ACTA BOT. ISL. 14: 59–70, 2004

Genetic and biological characteristics of *Typhula ishikariensis* from Northern Iceland

Tamotsu Hoshino[1,2], Michiko Kiriaki[1], Isao Yumoto[1,3] and Akira Kawakami[4]

[1] *National Institute of Advanced Industrial Science and Technology (AIST), 2-17-2-1, Tsukisamu-higashi, Toyohira-ku, Sapporo 062-8517, Japan.*

[2] *Division of Biological Sciences, Graduate School of Science, Hokkaido University, N10 W8, Kita-ku, Sapporo, Hokkaido 060-0810, Japan.*

[3] *Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, N9 W9, Kita-ku, Sapporo 060-0809, Japan.*

[4] *National Agricultural Research Center for Hokkaido Region, Hitsujigaoka 1, Toyohira-ku, Sapporo 062-8555, Japan.*

ABSTRACT**:** Isolates of snow mold fungus, *Typhula ishikariensis*, from Akureyri in Northern Iceland were divided into two groups according to culture morphology and results of mating reactions with testers of *T. ishikariensis* biological species I and II from Japan and Norway. Isolates in both groups showed a similar mycelial growth pattern of thermal dependence. This is first report of the genetic characteristics of *T. ishikariensis* from Iceland.

INTRODUCTION

Snow mold fungi are psychrophilic or psychrotrophic fungal pathogens of perennial grasses and winter cereals in the Northern Hemisphere (HSIANG *et al.* 1999). *Microdochium nivale* (Fries) Samuel's & Hallett, *Sclerotinia borealis* Bub. & Vleug., *Typhula incarnata* Lasch ex. Fr. and *T. ishikariensis* Imai are typical snow mold fungi found in Nordic countries, including Denmark (where *M. nivale* and *T. incarnata* have been found: WELLING & JENSEN 1970), the Faroe Islands (where *M. nivale* and *T. incarnata* have been found: HOSHINO *et al.* 2004), Finland (JAMALAINEN 1949, 1957), Greenland (where *S. borealis*, *T. incarnata* and *T. ishikariensis* have been found: Hoshino *et al.* unpublished results), Iceland (where *M. nivale*, *T. incarnata* and *T. ishikariensis* have been found: KRISTINSSON & GUÐLEIFSSON 1976), Kola Peninsula in Russian Arctic (PETROV 1983), Norway (ÅRSVOLL 1975) including Svalbard (where *T. ishikariensis* and *S. borealis* have been found: HOSHINO *et al.* 2003) and Sweden (EKSTRAND 1955).

Most snow mold fungi in Scandinavia except for *T. ishikariensis* are airborne fungal pathogens. Basidiospores of *T. ishikariensis* were less effective propagules (CUNFER & BRUHL 1973, MATSUMOTO *et al.* 1982, MATSUMOTO & TAJIMI 1990). Therefore, *T. ishikariensis* has several infraspecific variations in geographical distribution. MATSUMOTO has divided *T. ishikariensis* in the Northern Hemisphere into two genetic types: biological species I and II (MATSUMOTO 1997, MATSUMOTO *et al.* 2001). Norwegian isolates have been divided into three groups (groups I, II and III) based on mating reactions and cultural morphologies (MATSUMOTO & TRONSMO 1995, MATSUMOTO *et al*. 1996). Biological species I include Norwegian strains of groups I and III, and biological species II include Norwegian group II. Strains of group I and II grow normally at 10˚C, but strains of group III show irregular growth at 10˚C. Cultural morphology of group III resembled that of other taxa at 0˚C (MATSUMOTO *et al*. 1996, HOSHINO *et al*. 1997). Group III strains showed higher degrees of frost resistance than did group I strains (HOSHINO *et al*. 1998, 2001). It is therefore thought that group III strains have higher adaptability to low temperature than do other groups. This hypothesis is supported by results showing that group III strains predominate in Finnmark, the northernmost part of Norway (MATSUMOTO & TRONSMO 1995, MATSUMOTO *et al*. 1996), Svalbard (HOSHINO *et al.* 2003), Greenland (HOSHINO *et al.* unpublished results) and central Siberia (HOSHINO *et al.* 2001).

T. ishikariensis has been found in and collected from Iceland (KRISTINSSON & GUÐLEIFSSON 1976), but there is not any information of the genetic characteristics or detailed information on the biological characteristics of *T. ishikariensis* in Iceland. Iceland is located in the central part of the Atlantic Ocean between Greenland and Scandinavia. ÅRSVOLL and SMITH (1978) showed that Norwegian isolates mated with isolates of biological species I (var. *ishikariensis*) and II (var. *idahoensis* and var. *canadiensis*) from North America. Therefore, elucidation of the genetic and biological characteristics of isolates from Iceland would be interesting from biogeographical viewpoints. In May 2000, we found and collected *T. ishikariensis* in Akureyri, Northern Iceland. In this paper, we describe the genetic and biological characteristics of isolates from Iceland.

