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# Fungi in bathwater and sludge of bathroom drainpipes

*1. Frequent isolation of* Exophiala *species*

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## Abstract

Samples of bathwater from 14 homes and 22 public bathhouses and sludge in drainpipes from 19 household bathrooms were plated out onto potato dextrose agar supplemented with chloramphenicol. Several media were used to study colony morphology of the isolates and the thermotolerance and alkaline tolerance of each isolate were examined.

Eleven sludge samples produced 12 isolates of *Exophiala jeanselmei*, 2 of *E. dermatitidis* and 1 of *E. moniliae*. Five household bathwater samples produced 2 isolates of *E. jeanselmei*, 4 of *E. dermatitidis* and 1 of *E. alcalophila*. One isolate of *E. jeanselmei*, 2 of *E. dermatitidis*, 3 of *E. moniliae* and 2 of unidentified *Exophiala* species were recovered from 6 samples of the bathwater dissolving 'Chinese medicine' in the bathtubs of public bathhouses. One isolate of *E. jeanselmei* was recovered from the 15 samples of bathwater from public bathhouses. Bathwater and sludge in bathroom drainpipes may be an important habitat of *Exophiala* species.

## Introduction

Although the genus *Exophiala* Carmichael is important in medical mycology, the habitat of *Exophiala* species has not been adequately clarified in the past. *Exophiala jeanselmei* (Langeron) McGinnis et Padhye, *E. dermatitidis* (Kano) de Hoog, *E. moniliae* de Hoog, *E. spinifera* (Nielsen et Conant) McGinnis and *E. alcalophila* Goto et Sugiyama have been isolated from non-living woody materials (1, 4, 6, 7, 9), soil (1, 4, 5), sludge, activated sludge (3), polluted water (2), nest of birds, wasps and armadillos (1, 9), or visceral organs of bats (12). In the human home environment, *E. jeanselmei* has been isolated from a furniture and freezer support (4) and *E. dermatitidis* from a humidifier (11). However, there have been no reports of fungi in bathwater or sludge of bathroom drainpipes.

In recent years the Japanese way of life has been greatly influenced by the West. Many indigenous traditions survive, however, and these include bathing customs. Unlike Western people, the Japanese share bathwater but wash thoroughly before entering the bathtub. As a result most fungi on the skin surface are washed off in the waste water which runs into bathroom drainpipes, and most of the remaining fungi are washed off in the bathwater. Therefore, it is likely that any fungi in bathwater or sludge originate from the skin surface. In addition, as the bathwater is warmed to over 42 °C for a few hours each day, these fungi may be thermotolerant. It is an interesting ecological question of what kinds of fungi survive and multiply in bathwater, waste water and sludge of bathroom drainpipes which contain soap, surface active compounds (shampoo, hair rinse), keratin substance, cutaneous fatty acid and perspiration, and these fungi may also present an important problem for public health.

In the present work the authors tried to clarify which fungi occur in bathwater and sludge of bathroom drainpipes, and frequently isolated *Exophia-*

*la* species. Their morphology, thermotolerance and alkaline tolerance are accordingly described.

## Materials and methods

Samples were collected in Tokyo, Chiba, Kanagawa, Tochigi, Shizuoka and Nara Prefectures in winter, between December 15, 1983 and March 14, 1984. Fourteen samples of bathwater were collected from 14 homes after families had bathed. Twenty-two samples of bathwater were collected from 22 public bathhouses. In 7 of these 22 samples so-called 'Chinese medicine' or 'Rensen-Simucan' ('Ninjin Jitsubosan' in Japanese) was dissolved in the bathwater. Nineteen samples of sludge were collected from 19 households by scratching out the inside of the drainpipes or the back of the caps using sterile cotton balls.

The floors and walls of the household bathrooms examined were tiled, and the bathtubs were made of wood, plastics, stainless steel or enamelled iron. The bathtubs, floors and walls of the bathrooms of the public bathhouses were tiled. Each sample was preserved at 4 °C until examined.

