

タイの植物葉圏における射出胞子形成酵母の分離同定

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Ballistoconidium-Forming Yeasts Found in the Phyllosphere of Thailand

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One hundred and thirty-six strains of ballistoconidium-forming yeasts were isolated from 54 plant samples collected in Thailand in 1987 and 1990. They were identified as 21 species in the genera *Bensingtonia*, *Bullera*, *Kockovaella*, *Sporidiobolus*, *Sporobolomyces*, and *Tilletiopsis*. Twelve of them were found to represent hitherto undescribed species. Strains assigned to undescribed species occupied 22.8% of the isolates. Only 8 species, including 2 new ones, were commonly isolated in 1987 and 1990. This clearly suggests that many undescribed ballistoconidium-forming yeasts are living in the phyllosphere of Thailand. In the isolation study in 1987, yeasts were isolated at 23°C and 30°C and identified as 16 species. The number of isolated species was higher at 23°C than at 30°C, i.e., 12 at 23°C and 8 at 30°C, though Thailand is located in the tropical region, and the atmospheric temperature was more than 35°C in the daytime, and 24°C was the minimum from midnight to dawn, during the period of collection of plant materials from which yeasts were isolated.

Key words : ballistoconidium-forming yeasts, yeasts in Thai phyllosphere, yeasts on plant leaves

INTRODUCTION

In the course of a survey of ballistoconidium-forming yeasts associated with plant leaves, 372 strains of yeasts were isolated in 1987 and 1990 from plants collected in Thailand. One hundred and thirty-six of them produced ballistoconidia and were classified into 21 species, of which 12 re-

presented new species and were described as *Bensingtonia musae* (36), *Bullera penniseticola* (33), *Kockovaella thailandica* (22), *K. imperatae* (22), *K. sacchari* (33), *Sporobolomyces blumeae* (34), *S. nylandii* (34), *S. poonsookiae* (34), *S. vermiculatus* (34), *Tilletiopsis dextrii* (35), *T. oryzicola* (35), and *T. penniseti* (35). The genus *Kockovaella* was established based on these Thai isolates, and it was characterized by the production of three kinds of conidia : ballistoconidia, stalked conidia, and budding yeast cells (22).

This paper discusses the isolation and identification of these ballistoconidium-forming yeasts living in Thailand.

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MATERIALS AND METHODS

Plant leaves for the isolation of yeasts

In 1987, plant samples were collected in forests, fields, rice fields, and roadsides, in the northwestern suburbs and urban areas of Bangkok, including markets and a house garden. In 1990, plants were collected along the southeastern seacoast, from Bangkok to Pattaya. The samples examined are shown in Tables 1 and 2.

Isolation of yeasts

The isolation of yeasts was carried out at the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok. Yeasts were isolated by the ballistoconidium-fall method with YM agar (Difco Labs., Detroit) plates, without any antibacterial agents. As described by Nakase and Takashima (26), YM agar plates were replaced every day with new ones, to avoid inhibition of growth of slow-growing yeasts, and yeasts with minor populations, by rapidly-growing ballistoconidium-forming fungi, such as *Tilletiopsis* spp. and *Itersonilia* spp. Yeasts were isolated at 23°C and 30°C in the isolation study in 1987, and at 25°C in the isolation study in 1990.

Isolated yeasts were purified by the conventional streaking technique, and they were preserved at -80°C suspended in YM broth (Difco Labs., Detroit) containing 10% (w/v) glycerol just after purification, to prevent the death of cultures and the loss of ballistoconidium-forming ability. Isolated yeasts were examined as to their colonial morphology on YM agar and Potato Dextrose agar (Difco Labs., Detroit), by visual inspection and with a stereomicroscope. Only one isolate was selected when several isolates with the same morphology were recovered from the same sample.

Examination of ballistoconidium-forming abilities

The formation of ballistoconidia was examined immediately after the purification. Yeasts were streaked on Corn Meal agar (Difco Labs., Detroit) plates and incubated at 17°C in the dark for 2-5 days, and then the plates were transferred to room temperature (below 25°C, light and dark) and incubated for up to 4 weeks. The formation of ballistoconidia was examined every day with a stereomicroscope. Ballistoconidia were collected on a glass slide for from 5 hrs to overnight, depending on the