MATERIALS AND METHODS

Isolation of *T. ishikariensis* from overwintering grass leaves.

Fungal sclerotia were collected from decayed leaves or stems of *Achuatherum pekinese*, *Dianthus monspessulanus, Elymus fibrosus*, *Luzula maxima* and *L. lutea* from the Akureyri Botanical Gardens on May 19-21, 2000 (Fig. 1). The fungal sclerotia were placed in paper envelopes and dried at room temperature during transportation. In the laboratory, the fungal sclerotia were surface-sterilized in 70% (v/v) ethanol and 0.5% (as active chlorine) sodium hypochlorite solution and thoroughly washed with sterilized distilled water. They were then cut with sterilized razor blades, placed on potato dextrose agar (PDA, Difco, Becton Dickinson Microbiology Systems, MD, USA) so that cut surfaces were in contact

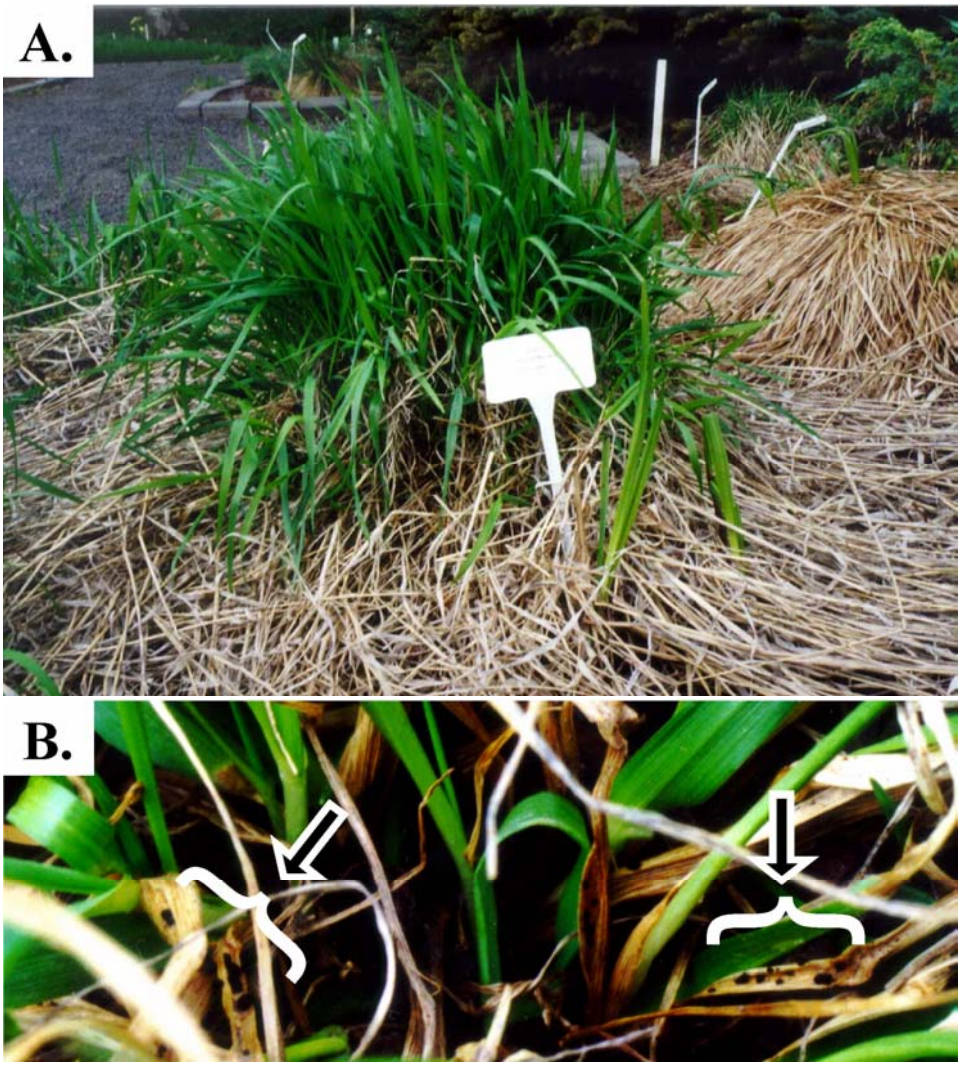

FIGURE 1. Sclerotia on overwintering grass leaves collected in Akureyri, Northern Iceland. A. Host plants in a botanical garden. B. Sclerotia of *T. ishikariensis*.

with the agar, and incubated at 5˚C. Mycelia from growing margins of colonies were transferred to new PDA plates (9 cm in diameter). All isolates were maintained on PDA slant cultures at 0˚C.

