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Filamentous Soil Fungi from Ny-Ålesund, Spitsbergen, and Screening for Extracellular Enzymes

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ABSTRACT. Soil filamentous fungi from Ny-Ålesund, Spitsbergen, were studied. A total of 30 fungal isolates were identified by morpho-taxonomy, and the identity of some morpho-taxonomically complex isolates was authenticated by ITS1-5.8S and ITS2 rDNA domain sequence similarity. The isolates belonged to 19 species under 14 genera (*Acremonium*, *Arthrinium*, *Aspergillus*, *Cladosporium*, *Corynespora*, *Emericella*, *Geomyces*, *Mortierella*, *Mucor*, *Myrothecium*, *Penicillium*, *Phialophora*, *Preussia*, *Xylaria*). To the best of our knowledge, *Acremonium roseolum*, *Aspergillus aculeatus*, *Emericella nidulans*, and *Preussia* sp. are the first northernmost records from Arctic soils. The viable fungal count in different soil samples varied from 0.5×10^4 to 2.0×10^5 g^{-1}. Species richness in different soil samples was also calculated. *Mortierella* was one of the most dominant genera in Arctic soils. A temperature tolerance study was carried out for all the isolates, and representative species were screened for their extracellular enzyme activity (amylase, cellulase, phosphatase, and pectinase) at 4˚C and 20˚C. Among the 30 isolates, seven showed cellulolytic activity, two were phosphate solubilizers, three had amylolytic activity, and only one showed pectinolytic activity on solid media. CMCase (β1, 4-endoglucanase) activity was quantified in seven isolates that exhibited positive activity during preliminary screening. The records of enzyme activity for amylases, pectinases, and cellulases are the first from the fungi of Spitsbergen. The present study indicates the dominance in Ny-Ålesund of cellulolytic strains, which may serve as potent decomposers in Arctic tundra. These isolates may be used to facilitate the mineralization of cellulolytic wastes generated by human activities in colder hilly areas across the world, including the Himalayas in India.

Key words: mycology, diversity, bioprospecting, Svalbard, β1, 4-endoglucanase, Arctic

RÉSUMÉ. Nous avons étudié des champignons telluriques filamenteux de Ny-Ålesund, Spitzberg. Grâce à la morpho-taxonomie, nous avons identifié 30 isolats fongiques, et l'identité de certains complexes d'isolats morpho-taxonomiques a été authentifiée au moyen des similarités des séquences de domaines ITS1-5.8S et ITS2 DNAr. Les isolats relevaient de 19 espèces faisant partie de 14 genres (*Acremonium*, *Arthrinium*, *Aspergillus*, *Cladosporium*, *Corynespora*, *Emericella*, *Geomyces*, *Mortierella*, *Mucor*, *Myrothecium*, *Penicillium*, *Phialophora*, *Preussia*, *Xylaria*). Au meilleur de nos connaissances, *Acremonium roseolum*, *Aspergillus aculeatus*, *Emericella nidulans* et *Preussia* sp. constituent les premiers enregistrements aussi nordiques des sols arctiques. Le dénombrement viable de champignons dans différents échantillons de sol variait de $0{,}5 \times 10^4$ à $2{,}0 \times 10^5$ g^{-1}. Nous avons également calculé la diversité des espèces prélevées dans différents échantillons de sol. Le genre *Mortierella* était l'un des plus dominants des sols arctiques. Nous avons étudié la tolérance à la température de tous les isolats, et des espèces représentatives ont été examinées du point de vue de l'activité enzymatique extracellulaire (amylase, cellulase, phosphatase et pectinase) à 4 ˚C et 20 ˚C. Parmi les 30 isolats, sept présentaient de l'activité cellulolytique, deux étaient des solubilisants du phosphate, trois présentaient de l'activité amylolytique et seulement un présentait de l'activité pectolytique dans le cas des solides. L'activité CMCase (β1, 4-endoglucanase) a été quantifiée dans sept isolats qui affichaient une activité positive au cours de l'examen préliminaire. Il s'agissait de la première fois que de l'activité enzymatique pour les amylases, pectinases et cellulases a été détectée dans les champignons de Spitzberg. Cette étude indique la dominance de souches cellulolytiques à Ny-Ålesund, souches qui peuvent servir de décomposeurs puissants dans la toundra arctique. Ces isolats peuvent servir à faciliter la minéralisation des déchets cellulolytiques émanant des activités humaines dans les régions montagneuses plus froides du monde entier, y compris l'Himalaya, en Inde.

