Polar Biosci., **12**, 68–75, 1999

A FILAMENTOUS FUNGUS, *PYTHIUM ULTIMUM* TROW VAR. *ULTIMUM*, ISOLATED FROM MORIBUND MOSS COLONIES FROM SVALBARD, NORTHERN ISLANDS OF NORWAY

Tamotsu HOSHINO[1], Motoaki TOJO[2], Gen OKADA[3],
Hiroshi KANDA[4], Satoru OHGIYA[1] and Kozo ISHIZAKI[1]

[1]*Bioscience and Chemistry Division, Hokkaido National Industrial Research Institute, 2-1, 2-jo, 17-chome, Tsukisamu-higashi, Toyohira-ku, Sapporo 062-8517*
[2]*College of Agriculture, Osaka Prefecture University, 1-1, Gakuen-cho, Sakai 599-8531*
[3]*Physical and Chemical Institute (RIKEN), 2-1, Hirosawa, Wako 351-0001*
[4]*National Institute of Polar Research, Kaga 1-chome, Itabashi-ku, Tokyo 173-8515*

Abstract: A fungus, *Pythium ultimum* TROW var. *ultimum*, was isolated from colonies of moribund moss, *Sanionia uncinata* (HEDW.) LOESKE, in Svalbard. This is the first report of isolation of *P. ultimum* var. *ultimum* from the Arctic Zone. This fungus showed possible moss pathogenic activity. The taxonomic, morphological and ecological characteristics are described here. In addition, the mycelial growth of this isolate is compared with that of isolates from the Temperate Zone, and its physiological characteristics are discussed.

key words: moss disease, *Pythium*, *Sanionia* , Svalbard

Introduction

Moss vegetation plays an important role as a producer in early stages of primary succession in the polar regions (MILES and WALTO, 1993). On the other hand, microorganisms, especially fungi in polar regions, are well known as decomposers of mosses and higher plants (ROBINSON and WOOKEY, 1997). Fungi have been reported to actively attack mosses growing on islands in polar regions: Jan Mayen, an island in the Greenland Sea (WILSON, 1951); Ellesmere Island, northern Canada (LONGTON, 1973); Svalbard, northern Norway (RIDLEY *et al*., 1979); Signy Island, South Orkney Islands (FENTON, 1983; LONGTON, 1973). Some fungi, such as *Thyronectria antarctica* var. *hyperantarctica*, an undetermined plectomycete (HAWKSWORTH, 1973; LONGTON, 1985), *Coleroa turfosorum, Bryosphaeria megaspora* and *Epibryon chorisodontii* (FENTON, 1983; PEGLER *et al*., 1980), that may cause parasitic disease in mosses have been isolated from various regions of the Antarctic Zone. However, there have been very few ecological studies on moss pathogenic fungi in the Arctic region. WILSON (1951) studied infections of *Rhacomitrium* carpet on Jan Mayen Island and reported that the moss disease was caused by an unidentified basidomycete. Therefore, fungi also play an important ecological role in polar regions. Some fungi may cause not only disintegration of dead moss leaves but also infection of the moss colony during decomposition process and pathogenic fungi kill the living mosses in polar regions. In this study, we aimed to elucidate the mycological characters of moss pathogenic fungi in the Arctic Zone.

Materials and Methods

Isolation of fungi from moribund moss colonies

Moribund moss leaves were collected to isolate parasitic fungi in Longyearbyen, Svalbard on June 15, 1997. The leaves were put in sterilized test tubes and were kept at *ca.* 4°C in Styrene boxes containing ice bags during transportation. Isolation of fungi was carried out at the University Courses on Svalbard (UNIS, Longyearbyen). The moss leaves were removed from the test tubes and immediately placed on potato dextrose agar (PDA, Difco) plates. Fungi growing on the moss leaves were cultivated at 4°C for 2 weeks and at 15°C for 3 days. A single fungal colony was selected from each sample and subcultured on PDA slants at 4°C.

