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FULL PAPER

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Physiological characteristics of 13 common fungal species in bathrooms

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Abstract To clarify the factors affecting fungal contamination in bathrooms, the growth of 13 common fungal species (13 isolates) in bathrooms was studied under various environmental conditions. Most of the fungi examined grew on media of 0.01% and 0.05% sodium fatty acid and on media of 0.01% anion surfactant. On media of non-ion surfactant, however, growth varied from species to species. Fungi found commonly in bathrooms can be divided into two groups. The first group, including six species – *Cladophialophora boppii*, *Exophiala spinifera*, *E. salmonis*, *Phialophora europaea*, *Phoma herbarum*, and *Scolecobasidium constrictum* – grew on media of 0.01%, 0.05%, and 0.25% non-ion surfactant, with the latter five species also growing on alkali medium. Most of them did not grow at 33°C, or on media with 10% NaCl, however. Fungi of these six species, identified using DNA and morphological analysis, were common in bathrooms, but not in other indoor environments, for example, in house dust or on windows. The second group contained seven species including *Aureobasidium* sp., *Cladosporium cladosporioides*, and *Fusarium* sp., which were common both in house dust and in bathrooms; they did not grow on media of 0.05% or 0.25% non-ion surfactant, but most grew comparatively fast on normal medium (1/4 PDA), and were able to grow on media with 10% NaCl and also at 33°C. The characteristic fungi found in bathrooms were able to exploit surfactant but were unable to grow well under comparatively dry or high-temperature conditions.

Key words Drought · Fungal contamination · Shampoo · Temperature

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Introduction

The Japanese style of bathing maintains bathrooms at a high level of humidity for long periods, and much condensed moisture accumulates and flows down the walls of the bathroom. These bathroom conditions promote fungal growth, and biodeterioration and discoloration by fungal contamination constitutes a hygiene problem and spoils the appearance of bathrooms (Moriyama et al. 1992; Ara et al. 2004).

Fungal contamination on bathroom walls was examined in a previous study in which the amount of mold was reported to be similar in summer and winter (Hamada and Fujita 1999). This contamination seems to be proportional to the quantity of moisture and soap used for bathing (Hamada and Fujita 2000). *Exophiala* and *Cladosporium*, which have dark hyphae, were the predominant fungi, followed by *Phoma* and *Scolecobasidium* (Hamada and Abe 2008).

Exophiala and *Scolecobasidium*, often found in bathrooms, were reported to also be encountered frequently in washing machines in which detergents are regularly used for laundering (Hamada 2004), but are rarely found in either outdoor or other indoor environments. Moreover, *Exophiala* and *Scolecobasidium*, unlike *Cladosporium* and *Aureobasidium*, seem to utilize the non-ion surfactant component of detergents as a nutrient (Hamada 2005).

Exophiala, *Phoma*, *Scolecobasidium*, and *Cladophialophora* are more common inside than outside bathrooms (Hamada and Abe 2008). In our preliminary studies, *Phialophora* was also often found in bathrooms. We are interested in the physiological characteristics of these five genera of fungi, which are often found in bathrooms. Although the fungal flora of bathrooms is unique, the nutritional requirements of common bathroom fungi, including these five genera, remain unknown.

Some species of *Exophiala* found in bathrooms are reported to be heat- and/or alkali tolerant (Goto et al. 1981; Nishimura et al. 1987). Moreover, these fungi are thought to often be pathogenic and harmful to health. Although

Table 1. Effect of incubation temperature on common fungi in bathrooms

Temperature				25°C	30°C			33°C			35°C		
Fungal species	Strain no.	Sequence no.	Times[a]	Color	Percentage	Color	Size	Percentage	Color	Size	Percentage	Color	Size
Acremonium sp.	NH1107		10 000	White	91	+	+	115	+	+	0		
Alternaria sp.	NH1106		1 000	Dark	98	+	+	102	+	+	83	+	−
Aureobasidium sp.	NH1326		10 000	Black	98	+	+	70	±	+	33	±	±
Cladosporium cladosporioides	JCM 15937	AB456576	10 000	Dark	70	+	±	0			0		
Cladophialophora boppii[b]	JCM 15938	AB456577	100	Dark	96	+	+	0			0		
Exophiala spinifera[b]	JCM 15939	AB456580	10 000	Dark	84	+	+	16	+	−	0		
Exophiala salmonis[b]	JCM 15940	AB456581	100	Dark	108	+	±	0			0		
Fusarium sp.	NH471		1 000	Purple	88	+	+	93	+	+	4	−	±
Paecilomyces sp.	NH716		10 000	Pink	103	+	+	77	+	+	25	−	±
Phialophora europaea[b]	JCM 15941	AB456578	10 000	Dark	76	+	+	96	+	±	0		
Phoma herbarum[b]	JCM 15942	AB456575	10 000	Dark	88	+	+	104	±	±	0		
Scolecobasidium constrictum[b]	JCM 15943	AB456579	100	Brown	96	+	±	0			0		
Rhodotorula sp.	NH6106		100 000	Pink	102	+	+	7	+	−	0		

The sequence number of JCM 15937–15943 was registered as AB456575–456581
[a]Times indicate levels of dilution for counting 10–100 colonies on each medium
[b]Fungus more common inside bathrooms than outside

bathrooms are humid and warm environments, the environmental factors affecting the growth of common bathroom fungi are still unknown.

The 13 species of fungi have been predominantly isolated from bathroom walls or floors (Hamada and Abe 2008), and their growth characteristics in culture on various media and under various environmental conditions were comprehensively examined and compared in this study. With a view to reducing fungal contamination in bathrooms, the factors determining bathroom fungal flora are discussed.

Materials and methods

Identification of 13 species of fungi

Thirteen fungi examined in this study were identified morphologically using a monograph of fungi (de Hoog et al. 2000; Samson et al. 2000). Identification by genetic and morphological characteristics was attempted in six species of fungi more common inside bathrooms than outside and one species of *Cladosporium* commonly found in bathrooms.

