European Journal of Plant Pathology **107:** 761–765, 2001.

Short communication

Occurrence of *Pythium ultimum* var. *ultimum* in a greenhouse on Spitsbergen Island, Svalbard

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Accepted 5 July 2001

Key words: carrot, damping off, rDNA ITS sequences, the Arctic zone

Abstract

Pythium ultimum var. *ultimum* was isolated from carrot seedlings with damping off and from soil used for growing the plant in a greenhouse on Spitsbergen Island, Svalbard. The fungus caused severe damping off of carrot, cucumber and tomato seedlings after artificial inoculation. The rDNA internal transcribed spacer sequences of the Svalbard isolate were identical to those of Canadian and Japanese isolates of *P. ultimum* var. *ultimum*. The results suggest that the pathogen in the greenhouse on Svalbard was probably introduced from temperate regions through contaminated plants and/or soil imported to the island. This is the first record of *P. ultimum* var. *ultimum* within the Arctic zone.

Vegetables cultivated in greenhouses can be affected by damping off and root rot diseases caused by *Pythium* spp. Diseases caused by these fungi are more common in regions with warm climates (van der Plaats-Niterink, 1981), but they have also caused damage in greenhouses in colder regions. *P. aphanidermatum* (Edson) Fitzp. and *P. irregulare* Buisman have been found in British Columbia, Canada (Favrin et al., 1988), and *P. aphanidermatum*, *P. irregulare*, *P. paroecandrum* Drechsler and *P. ultimum* Trow var. *ultimum* in Norway (Herrero, 2000). High soil temperatures and abundant moisture, identified as the two most important factors for infection by *Pythium* spp. (Hendrix and Campbell, 1973), are common in greenhouses. Such environments may contribute to the introduction of *Pythium* spp. to colder regions, but specific cases have rarely been demonstrated. Barentsburg is a Russian mining town on Spitsbergen Island, Svalbard in the Arctic zone and has a greenhouse for cultivating vegetables for the self-sufficiency of the town (Gnilorbova and Ivanova, 1988). In the summer of 1999, severe damping off of carrot seedlings was observed in the greenhouse. *P. ultimum* var. *ultimum*, an important temperate-region pathogen on many plants (van der Plaats-Niterink, 1981), was consistently isolated from diseased plants. Damping off caused by *P. ultimum* var. *ultimum* is not only important to the local economy, but is also notable for its epidemiology in the Arctic zone. Our study elucidates that a warm greenhouse allows the pathogen to widen its distribution area into the Arctic zone. The morphology, pathogenicity and sequence of the rDNA internal transcribed spacer (ITS) gene of the isolate of *P. ultimum* var. *ultimum* in the greenhouse on Svalbard will be described.

The roots of carrot seedlings with damping off disease were collected from a greenhouse in Barentsburg (78°4′ N, 14°14′ E), Spitsbergen Island, Svalbard on August 5, 1999. The plants were cultivated in a concrete frame containing a sand-based soil. Samples of the damaged plants were washed in tap water, air dried and incubated on water agar plates at 15–20 °C. Fungal mycelia growing on the plates were transferred to grass leaf culture (Martin, 1992) and corn meal agar (CMA; Difco). Morphological identification was based on the keys of van der Plaats-Niterink (1981). Eleven *Pythium* isolates were obtained from 10 root samples of

