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Growth rate of fungi in bathrooms —Experimental survey—

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Factors promoting fungal contamination of the cement jointing between bathroom tiles were studied in the laboratory. Under continuous wet conditions, the growth of the yeast *Rhodotolura* and *Candida* on cement was detected from the fourth day of the experiment. Following the rapid growth and decline of the yeast, growth of the mold *Paecilomyces* was detected on the 12th day. The application of soap or malt extract to the cement promoted the growth of *Paecilomyces*. Prolongation of dry conditions delayed the growth of both yeast and mold; under these conditions, *Cladosporium*, one of the most common molds in household bathrooms, was detected instead of *Paecilomyces*. Colonies of *Cladosporium* were observed along cracks in the cement. On all cement examined, a succession of mycological flora from yeast to mold was found, although fungal genera varied with culture conditions.

Key Words——bathroom; *Cladosporium*; fungal contamination; *Paecilomyces*; yeast.

How long after cleaning does it take for fungi to colonize the cement between bathroom tiles? What factors control fungal contamination in bathrooms? Exact answers to these questions would be useful, but are very difficult to establish and have, to our knowledge, been attempted by few studies.

In a previous paper (Hamada and Fujita, 1999a), fungal contamination in household bathrooms was surveyed. *Cladosporium* Link and *Exophiala* J. W. Carmich. are the predominant mold, while *Rhodotorula* F. C. Harrison is the predominant yeast. Moisture and nutrients, including soap, seem to promote contamination. To control fungal contamination, daily drying of bathroom was found to be essential (Hamada and Fujita, 1999b). However, the growth of fungi after cleaning in bathrooms and factors controlling that growth are still obscure.

In the laboratory we usually culture fungi on petridishes with agar. However, the growth of fungi varies greatly with the substrate. For example, it takes about two months for *Phoma* Sacc. to cover a 25 mm square piece of cement (Moriyama, 1999), but only about 2 wk to cover an agar of 9 cm diam. We examined the growth of fungi on cement rather than agar.

In a model experiment, we tested various treatments on cement between tiles, including fungal inoculation and application of nutrient. Changes over time in the fungal flora were compared under various culture conditions in order to elucidate the factors causing fungal contamination of bathrooms.

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Materials and Methods

Substrates and Treatments Four 10 cm square tiles were fixed to each board, and jointed with cement of about 1.0 cm width in a cross-shape. Cement not containing fungicide was prepared for the experiments, and was adjusted to about pH 7.0 by bubbling with CO_2 for more than 1 wk. After drying and sterilizing their surfaces with 80% ethanol, four boards with tiles and cement were used in each experiment.

Each board was submerged in a separate bowl of distilled water for 30 min a day to wet the cement. Fresh distilled water was used every day. After removal from the water, the cement samples were treated variously with sterilized water (Experiment 1), a solution of 0.01% soap (Experiment 2), or 0.1% malt extract (Experiment 3), applied every day with tip of cotton (Promedia ST-15; ELMEX). Every other day, about 1,000 spores of *Cladosporium* or *Exophiala* were then inoculated onto the cement, using another tip of cotton. After treatment, the boards were placed in a plastic box controlled to 25°C and 90–100% relative humidity by water stored in the box, and left there for 23.5 h a day. In Experiment 4, the boards were treated as in Experiment 1, but the lid of the box was removed after 4 h so that humidity was reduced to 60–75% for the remaining 19.5 h.

Sampling of fungi was performed before treatment every four days for 32 d (Experiments 1–3) or every eight days for 64 d (Experiment 4).

Sampling and culturing methods To examine fungal flora, a swabbing kit (Pro-media ST-15, ELMEX) was used, as described in the previous paper (Hamada and

Fujita, 1999a). The two intersecting strips of cement (20 cm × 1 cm) between the four tiles were swabbed once with a cotton tip under constant pressure and at low speed. After sampling, the tip was replaced in the kit bottle with 10 ml of physiological salt solution. The kits containing the samples were stored in a refrigerator, and examined within a few days.

After shaking, 0.5 ml of the 10 ml of suspension in the kit was used for cultivation. If necessary, the suspension was diluted 200 or 2,000 times for counting the fungal colonies. A 0.5-ml aliquot of the diluted suspension was poured and spread on each of two petri-dishes with 25 ml of PDA (Potato Dextrose Agar, Difco) medium containing 50 mg of chloramphenicol/L.

After inoculation, the media were incubated at 25°C for 6–8 d and the fungal colonies counted and identified as described previously (Hamada and Fujita, 1999a). Total CFU detected on the cement was examined in Experiments 1–4.

