# 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 2$^{nd}$ edition edited by Lodder (1970), 394 species belonging to 39 genera were listed. The 3$^{rd}$ 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**<br>**Order**<br>**Family**[a]<br>**Genus** | **Family**[a]<br>**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**<br>**Order**<br>**Family**<br>**Genus** | **Order**<br>**Family**<br>**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<br>5' TGGAATTACCGCGGCTGCTGGCACC 3'<br>5' CCGTCAATTCCTTTAAGTTTCAGCC 3'<br>5' TCTGGGCCGCACGCGCGCTACACTG 3'<br>5' GACGGGCGGTGTGTACAAAGGGCAG 3' | 1750 bp | Suh *et al*. (1996) |
| 18S rDNA total | Sequencing of PCR product<br>NS1-5' GTAGTCATATGCTTGTCTC 3'<br>NS8-5' TCCGCAGGTTCACCTACGGA 3'<br>Basid2-5' CTGTTAAGACTACAACGGAGCAGGC 3'<br>Basid3-5' AGAGTGTTCAAAGCAGGA 3' | 1800 bp | Swann and Taylor (1993) |
| 18S rDNA<br>18S-1627<br>(1451-1618) | Direct sequencing<br>5' ACGGGCGGTGTGTAC 3' | 167-172 bp | Guého *et al*. (1990) |
| 25S rDNA<br>25S-635<br>(D2, B, V3 or 493-622) | Direct sequencing<br>5' GGTCCGTGTTTCAAGACGG 3' | 200-300 bp | Guého *et al*. (1990) |
| 25S rDNA<br>25S-1841<br>(1611-1835) | Direct sequencing<br>5' TTGGAGACCTGCTGCGG 3' | 225 bp | Guého *et al*. (1990) |
| 25S rDNA D1/D2 | Sequencing of PCR product<br>NL1-5' GCATATCAATAAGCGGAGGAAAAG 3'<br>NL4-5' GGTCCGTGTTTCAAGACGG 3' | 600 bp | Kurtzman and Robnett (1997) |
| ITS | Sequencing of PCR product<br>ITS1-5' TCCGTAGGTGAACCTGCGG 3'<br>or ITS5-5' GGAAGTAAAAGTCGTAACAAGG 3'<br>ITS4-5' TCCTCCGCTTATTGATATGC 3' | Variable (<400->800 bp) | James *et al*. (1996) |
| IGS[c] | Sequencing of PCR product<br>AC10-5' GAGGCTAGAGGTGCCAG 3'<br>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)

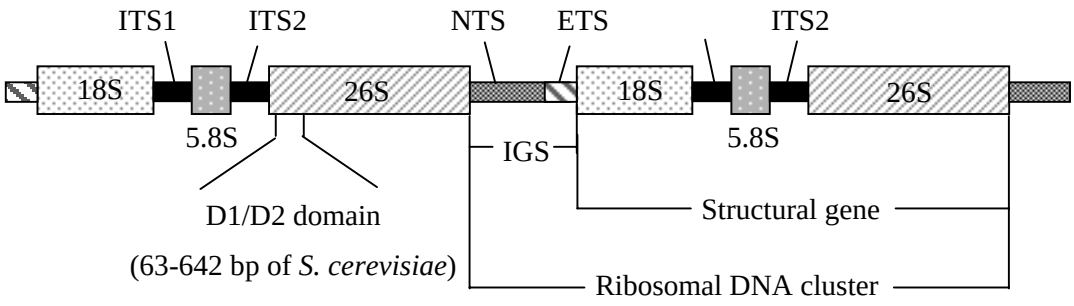


Figure 1 Structure of the ribosomal RNA gene cluster of yeasts. The cluster is split into coding (18S, 5.8S and 26S genes) and non-coding (Internal Transcribed Spacer or ITS and Inter-Genic Spacer or IGS. IGS consist of Non-Transcribed Spacer (NTS) and External Transcribed Spacer (ETS) regions.

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) |