Mots clés : mycologie, diversité, bioprospection, Svalbard, β1, 4-endoglucanase, Arctique

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INTRODUCTION

Around 2.3% of the world's fungal biota exists in the Arctic. In this region, fungi have been isolated from various substrates and habitats (Ivarson, 1965; Reeve et al., 2002; Säwström et al., 2002; Callaghan et al., 2004; Ozerskaya et al., 2009; Pathan et al., 2009). However, fungal diversity in Arctic soils has been investigated only to a limited extent.

Mycological exploration in Svalbard began with the studies of Karsten (1872), Lind (1928), Hagen (1941), Kobayashi et al. (1968), Zabawski (1976), and Tamotsu et al. (1999). Recently, the diversity of fungi in soils of Bellsund, Svalbard, has been studied (Kurek et al., 2007), and new genera and species have been described from the region (Pang et al., 2008, 2009). Elvebakk et al. (1996), in their comprehensive account of known Svalbard fungi, list 389 species belonging to Myxomycota, Oomycota, Chytridiomycota, Zygomycota, Ascomycota, Deuteromycota, and Basidiomycota. However, the authors imply that the mycobiota studies of the region are only fragmentary and that these 389 species represent a very small part of the actual mycobiota. A recent catalogue of "macro-and micromycetes recorded for Norway and Svalbard" (Aarnæs, 2002) also indicates that diverse groups of fungi exist in the area.

Arctic fungi find applications in the field of biotechnology because they produce substances such as enzymes, polyunsaturated fatty acids, antifreeze proteins, and secondary metabolites (Feller and Gerday, 2003; Hoshino et al., 2003; Frisvad et al., 2006; Frisvad, 2008; Leary, 2008). As decomposers, these fungi also form an important part of the nutrient cycle (Ludley and Robinson, 2008). Though cold-active enzymes such as amylases, catalase, cellulases, invertase, lactase, lipases, pectinases, and proteases produced by Arctic fungal strains find potential applications in the food, medicine, and detergent industries, as of now only catalase (Fiedurek et al., 2003) and invertase (Skowronek et al., 2003) have been recorded from Spitsbergen fungi.

The present paper focuses on studying the soil filamentous fungi of Ny-Ålesund and screening the isolates for production of extracellular enzymes.

MATERIALS AND METHODS

Study Site and Sampling Methods

Ny-Ålesund is on the west coast of Spitsbergen, the largest island of the Svalbard archipelago. Topographical features of Ny-Ålesund include the eastern and western glaciers, terminal moraines, and glacial streams and rivers flowing northward to Kongsfjord. Within the marine terraces, gravelly and stony plains are dominant. The sampling sites are situated in different habitats, such as near a glacier, a wetland, and a plain (Fig. 1). The mean temperature is -14˚C in the coldest month (February) and +5˚C in the warmest month (July). The soils of the area are loose and poorly developed (Klimowicz and Uziak, 1988).

In the present study, soil samples were collected from the Ny-Ålesund region (78˚55′ N, 11˚56′ E) during the Indian Arctic Expedition in 2007. Three 100 g samples were collected at 5 cm depth from each of four different locations: S1, S2, S3, and S4. Collection site S1, located at 36 m near the Austre Brøggerbreen glacier, had fragmentary moss vegetation. Site S2 was at a higher altitude (60 m) and had a diverse plant population dominated by *Sanionia uncinata* (moss) and flowering plants such as *Deschampsia alpina* and *Dryas octopetala*. Site S3, located on a low-lying plain, had scanty moss and lichen vegetation, while S4, located near the coast, had moss, lichen, and *Dryas* sp. vegetation. The samples were placed in sterile ampules and stored at -20˚C until studied.