Table 1. Plant samples for the isolation of yeasts collected in 1987

Sample No.	Name of plants
Group A : Northwestern suburb of Bangkok (near Ayuthaya), Dec. 17, 1987	
1,2	<i>Pennisetum pedicellatum</i> (feather pen-nisetum)
3	<i>Molochina corchorifolia</i>
4	<i>Oryza sativa</i>
5	<i>Eleusin indica</i>
6	<i>Imperata cylindrica</i>
7	<i>Typha angustifolia</i>
8	<i>Samanea saman</i>
9	<i>Oryza</i> sp.
10	<i>Oryza sativa</i>
Group B : Markets in Bangkok, Dec. 21, 1987	
11	<i>Ananas comosus</i> (pineapple)
12	<i>Musa sapientum</i> (leaf of banana)
13	<i>Musa sapientum</i> (flower of banana)
14	<i>Trapa bicornis</i>
16	<i>Benincasa hispida</i>
17	<i>Daucus carota</i> subsp. <i>sativus</i> (leaf of carrot)
18	<i>Cocos nucifera</i>
19	<i>Musa sapientum</i> (leaf of banana)
Group C : Roadside in Bangkok, Dec. 21, 1987	
15	<i>Typha angustifolia</i>
20	<i>Musa paradisiaca</i> (leaf of banana)
21	<i>Achras zapota</i> (leaf)
Group D : House garden in Bangkok, Dec. 22, 1987	
22	<i>Citrus hystrix</i> (citrus)
23	<i>Carica papaya</i> (papaya)
24	<i>Psidium gajava</i> (guava leaves)
25	<i>Solanum melongena</i> (a kind of egg plant)
26	<i>Capsicum frutescens</i> (chili)
27	<i>Musa sapientum</i> (leaf of banana)
Group E : Campus of Thailand Institute of Scientific and Technological Research, Bangkok, Dec. 22, 1987	
28, 29, 30	<i>Pinus</i> sp.
Group F : Northwestern suburb of Bangkok, Dec. 23, 1987	
31	<i>Oryza sativa</i>
32	<i>Saccharum spontaneum</i>
33	<i>Imperata cylindrica</i>
34, 35	<i>Phragmites karta</i>
36, 37, 38	<i>Oryza sativa</i>
39	<i>Cyperus superinus</i>
40, 41, 42	<i>Oryza sativa</i>

Table 2. Plant samples for the isolation of yeasts collected in 1990

Sample no.	Name of plants	Sample no.	Name of plants
1	<i>Imperata cylindrica</i>	21	<i>Neyraudia reynaudiana</i>
2	<i>Artabotrys siamensis</i>	22	<i>Echinochloa colonum</i>
3	<i>Thypha anguitifolia</i>	23	<i>Pennisetum</i> sp.
4	<i>Fimbristylis</i> sp.	24	<i>Mangifera indicalin</i>
5	<i>Blumea</i> sp.	25	<i>Saccharum officinarum</i>
6	<i>Cyperus</i> sp.	26	<i>Imperata cylindrica</i>
7	<i>Eragrotis</i> sp.	27	<i>Eucalyptus</i> sp.
8	<i>Pennisetum</i> sp.	28	<i>Merrymia</i> sp.
9	<i>Dactyloctenium aegyptium</i>	29	<i>Ageratum conyzoides</i>
10	<i>Imperata cylindrica</i>	30	<i>Cyperus cyperinus</i>
11	<i>Pennisetum</i> sp.	31	<i>Eucalyptus</i> sp.
12	<i>Melaleuca leucadendra</i>	32	<i>Ageratum conyzoides</i>
13	<i>Syncdrella nodiflora</i>	33	<i>Pennisetum pedicellatum</i>
14	<i>Aristida balansae</i>	34	<i>Cassia siamea</i>
15	<i>Grewia</i> sp.	35	<i>Pennisetum pedicellatum</i>
16,17	<i>Antigonon leptopus</i>	36	<i>Cyperus cyperinus</i>
18	<i>Musa paradisiaca</i>	37, 38	<i>Neyraudia reynaudiana</i>
19, 20	<i>Oryza sativa</i>	39, 40	<i>Oryza sativa</i>

Collected on Nov. 22, 1990, along the southeastern seacoast from Bangkok to Pataya.

amount of produced ballistoconidia that was estimated by examining the surface of colonies with stereomicroscope, and then they were examined microscopically.

Identification of yeasts

Yeasts were identified based on morphological, physiological, and biochemical properties according to the methods described by van der Walt and Yarrow (37). Chemotaxonomic and molecular phylogenetic examinations were carried out when required for correct identification (22, 33~36). Final decisions on differences between respective taxonomic rank species were made based on the DNA-DNA hybridization experiment among related species (22, 33~36) that were deduced based on the analysis of nucleotide sequences of small subunit ribosomal DNAs (SSU rDNAs) and/or ITS1-5.8S-ITS2 regions (22, 33~36).

RESULTS AND DISCUSSION

Isolation and identification of yeasts

A total of 372 strains of yeasts were isolated from plant samples collected in 1987 and 1990. One hundred and thirty-six of them produced ballis-

toconidia, and these were identified as 21 species : nine known species and 12 new ones (Table 3). Eight of them were commonly isolated in 1987 and 1990, but the remaining 13 species were isolated in either year. We did not isolate *Tilletiopsis* strains in 1990, though many *Tilletiopsis*-like colonies appeared on the isolation media, so common species in 1987 and 1990, it can be assumed, would have been increased if we had isolated these colonies in 1990.

