Invited Review

Expanding world of ballistosporous yeasts: Distribution in the phyllosphere, systematics and phylogeny

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Ecological and systematic aspects of ballistosporous yeasts inhabiting the phyllosphere of the Asia-Pacific region were reviewed in the light of recent advances. In the past 20 years, the extensive isolation studies of ballistosporous yeasts were carried out in the Asia-Pacific region, and the following new species were found in the phyllosphere of the main island of Japan, of the Ogasawara Islands in the Pacific Ocean, and of Thailand, New Zealand, and the Yunnan Province of China: Bensingtonia ingoldii, B. intermedia, B. miscanthi, B. naganoensis, B. musae, B. sakaguchii, B. subrosea, B. thailandica, and B. yamatoana; Bullera boninensis, B. coprosmensis, B. hanna, B. huiaensis, B. miyagiana, B. mrakii, B. oryzae, B. pennisetica, B. pseudoalba, B. schimicola, B. sinensis, B. unica, B. variabilis, and B. waltii; Kockovaella imperatae, K. machilophila, K. phaffii, K. sacchari, K. schimae, and K. thailandica; Sporobolomyces blumeae, S. coprosmae, S. coprosmicola, S. dimmenae, S. draycophyllus, S. falcatus, S. griseoflavus, S. inositolphilus, S. lactophilus, S. linderae, S. novazealandicus, S. nylandii, S. oryzae, S. poonsookiae, S. ruber, S. sasicola, S. subbrunneus, S. taupoensis, S. vermiculatus, S. xanthus, and S. yunnanensis; and Udeniomyces megalosporus. Yeast species containing Q-10(H₂), a monohydrated ubiquinone, have long been considered to be rare yeasts; however, these yeasts were proved to be common in the tropical and subtropical phyllosphere of Asia. The chemotaxonomic and molecular phylogenetic studies based on the sequence analysis of 18S rDNA revealed the heterogeneity of ballistosporous yeasts, which comprised species with polyphyletic phylogenetic origins. A new phylogenetic cluster, Subbrunneus, was newly found in the class Urediniomycetes as the fifth phylogenetical cluster of this class, based on the analysis of 18S rDNA sequences. The Subbrunneus cluster comprises four ballistosporous yeast species found in the phyllosphere of Japan and New Zealand. The future perspectives of basidiomycetous yeast systematics are discussed.

Key Words——ballistosporous yeasts in phyllosphere; distribution of ballistosporous yeasts; phylogeny of ballistosporous yeasts; systematics of ballistosporous yeasts

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Introduction

Phaff and Starmer (1987) stated in their review on yeasts associated with plants, insects, and soil that the external surface of the leaf as an environment for microorganisms has been termed the phyllosphere or phylloplane. Its use as a habitat for yeasts has been
recognized more recently than the interior of flowers or the fruits of higher plants. Last and Price (1969) stated that the phyllosphere is a good substrate for saprophytic organisms, and cereal leaves were an especially suitable environment for species of Sporobolomyces, an anamorphic genus of ballistosporous yeasts. Indeed, ballistosporous yeasts are commonly found on the leaf surfaces of trees and on grasses.

Ballistosporous yeasts are a group of yeasts that produce conidia called ballistospores, or ballistoconidia, which are produced at the end of sterigmata and are forcibly discharged at maturation. These kinds of yeasts were found more than 100 years ago, but the progress of systematics had been much delayed. Since the classical work of Derx (1930), no prominent work has been reported on the isolation and systematics of ballistosporous yeasts until recent years. However, many new ballistosporous yeasts have been found in the phyllosphere in the past 20 years. This stimulated the progress of the systematic studies of this group of yeasts. This paper discusses the ballistosporous yeasts found in the phyllosphere of the Asia-Pacific region from the ecological and systematic points of view.

1. Ballistosporous Yeasts Found in the Phyllosphere of the Main Island of Japan

In Japan, the taxonomic study of ballistosporous yeasts started about 70 years ago by Saito (1922) and Okunuki (1931), and their strains were provided to Dutch researchers (Lodder and Kreger-van Rij, 1952). In the series of a study on red yeasts, Yamazaki and Morishita (1941) and Yamazaki and Fuji (1950) isolated ballistosporous yeasts of the genera Sporobolomyces and Bullera. Tubaki (1952) described new taxa of ballistosporous yeastlike fungal genus Tilletiopsis, Tilletiopsis crenea, Tilletiopsis lilacina, and Tilletiopsis minor var. flav. Iizuka and Goto (1965) described Sporobolomyces japonica, which was isolated from the oil brine of an oil field in Japan.

In 1971, Yoshizawa and Komagata isolated 403 strains of ballistosporous yeasts from various plants collected in a suburb of Tokyo, but they did not publish their study. After 16 years, part of it was published after a reexamination of several taxonomic properties, i.e., 88 strains were identified as 10 species in the genera Bullera, Sporidiobolus, Sporobolomyces, and Tilletiopsis (Yoshizawa et al., 1987). The result is shown in Table 1 under up-to-date scientific names.

Nakase and Suzuki (1985) reported the isolation of yeasts from 43 samples of Oryza sativa (rice plant) that were collected in Kanagawa Prefecture, on the main island of Japan in February and March 1984. Ninety-four ballistosporous yeasts were isolated from 37 samples (86%) and identified as 14 species—6 known ones and 8 new ones—in the genera Bullera, Sporobolomyces, and Tilletiopsis. Five isolates did not produce ballistospores, but they were identified as Bullera crocea because the taxonomic characteristics other than the lack of ballistosporous ability coincided well with this species.

Four of eight new species were described as Bullera intermedia, Bullera oryzae, Sporobolomyces oryzicola, and Sporobolomyces subbrunneus, based on traditional and chemotaxonomic investigations (Nakase and Suzuki, 1985, 1986b). Two were described as Bullera derxii and Bullera pseudoalba together with isolates from Miscanthus sinensis (Nakase and Suzuki, 1986a), and the other two were described as Bullera megalospora (Nakase and Suzuki, 1986c) and Bullera variabilis (Nakase and Suzuki, 1987e), together with the isolates from several other plants. Later, Bullera derxii was found to be synonymous with Bullera sinensis (Nakase et al., 1990c), Bullera megalospora was transferred to a newly established genus Udeniomyces (Nakase and Takematsu, 1992), and Bullera intermedia was transferred to the genus Sporobolomyces (Nakase and Suzuki, 1987b), then to the genus Bensingtonia because this species had Q-9 as the major ubiquinone (Nakase and Boekhout, 1988). Ballistosporous yeasts isolated from Oryza sativa are shown in Table 1 under up-to-date scientific names.

The most popular species was Sporobolomyces roseus, which was isolated from 72.1% of the samples of Oryza sativa examined, followed by Udeniomyces megalosporus (34.9%), Bullera crocea (30.2%), and Bullera alba (27.9%).

Nakase and Suzuki (1987b) examined 48 samples of Miscanthus sinensis, a popular plant in Japan, collected in March and April 1985 in Kanagawa and Saitama Prefectures on the main island of Japan, and isolated 101 strains of ballistosporous yeasts from 83.3% of plants examined. These strains were identified as 16 species in the genera Bullera, Sporobolomyces, and Tilletiopsis.
Three new species, *Sporobolomyces miscanthi*, *Sporobolomyces weijmanii*, and *Sporobolomyces subroseus*, were described, based on the isolates from *Miscanthus sinensis*. Another new species, *Sporobolomyces yamatoanus*, was described together with the isolates from *Sasa sp.*, *Benzoin obtusilobum*, *Lyonia neziki*, *Rhus trichocarpa*, and *Vitis ficifolia var. lobata* (Nakase et al., 1987b). Yamada and Nakagawa (1988), however, regarded *Sporobolomyces weijmanii* as a synonym of *Sporobolomyces intermedius* based on the electrophoretic comparison of enzymes. These four species were later transferred to the genus *Bensingtonia* because they had Q-9 as the major ubiquinone (Nakase and Boekhout, 1988). Ballistosporous yeasts isolated from *Miscanthus sinensis* are shown in Table 1 under up-to-date scientific names.

