Isolation of *Trichoderma* spp. at Low Temperatures from Tennessee and Alaska Soils

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ABSTRACT

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Trichoderma spp. were isolated from soils from Tennessee and Alaska on a selective medium at 25, 12, or 10 C. Cold-tolerant species (those isolated at 10 or 12 C) were *T. pseudokoningii* and *T. harzianum* from Tennessee and *T. viride* from Alaska. Densities of cold-tolerant propagules were about seven times greater in Alaska soils than in Tennessee soils. Cold-tolerant strains of *T. viride* are widespread in Alaska; they were isolated from soils from southern, central, and northern Alaska.

Use of *Trichoderma* spp. as agents for biological control of plant diseases is well documented. Reductions in severity have been obtained with diseases caused by *Rhizoctonia solani* Kühn, *Pythium* spp., *Sclerotium rolfsii* Sacc., *Armillaria mellea* (Vahl ex Fr.) Kummer, *Sclerotium cepivorum* Berk., and *Verticillium dahliae* Kleb. In addition, the suppressiveness of certain soils to soilborne pathogens has been attributed to native or introduced *Trichoderma* spp. (3,14). Limited success has been obtained with diseases that are more severe under conditions of low temperature. Thus, *Trichoderma hamatum* (Bon.) Bain. protected pea seedlings from damping-off induced by *Pythium* spp. at temperatures of 17–30 C but not 12 C (8). Isolates of *Trichoderma* capable of growing at low temperatures may be effective in suppression of diseases that are severe at low temperatures. Tronsmo and Dennis (17) found that strains of several species of *Trichoderma* produced both nonvolatile and volatile fungal inhibitors at 10 C. In another study, *T. koningii* Oud. (T-8) grew faster at 15 and 20 C than did *T. harzianum* Rifai (T-12) and provided better protection of pea seed from rot than did T-12 both in growth chamber tests at 25 C and in field tests where lower temperatures occurred (7). Soilborne diseases that are most severe at 20 C or lower include damping-off and root rots of plants caused by *Pythium* spp., damping-off diseases caused by "low-temperature" strains of *R. solani*, late blight of potato, and snow mold of cereals and grasses caused by *Fusarium nivale* (Fr.) Ces. (1,9,10,18).

The objectives of this study were to determine if cold-tolerant strains of *Trichoderma* spp. could be isolated from Alaska soils and to compare their identi-

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ties and isolation frequencies with cold-tolerant strains from western Tennessee soils.

MATERIALS AND METHODS

Soil samples were collected in western Tennessee from two cultivated fields in June and from 11 fields in October 1985. Fifteen or more subsamples from each field were taken with a hand trowel to a depth of 8–15 cm. Subsamples were bulked by field and stored at room temperature in sealed plastic bags.

During July and August 1985, soil samples were collected from 520 sites in southern and central Alaska by the second author. Additional samples were collected by Jay McKendrick, Alaska Agricultural and Forestry Experiment Station, Palmer, from 20 sites in the Prudhoe Bay area of northern Alaska. Samples were also collected from seven sites near Ambler, Noorvik, and Kotzebue in northwestern Alaska by Gina Delucchi, Mannilaq Native Association. Most of the samples were taken from sites under natural vegetation. Nine were from cultivated fields, of which two samples were from southern Alaska and seven were from potato fields in northwestern Alaska. Each sample consisted of about 1 kg of soil collected with a shovel or soil-sampling tube to a depth of 30 cm (or to permafrost if less than 30 cm). Samples were placed in plastic bags and air-mailed to the Plant Pathology Laboratory at Knoxville, TN, where they were stored at room temperature. Eighty-two of the samples were selected for assay of *Trichoderma* spp. (Fig. 1). Selected samples were chosen to reflect diverse vegetation characteristics and/or locations.