Potato dextrose agar (Difco, Detroit, Michigan, U.S.A.) (PDA) supplemented with chloramphenicol at a rate of 100 μg/ml was used as a medium for isolation. Ten plates were allotted to each sludge sample. The sludge on the cotton balls was directly and successively smeared on 10 plates. When the same colonies grew on one or more plates, they were recorded as an isolate. Five sterilized test tubes were provided for each bathwater sample, and 10 ml of the sample was put in each of the 5 tubes. After centrifuging at 3000 rpm for 20 minutes, most of the water was removed and the remaining water (0.1 to 0.2 ml) pipetted and dropped at 7 equidistant points on a plate. The plates were then put into a 27 °C incubator and observed every other day for 3 weeks. Fungi growing on the plates were isolated and identified.

PDA, Sabouraud's dextrose (2% w/v) agar (SDA), corn meal agar (Difco) (CMA), Czapek Dox agar (Eiken, Tokyo, Japan) (CDA) and brain heart infusion agar (Difco) (BHIA) supplemented with dextrose at a rate of 1% (w/v) were used to study colony morphology. Slide cultures of the isolates were made using PDA and CMA and were incubated for 2 weeks at 27 °C. Specimens for scanning electron microscopy were prepared from colonies on CMA. Thermotolerance was assessed by presence or absence of growth on PDA and SDA after 2 weeks with incubation at 27, 37, 40 and 42 °C. Alkaline tolerance was tested by the method employed by Goto *et al.* (5), judged after 3 weeks.

## Results

### *Isolates of the genus* Exophiala

Results are shown in Tables 1 and 2. Fifteen isolates of *Exophiala* species were obtained from 11 of the 19 samples of the drainpipe sludge. Twelve were *E. jeanselmei*, 2 *E. dermatitidis* and 1 *E. moniliae*. Many colonies of *E. jeanselmei* were recovered from the 11 samples. Both *E. jeanselmei* and *E. dermatitidis* were recovered from 2 samples each. Two varieties of *E. jeanselmei* were isolated from another sample. Seven isolates of *Exophiala* species were isolated from 5 of the 14 bathwater samples from household bathtubs. There was no relationship between the frequency of isolation of *Exophiala* species and the materials of which the bathtubs were made.

These isolates comprised 2 of *E. jeanselmei*, 4 of *E. dermatitidis* and 1 of *E. alcalophila*. Both *E. jeanselmei* and *E. dermatitidis* were recovered from 1 of the 5 samples and two varieties of *E. dermatiti-*

*Table 1.* Number of *Exophiala* spp. isolated from sludge in bathroom drainpipes and from bathwater.

| Type of samples | Number of samples | Total number of isolates | Number of *Exophiala* spp. |
|---|---|---|---|
| Sludge in bathroom drainpipes | 19 | 115 | 15 |
| Bathwater from households | 14 | 59 | 7 |
| Medicated bathwater from public bathhouses | 7 | 53 | 8 |
| Bathwater from public bathhouses | 15 | 28 | 1 |
| Total | 55 | 255 | 31 |

*Table 2. Exophiala* spp. isolated from sludge in bathroom drainpipes and from bathwater.

| Type of samples | Number of samples | | Number of isolates (*E.j., E.d., E.m., E.a., Exo.* sp.) |
|---|---|---|---|
| | Tested | Positive | |
| Sludge in bathroom drainpipes | 19 | 11 | 15* (12, 2, 1, 0, 0) |
| Bathwater from households | 14 | 5 | 7* (2, 4, 0, 1, 0) |
| Medicated bathwater from public bathhouses | 7 | 6 | 8* (1, 2, 3, 0, 2) |
| Bathwater from public bathhouses | 15 | 1 | 1 (1, 0, 0, 0, 0) |
| Total | 55 | 23 | 31 (16, 8, 4, 1, 2) |

* Two species or two varieties of a species were isolated from same samples. *E.j.: E. jeanselmei; E.d.: E. dermatitidis; E.m.: E. moniliae; E.a.: E. alcalophila; Exo.* sp.: *Exophiala* species, not identified.

*dis* were isolated from another sample. Eight isolates of *Exophiala* species were recovered from the public bathhouses from 6 samples of the bathwater in which 'Chinese medicine' was dissolved. One isolate was *E. jeanselmei*, 2 were *E. dermatitidis*, 3 were *E. moniliae* and 2 were unidentified *Exophiala* species. Two varieties of *E. dermatitidis* were isolated from 1 of the 6 samples and both *E. jeanselmei* and *E. moniliae* were recovered from another sample. An isolate of *E. jeanselmei* was recovered from 1 of the 15 samples of the bathwater from the public bathhouses.