Morphology

The morphological characteristics examined were: dimensions of oogonia, oospores and hyphal swelling, and width of oospore wall thickness and main hyphae. Percentages of terminal oogonia and monoclinous antheridia were also determined. Isolates were pre-grown on corn meal agar (CMA, Difco) plates at 25°C, and then agar discs of 7 mm in diameter were taken from actively growing colony margins and inoculated onto CMA plates. Cultures were incubated for 7 to 30 days at 15°C for morphological observations. The isolates were also grown on grass blades for 3 to 7 days at 5 and 20°C to determine zoospore production and percentages of monoclinous antheridia (MARTIN, 1992). At least 30 organs were examined for each isolate for all characteristics.

Growth temperature

Mycelial discs 5 mm in diameter were cut from the margin of an actively growing colony, transferred to the centers of PDA plates (9 cm in diameter), and inoculated at 9 different temperatures from 0 to 40°C, in duplicate. After 1, 2 and 3 days of inoculation, the colony diameters were determined. The linear mycelial growth rate per day was calculated after the initial lag period.

Results and Discussion

Fungal infection patterns

The moss carpets of *Sanionia uncinata* (HEDW.) LOESKE [= *Drepanocladus uncinata* (HEDW.) WARNST.] were spread over wet or submerged areas near Longyearbyen, Svalbard. Moribund moss colonies were sometimes found in the moss carpets after the snow bed had melted. Fungal infections were seen in irregular patches along a snowmelt stream (Fig. 1A) or forming a circular pattern (Fig. 1B). Mycelia on moss shoots were visible to the naked eye immediately after thawing. In Antarctica, many fungal infections in moss are observed in moss vegetation near the seashore (HOSHINO *et al.*, unpublished results).

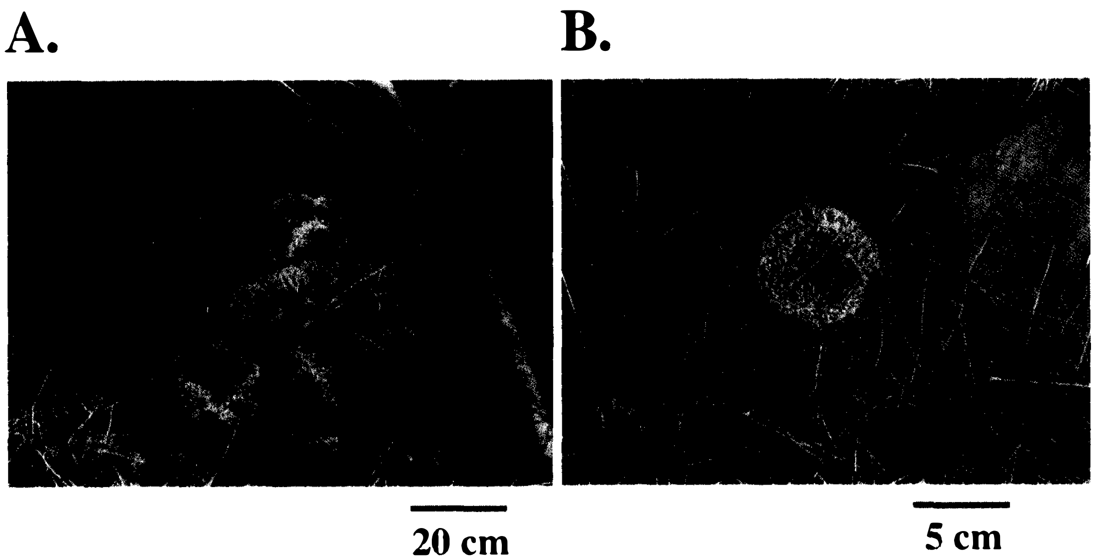

Fig. 1. Fungal infection of *Sanionia uncinata* in Longyearbyen, Svalbard.
A. fungal infection in Hagen area, Longyearbyen. B. fungal infection in Lia area, Longyearbyen.

However, we could not find any obvious disease in moss along the coasts of Longyearbyen and Ny-Ålesund.