DNA extraction, PCR amplification, and homology searching

Genomic DNA from each mycelium sample was extracted and purified using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed using the primers ITS1 and ITS4, which amplify the region of the internal transcribed spacers (ITS) 1 and -2, including the 5.8S ribosomal RNA gene (5.8S rRNA), as reported previously (Hamada et al. 2006). The PCR products were gel-purified using a QIAquick Gel Extraction Kit (Qiagen) and sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (PE Applied Biosystems, Foster City, CA, USA) on an ABI 3130 automated sequencer (PE Applied Biosystems). PCR products were sequenced in both directions using the primers mentioned above. Homology searching of the sequences obtained was performed using the FASTA program on the DDBJ (DNA Data Bank of Japan) website (http://www.ddbj.nig.ac.jp/search/fasta-j.html). The sequences of the isolates obtained in the present study have been deposited in the DDBJ under accession numbers AB456575–AB456581, and those voucher cultures were also deposited in the JCM (Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, Wako, Saitama) (Table 1).

Fungal species and strain

Many fungal strains were collected from bathroom walls or floors in the previous study (Hamada and Abe 2008). The

following 13 species of fungi, predominant in bathrooms, were selected in this study and subjected to examination of growth characteristics: *Acremonium* sp. (N. Hamada NH1107), *Alternaria* sp. (NH1106), *Aureobasidium* sp. (NH1326), *Cladosporium cladosporioides* (Fres.) G.A. de Vries (NH1191, JCM 15937, *Cladophialophora boppii* (Borelli) de Hoog, Kwon-Chung & McGinnis (NH7009, JCM 15938), *Exophiala spinifera* (H.S. Nielson & Conant) McGinnis (NH917, JCM 15939), *Exophiala salmonis* J.W. Carmich. (NH119, JCM 15940), *Fusarium* sp. (NH471), *Paecilomyces* sp. (NH716), *Phialophora europaea* de Hoog, Mayser & Haase (NH 228, JCM 15941), *Phoma herbarum* Westend. (NH1014, JCM 15942), *Scolecobasidium constrictum* E.V. Abbott (NH9105, JCM 15943), and *Rhodotorula* sp. (NH6061).

Culture conditions used for comparison

After preculture of colonies of each strain for 2–3 weeks on slants, blocks of spores and mycelia of about 5 × 5 mm were collected with a sterilized pin, added to 50 ml sterilized water in an Erlenmeyer flask, and separated uniformly for about 30 s with a mixer (TM-101; Iwaki, Tokyo, Japan). This suspension was designated as 100 times diluted solution; it was diluted 10, 100, and 1000 times and designated as 1000, 10 000, and 100 000 times. The 0.5-ml suspensions of the spores of the 13 fungi were then poured and spread uniformly on two Petri dishes each with various media. About 12 ml 1/4 PDA (potato dextrose agar), which consists of 9.8 g/l PDA (BD Difco, Sparks, MD, USA) and 11.3 g/l Bacto-agar (BD Difco), was used as the standard medium for the growth studies; in contrast to PDA, 1/4 PDA suppresses the growth of *Rhodotorula* and makes it easier to detect dark fungi growing slowly (Hamada and Abe 2008).

Growth characteristics were evaluated with the count, color, and size of colonies detected on the media cultured under various conditions. To count the number of colonies found on each medium, media with 10–100 colonies were selected from among the cultures inoculated at various levels of dilution. The deviations of colony numbers on each two Petri dishes were within 80%, so those averages were used for calculation.

Each fungus was cultured for 8–14 days in an incubator maintained variously at temperatures of 25°, 30°, 33°, 35°, and 37°C. The temperature deviation was within 1.0°C. The incubator used was the MTH-2200 (Sanyo, Osaka, Japan). To prevent the media from drying up, the cultures at 30°, 33°, 35°, and 37°C were wrapped in plastic bags.

A culture of 1/4 PDA with 0%, 5%, 10%, or 15% NaCl added was used to examine the tolerance of fungi to drought (Sterflinger 1998). For comparison of the effect of NaCl, each fungus was incubated in darkness for 8–14 days at 25° ± 1°C.

A culture of 1/4 PDA was also used as the acidic medium to examine the tolerance of fungi to alkali. To adjust the pH value of the medium to an alkaline level, 3 g/l Na_2CO_3 and 3 g/l $NaH_2PO_4 \cdot 2H_2O$ were added to 1/4 PDA using a modified version of the method of Nagai et al. (1998). The pH of 1/4 PDA was 6.0 and that of the alkaline 1/4 PDA 9.7.

Three components of common commercial detergents were used as culture media: sodium oleate (SO; Kishida Chemicals, Osaka, Japan), a sodium fatty acid and one of the main components of soap; AE [Nikkol BL-9EX; polyoxyethylene-(9)-lauryl ether; Wako Pure Chemicals, Osaka, Japan], a non-ion surfactant and a component of shampoo; and LAS [C12, sodium dodecyl (= lauryl) benzenesulfonate; Kanto Chemicals, Tokyo, Japan), an anion surfactant and another component of shampoo. These substances were used to compare the nutrient effect on the growth of the fungi examined. These three surfactant components were each adjusted to 0.01%, 0.05%, and 0.25% (0.10, 0.50, and 2.5 g/l) and added to 15 g/l Bacto-agar.

The fungi inoculated onto the Petri dishes with the various added surfactant components were incubated at 25° ± 1°C for 12–16 days.

The fungal count of each species shown in the tables is expressed as a percentage of the colony count detected on 1/4 PDA at 25°C under the corresponding conditions.