*Morphology and development of* Exophiala *species*

Isolates of *E. dermatitidis* formed yeast-like colonies on PDA, SDA and CMA at early stages of growth. Later, in 6 of the 8 isolates mycelial growth appeared at the periphery of the yeast-like colonies. Colonies on PDA were covered with thin tufts of aerial mycelium. All 8 isolates formed chocolate brown pasty colonies on BHIA, while on CDA they grew only slightly.

Conidiogenous cells of *E. dermatitidis* bore a succession of circular scars (annellations) (Fig. 1-1). One to 5 fertile tips were observed at the apex and subapical region of each bottle- or jar-shaped conidiogenous cell, and annellations were clearly observed on the wall of the tips. These annellated tips were the shortest of all *Exophiala* species and were a little thicker than those of isolates of *E. jeanselmei.* They occurred nonsynchronously. Conidia were also produced from small projections with annellations on the walls of hyphae. Conidia were spherical or ellipsoid. Occasionally daughter conidia were produced directly from mother conidia. In yeast-like growth individual cells also functioned as conidiogenous cells producing conidia from 1 to 5 short fertile tips bearing a few annellations.

The isolates of *E. jeanselmei* grew well on all media except for BHIA. Colonies were greenish grey to black, and later floccose. In one the colony was greyish brown at early stages of growth, but later became black. Conidiogenous cells occurred terminally or laterally on hyphae, and also bore annellations (Fig. 1-2). Tips bearing annellations were slightly longer and more slender than those of *E. dermatitidis.* Isolates of *E. jeanselmei* are similar to those of *E. dermatitidis* under the light microscope.

The 4 isolates of *E. moniliae* grew well on the 5 media. Colonies were black, viscous, yeast-like at early stages of growth and later with the peripheries covered with greenish grey aerial mycelium tufts. Conidiogenous cells bore annellations (Fig. 1-3). Allantoid or banana-shaped conidia and dark brown, small, globose conidiogenous cells with slender fertile tips bearing annellations were characteristic in this species.

The isolate of *E. alcalophila* formed a dark brown to black pasty colony on all media except CDA on which it scarcely grew. Microscopically, the colony consisted of globose or subglobose yeast-like cells with a few moniliform hyphae (Fig. 1-4). These cells produced more cells from one to several fertile points. Each point became a short cylindrical tip with 1 to 5 annellations as it produced more cells (Fig. 1-5). Isthmi were often observed between connected yeast cells.

