Studies on a saprophyte of *Exophiala dermatitidis* isolated from a humidifier

K. Nishimura & M. Miyaji
Department of Food Hygiene, Research Institute for Chemobiodynamics, Chiba University, 1-8-1 Inohana, Chiba, 280 Japan

Abstract

Black yeast (MM-7) isolated from a humidifier was studied morphologically, biologically and serologically. Furthermore, its pathogenicity was compared with that of four human isolates of *Exophiala dermatitidis.*

The MM-7 is dimorphic and its growth at 37 °C was better than that at 27 °C. Giant colonies of the MM-7 were very similar to those of the four human isolates. Microscopically, hyphae were pale brown, slender or toruloid. Cylindrical, bottle- or flaskshaped conidiogenous cells arose from the tips and sides of the hyphae. Conidiogenous foci were also seen as small projections at the lateral walls of hyphae. One to four projections were seen at the conidiogenous apices by scanning electron microscopy. Annellation could be observed clearly on them. It was also seen in all of the human isolates of *E. dermatitidis* used for reference. Conidia were globose to subglobose, one celled, smooth, hyaline to brown.

The MM-7 utilized all carbon compounds examined except lactose, melibiose and raffinose. It split arbutin, but did not hydrolyze starch. It utilized neither potassium nitrate, nor hydrolyzed skim milk and gelatin.

The GC content of the MM-7 (56.6%) was almost the same as that of Kano's isolate (58%) and titers of agglutinin of the anti-*E. dermatitidis* serum to the MM-7 and four isolates of the fungus were 512-fold.

From these morphological, biological and serological examinations the MM-7 was identified as *E. dermatitidis* (Kano) de Hoog.

As far as pathogenicity is concerned, the MM-7 showed the strongest pathogenicity of all. Two of the ten mice inoculated intravenously with 5×10^6 cells of the MM-7 died on the 6th and 7th day, and the fungus was recovered from various organs. Histopathologically, the brains were affected severely. A large number of polymorphonuclear leucocytes accumulated around short hyphae and yeast cells to form micro-abscesses. Some micro-granulomatous lesions with a few yeast cells were also observed. Seven of the surviving eight mice showed nervous symptoms. The MM-7 was recovered from the brains of the four mice sacrificed on the 30th day. Some granulomatous lesions with a few yeast cells were recognized in the tissues.

Introduction

In 1937, Kano isolated a new species of dematiaceous fungus from a patient with chromomycosis and termed it *Hormiscium dermatitidis* (13). Since then, this fungus has been isolated little by little as one of the causative agents of the disease.

Interestingly, most isolates have been isolated in Japan and therefore chromomycosis caused by this fungus seems to be a disease peculiar to there.

Up to now 21 patients have been infected with the fungus in Japan (8). Even though the number is not that high, more than one third of them died because of its severe ability to invade the central nervous

Mycopathologia 77, 173–181 (1982). 0301-486x/82/0773-0173/$1.80.

system and liver. Therefore, it is very important to clarify its mode of life in nature and to investigate its pathogenicity.

There have been three papers (2, 5, 22) on isolation of the fungus from nature. However, none are from Japan.

A few years ago, five Sabouraud's dextrose (2%) agar (SDA) plates were brought to the authors' laboratory by a physician. He had examined a patient with hypersensitive pneumonitis living on Hokkaido island which is located in the northern part of the Japanese archipelago. The patient became aware that he suffered from asthma attacks whenever the humidifier in his living room was turned on. Therefore, the physician assumed that any of the microorganisms dispersed by the humidifier might have caused allergic reactions in the patient.

A considerable number of black yeast colonies grew among various other colonies on the plates.

In this study the black yeast was examined morphologically, biologically, serologically and pathologically and was compared with isolates of *Exophiala dermatitidis, E. jeanselmei, E. gougerotii, E. spinifera* and *Aureobasidium pullulans.*

Materials and methods

Isolation of saprophytic black yeast: Three SDA plates were exposed to vapor being dispersed by the humidifier for a few seconds and two plates were smeared with small sterile cotton balls, with which the inside of the apparatus had been swept. These plates were incubated at 27 °C for 7 days. Various colonies of molds, yeasts and bacteria grew on the plates. Yeast-like colonies could be divided into eight species, and one of them was black yeast. It was termed tentatively MM-7. This time the MM-7 became an object of investigation.

Reference fungi: Four isolates of *E. dermatitidis* and an isolate of *E. gougerotii, E. jeanselmei, E. spinifera* or *A. pullulans* were used for reference fungi (Table 1). All these fungi except *A. pullulans* were isolated from patients.

Giant colony: Four kinds of media such as SDA, corn meal agar (CMA), potato dextrose (1%) agar (PDA) and brain heart infusion dextrose (1%) agar (BHI) were used for observation of the giant colonies. The plates inoculated with each isolate were incubated at 27 °C or 37 °C for 21 days.

Slide culture: Slide cultures using SDA and CMA as media were made for microscopical observation. They were incubated at 27 °C for 14 days.