The most popular species associated with *Miscanthus sinensis* was *Sporobolomyces roseus*, which was found in 70.8% of samples, followed by *Udeniomyces megalosporus* (33.3%) and *Bullera crocea* (31.3%). Unlike *Oryza sativa*, the frequency of isolation of *Bullera alba* from *Miscanthus sinensis* was not so high (Table 1).

In October 1986, eleven samples of various plants were collected at Mt. Fuji, and yeasts living on the leaves were investigated. Fifty-four strains of ballistosporous yeasts and yeastlike fungi were isolated from 10 samples (90.9%) and identified as 14 species in the genera *Bullera*, *Bensingtonia*, *Sporobolomyces*, and *Tilletiopsis*, and in a new genus, *Ballistosporomyces* (Nakase and Takashima, 1993). The latter genus was proposed based on the finding that two isolates from Mt. Fuji produced ballistospores and stalked conidia, but not budding yeast cells (Nakase et al., 1989b). This combination of conidiogenesis had not been reported so far. Two new species, *Ballistosporomyces ruber* and *Ballistosporomyces xanthus*, were included in the genus. However, Boekhout (1991) transferred these two species to the genus *Sporobolomyces* based on the observation of conidia resembling stalked conidia in several strains of known ballistosporous yeasts.

Besides the *Ballistosporomyces* species, *Sporobolomyces griseoflavus* (Nakase and Suzuki, 1987a) and *Sporobolomyces linderae* (Nakase et al., 1994) were described as new species. Thirteen strains of ballistosporous yeastlike fungi were identified as five species in the genus *Tilletiopsis*. Ballistosporous yeasts and yeastlike fungi isolated from Mt. Fuji are shown in Table 2 under up-to-date scientific names.

Nakase and co-workers carried out further isolation studies of ballistosporous yeasts in the phyllosphere of the main island of Japan and found 13 species in the genera *Bullera*, *Udeniomyces*, *Bensingtonia*, and *Sporobolomyces*. These species are shown in Table 3 under up-to-date scientific names. Six new species, *Sporobolomyces naganoensis* (Nakase and Suzuki, 2000) and *Sporobolomyces wakayamaensis* (Nakase et al., 2000), were described as new species. The isolates from *Oryza sativa* and *Miscanthus sinensis* collected on the main island of Japan are shown in Table 1.

### Table 1. Ballistosporous yeasts found in leaves of *Oryza sativa* and *Miscanthus sinensis* collected on the main island of Japan.

<table>
<thead>
<tr>
<th>Species</th>
<th>Oryza sativa</th>
<th>Miscanthus sinensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of strains isolated</td>
<td>Frequency of isolation (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No. of strains isolated</td>
</tr>
<tr>
<td>Hymenomycetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bullera</em></td>
<td>35</td>
<td>53.5</td>
</tr>
<tr>
<td><em>B. alba</em></td>
<td>15</td>
<td>27.9</td>
</tr>
<tr>
<td><em>B. crocea</em></td>
<td>13</td>
<td>30.2</td>
</tr>
<tr>
<td><em>B. oryzae</em></td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td><em>B. pseudoalba</em></td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td><em>B. sinensis</em></td>
<td>2</td>
<td>4.7</td>
</tr>
<tr>
<td><em>B. variabilis</em></td>
<td>3</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Udeniomyces</em></td>
<td>19</td>
<td>37.2</td>
</tr>
<tr>
<td><em>U. megalosporus</em></td>
<td>16</td>
<td>34.9</td>
</tr>
<tr>
<td><em>U. pyricola</em></td>
<td>3</td>
<td>4.7</td>
</tr>
<tr>
<td>Urediniomycetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bensingtonia</em></td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td><em>B. intermedia</em></td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td><em>B. miscanthi</em></td>
<td>2</td>
<td>4.2</td>
</tr>
<tr>
<td><em>B. subrosea</em></td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td><em>B. yamatoana</em></td>
<td>2</td>
<td>4.2</td>
</tr>
<tr>
<td><em>Sporobolomyces</em></td>
<td>41</td>
<td>79.1</td>
</tr>
<tr>
<td><em>S. oryzicola</em></td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td><em>S. roseus</em></td>
<td>35</td>
<td>72.1</td>
</tr>
<tr>
<td><em>S. salmonicolor</em></td>
<td>2</td>
<td>4.2</td>
</tr>
<tr>
<td><em>S. shibatanus</em></td>
<td>4</td>
<td>9.3</td>
</tr>
<tr>
<td><em>S. subbrunneus</em></td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td>Ustilaginomycetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tilletiopsis</em></td>
<td>3</td>
<td>7.0</td>
</tr>
<tr>
<td><em>T. lilacina</em></td>
<td>3</td>
<td>7.0</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>86.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Frequency of isolation (%) = (no. of samples from which that species was isolated)/(no. of samples examined) × 100.
1987d), Bullera miyagiana (Nakase et al., 1990a), Sporobolomyces inositophilus (Nakase and Suzuki, 1987c), Sporobolomyces sasicola (Nakase and Suzuki, 1987a), and Sporobolomyces falcatus (Nakase et al., 1987a), and Sporobolomyces lactophilus (Nakase et al., 1990b) were included in these isolates. Among these new yeasts, strains assigned to Bullera miyagiana and Sporobolomyces lactophilus were isolated by R. J. Bandoni from Abies firma in Miyagi Prefecture on the main island of Japan and sent to me for identification. Later, Sporobolomyces naganoensis was transferred to the genus Bensingtonia because it had Q-9 as the major ubiquinone (Nakase and Boekhout, 1988).

2. Ballistosporous Yeasts Found in the Phyllosphere of Ogasawara Islands in the Pacific Ocean

The Ogasawara (Bonin) Islands are isolated islands in a subtropical region of the Pacific Ocean about 1,000 km from Tokyo and are known to have a unique flora of higher plants. In the autumn of 1994, we received various plants collected in these islands from T. Sato and examined the ballistosporous yeasts. A total of 197 strains were isolated from 24 samples (66.7%) of 36 examined, though the number of colonies appearing on agar media was usually small, probably because collected samples had been exposed to much wind from the sea (T. Sato, personal communication).

These strains were classified into 44 groups based on the morphology of ballistospores, physiological properties, the presence or absence of xylose in the cells, and the ubiquinone types, and a strain was then selected from each group and used for chemotaxonomic and molecular phylogenetic study (Nakase et al., unpublished).

Forty-six strains of the genus Bullera isolated from 10 samples (27.8%) were identified as six species

<table>
<thead>
<tr>
<th>Table 2. Ballistosporous yeasts and yeastlike fungi found in plants collected on Mt. Fuji on the main island of Japan.</th>
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</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
</tr>
<tr>
<td>Plant samples for the isolation of yeasts&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Hymenomycetes</td>
</tr>
<tr>
<td><em>Bullera</em></td>
</tr>
<tr>
<td><em>B. alba</em></td>
</tr>
<tr>
<td><em>B. crocea</em></td>
</tr>
<tr>
<td><em>B. variabilis</em></td>
</tr>
<tr>
<td>Urediniomycetes</td>
</tr>
<tr>
<td><em>Bensingtonia</em></td>
</tr>
<tr>
<td><em>B. yamatoana</em></td>
</tr>
<tr>
<td>Ustilaginomycetes</td>
</tr>
<tr>
<td><em>Sporobolomyces</em></td>
</tr>
<tr>
<td><em>S. griseoflavus</em></td>
</tr>
<tr>
<td><em>S. linderae</em></td>
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<tr>
<td><em>S. roseus</em></td>
</tr>
<tr>
<td><em>S. ruber</em></td>
</tr>
<tr>
<td><em>S. xanthus</em></td>
</tr>
<tr>
<td><em>Tilletiopsis</em></td>
</tr>
<tr>
<td><em>T. lilacina</em></td>
</tr>
<tr>
<td><em>T. minor</em></td>
</tr>
<tr>
<td><em>T. pallescens</em></td>
</tr>
<tr>
<td><em>Tilletiopsis</em> sp.</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<sup>a</sup> Alphabet letters indicate samples, and numerals indicate the number of strains isolated.
<sup>b</sup> Frequency of isolation (%) = (no. of samples from which that species was isolated)/(no. of samples examined) × 100.
Three species were described as new, *Bullera boninensis*, *Bullera schimicola*, and *Bullera waltii* (Sugita et al., 1999). *Bullera* sp. 1 and *Bullera* sp. 2 seem to represent respective new species, and *Bullera* sp. 3 is closely related to *Bullera variabilis*, a taxonomically heterogeneous species supposedly comprising several different species (Nakase and Suzuki, 1987e).

