# Airborne microorganisms in the indoor environment of Syowa Station in Antarctica

Hiroyuki Nakashima<sup>1</sup>, Nobuo Hamada<sup>2</sup> and Takashi Yamanouchi<sup>3</sup>

 <sup>1</sup>Kurume National College of Technology, 1–1–1, Komorino, Kurume 830-8555
 <sup>2</sup>Osaka City Institute of Public Health and Environmental Sciences, 8–34, Tojo-cho, Tennoji, Osaka 543-0026
 <sup>3</sup>National Institute of Polar Research, Kaga 1-chome, Itabashi-ku, Tokyo 173-8515

(Received April 30, 2003; Accepted August 25, 2003)

*Abstract:* Airborne bacterial and fungal numbers in the buildings of Syowa Station in Antarctica were examined for 9 months in 2001. The number of bacteria or fungi was less than 20 or  $70/m^3$  in the dining room and washroom. The average number of bacteria or fungi was less than 1/50 or 1/5 of those in Japan and Europe, respectively, and remained constant regardless of season. The number of airborne microorganisms appeared to depend on drying of the indoor environment by the use of air-conditioners.

key words: Antarctica, airborne microorganisms, indoor environment, fungi, bacteria

# 1. Introduction

Airborne microorganisms, including bacteria and fungi, are generally responsible for infectious or allergic disorders, and microbiological contamination on foods and in houses (Pastuszka *et al.*, 2000). Thus, contamination is important from the viewpoint of hygiene. In addition, the number of airborne microorganisms is an indicator of the indoor environment. For example, a large number of airborne fungi were detected in rooms where the carpets used were contaminated heavily by fungi. The source of microbiological contamination was suggested to be the interior, floor or wall (Hamada and Yamada, 1995).

In general, higher temperature appears to increase the microbiological number. In Japan and Europe, the number of airborne fungi generally increases in summer, and decreases in winter (Matsuda, 1969; Giorgio *et al.*, 1996). However, the annual variation in airborne microorganisms is smaller indoors than outdoors, because the indoor environment, for example temperature, is comparatively stable compared with the outdoor environment (Ebner *et al.*, 1992). Humidity is also an important factor to control the microbiological number. Rooms located on higher floors are generally drier, so the number of xerophilic fungi colonizing the upstairs was significantly more than that downstairs (Hamada and Yamada, 1995).

The climate in Antarctica is characterized by both extremely low temperature and humidity (Longton, 1988). The airborne microbiological numbers under indoor environments in Antarctica have not been studied.

In this study, the airborne fungal and bacterial numbers at Syowa Station in Antarctica were examined using a slit sampler. The dining room and washroom, used by many occupants and where a large microbiological number was generally recognized (Sessa *et al.*, 2002), were examined. The airborne microbiological number in Syowa Station is compared with those in Japan and Europe, having different climatic characteristic, and the factors affecting the microbiological numbers are discussed.

# 2. Materials and methods

# 2.1. Environmental profile of sampling sites

The dining room with 5 tables,  $75.6 \text{ m}^2$  located on the third floor in the main building, and washroom with 3 basins,  $9.25 \text{ m}^2$  is on the second floor in the adjoining building in Syowa Station ( $69^{\circ}00'\text{S}$ ,  $39^{\circ}35'\text{E}$ ), respectively, and their indoor environments are constantly controlled by air-conditioners. This building is enclosed, and separated from the outdoor conditions of Antarctica by a stainless steel door. The occurrence of condensation on the inside wall was not found due to the insulated structure of the building. The material of the interior floor was wood or linoleum.

Indoor temperature ranged from 21 to  $25^{\circ}$ C in the dining room and from 21 to  $31^{\circ}$ C in the washroom, while the outdoor temperature ranged from -32.4 to  $0.7^{\circ}$ C between April and December of 2001. Indoor humidity ranged from 11 to 28% in the dining room, and ranged from 9 to 26% in the washroom. More than 20 occupants per hour during 12:00–13:00, and at other times less than 10 occupants, used the dining room. More than 20 occupants per hour during 08:00–09:00, and at other periods less than 10 occupants, used the washroom.