Production of basidiocarps.

Sclerotia that were formed in oatmeal agar (Difco) plates were placed in glass dishes (55 mm in I.D. x 45 mm in height) containing humid unsterile commercial artificial soil (Hokkai Sankyo Co. Ltd., Kita-Hiroshima, Japan) and sea sand (14-20 mesh, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Sclerotia were

incubated under light conditions of 12 h on (10˚C) and 12 h off (4˚C) for 2 months.

Mating experiments.
Basidiocarps were soaked separately in 500 µl autoclaved water in test tubes and kept overnight in a refrigerator (4˚C). The test tubes were shaken to remove basidiospores from the basidiocarps. The basidiospore suspension was appropriately thinned, spread on PDA plates containing lactic acid, and incubated at 10˚C for two weeks. Colonies with smooth hyphae (monokaryons) were subcultured on PDA slants at 0˚C.

Monokaryon of tester isolates (Table 2) and dikaryon of collected isolates were paired on PDA plates and incubated at 10˚C for 14 days, and a small agar block was cut out from monokaryon colonies near the junction of colonies and transplanted to another PDA plate. Growth from the block was then examined for the presence of clamp connections on hyphae 5 to 7 days later. The presence of clamp connections on hyphae was the criterion of compatibility of monokaryons with tester isolates.

TABLE 1. Basidiospore size of *Typhula ishikariensis* from Northern Iceland

	Length of basidiospores (µm)	Widths of basidiospores (µm)	Lenght/width
Tested isolates			
Large brown sclerotia forming isolates			
AKR-1	9.5 - 11.3 (av. 10.3)	3.0 - 4.1 (av. 3.5)	2.94
AKR-3	7.2 - 10.1 (av. 8.7)	3.5 - 4.7 (av. 4.4)	1.97
AKR-4		3.2 - 4.9 (av. 4.5)	1.96
Small black sclerotia forming isolates			
AKR-2	12.0 - 13.9 (av. 12.6)	3.6 - 4.8 (av. 4.2)	3.00
AKR-5	10.1 - 12.2 (av. 11.8)	2.9 - 4.3 (av. 3.6)	3.28

Each characteristic was examined using at least 10 basidiospores

Growth temperature of mycelia.
Mycelial discs of 5 mm in diameter were cut from the margin of an actively growing colony, transferred to the centers of PDA plates, and inoculated at 5 different temperatures from 0 to 20˚C, in duplicate. After 1, 2 and 3 weeks of inoculation, the colony diameters were measured. The linear mycelial growth rate per week was calculated after the initial lag period.