Sixteen strains isolated from three samples (8.3%) represented three new species of *Kockovaella* and were described as *Kockovaella machilophila*, *Kockovaella phaffii*, and *Kockovaella schimae* (Cañete-Gibas et al., 1998). Two strains of *Bensingtonia*, which were isolated from the same sample, were found to represent a new species and were described as *Bensingtonia sakaguchii* (Sugita et al., 1997).

A total of 133 strains were assigned to the genus *Sporobolomyces*, in which 103 had Q-10 as the major ubiquinone, and the remaining 30 were found to have Q-10(H2) as the major ubiquinone homologue. The latter strains were found in 10 samples (27.8%) of the plants examined. These 30 strains were classified into 14 groups based on the characteristics mentioned above. The representative 14 strains from respective

(Tables 4). Three were described as new species, *Bullera boninensis*, *Bullera schimicola*, and *Bullera waltii* (Sugita et al., 1999). *Bullera* sp. 1 and *Bullera* sp. 2 seem to represent respective new species, and *Bullera* sp. 3 is closely related to *Bullera variabilis*, a taxonomically heterogeneous species supposedly comprising several different species (Nakase and Suzuki, 1987e).

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groups constituted a cluster, which was close to a cluster where *Erythrobasidium hasegawianum* and *Sporobolomyces elongatus*, the known species of Q-10(H₂)-containing yeasts, were located (Fig. 1). These 14 strains were found to represent four different species based on DNA-DNA hybridization experiment, and one demonstrated a sexual stage (Hamamoto et al., unpublished).

Besides the ballistosporous yeasts, two strains of nonballistosporous, stalked conidium-producing yeasts, were also isolated from a plant leaf collected in the phyllosphere of the Ogasawara Islands. These yeasts represented two new species and were named *Fellomyces distylii* and *Fellomyces ogasawarenisis*.
3. Ballistosporous Yeasts Found in the Phyllosphere of Thailand

Ballistosporous yeasts were isolated from plant materials collected in Thailand in 1987, 1990, and 1996. In 1987, forty-two samples of various plants were collected in the forests, fields, rice fields, and roadsides of the western suburbs of Bangkok and near Ayuthaya, and also in the urban areas of Bangkok, including markets. Yeasts were isolated from these samples at 23 and 30°C. Sixty-three strains of ballistosporous yeasts were isolated from 21 samples (50%) examined. The frequency of isolation of ballistosporous yeasts reached 81.2% when samples collected in the suburbs of Bangkok and Ayuthaya were employed, but it was very low (15%) when samples collected in the urban areas of Bangkok, including markets, were examined.

Sixty-three isolates were identified as 16 species in the genera Bullera, Kockovaella, Bensingtonia, Sporidiobolus, Sporobolomyces, and Tilletiopsis (Table 5). Three strains produced ballistospores, stalked conidia, and budding yeast cells. This combination of the conidiogenesis has not been found so far; therefore a new genus Kockovaella was proposed for these strains. Two species, Kockovaella imperatae and Kockovaella thailandica, were included in the genus (Nakase et al., 1991). Besides the new species of Kockovaella, Sporobolomyces nylandii and Sporobolomyces vermiculatus were also described as new species (Takashima and Nakase, 2000). Four strains of yeastlike fungi were assigned to the genus Tilletiopsis. Three were found to represent three new species (Takashima and Nakase, unpublished).

In 1990, 73 strains of ballistosporous yeasts were isolated from 33 plant materials (82.5%) out of 40 collected along the seacoast southeast of Bangkok. The isolation of yeasts was carried out at 25°C. These yeasts were identified as 3 species in the genera Bullera, Kockovaella, Bensingtonia, and Sporidiobolus/ Sporobolomyces (Table 5). Bensingtonia musae (Takashima et al., 1995b), Bullera penncetiocola (Takashima and Nakase, 1998), Kockovaella sacchari (Takashima and Nakase, 1998), Sporobolomyces blumeae (Takashima and Nakase, 2000), and Sporobolomyces poonsookiae (Takashima and Nakase, 2000) were described as new species. In this isolation study, we did not isolate Tilletiopsis strains, though they are commonly found in the samples.

A total of 21 species in the five genera of ballistosporous yeasts and yeastlike fungi were isolated in the study of 1987 and 1990, and 12 species represented new taxa.

In 1996, plant samples were collected in a tropical rain forest of the Sakaerat Environmental Research Station, Thailand Institute of Scientific and Technological Research, in Nakhon Ratchasima Province, a northeastern region of Thailand, about 210 km from Bangkok. One hundred and fifty-two ballistosporous yeasts were isolated from 88.5% of samples examined (Fungsin et al., 1999). These yeasts were assigned to the genera Bullera (55 strains), Kockovaella (3), Bensingtonia (10), Sporobolomyces (66), and Tilletiopsis (18). Nineteen strains assigned to Sporobolomyces had Q-10(H2) as the major ubiquinone and were recovered from 57.7% of the samples examined (Table 6). Although the identification is not completed yet, Bensingtonia thailandica was already described as a new species (Fungsin et al., 2000), and an additional 6 new species were found in the genera Kockovaella and Sporobolomyces (Fungsin et al., unpublished).

4. Ballistosporous Yeasts Found in the Phyllosphere of New Zealand and Tasmania Island, Australia

In May 1987, we isolated ballistosporous yeasts from 58 samples of various plants in New Zealand and Tasmania Island, Australia, that were collected by J. Sugiyama. These samples were sent to the Japan Collection of Microorganisms by airmail within a week after the collection and without delay were used for the isolation study of yeasts.

Ballistosporous yeasts were isolated from 22 samples (75.7%) of 29 New Zealand plants examined. Sixty-seven selected strains were identified as 22 species in the genera Bensingtonia, Bullera, Sporobolomyces, and Udeniomyces (Table 6).

Twenty-nine strains of Bullera were identified as eight species. Five were found to represent new species and were described as Bullera coprosmiensis, Bullera hannae, Bullera huiaensis, Bullera mrakii, and Bullera unica (Hamamoto and Nakase, 1996). Thirty-five strains of Sporobolomyces were identified as 11 species. Six were found to represent new species and were described as Sporobolomyces coprosmiae, Sporobolomyces copromicola, Sporobolomyces dimmenae, Sporobolomyces
Two strains of *Bensingtonia* were found to represent two different species. The one represented a new species and was named *Bensingtonia ingoldii* (Nakase et al., 1989a). The other was found to be a second strain of *B. naganoensis*. Only one *Udeniomyces* strain was isolated from New Zealand plants and was assigned to *Udeniomyces pyricola*.

*Sporobolomyces roseus* was found to be the most popular ballistosporous yeast species in the phyllosphere of New Zealand and was found in 37.9% of the samples examined, followed by *B. alba* (24.1%), *S. dimmenae* (17.2%), *B. crocea* (10.3%), *B. mrakii* (10.3%), *B. variabilis* (10.3%), and *S. novazealandicus*.
Di Menna (1959), an early investigator of yeasts inhabiting the phyllosphere, also reported the isolation of ballistosporous yeasts, *S. roseus*, *Sporobolomyces odorus* (synonym of *S. salmonicolor*), *Sporobolomyces pararoseus* (synonym of *Sporidiobolus pararoseus*: teleomorph of *S. shibatanus*), and *B. alba*, from pasture leaves in New Zealand.

Ballistosporous yeasts were isolated from 10 samples (34.5%) of 29 Tasmanian plants examined. These plants were assumed to be exposed to a bad condition during transportation because yeast colonies appearing on the agar plates were usually few. Nineteen isolated strains were identified as five species in the three genera. All belonged to the known species (Table 6). *Sporobolomyces roseus* was found in 31% of the samples examined.