Before converting counts of fungi into a logarithmic scale, each count was increased by 10, as this was the detection limit. Logarithmic averages of the counts in the samples examined were calculated. To give a readily understandable number, the logarithmic number is converted back into a natural number in the figures and table.

Results

Experiment 1

Although spores of *Cladosporium* and *Exophiala* were inoculated, detection of these genera was limited to a few samples and totaled less than 40 colonies. No yeast was inoculated, but yeast was detected on all four boards examined on the 4th day of culture (Fig. 1). The yeast population increased dramatically to reach about 9.7×10^5 (CFU) on the 8th day, and 1.5×10^6 on the 12th day. This number fell to less than 20,000 on the 16th day and decreased gradually thereafter. Most of the yeast was *Rhodotorula* or *Candida* Berkhout, and the ratio of the two populations remained similar throughout the experiment (Fig. 1; Table 1).

Paecilomyces Bainier, not inoculated, was detected on three of the four boards examined on the 12th day, and its population was about 100. This increased gradually to about 7,000 on the 32nd day. No increase in

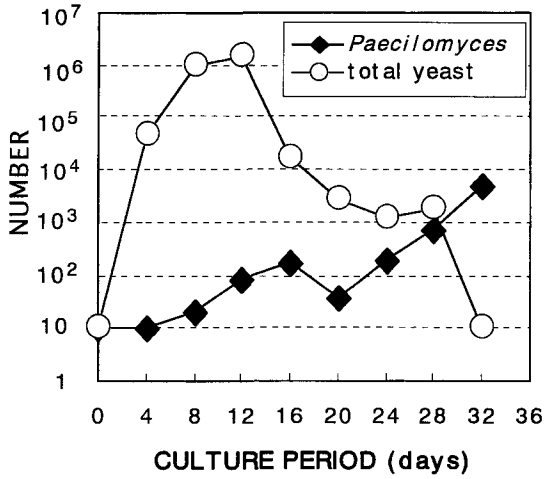

Fig. 1. Growth of mold and yeast on cement jointing tiles. Numbers of *Paecilomyces* and total yeast collected at each sampling are indicated. Each board with cement and tiles was left in the wet box all day.

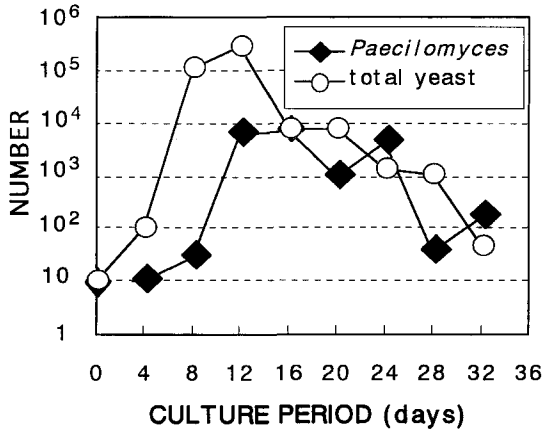

Fig. 2. Effect of soap on growth of mold and yeast on cement jointing tiles. Numbers of *Paecilomyces* and total yeast collected at each sampling are indicated. A 0.01% soap solution was applied every day. Each board with cement and tiles was left in the wet box all day.

Table 1. Yeast flora under various culture conditions (CFU).

Applied solution	Period (d)	*Rhodotolura*	*Candida*	Others	Total
0% nutrient	12	552000	1480000	10	1490000
(Experiment 1)	24	168	230	32	1230
0.01% soap	12	141000	88400	36200	281000
(Experiment 2)	24	50	1070	25	1340
0.1% malt ext.	12	948	15200	10	17700
(Experiment 3)	24	186	3860	10	4040
0%, wet-dry	24	10	179	17	194
(Experiment 4)	48	1630	13400	10	21300

Yeast was counted on the 12th and 24th day, or on the 24th and 48th day.
Logarithmic averages calculated were converted into natural numbers.

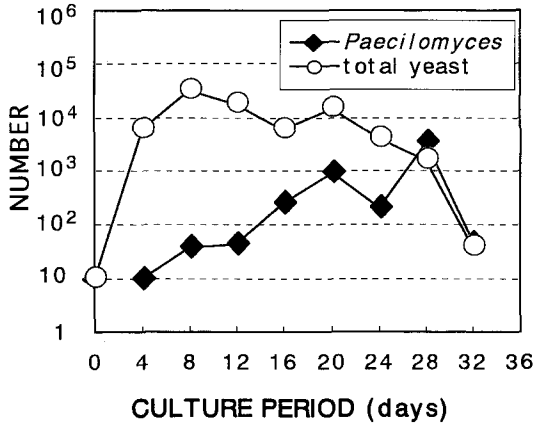

Fig. 3. Effect of malt extract on growth of mold and yeast on cement jointing tiles. Numbers of *Paecilomyces* and total yeast collected at each sampling are indicated. A 0.1% malt extract solution was applied every day. Each board with cement and tiles was left in the wet box all day.

other molds was detected during the experiment.

