-	w	-	-	37-38	Thiamine
ST-306	+	-	-	-	-	-	-	-	nd	-	-	-	-	-	-	36-37	Inositol, niacin, pantothenate and pyridoxine
ST-311	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	34-35	Biotin, pyridoxine and thiamine
ST-314	l/-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	32-33	Biotin, pyridoxine
ST-315	l/-	l/-	-	-	-	-	-	-	+	-	lw	-	lw	-	-	35-36	Biotin, pyridoxine
ST-318	-	-	-	-	-	-	-	-	-	-	-	-	-	-	s	35-36	Thiamine
ST-320	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	34-35	Biotin, pyridoxine and thiamine
ST-328	+	+	-	-	-	-	-	-	nd	nd	-	-	w	-	-	38-39	Biotin and thiamine
ST-329	+	+	-	-	-	-	-	-	nd	nd	-	-	w	-	-	38-39	Biotin and thiamine
ST-330	+	-	-	-	-	-	-	+	+	-	-	-	w	-	-	31-32	-
ST-331	+	+	-	-	-	-	-	+	nd	-	-	-	w	-	-	38-39	-
ST-333	+	+	-	-	-	-	-	-	nd	-	-	-	-	-	-	38-39	Thiamine

Appendix Table B3 (Continued)

Strain	Fermentation							Other Tests									
	Glucose	Galactose	Sucrose	Maltose	Lactose	Rafinose	Melibiose	Vitamin free medium	50% Glucose	Starch formation	Gelatin liquefaction	0.1% Cycloheximide	Acid formation	Lipase	Urease	Maximum growth temperature (°C)	Vitamin Requirement
ST-334	+	-	-	-	-	-	-	nd	-	-	-	-	w	-	-	36-37	nd
ST-335	+	s	-	-	-	-	-	-	nd	-	-	-	-	-	-	37-38	Biotin
ST-337	-	-	-	-	-	-	-	-	nd	-	-	-	-	-	-	32-33	Biotin and thiamine
ST-338	-	-	-	-	-	-	-	+	nd	-	-	-	-	-	-	31-32	-
ST-339	w	-	-	-	-	-	-	+	-	-	-	-	-	-	-	31-32	-
ST-343	+	+	-	-	-	-	-	-	nd	-	-	-	lw	-	-	35-36	Pantothenate, pyridoxine and thiamine
ST-358	+	vw	-	-	-	-	-	-	nd	nd	-	-	l	-	-	35-36	Biotin
ST-365	+	+	-	-	-	-	-	+	+	-	-	-	w	-	-	37-38	Thiamine
ST-366	+	+	-	-	-	-	-	+	+	-	-	-	w	-	-	37-38	Thiamine
ST-370	+	s	-	-	-	-	-	+	+	-	-	-	lw	-	-	35-36	Biotin
ST-377	+	-	-	-	-	-	-	+	nd	nd	lw	-	-	-	-	38-39	Biotin
ST-380	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	nd	nd
ST-388	+	-	+	+	-	+	-	+	+	-	-	-	-	-	-	37-38	Biotin and thiamine
ST-390	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	nd	Biotin and thiamine
ST-391	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	34-35	nd
ST-394	+	+	-	-	-	-	-	-	nd	nd	-	-	-	-	-	40-41	Pantothenate, niacin, pyridoxine and thiamine

Appendix Table B3 (Continued)

Strain	Fermentation							Other Tests									
	Glucose	Galactose	Sucrose	Maltose	Lactose	Rafinose	Melibiose	Vitamin free medium	50% Glucose	Starch formation	Gelatin liquefaction	0.1% Cycloheximide	Acid formation	Lipase	Urease	Maximum growth temperature (°C)	Vitamin Requirement
ST-422	w	-	-	-	-	-	-	+	+	-	-	-	-	-	-	nd	nd
ST-431	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	35-36	Biotin
ST-433	+	-	-	-	-	-	-	-	w	nd	-	-	-	nd	-	37-38	Piridoxine and thiamine
ST-445	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34-35	nd
ST-449	+	-	-	-	-	-	-	-	nd	-	-	-	-	nd	-	41-42	Niacin, pyridoxin and thiamine
ST-451	+	-	+	-	-	-	-	-	nd	nd	-	-	-	nd	-	36-37	Pyridoxine and thiamine
ST-464	+	-	-	-	-	-	-	-	nd	nd	-	-	-	nd	-	36-37	Inositol, niacin, pantothenate, pyridoxine
ST-476	+	-	-	-	-	-	-	-	nd	nd	-	-	-	nd	-	33-34	Inositol, niacin, pantothenate, pyridoxine and thiamine
ST-490	+	-	+	s	-	-	-	-	nd	-	lw	-	-	nd	-	39-40	Thiamine
ST-493	+	-	-	-	-	-	-	-	nd	nd	-	-	-	nd	-	36-37	Inositol, niacin, pantothenate, pyridoxine and thiamine
ST-533	+	-	+	lw	-	-	-	-	nd	-	w	-	lw	nd	-	38-39	Thiamine
ST-536	+	-	+	lw	-	-	-	-	nd	-	w	-	lw	nd	-	38-39	Thiamine
ST-613	+	-	-	-	-	-	-	-	nd	-	-	-	-	nd	-	37-38	Inositol, niacin, pantothenate, pyridoxine and thiamine