Note

Effect of Temperature and Water Activity on the Growth of *Cladosporium sphaerospermum* and *Cladosporium cladosporioides*

MAKI AIHARA¹*, TATSUAKI TANAKA², TOSHIKO OHTA³
AND KOSUKE TAKATORI¹

¹National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501,

²Ochanomizu University, 2-1-1, Otsuka, Bunkyo-ku, Tokyo 112-8610 and

³Sagami Women's University, 2-1-1, Bunkyo, Sagami-hara-city, Kanagawa 228-8533, Japan

Received 4 October 2000/Accepted 25 February 2002

The effects of temperature and water activity (Aw) were studied on the growth activity of *Cladosporium sphaerospermum* and *C. cladosporioides*. High growth was noted in *C. sphaerospermum* at high temperatures and in *C. cladosporioides* at low temperatures, but the growth activity of both *C. sphaerospermum* and *C. cladosporioides* was similar at 23–30°C. With regard to Aw, growth of *C. cladosporioides* was greater than that of *C. sphaerospermum* at 10–23°C. At 32–36°C, higher activities were shown in *C. sphaerospermum*. *C. sphaerospermum* and *C. cladosporioides* showed morphological changes such as dark brown hyphae with thick and cigar shaped cells, and irregularly swollen cells at low Aw. *C. sphaerospermum* showed a broader range of growth temperature and Aw than *C. cladosporioides*.

Key words : *Cladosporium sphaerospermum*/*Cladosporium cladosporioides*/ Temperature/Water activity/Growth rate.

There are many kinds of fungi in dwelling environments. One of the predominant species is *Cladosporium*, and it can be isolated from the air, wall surfaces and home materials (Aihara et al., 2000a, 2000b, and 2001, Grant et al., 1989; Hunter et al., 1988). Though we have conducted an ecological investigation of *Cladosporium* in dwelling environments, there is little detailed information on the biological characteristics of *Cladosporium*, especially *C. sphaerospermum* and *C. cladosporioides* (Kawai et al., 1990). Since *Cladosporium* showed predominance with regard to distribution and population in dwelling environments, biodeterioration caused by *Cladosporium* also occurs in such environments (Moriyama et al., 1992). Therefore, *Cladosporium* caused biodeterioration is a very important concern. In

this paper, temperature and water activity (Aw) as physical factors, were briefly investigated in terms of their effects on *C. sphaerospermum* and *C. cladosporioides*.

The fungi used were five species of *C. sphaerospermum* MAF-23, 38, 61, 63, and 492 and five of *C. cladosporioides* MAF-24, 39, 58, 563, and 636, isolated from dwelling environments.

Potato dextrose agar (PDA, Nissui Co.Ltd.), M40Y, M70Y, M100Y, MY10G, MY20G, MY30G, MY40G, MY55G and MY80G (Uchiyama et al, 1994) were used as media. Each type of media was supplemented with 50 µg/ml chloramphenicol and poured into 90mm-diameter sterile Petri dishes. The components and water activity (Aw) of these types of media are shown in Table 1. The Aw of each medium was measured with Hygroskop (Rotronic Co. Ltd.).

Ten- to 14-day-old colonies on PDA were used for all experiments. Spores of *Cladosporium* were

*Corresponding author. Tel: +81-3-3700-9048, Fax: +81-3-3700-9048.

TABLE 1. The components of medium used and Aw.