One hundred and forty-nine yeast strains were isolated from 32 samples (76.2%) of 42 examined in 1987, and ballistoconidium-forming yeasts were found in 21 samples (50%). Sixty-three isolated ballistoconidium-forming yeasts were identified as 16 species, i.e., nine known species and seven unknown ones, in the genera *Bullera*, *Sporidiobolus*, *Sporobolomyces*, and *Tilletiopsis* (Tables 3 and 4). Unknown species were described as respective new species (22, 34, 35). The most frequently isolated species was *S. shibatani* which was found in 21.4% of plant samples examined, followed by *B. sinensis* (19%), *B. crocea* (16.7%), and *S. salmonicolor* (11.9%) (Table 3).

Two hundred and twenty-four yeast strains were isolated from 39 samples (97.5%) of 40 examined in

Table 3. Ballistoconidium-forming yeasts isolated from plants collected in Thailand

Species	1987		1990	
	No. of strains isolated	Frequency of isolation (%)*	No. of strains isolated	Frequency of isolation (%)*
<i>Bensingtonia musae</i> **			1	2.5
<i>Bullera alba</i>	1	2.4		
<i>Bullera crocea</i>	8	16.7	2	5.0
<i>Bullera peniseticola</i> **			1	2.5
<i>Bullera pseudoalba</i>	4	9.5	3	7.5
<i>Bullera sinensis</i>	11	19.0	30	52.5
<i>Bullera variabilis</i>	5	9.5		
<i>Kockovaella imperatae</i> **	1	2.4		
<i>Kockovaella sacchari</i> **			1	2.5
<i>Kockovaella thailandica</i> **	2	4.8	2	5.0
<i>Sporidiobolus ruineniae</i>	1	2.4	8	17.5
<i>Sporobolomyces blumeae</i> **			1	2.5
<i>Sporobolomyces nylandii</i> **	3	7.1		
<i>Sporobolomyces poonsookiae</i> **			8	15.0
<i>Sporobolomyces salmonicolor</i>	6	11.9	2	2.5
<i>Sporobolomyces shibatanus</i>	10	21.4	13	30.0
<i>Sporobolomyces vermiculatus</i> **	7	7.1	1	2.5
<i>Tilletiopsis dextrii</i> **	1	2.4		
<i>Tilletiopsis flava</i>	1	2.4		
<i>Tilletiopsis oryzicola</i> **	1	2.4		
<i>Tilletiopsis penniseti</i> **	1	2.4		
Total	63		73	

* Frequency of isolation

= (No. of samples from which that species was isolated) / (No. of samples examined) × 100 (%)

** New species found in this study (22, 33~36)

1990. Ballistoconidium-forming yeasts were found in 33 plant samples (82.5%). Seventy-three isolated ballistoconidium-forming yeasts were identified as 13 species, i.e., eight known species and five unknown ones, in the genera *Bensingtonia*, *Bullera*, *Kockovaella*, *Sporidiobolus*, and *Sporobolomyces* (Tables 3 and 5). Unknown species were described as respective new species (33~35). The most frequently isolated species was *B. sinensis*, which was found in 52.5% of plant samples examined, followed by *S. shibatanus* (30%), *S. ruineniae* (17.5%), and *S. poonsookiae* (15%) (Table 3). The frequencies of isolation of *B. sinensis* and *S. shibatanus* were high in both 1987 and 1990, but those of *B. crocea*, *S. ruineniae*, and *S. poonsookiae* were different in the respective years (Table 3).

In the isolation study in 1987, the frequency of isolation of ballistoconidium-forming yeasts was high (81.2%) from samples collected in the suburbs of Bangkok (groups A and F), but the frequency was very low (0% in groups D and E, 25% in group B, and 33.3% in group C; average 15%) from samples collected in the urban areas of Bangkok, including markets and a house garden (Tables 1 and 4). It is not easy to explain why the frequency of isolation was low from the samples collected in urban areas. The only possible explanation is an effect of air pollution in urban areas of Bangkok, caused by a large amount of waste gas generated from automobiles.

Dowding and Richardson (7) reported that leaf yeasts decreased in air-polluted areas, and that this

Table 4. Distribution of ballistoconidium-forming yeasts on plants in Thailand (1987)

Species isolated	Samples from which ballistoconidium-forming yeasts were isolated*																No. of strains isolated						
	1	6	7	8	9	10	11	15	19	31	32	33	34	35	36	37	38	39	40	41	42	at 23°C	at 30°C
<i>B. alba</i>					○																	1	0
<i>B. crocea</i>					○	○					●		○				●	○	○			6	2
<i>B. pseudoalba</i>		○							○		○			○								4	0
<i>B. sinensis</i>	○								○	○	◎			○		○		○	○			10	1
<i>B. variabilis</i>									○		○							○		○		5	0
<i>K. imperatae</i>												●										0	1
<i>K. thailandica</i>						○						○										2	0
<i>S. ruineniae</i>									○													1	0
<i>S. nylandii</i>											○	○			○							3	0
<i>S. salmonicolor</i>										○		○			○		○			○		6	0
<i>S. shibatanus</i>	○		○	○	○	○			○	◎					●			○				8	2
<i>S. vermiculatus</i>		○									◎				○							5	2
<i>T. derxii</i>																				●		0	1
<i>T. flava</i>												●										0	1
<i>T. oryzicola</i>																					●	0	1
<i>T. penniseti</i>	○																					1	0
Total																					52	11	