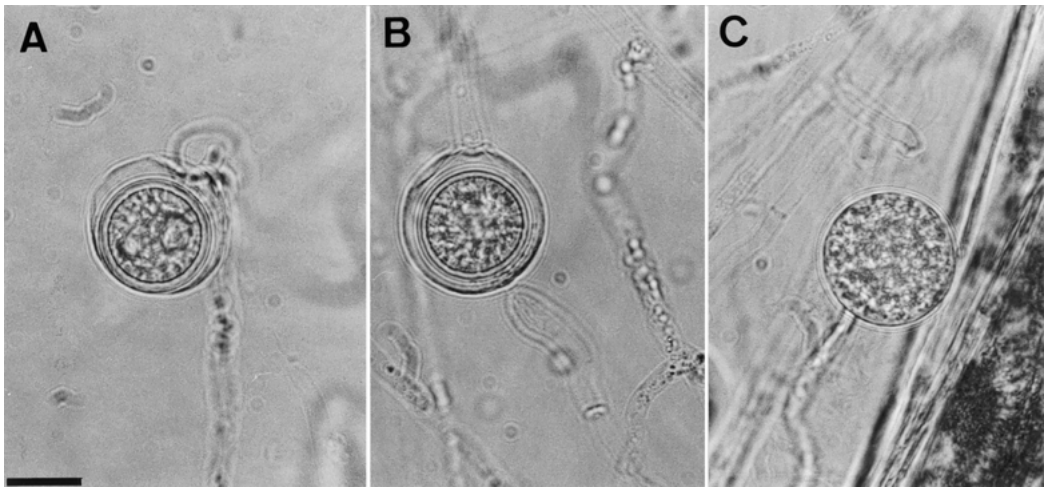

Figure 1. Sexual organs and hyphal swellings of *Pythium ultimum* var. *ultimum*, cause of damping off of carrot seedlings in a greenhouse on Svalbard. (A) Terminal oogonium and aplerotic oospore with monoclinous antheridium. (B) Terminal oogonium and aplerotic oospore with diclinous antheridium. (C) Terminal globose hyphal swellings.

plants with damping off disease. All the isolates were morphologically similar. One isolate (OPU451 = CBS 102344) was selected for detailed morphological analysis. Thirty for each of the structures described were examined with light microscopy. The main hyphae were up to 11 μm wide. Oogonia were terminal or intercalary, globose, smooth-walled, 15.1–23.8 μm (mean = 20.9 μm) in diameter (Figure 1A and B). Antheridia were terminal, 1 or rarely 2 per oogonium, sac-like, mostly monoclinous originating from immediately below the oogonium (Figure 1A), sometime diclinous (Figure 1B). Oospores were single, aplerotic, globose, 12.5–19.8 μm (mean 17.0 μm) diameter. The thickness of the oospore wall ranged from 1.0 to 1.7 μm (mean 1.5 μm). Hyphal swellings were globose or subglobose, terminal or intercalary, 9.3–22 × 7.5–22 μm (Figure 1C). Zoospores were not formed at 5, 10, 15 and 20 °C on transfer of the grass leaf culture to water. Optimal growth of mycelia occurred at 25 °C, the minimum temperature for the growth was 4 °C, and the maximum was 34 °C. The daily growth rates were 35.2 mm at 25 °C, 1.2 mm at 4 °C, and 27.5 mm at 34 °C on potato carrot agar (PCA). Colonies on CMA were submerged and had a radiate pattern, but on PCA formed cottony, aerial mycelium without a special pattern. Based on these characteristics, isolate OPU451 was identified as *P. ultimum* Trow var. *ultimum* and it was concluded that all the isolates were the same species.

Pathogenicity of isolate OPU451 was examined by the methods of Herrero (2000) with slight modifications on carrot (*Daucus carota* L. cv. Nantes duke), cucumber (*Cucumis sativus* L. cv. Rhinsk drue) and tomato (*Lycopersicon esculentum* Mill. cv. Totem). Eight seeds of each host were sown in a pot ($8 \times 8\,cm^2$) containing a fertilized, limed peat mixture (P-jord, Emmaljunga Torvmull AB, Sweden). The pots were placed in a growth chamber at 21 ± 1 °C with continuous light (46–65 $\mu mol\,m^{-2}\,s^{-1}$ measured at the level of the plants) and irrigated daily with tap water. When most of the seeds had germinated and had open cotyledons, the plants were thinned

Table 1. Pathogenicity of *Pythium ultimum* var. *ultimum*, isolate OPU 451, isolated from a greenhouse in the Svalbard after artificial inoculation on carrot, cucumber and tomato

Host	Mortality (%)[a]	
	Inoculated	Noninoculated
Carrot	37.5 ± 14.1	0
Cucumber	95.3 ± 3.8	0
Tomato	20.8 ± 9.2	0

[a]Mortality was evaluated in the growth chamber at 21 ± 1 °C with continuous light (46–65 $\mu mol\,m^{-2}\,s^{-1}$ measured at plant level). Six pots were used for each host plant. Each pot held 4 seedlings of the plant. Plants were inoculated 6 days after sowing for carrot, 4 days after sowing for cucumber and tomato. The mortality was recorded as the number of seedlings showing damping off 4 days after inoculation. ± represents standard errors ($n = 6$).

to 4 plants per pot. Plants were inoculated 6 days after sowing for carrot and 4 days after sowing for cucumber and tomato. Agar plugs (10-mm diameter) from cultures grown at 25 °C for 48 h on potato dextrose agar (PDA; Difco) plates were used for the inoculation. A plug was placed near each seedling, avoiding direct contact. Six pots of plants for each host were inoculated. Six pots with noninoculated seedlings acted as controls. The pots were randomized within the chamber. The number of seedlings with damping off was scored and rated as percentage mortality 4 days after inoculation. The isolate caused damping off of carrot, cucumber and tomato seedlings within 3 days after inoculation. Cucumber had the most severe symptoms (Table 1).