Component	Medium									
	PDA (0.97) ^a	M40Y (0.95)	M70Y (0.93)	M100Y (0.91)	MY10G (0.90)	MY20G (0.87)	MY30G (0.85)	MY40G (0.83)	MY55G (0.80)	MY80G (0.75)
Potato dextrose agar	3.9 ^b									
Malt extract		2	2	2	2	2	2	2	2	2
Yeast extract		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sucrose		40	70	100	100	100	100	100	100	100
Glycerol					10	20	30	40	55	80
Agar		2	2	2	2.8	2.8	2.8	2.8	2.8	2.8
Distilled water	100 ^c	100	100	100	100	100	100	100	100	100

^aAw.^bg.^cml.

inoculated on the center of the agar plates. The incubation temperatures were 5, 10, 15, 23, 25, 28, 30, 32, 34 and 36°C. Agar plates were then sealed with adhesive cellulose tape. After 28 days of incubation, the colony diameter was measured with a vernier caliper. Morphological changes of the growing *Cladosporium* hyphae under low Aw condition were also investigated in detail.

The minimum Aw needed for the growth of *C. sphaerospermum* and *C. cladosporioides* was investi-

gated in the germination and spore production at 5-36°C after incubation (Table 2). Regarding germination, the minimum Aw for *C. sphaerospermum* and *C. cladosporioides* ranged from 0.80-0.91 and 0.85-0.91, respectively. Regarding spore production, the minimum Aw for *C. sphaerospermum* was 0.80-0.91 and that for *C. cladosporioides* was 0.87-0.95. From these results, *C. sphaerospermum* was shown with a slightly higher activity at low Aw.

To describe the growth activities of *C. sphaero-*

TABLE 2. Minimum Aw required for the germination and spore production at each temperature.

Item	Strain	Temperature (°C)										
		5	10	15	20	23	25	28	30	32	34	36
Germination	<i>C. sphaerospermum</i>	0.90 ^a	0.85	0.83	0.83	0.80	0.80	0.83	0.83	0.85	0.83	0.91
	<i>C. cladosporioides</i>	0.90	0.85	0.85	0.87	0.85	0.85	0.90	0.87	0.91	0.90	—
Spore production	<i>C. sphaerospermum</i>	0.93	0.91	0.85	0.85	0.80	0.80	0.83	0.83	0.85	0.85	—
	<i>C. cladosporioides</i>	0.90	0.90	0.87	0.90	0.90	0.90	0.91	0.91	0.93	0.95	—

^aMean value of Aw of 5 strains. **FIG. 1.** Isoleth of temperature and water activity affecting the growth rate of *C. sphaerospermum* (A) and *C. cladosporioides* (B).

spermum and *C. cladosporioides* as affected by temperature and Aw, the growth rate (GR) was defined as follows: semidiameter (mm) / incubation period (day) (Smith et al., 1982). The isopleth on temperature and Aw in *C. sphaerospermum* and *C. cladosporioides* is shown in Fig.1. The GR of *C. sphaerospermum* and *C. cladosporioides* was less than 0.25 at Aw0.83 and 0.88, respectively, at all temperatures. At Aw0.95 and 28°C, the highest value of GR at 2.72 was shown. The GR of *C. cladosporioides* was less than 0.2 at Aw0.87, but showed a high value when Aw was 0.93-0.97.

Though there was no difference at Aw0.93-0.97 and 23-30°C in the growth of *C. sphaerospermum* and *C.*

cladosporioides, the former could grow at a lower Aw; GR was less than 0.25 at Aw0.83. The GR of the latter was less than 0.75 at 15°C. At a low temperature, *C. cladosporioides* had a high GR, which was 1.50 at 15°C.

From these results, it was thought that *C. sphaerospermum* could grow more easily than *C. cladosporioides* at high temperatures.

In the colony findings for various temperature and Aw conditions, *C. sphaerospermum* differed remarkably from *C. cladosporioides*. Also it was confirmed that *C. sphaerospermum* was more active than *C. cladosporioides* at a wide range of Aw. For example, Fig. 2 shows the colony growth patterns for *C.*

FIG. 2. Colony growth of *C. sphaerospermum* (A) and *C. cladosporioides* (B) at various Aw at 25°C.

FIG. 3. Morphological changes of *C. sphaerospermum* (A) and *C. cladosporioides* (B) at 25°C and Aw of 0.85 after 28 days. Scale bar indicates 10 μ m.

sphaerospermum and *C. cladosporioides* at 25°C.