Soil Analysis

For chemical analysis, the soil samples were air-dried, crushed gently using a wooden mortar and pestle, and passed through a 1 mm sieve. Soil pH was measured in a soil: water solution (1:2.5). Organic carbon was determined by the wet digestion method (Walkley and Black, 1934), and available/mineralizable nitrogen by the method of Subbiah and Asija (1956), using the VAP 30 distillation apparatus (Gerhardt). Available phosphorus was extracted using sodium bicarbonate and estimated spectrophotometrically following Bray and Kurtz (1945). Calcium, magnesium, sodium, and potassium were extracted from 5 g of soil using 25 ml neutral normal ammonium acetate. Potassium and sodium were estimated using a flame photometer, and calcium and magnesium, using an atomic absorption spectrophotometer (Perkin Elmer Analyst 100 model).

Isolation of Fungi and Their Growth Characteristics

The soil samples were defrosted overnight at 4˚C. To isolate the fungi, soil dilution (Waksman, 1916) and the soil plate method (Warcup, 1960) were used on five different culture media: Malt Extract Agar (MEA), Corn Meal Agar (CMA), Potato Dextrose Agar (PDA), Martin Rose Bengal Agar (MRB), and Czapek's Dox Agar (CzA). Streptomycin was added to culture media to prevent bacterial growth. Three extracts from each soil sample were sprinkled simultaneously on solidified media plates (one plate each of five culture media). The plates incubated for 27 days, at 4˚C for 20 days and at 15˚C for the last 7 days. The growing fungal colonies having different morphological features were purified and transferred onto the PDA slants (potato dextrose agar medium solidified in a test tube at about a 35˚ slant to provide more surface area for fungal growth) for detailed study. Sporulating cultures were identified on the basis of morpho-taxonomic characters with the help of standard literature (Barnett, 1960; Rapper and Fennell, 1965; Ellis, 1971, 1976; von Arx, 1974; Barron, 1977; Pitt, 1979; Carmichael et al., 1980; Domsch et al., 1980; Samson and Frisvad, 2004; Kirk et al., 2008). For morpho-taxonomical studies, fungal mounts, prepared on slides using lactophenol-cotton