A 1:100 (w/v) dilution of each sample with sterile, distilled water was prepared, and 1 ml of the dilution was applied to the surface of the selective medium described by Papavizas and Lumsden (15) in each of 10 petri plates. The plates were rotated to distribute soil particles over the surface, placed in paper bags, and incubated at 10, 12, or 25 C. After 10 days at 25 C or after 35 days at 10 and 12 C, the plates were examined and colonies suspected as *Trichoderma* spp. were transferred to culture tubes containing a weak cornmeal agar medium prepared with 10 g/L of cornmeal (11). Identification of *Trichoderma* to species aggregates was according to Rifai (16).

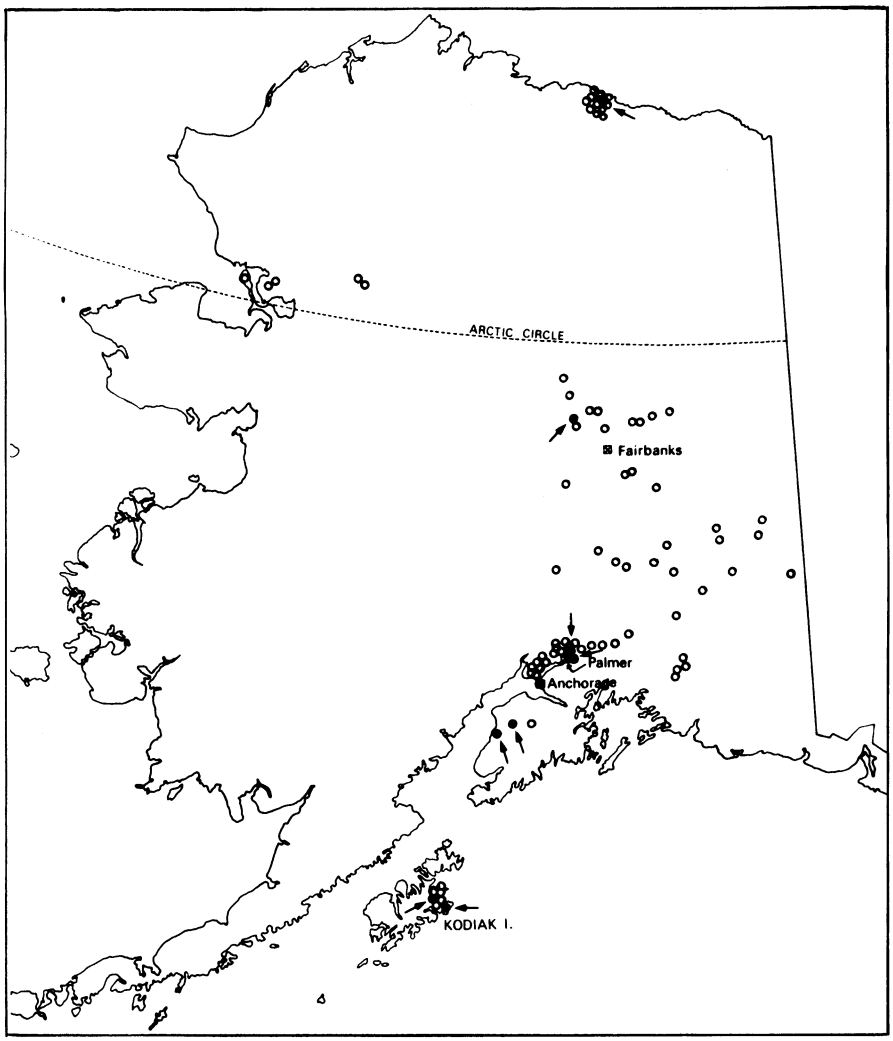

Fig. 1. Distribution of Alaska soils from which *Trichoderma viride* was isolated at 10 C: • (with arrow) = *T. viride*-positive site, and o = *T. viride*-negative site.