Wilson (1951) reported a large number of concentric bands in dead moss from Jan Mayen Island. Ridley *et al.* (1979) reported similar concentric bands from Reindalen, Svalbard, although the host moss was not described in their brief report. They reported that there were many concentric fungal rings in the Arctic moss, one of the rings reaching 1 m in diameter. However, we observed only irregular patches and small circular patterns of fungal infections in samples of *Sanionia uncinata* collected in Longyearbyen and Ny-Ålesund. Large concentric rings were not found in any of our samples of *Sanionia uncinata*. Therefore, it is thought that the parasitic fungus differs from that in their reports.

Identification of fungus isolated from moribund moss

Figure 2 shows microscopic images of a moribund leaf of *Sanionia uncinata*. Mycelia grew well on the moss leaf and were intertwisted at the growth point of the moss. We obtained many fungal isolates from moribund moss leaves. However, the morphological characteristics of the mycelia were the same. Hyphae of the isolate did not have clamp connections and showed fungal colonies on PDA with a radial pattern (Fig. 2a).

Morphology and dimensions of sexual and asexual organs of isolate from Svalbard and the related data of *Pythium ultimum* Trow var. *ultimum* from Van der Plaats-Niterink (1981) are presented in Table 1. Typical morphology of sexual organs and hyphal swellings of the isolate were shown in Fig. 3. Oogonia were smooth-walled and mostly terminal. Diameters of oogonia and oospores averaged 23.0 ± 1.7 and 20.1 ± 1.7 μm, respectively. The thicknesses of oospore walls averaged 1.5 ± 0.3 μm. Antheridia were sac-like in form, mostly monoclinous and originated from immediately below the