* Isolated at : ○, 23°C ; ●, 30°C ; ◎, 23°C and 30°C. Sample numbers are the same as Table 1.

kind of yeasts, especially *S. roseus*, can be employed as an indicator for air pollution in a city. Apparently, repeated isolation studies will be required to clarify if the low frequency of ballistoconidium-forming yeasts in urban area was caused by air pollution, because no clear report has been published on the sensitivity of ballistoconidium-forming yeasts other than *S. roseus*.

The genus *Bullera*

Sixty-five strains of *Bullera* were identified as six species : five known species and a new one (Table 3). Among them, *B. alba*, *B. crocea*, and *B. variabilis* were also found on the main island of Japan and in New Zealand (19). *Bullera pseudoalba* and *B. sinensis* were found in Thailand and on the main island of Japan, but not in New Zealand (19). *Bullera sinensis* was isolated at high frequency both in 1987 and 1990 (Table 3). This species was found in China (15), Japan (19), and Taiwan (Nakase, unpublished), in the North Temperate Zone, but not in New Zealand (13), in the South Temperate Zone. *Bullera alba*, the type species of the genus and anamorph of *Bulleromyces albus*, was suggested to be a minor species in Thailand (Table 3). This species is a common

species found in various kinds of substrates in many countries in the Temperate Zones (4). In spite of a young species found in 1983 in United Kingdom (5), *B. crocea* has been found in Canada, China, Japan, New Zealand, and Tasmania Island in Australia (20). This species is assumed to be widely distributed on the earth. *Bullera pseudoalba* was found in 1986 in Japan, and Thailand is the second locality of the species. Probably it is widely distributed on the earth, because it was found in two countries where the climate is quite different. Nakase and Suzuki (25) reported heterogeneity of *B. variabilis* when they described this species. Bai et al. (1) studied this species based on ribosomal DNA sequences and DNA-DNA relatedness, and separated it into five species : *B. variabilis*, *B. mrakii*, and three hitherto undescribed species. They found that one of four strains of *B. variabilis* isolated from New Zealand (12) was identified as *B. mrakii*. Possibly, the remaining three strains from New Zealand belong to the latter species.

The remaining species represented a new species and was named *B. penniseticola* (33). After its description, this species was found to produce stalked conidia (J. A. Barnett, personal communica-

tion), such that it might preferably be included in the genus *Kockovaella*. In their monograph of yeasts, Barnett et al. (2) showed a picture of stalked conidium-like cells but did not refer to the production of stalked conidia. The morphology of these conidia, however, is somewhat different from those of *Kockovaella*. Furthermore, in the phylogenetic tree based on SSU rDNA sequences, *B. penniseticola* is located in the cluster including *Bulleromyces albus* (teleomorph of *Bullera alba*) but not in the cluster in which stalked conidium-forming yeasts: *Kockovaella*, *Fellomyces*, and *Sterigmatosporidium*, are located (33). Further detailed studies will be needed to determine the proper taxonomic position of *B. penniseticola*.

The genus *Kockovaella*

Six strains of *Kockovaella* were identified as three new species (Table 3). The genus *Kockovaella* was proposed based on two species isolated in Thailand: *K. thailandica* and *K. imperatae* (22), and then another new species, *K. sacchari* (33), was described. Recently, Fungsin et al. (10) reported the isolation of an additional new species of *Kockovaella* from the phyllosphere of Thailand. Further, several new species have been described in the genus based on isolates from plants collected in the Ogasawara (Bonin) Islands, Japan, isolated islands in the subtropical region in the Pacific Ocean (6), and Vietnam (18). Species common to two or more countries have not been reported so far in the genus.

The genera *Sporidiobolus* and *Sporobolomyces*

Sixty strains of the genus *Sporidiobolus*, and its anamorphic counterpart *Sporobolomyces*, were identified as seven species: three known species and four new ones (Table 3). The unknown species were described as respective new species (34). *S. shibatanus* (anamorph of *Sporidiobolus pararoseus*) and *S. salmonicolor* (anamorph of *Sporidiobolus salmonicolor*) are considered common species in the world, and they are found in several Asian countries, including Japan, New Zealand, Mainland China (19), and Taiwan (unpublished). The former species was isolated at high frequencies in both 1987 and 1990 (Table 3). Li (16) reported that *S. shibatanus* is the most frequently isolated ballistoconidium-forming species from plants collected in China.