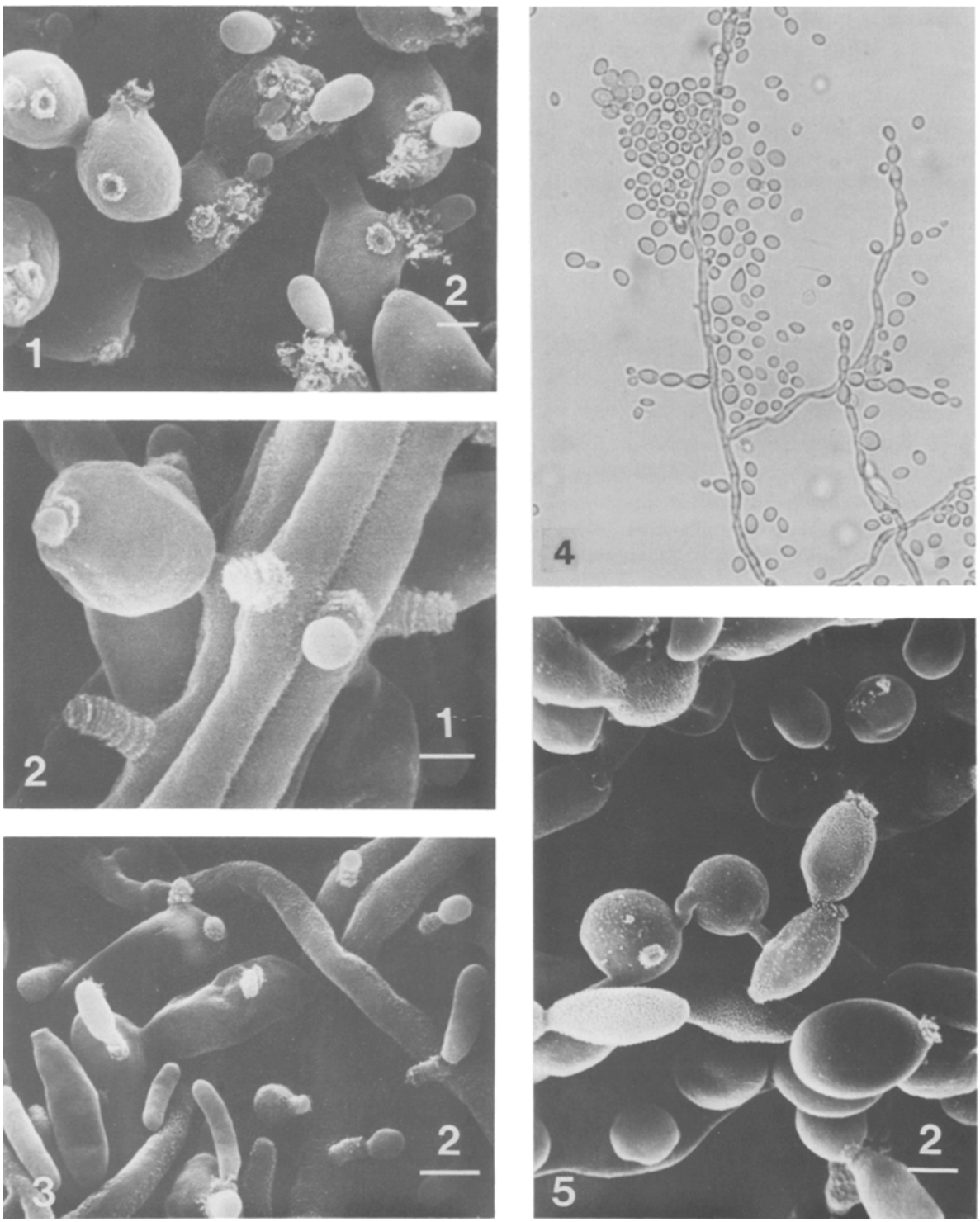


*Fig. 1.* Scanning electron micrographs (1-3, 5) and a light micrograph (4) of *Exophiala* species.
1. *E. dermatitidis* (HB 1801) isolated from a sample of household bathwater. Yeast form. 2. *E. jeanselmei* (HB 1803) isolated from a sample of household bathwater. Fertile tips bearing annellations on hyphae and a conidiogenous cell. 3. *E. moniliae* (D 4405) isolated from the sludge in a bathroom drainpipe. Slender fertile tips with annellations, and allantoid and banana-shaped conidia. 4, 5. *E. alcalophila* (HB 2706) isolated from a sample of household bathwater. Moniliform hyphae and isthmi connecting yeast cells.

*Thermotolerance*

Results are shown in Tables 3 and 4. The 8 isolates of *E. dermatitidis* grew on PDA and SDA at 42 °C. Two of the 4 isolates of *E. moniliae* grew at 40 °C. Two of the 16 isolates of *E. jeanselmei* grew at 37 °C. Table 4 shows survival after incubation at 42 °C. When the 4 isolates of *E. moniliae* were reincubated at 27 °C after 7 days' incubation at 42 °C, they had survived and began to grow. Two of the 16 isolates of *E. jeanselmei* survived after 5 days' incubation at 42 °C and 12 of the other fourteen survived after one to 3 days' incubation at 42 °C. The isolate of *E. alcalophila* survived after 24 hours' incubation at 42 °C.

*Alkaline tolerance*

The isolate of *E. alcalophila* grew at pH 10.4 as shown in Table 5. Thirteen of the 16 isolates of *E. jeanselmei* scarcely grew at pH 10.4. No isolate of *E. dermatitidis* or *E. moniliae* grew at pH 10.4.