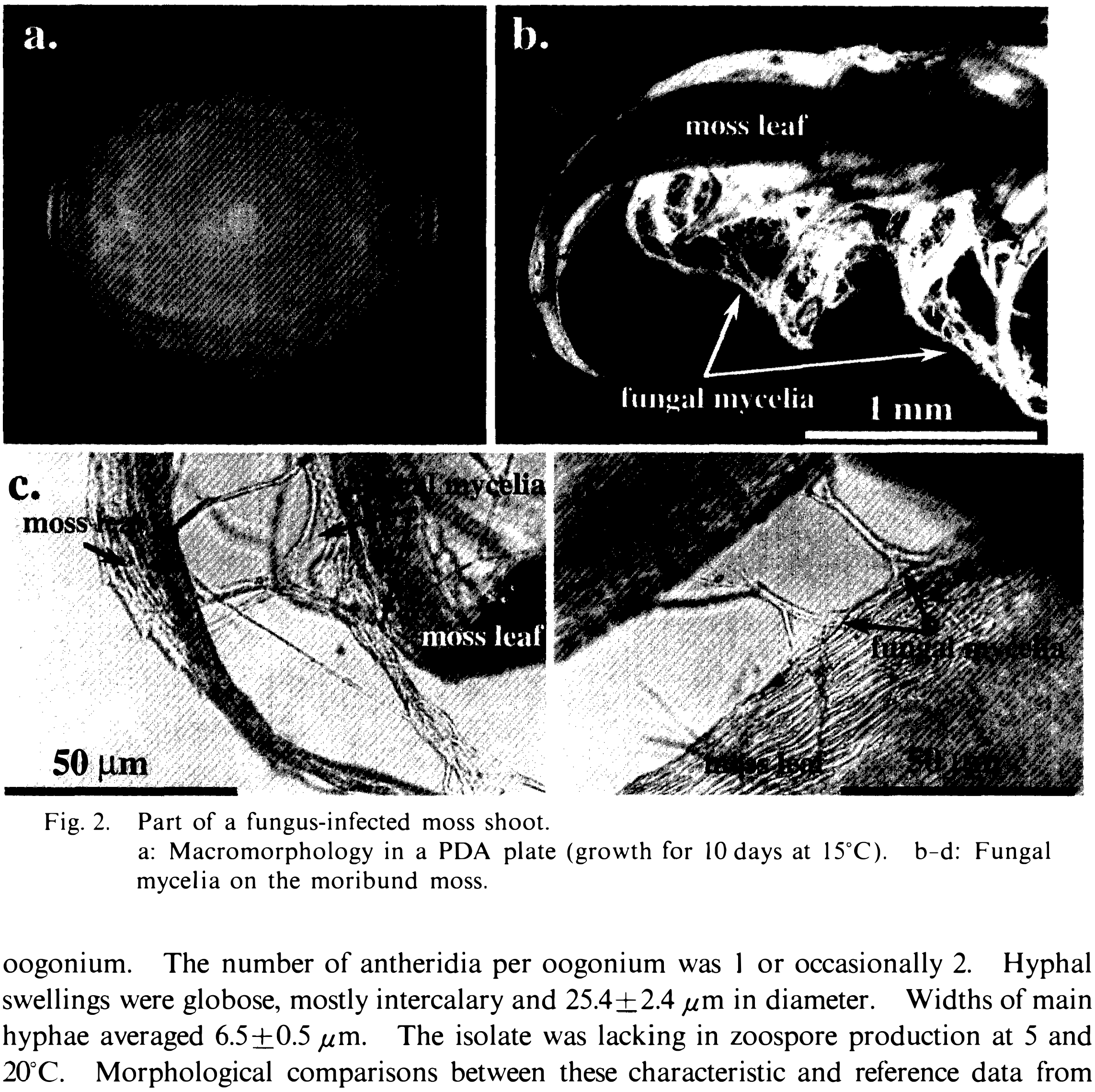

Fig. 2. Part of a fungus-infected moss shoot.
a: Macromorphology in a PDA plate (growth for 10 days at 15°C). b–d: Fungal mycelia on the moribund moss.

oogonium. The number of antheridia per oogonium was 1 or occasionally 2. Hyphal swellings were globose, mostly intercalary and 25.4±2.4 μm in diameter. Widths of main hyphae averaged 6.5±0.5 μm. The isolate was lacking in zoospore production at 5 and 20°C. Morphological comparisons between these characteristic and reference data from VAN DER PLAATS-NITERINK (1981) indicate that the isolate was *P. ultimum* var. *ultimum*.

Pythium ultimum is one of the commonest *Pythium* species in soil. *P. ultimum* var. *ultimum* is known as cosmopolitan and grows in cool to moderately warm climates (VAN DER PLAATS-NITERINK, 1981). Other *Pythium* strains have been isolated from Svalbard (*Pythium* spp.; GAERTNER, 1954), Greenland (*P. monospermum, P. graminicolum, P. irregularae* and *Pythium* spp.; HÖHNK, 1960), Alaska (*P. carolinianum*; KOBAYASHI *et al*., 1967) and Devon Island (*P. irregularae* and *Pythium* spp.; BOOTH and BARRETT, 1971). *P. ultimum* var. *ultimum* has been isolated in Iceland, in the subarctic zone (JOHNSON, 1971). Previous isolated fungi of Svalbard were summarized by ELVEBAKK *et al.* (1996). However, this fungus has not been isolated from either polar region. Therefore, this is the first report of *P. ultimum* var. *ultimum* being isolated from Svalbard, which is in the Arctic Zone.

Pythium ultimum var. *ultimum* isolated from Temperate Zone countries, is a severe parasite to many cultured plants whereas it has never been reported that this fungus has pathogenic activity in mosses. However, this Arctic isolate showed possible moss path-

Table 1. Comparion of morphological characteristics of isolate from Svalbard and *Pythium ultimum* var. *ultimum*.