Sporidiobolus ruineniae was isolated both in 1987 and 1990 (Table 3). This species was described based on isolates from the phyllosphere of tropical foliage in Indonesia (27, 29). Sugiyama and Goto (32) reported the isolation of a new species, *Sporobolomyces coprophilus*, from goat dung in Pakistan. Kurtzman and Fell (14) regarded this species as a variety of *S. ruineniae*. In the 4th edition of *The Yeasts, a Taxonomic Study*, Statzell-Tallman and Fell (31) dealt with this variety as synonymous with *S. ruineniae*, because both taxa had the identical sequence alignments in a partial region of the large subunit rDNA (LSU rDNA) (9). Probably, *S. ruineniae* is common in the tropics but not in the Temperate Zones.

A strain from Jamaica, which was invalidly described as *Sporidiobolus microsporus*, was once regarded as a synonym with *S. ruineniae* based on the morphological and physiological similarities (3), and this classification was adopted in *The Yeasts, a Taxonomic Study*, 4th ed., published in 1998 (31), with a comment that the LSU rDNA sequence suggested a difference between these two species. Fell et al. (8) reported that *S. microsporus* was a different species from *S. ruineniae*, based on the difference of several phenotypic characteristics and analysis of a partial sequence of LSU rDNA, and they provided the Latin diagnosis and standard description to validate this species. Takashima and Nakase (34) found a difference between *S. ruineniae* and *S. microsporus* based on the sequence of SSU rDNAs, and proved distinctness of *S. microsporus* from *S. ruineniae* by a DNA-DNA reassociation experiment.

The remaining four species represented respective new species and were named *S. blumeae*, *S. nylandii*, *S. poonsookiae*, and *S. vermiculatus* (34).

Sporobolomyces roseus was not isolated in the studies in 1987 and 1990. This species is the most common ballistoconidium-forming yeast species associated with plant leaves in the Temperate Zones. In Japan, for example, this species was isolated at high frequency from plant leaves: 72.1% of rice plants in Kanagawa Prefecture (23), 70.8% of *Miscanthus sinensis* in Kanagawa and Saitama Prefectures (24), 35.8% of various plants in the suburbs of Tokyo (39), and 45.5% of various plants at Mt. Fuji (26). According to Phaff and Starmer (28), J. F. T. Spencer and H. J. Phaff studied the

yeasts present in various flowers from Saskatoon, Saskatchewan, Canada; Fort Smith, Northwest Territory, Canada, and the Davis area, California, U.S.A. They found that the effect of geography on the species recovered was minimal, and *S. roseus* was a common species in flowers. On the other hand, Ruinen (29) found each strain of this species among the strains isolated from plants collected in Indonesia, Surinam in South America, and Ivory Coast in Africa. Probably *S. roseus* is rich in the Temperate Zones and poor in the tropics, though the possible effect of air pollution cannot be neglected in the case of Bangkok.

The genus *Bensingtonia*

A strain of *Bensingtonia* represented a new species and was described as *B. musae* (36). The number of species of the genus is steadily increasing; 11 species have been described so far. Recently, a 12th species of the genus, isolated from Thai plants, was described as *B. thailandica* (11).

The genus *Tilletiopsis*

In the isolation study in 1987 and 1990, we decided not to isolate *Tilletiopsis* strains, because *Tilletiopsis* belongs to yeast-like fungus but not to true yeast. However, strains of certain species of this genus are sometimes difficult to distinguish from species of *Sporobolomyces*. As a result, we isolated four strains in the study in 1987. These strains were identified as four species: one known species and three new ones (Table 3). The three new species were described as *T. derxii*, *T. oryzicola*, and *T. penniseti* (35). Ballistoconidia of *T. oryzicola* showed some resemblance to those of certain species of *Sporobolomyces*, but this species is located in the cluster of *Tilletiopsis*. In the study in 1990, we found many colonies showing the typical appearance of *Tilletiopsis*, though we did not isolate them. *Tilletiopsis* strains are considered common fungi in Thailand.

Isolation temperature and the species recovered

As mentioned, the isolation study in 1987 was carried out at 23°C and 30°C, and 16 species were isolated; i.e., 12 at 23°C and eight at 30°C. Five species were isolated both at 23°C and 30°C (Table 4). It is not easy to explain this fact because, when we collected plant samples, the atmospheric temperature was more than 35°C in the daytime, and 24°C

was the minimum temperature from midnight to dawn. Probably yeasts with minor populations and slowly growing yeasts could not produce colonies at 30°C, due to inhibition by rapidly growing ballistoconidium-forming fungi, such as *Tilletiopsis* spp. and *Itersonilia* spp., which quickly covered the entire surface of agar plates. Since all of the strains isolated at 23°C could grow at 25°C, the isolation study in 1990 was carried out at 25°C.