As a rule, we did not isolate strains supposedly belonging to the yeastlike genus *Tilletiopsis* from plants in New Zealand and Tasmania Island, though these strains often appeared on agar plates. However, several colonies of *Tilletiopsis* with a yeastlike appearance were isolated by chance. One of these strains was identified as *Tilletiopsis washingtonensis* (Hamamoto and Nakase, 2000).

Li (1982) reported the isolation of *Bullera* species from leaves of wheat and corn and from insects. She found two new taxa, *Bullera sinensis* and *Bullera alba* var. *lactis*, besides *Bullera alba*. Further, Li (1988) studied 70 strains of ballistosporous yeasts isolated from various plants and identified them as *Sporobolomyces shibatanus* (45 strains), *Sporobolomyces salmonicolor* (3), *Sporobolomyces roseus* (1), *Sporobolomyces sp.* (4), *Bullera alba* (4), *Bullera alba* var. *lactis* (1), *Bullera sinensis* (10) and *Tilletiopsis* sp. (2).

In 1996, Bai et al. (unpublished) examined 43 plant materials collected in Yunnan Province in China and isolated 670 strains of yeasts. Some of these isolates were taxonomically studied after an examination of their ballistosporous abilities. They found a strain of *Bensingtonia yamatoana* whose physiological characteristics differed from strains found in Japan (Bai et al., 1999b). This is the first report on the isolation of this species outside Japan.

A new species, *Sporobolomyces yunnanensis*, was described based on a Yunnan isolate (Bai et al., 2000). This species is the third yeast species containing Q-10(H₂) as the major ubiquinone homologue. Furthermore, three new species of *Bullera* were recognized among the Yunnan isolates (Bai et al., 1999a).

Ruinen (1963) examined yeasts living in the phyllo-

### Table 6. Ballistosporous yeasts found in plants collected in New Zealand and on Tasmania Island, Australia.

<table>
<thead>
<tr>
<th>Species</th>
<th>New Zealand</th>
<th></th>
<th>Tasmania Island, Australia</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of strains</td>
<td>Frequency of isolation (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No. of strains</td>
<td>Frequency of isolation (%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
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<tr>
<td><em>Bullera</em></td>
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<td>34.5</td>
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<td>6.9</td>
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<tr>
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<td>24.1</td>
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<td>3.4</td>
</tr>
<tr>
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<td>1</td>
<td>3.4</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>10.3</td>
<td>1</td>
<td>3.4</td>
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<td>3.4</td>
<td></td>
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<td>3.4</td>
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<td></td>
</tr>
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<td>4</td>
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<td></td>
<td></td>
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<td><em>B. variabilis</em></td>
<td>4</td>
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<td></td>
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<td><strong>Udeniomyces</strong></td>
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<td>3.4</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>3.4</td>
<td>1</td>
<td>3.4</td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td>3.4</td>
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</tr>
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<td><em>S. inostophitius</em></td>
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<td></td>
</tr>
<tr>
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<td><em>S. shibatanus</em></td>
<td>3</td>
<td>10.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. taupoensis</em></td>
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<td>3.4</td>
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<tr>
<td><strong>Total</strong></td>
<td>67</td>
<td>75.7</td>
<td>19</td>
<td>34.5</td>
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</table>

<sup>a</sup> Frequency of isolation (%) = (no. of samples from which that yeast was isolated)/(no. of samples examined) × 100.
sphere of Indonesia and found seven strains of ballistosporous yeasts. She provisionally classified six strains as *Sporobolomyces salmonicolor*. She pointed out, however, that the presence in these strains of a buckled mycelium with chlamydospores would make the identification with the genus *Sporidiobolus* even more plausible. Phaff (1970) described these strains as *Sporidiobolus ruinenii*, and Holzschu et al. (1981) validated this species. It is now listed in The Yeasts, a Taxonomic Study, 4th ed., as *Sporidiobolus ruineniae* (Statzell-Tallman and Fell, 1998). The remaining one strain was identified as *Sporobolomyces roseus*.

In 1996, Haryono et al. collected 40 plant materials in the suburbs of Yogyakarta, Indonesia, and isolated more than 250 strains of yeasts (Haryono et al., unpublished). Seventy of them isolated from the Kaliurang area, 900 to 1,000 m above sea level, were examined for their ballistosporous abilities, and 61 strains were found to be ballistosporous. These strains were assigned to the genera *Bullera* (11 strains), *Bensingtonia* (14), and *Sporobolomyces* (36) (Haryono et al., 1998). Among strains assigned to *Sporobolomyces*, 8 had Q-10(H2) as the major ubiquinone. They reported that these Q-10(H2)-containing strains represented a different species from the known Q-10(H2)-containing species, *Erythrobasidium hasegawianum* and *Sporobolomyces elongatus* (Yamada, 1998).

Luong et al. (unpublished) isolated 121 strains of ballistosporous yeasts from 20 plant materials collected in the Cuc Phuong National Forest in Vietnam. Ballistosporous yeasts were isolated from all samples examined. They studied 85 selected strains and assigned them to the genera *Bullera* (39 strains), *Kockovaelia* (5), *Sporobolomyces* (39), and *Tilletiopsis* (2). Five strains assigned to *Sporobolomyces* had Q-10(H2) as the major ubiquinone homologue. Among 5 strains assigned to *Kockovaelia*, 4 were proved to represent 3 new species, but further study was required about the taxonomic position of the remaining strain (Luong et al., 1999). Later, it was proved to represent a new species (Luong et al., unpublished).

6. **Ballistosporous Yeasts Containing Q-10(H2) Found in the Asian Phyllosphere**

The yeasts and yeastlike fungi contain ubiquinone or coenzyme Q as a respiratory component. Ubiquinone homologues are useful for classifying yeasts and yeastlike fungi at the generic level (Yamada and Kondo, 1973; Yamada et al., 1976). The ubiquinone homologues of these microorganisms are recognized to be Q-5 through Q-10. A dehydrated isoprenoid side-chain ubiquinone homologue, Q-10(H2), was found in basidiomycetous yeasts *Erythrobasidium hasegawianum* and *Sporobolomyces elongatus* (Yamada, 1998).

Recent isolation studies of ballistosporous yeasts carried out by our research group revealed that Q-10(H2)-containing yeasts are not rare in the phyllosphere of tropical and subtropical regions of Asia (Nakase, 1998). As shown in Table 7, these kinds of yeasts were isolated from 27.8% of plants collected in the Ogasawara Islands and from 50% collected in Indonesia. In Thailand, Q-10(H2)-containing yeasts were isolated from 57.7% of plants examined in 1996, but they were not obtained in the isolation studies carried out in 1987 and 1990. This fact is not easy to explain. One possible explanation is the effect of air pollution. Ballistosporous yeasts were reported to be sensitive to air pollution and able to use the indicator of an air pollution of the city (Dowdings and Richardson, 1990). In 1987 and 1990, samples were collected in the suburban and urban areas of Bangkok, and in 1996 samples were collected in a tropical rain forest of the Environmental Research Station of the Thailand Institute of Scientific and Technological Research in Nakhon Ratchasima Province, about 210 km from Bangkok. As mentioned, the frequency of the isolation of ballistosporous yeasts was very low in the samples collected in the urban areas of Bangkok, including markets, suggesting the effect of air pollution on the population of ballistosporous yeasts. If Q-10(H2)-containing yeasts are more sensitive to air pollution than other kinds of ballistosporous yeasts, the difference between 1996 and 1987/1990 can be explained. Apparently, a further detailed examination is required to explain this phenomenon because there is no data about the sensitivity of Q-10(H2)-containing ballistosporous yeasts to the polluted air.

