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Psychrophylic Deuteromycetes from alpine habitats

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Summary: Five species of Deuteromycetes and five sterile mycelia are described from glacier cones, snow fields and from an alpine pond at the glacier front. The temperature requirements of the isolates and their ability to degrade cellulose, lignin, proteins, and starch have been tested. All species can be described as psychrophilic. The optimum growth temperature lies at ca 10° C and the upper limit for growth at ca 20° C. With the exception of *Myrioconium* sp. 2, the hyphae of all isolates cannot survive temperatures above 25° C for long periods of time. A spore survival experiment has shown that the conidia of *Leptodontidium obscurum* and *Myrioconium* sp. 1 are killed by an exposure to 25° C for eight days, whereas conidia of *Microsphaeropsis* sp. and *Myrioconium* sp. 2 remain viable after 30 days at 25° C. Conidia of *Heteroconium* cf. *chaetospira* remain viable after short periods of exposure to 25° C, but they are killed after 30 days at this temperature. Degradation of cellulose, starch, and protein is common among the isolates studied. The production of peroxidases has been demonstrated for 13 out of 18 isolates.

Zusammenfassung: Es werden fünf Deuteromyceten-Arten und fünf sterile Myzelien beschrieben, die aus Gletschervorfeldern, Schneefeldern und aus einem Teich am Gletscherende isoliert worden sind. Substratabbauvermögen und Temperaturansprüche der Isolate sind getestet worden. Alle Arten sind psychrophil, mit einem Temperaturoptimum um ca 10° C und -maximum um ca 20° C. Mit der Ausnahme von *Myrioconium* sp. 2 können die Hyphen keines Isolates bei Temperaturen oberhalb 25° C während längerer Zeit überleben. Konidien von *Leptodontidium obscurum* und *Myrioconium* sp. 1 sind nach 8 Tagen bei 25° C nicht mehr keimfähig; Konidien von *Microsphaeropsis* sp. und *Myrioconium* sp. 2 keimen immer noch, nachdem sie 30 Tage bei 25° C inkubiert worden sind. Konidien von *Heteroconium* cf. *chaetospira* dagegen sind nicht mehr keimfähig. Die meisten Stämme sind in der Lage, sowohl Cellulose und Stärke als auch Proteine abzubauen. Peroxidasen werden von 13 der 18 untersuchten Stämme gebildet.

Résumé: Cinq espèces de Déuteromycètes et cinq mycélia stérils, isolés de cones glaciaires, de champs de neige et d'un lac alpin au voisinage d'un glacier, sont décrites. Les conditions requises de température des isolats ainsi que l'aptitude à dégrader la cellulose, la lignine, les protéines et l'amidon ont été testées. Toutes les espèces peuvent être classifiées comme psychrophiles. L'optimum de température pour la croissance se situe à env. 10° C et la limite supérieure à env. 20° C. A l'exception de *Myrioconium* sp. 2, les hyphes de chaque souche ne survivent pas à des températures supérieures à 25° C durant une periode prolongée. Une incubation durant 8 jours à 25° C tue les conidies de *Leptodontidium obscurum* et *Myrioconium* sp. 1

alors que celles de *Microsphaeropsis* sp. et *Myrioconium* sp. 2 restent viables après 30 jours à 25° C. Les conidies d'*Heteroconium* cf. *chaetospira* conservent leur viabilité après de courtes périodes d'incubation à 25° C mais ne sont plus viables après 30 jours d'incubation à cette température. La dégradation de la cellulose, de l'amidon et des protéines parmi les souches étudiées est commune. La production de peroxidases a été démontrée pour 13 des 18 isolats.

Introduction

Soil and water micro-organisms from the alpine regions may be exposed to widely fluctuating temperatures. Communities sharing the same habitat have adapted to these conditions and include eurythermal as well as stenothermal species, with thermophilic, thermotolerant, mesophilic and psychrophilic taxa.

Most of the studies made on arctic-alpine microorganisms are limited to the analysis of bacterial (Boyd & Boyd 1972; Lauwers & Heinen 1982) and yeast communities (e.g., Babieva & Azieva 1980; Vishniac 1988). A limited number of investigations has been devoted to larger fungi (e.g., Laursen & Ammirati 1981, 1982; Watling 1983) and only scant information exists on microfungi isolated from cold habitats (Dowding & Widden 1974; Gray 1982; Gray et al. 1982; Hurst et al. 1983; Latter & Heal 1971; Pugh & Allsopp 1982).