The ratio of new species in the isolates

In the isolation study carried out in 1987 and 1990, strains assigned to new species occupied 22.8% of isolates: 25.4% in 1987 and 20.5% in 1990, respectively. Among 12 new species, two species (8.8% of isolates): *Kockovaella thailandica* and *Sporobolomyces vermiculatus*, were commonly found in 1987 and 1990, but the remaining ten species were found in either year.

In our isolation studies in Japan in 1984 and 1985, plant samples were collected in areas near Tokyo (23, 24). Two hundred strains isolated were assigned to 16 species, including six known species and ten new species. Strains assigned to new species occupied 29.5% of isolates: 24.2% in 1984 and 34.7% in 1985, respectively (19). Among the ten new species, five species (26% of isolates) were commonly found in 1984 and 1985. The ratio of strains assigned as new species in the total of isolates is slightly higher in Japan than in Thailand, but this can be regarded as within a range of deviation. However, the ratio of new species commonly found in both years is apparently lower in Thailand compared than in Japan. This fact suggests a higher possibility of finding additional new species in Thailand than in Japan. Further, we could not isolate slow growers in Thailand, because of the limited length of the incubation experiment. We could have isolate more new species if we had had similar days for isolation as in Japan.

Ubiquinone systems of the isolates

Among ballistoconidium-forming yeasts isolated in 1987 and 1990, a strain: K-304, had Q-9 as the major component of ubiquinone, and the strain was assigned to the genus *Bensingtonia*, which was characterized by this ubiquinone, like *B. musae* (36). Eleven of the remaining species had Q-10 and were assigned to the genera *Bullera*, *Kockovaella*, *Spor-*

idiobolus, *Sporobolomyces*, and *Tilletiopsis* (22, 33~35). No yeast was found to have Q-10 (H₂) as the major ubiquinone.

Yeast species having Q-10 (H₂) have long been considered rare among yeasts. Only two strains belonging to two species: *Erythrobasidium hasegawianum* and *Sporobolomyces elongatus*, were reported to have this ubiquinone homologue as the major component (38). The former was isolated from an old culture of beer yeast, in Philadelphia, U.S.A. (4), and the latter was from a leaf of *Callistemon viminalis*, in Australia (30). Recent isolation studies of ballistoconidium-forming yeasts, however, proved that Q-10 (H₂)-containing ballistoconidium-forming yeasts were widely distributed in tropical and subtropical regions in East Asia; the Ogasawara Islands in the Pacific Ocean (21), Indonesia (13), and Vietnam (17).

In 1996, Fungsin et al. (10) isolated 152 strains of ballistoconidium-forming yeasts from plants collected in a tropical rain forest in the Sakaerat Environmental Research Station in Nakhon Ratchasima Province, a northeastern region of Thailand, about 210 km from Bangkok. They found 19 strains of Q-10 (H₂)-containing yeasts, which occupied 12.5% of the isolates and found in 57.7% of the plants examined. It is not easy to explain why Q-10 (H₂)-containing yeasts could not be recovered in the isolation studies in 1987 and 1990, while they were found at high frequency in 1996. Further isolation studies will be required to clarify this point.

The number of species found on a leaf sample

In the isolation study in 1987 and 1990, one to four species, for an average of 2.1 species, were isolated from each of 54 samples from which ballistoconidium-forming yeasts were isolated (Tables 4 and 5). These numbers are slightly lower than those found in the isolation studies carried out in Japan (23, 24). In Japan, in the case of rice plants (*Oryza sativa*), one to six species, for an average of 2.4 species, were isolated from 27 samples from which ballistoconidium-forming yeasts were isolated (23), and in the case of *Miscanthus sinensis*, one to seven species, for an average of 2.4 species, were isolated (24). Probably this was caused by differences in length of incubation. As mentioned above, the length of incubation of agar plates after the collection of ballistoconidia in Thailand was shorter than

that in Japan, due to the restricted funds for the staying of Japanese researchers in Thailand. We believe we could have isolated several more slowly growing yeast strains representing new species, if we could have had an adequate incubation period.

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REFERENCES

1. Bai, F.-Y., Takashima, M. and Nakase, T. Phylogenetic analysis of strains originally assigned to the species *Bullera variabilis*: descriptions of *Bullera pseudohuiaensis* sp. nov., *Bullera komagatae* sp. nov. and *Bullera pseudoschimicola* sp. nov. Int. J. Syst. Evol. Microbiol. accepted.
2. Barnett, J. A., Payne, R. W. and Yarrow, D. Yeasts: Characteristics and Identification, 3rd ed., Cambridge University Press, Cambridge, p. 122 (2000).
3. Boekhout, T. A revision of ballistoconidia-forming yeasts and fungi. Stud. Mycol. **33**: 1-194 (1991).
4. Boekhout, T. *Bulleromyces* Boekhout & Fonseca. In Kurtzman, C. P. and Fell, J. W. (eds.), The Yeasts, a Taxonomic Study, 4th ed., pp. 641-642, Elsevier, Amsterdam (1998).
5. Buhagiar, R.M.W., Yarrow, D. and Barnett, J.A. *Bullera crocea* and *Bullera armeniaca*, two new yeasts from fruit and vegetables. J. Gen. Microbiol. **129**: 3149-3155 (1983).
6. Cañete-Gibas, C. F., Takashima, M., Sugita, T. and Nakase, T. Three new species of *Kockovaella* isolated from plants collected in the Ogasawara Islands. J. Gen. Appl. Microbiol. **44**: 11-18 (1998).