### 2.2. Air sampling

Air was sampled once at the beginning of each month between April and December of 2001. Sampling was performed between 08:30 and 16:00, when some occupants used these rooms.

Airborne microorganisms were collected for 30 min at each sampling by using a slit sampler (model 220J M/G air sampler; Sumilon Co., Tokyo, Japan) with a clock motor



Fig. 1. Schema of slit sampler (M/G air sampler).

rotating the Petri dish once per  $30 \min$  (Fig. 1). This model absorbs  $1.698 \text{ m}^3$  of air per hour through a 0.152 mm-wide slit positioned 2 mm above the agar surface, and blows it onto 60 ml agar in the Petri dish with a diameter of 150 mm where all microorganisms are trapped under reduced pressure. The mouth of the sampler was located at about 30 cm height above the floor in the dining room and the washroom of Syowa Station.

Samples were collected once each for counting bacteria, general fungi and xerophilic fungi. Bacteria and general fungi were sampled for 9 months (April to December), xerophilic fungi for only 7 months (June to December) due to insufficient preparation.

### 2.3. Detection of bacteria and fungi

To detect microorganisms, three kinds of media were used. To detect general bacteria, a medium consisting of triptone (10 g, Difco), beef extract (10 g, Nissui Co.), sodium chloride (1.5 g) and agar (15 g), adjusted to pH 7.0 with 1 N sodium hydroxide, per liter of distilled water, was used. For detecting general fungi, PDA (potato dextrose agar) medium (Nihonseiyaku Co.), added with 50 $\mu$ g of chloramphenicol per liter, was used. For detecting xerophilic fungi, PDA medium with 20% sucrose was used. All media were autoclaved at 121°C for 15 min before the experiment.

The Petri dishes used for air-sampling were incubated at  $37^{\circ}$ C for 1 or 2 days to detect bacteria, and at  $25^{\circ}$ C for 3 or 4 days to detect fungi. For identification of fungal genus, fungal colonies without spores on the PDA medium were isolated and cultured on the PDA medium for more than 10 days.

Bacterial and fungal numbers were expressed as bacterial and fungal counts per unit volume of air (CFU/ $m^3$ ) in room interiors.

# 3. Results

The largest number of bacteria in the dining room was  $14.1/m^3$  in September, the smallest was  $3.5/m^3$  in October and November (Fig. 2). The largest number of bacteria in the washroom was  $14.1/m^3$  in April and May, and the smallest was  $4.7/m^3$  in



Fig. 2. Monthly variation of airborne bacterial number in the dining room and in the washroom.



Fig. 3. Monthly variation of airborne fungal number in the dining room and in the washroom.

Table 1. Average number of airborne microorganisms  $(/m^3)$ .

	Dining room	Washroom	
	avg±SE	avg±SE	
Bacteria	$7.3 \pm 1.2$	10.6±1.0*	
General fungi	$16.7 \pm 7.7$	$7.1 \pm 2.1$	
Xerophilic fungi	$7.2 \pm 2.7$	$20.3 \pm 6.2*$	

An asterisk indicates significantly larger, when difference between averages is larger than SE (standard error).

December. The average numbers of airborne bacteria in the dining room and washroom were  $7.3/m^3$  and  $10.6/m^3$ , respectively (Table 1). The average number of airborne bacteria in the washroom was significantly larger than that in the dining room.

The largest number of general airborne fungi in the dining room was  $62.4/m^3$  in August, and the smallest was  $0.6/m^3$  in July and September (Fig. 3). The largest number of fungi in the washroom was  $21.2/m^3$  in July and the smallest was zero in May. The average numbers of general fungi in the dining room and washroom, detected using PDA, were  $16.7/m^3$  and  $7.1/m^3$ , respectively (Table 1). Although comparatively large numbers were found in the dining room in some months (August and October), no significant difference was found between the dining room and washroom (Table 1).