Temperature study: Growth of these isolates on SDA, CMA and PDA at 37 °C for 14 days was compared with that at 27 °C. A standard judgment is determined as follows. The ratio of the colony diameter at 37 °C to that at 27 °C $\geqslant 75\% \rightarrow +4, 75\%$

Table 1. Isolates used for reference.

Isolates		History
Exophiala dermatitidis	1184	CBS 207.35 (Kano's isolate). Received from Dr. Fukushiro (Kanazawa Medical Univ.).
Exophiala dermatitidis	SM-1506	Isolated from the pharynx of a patient with chromomycosis (14). Received from Dr. Hironaga (Shiga Univ. of Medical Science).
Exophiala dermatitidis	SM-1518	Isolated from a patient with chromomycosis (26). Received from Dr. Hironaga (Shiga Univ. of Medical Science).
Exophiala dermatitidis	SM-1519A	Isolated from a patient with chromomycosis (25). Received from Dr. Hironaga (Shiga Univ. of Medical Science).
Exophiala gougerotii	999	Received from Dr. Fukushiro (Kanazawa Medical Univ.).
Exophiala jeanselmei	1171	Received from Dr. Fukushiro (Kanazawa Medical Univ.).
Exophiala spinifera	2043	Duke University-3342. Received from Dr. Fukushiro (Kanazawa Medical Univ.).
Aureobasidium pullulans	ATCC-15233	

$>$ the ratio $\geqq 50\% \rightarrow +3$, $50\% \geqq$ it $> 25\% \rightarrow +2$, $25\% \geqq$ it $\rightarrow +1$.

Scanning electron microscopy: Colonies of the MM-7 and four human isolates of *E. dermatitidis,* which were cultured on CMA at 27 °C for 14 days, were cut into small blocks and put in 5% glutaraldehyde. After being fixed at 3 °C for 4 hours and then in 1% osmium tetroxide at 3 °C for 17 hours, the blocks were dehydrated by a series of gradient alcohols and isoamyl acetate, then dried with a Critical Point Dryer HCP-1 (Hitachi), coated with gold-palladium using an ION Cleaner IB-1 (Eiko Engineering Ltd.) and observed by a scanning electron microscope HFS-2 (Hitachi).

Assimilation tests of carbon compounds: Carbon assimilation by each isolate was auxanographically examined (15). Glucose, fructose, xylose, galactose, mannose, arabinose, mannitol, maltose, saccharose, lactose, melibiose and raffinose were used as carbon sources. Results were judged for 7 days. The hydrolysis of starch was examined using a medium composed of 0.2% soluble starch (Difco), 0.6% dextrose, 1% polypeptone and 1.5% agar. Results were judged with an iodostarch reaction on the medium incubated at 27 °C for 2 weeks.

Splitting of glucoside: The splitting of glucoside was examined using arbutin agar (15). The plates inoculated with each isolate were incubated at 27 °C for 3 weeks.

Assimilation tests of nitrogen compounds: Assimilation of potassium nitrate was auxanographically examined (15). The plates were incubated at 27 °C and observations were made until the 7th day. The hydrolysis of skim milk was examined using a medium composed of 5% skim milk (Difco) and 1.5% agar. The plates inoculated with each isolate were incubated at 27 °C for 3 weeks. The hydrolysis of gelatin was tested using Czapek-Dox solution supplemented with 0.1% yeast extract (Difco) and 15% gelatin (Difco). Each isolate was inoculated into the replicate test tubes containing 10 ml of the medium and cultured at 20 °C for 10 weeks.

Hydrolysis of urea: A urease test was done using Christensen's urea agar (15). The medium inoculated with each isolate was cultured at 27 °C for 5 weeks.

Determination of deoxyribonucleic acid base composition: The guanine (G) and cytosine (C) contents of the MM-7, Kano's isolate of *E. dermatitidis* (1 184), *E. spinifera, E- jeanselmei* and *E. gougerotii* were examined. Yeast cells of the former three were harvested from colonies cultured on BHI agar slants at 37 °C for 3 days. The latter two were inoculated into BHI broth and cultured at 27 °C for 7 days. Then, hyphae of each isolate were collected from the broth by centrifugation, washed three times with phosphate buffered saline (1/15 M, pH 7.4) and dried with acetone. After being ground with quarz sand, DNA of each isolate was extracted according to Marmur's procedure (16). Then, the obtained DNA was hydrolyzed using the procedure of Hershey *et al.* (11). Thin-layer chromatographic separation of the hydrolysates was performed with the procedure of Grippo *et al.* (10). The nucleic acid bases separated on a thin-layer, microcrystalline cellulose 'Avicel', were measured at 270 nm with a Dualwave-length TLC Scanner, CS-910 (Shimadzu).