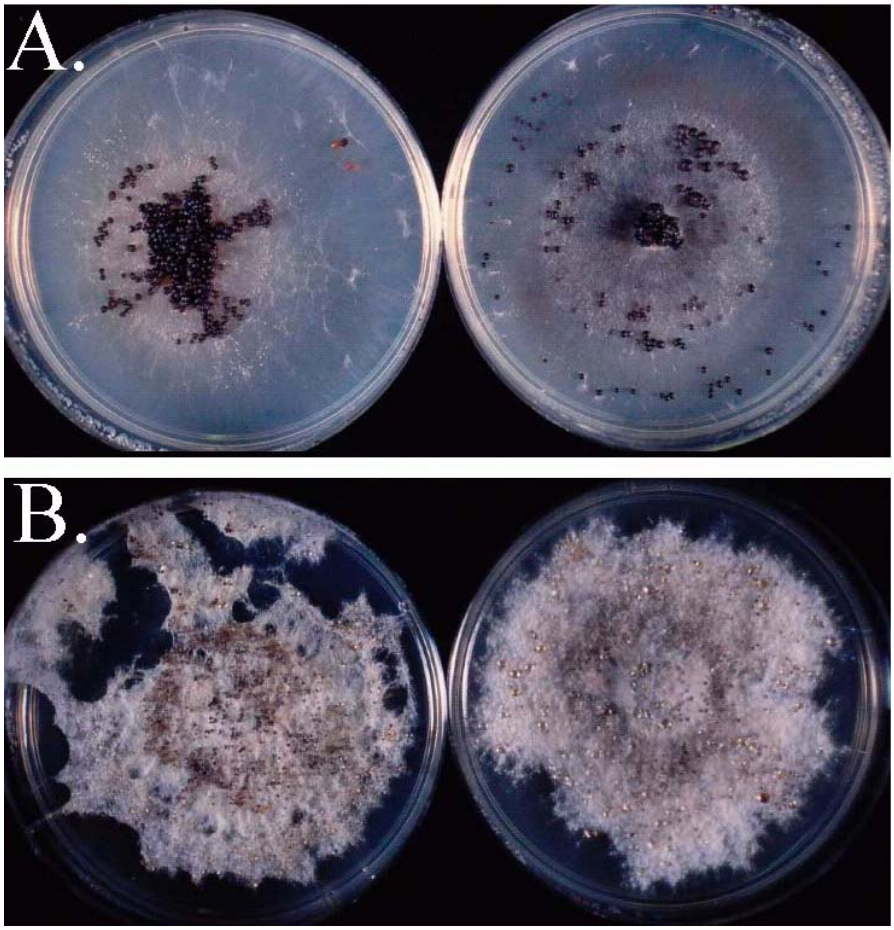

FIGURE 2. Macromorphologies of fungal colonies of isolates from Akureyri. A. Large brown sclerotia forming strains. An isolate of AKR-1 is shown on the left and an isolate of AKR-3 is shown on the right. B. Small and black sclerotia forming strains. An isolate of AKR-2 is shown on the left and an isolate of AKR-5 is shown on the right.

RESULTS AND DISCUSSION

Snow mold diseases of *Typhula ishikariensis* in overwintering grasses in Northern Iceland.

Three pathogenic *Typhula* species, *T. incarnata*, *T. ishikariensis* and *T. phacorrhiza*, have been found in Iceland (KRISTINSSON & GUÐLEIFSSON 1976). We found a few infections of *T. ishikariensis* in Akureyri, and we found fungal sclerotia in overwintering leaves of *Achuatherum pekinese*, *Dianthus monspessulanus, Elymus*