As mentioned above, Q-10(H2)-containing yeasts were also isolated in Vietnam and China. Probably, Q-10(H2)-containing ballistosporous yeasts are widely distributed in the phyllosphere of the tropical and subtropical regions of Asia.
7. Regional Difference of Ballistosporous Yeast Flora in the Phyllosphere

The role of ballistosporous yeasts in the phyllosphere is not clear. No clear report exists on the pathogenicity, parasitism, or symbiosis of ballistosporous yeasts. The only exception is *Sporobolomyces roseus*, which was reported as a causal agent of citrus pseudo greasy spot (Koizumi, 1986; Koizumi and Kuhara, 1984). Probably, ballistosporous yeasts are saprophytically living in the phyllosphere, with rare exceptions. In the series of isolation studies of ballistosporous yeasts living in the phyllosphere, we did not notice a relationship between the kinds of yeasts and the kinds of plants, though the study is still in an early stage.

On the other hand, the regional difference was suggested in the yeast flora of the phyllosphere. This is probably caused by the differences of temperature and geography. The papers published so far are not enough to discuss this matter, and extensive isolation studies are required to learn regional characteristics of the yeast flora of the phyllosphere of Asia.

Table 8 compares the ballistosporous yeasts found in the phyllospheres of the main island of Japan, which is located in the North Temperate Zone, Thailand in the tropics, and New Zealand in the South Temperate Zone. Except for the *Tilletiopsis* species, 49 species were isolated in these regions. Among them, 12 were commonly found in two or three countries. Namely, 4 were commonly found in the main island of Japan, and in Thailand and New Zealand, 7 on the main island of Japan and in Thailand, 9 on the main island of Japan and in New Zealand, and 4 in Thailand and New Zealand. Fifteen of the remaining 37 species were found on the main island of Japan, 10 in Thailand, and 12 in New Zealand.

*Sporobolomyces shibatanus* (Teleomorph: *Sporidiobolus pararoseus*), *Bullera alba*, *Bullera crocea*, and *Bullera variabilis* were commonly found in three countries. *Sporobolomyces shibatanus* and *Bullera alba* were described about 70 years ago and are very popular species in the phyllosphere. The former species was reported to occupy 84% of the population of ballistosporous yeasts isolated from the phyllosphere of China (Li, 1988). These species are considered to live in the worldwide phyllosphere. In spite of a relatively young species reported in 1983 (Buhagiar et al., 1983), *Bullera crocea* was found in Japan, China, Canada, Thailand, and New Zealand and considered to be widely distributed in the earth’s phyllosphere. *Bullera variabilis* is a heterogeneous species supposedly consisting of several different species (Nakase and Suzuki, 1987e) so that a taxonomic reexamination is required before we discuss the distribution of this species.

Five species, *Bensingtonia naganoensis*, *Sporobolomyces roseus*, *Sporobolomyces inositophilus*, *Sporobolomyces sasicola*, and *Udeniomyces pyricola*, were commonly found on the main island of Japan and in New Zealand, but not in Thailand.

*Sporobolomyces roseus* is a popular yeast species commonly found in the phyllosphere in the Temperate Zone. On the main island of Japan, it was found in 35.8% of plants collected in the suburban Tokyo (Yoshizawa et al., 1987), 72.1% of *Oryza sativa* in Kanagawa Prefecture (Nakase and Suzuki, 1985), 70.8% of *Miscanthus sinensis* in Kanagawa and Saitama Prefectures (Nakase and Suzuki, 1987b), and 45.5% of plants collected at Mt. Fuji (Nakase and Takashima, 1993). Furthermore, this species was isolated from 37.9% of plants collected in New Zealand and 31% of plants collected on Tasmania Island of...
Australia (Nakase, unpublished). Di Menna (1959) reported the isolation of this species on pasture leaves in New Zealand. It is also found in Siberia, Russia (Golubev, personal communication), and China (Bai, personal communication). This species is believed to be distributed in a wide area within the Temperate Zone and probably in the Frigid Zone.

On the other hand, *Sporobolomyces roseus* was not found in the phyllosphere of Thailand (Takashima et al., 1998). It seems not to be found on the Ogasawara Islands in the Pacific Ocean, in Kaliurang, Indonesia, and Vietnam, though the identification experiment is not completed yet (Nakase et al., unpublished). It should be noted, however, that Ruinen (1963) found each strain of this species among strains isolated from plant samples collected in Indonesia (Bogor), Surinam in South America, and the Ivory Coast in Africa. This species seems to be rich in the Temperate Zone and probably in the Frigid Zone, but rare in the tropics.

The genus *Udeniomyces* comprises 3 species, *U. puniceus*, *U. pyricola*, and *U. megalosporus* (Nakase and Takematsu, 1992). *Udeniomyces pyricola* is the most popular. This species was first found in Switzerland as *Bullera pyricola* (Stadelmann, 1975), then recorded on the main island of Japan (Nakase and Suzuki, 1985, 1987b), Canada (as *Bullera grandispora*) (Johri and Bandoni, 1984), New Zealand, Tasmania Island of Australia, and Hungary (Nakase, unpublished). *Udeniomyces megalosporus* is a common yeast in the phyllosphere of Japan, but has not yet been found in other countries. *Udeniomyces puniceus* was first isolated from a frozen fish in the market in Japan and described as *Candida punicea* (Komagata and Nakase, 1965). According to Hernandez-Saaavedra et al. (1992), this species occupied 20% of the isolates from seawater collected in the Pacific Ocean off the west coast of Baja California, Mexico. However, this species has not yet been found in the phyllosphere. All the *Udeniomyces* species are psychrophilic and have maximum growth temperatures of below 24°C; thus they are assumed to be distributed in the Temperate Zone and probably also in the Frigid Zone.

*Bensingtonia naganoensis*, *Sporobolomyces inositophilus*, and *Sporobolomyces sasicola* are young species found in the 1980s and have been found in no country except Japan and New Zealand. Since these species were found in the North and South Temperate Zones, geographically far apart, they are assumed to be widely distributed in the phyllosphere in both Temperate Zones.

Among 39 species of ballistosporous yeasts isolated on the main island of Japan and in New Zealand, only 9 were commonly found in these two countries, and the remaining 30 were found only in one or the other of these countries. Both countries are in the Temperate Zone, but in different hemispheres. In the isolation of ballistosporous yeasts from plants in New Zealand, only 29 plant samples were employed. This study thus does not represent the whole figure of ballistosporous yeast flora in the phyllosphere of New Zealand. Di Menna (1959) reported the isolation of only a few ballistosporous species from pasture leaves of New Zealand in spite of her extensive study, probably because of the primitive taxonomy at that time. I am assuming that the species common to Japan and New Zealand may increase when we repeat the isolation study, but new species in different countries are expected to increase.

In our study, three species, *Sporobolomyces salmonicolor* (Teleomorph: *Sporidiobolus salmonicolor*), *Bullera pseudoalba*, and *Bullera sinensis* were commonly found on the main island of Japan and in Thailand, but not in New Zealand. Di Menna (1959), however, reported the isolation of *Sporobolomyces odorus* (synonym of *Sporobolomyces salmonicolor*) from New Zealand plants. *Sporobolomyces salmonicolor* is the oldest species of ballistosporous yeast found more than 100 years ago and widely distributed throughout the world. *Bullera pseudoalba* and *Bullera sinensis* are young species found in the 1980s, so further study is required about the distribution of these species.

*Sporidiobolus ruineniae* was first found in Indonesia (Holzscheu et al., 1981; Phaff, 1970; Ruinen, 1963). It was isolated from 2.4 and 17.5% of plants collected in Thailand (Table 5), but not in the Temperate Zone (Tables 1–3, 6). This species seems to be tropical yeast, though further detailed study is required.

This suggests that yeast flora of the tropical phyllosphere is different from that of the Temperate Zones, and that the yeast flora of the North Temperate Zone is different from that of the South Temperate Zone. The difference seems to be bigger between the tropics and the Temperate Zones than between the North and South Temperate Zones.

Ballistosporous yeast flora in the Ogasawara Islands seems to be quite different from other regions of Asia. These islands are in the subtropical Pacific Ocean.
about 1,000 km from Tokyo and are known to have a unique flora of higher plants. As mentioned above, 94 hitherto identified strains of isolates from the Ogasawara Islands represented 14 new species (Table 4). Only 1 species having Q-10(H2) seems to be common to Thailand (Fungsin et al., unpublished). Besides ballistosporous yeasts, 2 strains of nonballistosporous, stalked conidium-forming yeasts isolated from the Ogasawara Islands were found to represent 2 new species of *Fellomyces* (Hamamoto et al., 1998).