7. Dowding, P. and Richardson, D. H. S. Leafyeasts as indicators of air quality in Europe. *Environ. Pollut.* **66** : 223-235 (1990).
8. Fell, J. W., Blatt, G. M. and Stutzell-Tallman, A. Validation of the basidiomycetous yeast, *Sporidiobolus microsporus* sp. nov., based on phenotypic and molecular analyses. *Antonie van Leeuwenhoek* **74** : 265-270 (1998).
9. Fell, J. W., Stutzell-Tallman, A., Lutz, M. J. and Kurtzman, C. P. Partial rRNA sequences in marine yeasts : a model for identification of marine eukaryotes. *Mol. Mar. Biol. Biotechnol.* **1** : 175-186 (1992).
10. Fungsin, B., Hamamoto, M., Arunpairojana, V., Atthasampunna, P. and Nakase, T. Systematics of ballistoconidium-forming yeasts isolated from plants in Thailand, and proposal of *Bensingtonia thailandica* sp. nov. *Int. Conf. Asian Network on Microbial Research*, Chiang Mai, Nov., pp.862-869 (1999).
11. Fungsin, B., Hamamoto, M., Arunpairojana, V., Sukhumavasi, J., Atthasampunna, P. and Nakase, T. *Bensingtonia thailandica* sp. nov., a novel basidiomycetous yeast species isolated from plant leaves in Thailand. *Int. J. Syst. Evol. Microbiol.* **51** : 1209-1213 (2001).
12. Hamamoto, M. and Nakase, T. Ballistospore yeasts found on the surface of plant materials collected in New Zealand. The genera *Bensingtonia* and *Bullera* with descriptions of five new species. *Antonie van Leeuwenhoek* **69** : 279-291 (1996).
13. Haryono, B., Hamamoto, M., Kuswanto, K.R. and Nakase, T. Systematics of ballistoconidium-forming yeasts isolated from plants in Indonesia. *Asian Network on Microbial Researches. Int. Conf. Asian Network on Microbial Researches*, Yogyakarta, Nov., pp. 223-231 (1998).
14. Kurtzman, C. P. and Fell, J. W. Molecular relatedness between the basidiomycetous yeasts *Sporidiobolus ruinenii* and *Sporobolomyces coprophilus*. *Mycologia* **83** : 107-110 (1991).
15. Li, M.-X. Studies on Sporobolomycetaceae I. Taxonomy of *Bullera*. *Acta Microbiol. Sinica* **22** : 17-25 (1982).
16. Li, M.-X. Study on Sporobolomycetaceae II. Ballistospore-forming yeasts found on leaves in China and species in *Sporobolomyces*. *Acta Mycol. Sinica* **7** : 216-220 (1988).
17. Luong, D. T., Takashima, M., Ty, P. V., Dung, N. L., and Nakase, T. Ballistoconidiogenous yeasts living in the phyllosphere in Vietnam. *Int. Conf. Asian Network on Microbial Research*, Chiang Mai, Nov., pp. 578-588 (1999).
18. Luong, D. T., Takashima, M., Ty, P. V., Dung, N. L., and Nakase, T. Four new species of *Kockovaella* isolated from plant leaves collected in Vietnam. *J. Gen. Appl. Microbiol.* **46** : 297-310 (2000).
19. Nakase, T. Expanding world of ballistospore yeasts : Distribution in the phyllosphere, systematics and phylogeny. *J. Gen. Appl. Microbiol.* **46** : 189-216 (2000).
20. Nakase, T. Systematics and species diversity of yeasts. *Bull. Jpn. Mycol. Soc.* **41** : 119-136 (2000) (in Japanese).
21. Nakase, T. Asian network on microbial researches (ANMR) : Promotion of microbiology and biotechnology in Asian region. *Int. Conf. Asian Network on Microbial Researches*, Yogyakarta, Nov., pp. 1-6 (1998).
22. Nakase, T., Itoh, M., Takematsu, A., Mikata, K., Banno, I. and Yamada, Y. *Kockovaella*, a new ballistospore-forming anamorphic yeast genus. *J. Gen. Appl. Microbiol.* **37** : 175-197 (1991).
23. Nakase, T. and Suzuki, M. Ballistospore-forming yeasts found on the surface of the Japanese rice plant, *Oryza sativa* L. *J. Gen. Appl. Microbiol.* **31** : 457-474 (1985).
24. Nakase, T. and Suzuki, M. Studies on ballistospore-forming yeasts from the dead leaves of *Miscanthus sinensis* with descriptions of the new species *Sporobolomyces miscanthi*, *Sporobolomyces subroseus*, and *Sporobolomyces weijmanii*. *J. Gen. Appl. Microbiol.* **33** : 177-196 (1987).
25. Nakase, T. and Suzuki, M. *Bullera variabilis*, a new species of yeast with uniquely shaped ballistospores isolated from various plant materials. *J. Gen. Appl. Microbiol.* **33** : 343-354 (1987).
26. Nakase, T. and Takashima, M. A simple procedure for the high frequency isolation of new taxa of ballistospore yeasts living on the surfaces of plants. *Riken Review* **3** : 33-34 (1993).
27. Phaff, H.J. *Sporidiobolus* Nyland. *In* Lodder, J. (ed.), *The Yeasts, a Taxonomic Study*, 2nd ed., pp. 822-830, North-Holland Publ., Amsterdam (1970).
28. Phaff, H. J. and Starmer, W. T. Yeasts associated with plants, insects and soil. *In* Rose, A. H. and Harrison, J. S. (eds.), *The Yeasts*, 2nd ed., Vol. 1., *Biology of Yeasts*, pp. 123-180, Academic Press,