Serological studies: Kano's isolate was used as an antigen. The isolate was inoculated into Sabouraud's dextrose broth and shaking-cultured at 27 °C for 7 days. After being sterilized with formalin, the broth was filtered through several layers of gauze to eliminate hyphal elements. The filtrate was centrifuged, and yeast cells were washed three times with the phosphate buffered saline solution. Then, yeast cells were resuspended in the solution to prepare 1% yeast cell suspension. Blood was exsanginated from two female white rabbits a week prior to sensitization and the serum was used as controls. The two rabbits were sensitized intravenously with the antigen according to the procedure of Nielsen & Conant (20). One week after the last injection antiserum was obtained from them. It was stored at −80 °C until usage. An agglutinin titer to living yeast cells of each isolate was determined by a slide agglutination method.

Studies on pathogenicity: Pathogenicity of the MM-7 and four human isolates of *E. dermatitides* was examined. These isolates were cultured on BHI agar slants at 37 °C. On the 4th day yeast cells of each isolate were harvested, washed with the phosphate buffered saline solution and resuspended in the solution. Then, 2.5×10^6/0.1 ml cell suspension was prepared by using a Thoma's hemacytometer. Fifty male ddY mice, weighing 18–20 g, were divided into five groups consisting of ten each. One group was allotted to each of the five cell suspensions. Each mouse was inoculated into a tail vein

with 0.2 ml of the cell suspension and observed for 30 days. Two dead mice and half of the mice surviving until the 30th day were necropsied and portions of the brains, hearts, lungs, livers, spleens and kidneys were cultured on BHI agar plates at 37 °C for 14 days. The remains of these organs, thymi, mesenteric lymph nodes and parts of small intestines were fixed in 10% formalin. Then, sections, stained with hematoxylin and eosin (H & E) or by the periodic acid-Schiff reactions (PAS), were prepared.

Results

Morphology: The growth of the MM-7 at 37 °C was better than that at 27 °C as shown in Table 2. The giant colony of the MM-7 was very similar to those of the four human isolates of *E. dermatitidis,* especially to the SM-1518. Three days after incubation on SDA, PDA and CMA at 27 °C or 37 °C, colonies of the MM-7 were yeast-like and had a smooth, dark, pasty appearance. Thereafter, superficial and submerged hyphae appeared gradually at the periphery of the colonies. Three weeks after incubation on SDA and PDA at 37 °C colonies were moist, flat, dark brown, and measured 33 and 36 mm in diameter, respectively. The centers of the colonies were still pasty, elevated slightly and sloped toward the border (Fig. 1). While being at 27 °C the colonies were partially covered with short aerial hyphae. Three weeks after incubation on CMA at 27 °C and 37 °C the colonies were black, flat and moist without aerial hyphae. When cultured on BHI agar, colonies were pasty and had a chocolate brown color, regardless of the temperature. When cultured on SDA or BHI agar, the agar around the colony was brown.

Microscopically, hyphae were pale brown, slender or toruloid. Cylindrical, bottle- or flask-shaped conidiogenous cells, which were deeply pigmented, arose from the tips and sides of hyphae

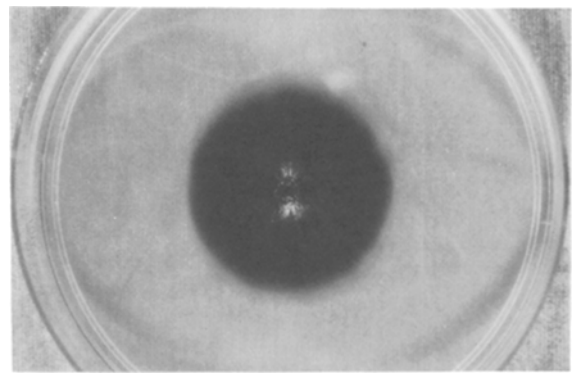

Fig. 1. Giant colony of *E. dermatitidis* MM-7 incubated on SDA at 37 °C for three weeks.

Table 2. Results of growth at 37 °C, biological examinations and GC content.

			Assimilation of carbon compounds																			
		Growth at 37 °C	Glucose	Fructose	Xylose	Galactose	Mannose	Rhamnose	Arabinose	Mannitol	Maltose	Saccharose	Lactose	Melibiose	Raffinose	Hydrolysis of starch	Splitting of arbutin	Assimilation of KNO_3	Hydrolysis of skim milk	Hydrolysis of gelatin	Hydrolysis of urea	GC content
E.[a] *dermatitidis*	MM-7	+4	+	+	+	±	+	+	+	+	+	+	–	–	–	–	+	–	–	–	+	56.6%
E. dermatitidis	1184	+4	+	+	+	+	+	+	+	±	+	+	–	–	–	–	±	–	–	–	+	58.0%
E. dermatitidis	SM-1506	+4	+	+	+	+	+	–	+	+	+	+	–	–	–	–	±	–	–	–	+	
E. dermatitidis	SM-1518	+4	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	–	+	
E. dermatitidis	SM-1519A	+4	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	–	+	
E. gougerotii	999	+2	+	+	+	+	+	+	+	+	+	+	–	–	+	–	±	+	–	–	+	52.6%
E. jeanselmei	1171	–[c]	+	+	+	±	+	±	+	+	+	+	–	–	–	–	–	+	–	–	+	54.6%
E. spinifera	2043	+2[d]	+	+	+	±	+	±	+	+	+	+	–	+	+	–	–	+	–	–	+	59.7%
A.[b] *pullulans*	ATCC-15233	+1	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	