## Discussion

There have been no reports of fungi in bathwater and sludge in bathroom drainpipes. Yoshimura (personal communication) has isolated *Alternaria, Cladosporium, Phoma, Aureobasidium, Ochroconis* and *Nigrospora* from black stains on the ceilings and walls of bathrooms. We also isolated several isolates of *Phoma, Aureobasidium, Cladosporium* and *Ochroconis* from bathwater and sludge (data not shown). The present investigation shows, however, that the fungi inhabiting bathwater and sludge are different from those of ceilings and walls of bathrooms. Bathwater and sludge of drainpipes are clearly important habitats of *Exophiala* species. Many colonies of *E. jeanselmei* were recovered from 58% of sludge samples indicating that the species is a main member of this fungal community and that sludge is an important habitat for the species. One or 2 species of *Exophiala* were isolated from 5 out of 14 household bathwater samples. Interestingly, *E. dermatitidis* was isolated from bathwater more than from sludge, which may relate to its thermotolerance.

*Table 3.* Thermotolerance of *Exophiala* spp. isolated from sludge in bathroom drainpipes and from bathwater.

| Species and number of assayed isolates | Number of isolates growing at | | | |
|---|---|---|---|---|
| | 27 °C | 37 °C | 40 °C | 42 °C |
| *E. dermatitidis* | | | | |
| 6 from bathwater | 6 | 6 | 6 | 6 |
| 2 from sludge | 2 | 2 | 2 | 2 |
| *E. moniliae* | | | | |
| 3 from bathwater | 3 | 3 | 2 | 0 |
| 1 from sludge | 1 | 1 | 0 | 0 |
| *E. jeanselmei* | | | | |
| 4 from bathwater | 4 | 2 | 0 | 0 |
| 12 from sludge | 12 | 0 | 0 | 0 |
| *E. alcalophila* | | | | |
| 1 from bathwater | 1 | 0 | 0 | 0 |
| *Exophiala spp.* | | | | |
| *2 from bathwater* | *2* | *2* | *1* | *0* |

*Table 4.* Growth at 27 °C after incubation at 42 °C.

| Isolate | | | Incubation time at 42 °C | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 15 h | 20 h | 24 h | 2 d | 3 d | 5 d | 7 d | 14 d |
| *E. moniliae* | PB | 3701 | + | + | + | + | + | + | + | − |
| | PB | 4801 | + | + | + | + | + | + | + | − |
| | PB | 5001 | + | + | + | + | + | + | + | − |
| | D | 4405 | + | + | + | + | + | + | + | − |
| *E. jeanselmei* | PB | 3711 | + | + | + | + | + | + | − | − |
| | PB | 4101 | + | + | + | + | + | + | − | − |
| | D | 0307 | + | + | + | + | + | − | − | − |
| | D | 1205 | + | + | + | + | + | − | − | − |
| | D | 0607 | + | + | + | + | − | − | − | − |
| | D | 0701 | + | + | + | + | − | − | − | − |
| | D | 1601 | + | + | + | + | − | − | − | − |
| | D | 3502 | + | + | + | + | − | − | − | − |
| | D | 5302 | + | + | + | + | − | − | − | − |
| | D | 0703 | + | + | + | − | − | − | − | − |
| | D | 0907 | + | + | + | − | − | − | − | − |
| | D | 1706 | + | + | + | − | − | − | − | − |
| | HB | 1803 | + | + | + | − | − | − | − | − |
| | D | 2602 | + | + | + | − | − | − | − | − |
| | HB | 1104 | + | + | − | − | − | − | − | − |
| | D | 3501 | + | + | − | − | − | − | − | − |
| *E. alcalophila* | HB | 2706 | + | + | + | − | − | − | − | − |
| *Exophiala* spp. | PB | 2501 | + | + | + | + | + | + | + | + |
| | PB | 4011 | + | + | + | + | + | + | + | − |

The assay was performed using SDA and PDA.