Sakaguchi and Ohtani (1939) found *Schizosaccharomyces pombe* in spontaneously fermented sugarcane molasses in the manufacture of Shochu, a distilled alcoholic beverage in the Ogasawara Islands. This fission yeast was widely found in fermented sugarcane molasses in Southeast Asian countries such as Indonesia and Vietnam. This suggests that accompanied by the progress of the isolation study, yeast species common to other regions of Asia will increase in the Ogasawara Islands. Yeast species which are unique in these islands, however, are expected to increase more and more. The geographic isolation may strongly affect the yeast flora in the phyllosphere.

In the series of the isolation study of ballistosporous yeasts, 17°C was usually chosen to prevent disturbance by the rapidly growing dominant yeasts and filamentous fungi. In Thailand, however, 23 and 30°C were employed in the isolation study in 1987 because Thailand is in the tropics, and it was not easy to use the incubator for 17°C. Among 16 species isolated from Thai plants, only 4 are commonly isolated at 23 and 30°C, and the remaining 12 species were isolated

<table>
<thead>
<tr>
<th>Species</th>
<th>Japan (main island)</th>
<th>Thailand</th>
<th>New Zealand</th>
<th>Species</th>
<th>Japan (main island)</th>
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<th>New Zealand</th>
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Table 8. Ballistosporous yeasts found in plants collected on the main island of Japan, in Thailand, and in New Zealand.
at one or the other temperatures, 8 at 23°C and 4 at 30°C (Table 5). Based on this observation, we used 17 to 25°C for the isolation study even if we employed plant samples collected in the tropics and subtropics, together with the frequent replacement of agar plates for collecting ballistospores discharged from plant materials, to prevent disturbance by rapid growers.

8. New Ballistosporous Yeasts Found in Regions Other than the Asia-Pacific Region

In the past 20 years, several new ballistosporous yeasts were also found in several regions besides the Asia-Pacific region. Buhagiar et al. (1983) reported Bullera crocea and Bullera armeniaca, which produce orange colonies. These species were isolated from fruit and vegetables in the United Kingdom. Shivas and Rodrigues de Miranda (1983) found Sporobolomyces elongatus on a leaf in Australia. This species was later found to have Q-10(H₂) as the major ubiquinone (Nakase and Suzuki, 1986d). Johri and Bandoni (1984) studied ballistosporous yeasts isolated from plants in Canada and described Bullera aurantiaca, Bullera globospora, and Bullera salicina. Later, Bullera aurantiaca was regarded as a synonym of Bullera crocea (Boekhout, 1991), and Bullera salicina was transferred to Sporobolomyces (Nakase and Itoh, 1988). Ingold (1986) described Bensingtonia ciliata gen. nov. sp. nov., based on a strain isolated from a fruit body of Auricularia auricula-judae, in the United Kingdom. Boekhout (1987) found that this species had Q-9 as the major ubiquinone.

Three new species, Sporobolomyces kluyver-nielli (van der Walt et al., 1986), Sporobolomyces phyllomatis (van der Walt et al., 1988), and Sporobolomyces phyliladus (van der Walt et al., 1989), were found in the phyllosphere of South Africa. The latter species was transferred to the genus Bensingtonia because it had Q-9 as the major ubiquinone (Yamada et al., 1988). Nakase and Suzuki (1988) described Sporobolomyces yuccicola based on a strain isolated by Bandoni from a plant in Canada. This species, containing Q-9, was transferred to Bensingtonia (Nakase and Boekhout, 1988). Sláviková and Grabiñska-Loniewska (1992) described Sporobolomyces lactosus, which was isolated from activated sludge-treating wastes of the petrochemical industry in Poland.

Recently, Sampaio et al. (1999) isolated a ballistosporous yeast from an old fruit body of Exidiopsis sp. in Portugal and placed it in the genus Kurtzmanomyces, as Kurtzmanomyces insolitus, after emendation of the diagnosis of the genus to permit the inclusion of ballistosporous yeasts.

9. Systematics and Phylogeny of Ballistosporous Yeasts

Since the publication of The Yeasts, a Taxonomic Study, 3rd ed. (Kreger-van Rij, 1984), many new species were found in ballistosporous yeasts. As a result, the number of species included in The Yeasts, a Taxonomic Study, 4th ed. (Kurtzman and Fell, 1998) increased greatly and reached 47, excluding the duplication of teleomorphs and anamorphs: 1 in Bulleromyces (Boekhout, 1998a); 4 in Sporidiobolus (Statzell-Tallman and Fell, 1998); 9 in Bensingtonia (Boekhout and Nakase, 1998a); 13 in Bullera, including 3 Udeniomyces species (Boekhout and Nakase, 1998b); 2 in Kockovaella (Nakase and Banno, 1998); and 21 in Sporobolomyces (Boekhout and Nakase, 1998c). Moreover, 6 species were included in the ballistosporous yeast-like fungal genus Tilletiopsis (Boekhout, 1998b).

However, 28 species isolated from the phyllosphere of the Asia-Pacific region and 2 found in Europe were not included in this book because of the delay it would have caused in its publication, though most of them were described before 1998. These species are summarized in Table 9, so 77 species are now recognized in ballistosporous yeasts, excluding the duplication of teleomorphs and anamorphs. This dramatic increase of ballistosporous yeast species was accompanied by an increase of their heterogeneity.

As shown in Fig. 2, the G+C value of DNA of ballistosporous yeasts is widely distributed from 39 to 69 mol%. The mol% G+C of the species belonging to the Hymenomycetes, were somewhat lower than those of the Urediniomycetes, but the range of distribution is wide in both classes. This clearly suggests the phylogenetic diversity of ballistosporous yeasts.

Together with trials for the finding of teleomorphs, chemotaxonomic and molecular phylogenetic studies became the mainstream of modern research of yeast systematics. Basidiomycetous yeasts, including ballistosporous yeasts, have been studied from the viewpoint of carbohydrate composition of whole cells and cell walls (Nakase et al., 1995b; Prillinger et al., 1990, 1991a, b, 1993; Sugiyama et al., 1985; Suzuki and

Phylogenetic analyses of the nucleotide sequences of ribosomal DNAs (rDNAs), especially those of 18S rDNA and D1/D2 domain of 26S rDNA, were extensively studied (Boekhout et al., 1995; Hamamoto and Nakase, 2000; Nakase et al., 1993a, 1995a; Suh and Nakase, 1995; Suh and Sugiyama, 1993, 1994; Sugiyama and Suh, 1993; Suh et al., 1996a, b, c; Swann and Taylor, 1993, 1995a, b; Takashima and Nakase, 1996, 1999; Takashima et al., 1995a; Yamada...
et al., 1990a, b). Recently, Fell et al. (2000) published an exhaustive paper on the sequences of the D1/D2 domain of basidiomycetous yeasts and showed the whole profile of the phylogenetical interrelationships of these yeasts.

Despite recent extensive studies on the systematics and molecular phylogeny of ballistosporous yeasts and related basidiomycetous taxa, the systematics of ballistosporous yeasts is still in the primitive stage. The genera of these yeasts now recognized are shown in Table 10 with salient characteristics. The genera enumerated in this table, however, especially the anamorphic ones, are often very heterogeneous, comprising species with polyphyletic phylogenetic origins as discussed below. Several reviews have been published about the systematics and phylogeny of basidiomycetous yeasts, including ballistosporous yeasts (Boekhout et al., 1993, 1998; Fell et al., 2000; Nakase, 1989; Nakase et al., 1991, 1993b, 1995a; Sugiyama, 1998; van der Walt, 1987), and the latest taxonomic system of basidiomycetous yeasts, was summarized in The Yeasts, a Taxonomic Study, 4th ed. (Boekhout et al., 1998). Thus only the outline of the systematics and phylogeny of ballistosporous yeasts and related taxa are discussed in this paper.