The presence of psychrophilic or psychrotrophic fungi in arctic soils has been demonstrated by several authors (Latter & Heal 1971; Dowding & Widden 1974). To our knowledge, however, no report exists on cold tolerant or psychrophilic ascomycetes and deuteromycetes isolated from alpine soils.

This preliminary study reports some species of deuteromycetes which have been isolated from glacier cones, snow fields and from an alpine pond at the glacier front. The temperature requirements of the isolates and their ability to degrade cellulose, lignin, proteins, and starch have been tested.

Material and methods

Samples were taken from eight sites. In 1981 a sample composed of soil and plant debris (mainly moss remains and algae) covered by the melting water from a large snow field near Lei Alv (Fex valley, Canton Grison, Switzerland) was collected; in 1983 three samples were taken from sites situated on the Grindelwald glacier (Canton Berne, Switzerland), two from the bed of an effluent creek and one from a pond located approx. 1.6 km from the same glacier; in 1984 one additional sample was taken from the Grüebu glacier, above Saas-Balen (Canton Wallis, Switzerland). Soil and water temperatures were measured in each case and found to be less than 2° C except for the sample collected from the pond near the Grindelwald glacier (8° C).

Several grams of each sample were collected with a sterile spatula, placed in sterile plastic vials kept in an ice-cooled box and processed within 24 hours. Soil dilution plates containing 2% malt extract, 0.4% yeast extract and 2% agar, supplemented with 50 mg/L Tetracyclin were prepared.

Table 1. Fungal species and sterile strains isolated. G1-G3: Grindelwald glacier, sites 1 to 3; CG: effluent creek of Grindelwald glacier; P: Pond near Grindelwald glacier;GR: Grüebu glacier; LA: Lei Alv. The number of strains studied for each species is given.

Taxon	G1	G2	G3	CG	P	GR	LA
Heteroconium cf. *chaetospira*	—	1	—	—	—	—	—
Leptodontidium obscurum	—	—	—	—	—	1	1
Microsphaeropsis sp.	—	—	—	1	—	—	—
Myrioconium sp. 1	2	5	1	—	—	—	—
Myrioconium sp. 2	—	—	—	1	—	—	—
sterile #1 to 5	—	3	—	1	1	—	—

The plates were incubated for ten weeks at 4° C and the growing fungal colonies isolated onto 2% malt extract agar slants. Yeasts, yeast-like organisms and bacteria were discarded: no colony counts were performed. The slants were incubated for three to ten weeks at 4° C until substantial growth was observed. For each fungal taxon or morphologically distinctive mycelium isolated from a given sample only few strains were kept. These were then transferred onto fresh slants and incubated at 4, 15, and 24° C. Only those strains with optimum growth temperatures at 4 or 15° C were kept for further investigations.

Growth was studied on 2% malt extract agar (MA), potato dextrose agar (PDA: Difco), cornmeal agar (CMA: Difco), and on Czapek-Dox agar (Czapek: Oxoid).

To test temperature requirements 90 mm Petri dishes containing 2% malt extract agar were inoculated with a disk of mycelium 5 mm diam., taken from the margin of a four week old culture grown on the same medium. The plates were incubated at -3, 0, 3, 7, 10, 15, 20, and 25° C in the dark and checked after 14 and 28 days. Measurements of colony diameters were made after 28 days. Cultures which failed to grow at a given temperature were transferred at the end of the experiment to the optimum growth temperature.

A spore survival test was conducted for all sporulating isolates. Five hundred µL of a sterile conidial suspension containing approx. 10^7 conidia/mL were spread onto 90 mm MA Petri dishes. Three replicates each were incubated at 10° C and at 25° C. Germination was checked after 1, 8, 14, and 30 days: since no germination of conidia ever occurred at 25° C, one plate of each isolate was transferred from 25° C to 10° C after each checking.