[a] *Exophiala;* [b] *Aureobasidium;* [c] SDA was used as medium; [d] PDA and CMA were used as media

(Figs. 2–1, 3). Conidiogenous foci were also seen as small projections with or without serrate markings at the lateral walls of hyphae (Figs. 2–2, 3). One to four projections were seen at the conidiogenous apices by scanning electron microscopy (Figs. 3–1, 2). In a higher magnification of these projections, annellations could be clearly observed on them. Annellations were also seen in all of the human isolates of *E. dermatitidis* used for reference (Fig. 4–1~3). Conidia were globose to subglobose, one celled, smooth, hyaline to brown. They were produced exogenously and basipetally from annellides and aggregated in clusters. Sometimes, these annelloconidia secondarily produced blastoconida (Fig. 4–1). In old cultures some yeast cells with small projections were observed. These projections were formed by a process in which daughter cells were continuously produced at the proliferation focus of the mother cells.

Biological examination: The results are shown in Table 2. Concerning assimilation of carbon compounds, the MM-7 utilized all carbon compounds examined except lactose, melibiose and raffinose. It split arbutin, but did not hydrolyze starch. It utilized neither potassium nitrate, nor hydrolyzed skim milk and gelatin. These results coincided with those of the human isolates of *E. dermatitidis*. *dermatitidis*.

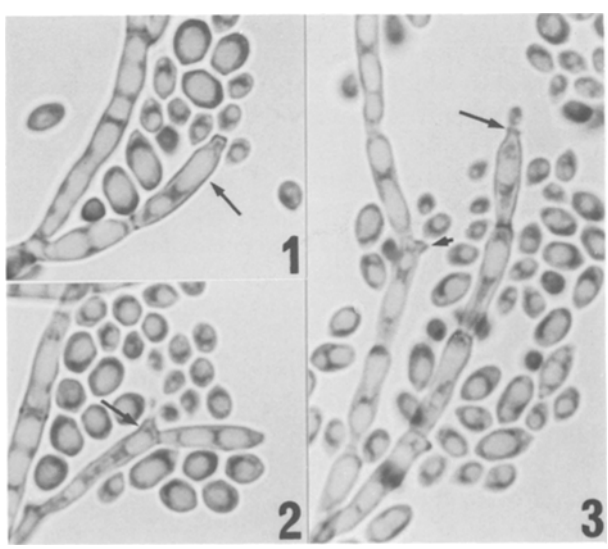

Fig. 2. Microphotographs of *E. dermatitidis* MM-7. (2–1) Terminal conidiogenous cell (arrow) on a lateral branch. (2–2) Intercalary conidiogenous cell (arrow) produces conidia consecutively. (2–3) Conidial formation from a projection on a toruloid hypha (short arrow) and conidiogenous cell with a rudimentary collarette (long arrow).

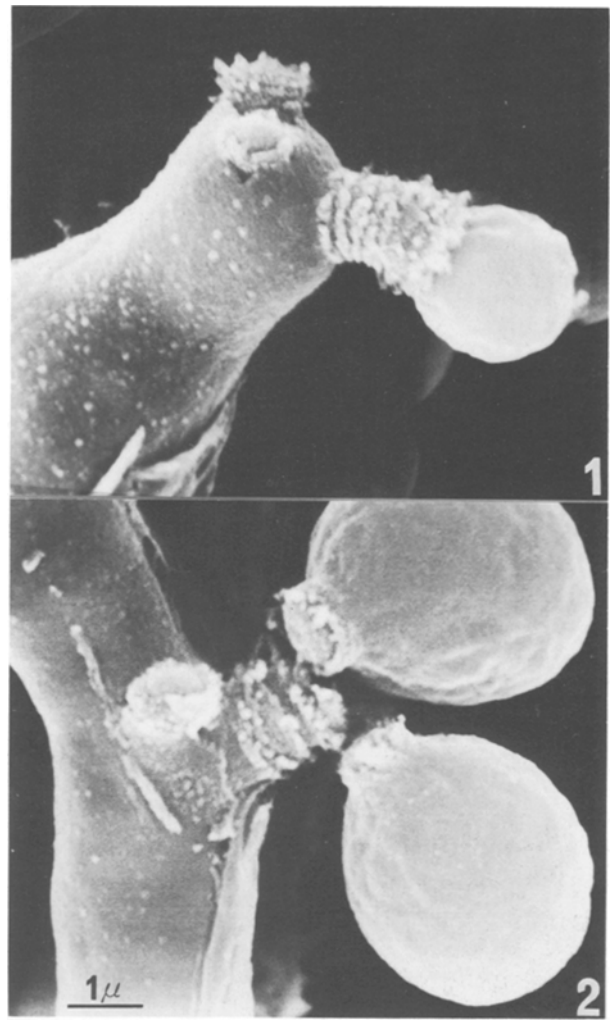

Fig. 3. Scanning electron microphotographs of *E. dermatitidis* MM-7. (3–1) Annellation. Three tips are formed on an annellide. (3–2) Annellate tips on a hypha.