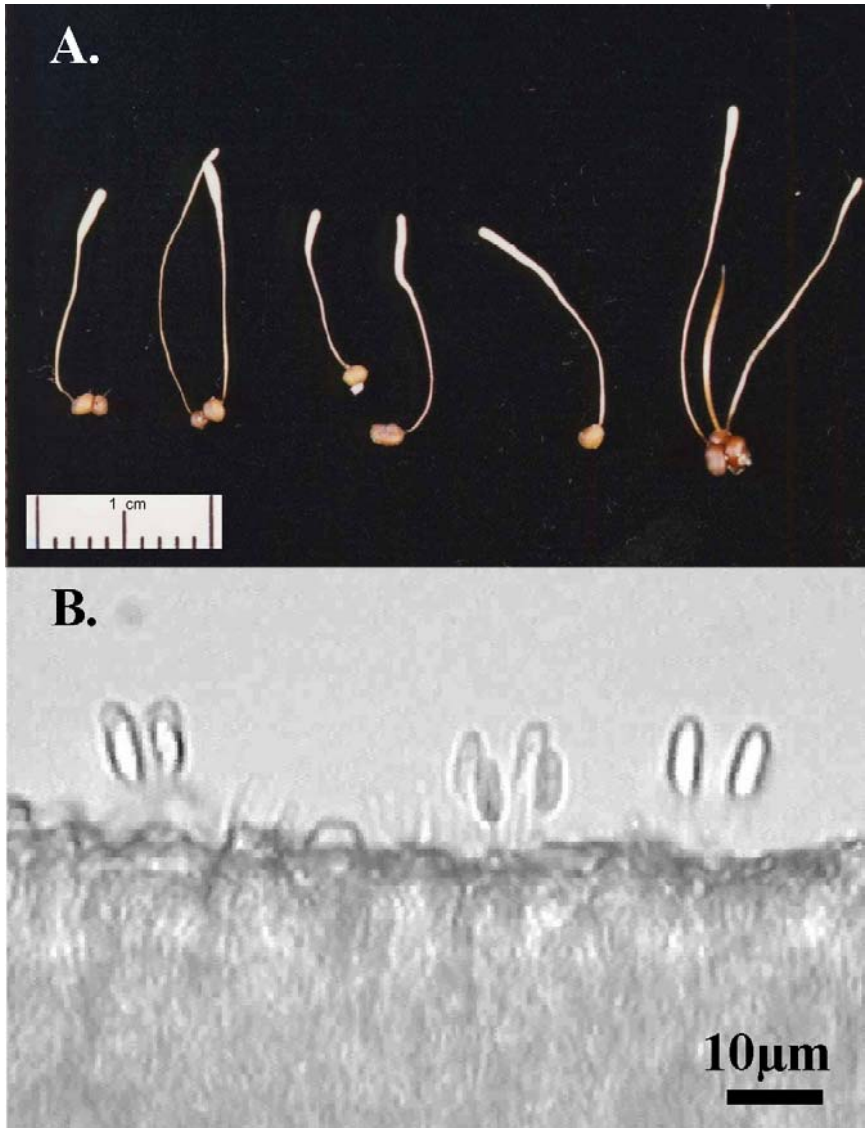

FIGURE 3. Basidiocarps and basidiospores of large brown sclerotia forming isolate (AKR-1) from Akureyri. A. Basidiocarps. B. Basidiospores with basidia.

fibrosus, *Luzula maxima* and *L. lutea* from Akureyri Botanical Gardens. The sclerotia were dark brown to black in color and globular to oval in shape and had formed on and in the leaves (Fig. 1). The diameter of collected sclerotia was 1.15 × 0.96 mm. The above description of the morphology of sclerotia is almost the same as that by KRISTINSSON & GUÐLEIFSSON (1976). *T. incarnata* was found throughout Iceland but *T. ishikariensis* was rarely found and *T. phacorrhiza* were

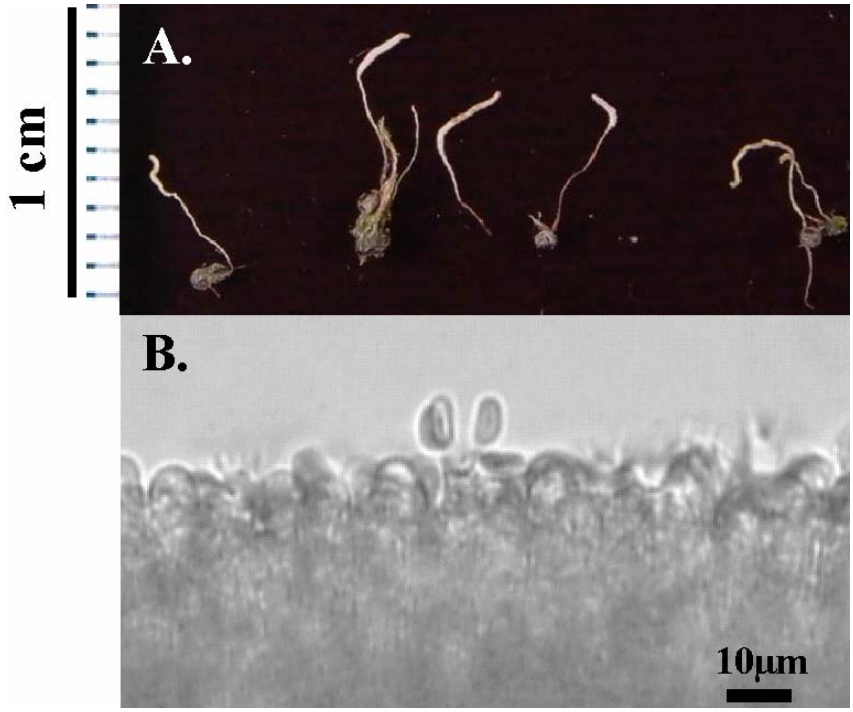

FIGURE 4. Basidiocarps and basidiospores of small black sclerotia forming isolate (AKR-5) from Akureyri. A. Basidiocarps. B. Basidiospores with basidia.

never found in our research expedition. We collected sclerotia of *T. ishikariensis* attached to five kinds of host plants in Akureyri Botanical Garden. All host plants of this fungus had been only slightly damaged by fungal infections, suggesting that snow mold diseases caused by *T. ishikariensis* do not have a great impact on overwintering grasses in Iceland.

Morphological characteristics of *T. ishikariensis* in Northern Iceland.
Macromorphologies of fungal colonies of collected isolates are shown in Fig. 2. The isolates were divided two types based on colony morphology and sclerotium size. Fungal colonies of three isolates (AKR-1, AKR-3, AKR-4) in PDA showed scatter hyphal extension (Fig. 2 A.). Sclerotia matured on PDA plates were globe-shaped and light to dark brown in color. The sizes of the sclerotia ranged from 0.65 to 1.83 mm, and the mean dry weight was 0.88 mg. Those colony and sclerotia morphologies are similar to those of Norwegian group I (biological species I).