Experiment 2

The fungal flora on the cement treated with 0.01% soap solution is shown in Fig. 2. The yeast population rose to about 2.8×10^5 on the 12th day and decreased thereafter. This peak coincided with that on the cement treated with sterilized water, but its maximal level was about one-fifth of that with the sterilized water (Fig. 1). The yeast population consisted of *Rhodotolura* and *Candida* in roughly equal numbers (Table 1). *Cryptococcus* Kütz. was also found.

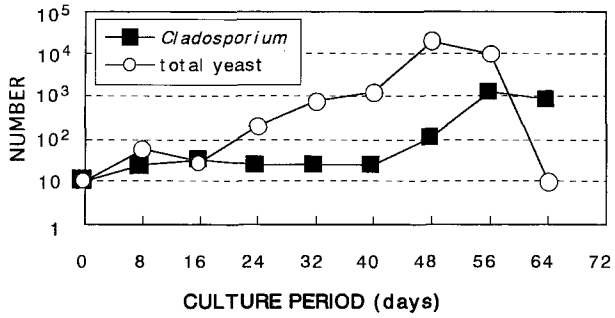

Fig. 4. Effect of dry conditions on growth of mold and yeast on cement jointing tiles. Numbers of *Cladosporium* and total yeast collected at each sampling are indicated. Each board with cement and tiles was left in the box with lid for 4 h a day, and without lid for 19.5 h.

The population of *Paecilomyces* was about 6,000 on the 12th day and maintained a level of more than 900 for the following 12 d, then decreased steeply on the 28th day (Fig. 2). *Paecilomyces* grew more quickly on cement with soap solution than with sterilized water. Other than *Paecilomyces*, counts of less than 100 of *Trichoderma* Pers. and *Acremonium* Link were detected after the 24th day.

Experiment 3

Figure 3 shows the effect of malt extract. The dominant fungal flora changed from yeast to mold with the progress of the culture period, as in Experiments 1 and 2. However, the yeast population peaked at about 30,000 on the 8th day and decreased gradually thereafter. The peak level of the yeast population was one-

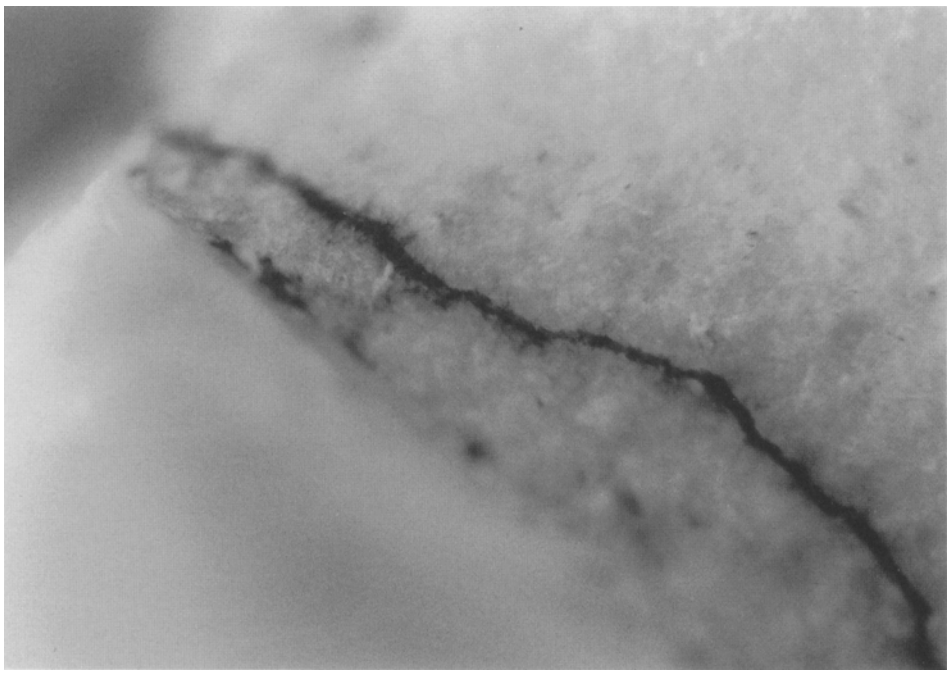

Fig. 5. Colonies of *Cladosporium* growing along a crack in cement on the 64th day after the start of experiment. Width of cement is approximately 1 cm.

fiftieth of that in Experiment 1. Unlike in Experiments 1 and 2, the predominant yeast was *Candida*, with *Rhodotolura* as a minor component (Table 1).