Deoxyribonucleic acid base composition: The GC content of the MM-7 (56.6%) was almost the same as that of Kano's isolate (58%). Those of *E. gougerotii*, *E. jeanselmei* and *E. spinifera* were 52.6, 54.6 and 59.7%, respectively. These results seem to indicate that it may be impossible to differentiate the four species of *Exophiala* from each other due to their GC contents.

Agglutinin titer: Titers of agglutinin of the anti-*E. dermatitidis* serum to the MM-7 and four

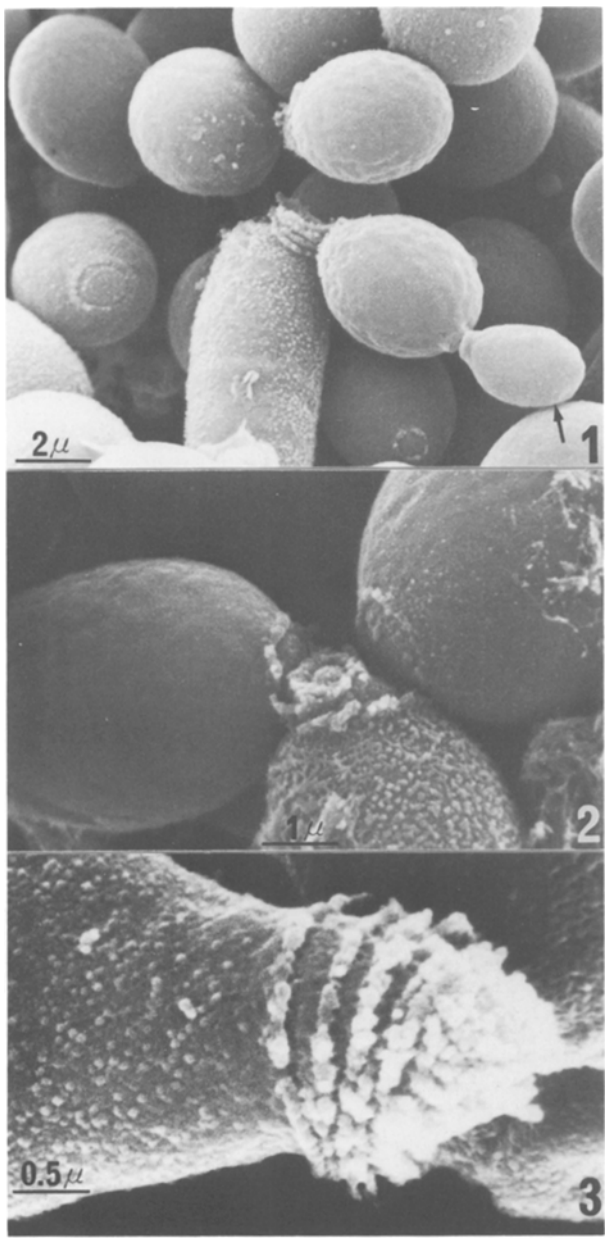

Fig. 4. Scanning electron-microphotographs of human isolates of *E. dermatitidis*. (4–1) Annellide and annelloconidia (Kano's isolate). Conidium (arrow) buds from an annelloconidium secondarily. (4–2) Conidium of the right side is separating from a lower scar and one of the right side, from an upper scar (Kano's isolate). (4–3) Annellation (SM-1519A).

isolates of *E. dermatitidis* were 512-fold. Those to *E. gougerotii, E. jeanselmei* and *E. spinifera* were 4-, 8- and 2-fold, respectively. An agglutinin titer to *A. pullulans* was 256-fold (Table 3).

Table 3. Agglutinin titer of anti-*Exophiala dermatitidis* (1184) rabbit serum.

Isolates		Anti-serum	Control
E. dermatitidis	MM-7	512	–
E. dermatitidis	1184	512	–
E. dermatitidis	SM-1506	512	–
E. dermatitidis	SM-1518	512	–
E. dermatitidis	SM-1519A	512	–
E. gougerotii	999	4	–
E. jeanselmei	1171	8	2
E. spinifera	2043	2	–
A. pullulans*	ATCC-15233	256	2

* *Aureobasidium*

From the morphological, biological and serological examinations the authors identified the MM-7 as *E. dermatitidis* (Kano) de Hoog (3).

Pathogenicity: Pathogenicity of the MM-7 and the four human isolates of *E. dermatitidis* to mice (ddY strain) is shown in Table 4 and Fig. 5-1, 2. From the 4th to 5th day, regardless of the isolates, the mice languished under the infection and lost in weight. This tendency was conspicuous in the mice inoculated with the MM-7, SM-1518 and SM-1519A. In the mice inoculated with the MM-7, two mice died on the 6th and 7th day. After the 7th day conditions of all the mice took a turn for the better, namely their coat became more lustrous and they gained weight. After the 9th day torticollis or abnormal movement began to show in several mice. These central nervous symptoms were observed in seven of the eight survivals inoculated with the MM-7, seven of the ten mice inoculated with the SM-1518, six of the ten mice inoculated with the SM-1519A and SM-1506. However, there was no dead mouse except the two mentioned above during the experiment.