Regarding morphological changes in *C. sphaerospermum* and *C. cladosporioides* under low Aw conditions irregular forms were seen. Under low Aw conditions, *C. sphaerospermum* and *C. cladosporioides* showed irregular hyphae with irregular, dark brown with thick and cigar shaped cells, and irregularly swollen cells at Aw0.85 for 28 days at 25°C (Fig. 3). In general, *Cladosporium* hyphae grows with a constant width and septa in agar media. However, on natural materials, it grows with irregular morphology. Therefore, these hyphae in Fig. 3 are influenced by the abnormal environments such as low Aw. It shows that *Cladosporium* hyphae sometimes take abnormal forms like other fungi.

Some reports describe that *Cladosporium* is a fungus the minimum Aw to be 0.84-0.86 for growth (Grant et al., 1989; Magan et al., 1984; Pelhate, 1967). The optimal growth temperature was shown to be in the range of 20-30°C and *Cladosporium* is apparently sensitive to heat (Kawai et al., 1990). The predominant contaminant in house environments is *Cladosporium*, especially *C. sphaerospermum* and *C. cladosporioides* (Aihara et al., 2001). As factors related to fungal adherence to and penetration into housing materials, it was speculated that Aw and temperature are connected with fungal contamination. This paper focused on the main contamination-causing species in *Cladosporium* such as *C. sphaerospermum* and *C. cladosporioides*. It was confirmed that *C. sphaerospermum* has a higher and also wider growth activity than *C. cladosporioides* with regard to the effects of the temperature and Aw.

REFERENCES

- Aihara, M., Tanaka, T., and Takatori, K. (2001) *Cladosporium* as the main fungal contaminant of locations in dwelling environments. *Biocontrol Sci.*, **6**, 49-52.
- Aihara, M., Tanaka, T., and Takatori, K. (2000a) Monthly changes of fungi detected from the indoor environments of a house structure (in Japanese). *Bokin Bobai*, **28**, 3-8.
- Aihara, M., Tanaka, T., and Takatori, K. (2000b) Fungal CFU and distribution on the upper, middle and lower vertical points of house walls (in Japanese). *Bokin Bobai*, **28**, 421-426.
- Grant, C., Hunter, C. A., Flannigan, B., and Bravery, A. F. (1989) The moisture requirements of moulds isolated from domestic dwellings. *Int. Biodeterior.*, **25**, 259-284.
- Hunter C. A., Grant, C., Flannigan, B., and Bravery, A. F. (1988) Mould in buildings: the air spora of domestic dwellings. *Int. Biodeterior.*, **24**, 81-101.
- Kawai, S., Takatori, K., and Ohtaki, T. (1990) Heat resistance of *Cladosporium* isolated from laboratory animal facilities (in Japanese). *Exp. Anim.*, **39** (3), 319-323.
- Magan, N., and Lacey, J. (1984) Effect of temperature and pH on water relations of field and storage fungi. *Trans. Br. mycol. Soc.*, **82** (1), 71-81.
- Moriyama, Y., Nawata, N., Tsuda, T., and Nitta, M. (1992) Occurrence of moulds in Japanese bathrooms. *Int. Biodeterior. Biodegrad.*, **30**, 47-55.
- Pelhate, J. (1967) Determination of water requirements in grain storage fungi. *Mycopathol. Mycol. Appl.*, **36**, 117-127.
- Smith, S. L. and Hill, T. (1982) Influence of temperature and water activity on germination and growth of *Aspergillus restrictus* and *A. versicolor*. *Trans. Br. mycol. Soc.* **79**, 558-560.
- Uchiyama, Y., Noguchi, T., Tsuchibe, M., Mieno, Y., Kawashima, T., and Takatori, K. (1994) Studies on influence of temperature and relative humidity in fungal growth (in Japanese). *Bokin Bobai*, **22**, 397-401.