Ballistosporous yeasts are a heterogeneous group of yeasts widely distributed in the Basidiomycota. As shown in Fig. 3, ballistosporous yeasts are found in the 3 classes of the Basidiomycota; Urediniomycetes, Hymenomycetes, and Ustilaginomycetes, in the phylogenetic tree based on the analysis of 18S rDNA sequences. They constitute many clusters, often with nonballistosporous yeasts, yeastlike fungi, and filamentous fungi. Indeed, ballistosporous yeasts are a heterogeneous group of basidiomycetous yeasts with phylogenetically polyphyletic origins.

The class Urediniomycetes includes four ballistosporous yeast genera; two teleomorphic genera, Sporidiobolus and Mastigobasidium, and two anamorphic genera, Bensingtonia and Sporobolomyces (Fig. 4). Sporidiobolus is a telemorph of Sporobolomyces, and the establishment of Mastigobasidium was based on the observation of a sexual state of Bensingtonia intermedia (Golubev, 1999). The class Urediniomycetes comprises five clusters; Agaricostilbum/Bensingtonia, Subbrunneus, Sporidiales, Urediniales, and Erythrobasidium. Ballistosporous yeasts are distributed in Agaricostilbum/Bensingtonia, Subbrunneus, Sporidiales, and Erythrobasidium, but not in the Urediniales.

The species of the genus Sporobolomyces are distributed in four clusters, and species of the genus Bensingtonia are distributed in two clusters, mixed with each other and with species of nonballistosporous yeasts and fungi. This clearly indicates that these anamorphic genera are heterogeneous and are phylogenetically polyphyletic. Species of the genus Sporidiobolus are found in the Sporidiales cluster. Sporidiobolus microsporus nom. nud. isolated in Jamaica was regarded as a synonym of S. ruineniae (Statzell-Tallman and Fell, 1998). Fell et al. (1998), however, found that this species is a distinct species and validated it. Sporobolomyces coprophilus, which was isolated from goat dung in Pakistan (Sugiyama and Goto, 1967), was dealt with as a variety of S. ruineniae by Kurtzman and Fell (1991) and was then considered as a synonym of S. ruineniae (Statzell-Tallman and Fell, 1998). Probably the species of Sporobolomyces in the Agaricostilbum/Bensingtonia, Subbrunneus, and Erythrobasidium clusters may have teleomorphs different from Sporidiobolus. Moreover, the teleomorphic genus Mastigobasidium, proposed for a telemorph of Bensingtonia intermedia, seems to be a telemorph of certain Bensingtonia species, but not all.

The Subbrunneus cluster comprises four Sporobolomyces species. This cluster was not included in the classification by Swann and Taylor (1995a, b) but found by Hamamoto and Nakase (2000) based on yeasts recently found in Japan (Nakase and Suzuki,
Table 10. Salient characteristics of ballistosporous yeasts and related genera of basidiomycetous yeasts.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Basidial form</th>
<th>Teliospore</th>
<th>Septal pore structure</th>
<th>Ballistospore</th>
<th>Stalked conidium</th>
<th>Budding cell</th>
<th>Xylose in cell wall</th>
<th>Major ubiquinone</th>
<th>Anamorph</th>
</tr>
</thead>
<tbody>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q9</td>
<td>Malassezia</td>
</tr>
<tr>
<td>Tilletiaria</td>
<td>phragmo</td>
<td>+</td>
<td>primitive dolipore</td>
<td>+</td>
<td></td>
<td></td>
<td>-</td>
<td>Q9</td>
<td>Tilletiopsis</td>
</tr>
<tr>
<td>Entyloma?</td>
<td>holo</td>
<td></td>
<td>simple pore</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Q10</td>
<td>Sympodidomycopsis</td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q10</td>
<td>Sterigmatomyces</td>
</tr>
<tr>
<td>Ustilago?</td>
<td>phragmo</td>
<td>+</td>
<td>poreless</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q10</td>
<td>Pseudozyma</td>
</tr>
<tr>
<td>Mastigobasidium</td>
<td>phragmo</td>
<td>+</td>
<td></td>
<td>+</td>
<td>-/+</td>
<td>+</td>
<td></td>
<td>Q9</td>
<td>Bensingtonia</td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q10</td>
<td>Kurtzmanomyces</td>
</tr>
<tr>
<td>Leucosporidium</td>
<td>phragmo</td>
<td>+</td>
<td>simple pore</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q9/Q10</td>
<td>Rhodotorula</td>
</tr>
<tr>
<td>Rhodosporidium</td>
<td>phragmo</td>
<td>+</td>
<td>simple pore</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q9</td>
<td>Sporobolomyces</td>
</tr>
<tr>
<td>Kondoa</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Q10</td>
<td>?</td>
</tr>
<tr>
<td>Sporidiobolus</td>
<td>holo/phragmo</td>
<td>+</td>
<td></td>
<td>+</td>
<td>-/+</td>
<td>-/+</td>
<td></td>
<td>Q10(H2)</td>
<td>?</td>
</tr>
<tr>
<td>Erythrobasidium</td>
<td>holo</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q10</td>
<td>?</td>
</tr>
<tr>
<td>Cystofilobasidium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q9</td>
<td>Itersonilia</td>
</tr>
<tr>
<td>Mrakia</td>
<td>holo</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q8</td>
<td></td>
</tr>
<tr>
<td>Filobasidium</td>
<td></td>
<td></td>
<td>dolipore</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q9, Q10</td>
<td>Cryptococcus</td>
</tr>
<tr>
<td>Filobasidiella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q10</td>
<td></td>
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<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q9/Q10</td>
<td>Trichosporon</td>
</tr>
<tr>
<td>Bulleromyces</td>
<td>phragmo</td>
<td>-</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Q9</td>
<td>Bullera</td>
</tr>
<tr>
<td>Xanthophyllomyces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q10</td>
<td>Phaffia</td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q10</td>
<td>Udeniomyces</td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q10</td>
<td>Kockovaella</td>
</tr>
<tr>
<td>Sterigmatosporidium</td>
<td>holo</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Q9</td>
<td>Fellomyces</td>
</tr>
</tbody>
</table>

*: Unknown.
Fig. 3. Phylogenetic relationships among ballistosporous yeasts and related taxa deduced from the analysis of 18S rDNA sequences.

The tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) based on Kimura’s two-parameter distance (Kimura, 1980). Numerals represent the percentages from 100 replicate bootstrap samplings (Felsenstein, 1985).
1985; Nakase et al., 1994) and in New Zealand (Hamamoto and Nakase, 1995). This cluster is well separated from the other four clusters with enough phylogenetic distance. It probably represents a new order to be established in the class.

The major sugar component of the cell wall in the Urediniomycetes is mannose, and glucose is included as the second component, but xylose is not detected. Fucose is detected in species of the Agaricostilbum/Bensingtonia, Subbrunneus and Sporidiales clusters, but not in the Erythrobasidium cluster (Takashima et al., 2000). This coincided well with the serological analyses of the genera Sporobolomyces and Rhodotorula by Tsuchiya et al. (1957, 1969), who found two independent groups of species without cross-reaction.

The class Hymenomycetes comprises four ballistosporous genera; a teleomorphic genus Bulleromyces and three anamorphic genera, Bullera, Kockovaella, and Udeniomycetes. The species of these ballistosporous genera and related nonballistosporous taxa in the class Hymenomycetes are in seven lineages

Fig. 4. Phylogenetic tree of yeasts in the class Urediniomycetes based on 18S rDNA sequences. The tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) based on Kimura's two-parameter distance (Kimura, 1980). Numerals represent the percentages from 1,000 replicate bootstrap samplings (Felsenstein, 1985). Reproduced from Takashima et al. (2000) by permission of the editor.
Ballistosporous yeasts are distributed in the *C. luteolus, Filobasidiella, Bulleromyces, Sterigmatosporidium* and *Cystofilobasidiales* (Fell et al., 1999) lineages, but not in the *Filobasidium* and *C. humicolicus-Trichosporon* lineages (Takashima and Nakase, 1999). Recently, Fell et al. (2000) proposed a new order Trichosporonales for *C. humicolicus-Trichosporon* lineage. More recently, we found that two strains of ballistosporous yeasts isolated in the phyllosphere of Taiwan are in the latter two lineages (Nakase et al., unpublished).