Cellulose degradation was tested as described by Smith (1977), using Cellulose Azure (Calbiochem no. 219481) as substrate. The oxidation of phenolic compounds was investigated using the Bavendamm test (Nobles 1965) as modified by Carroll & Petrini (1983). Amylolytic activity was tested by the method of Kjøller & Struwe (1980) with 1% corn starch as a substrate. Determination of protease activity (degradation of Promine-D) followed the method described by Petrini et al. (1984).

A culture of each species used in this study is deposited in the SANDOZ fungus collection. Dried specimens are kept at ZT.

Results

Taxonomic position of the strains isolated

Eighteen psychrophilic fungal isolates were obtained. Thirteen of them sporulated and could be assigned to five distinct taxa (Table 1). The other five isolates could not be differentiated by morphological characters but showed distinct physiological requirements and are referred to as sterile strains #1–#5 throughout this paper. Species descriptions that matched the morphology of the isolates could not be found in the literature. Although they may be new species, we feel that additional specimens should be examined before any formal description is made and we have chosen to only describe their cultural and morphological characters.

Heteroconium **cf** ***chaetospira*** (Grove) M.B. Ellis (Fig. 1e).

Cultures on MA 35 mm diam after 10 d at 10° C, in the centre grey-brown to olivaceous, with felty to woolly aerial mycelium, at the margin black, slimy and mycelium immersed in the substrate, margin strongly lobed; reverse black; on CMA culture grey-brown, margin entire and aerial mycelium appressed; on PDA olivaceous, margin entire and aerial mycelium appressed; on Czapek grey-brown, margin lobed and aerial mycelium appressed. Fructifications abundant all over the surface. Conidiogenesis holoblastic, conidia borne directly on the hyphae, (14)18–30 x 2.5–3 µm, 1–3–septate, narrowly cylindrical, tapering towards both truncate ends, hyaline, in acropetal chains.

The isolates are morphologically close to *H. chaetospira* but their straight conidia and their peculiar habitat do not allow unequivocal assignation to this taxon.

Leptodontidium obscurum (de Hoog) de Hoog (Fig. 1a, b).

Cultures on MA up to 20 mm diam. after 10 d at 10° C, white with dark grey to olivaceous areas and a smooth to shiny surface, appressed to the substrate, margin entire, aerial mycelium usually absent, when present white and felty; reverse cream-coloured; on CMA, PDA and Czapek white, margin entire, aerial mycelium absent; sporulating only on MA and CMA. Fructifications first in restricted dark green to olivaceous spots, later expanding over larger areas. Conidiophores up to 30 µm, branched, hyaline, conidiogenesis holoblastic, sympodial, conidiogenous cells 7–16 x 2 µm, cylindrical, tapering towards the apex, hyaline. Conidia 3–4 x 2.5–3 µm, ovoid with truncate base, dark green to olivaceous, thick-walled, sometimes 1–2–catenate, produced apically in a more or less sympodial order.

The conidiogenesis in these isolates could be at first sight be interpreted as enteroblastic, thus suggesting species of *Acremonium* Sect. *Gliomastix* (Gams 1971). Closer inspection of the conidiogenous cells, however, reveals that it is holoblastic, although no scars can be seen on the conidia or on the conidiogenous cells. The colour of the conidia does not correspond to that of the original description. The dark, catenate chlamydospores typical for *L. obscurum* (de Hoog 1977) have never been observed during this study.

Microsphaeropsis sp. (Fig. 1c, d).

Cultures on MA and CMA less than 20 mm diam after 10 d at 10° C, cream-coloured to hyaline, with lobed margin, aerial mycelium absent; reverse in the centre black, white to hyaline towards the margin; on PDA white, margin entire, with woolly aerial mycelium; on Czapek white, margin entire, aerial mycelium absent. Conidiomata on MA up to 400 µm in diameter, ovoid to globose, peridium brown with textura prismatica, cells 9–14 x 7–11 µm, uniloculate, developing in the centre of the colony. Conidiophores undifferentiated, conidiogenesis enteroblastic, phialidic, conidiogenous cells 3.5–10 x 3–5 µm, broadly cylindrical, tapering towards the apex, with small collarette, hyaline. Conidia 6–8.5 x 3–4 µm, oblong with rounded ends, light brown to brown.