Table 1. Effect of incubation temperature on isolation of *Trichoderma* spp. and other fungi from Tennessee soils on a *Trichoderma*-selective medium[a]

		Colonies of fungi per plate[b]		Colonies of *Trichoderma* per plate[b]	
Soil no.	Crop	10 C	25 C	10 C	25 C
Am-1	Cotton	127	158	0.0	40
Am-2	Soybean	49	185	0.0	12
Am-3	Soybean	181	299	0.0	4
Am-4	Soybean	35	173	0.3	3
J-1	Cotton	6	116	0.0	2
J-2	Clean fallow	10	163	0.0	2
J-3	Cotton	23	213	0.0	7
J-4	Cotton	6	387	0.0	20
J-5	Cotton	9	168	0.0	7
M-1	Soybean	17	137	0.0	11
M-2	Cotton	39	157	0.0	6

[a] Isolated on Papavizas and Lumsden's (15) selective agar medium; 1 ml of a 1:100 (w/v) dilution of soil with water was spread over the medium surface in each plate.
[b] Isolation plates were incubated for 35 days at 10 C and for 10 days at 25 C. Values are means of 10 plates each. Colonies per plate × 100 = colonies per gram of soil.

RESULTS

An initial preliminary assay of two soil samples collected in June 1985 from western Tennessee was performed at 12 C. Two colonies of *T. pseudokoningii* Rifai were among 50 plates of a 1:100 dilution of soil Am-1, and 12 colonies of *T. harzianum* were among 50 plates of soil J-5. Subsequent assays for cold-tolerant strains of *Trichoderma* were conducted at 10 C.

Assays of soils collected from 11 fields in western Tennessee in October 1985 yielded an average of 196 colonies of fungi per plate on the selective medium after 10 days at 25 C (Table 1). An average of 10 colonies per plate were identified as *Trichoderma* spp. Considerably fewer fungal colonies (a mean of 46 per plate, a 77% reduction) developed on plates incubated at 10 C after 35 days of incubation. Three colonies, identified as *T. pseudokoningii*, were isolated; all three were from soil Am-4.

After 35 days of incubation at 10 or 12 C, most of the colonies of *Trichoderma* were 10–18 mm in diameter. Green or yellow-green sporulating areas were

Table 2. Sites and locations of Alaska soils from which *Trichoderma viride* was isolated on selective agar medium incubated at 10 C for 35 days[a]

Soil no.	Colony-forming units of *Trichoderma*/g soil	Site characteristics	Location
23	410	Willow, sandy soil with gravel	Matamuska river bank, 1.6 km E of Palmer
26	460	Mixed grasses, meadow	Knik River Road, 10 km SE of Palmer
123	640	Squirrel-tail and other grasses, meadow	19 km N of Minto
258	10	Vetch, ocean beach	Kodiak Island, 5 km S of Kodiak
286	10	Fescue and other grasses, meadow	Kodiak Island, 3 km W of Kodiak
345	70	Rye hayfield, cultivated	Kenai Peninsula, Scott Lake Recreation Area
352	10	Spruce forest, with understory of rose and other shrubs	Kenai Peninsula, Skyland Trail
521	150	Grass (*Arctophylla fulva*), wet tundra	Prudhoe Bay, West Dock area

[a] Isolated on Papavizas and Lumsden's (15) selective medium; 1 ml of a 1:100 dilution (w/v) of soil with water was spread over the medium surface in each plate. Ten plates of each soil were assayed.

sometimes visible on colony surfaces, more often on plates incubated at 12 C. On plates incubated at 10 C, it was often necessary to examine colonies microscopically (100× with transmitted light) to discern sporulation. Spores could not be detected on some colonies. Nonsporulating colonies were usually thin and flat, white to very light tan, and slightly opaque.