Results

Homology searching of ITS regions of dark fungi

The predicted size fragment (approximately 500–600 bp) was successfully amplified by PCR in black yeast isolate (data not shown). Homology searching of the partial sequences obtained showed that the sequences of isolates NH119, NH917, NH7009, NH228, NH1191, NH1014, and NH9105 had homology of 99.7%, 99.8%, 100%, 100%, 100%, 97.5%, and 75% with *Exophiala salmonis* (AY213652), *E. spinifera* (AM176734), *Cladophialophora boppii* (AB109182), *Phialophora europaea* (EF540756), *Cladosporium cladosporioides* (AY361968), *Phoma herbarum* (AY337712), and *Scolecobasidium constrictum* (DQ307327), respectively.

Morphological characteristics of seven species of dark fungi

The color of colony of 13 species cultured at 25°C is shown in Table 1. Seven black fungi cultured on a slant showed dark under microscopic observation and were identified morphologically as well as genetically. Six other genera of molds were identified morphologically. *Acremonium* sp. has phialides arising from bundled aerial hyphae; *Alternaria* sp. has dark conidia with both cross and longitudinal septa; *Aureobasidium* sp. has hyaline mycelia when young, becoming dark with age; *Fusarium* sp. has canoe-shaped macroconidia; *Paecilomyces* sp. has penicillate conidiophores; *Rhodotorula* sp. has hyaline, 1-celled, and ovoid conidia by budding.

Effect of temperature, NaCl, and pH on fungal growth

The percentage colony count of the 13 species cultured at 30°C ranged from 70% to 108% and was similar to that at 25°C (see Table 1), but the colonies of *C. cladosporioides*, *E. salmonis*, and *S. constrictum* at 30°C were smaller than at 25°C (Fig. 1A). The color and size of the other species at 25° and 30°C were similar.

At 33°C, no colonies of *C. cladosporioides*, *C. boppii*, *E. salmonis*, or *S. constrictum* were detected (Table 1), and the number and size of colonies of *E. spinifera* were also much smaller than at 30°C, with a percentage of 16%. The number of colonies of *P. herbarum* was similar to that at 30°C, but the color of the colonies was lighter (Fig. 1B). The colonies of common bathroom fungi thus were smaller or not detectable at 33°C.

At 35°C, colonies were detected for *Alternaria* sp., *Aureobasidium* sp., *Fusarium* sp., and *Paecilomyces* sp. Each of the colonies was smaller and lighter than at 30° or 33°C. At 37°C, no colonies of the 13 species examined were detected.

Although colonies of all 13 species were detected on media with 5% NaCl, the number and size of colonies of *C. boppii* and *P. europaea* were smaller than on media without NaCl (Table 2). No colonies of the six fungal species *C. boppii*, *E. spinifera*, *E. salmonis*, *Paecilomyces* sp., *P. europaea*, and *S. constrictum* were detected on medium with 10% NaCl. In the seven fungi for which colonies were detected on media with 10% NaCl, the number of colonies ranged from 50% to 100% of the count on media without NaCl, but the colonies of most species were smaller and lighter than on media with 0% or 5% NaCl (Fig. 2). Only *Alternaria* sp. and *C. cladosporioides* were detected on media with 15% NaCl (Table 2), and the size of the colonies detected was smaller than on media with 10% NaCl.

In the four fungi *E. spinifera*, *E. salmonis*, *P. herbarum*, and *S. constrictum*, the colony size and color were approximately similar on media of pH 6.0 and pH 9.7 (Table 3, Fig. 3), with the number of colonies at pH 9.7 ranging from 70% to 107% of that at pH 6.0. On the other hand, the size of colonies of the four fungi *Alternaria* sp., *Aureobasidium* sp., *C. cladosporioides*, and *Rhodotorula* sp. was much smaller than at pH 6.0, whereas the color of colonies of *Aureobasidium* sp. and *Fusarium* sp. was much lighter than on media

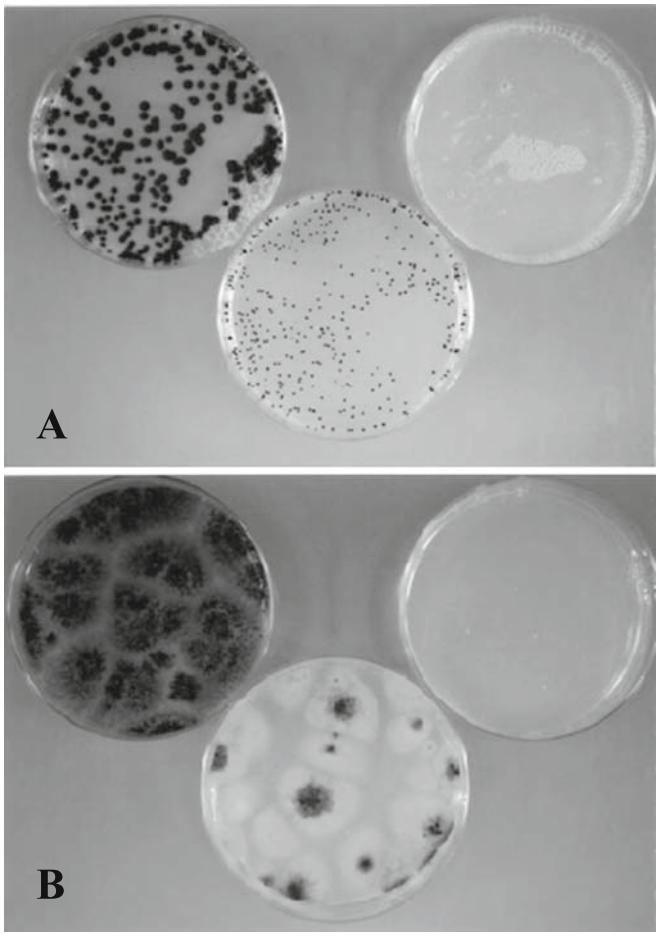

Fig. 1. Effect of temperature for *Exophiala salmonis* (JCM 15940; **A**) and *Phoma herbarum* (JCM 15942; **B**) on 1/4 potato dextrose agar (PDA) for 10 days. **A** *Left*, 25°C; *center*, 30°C; *right*, 33°C. **B** *Left*, 25°C; *center*, 33°C; *right*, 35°C