Sutton (1971) has discussed in detail the nomenclatural status of *Coniothyrium* Cda. and *Microsphaeropsis* Höhnel. He has proposed to use *Microsphaeropsis* for species with phialidic conidiogenesis and retain only species with annellides in *Coniothyrium*. Since more than 800 species have been described in the *Microsphaeropsis/Coniothyrium* complex, we refrain from describing a new species until a major revision of both genera is available.

Myrioconium sp. 1 (Fig. 2a, b).

on MA 25 mm diam after 10 d at 10° C, cream-coloured to apricot, with smooth and slightly shiny surface, appressed to the substrate, margin entire, aerial mycelium usually absent or poorly developed around the inoculum, white to cream-coloured; reverse cream-coloured to apricot; fructifications all over the colony; on CMA white to cream-coloured, margin entire, aerial mycelium absent, rarely present and then scant, appressed; on PDA white to pink or apricot, margin entire and aerial mycelium appressed; on Czapek white to cream-coloured, margin entire, aerial mycelium usually absent, when present scant and woolly. Conidiophore stipes not differentiated, conidiogenesis enteroblastic, phialidic, conidiogenous cells 5–12 x 2–4 µm (without collarette), ampulliform to cylindrical, hyaline, with funnel-shaped, hyaline, 2–4 x 1.5–4 µm long collarette, developing singly or a few together on undifferentiated hyphae or on up to 10 µm thick hyphal strands. Conidia (2)3–3.5 x 2–3 µm, ovoid to ellipsoidal, with small truncate base, hyaline, borne singly, sometimes aggregated in droplets.