Table 4 shows the results of the recoveries from various internal organs of the mice sacrificed 30 days post infection and from those of the two dead mice. The MM-7 and SM-1518 were recovered from all the brains of the sacrificed mice. The SM-1506 was recovered from the hearts, livers and spleens of all the mice.

Histopathologically, in the two dead mice, the brains were affected severely. A large number of polymorphonuclear leucocytes accumulated around short hyphae and yeast cells to form micro-abscesses. Some micro-granulomatous lesions with

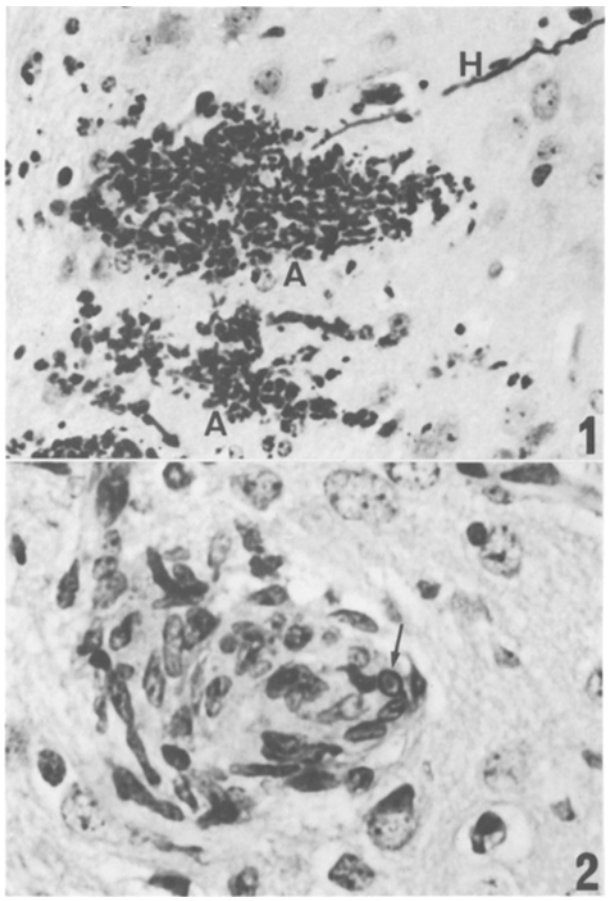

Fig. 5. Brains of mice inoculated intravenously with 5×10^6 yeast cells of the MM-7 (PAS). (5–1) Brain of the mouse dying on the 6th day. H: Hypha. A: Abscess. (5–2) Brain of the mouse sacrificed 30 days after inoculation. Micro-granuloma with a yeast cell (arrow).

a few yeast cells consisting chiefly of mononuclear cells were also observed in the tissues. Furthermore, there were several hyphae and yeast cells without cellular response. The livers of the mice were very atrophic and a few micro-abscesses and micro-granulomata with yeast cells were formed in the tissues. There were some Kupffer cells engulfing yeast cells which retained a good shape. In the spleens a few solitary yeast cells were observed without cell reaction. In the lungs a few micro-abscesses with yeast cells were formed and in the hearts a few of small clumps of yeast cells, without cellular response, were observed. In the kidneys there were no lesions, even though a few yeast cells were recognized in the tubuli.

A few micro-granulomata, consisting chiefly of mononuclear cells, were formed in the brains of the mice sacrificed 30 days after inoculation of the isolates except Kano's isolate. Yeast cells were recognized in most of the granulomata. In the livers of all of the mice a few granulomatous lesions without yeast cells were also formed. Several Kupffer cells without cellular response engulfed intact yeast cells. A few solitary yeast cells engulfed by macrophages were recognized in the spleens of the mice injected with the MM-7, SM-1506, SM-1518 and SM-1519A. Even though the MM-7, SM-1506, SM-1518 and SM-1519A were recovered from the hearts, lungs or kidneys of some of the mice, there were no lesions histopathologically.

Table 4. Recovery of *Exophiala dermatitidis* from various organs of two dead mice and those sacrificed 30 days after inoculation.

Isolates		Brain		Heart		Lung		Liver		Spleen		Kidney	
E. dermatitidis	MM-7	4/4	2/2*	0/4	2/2*	0/4	1/2*	1/4	2/2*	1/4	2/2*	2/4	2/2*
E. dermatitidis	1184	1/5		0/5		0/5		1/5		0/5		0/5	
E. dermatitidis	SM-1506	2/5		5/5		2/5		5/5		5/5		3/5	
E. dermatitidis	SM-1518	5/5		2/5		2/5		1/5		1/5		4/5	
E. dermatitidis	SM-1519A	1/5		1/5		1/5		0/5		2/5		2/5	

* Dead mice
A denominator indicates the number of mice sacrificed
A numerator indicates the number of positive organs

Discussion

The first isolation of *E. dermatitidis* from nature was reported in Uruguay by Conti-Díaz *et al.* (2) who isolated several isolates from wasp nest, fallen palm tree and soil with plant debris. In Virginia (USA) Dixon *et al.* (5) isolated saprophytes of the fungus from sawdust, wood, rotten wood and bark, and in Brasil Reiss & Mok (22), from the livers, lungs and spleens of healthy wild bats. Frank & Roester (7) reported several isolates of *E. dermatitidis* from spontaneous mycotic lesions of toads *(Bufo alvarius)* in Germany. However, their isolates did not grow at 37 °C and there was no microscopical description in their paper.