Other types of isolates (AKR-2 and AKR-5) showed thick colonies and formed many aerial mycelia (Fig. 2 B.). Matured sclerotia were globe-shape and black in color. Sclerotium size was smaller than those of previous described strains. Diameters of the sclerotia were less than 1.5 mm (average size: 0.75 mm) and the mean dry weight was 0.19 mg. Those colony and sclerotia morphologies

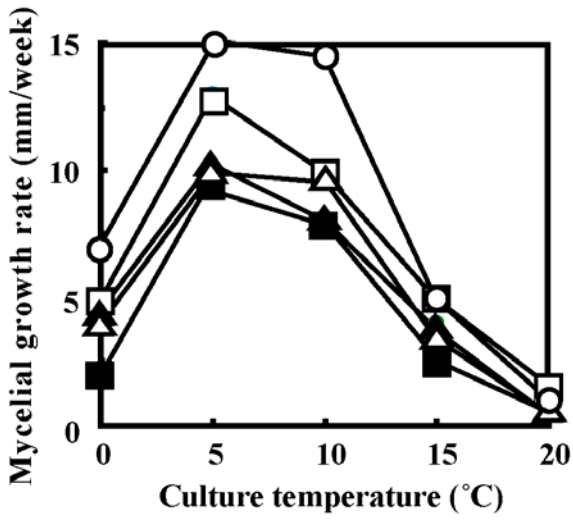

FIGURE 5. Effects of culture temperature on mycelial growth of *Typhula ishikariensis* from Akureyri. Open circles: AKR-1; open triangles: AKR-3; open squares: AKR-4; closed triangles: AKR-2; closed squares: AKR-5.

are similar to those of Norwegian group II and *T. ishikariensis* var. *canadiensis* (biological species II).

We succeeded in preparing basidiocarps of isolates that formed large brown sclerotia (AKR-1, AKR-3 and AKR-4) and small black sclerotia (AKR-2 and AKR-5). Basidiocarps and basidiospores of AKR-1 and AKR-5 are shown in Fig. 3 and Fig. 4, respectively. The basidiocarps of large brown sclerotia were 1.1 - 2.5 cm in height and separated from sclerotia. The heads of the basidiocarps were 2.5 - 5.5 mm long, 0.5 - 1.2 mm thick, long clavate or nearly linear, ivory white. Basidia had 4 basidiospores. Basidiospores of AKR-1 were ellipsoidal 10.3 × 3.5 µm, smooth. The basidiocarps of small black sclerotia that were smaller than those of large brown sclerotia, were 0.5 - 0.8 mm in height and also separated from sclerotia. The heads of basidiocarps were 1.0 - 2.1 mm long, 0.1 - 0.2 mm thick, long clavate or nearly linear, grayish white to gray white. Basidia had 4 basidiospores. Basidiospores of AKR-5 were ellipsoidal 11.8 × 3.7 µm, smooth.

Those morphological dimensions are similar to those reported by IMAI (1930), REMSBERG (syn. *T. idahoensis*, 1940) and EKSTRAND (syn. *T. borealis* and syn. *T. hyperborea*, 1955). EKSTRAND (1955) reported that the widths of basidiospores of *T. borealis* and *T. hyperborea* were different. Basidiospores of AKR-1, AKR-2 and AKR-5 were similar to those of *T. borealis*, and those of AKR-3 and AKR-4 were *T. hyperborea* type (Table 1). The specimens of fungal sclerotia and basidiocarps are kept in Akureyri Division, Icelandic Institute of Natural History.

Genetic characteristics of *T. ishikariensis* in Northern Iceland.

Isolates of AKR-1, AKR-3 and AKR-4 (large brown sclerotia forming strains) mated with tester monokaryons of biological species I, including Norwegian group III, and isolates of AKR-2 and AKR-5 (small black sclerotia forming strains) mated with biological species II (Table 2). Therefore, large brown sclerotia forming strains such as AKR-1, AKR-3 and AKR-4 belong to biological species I and small black sclerotia-forming strains such as AKR-2 and AKR-5 are

TABLE 2. Mating reaction of *Typhula ishikariensis* from Northern Iceland.

		Tested isolates				
Tester monocaryons	locality / genotype	AKR-1	AKR-2	AKR-3	AKR-4	AKR-5
Biological species I						
2-5BS-1	Norway/group I	+	–	–	–	–
4-6S-16	Norway/group III	+	–	+	+	–
PR7-6-7	Japan/biotype A	+	–	+	+	–
PR9-4-3	Japan/biotype A	+	–	+	+	–
92-32 m1	Russia/var. idahoensis	+	–	+	+	–
Biological species II						
4-3S-5	Norway/group II	–	+	–	–	+
35-8	Japan/biotype B	–	–	–	–	–
8-2	Japan/biotype B	–	+	–	–	+
Isolates from Northern Iceland						
monocaryon from AKR-1		+	–	+	+	±

+ : vigorous hyphae with clamps profuced; – : vigorous hyphae without clamps; ± : vigorous hyphae with a few clamps.

biological species II. Basidiospore morphologies of AKR-1 and other isolates mated with biological species I testers were different. Basidiospores of AKR-1, AKR-2 and AKR-5 were similar to *T. hyperborea*, and those of AKR-3 and AKR-4 were *T. borealis* type (according to the classification of EKSTRAND 1955). However, all isolates of dikaryons showed a similar mating pattern, and monokaryon from AKR-1 mated with dikaryons of AKR-3 and AKR-4. Therefore, spore size is not good marker of subgroups in *T. ishikariensis*.