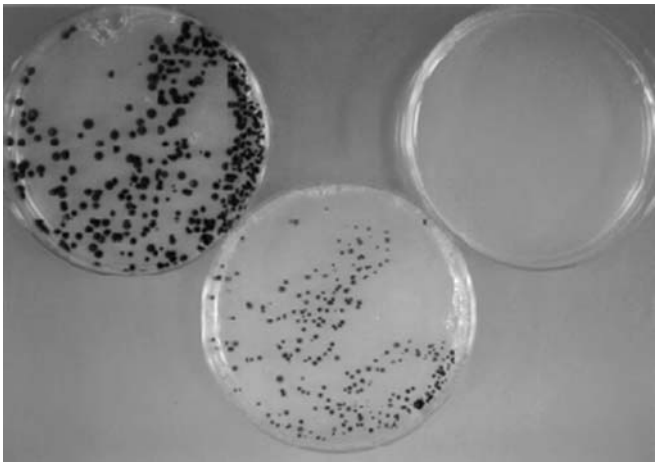

Fig. 2. Effect of media added to NaCl for *Exophiala spinifera* (JCM 15939) on 1/4 PDA for 12 days. *Left*, 0% NaCl; *center*, 5% NaCl; *right*, 10% NaCl

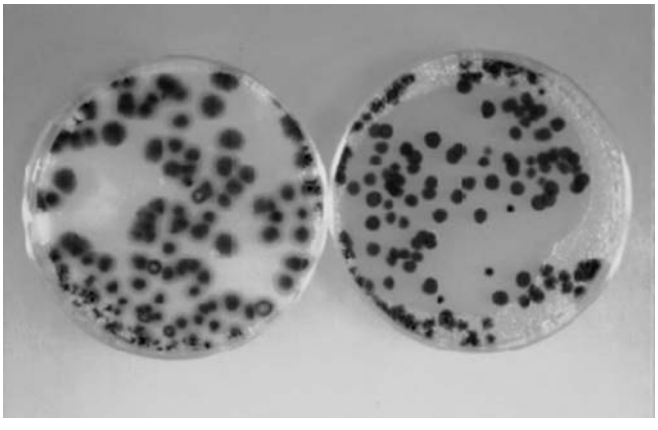

Fig. 3. Effect of pH for *Scolocobasidium constrictum* (JCM 15943) on 1/4 PDA for 12 days. *Left*, pH 6.0; *right*, pH 9.7

Table 2. Effect of desiccation on common fungi in bathrooms

Fungal species	Strain no.	Times[a]	0% NaCl	5% NaCl			10% NaCl			15% NaCl		
			Color	Percentage[b]	Color[c]	Size[d]	Percentage[b]	Color[c]	Size[d]	Percentage[b]	Color[c]	Size[d]
Acremonium sp.	NH1107	10000	White	98	+	+	79	+	±	0		
Alternaria sp.	NH1106	1000	Dark	84	+	+	72	+	±	6	+	–
Aureobasidium sp.	NH1326	10000	Black	103	–	+	84	–	–	0		
Cladosporium cladosporioides	JCM 15937	10000	Dark	114	+	+	100	+	+	54	±	±
Cladophialophora boppii[e]	JCM 15938	100	Dark	28	+	–	0			0		
Exophiala spinifera[e]	JCM 15939	10000	Dark	84	+	±	0			0		
Exophiala salmonis[e]	JCM 15940	100	Dark	59	+	±	0			0		
Fusarium sp.	NH471	1000	Purple	102	±	+	50	–	±	0		
Paecilomyces sp.	NH716	10000	Pink	70	±	±	0			0		
Phialophora europaea[e]	JCM 15941	10000	Dark	91	+	–	0			0		
Phoma herbarum[e]	JCM 15942	10000	Dark	98	+	+	53	±	±	0		
Scolocobasidium constrictum[e]	JCM 15943	100	Brown	28	+	±	0			0		
Rhodotorula sp.	NH6106	100000	Pink	96	±	±	85	±	–	0		

[a] Times indicate levels of dilution for counting 10–100 colonies on each medium
[b] Percentage figure indicates percentage of fungal count on 1/4 PDA media without NaCl
[c] Color of fungi compared with that on ¼ PDA and assessed as follows: +, same; ±, lighter; –, much lighter
[d] Size of colonies compared with that on ¼ PDA and assessed as follows: +, same; ±, smaller; –, much smaller
[e] Fungus more common inside bathrooms than outside

of pH 6.0. In *C. boppii* and *Paecilomyces* sp., no colonies were detected on media of pH 9.7. Apart from *C. boppii*, common bathroom fungi grew at a similar speed on media of pH 9.7 and pH 6.0.

Effect of surfactant to fungal growth

The effect of the sodium fatty acid SO on the growth of the 13 fungi was examined (Table 4). Colonies of all species examined were detected on media of 0.01% and 0.05% SO within 16 days. The percentage colony count on media of 0.05% SO, for example, compared to 1/4 PDA ranged from 76% to 114% and remained similar regardless of the fungal species. For example, the colony count of *C. boppii* on 0.05% SO medium was about 85% of that on 1/4 PDA, although in the former culture the colonies were smaller and more obscure (Fig. 4).

On the other hand, the level of dilution and the number of fungal spores collected per colony varied among species. For example, spores of *E. spinifera* were diluted 10000 times to detect countable colonies on each Petri dish whereas spores of *E. salmonis* were diluted only 100 times (Tables 1, 4).

The color of the colonies of most of the fungi on 0.05% SO media was darker than on 0.01% SO, but colonies of *Fusarium* sp. and *Rhodotorula* sp. on 0.05% SO were both lighter and smaller than on 0.01% SO.