To investigate fungal contamination of the soil in the greenhouse, the soil used for growing carrot was examined by the baiting method described by Watababe (1989) with slight modifications. At least 10 soil cores (2 cm diam. × 3 cm long) were collected at 10-cm intervals from the site on August 5, 1999. Two hundred gram of the soil was combined as a composite sample and

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Svalbard  ccacacttta aaaaactgtc cacgtgaact gtaagcaagt ctagcgctgt gactgagctg
Canada    .......... .......... .......... .......... .......... ..........
Japan     .......... .......... .......... .......... .......... ..........

          gtgttttcat ttttggacac tggaacggga gtcagcagga cgaaggttgg tctgttgtaa
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          tgcaagttat gatggactag ctgatgaact tttgttttta aacccttacc taaatactga
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          tttatactgt ggggacgaa  gtccttgctt ttactagata acaactttca gcagtggatg
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          tctaggctcg cacatcgatg aagaacgctg cgaactgcga tacgtaatgc gaattgcaga
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          attcagtgag tcatcgaaat tttgaacgca tattgcactt tcgggttatg cctggaagta
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          tgtctgtatc agtgtccgta aatcaaactt gcctttcttt ttctgtgtag tcagggatgg
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          aatgtgcaga tgtgaagtgt ctcgcatggt tgcgttcgtt ttttcgatcg agaatctgtc
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          gagtcctttt aaatggacac ggtcttttct atggtttcta tgaagtgtaa tggttggaag
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          gcagtgattt tcggattgct ggcggctttt ggcgacttcg gtatgaacgt atggagacta
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          gctcaattcg tggtatgtta ggcttcggct cgacaatgtt gcgtaattgt gtgtggtctt
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          tgtttgtgcc ttgaggtgta ctagaggttg tcggtttgaa ccgtaagtga ttgtttagta
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          gagcatttc  acgatgtatg gagacgctgc atttagttgc gtagagagat tgatttggga
          .......... .......... .......... .......... .......... ..........
          .......... .......... .......... .......... .......... ..........

          aattttgtat cattgtcaat tgcaagattg tgtatggtat ctcaa
          .......... .......... .......... .......... .....
          .......... .......... .......... .......... .....
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Figure 2. Alignment of the sequences of ITS1-2 and 5.8 S rDNA region of *Pythium ultimum* var. *ultimum* isolated from Svalbard (isolate OPU451), Canada (isolate CBS730.94) and Japan (isolate OPU407). Residues identical to the sequence of Svalbard isolate are indicated as dots.

kept at 5–10 °C for 2 weeks. Sixty mililiter of the sample was taken from the composite sample and poured into two Petri dishes. Twenty cucumber seeds (cv. Rhinsk drue) were buried in each dish. The soil was moistened with distilled water and incubated at 15 °C for 3 days. After incubation, seeds were washed in tap water for 1 h, air dried, and incubated on the *Pythium*-selective medium (10 mg pimaricin, 250 mg ampicillin, 10 mg rifampicin, 100 mg pentachloronitrobenzene and 17 g CMA powder (Difco) per liter of deionized water) (Kannwischer and Michell, 1978) at 15 °C for up to 2 days. All 40 isolates obtained from 40 bait seeds were identified as *P. ultimum* var. *ultimum*.