Eight of the 82 Alaska soil samples yielded colonies of *Trichoderma* on plates incubated for 35 days at 10 C (Table 2, Fig. 1). Site and vegetation characteristics were diverse, ranging from an ocean beach with vetch, to a spruce forest, to a grassy wet tundra. *Trichoderma*-positive soils in southern Alaska were from Kodiak Island, Kenai Peninsula, and the Palmer area. One sample from central Alaska, near Minto (northwest of Fairbanks), contained 640 propagules per gram, the highest concentration in any of the soils assayed at low temperature from Alaska or Tennessee. One of the 12 samples assayed from the North Slope (Prudhoe Bay area) contained *Trichoderma*. *Trichoderma* was not isolated from the five samples assayed from northwestern Alaska. Colonies of *Trichoderma* from the eight soils were transferred to pure culture and examined microscopically. Forty-five such isolates were examined and all were identified as *T. viride* Pers. ex S. F. Gray.

When compared with soils from Tennessee, Alaska soils contained about twice as many propagules of cold-tolerant fungi capable of growing on the selective medium (Table 3). The two states differed considerably in quantities of cold-tolerant propagules of *Trichoderma* in *Trichoderma*-positive soils (seven times greater in Alaska soils). Also, on the basis of total weights of all soils used in the assays, quantities of cold-tolerant propagules of *Trichoderma* were about seven times greater in Alaska than in soils in Tennessee.

DISCUSSION

Considerable numbers of fungal colonies other than *Trichoderma* developed on Papavizas and Lumsden's selective medium (15). Most were 2 mm or less in diameter, especially on isolation plates containing 50 or more colonies. Interference with identification of *Trichoderma* was minimal. Colonies of *Trichoderma* were larger, had green or yellow-green sporulating areas (especially at 12 C), or had distinctive flat, opaque surfaces. Isolation plates of soil Am-1, for example, had an average of 158 colonies of fungi, yet 40 of these were easily identified as *Trichoderma*.

Cold-tolerant species of *Trichoderma* differed in Tennessee and Alaska. Two species, *T. pseudokoningii* and *T. harzianum*, were isolated from Tennessee soils at 10 and 12 C, respectively. Only one species, *T. viride*, was isolated from Alaska soils. There are few reports of *Trichoderma* isolated from Alaska soils. *T. viride* was isolated from soil in southeastern Alaska near Juneau (5), from soil within the Katmai National Monument in southwestern Alaska (2), and from a maritime tundra soil in southwestern Alaska (4). As far as we can ascertain, species other than *T. viride* have not been reported from Alaska. From studies of soil fungi found in alpine and in lowland areas of Virginia, North Carolina, and the state of Washington, Danielson and Davey (6) concluded that *T. viride* is restricted to regions with cool soils and that *T. harzianum* is commonly found in soils of warmer climates.

In previous studies of fungi from arctic or antarctic soils, *Trichoderma* was not isolated from Barrow, Alaska (12), Devon Island, Northwest Territories, Canada (19), or Signy, South Orkney (13). To our knowledge, isolation of *T. viride* in the present study from a tundra soil at Prudhoe Bay represents the first report of *Trichoderma* north of latitude 70° N.

Cold-tolerant strains of *T. viride* apparently are widespread in Alaska soils. They are more prevalent in southern Alaska but were found in soil in central Alaska and in a tundra soil in extreme northern Alaska. Cold-tolerant strains of *Trichoderma* spp. also occur in soils in regions of warmer climates. Whether cold tolerance is linked to increased biocontrol potential at lower temperatures is yet to be determined.

Table 3. Summary of comparative values for isolation of *Trichoderma* spp. on a selective medium at 10 C from Tennessee and Alaska soils[a]

Variable	Tennessee soils	Alaska soils
Soils assayed (no.)	11	82
Soils from which *Trichoderma* spp. were isolated (no.)	1	8
Fungal colonies/plate:		
Minimum	6	1
Maximum	181	276
Mean	46	103
Colony-forming units of *Trichoderma*/g in *Trichoderma*-positive soils (mean/soil)	30	220
Colony-forming units of *Trichoderma*/g in all soils[b]	3	21

[a] Isolated on Papavizas and Lumsden's (15) selective agar medium; 1 ml of a 1:100 dilution (w/v) of soil with water was spread over the medium surface in each plate.
[b] Based on total weights of all soils used in dilution plates.

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