- London (1987).
29. Ruinen, J. The phyllosphere. II. Yeasts from the phyllosphere of tropical foliage. *Antonie van Leeuwenhoek* **29** : 425-438 (1963).
 30. Shivas, R. G. and Rodrigues de Miranda, L. Two new species of the genus *Sporobolomyces* and a new *Rhodotorula* species from leaf surfaces. *Antonie van Leeuwenhoek* **49** : 159-166 (1983).
 31. Statzell-Tallmann, A. and Fell, J. W. *Sporidiobolus* Nyland. In Kurtzman, C.P. and Fell, J. W. (eds.), *The Yeasts, a Taxonomic Study*, 4th ed., pp. 693-699, Elsevier, Amsterdam (1998).
 32. Sugiyama, J. and Goto, S. *Coprophilus* fungi from Karakorum I. *Jpn. Bot.* **42** : 75-84 (1967).
 33. Takashima, M. and Nakase, T. *Bullera penniseticola* sp. nov. and *Kockovaella sacchari* sp. nov., two new yeast species isolated from plants in Thailand. *Int. J. Syst. Bacteriol.* **48** : 1025-1030 (1998).
 34. Takashima, M. and Nakase, T. Four new species of the genus *Sporobolomyces* isolated from leaves in Thailand. *Mycoscience* **41** : 357-369 (2000).
 35. Takashima, M. and Nakase, T. *Tilletiopsis derxii*, *Tilletiopsis oryficola* and *Tilletiopsis penniseti*, three new species of the ustilagonomycetous anamorphic genus *Tilletiopsis* isolated from leaves in Thailand. *Antonie van Leeuwenhoek*, in press.
 36. Takashima, M., Suh, S.-O. and Nakase, T. *Bensingtonia musae* sp. nov. isolated from a dead leaf of *Musa paradisiaca* and its phylogenetic relationship among basidiomycetous yeasts. *J. Gen. Appl. Microbiol.* **41** : 143-151 (1995).
 37. van der Walt, J. P. and Yarrow, D. Methods for the isolation, maintenance, classification and identification of yeasts, In Kreger-van Rij, N.J.W. (ed.), *The Yeasts, a Taxonomic Study*, 3rd ed., pp.95-105, Elsevier, Amsterdam (1984).
 38. Yamada, Y. Identification of coenzyme Q (ubiquinone) homologs. In Kurtzman, C.P. and Fell, J. W. (eds.), *The Yeasts, a Taxonomic Study*, 4th ed., pp.101-102, Elsevier, Amsterdam (1998).
 39. Yoshizawa, M., Nakase, T., Komagata, K. and Iizuka, H. Ballistospore-forming yeasts found in various plant materials in Japan. *JFCC Bull.* **3** : 8-16 (1987) (in Japanese).

タイの植物葉圏における射出胞子形成酵母の分離同定

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1987年および1990年にタイで採集した植物54点から136株の射出胞子形成酵母を分離し、*Bensingtonia* 属、*Bullera* 属、*Kockovaella* 属、*Sporidiobolus* 属、*Sporobolomyces* 属および *Tilletiopsis* 属の21種に同定した。このうち12種は新種であり、新種に属する酵母は分離株の22.8%を占めた。21種のうち、2新種を含む8種のみが1987年と1990年に共通に分離された。この事実はタイの植物葉圏には多くの未知射出胞子形成酵母が生息していることを示唆している。1987年の分離は23°Cおよび30°Cで行い、分離株は16種に同定された。熱帯地域にあるタイでは試料採集時の気温は日中で35°Cを越え、深夜から夜明け前にかけての最低気温は24°Cであったが、分離された種は23°Cのほうが多く12種を数え、30°Cでは8種のみであった。