To confirm the species identification of the isolate of *P. ultimum* var. *ultimum*, sequences of rDNA ITS regions were examined and compared to those of temperate-region isolates of the species. The fungal isolates used were: OPU451 described earlier, CBS730.94 from bean in Canada and OPU407 from spinach in Japan. The ITS regions containing ITS1 and ITS2 and intervening 5.8 S rDNA were amplified by using a Parkin Elmer 9700 thermal cycler (Parkin Elmer Cetus Corporation, Emeryville, CA). The primers ITS5 and ITS4 (White et al., 1990) were used. The amplification program consisted of pre-denaturalization at 95 °C for 5 min, 35 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, and final incubation at 72 °C for 7 min to complete the last extension. The final products were purified using the QIAquick PCR Purification Kit (QIAGEN, CA). Sequence reactions were performed using primers ITS1, ITS2, ITS3 and ITS4 (White et al., 1990). The reactions were run on a 310 DNA sequencer (Parkin Elmer Cetus Corporation, Emervyville, CA). The sequences of the ITS 1-2 and 5.8 S rDNA regions of the Svalbard isolates were identical to those of Canadian and Japanese isolates of *P. ultimum* var. *ultimum* (Figure 2). The main features of the sequences are: 825 bp; 194A; 133C, 217G and 281T. Location ITS1 from nucleotide 1 to 218; gene 5.8 S rRNA from nucleotide 219 to 380; ITS2 from nucleotide 381 to 825. It has been deposited in the European Molecular Biology Laboratory (EMBL) as AJ319725.

It has been shown that *P. ultimum* var. *ultimum* occurred in the greenhouse on Spitsbergen Island, Svalbard. The species was frequently found on diseased carrot seedlings and in soil used for growing the plant in the greenhouse. This species has also been isolated from soil and water in Iceland in the subarctic zone (Johnson, 1971). To our knowledge, this is the first record of *P. ultimum* var. *ultimum* in the Arctic zone.

Cardinal temperatures for hyphal growth and the sequences of the rDNA ITS gene of the Svalbard isolate of *P. ultimum* var. *ultimum* corresponded with those of temperate-region isolates of the species. In other investigations, the species has not been found in soils and moss colonies (*Sanionia uncinata* (Hedw.) Loeske) in natural environments in Svalbard (Tojo, unpublished). *P. ultimum* var. *ultimum* in the greenhouse on Svalbard, therefore, was probably introduced from temperate regions through contaminated plant and/or soil imported to the island. Although several *Pythium* spp. have commonly been found in greenhouses of Canada (Favrin et al., 1988) and Norway (Herrero, 2000), no *Pythium* spp. other than *P. ultimum* var. *ultimum* was found in the greenhouse on Svalbard. This suggests that the pathogen spread inside the greenhouse from very limited source such as a few numbers of the diseased plants. Considering the severe cold stress and absence of host plants in the natural environment (Hisdal, 1998), the pathogen is only likely to survive on Svalbard under greenhouse conditions.

Acknowledgements

This study was supported in part from Japan Society for the Promotion of Science by the grant-in-aid for scientific research (No. 13780437).

References

Favrin RJ, Rahe JE and Mauza B (1988) *Pythium* spp. associated with crown rot of cucumbers in British Colombia greenhouses. Plant Disease 72: 683–687

Gnilorbova N and Ivanova G (1988) Pod Nebom Arktiki. Planeta, Moscow

Hisdal V (1998) Svalbard Nature and History. Norsk Polarinstitutt, Oslo

Hendrix FF Jr and Campbell WA (1973) Pythiums as plant pathogens. Annual Review of Phytopathology 73: 77–98

Herrero ML (2000) *Pythium* spp. and *Phytophthora* spp. in some horticultural crops in Norway. PhD Thesis of Agricultural University of Norway

Johnson TW Jr (1971) Aquatic fungi of Iceland: *Pythium*. Mycologia: 517–536

Kannwischer ME and Michell DJ (1978) The influence of a fungicide on the epidemiology of black shank of tobacco. Phytopathology 68: 1760–1765

Martin FN (1992) *Pythium*. In: Singleton LL, Mihail JD and Rush CM (eds) Methods for research on soilborne phytopathogenic fungi (pp 39–49) APS Press, St. Paul

van der Plaats-Niterink, AJ (1981) Monograph of the genus *Pythium*. Studies in Mycology 21: 1–242

Watanabe T (1989) Kinds, distribution, and pathogenicity of *Pythium* species isolated from soils of Kyushu island in Japan. Annals of the Phytopathological Society of Japan 68: 697–698

White TJ, Bruns T, Lee S and Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ and White TJ (eds) PCR Protocols. A Guide to Methods and Applications (pp 315–322) Academic Press, San Diego