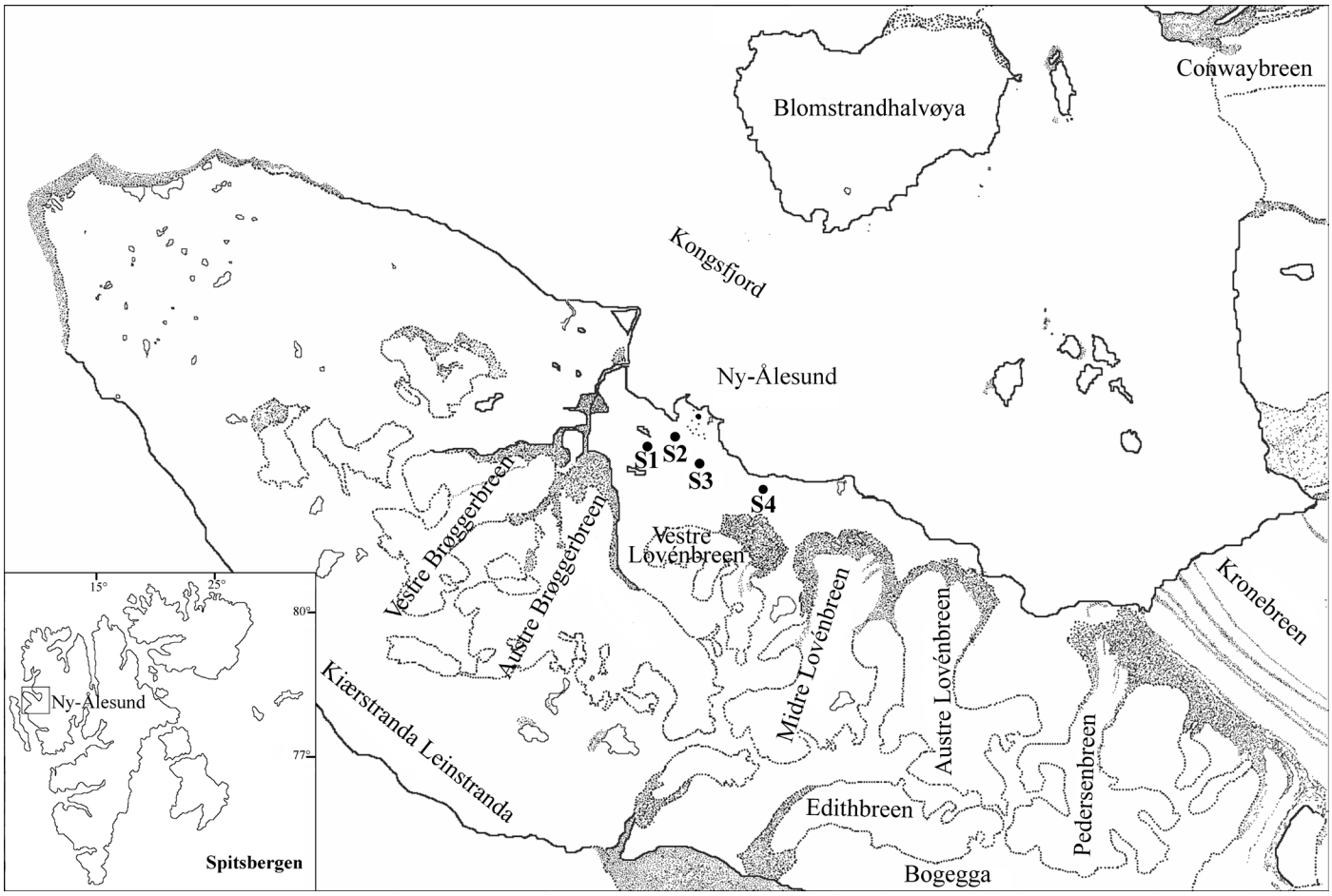

FIG. 1. Map showing the locations of sampling sites (•) in Ny-Ålesund, Spitsbergen.

blue as a mounting medium, were observed under OLYMPUS CX-41, BX-51, and IX-71 microscopes. Photomicrographs of isolates were taken by using OLYMPUS BX-51 and DP-70. Isolates whose identity could not be confirmed by morpho-taxonomy were subjected to sequence analysis of ITS1-5.8S and ITS2 of the rDNA region. All identified pure cultures were maintained on PDA slants and deposited at the National Fungal Culture Collection of India (NFCCI-WDCM 932) in Pune, India.

Temperature tolerance of the isolated fungi was determined by cultivating them on PDA medium at 4, 10, 15, 20, 25, and 30˚C. The diameter of the three sets of growing colonies was measured, and the optimal temperature for growth was observed.

DNA Extraction, Amplification, and Sequencing

DNA was extracted from cultures grown on PDA plates for two weeks at 28˚C by first homogenizing the mycelia in FastPrep®24 tissue homogenizer (MP Biomedicals Gmbh, Germany) and then using the CTAB method (Graeser et al., 1999). The universal primers ITS4 (5’ TCC TCC GCT TAT TGA TAT GC 3’) and ITS5 (5’ GGA AGT AAA AGT CGTAAC AAG G 3’) were used to amplify a DNA fragment of about 700 bp of the rDNA gene (White et al., 1990). The PCR mixture contained reaction buffer (10 mM Tris-HCl pH 8.0 - 50 mM KCl- 1.5 mM $MgCl_2$), 200 µM each of dNTPs (Genei, Bangalore, India), 50 pmol of each primer (ITS4 and ITS5), 1U of Taq polymerase (Genei, Bangalore, India), and 25 ng of template DNA. Samples were overlaid with sterile mineral oil and amplified through 30 cycles in a thermocycler (Eppendorf MastercyclerAG, Hamberg, Germany) as follows: initial denaturation for 5 min at 95˚C, denaturation for 1 min at 95˚C, annealing for 1 min at 56˚C, and extension for 1 min at 72˚C. This procedure was followed by a final extension step for 10 min at 72˚C. The resulting PCR product was checked on 1.2% agarose gel. PCR products were cleaned with an Axygen PCR cleanup kit (Axygen Scientific Inc, CA, USA) and sequenced using primers ITS4 and ITS5 (White et al., 1990) on an automated DNA Sequencer ABI 3130 (Applied Biosystems, USA).