The number of colonies on 0.25% SO media varied among species. No colonies of *C. boppii*, *Fusarium* sp., *Paecilomyces* sp., or *Rhodotorula* sp. were detected on 0.25% SO, but the percentage colony count of *Alternaria* sp., *E. spinifera*, *E. salmonis*, *P. europaea*, *P. herbarum*, and *S. constrictum* on 0.25% SO media ranged from 64% to 119% and was similar to that on 0.05% SO (Fig. 5). The colony size of *P. herbarum* and *S. constrictum* was similar on 0.25% SO, although that of the other four fungal species was smaller.

The growth of fungal colonies on media of the non-ion surfactant AE varied among species (Table 5). Colonies of

Fig. 4. Effect of surfactant medium for *Cladophialophora boppii* (JCM 15938) cultured for 12 days. *Left*, 1/4 PDA; *right*, 0.05% SO medium

Table 3. Effect of pH on common fungi in bathrooms

Fungal species	Strain no.	Times[a]	pH 6.0	pH 9.7		
			Color	Percentage[b]	Color[c]	Size[d]
Acremonium sp.	NH1107	10000	White	127	+	±
Alternaria sp.	NH1106	1000	Dark	65	±	–
Aureobasidium sp.	NH1326	10000	Black	105	–	–
Cladosporium cladosporiodes	JCM 15937	10000	Dark	83	+	–
Cladophialophora boppii[e]	JCM 15938	100	Dark	0		
Exophiala spinifera[e]	JCM 15939	10000	Dark	100	+	+
Exophiala salmonis[e]	JCM 15940	100	Dark	89	+	+
Fusarium sp.	NH471	1000	Purple	50	–	±
Paecilomyces sp.	NH716	10000	Pink	0		
Phialophora europaea[e]	JCM 15941	10000	Dark	94	+	±
Phoma herbarum[e]	JCM 15942	10000	Dark	70	++	+
Scolocobasidium constrictum[e]	JCM 15943	100	Brown	107	+	+
Rhodotorula sp.	NH6106	100000	Pink	13	+	–

[a] Times indicate levels of dilution for counting 10–100 colonies on each medium
[b] Percentage figure indicates percentage of fungal count on 1/4 PDA of pH 6.0
[c] Color of fungi compared with that on media of pH 6.0 and assessed as follows: ++, darker; +, same; ±, lighter; –, much lighter
[d] Size of colonies compared with that on media of pH 6.0 and assessed as follows: +, same; ±, smaller; –, much smaller
[e] Fungus more common inside bathrooms than outside

Table 4. Effect of sodium fatty acid (SO) on common fungi in bathrooms

Fungal species	Strain no.	Times[a]	0.01% SO			0.05% SO			0.25% SO		
			Percentage[b]	Color[c]	Size[d]	Percentage[b]	Color	Size[d]	Percentage[b]	Color[c]	Size[d]
Acremonium sp.	NH1107	10000	105	±	+	95	White	+	5	±	±
Alternaria sp.	NH1106	1000	95	±	+	110	Brown	+	119	++	±
Aureobasidium sp.	NH1326	10000	79	±	+	85	Dark	+	32	++	±
Cladosporium cladosporiodes	JCM 15937	10000	109	±	+	114	Dark	+	19	+	±
Cladophialophora boppii[e]	JCM 15938	100	96	±	+	85	Dark	+	0		
Exophiala spinifera[e]	JCM 15939	10000	83	±	+	98	Dark	+	75	++	±
Exophiala salmonis[e]	JCM 15940	100	113	±	+	107	Dark	+	93	+	±
Fusarium sp.	NH471	1000	78	++	++	81	Pale	+	0		
Paecilomyces sp.	NH716	10000	89	±	++	76	Pale	+	0		
Phialophora europaea[e]	JCM 15941	10000	91	±	+	85	Dark	+	73	+	±
Phoma herbarum[e]	JCM 15942	10000	68	±	+	92	Dark	+	64	+	+
Scolocobasidium constrictum[e]	JCM 15943	100	77	±	±	85	Brown	+	72	+	+
Rhodotorula sp.	NH6106	100000	85	++	++	87	Pink	+	0		

[a] Times indicate levels of dilution for counting 10–100 colonies on each medium
[b] Percentage figure indicates percentage of fungal count on 1/4 PDA at 25°C (Table 1)
[c] Color of fungi compared with that on 0.05% SO and assessed as follows: ++, darker; +, same; ±, lighter
[d] Size of colonies compared with that on 0.05% SO and assessed as follows: ++, larger; +, same; ±, smaller
[e] Fungus more common inside bathrooms than outside

Table 5. Effect of non-ion surfactant (AE) on common fungi in bathrooms

Fungal species	Strain no.	Times[a]	0.01% AE			0.05% AE			0.25% AE		
			Percentage[b]	Color[c]	Size[d]	Percentage[b]	Color	Size[d]	Percentage[b]	Color[c]	Size[d]
Acremonium sp.	NH1107	10000	84	White		0			0		
Alternaria sp.	NH1106	1000	119	+	+	124	Brown	+	67	±	±
Aureobasidium sp.	NH1326	10000	4	White		0			0		
Cladosporium cladosporiodes	JCM 15937	10000	0			0			0		
Cladophialophora boppii[e]	JCM 15938	100	71	±	+	62	Dark	+	65	+	+
Exophiala spinifera[e]	JCM 15939	10000	88	±	+	65	Dark	+	53	+	+
Exophiala salmonis[e]	JCM 15940	100	111	±	+	116	Dark	+	60	+	+
Fusarium sp.	NH471	1000	0			0			0		
Paecilomyces sp.	NH716	10000	0			0			0		
Phialophora europaea[e]	JCM 15941	10000	112	±	±	92	Dark	+	72	++	+
Phoma herbarum[e]	JCM 15942	10000	96	±	+	92	Dark	+	60	+	±
Scolocobasidium constrictum[e]	JCM 15943	100	78	±	±	80	Brown	+	71	++	+
Rhodotorula sp.	NH6106	100000	0			0			0		