	Isolate from Svalbard[1)]	*P. ultimum* var. *ultimum*[2)]
Oogonium		
Morphology	Terminal: 93%, intercalary: 7%, globose, smooth-walled	Terminal, sometimes intercalary, globose, smooth-walled
Diameter (μm)	av. 23.0 (SD=1.7)	(14-)20-24(-25) (av. 21.5)
Antheridium		
Morphology	Monoclinous: 93%, diclinous: 7%, sac-like, originating from immediately below the oogonium, sometime hypogynous	Mostly monoclinous, sac-like, originating from immediately below the oogonium, sometime hypogynous
Number per oogonium	1(-2)	1(-3)
Oospore	Single, aplerotic, globose	Single, aplerotic, globose
Diameter (μm)	av. 20.1 (SD=1.7)	(12-)17-20(-21) (av. 18)
Wall thickness (μm)	av. 1.5 (SD=0.3)	Ofter 2 or more
Hyphal swelling		
Morphology	Intercalary, sometimes terminal globose	Intercalary, sometimes terminal globose
Diameter (μm)	av. 25.4 (SD=2.4)	20-25(-29)
Width of main hyphae (μm)	av. 6.5 (SD=0.5)	<11
Zoospore	Not produced	Not or very rarely produced at 5°C

[1)] Each characteristic was examined using at least 30 organs.
[2)] Data from VAN DER PLAATS-NITERRINK (1981).

ogenic activity. In Svalbard, agricultural activities are very low and moss is one of the dominant species of vegetation. Therefore, this Arctic isolate probably has a wide host range against the environmental conditions of Svalbard.

In the Eastern Canadian Arctic, it has been reported that *P. irregularae* and *Pythium* spp. are species of mesic habitats (BOOTH and BARRETT, 1976). We isolated *P. ultimum* var. *ultimum* from many moribund moss colonies occurring in wet or submerged areas immediately after snowmelt. This result is probably related to the fact that the genus *Pythium* belongs to Oomycota. Many species that belong to Oomycota prefer to reproduce in submerged conditions.

The morphological characteristics of the Arctic isolate corresponded with those of Temperate isolates (Table 1). However, the Arctic isolate showed a different response to temperature (Fig. 4). This isolate grew at 0°C but the Temperate isolate did not grow at 0°C (same conditions as under snow cover). On the other hand, the mycelial growth rate of this isolate, compared with that of isolates from Temperate Zone countries (VAN DER PLAATS-NITERINK, 1981; TOJO *et al*., 1998), was very low at temperatures above 15°C. However, the optimal growth temperature of this isolate did not differ from that of isolates from Temperate Zone countries. These results suggest that the Arctic isolate originally developed from Temperate Zone isolates and adapted to grow under the Arctic conditions.

Oomycota including the genus *Pythium* has less resistance to freezing than do other