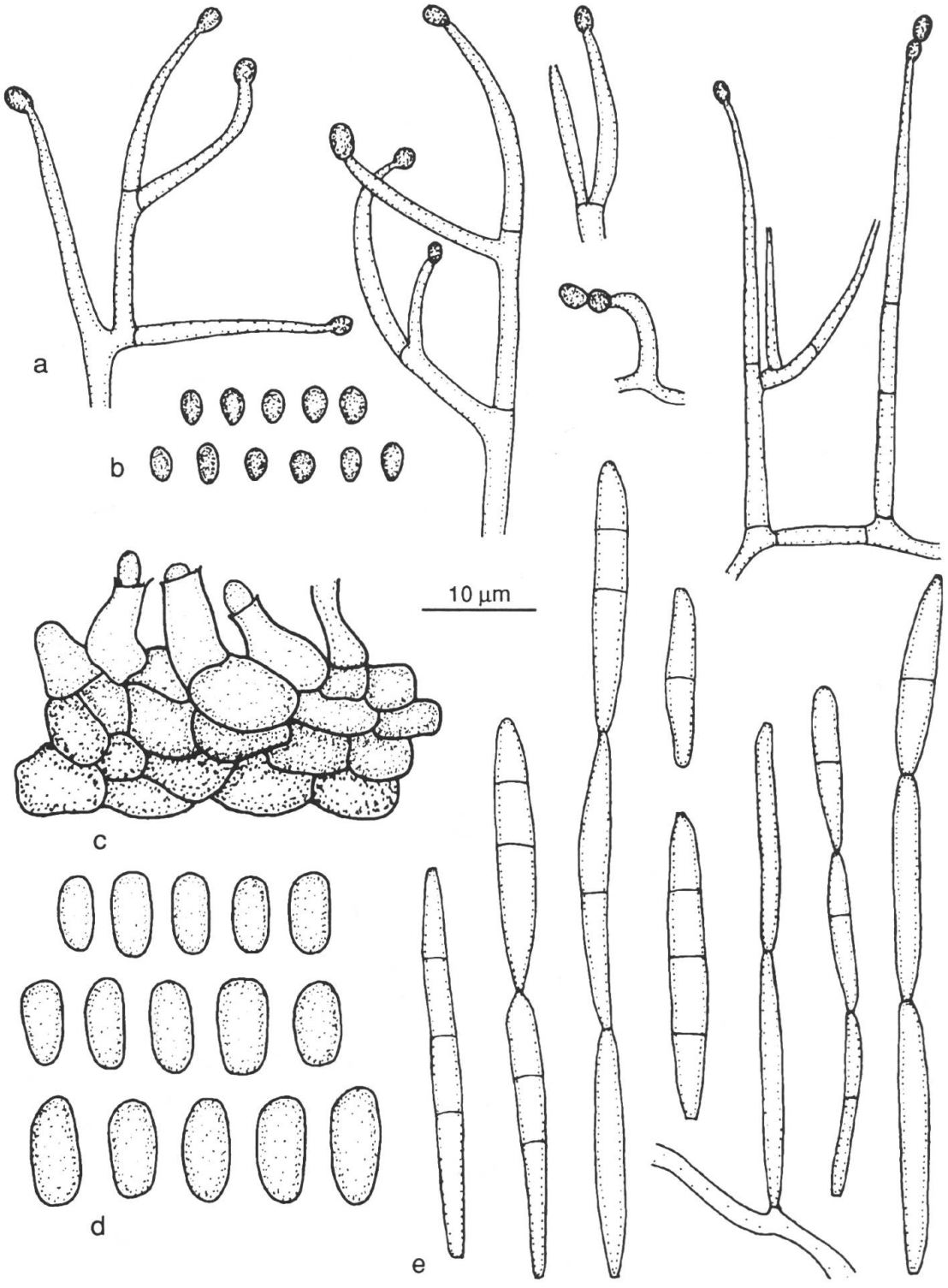

Figure 1: *Leptodontidium obscurum*: a, conidiophores; b, conidia. – *Microsphaeropsis* spec.: c, conidiogenous cells; d, conidia. – *Heteroconium* cf. *chaetospira:* e, conidia and fertile hypha.