[a] Times indicate levels of dilution for counting 10–100 colonies on each medium
[b] Percentage figure indicates percentage of fungal count on 1/4 PDA at 25°C (Table 1)
[c] Color of fungi compared with that on 0.05% AE and assessed as follows: ++, darker; +, same; ±, lighter
[d] Size of colonies compared with that on 0.05% AE and assessed as follows: +, same; ±, smaller; –, much smaller
[e] Fungus more common inside bathrooms than outside

Alternaria sp., *C. boppii*, *E. spinifera*, *E. salmonis*, *P. europaea*, *P. herbarum*, and *S. constrictum* were found on 0.01%, 0.05%, and 0.25% AE media, for example, with the percentage colony count ranging from 53% to 72% on 0.25% AE. On the other hand, no colonies of *C. cladosporioides*, *Fusarium* sp., *Paecilomyces* sp., or *Rhodotorula* sp. were found on 0.01%, 0.05%, or 0.25% AE.

The color of the colonies of *C. boppii*, *E. spinifera*, *E. salmonis*, *P. europaea*, *P. herbarum*, and *S. constrictum* was darker on 0.05% AE than on 0.01% AE whereas that of colonies on 0.25% AE was similar to or darker than that on 0.05% AE (Fig. 6A). In *Alternaria* sp., however, colonies were lighter in color on 0.25% AE than on 0.01% AE or 0.05% AE (Fig. 6B). On the other hand, the colony size of common bathroom fungi was thus generally similar regardless of AE concentration.

With the exception of *Aureobasidium* sp. and *P. europaea*, colonies of all fungi were found on 0.01% media of the anion surfactant LAS, but no colonies were found on 0.05% or 0.25% LAS (Table 6). The percentage colony count of fungi other than *Rhodotorula* sp. ranged from 56 to 119% on 0.01% LAS and was similar to that on the other

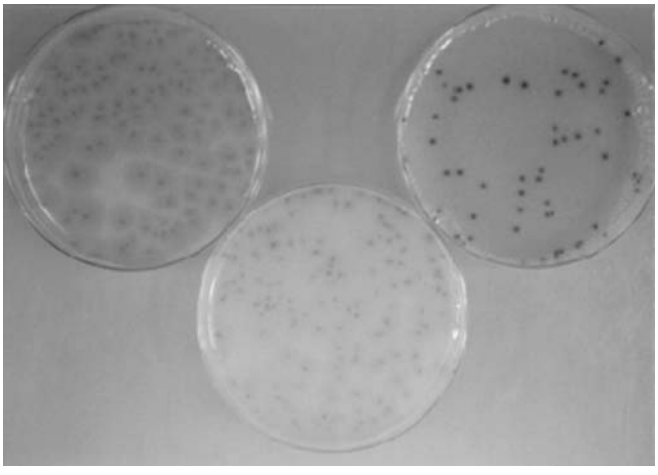

Fig. 5. Effect of SO (sodium fatty acid) medium for *Exophiala spinifera* (JCM 15939) cultured for 14 days. *Left*, 0.01% SO; *center*, 0.05% SO; *right*, 0.25% SO

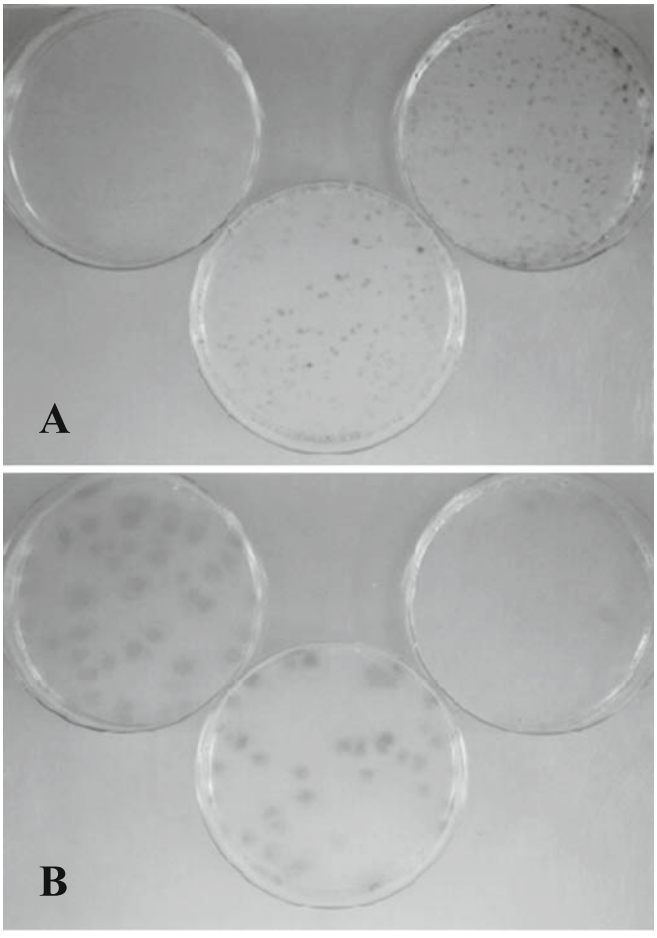

Fig. 6. Effect of AE (nonion surfactant) medium for *Phialophora europaea* (JCM 15941; **A**) and *Alternaria* sp.(NH1106; **B**) cultured for 14 days. *Left*, 0.01% AE; *center*, 0.05% AE; *right*, 0.25% AE