Even though the authors' isolate (MM-7) was isolated from a humidifier, it showed stronger pathogenicity to mice than the human isolates. This result is very important from the standpoint of public health, because humidifiers are used in many households. It will be necessary for mycologists to pay more attention to humidifiers as a source of thermophilic pathogenic fungi.

The results of the morphological, biological and serological examinations of the MM-7 were almost the same as those of the human isolates of *E. dermatitidis*. The MM-7 could be easily differentiated from *E. gougerotii, E. jeanselmei* and *E. spinifera* due to their morphology, growth at 37 °C and utilization for KNO_3. It could also be differentiated from *A. pullulans* from morphology, assimilation tests of carbon and nitrogen compounds.

An agglutinin titer in the anti-*E. dermatitidis* serum to the MM-7 was 512-fold which was the same as that of the human isolates of *E. dermatitidis*. This result was different from that of Mok & Luizâo (19), who described that there was no cross-reaction between the bat and human isolate antigens. Even though the anti-serum also showed a cross-reaction to *E. jeanselmei, E. gougerotii* and *E. spinifera,* their titers were very low. On the other hand, yeast like cells of *A. pullulans* were agglutinated in the antiserum diluted to 256-fold. According to Nielsen & Conant (20), their anti-*E. dermatitidis* serum showed a cross-reaction to *E. jeanselmei* and *A. pullulans*. Even though Gordon & Al-Doory (9) did not admit cross-reactions in their fluorescent antigen-antibody reaction between *E. dermatitidis* and *A. pullulans,* it seems to be difficult to differentiate *E. dermatitidis* from *A. pullulans* serologically from the above mentioned results.

There have been a few reports on the pathogenicity of *E. dermatitidis*. Kano (13) inoculated his isolate into the peritoneal cavities and subcutaneous tissues of both mice and guinea pigs, and into the testes of rabbits, and abscesses and granulomata were formed in these tissues. Shimazono *et al.* (23) injected their isolate intravenously into mice, and noticed many micro-abscesses in various vescera, especially in the brains where they observed abundant hyphal elements. Even though their description was very short, it is the first description on the neurotropism of *E. dermatitidis*. Tsai *et al.* (24) intravenously inoculated 2×10^7 cells of their isolate into mice and observed abscesses in the brains, kidneys and spleens. Jotisankata *et al.* (12) compared the pathogenicity of six isolates of the fungus to cortison-treated mice. According to them lesions were formed in the lungs, brains, kidneys and on the serosal surface of the peritoneum. Furthermore, they described that Kano's isolate showed the weakest pathogenicity and a retroculture of the fungus from the brain did not succeed. According to Mok & Luizâo (19), the isolates from wild bats possessed a potential to be pathogenic to mice. Histopathologically, however, they could not detect any fungal element in the tissues of the various internal organs.

In this experiment two of the ten mice inoculated intravenously with 5×10^6 cells of the MM-7 died on the 6th and 7th day, and the fungus was recovered from various organs of the dead mice. Histopathologically, their brains were affected severely. Seven of the survival mice showed nervous symptoms. When four mice were sacrificed on the 30th day, the MM-7 was recovered from the brains of the four mice where some granulomatous lesions with a few yeast cells were recognized. A few microfoci were formed in the liver of these mice. The MM-7 showed the strongest pathogenicity of all and just as in the report by Jotisankata *et al.* (12), the pathogenicity of Kano's isolate was the weakest of all.

The authors would like to express their reason for using genus *'Exophiala'*. Even though the fungus had various synonyms, the binominal *'Phialophora dermatitidis* (Kano) Emmons' (6) was supported by many mycologists before. However,

McGinnis (17, 18) insisted that conidia of the fungus were produced from phialides without collarette and introduced a new genus *'Wangiella'* instead of *'Phialophora'*. Cole (1) observed the condial ontogeny of various species of black yeast using a scanning electron microscope and agreeded with McGinnis' opinion. Dixon & Shadomy (4) also supported McGinnis.

As reported at the 23rd Japanese Medical Mycology Conference held in Tokyo in 1979, the authors (21) have already recognized annellation on the conidiogenous cells of the five isolates of *E. dermatitidis,* which were used in this experiment, by using a scanning electron microscope. Since then, they have insisted that *'Exophiala'* advocated by de Hoog (3) is better than *'Wangiella'*.