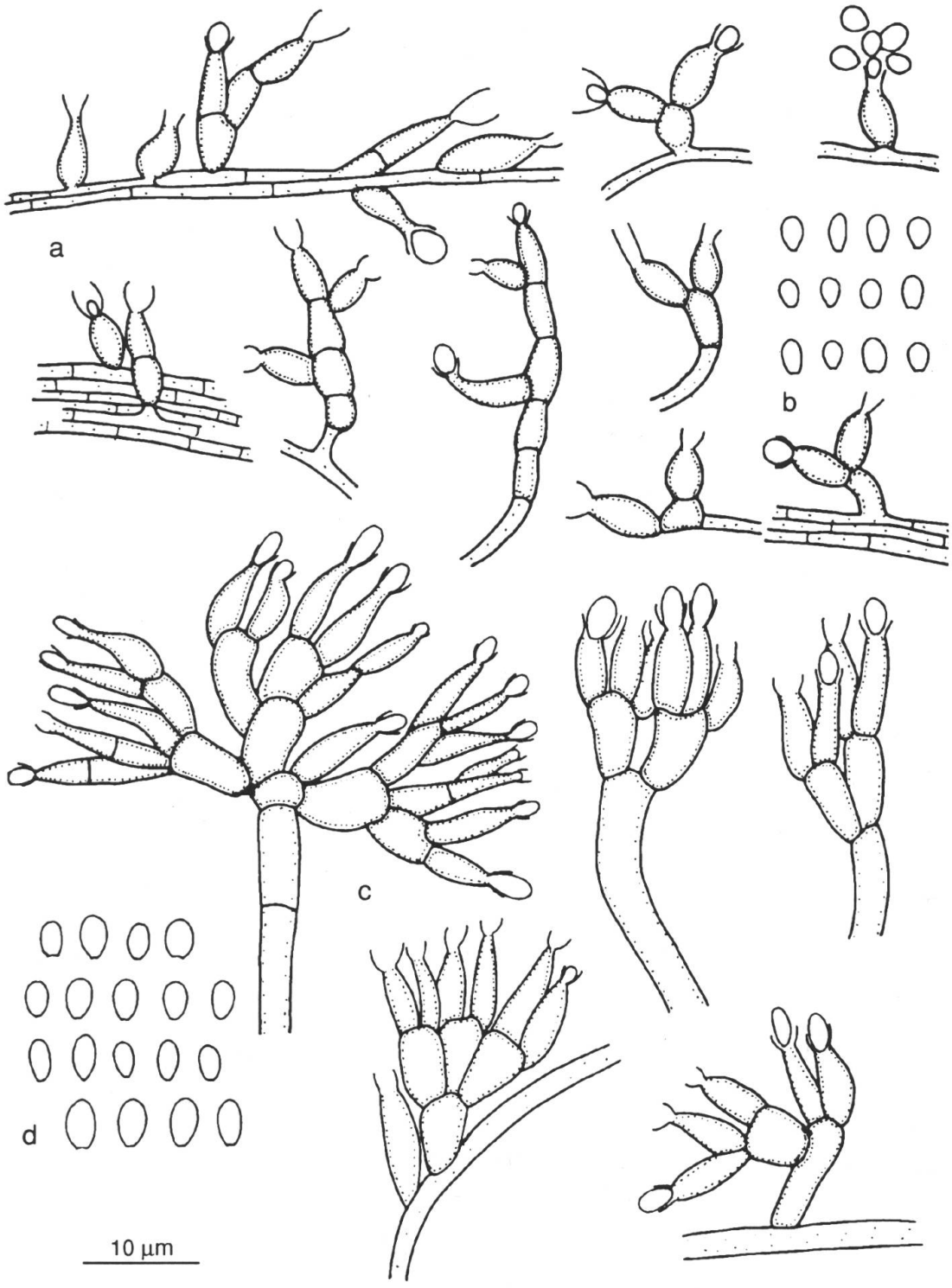

Figure 2: *Myrioconium* sp. 1: a, conidiogenous cells and conidiophores; b, conidia. – *Myrioconium* sp. 2: c, conidiophores; d, conidia.

Myrioconium Syd. is the spermatial state of some *Sclerotinia* species (von Arx 1974) and is quite similar to some *Phialophora* spp. *Myrioconium* cultures, however, are usually light-coloured. No synanamorphic form (*Botrytis* Pers.: Fr.) or sclerotia were formed in our isolates.

Myrioconium sp. 2 (Fig. 2c, d).

Cultures on MA up to 50 mm diam after 10 d at 10°C, white to cream-coloured, with smooth and slightly shiny surface, appressed to the substrate, margin entire, aerial mycelium poorly developed, felty; reverse cream-coloured; fructifications in restricted areas, appearing as white slimy droplets. On CMA and Czapek culture white, margin entire, aerial mycelium absent; on PDA white, margin entire, aerial mycelium appressed. Conidiophores up to 30 µm, short, brush-like, developing on single hyphae, conidiogenesis enteroblastic, phialidic, conidiogeneous cells 6–10(12) x 2.5–4 µm (without collarette), cylindrical to ampulliform, hyaline, with funnel-shaped, 3–6 x 4–5 µm long collarette. Conidia 3–4 x 2.5–3 µm, ovoid to ellipsoidal, with small truncate base, hyaline, borne singly.

Gams & Holubová-Jechová (1976) described *Phialophora brachyconia* W. Gams and *P. hyalina* W. Gams as having cultures similar to the one formed by our isolate, but these two taxa are clearly distinct because of their morphological characters (both form conidia in chains) and different optimum growth temperature.

Physiological tests

When the criteria of Morita (1975) for psychrophilic and psychrotrophic bacteria are applied to the strains studied, all species can be described as strictly psychrophilic.The optimum growth temperature lies at 10° C and the upper limit for growth at ca. 20° C. With the exception of *Myrioconium* sp. 2, the hyphae of all strains cannot survive temperatures above 25° C for prolonged periods of time (Table 2). The conidial survival experiment confirms a better heat tolerance of *Myrioconium* sp. 2. The conidia of *Leptodontidium obscurum* and of *Myrioconium* sp. 1 are killed by exposure to 25° C for eight days, whereas more than 70% of the conidia of *Microsphaeropsis* sp. and *Myrioconium* sp. 2 are still viable after 30 days at 25° C. Conidia of *Heteroconium* cf. *chaetospira* can tolerate short periods of exposure to 25° C, but this temperature is lethal to all conidia after 30 days (Table 3). Almost all fungi were able to degrade at least two of the substrates used in the substrate utilization tests. The production of peroxidases, as demonstrated by the Bavendamm test, has been shown for 13 out of 18 isolates (Table 4). Intraspecific variation in the substrate utilization by conspecific fungal isolates reflects genetic heterogeneity within the fungal populations studied (Carroll & Petrini 1983).

Table 2. Temperature requirements of the fungal species isolated.