Table 6. Effect of anion surfactant (LAS) on common fungi in bathrooms

Fungal species	Strain no.	Times[a]	0.01% LAS			0.05% LAS			0.25% LAS		
			Percentage[b]	Color	Size[c]	Percentage[b]	Color	Size[c]	Percentage[b]	Color	Size[c]
Acremonium sp.	NH1107	10000	81	White	±	0			0		
Alternaria sp.	NH1106	1000	119	Brown	±	0			0		
Aureobasidium sp.	NH1326	10000	0			0			0		
Cladosporium cladosporiodes	JCM 15937	10000	109	Dark	–	0			0		
Cladophialophora boppii[d]	JCM 15938	100	77	Dark	–	0			0		
Exophiala spinifera[d]	JCM 15939	10000	118	Dark	±	0			0		
Exophiala salmonis[d]	JCM 15940	100	110	Dark	±	0			0		
Fusarium sp.	NH471	1000	56	Pale	±	0			0		
Paecilomyces sp.	NH716	10000	102	Pale	±	0			0		
Phialophora europaea[d]	JCM 15941	10000	0			0			0		
Phoma herbarum[d]	JCM 15942	10000	72	Dark	±	0			0		
Scolocobasidium constrictum[d]	JCM 15943	100	83	Brown	–	0			0		
Rhodotorula sp.	NH6106	100000	39	Pink	±	0			0		

[a] Times indicate levels of dilution for counting 10–100 colonies on each medium
[b] Percentage figure indicates percentage of fungal count on 1/4 PDA at 25°C (Table 1)
[c] Size of colonies compared with that on 0.01% SO and assessed as follows: +, same; ±, smaller; –, much smaller
[d] Fungus more common inside bathrooms than outside

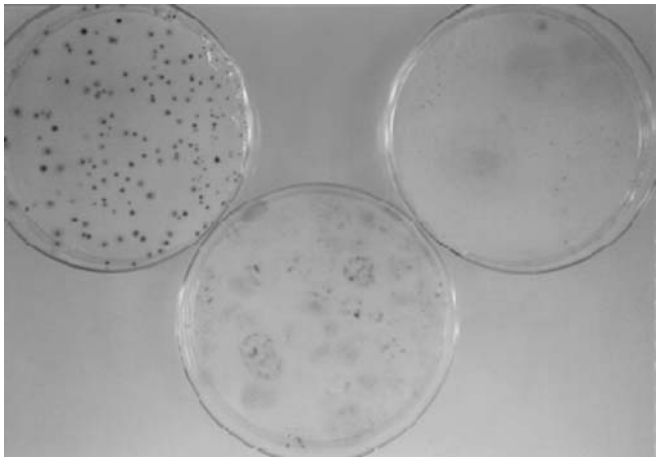

Fig. 7. Effect of three surfactant media for *Phoma herbarum* (JCM 15942) cultured for 14 days. *Left*, 0.01% LAS (anion surfactant); *center*, 0.01% SO; *right*, 0.01% AE

surfactant media, 0.01% SO and 0.01% AE (Fig. 7). The size of the colonies was however smaller than on the other media and the color darker. Microscopically, the length of hyphae appeared reduced and the blocks of spores condensed.

Discussion

Environmental conditions on the walls of bathrooms are found to be very humid, as well as on windows of the household where much moisture is accumulating and condensing in winter (Hamada 2006). *Cladosporium cladosporioides* and *Aureobasidium* sp. are the predominant fungi on household windows, whereas *S. constrictum*, *C. boppii*, and *P. europaea* are found often in bathrooms. The more common fungi in bathrooms were different from those found on windows. Factors other than moisture, for example, nutrients, are thought to affect fungal contamination in bathrooms.

Detergent adhering to the inside of washing machines is reported to be a nutrient for *E. spinifera* and *S. constrictum* (Hamada 2005). *Exophiala spinifera*, *P. herbarum*, *S. constrictum*, and *C. cladosporioides* are common in both bathrooms and washing machines (Hamada 2004; Hamada and Abe 2008).

In contrast to sodium fatty acid promoting the growth of all fungi (see Table 4), non-ion surfactant seem to provide nutrients for a number of fungi regardless of concentration (see Table 5). Non-ion surfactant promoted the growth of the more common fungi in bathrooms. Shampoo containing non-ion surfactant is therefore thought to have a clearer effect on fungal flora in bathrooms than soap or sodium fatty acid.

Soap seems to be used more frequently in bathrooms than in washing machines. *Phoma herbarum* was found more frequently in bathrooms than in washing machines (Hamada 2004; Hamada and Abe 2008) and the amount of *P. herbarum* found in washing machines using soap was about eight times larger than in those using synthetic detergent (Hamada 2005). *Phoma herbarum*, moreover, seem to be tolerant of alkali (see Table 3). Thus, soap also seems to have a great effect on fungal contamination, including *P. herbarum*, in bathrooms.

The 0.25% AE (non-ion surfactant) medium is equivalent to about 15 times the concentration of detergent solution used for washing clothes in washing machines (Hamada 2005). The fungi that grow on 0.25% AE or 0.25% SO media would seem therefore to make use of shampoo or soap adherent to or condensed on the walls and floor.

The six species that grew on 0.25% AE media (see Table 5), namely, *C. boppii*, *E. spinifera*, *E. salmonis*, *P. europaea, P. herbarum*, and *S. constrictum*, are found more frequently in bathrooms than in other household environments (Hamada and Abe 2008). The growth of *Alternaria* sp., however, which is also found often in household environments, seems to be decreased on 0.25% AE media. Thus, *Alternaria* sp., in contrast to the other six species, seems to be not suited to the bathroom environment.

Keratin derived from skin is reported as a nutrient for fungi growing in bathrooms (Lee et al. 2007). However, the quantities stored on walls and floors are difficult to estimate and its effect on fungal flora is at present unknown.

As nutritional conditions for fungi in bathrooms were thought to be poor, 1/4 PDA was used to reduce the nutrient level in the media in the present study. All fungi grew more slowly on surfactant media than on 1/4 PDA (see Fig. 4), but the number of colonies on surfactant was similar to that on 1/4 PDA. Surfactants are thus thought not to inhibit fungal germination, although they are not as good a nutrient for fungi as 1/4 PDA.