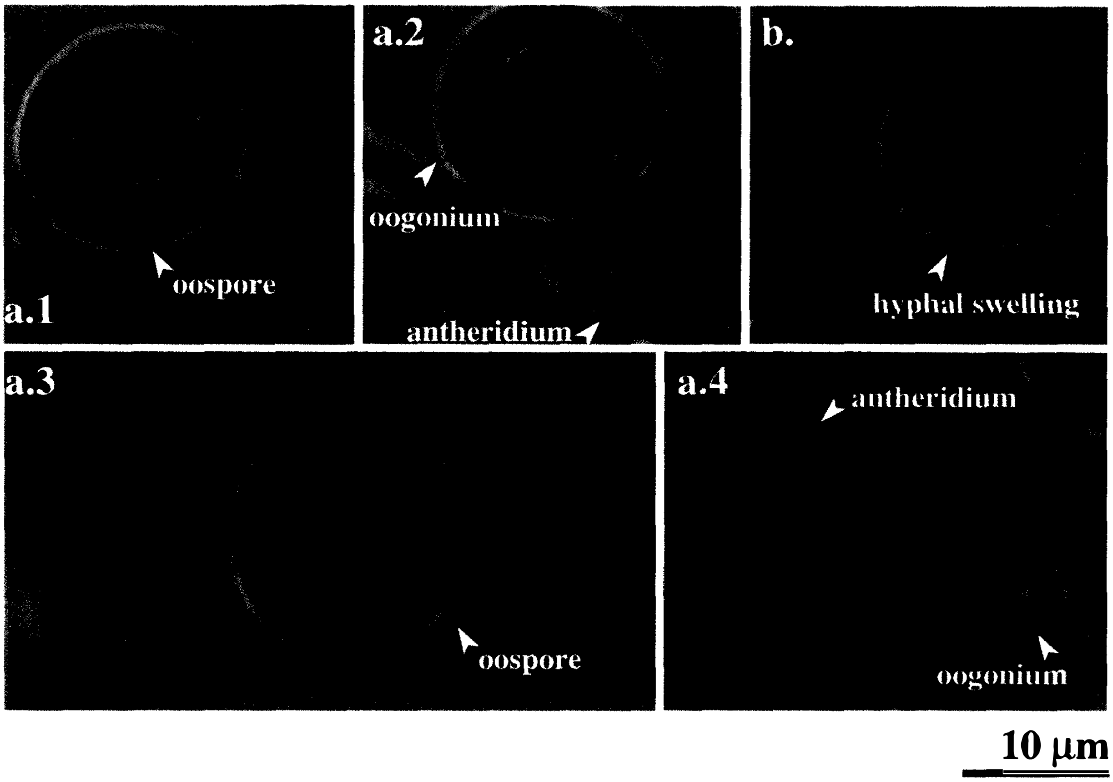

Fig. 3. Sexual organs and hyphal swelling of the Arctic isolate *Pythium ultimum* var. *ultimum*.
a.1. Terminal oogonium and aplerotic oospore. a.2. Terminal oogonium and monoclinous antheridium. a.3. Intercalary oogonium. a.4. Sac-like monoclinous antheridium. b. Terminal hyphal swelling. Scale = 10 μm.

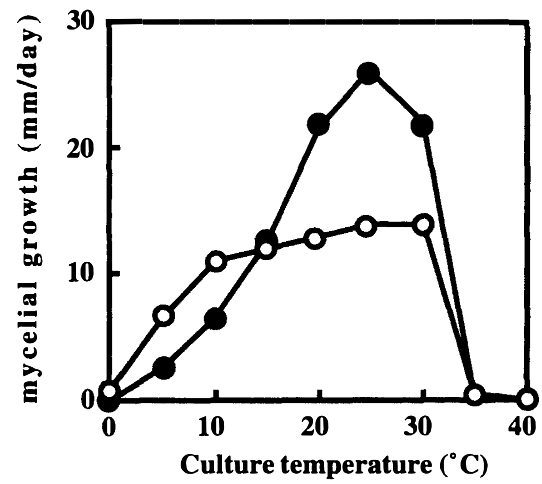

Fig. 4. Effects of cultural temperature on mycelial growth of Arctic and moderate zone isolates, *Pythium ultimum* var. *ultimum*.
Experiments using Arctic (○) and Temperate Zone (●) isolates were conducted in duplicate. After 1, 2 and 3 days of inoculation, the colony diameters were determined. The linear mycelial growth rate per day was calculated after the initial lag period. The Temperate Zone isolate is strain OPU407, which was isolated at the university farm of Osaka Prefecture University, Sakai, Japan (Tojo *et al*., 1998).

fungi (SAUVE and MITCHELL, 1977). However, the Arctic strain *P. ultimum* var. *ultimum* must survive severe winter conditions in Svalbard. Further studies are necessary to elucidate the moss pathogenic activity and the freezing-resistance mechanisms of Arctic *P. ultimum* var. *ultimum*.

Acknowledgments

We thank AIST and STA for their financial support of our research project. We are indebted to Prof. Dr. S. SPJELKAVIK, UNIS (Norway) and Dr. S. USHIO, National Institute of Polar Research (Japan) for their technical support in the collection of moribund moss colonies in Svalbard and the isolation of fungi from dead mosses. We are also grateful to Dr. N. MATSUMOTO, National Institute of Agro-Environmental Sciences (Japan) and Prof. Dr. A.M. TRONSMO, Norwegian Crop Research Institute for their valuable comments.

References

BOOTH, T. and BARRETT, P. (1971): Occurrence and distribution of zoosporic fungi from Devon Island, Canadian Eastern Arctic. Can. J. Bot., **49**, 359-369.

BOOTH, T. and BARRETT, P. (1976): Taxonomic and ecologic observations of zoosporic fungi in soils of a high-arctic ecosystem. Can. J. Bot., **54**, 533-538.