Acknowledgement

The authors would like to thank Dr. R. Fukushiro (Kanazawa Medical University) and Dr. M. Hironaga (Shiga University of Medical Science) for providing cultures.

References

1. Cole, G. T., 1978. Conidiogenesis in the black yeasts. The Black and White Yeasts, Pan American Health Organization, Washington, D.C., p. 66–78.
2. Conti-Díaz, I. A., Mackinnon, J. E. & Civilia, E., 1978. Isolation and identification of black yeasts from the external environment in Urguay. The black and White Yeasts, Pan American Health Organization, Washington, D.C., p. 109–114.
3. De Hoog, G. S., 1977. Studies in Mycology. No. 15 Rhinocladiella and allied genera, p. 118–119.
4. Dixon, D. M. & Shadomy, H. J., 1980. Taxonomy and morphology of dematiaceous fungi isolated from nature. Mycopathologia 70: 139–144.
5. Dixon, D. M., Shadomy, H. J. & Shadomy, S., 1980. Dematiaceous fungal pathogens isolated from nature. Mycopathologia 70: 153–161.
6. Emmons, C. W., 1966. Pathogenic dematiaceous fungi. Japan. J. Med. Mycol. 7: 233–245.
7. Frank, W. & Roester, U., 1970. Amphibien als Träger von Hormiscium (Hormodendrum) dermatitidis KANO, 1937, einem Erreger der Chromoblastomykose (Chromomykose) des Menschen. Zeitschrift für Tropenmedizin und Parasitologie 21: 93–108.
8. Fukushiro, R., 1977. Some considerations on infections by dematiaceous fungi, with special regard to chromomycosis. Japan. J. Med. Mycol. 18: 398–421.
9. Gordon, M. A. & Al-Doory, Y., 1965. Application of fluorescent-antibody procedures to the study of pathogenic dematiaceous fungi. II Serological relationships of the genus Fonsecaea. J. Bacteriol. 89: 551–556.
10. Grippo, P., Iaccarino, M., Rossi, M. & Scarano, E., 1965. Thinlayer chromatography of nucleotides, nucleosides & nucleic acid bases. Biochem. Biophys. Acta 95: 1–7.
11. Hershey, A. D., Dixon, J. & Chase, M., 1953. Nucleic acid economy in bacteria infected with bactrio-phage T_2. I. Purine and pyrimidine composition. J. General Physiol. 36: 777–789.
12. Jotisankasa, V., Nielsen Jr., H. S. & Conant, N. F., 1970. Phialophora dermatitidis: Its morphology and biology. Sabouraudia 8: 98–107.
13. Kano, K., 1937. Über die Chromoblastomykose durch einen noch nicht als pathogen beschriebenen Pilz: Hormiscium dermatitidis n. sp. Arch. Dermatol. Syph. 176: 282–294.
14. Kinka, S., Yamashita, K., Mizogami, I., Murakami, T. & Inoue, H., 1963. A case of chromoblastomycosis, with primary lesion on the pharynx, metastasized to the lungs and ear. Japan. J. Med. Mycol. 4: 71–80.
15. Lodder, J., 1970. The yeasts. 2nd ed. North-Holland Publishing Co.
16. Marmur, J., 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. 3: 208–218.
17. McGinnis, M. R., 1977. Wangiella, a new genus to accommodate Hormiscium dermatitidis. Mycotaxon 5: 353–363.
18. McGinnis, M. R., 1977. Wangiella dermatitidis, a correction. Mycotaxon 6: 367–369.
19. Mok, W. Y. & Luizâo, R. C. C., 1981. Serological analysis and pathogenic potentials of Wangiella dermatitidis isolated from bats. Mycopathologia 73: 93–99.
20. Nielsen, Jr. H. S. & Conant, N. F., 1967. Practical evaluation of antigenic relationships of yeast-like dematiaceous fungi. Sabouraudia 5: 283–294.
21. Nishimura, K., Miyaji, M. & Kawai, R., 1980. 'Phialophora' dermatitidis isolated from a humidifier. Japan. J. Med. Mycol. 21: 30.
22. Reiss, N. R. & Mok, W. Y., 1979. Wangiella dermatitidis isolated from bats in Manaus, Brazil. Sabouraudia 17: 213–218.
23. Shimazono, Y., Isaki, K., Torii, H., Otsuka, R. & Fukushiro, R., 1963. Brain abscess due to Hormodendrum dermatitidis (Kano) Conant, 1953. – Report of a case and review of the literature. – Folia Psychiatrica et Neurologica Japonica 17: 80–96.
24. Tsai, C. Y., Lü, Y. C., Wang, L. T., Hsu, T. L. & Sung, J. L., 1966. Systemic chromoblastomycosis due to Hormodendrum dermatitidis (Kano) Conant. Amer. J. Clinical Pathol. 46: 103–114.
25. Watanabe, S., Hayakawa, M. & Nabekura, K., 1964. Chromoblastomycosis. Japan. J. Dermatol. 74: 665.
26. Watanabe, S., Hisakawa, M. & Aoshima, T., 1976. A case of chromomycosis. Japan. J. Med. Mycol. 16: 231.