Cladosporium cladosporioides, *Aureobasidium* sp., and *Alternaria* sp., often detected in bathrooms, are able to also grow in tatami mats and carpets with low water activity (Hamada 1990) and to survive on windows of a household even in summer under dry, high-temperature conditions (Hamada 2006). *Alternaria* sp., *Aureobasidium* sp., *Fusarium* sp., and *Paecilomyces* sp. were found to grow at 35°C (see Table 1). Two fungi, *Alternaria* sp. and *C. cladosporioides*, are able to grow on media with 15% NaCl (see Table 2). Generally, common fungi in households are able to grow under more severe environmental conditions than fungi common only in bathrooms.

Acremonium sp. and *Rhodotorula* sp., as well as *Aureobasidium* sp., *Fusarium* sp., and *Paecilomyces* sp., are often found under humid conditions and grow comparatively fast on 1/4 PDA. However, the more common fungi in bathrooms generally grow more slowly on 1/4 PDA than the others examined in this study and seem to grow well only on the surfactant medium or a bathroom.

In the present study, high-temperature tolerance was not observed in certain species of *Exophiala*, despite bathroom fungi often being exposed to water of more than 37°C during bathing. This result differed from the findings of a study by Nishimura et al. (1987) in which fungal samples were chiefly collected from bathwater after bathing, in contrast to the present study, in which bathroom surfaces were swabbed with cotton tips. High-temperature-tolerant

species of *Exophiala*, for example, *E. dermatitidis*, did not appear to be common on bathroom walls or floors.

The dilution rate of spores seems to reflect approximately the number of spores per fungal colony, but the number of spores detected varied by species (see Table 1). To detect a similar number of spores, *C. boppii* and *S. constrictum*, with comparatively few spores per colony, were diluted 100 times, whereas *C. cladosporioides* and *P. herbarum*, with more spores, were diluted 10000 times. Even within the same genus, the number of spores of *E. salmonis* was about 1/100 that of *E. spinifera*. In a previous study (Hamada and Abe 2008), *E. spinifera* was found more frequently than *E. salmonis*, but it may be difficult to determine which of these two fungi plays the greater role as a cause of dirt by fungal mycelium.

When culture conditions become severe, the fungal colony seems to become smaller and lighter before becoming not detectable (Table 1). The color and size of the colony generally change in parallel fashion (see Table 5), but whether color or size of a fungal colony is more sensitive as the indicator of growth seems to vary with species or environmental factors.

The present study on the characteristics of more common fungi in bathrooms suggests methods for controlling fungal contamination in bathrooms. These fungi are able to utilize surfactant as a nutrient but to survive only under low-temperature and very wet conditions (see Tables 1, 2). Thus, washing away soap and shampoo, which provide nutrients for fungal contamination, is most important. Washing with hot water and treatment by drying in bathrooms are also thought to be effective in eliminating contamination. Use of a ventilation system would therefore also be likely to effectively control fungal contamination in bathrooms.

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References

Ara K, Aihara M, Ojima M, Toshima Y, Yabune C, Tokuda H, Kawai S, Ueda N, Tanaka T, Akiyama K, Takatori K (2004) Survey of fungal contamination in ordinary houses in Japan. Allergol Int 53:369–377

de Hoog GS, Guarro J, Gene J, Figueras MJ (2000) Atlas of clinical fungi, 2nd edn. Centraalbureau voor Schimmelcultures, Utrecht

Goto S, Aono R, Sugiyama J, Horikoshi K (1981) *Exophiala alcalophiala*, a new black, yeast-like hyphomycete with an accompanying *Phaeococcomyces alcalophilus* morph, and its physiological characteristics. Trans Mycol Soc Jpn 22:429–439

Hamada N (1990) Fungal flora in the house dust of dwellings. Trans Mycol Soc Jpn 31:237–247

Hamada N (2004) Dirt suspended in washing water and mold contamination (in Japanese). Seikatsu Eisei 48:124–130

Hamada N (2005) Characteristics of fungal growing inside washing machines (in Japanese). Seikatsu Eisei 49:108–113

Hamada N (2006) Condensation and fungal contamination on sash window (in Japanese). Seikatsu Eisei 50:69–75

Hamada N, Abe N (2008) Characteristics of recent fungal contamination in bathrooms: examination of fungal and yeasty flora (in Japanese). Seikatsu Eisei 52:98–106

Hamada N, Fujita T (1999) Fungal contamination in bathrooms: examination of fungal and yeasty flora (in Japanese). Bokin Bobai 27:351–358

Hamada N, Fujita T (2000) Growth rate of fungi in bathrooms: experimental survey. Mycoscience 41:297–301

Hamada N, Murakami T, Abe N (2006) Characteristics of fungi growing on window sashes. Seikatsu Eisei 50:510–515

Lee H, Kosuge J, Aihara M, Takatori K (2007) The effect of soap on the growth of major fungi contaminating the bathroom (in Japanese). Bokin Bobai 35:197–200

Moriyama Y, Nawata N, Tsuda T, Nitta M (1992) Occurrence of moulds in Japanese bathrooms. Int Biodeterior Biodegrad 30:47–55

Nagai K, Suzuki K, Okada G (1998) Studies on the distribution of alkalophilic and alkali-tolerant soil fungi II: fungal flora in two limestone caves in Japan. Mycoscience 39:293–298

Nishimura K, Miyaji M, Taguchi H, Tanaka R (1987) Fungi in bathwater and sludge of bathroom drainpipes. Mycopathologia 97:17–23

Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O (2000) Introduction to food- and airborne fungi, 6th edn. Centraalbureau voor Schimmelcultures, Utrecht

Sterflinger K (1998) Temperature and NaCl-tolerance of rock-inhabiting meristematic fungi. Antonie Leeuwenhoek 74:271–281