	Minimum	Optimum	Maximum	Lethal
Heteroconium cf. *chaetospira*	0	10	20	25
Leptodontidium obscurum	0	10	20	25
Microsphaeropsis sp.	3	10	20	25
Myrioconium sp. 1	0	10	20	25
Myrioconium sp. 2	-3	10	20	30
sterile # 1	3	10	20	25
sterile # 2	3	15	20	25
sterile # 3	-3	10	20	25
sterile # 4	-3	10	20	25
sterile # 5	-3	10	20	25

Table 3. Conidia survival experiment. Germination of conidia at 10° C, 25° C, and at 10° C after exposure to 25° C for 8, 14, and 30 days (10 trans). +: germination and growth of colonies; – no germination.

Taxon	10	25	10 trans		
			8 days	14 days	30 days
Heteroconium cf. *chaetospira*	+	–	+	+	–
Leptodontidium obscurum	+	–	–	–	–
Microsphaeropsis sp.	+	–	+	+	+
Myrioconium sp. 1	+	–	–	–	–
Myrioconium sp. 2	+	–	+	+	+

Discussion

The isolation method used allows good selection for mesophiles, psychrotrophs and psychrophilic organisms (see also Gow & Mills 1984). Incubation of dilution plates at low temperatures has been successfully used by other authors (e.g. Gray 1982; Gray *et al.* 1982) to isolate cold-tolerant nematophagous fungi from antarctic soils: however, subsequent incubation of the growing organisms at higher temperatures is necessary to distinguish true psychrophilic isolates from the psychrotrophic ones. Vishniac (1988) noted that psychrophily in yeasts can be a generic character. This seems not to be the case for most other fungi, because most species of *Leptodontidium*, *Microsphaeropsis* and *Myrioconium* are mainly mesophilic organisms (Gams 1971; Sutton 1980). Psychrophily very likely results from selection in cold ecosystems: nevertheless, psychrophilic organisms can be expected to co-exist with mesophilic organisms in soils and in plants of temperate regions, where their spores can survive warmer periods. This may be the case for *Leuconeurospora pulcherrima* (Winter) Malloch & Cain (von Arx 1978) and for species of *Nectria*, *Nectriella*, and *Hyponectria*, low-temperature tolerant ascomycetes

Table 4. Substrate utilization tests on psychrophilic fungi. Ratings are based on subjective estimates of relative degrees of activities. –, no activity; (+), very weak activity; +, moderate activity; ++, strong activity.

Taxon	Bavendamm	Cellulose	Protein	Starch
Heteroconium cf. *chaetospira*	+	–	–	–
Leptodontidium obscurum	–	+	+	+
Microsphaeropsis sp.	+	(+)	+	–*
Myrioconium sp. 1	+¶	+	+†	–
Myrioconium sp. 2	–	(+)	+	–
sterile # 1	+	+	+	+
sterile # 2	–	–	+	–
sterile # 3	–	–	+	–
sterile # 4	++	(+)	–	–
sterile # 5	+	+	+	–

¶: only 1 out of 8 strains positive †: 4 out of 8 strains positive * : 2 out of 8 strains positive

collected in alpine-boreal habitats (Samuels et al. 1984). Cellulose, starch, and lignin are resources likely to be widely available in the habitats investigated. It is, therefore, not surprising that almost all fungi studied were able to degrade at least two of these substrates. Thirteen out of 18 isolates produced peroxidases, as demonstrated by the Bavendamm test. If a positive correlation between lignin degradation and a positive Bavendamm reaction is assumed, up to 70% of the strains tested may be able to degrade lignin to some extent. In any case, they are able to oxidize phenolic compounds (Table 4) and their growth is not inhibited by them.

Flanagan & Scarborough (1974) stated that a very high percentage of tundra fungi are amylolytic and pectinolytic at room temperature. In their study incubation at less than 10° C depressed these activities, whilst cellulolysis and lignolysis were not adversely affected by low temperatures. Our data can be interpreted in the light of Flanagan & Scarborough's findings: the comparatively high substrate utilization activity by the isolates studied suggests that these fungi are probably able to continue litter degradation at very low temperatures. However, more detailed information on decomposition of litter is needed to understand the nutrient cycling in extreme ecosystems such as glacier cones and their immediate surroundings.

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