T. ishikariensis is widely distributed in Europe, including areas outside Scandinavia. This fungus has been found in Baltic countries, Northern Ukraine and the European part of Russia (summarized by PROTATOSOVA 1960, TKACEHNKO *et al.* 1997), Germany (ANDRES *et al.* 1987), Poland (DYNOWSKA 1983, 1984), and Switzerland (SCHMIDT 1976). However, there are very few reports of genetic characteristics of *T. ishikariensis* in Europe. MATSUMOTO *et al.* reported that biological species I and II are distributed in Norway (1996). However, biological species II has not been found in Baltic countries, Northern Ukraine and the European part of Russia (TKACEHNKO *et al.* 1997), Svalbard (HOSHINO *et al.* 2002) or west Greenland (HOSHINO *et al.* unpublished results). We found biological species II in Iceland. This is only the second record of biological species II in Europe.

Thermal dependence of mycelial growth of *T. ishikariensis* in Northern Iceland.

Figure 5 shows the thermal dependence of mycelial growth of isolates collected in Northern Iceland. All isolates belonging to both biological species I and II showed the same optimum growth temperature, 5˚C. Isolates of AKR-1, AKR-3 and AKR-4 mated with tester monokaryon of Norwegian group III (Table 2), whose dikaryons showed irregular growth at 10˚C. However, Icelandic isolates of biological species I did not show these changes in mycelial morphology at 10˚C. Mating results indicated that those isolates had the same genetic background as that of Norwegian group III strains. The same results were obtained from Siberian isolates (HOSHINO *et al.* 2001). Thus, it is thought that Icelandic isolates of biological species I remained their ability to grow in a relatively moderate temperature range (above 10˚C).

ACKNOWLEDGMENTS

We thank Dr. Hörður Kristinsson, Icelandic Institute of Natural History, and Dr. Bjarni Guðleifsson, The Agricultural Research Institute (RALA) for their technical support in the collection of *T. ishikariensis* in Akureyri. We also thank Dr. Naoyuki Matsumoto, the National Institute for Agro-Environmental Sciences (Tsukuba, Japan) and Prof. Dr. Anne Marte Tronsmo, The Norwegian Crop Research Institute for their valuable comments. T. Hoshino also thanks The Research Council of Norway and Agency of Industrial Science and Technology (AIST in Japan) for the financial support of our research project.

REFERENCES

ANDRES, H., HINDORF, H., FEHRMANN, H., & TRÄNGER-BORN, J. 1987. Untersuchungen zum Auftreten und zur Verbreitung von Typhula-Arten an Wintergetreide im östlichen Franken und Bayerischen Wald. — Z. Pflanzenkrank. Pflanzenschutz 94: 491-499.

CUNFER, B.M. & BRUEHL, G.W. 1973. Role of basidiospores as propagules and observations on sporophores of *Typhula idahoensis*. — Phytophathology 63: 115-120.

DYNOWSKA, M. 1983. Badania nad grzybami z rodzaju *Typhula* Fr. emend Karst. pochodzacymi z terenu województwa olsztynskiego I (in Polish). — Acta Mycol. 19: 283-296.

DYNOWSKA, M. 1984. Badania nad grzybami z rodzaju *Typhula* II. Właściwości biologiczne (in Polish). — Acta Mycol. 20: 71-194.

EKSTRAND, H. 1955. Höstsädens och vallgräsens överwintering. — Stat. Växtskyddsanst., Medd. 67: 1-125.

HOSHINO, T., GAARD, M., KIRIAKI, M. & YUMOTO, I. 2004. A snow mold fungus *Typhula incarnata* from the Faroe Islands. — Acta Bot. Isl. 14: 71-76.

HOSHINO, T., SAITO, I. & TRONSMO, A.M. 2003. Two new snow mold fungi from Svalbard. — Lidia 6: 30-32.