ELVEBAKK, A., GJÆRUM, H.B. and SIVERTSEN, S. (1996): Part 4. Fungi II. Myxomycota, Oomycota, Chytridiomycota, Zygomycota, Ascomycota, Deuteromycota, Basidiomycota: Uredinales and Ustilaginales, ed. by A. ELVEBAKK and P. PRESTRUD. Oslo, Norsk Polarinstitutt, 207-259.

FENTON, J.H.C. (1983): Concentric fungal rings in Antarctic moss communities. Trans. Br. Mycol. Soc., **80**, 413-420.

GAERTNER, A. (1954): Über das vorkommen niederer Erdphycomyceten in Afrika, Schweden und an einigen mitteleuropäischen Standorten. Arch. Mikrobiol., **21**, 4-56.

HAWKSWORTH, D.L. (1973): *Thyronectria antarctica* (Speg.) Seeler var. *hyperantarctica* D. Hawksw. var. nov. Br. Antarct. Surv. Bull., **32**, 51-53.

HÖHNK, W. (1960): Mikologische Notizen II. Phycomyceten von Island und Grönland. Veröff. Inst. Meeresforsch. Bremerhaven, **7**, 63-66.

JONSON, T.W., Jr. (1971): Aquatic fungi of Iceland: *Pyhtium*. Mycologia, **63**, 517-536.

KOBAYASHI, Y., HIRATSUKA, N., AOSHIMA, K., KORF, R., SONEDA, M., TUBAKI, K. and SUGIYAMA, J. (1967): Mycological studies of Alaskan Arctic. Ann. Rep. Inst. Ferment. Osaka, **3**, 1-138.

LONGTON, R.E. (1973): The occurrence of radial infection patterns in colonies of polar bryophytes. Br. Antarct. Surv. Bull., **32**, 41-49.

LONGTON, R.E. (1985): Terrestrial Habitats-Vegetation. Key Environments-Antarctica, ed. by W.N. BONNER and D.W.H. WALTON. Oxford, Pergamon, 73-105.

MARTIN, F.N. (1992): Pythium. Methods for Research on Soilborne Phytopathogenic Fungi, ed. by L.L. SIGLETON *et al.* St. Paul, APS Press, 39-49.

MILES, J. and WALTON, D.W.H. ed. (1993): Primary Succession on Land. Oxford, Blackwell.

PEGLER, D.N., SPOONER, B.M. and LEWIS SMITH, R.I. (1980): Higher fungi of Antarctica, the Subantarctic zone and the Falkland Islands. Kew Bull., **35**, 499-562.

RIDLEY, M., GILLOW, C., PERKINS, D. and OGILVIE, J. (1979): Oxford University expeditions to Svalbard 1978. Bull. Oxf. Univ. Explor. Club. New Series, Commem. Vol., 29-79.

ROBINSON, C.H. and WOOKEY, P.A. (1997): 2. Microbial ecology, decomposition and nutrient cycling. Ecology of Arctic Environments, ed. by S.J. WOODIN and M. MARQUISS. Oxford, Blackwell, 41-68.

SAUVE, R.J. and MITCHELL, D.J. (1977): An evaluation of methods for obtaining mycelium-free oospore of *Pythium aphanidermatum* and *P. myriotylum*. Can. J. Microbiol., **23**, 643-648.

TOJO, M., NAKAZONO, E., TSUSHIMA, S., MORIKAWA, T. and MATSUMOTO, N. (1998): Characterization of two morphological groups of isolates of *Pythium ultimum* var. *ultimum* in a vegetable field. Mycosience, **39**, 135-144.

VAN DER PLAATS-NITERINK, A.J. (1981): Monograph of the genus *Pythium*. Stud. Mycol., **21**, 1-241.

WILSON, J.W. (1951): Observations on concentric 'fairy rings' in Arctic moss mat. J. Ecol., **39**, 407-416.

(*Received April 16, 1998; Revised manuscript accepted October 21, 1998*)