*Table 5.* Effects of high pH on growth of *Exophiala* spp. isolated from sludge in bathroom drainpipes and from bathwater.

| Isolate | | | Growth at pH | | | |
|---|---|---|---|---|---|---|
| | | | 6.2 | 8.4 | 9.2 | 10.4 |
| *E. alcalophila* | HB | 2706 | + | + | + | + |
| *E. dermatitidis* | PB | 0101 | + | + | + | − |
| | PB | 0102 | + | + | + | − |
| | D | 0601 | + | + | + | − |
| | D | 0901 | + | + | + | − |
| | HB | 1801 | + | + | + | − |
| | HB | 5403 | + | + | + | − |
| | HB | 5406 | + | + | + | − |
| | HB | 5501 | + | + | + | − |
| *E. moniliae* | PB | 3701 | + | + | + | − |
| | D | 4405 | + | + | + | − |
| | PB | 4801 | + | + | + | − |
| | PB | 5001 | + | + | + | − |
| *E. jeanselmei* | D | 0307 | + | + | + | + |
| | D | 0607 | + | + | + | + |
| | D | 0701 | + | + | + | + |
| | D | 0703 | + | + | + | + |
| | D | 0907 | + | + | + | + |
| | HB | 1104 | + | + | + | + |
| | D | 1205 | + | + | + | + |
| | D | 1601 | + | + | + | + |
| | D | 1706 | + | + | + | + |
| | HB | 1803 | + | + | + | + |
| | D | 2602 | + | + | + | − |
| | D | 3501 | + | + | + | + |
| | D | 3502 | + | + | + | + |
| | PB | 3711 | + | + | + | − |
| | PB | 4101 | + | + | + | − |
| | D | 5302 | + | + | + | + |
| *Exophiala* spp. | PB | 2501 | + | + | − | − |
| | PB | 4011 | + | + | − | − |
| *E. alcalophila* | IAM | 12519 | + | + | + | + |
| *E. dermatitidis* | IFM | 4828 | + | + | + | − |
| *E. moniliae* | IFM | 4881 | + | + | + | − |
| *E. jeanselmei* | IFM | 4852 | + | + | + | − |

IAM: Institute of Applied Microbiology, the University of Tokyo, Tokyo, Japan.
IFM: Institute for Food Microbiology (at present the Research Institute for Chemobiodynamics, Chiba University, Chiba, Japan).

Infection by *E. moniliae* had not been reported in Japan until 1984 when Matsumoto *et al.* re-investigated stock cultures isolated from patients in Japan and identified as *E. dermatitidis* (10). They pointed out that 2 of them are in reality *E. moniliae*. Our investigation is the first report from Japan of the isolation of *E. moniliae* from the human environment.

Usually the temperature of bathwater in winter in Japan is 40–42 °C. *E. dermatitidis* grows at 42 °C. Even though *E. moniliae* and *E. jeanselmei* do not grow at 42 °C, the former survives for 7 days at 42 °C and the latter, for between 20 hours and 5 days at 42 °C.

Bathwater containing 'Chinese medicine' in public bathhouses is lower in temperature and richer in nutrient for fungi than ordinary bathwater. Indeed, *Exophiala* species were isolated from such bathwater more than from ordinary bathwater. From the view of public health further investigation is needed.

The main fungi inhabiting human skin are species of *Candida, Penicillium, Aspergillus* and *Cladosporium*, but two cultures of *E. jeanselmei* were isolated from the skin surface by Kazumori *et al.* (8). Most fungi on the skin surface probably do not survive in bathwater, but species of *Exophiala* may survive in the bathwater, soap scum, water deposits of bathtubs or sludge in bathroom drainpipes.

Furthermore, sludge is alkaline due to lather (pH 8 to 9). *E. jeanselmei*, which is a main member of the fungi in the sludge, grows well at pH 9.2 and even at pH 10.4. Except for the 2 isolates not identified, all isolates of *Exophiala* species grow at pH 9.2. Due to these physiological characteristics, *Exophiala* species may be able to compete effectively against the main skin-inhabiting fungi.

The Japanese enjoy taking baths, and many people take baths every day or every other day. In the case of patients with chromomycosis and phaeohyphomycosis lesions sometimes occur around the back, gluteal region or thigh covered with garments. These diseases occur due to implantation of a causal agent through a slight trauma. It is possible that the patients may be infected with *Exophiala* species while taking baths.

In conclusion, bathwater and sludge of bathroom drainpipes are important habitats for members of the genus *Exophiala*. They may even be more significant as the sources of *Exophiala* infections than woody materials, soil or nests of animals in Japan.

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