The average number of airborne xerophilic fungi, detected with PDA media with 20% sucrose added, were 7.2/m<sup>3</sup> and 20.3/m<sup>3</sup> in the dining room and washroom, respectively. The average number of these airborne fungi in the washroom was significantly larger than that in the dining room (Table 1). No correlation was found between the numbers of fungi detected with PDA medium and those detected with PDA medium with 20% sucrose added during the 7 months examined (Table 2). Fungal numbers detected by different media in the same room appeared to change independently. For example, in the washroom the peak of fungal number by PDA medium appeared in July, but that by PDA medium with 20% sucrose apeared in December.

No correlation was found in fungal numbers between the two rooms. The fungal number in the dining room had two peaks, in August and November, but that in the washroom had only one peak, in July (Fig. 3). When the monthly variations of the numbers of bacteria and fungi were compared, the variation of bacterial number was less than that of fungi (Figs. 2, 3).

As a preliminary survey, the relation of the frequency of use of the rooms and airborne fungal contamination was examined. However, no numerical difference in either airborne bacteria or fungi was found between the periods when occupants entered the room frequently and rarely.

Measuring airborne microorganisms outside Syowa Station was tried, but the freezing weather prevented the measurement.

*Penicillium* was the predominant fungus both in the dining room and washroom, followed by *Paecilomyces* and *Cladosporium*. About 50% of all colonies detected produced no spores.

When we compared the number of *Penicillium* among various seasons, they changed independently in the two rooms (Table 3). The maximum value of *Penicillium* was  $25.3/\text{m}^3$  in the dining room in November, and was  $13.5/\text{m}^3$  in the washroom in July.

Month	Dining room		Washroom	
	General fungi	Xerophilic fungi	General fungi	Xerophilic fungi
June	8.8	4.7	8.8	7.1
July	0.6	7.7	21.2	6.5
August	62.4	21.2	10.0	34.7
September	0.6	10.0	3.5	7.1
October	46.5	0.0	2.9	8.8
November	25.9	1.8	2.9	34.7
December	1.8	4.7	9.4	43.0

Table 2. Comparison of fungal number detected (/m<sup>3</sup>) using different media.

All samplings were performed in 2001.

Month	Dining room	Washroom
April	0.6	0.6
May	0.6	0.0
June	8.8	1.8
July	0.6	13.5
August	9.4	0.0
September	0.0	1.8
October	2.9	0.6
November	25.3	2.9
December	1.8	7.1

 

 Table 3. Seasonal variation in the number of airborne Penicillium (/m³).

All samplings were performed in 2001.

Aspergillus, including many xerophilic fungi, was not found on the PDA medium with 20% sucrose or the PDA medium without sucrose.

# 4. Discussion

Some studies on the number of indoor airborne microorganisms in Japan and Europe have been published previously, although air samplers used varied among those studies. In Japan, the average numbers of bacteria and fungi were  $682/m^3$  and  $480/m^3$  in summer at the dialysis facility of a hospital (Miki *et al.*, 2003). The annual mean number of airborne fungi was about  $100/m^3$  in the changing-room of a public bath, and about  $150/m^3$  at the food counter of a department store (Matsuda, 1969). In an office in France, the annual mean number of bacterial and fungal aerosols was  $447/m^3$  and  $113/m^3$ , respectively (Parat *et al.*, 1997).

The most outstanding result of the present study was that numbers of both airborne bacteria and fungi were very small in Syowa Station compared with those in Japan and Europe. For example, the average number of bacteria in the dining room and washroom,  $7.3/m^3$  and  $10.6/m^3$ , was less than 1/50 those in a hospital in Japan. Similarly, the fungal averages,  $16.7/m^3$  and  $7.1/m^3$ , were less than 1/5 that of the food counter or changing-room in Japan (Table 1).

We will discuss two environmental factors, temperature and humidity, controlling the small number of microorganisms.

In Japan, outdoor airborne fungal contamination appears to induce indoor fungal contamination, and the indoor airborne fungal number is greater in summer than in winter (Matsuda, 1969). In an alpine area of Austria, the annual average number of indoor airborne fungi has, however, been bound to be similar regardless of altitude, although the fungal average outdoors at an altitude of 1905 m was about 1/5 that outdoors at 582 m (Ebner *et al.*, 1992). Outdoor temperature may not affect the indoor airborne fungal number in an alpine area.

