

## 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.

| <b>Year</b>  | <b>Flowers</b> | <b>Insect frass</b> | <b>Leaves</b> | <b>Mosses</b> | <b>Mushrooms</b> | <b>Others</b> | <b>Total</b> |
|--------------|----------------|---------------------|---------------|---------------|------------------|---------------|--------------|
| 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           |
| <b>Total</b> | <b>24</b>      | <b>144</b>          | <b>54</b>     | <b>27</b>     | <b>28</b>        | <b>6</b>      | <b>283</b>   |

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

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

## **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 registered 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 |
|------------------------------------------------|----------------|-------------------------------------------------|----------------|
| <b>Ascomycetous yeasts</b>                     |                |                                                 |                |
| <i>Ambrosiozyma monospora</i>                  | 2              | <i>Saccharomyces cerevisiae</i>                 | 5              |
| <i>Aureobasidium pullulans</i>                 | 2              | <i>S. kluyveri</i>                              | 8              |
| <i>Blastobotrys catitulata</i>                 | 1              | <i>S. unisporus</i>                             | 2              |
| <i>Candida diversa</i>                         | 3              | <i>Stephanoascus smithiae</i>                   | 3              |
| <i>C. fukuyamaensis</i>                        | 3              | <i>Torulaspora delbrueckii</i>                  | 1              |
| <i>C. gotoi</i>                                | 2              | <i>Torulaspora</i> sp. IFO 11061                | 1              |
| <i>C. leandrae</i>                             | 1              | <i>Wililopsis saturnus</i> var. <i>mrakii</i>   | 1              |
| <i>C. natalensis</i>                           | 1              | <i>W. saturnus</i> var. <i>subsufficiens</i>    | 1              |
| <i>C. palmae</i>                               | 1              | <i>Zygosaccharomyces</i> sp. IFO 11070          | 1              |
| <i>C. parapsilosis</i>                         | 3              | <b>Total 17 genera, 39 species, 2 varieties</b> | <b>97</b>      |
| <i>C. rancensis</i>                            | 1              |                                                 |                |
| <i>C. sithepensis</i>                          | 1              | <b>Basidiomycetous yeasts</b>                   |                |
| <i>C. tropicalis</i>                           | 9              | <i>Bullera dendrophila</i>                      | 1              |
| <i>Candida</i> sp. NRRL Y-17456                | 4              | <i>Bullera sinensis</i>                         | 2              |
| <i>Candida</i> sp. UWO(PS)00-147.3             | 1              | <i>Cryptococcus heveanensis</i>                 | 8              |
| <i>Debaryomyces nepalensis</i>                 | 4              | <i>C. humicola</i>                              | 2              |
| <i>D. polymorphus</i>                          | 3              | <i>C. laurentii</i>                             | 3              |
| <i>D. polymorphus</i> var. <i>africanus</i>    | 1              | <i>Cryptococcus</i> sp. CBS 8372                | 1              |
| <i>D. vanrijiae</i> var. <i>yarrowii</i>       | 3              | <i>Exobasidium vexans</i>                       | 4              |
| <i>Debaryomyces</i> sp. NRRL-7804              | 1              | <i>Rhodospiridium paludigenum</i>               | 1              |
| <i>Geotrichum fragrans</i>                     | 2              | <i>R. toruloides</i>                            | 1              |
| <i>Hanseniaspora opuntiae</i>                  | 5              | <i>Rhodotorula nothofagi</i>                    | 1              |
| <i>H. vineae</i>                               | 1              | <i>Sporidiobolus ruineniae</i>                  | 4              |
| <i>Hanseniaspora</i> sp. CBS 8772              | 1              | <i>Sporobolomyces bannaensis</i>                | 1              |
| <i>Kloeckera lindneri</i>                      | 1              | <i>S. odoratus</i>                              | 3              |
| <i>Kluyveromyces lactis</i>                    | 2              | <i>S. poonsookiae</i>                           | 1              |
| <i>Kodamaea (Pichia) ohmeri</i>                | 3              | <i>Sporobolomyces</i> sp. TY-241 / TY-253       | 4              |
| <i>Metschnikowia koreensis</i>                 | 5              | <i>Tilletiopsis</i> sp. TY-2352                 | 1              |
| <i>Pichia nakazawae</i> var. <i>akitaensis</i> | 2              | <i>Trichosporon asahii</i>                      | 4              |
| <i>P. stipitis</i>                             | 2              | <b>Total 9 genera, 17 species</b>               | <b>42</b>      |
| <i>P. sydowiorum</i>                           | 2              |                                                 |                |
| <i>Pichia</i> sp. UWO(PS)99-305.1              | 1              |                                                 |                |