The species of the genus *Bullera* are found in three lineages, *C. luteolus, Filobasidiella*, and *Bulleromyces*. Two Taiwanese strains mentioned above are assigned to the genus *Bullera* according to the present definition of the genus. Therefore, the genus *Bullera* comprises species distributed in five different lineages. Apparently the taxonomic reinvestigation of the genus *Bullera* is required, together with the nonballistosporous counterpart genus *Cryptococcus*.

A teleomorphic genus *Bulleromyces* was established for the sexual state of *Bullera alba* (Boekhout et al., 1991). The *Bullera* species other than the *Bulleromyces* lineage are probably expected to have other kinds of teleomorphs. Stalked conidium-forming yeasts are in the *Sterigmatosporidium* lineage, except for *Tsuchiyaea wingfieldii*, which is in the *Filobasidiella* lineage in which *Bullera penniseticola* is also included (Fig. 5). *Bullera penniseticola* produces conidia resembling stalked conidia (Barnett, personal communication). The species of *Kockovella* are closely related to those of *Fellomyces*, a nonballistosporous yeast genus, so a detailed comparison is necessary to determine whether these two genera are well defined.

Nakase and Takematsu (1992) proposed a genus *Udeniomyces* for three *Bullera* species, *Bullera pyricola, Bullera punicea*, and *Bullera megalospora*, based on the observation that these yeasts were clearly differentiated from other members of *Bullera* in the production of large bilaterally symmetrical ballistospores. This genus was not accepted in The Yeasts, a Taxonomic Study, 4th ed. (Boekhout and Nakase, 1998b). In the editor's note in that monograph, however, it was stated as follows: "Based on nucleotide sequence analysis of 18S rDNA (Suh and Nakase, 1995) and partial sequences of 26S rDNA (G. Scorzetti and J. W. Fell, unpublished), *Udeniomyces* is related to *Cystofilobasidium* and *Mrakia* and well separated from *Bullera*. Consequently, the separate designation of *Udeniomyces* appears correct, but the information was received too late for inclusion in this edition." This genus was further characterized by the formation of annellidic scars (Boekhout, 1991).

The yeastlike ballistosporous genus *Tilletiopsis* is in the class Ustilaginomycetes (Fig. 3). According to The Yeasts, a Taxonomic Study, 4th ed., six species are recognized in *Tilletiopsis* in which *Tilletiopsis creema* and *Tilletiopsis lilacina* are regarded as synonyms of *Tilletiopsis washingtonensis* (Boekhout, 1998b). Recently, however, Hamamoto et al. (2000) reported that these two species represent distinct species, supporting the discussion of Yamazaki et al. (1985) based on the physiological properties and the electrophoretical comparison of enzymes.

Goto et al. (1969) described *Sporobolomyces antarcticus* based on a yeast isolated from the Antarctic. This species is now dealt with as a species of the nonballistosporous genus *Pseudozyma* (Boekhout, 1995; Boekhout and Fell, 1998), which is at a position close to *Tilletiopsis* (Fell et al., 2000). Because ballistosporous ability is easily lost during preservation, *S. antarcticus* is assumed to have lost its ballistosporous ability just after the description.

10. Future Perspectives

The introduction of new technologies and the isolation of new strains are the essential elements for the progress of microbial systematics. In the past 10 years, the sequence analysis of genes, especially the analyses of 18S rDNA and D1/D2 domain of 26S rDNA, became the common technology of systematic study of yeasts and fungi. As repeatedly mentioned, ballistosporous yeasts were proved to be a heterogeneous group of basidiomycetous yeasts distributed in all three classes in the Basidiomycota and are found in many clusters of the phylogenetic trees based on the sequence analysis of 18S rDNA and mixed with non-ballistosporous yeasts and fungi. The analysis of D1/D2 domain of 26S rDNA gives topologies similar to those based on 18S rDNA in the phylogenetic tree of basidiomycetous yeasts (Fell et al., 2000). Thus it is clearly indicated that the present taxonomic system for basidiomycetous yeasts is not rational and that the ballistosporous genera, especially anamorphic genera, comprise species with polyphyletic origins. Apparently ballistosporous ability has no phylogenetical significance, and a new system to be established for basid-
Fig. 5. Phylogenetic tree of ballistosporous yeasts and related taxa in the class Hymenomycetes based on 18S rDNA sequences.

The tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) based on Kimura’s two-parameter distance (Kimura, 1980). Numerals represent the percentages from 100 replicate bootstrap samplings (Felsenstein, 1985).
iomycetous yeasts should be constructed regardless of ballistosporous ability, though the morphology of ballistosporous has certain taxonomic significance.

The construction of a taxonomic system of higher taxa of basidiomycetous yeasts should be based on the analysis of sequences of several genes, including 18S and 26S rDNAs, ITS, ETS, and IGS regions of rDNA, type II topoisomerase, cytochromes, and elongation factors, but not on a single gene. Ideally, the definition of higher taxa should be based on clear phenotypic differences. This is not easily done, however, in yeasts, filamentous fungi, and mushrooms at present. For a while, we must admit the use of a signature sequence of certain genes for the definition of higher taxa, especially for the taxonomic rank genus.

I believe a definition of the taxonomic rank species should be based on a DNA-DNA hybridization experiment or allied molecular techniques, together with the interfertility test. For a definition of biological species, the interfertility is fundamentally important. This cannot, however, be applied to anamorphic yeasts or usually to homothallic species. Furthermore, the interfertility test is not often reproducible, especially in basidiomycetous yeasts. Fortunately a DNA-DNA hybridization experiment is usually parallel with the interfertility test. The DNA-DNA hybridization experiment is now the most reliable technology for the correct separation of the yeast species in which an interfertility test cannot be applied. Although the present techniques for a DNA-DNA hybridization experiment are laborious, a simple method will be soon developed. After the species has been correctly defined, a signature sequence of the species can be applicable to its rapid and correct identification. DNA chips will be employed for the identification of many strains and will contribute much to the ecological study of yeasts. It should be noted, however, that a correct definition of the species is the basis for these new technologies. We never neglect the fundamentals of systematics.

As I mentioned earlier, the use of phenotypic features is ideal for the definition of higher taxa of yeasts. In this sense, we must continue efforts to find features specific to respective higher taxa. We now lack poor data on the chemical structure of yeast cells. The qualitative and quantitative analyses of yeast cells will provide fruitful results to yeast systematics, and the study of the chemical structure of cell surfaces will provide more fruitful contributions to yeast systematics. The significance of an old serological study on the cell surface structure (Tsuchiya et al., 1957, 1969) was confirmed by the recent analysis of cell wall sugar composition (Takashima et al., 2000). An example of another possible approach to yeast systematics was shown in the CUG codon usage by the Candida species (Sugita and Nakase, 1999). Polyphasic approaches are necessary for the chemical and molecular systematic studies of yeasts.

Another important matter in the systematics is the finding of teleomorphs. By the development of molecular techniques, we can easily deduce the close relationships among strains. This provides taxonomists with a prediction for finding teleomorphs. Steady efforts are expected for the finding teleomorphs and the elucidation of life cycles of yeast species, which are now regarded as imperfect yeasts. Teleomorphs of basidiomycetous yeasts often produce mycelia. This provides us with a possibility for the study of fine structures of mycelia such as septal pores, besides the study of morphology of sexual organs.

I would like to close this paper by stressing the importance of isolation studies of yeasts inhabiting the natural environment. In past 20 years, the number of species of ballistosporous yeasts increased more than five times. This clearly indicates that a great many unknown yeast species are living in nature. I believe that biological systematics should not be the systematics of microorganisms already introduced to the laboratory, but the systematics of microorganisms living on earth, and that even if yeast species will be identified by in situ examinations of DNA in their habitat, cultured strains are required for detailed studies of the species because systematics is the overall understanding of organisms. Together with the introduction of new technologies, the isolation studies should be continually carried out for the sound development of yeast systematics.

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