Paecilomyces increased gradually until the 28th day, and its peak was about 5,000. The maximal number of *Paecilomyces* was similar to those Experiments 1 and 2. *Trichoderma* was also detected on the 24th day and increased gradually to about 200 on the 32nd day. Although results are not shown, the addition of 0.01% malt extract did not alter the growth of fungi, what is shown in Fig. 1.

Experiment 4

Figure 4 shows the effect of dry conditions on the fungal flora. The number of yeast detected was less than 1,000 until the 32nd day, increased gradually to about 20,000 on the 48th day, and decreased steeply from the 64th day. The number of *Candida* was about 10 times greater than that of *Rhodotolura* (Table 1). Unlike the cement cultured under continuous wet conditions, the growth of the mold *Cladosporium* was detected. No *Paecilomyces* or *Trichoderma* was detected, although *Fusarium* Link was detected with a count of less than 100 after the 40th day. *Cladosporium* was detected with a count of more than 100 on the 40th day, increasing to about 1,000 on the 56th day. Compared with the growth of yeast and mold under continuously wet conditions, the delay in attainment of the peak population was the most noteworthy feature (Fig. 4).

After detecting the contamination of *Cladosporium* (Fig. 4), we tried to observe colonies of *Cladosporium* on the cement using binoculars, and found dark colonies of mold along fine cracks in the cement (Fig. 5). These were confirmed to be *Cladosporium* under the microscope.

Throughout the experiments, no yeast colonies were observed by binoculars. In Experiments 1–3, no colonies of *Paecilomyces* were observed on the surface of cement, including in the cracks.

Discussion

Yeast was frequently detected in the experiments, although no pink colonies such as *Rhodotorula* were observed visually, as in the survey of household bathrooms (Hamada and Fujita, 1999a). Yeast is thus an important component of fungal contamination in bathrooms. Yeast grows first, and is followed by mold. The growth of yeast seems to induce contamination by mold. This "succession", common in the plant kingdom (Ricklefs, 1973), was newly found on the cement jointing between bathroom tiles.

The ecological succession of fungal flora on animal dung is also well known (Deacon, 1980; Kendrick, 1992). Zygomycetes appear first, then ascomycetes and imperfect fungi, and finally basidiomycetes. What factors control the succession on dung is still not clear. One factor is change in nutrient resources from readily accessible sugars to more complex carbon sources such as cellulose and lignin. On walls, yeast is a pioneer, growing in the poorest nutrient conditions. Mold, for example, *Paecilomyces* and *Cladosporium*, can grow on the cement using organic products accumulated by yeast (Figs. 1, 4). *Trichoderma* and *Fusarium* may follow *Paecilomyces*, although these molds are thought to have infected through the air.

Paecilomyces grew more quickly on cement treated with soap or malt extract than on cement deficient in nutrients (Figs. 1–3). *Paecilomyces* thus exploits soap or malt extract as a nutrient resource. Soap was thus confirmed to be a nutrient for fungi, as suggested in the household survey (Hamada and Fujita, 1999a).

In contrast, the addition of soap or malt extract remarkably inhibited the growth of yeast; in particular, the addition of malt extract reduced the peak population to one-fiftieth (Fig. 3). Reduction in the duration of wet condition would result in a slight increase in the osmotic pressure of moisture on the cement, which may suppress the growth of yeast, especially *Rhodotolura* (Fig. 4).

Paecilomyces rather than *Cladosporium* was the predominant mold under wet conditions. *Paecilomyces* is often found in water storage systems and can adapt to extremely wet conditions (Mori, 1981). *Cladosporium* is predominant under comparatively dry conditions; household bathrooms are not normally continuously wet.

It took about 2 mo for *Cladosporium* to colonize on cement (Fig. 4). Thus, the growth of mold on cement seems to be markedly slower than that on petri-dishes with agar. *Cladosporium* grows slower under comparatively dry conditions than *Paecilomyces* under wet conditions, although both molds grow at similar rates on petri-dishes. We emphasized previously that humidity affects fungal contamination in bathrooms (Hamada and Fujita, 1999b). Moisture conditions seem to determine not only the fungal population, but also the speed of fungal colonization (Fig. 4).

Appearance of cracks seemed to depend on repeated shrinkage and expansion of the board caused by alternate submersion and drying. This phenomenon may also occur in households. Cracks, appearing locally in the course of long-term use, provide damp patches, which induce fungal contamination. Moreover, the contaminating fungi may extend the crack deeper into the cement as well as into wood (Allsopp and Seal, 1986). Cracks may thus induce fungal contamination, even if the bathroom is kept comparatively dry. Treatment to preserve the cement surface is therefore important in avoiding fungal contamination.

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