Seasonal change of airborne microbiological number was not detected in the indoor environment of Syowa Station. The temperature is too cold for fungi to colonize outside Syowa Station even in summer, 0.7°C in December. Outdoor temperature appears to not be an environmental factor that induces the small number of microorganisms inside the building at Syowa Station.

On the other hand, the airborne microbiological number in an air-conditioned building was less than 1/5 that in a naturally ventilated building (Parat *et al.*, 1997). The humidity of the two rooms examined, controlled at 9-28%, is very low, compared with dwellings in Japan, where the humidity is often close to 100% and where much condensation occurs. This control of humidity appears to reduce the fungal contamination of the interior walls and floors of the buildings in Syowa Station.

More airborne fungi are found in dwellings having tatami or carpets than in rooms and offices without carpets (Hamada and Yamada, 1995). Therefore, wood or linoleum flooring without carpets in Syowa Station is thought to be another reason for the small number of airborne microorganisms.

Although bacteria are adapted to wetter conditions than fungi (Tsuruta and Udagawa, 1975), the smaller ratio of bacterial number in Syowa Station to that in Japan

67

than the ratio of fungal number supports the idea that the dry conditions in this building have reduced the microbiological contamination.

The fungal genus detected in this study was very unique. *Aspergillus*, one of the typical xelophilic fungi, was not found although other xerophilic fungi genuses were detected in these rooms (Table 1). There is a need to investigate airborne microorganisms in outdoor conditions in Antarctica. It has been accepted that *Penicillium*, adapted to cold environments (Udagawa, 1983), is predominant in indoor environments. Moreover, it is unclear why many of the fungal colonies did not produce spores. More studies are needed to elucidate these questions.

#### References

- Ebner, M.R., Haselwandter, K. and Frank, A. (1992): Indoor and outdoor incidence of airborne fungal allergens at low- and high-altitude alpine environments. Mycol. Res., 96, 117–124.
- Giorgio, C.D., Krempff, A., Guiraud, H., Binder, P., Tiret, C. and Dumenil, G. (1996): Atmospheric pollution by airborne microorganisms in the city of Marseilles. Atmos. Environ., 30, 155–160.
- Hamada, N. and Yamada, A. (1995): Airborne fungal contamination in dwellings. J. Antibact. Antifung. Agents, 23, 281–286.
- Longton, R.E. (1988): Biology of Polar Bryophytes and Lichens. Cambridge University Press, 391 p.
- Matsuda, Y. (1969): Studies on airborne fungal flora, Part II. Study on airborne fungal flora in Kobe-City. Bull. Kansai Med. Coll., **21**, 526–557.
- Miki, A., Kurosu, S., Taniai, E., Hayashi, F., Namiki, S., Namiki, T. and Shintani, H. (2003): Determination of microorganisms both airborne and fallen from the air at an artificial dialysis health care facility. J. Antibact. Antifung. Agents, **31**, 77–84.
- Parat, S., Perdrix, A., Fricker-H., H., Saude, I., Grillot, R. and Baconnier, P. (1997): Multivariate analysis comparing microbiological air content of an air-conditioned building and a naturally ventilated building over one year. Atmos. Environ., 31, 441–449.
- Pastuszka, J.S., Paw, U.K.T., Lis, D.O., Wlazlo, A. and Ulfig, K. (2000): Bacterial and fungal aerosol in indoor environment in Upper Silesia, Poland. Atmos. Environ., 34, 3833–3842.
- Sessa, R., Shiavoni, G., Santino, I., Altieri, A. and Pinelli, S. (2002): Microbiological indoor air quality in healthy buildings. New Microbiol., 25, 51–56.
- Tsuruta, O. and Udagawa, S. (1975): Kabi to Shokuhin (Food and Moulds). Tokyo, Ishiyaku Publisher, 382 p.
- Udagawa, S. (1983): Teionsei kabi-koubo (Psychrophilic fungi). Saishin Shokuhin Biseibutsu Seigyo-Sisutemu De-tashû (New Microbiological Control Data File in Food), ed. by S. Haruta *et al.* Tokyo, Science Forum, 114–122.