HOSHINO, T., TKACHENKO, O.B., TRONSMO, A.M., MORITA, N., OHGIYA, S., ISHIZAKI, K. & MATSUMOTO, N. 2001. Temperature sensitivity and freezing resistance among isolates of *Typhula ishikariensis* from Russia. — Icel. Agr. Sci. 14: 61-65.

HOSHINO, T., TRONSMO, A.M., MATSUMOTO, N., OHGIYA, S. & ISHIZAKI, K. 1997. Effect of temperature on growth and intracellular proteins of Norwegian *Typhula ishikariensis* isolates. — Acta Agric. Scand. Sect.B, Soil and Plant Sci. 47, 185-189.

HOSHINO, T., TRONSMO, A.M., MATSUMOTO, N., ARAKI, T., GEORGES, F., GODA, T., OHGIYA, S. & ISHIZAKI, K. 1998. Freezing resistance among isolates of psychrophilic fungus, *Typhula ishikariensis*, from Norway. — Proc. NIPR Symp. Polar Biol. 11, 112-118.

HSIANG, T, MATSUMOTO, N. & MILLETT, S.M. 1999. Biology and management of *Typhula* snow molds of turfgrass. — Plant Dis. 83: 788-798.

IMAI, S. 1930. On the Clavariaceæ of Japan. II. — Trans. Sapporo Nat. Hist. Soc. 11: 70-77.

JAMALAINEN, E.A. 1949. Overwintering of *Gramineae*-plants and parasitic fungi. I. *Sclerotinia borealis* Bubák & Vleugel. — J. Sci. Agr. Soc. Fin. 21: 125-142.

JAMALAINEN, E.A. 1957. Overwintering of *Gramineae*-plants and parasitic fungi. II. On the *Typhula* sp-.fungi in Finland. — J. Sci. Agr. Soc. Fin. 29: 75-81.

KRISTINSSON, H. & GUÐLEIFSSON, B.E. 1976. The activity of low temperature fungi under the snow cover in Iceland. — Acta Bot. Isl. 4: 44-57.

MATSUMOTO, N. 1997. Evolution and adaptations in snow mold fungi (in Japanese). — Soil Microorganisms 50: 13-19.

MATSUMOTO, N., SATO, T. & ARAKI, T. 1982. Biotype differentiation in the *Typhula ishikariensis* complex and their allopatry in Hokkaido. — Ann. Phytopathol. Soc. Jpn. 48: 275-280.

MATSUMOTO, N. & TAJIMI, A. 1990. Continuous variation within isolates of *Typhula ishikariensis* biotype B and C associated with habitat differences. — Can. J. Bot. 68: 1768-1773.

MATSUMOTO, N., TKACHENKO, O.B. & HOSHINO, T. 2001. The pathogenic species of *Typhula*. — Low Temperature Plant Microbe Interactions Under Snow. 49-59.

MATSUMOTO, N. & TRONSMO, A.M. 1995. Population structure of *Typhula ishikariensis* in meadows and pastures in Norway. — Acta Agric. Scand. Sect.B, Soil and Plant Sci. 45, 197-201.

MATSUMOTO, N., TRONSMO, A.M. & SHIMANUKI, T. 1996. Genetic and biological characteristics of *Typhula ishikariensis* isolates from Norway. — Eur. J. Plant Pathol. 102, 431-439.

PETROV, V.F. 1983. Pathogenic microflora of root growth in perennial grasses in Khibiny (in Russian). — Bull. Appl. Bot., Genet. Plant Breed. 82: 38-45.

PROTATOSOVA, E.G. 1960. Fungi in *Typhula* genus in the USSR (in Russian). — Bot. J. 45: 562-572.

REMSBERG, R.E. 1940. Studies in the genus *Typhula*. — Mycologia 40: 52-96.

SCHMIDT, D. 1976. Observations sur la pourrituer des neiges affectant les graminées. — Rev. Suisses Agric. 8: 8-15.

TKACHENKO, O.B., MATSUMOTO, N., & SHIMANUKI, T. 1987. Mating patterns of east European isolates of Typhula ishikariensis with isolates from distant region. — Mycol. Phytopathol. 31: 68-72.

WELLING, V.B. & JENSEN, A. 1970. Sneskimmel i græsplæner 1969/70. — Stat. Plantepathol. Forsøg. 451: 45-50.

ÅRSVOLL, K. 1975. Fungi causing winter damage on cultivated grasses in Norway. — Meld. Norg. Landbrukshøgskole 54 (9): 49 pp.

ÅRSVOLL, K & SMITH, J.D. 1978. *Typhula ishikariensis* and its varieties, var. *idahoensis* comb. nov. and var. *canadiensis* var. nov. — Can. J. Bot. 56: 348-364.