Screening for Extracellular Enzyme Activity

Extracellular enzyme activity was tested on solid media for all the isolated cultures. Ten-day-old fungal cultures grown on PDA medium were used as inoculum. Amylase activity was checked using the procedure given by Hankin and Anagnostakis (1975). The phosphate solubilizing ability of isolates was tested on Pikovskaya’s medium

(Pikovskaya, 1948). The isolates were screened for cellulolytic activity on carboxymethylcellulose (CMC) Congo red agar medium containing 0.2% $NaNO_3$, 0.05% K_2HPO_4, 0.025% $MgSO_4$, 0.2% CMC, 0.02% Congo red, and 1.7% agar. The plates were centrally inoculated and incubated for five days. For cellulolytic activity, the plates were flooded first with 0.1% Congo red for 15–20 minutes and then with 1 M NaCl for 15–20 minutes. The clearing zone around the colony was measured. All qualitative extracellular enzyme activities were assayed at 4–20˚C.

Determination of Endoglucanase Activity

The isolates that exhibited positive cellulolytic activity during preliminary screening were used for quantification of β1, 4-endoglucanase production in liquid broth. For this procedure, each culture was grown in 100 ml Reese liquid medium containing per litre 2.0 g KH_2PO_4, 0.3 g KCl, 0.3 g urea, 1.4 g $NH_4(SO_4)_2$, 0.3 g $MgSO_4.7H_2O$, 0.05 g Peptone, 0.1 g yeast extract and traces of $FeSO_4$, $MnSO_4$, and $ZnSO_4$. The medium was adjusted to pH 5.5, cellulose powder (0.5 g) was added, and the mixture was autoclaved. Tween 80 was used after autoclaving. The cultures were incubated on an orbital shaker (180 rpm) at 20˚C for 10 days. After incubation, the broth was filtered through glass wool and centrifuged at 6000 rpm for 15 minutes. The supernatants were assayed for their enzymatic activity. The soluble protein contents of the enzyme extract (supernatant) were determined by the method described by Lowry et al. (1951).

Endoglucanase (β1, 4-endoglucanase) activity was assayed by measuring the amount of reducing sugars released from carboxymethyl cellulose, as described by Ghose (1987). For this test, 0.5 ml of crude enzyme (supernatant) was incubated with 0.5 ml of 2% CMC in 0.05 M sodium citrate buffer (pH 4.8) at 20˚C for 30 minutes. The reaction was terminated by addition of 3 ml of dinitrosalicylic acid (DNSA) reagent. The amount of reducing sugars released from the substrate was estimated by spectrophotometer, using glucose as standard. The endoglucanase activity was defined in International Units (IU). One unit of enzymatic activity is defined as the amount of enzyme that releases 1 µmol of reducing sugars (measured as glucose) per ml per minute.

RESULTS

In total, 30 isolates were obtained from the soil samples on the basis of morphological identification and 18S rDNA gene sequence data. They belonged to 19 species under 14 genera (Table 1). We measured fungal abundance, expressed as colony-forming units (CFU) per gram of soil. The fungal abundance in the different soil samples (S1 - S4) varied from 0.5×10^4 to 2.0×10^5 cfu/g soil (Table 2), with S2 and S3 having higher CFU numbers than S1 and S4. The chemical characteristics of soil samples varied with their nutrient components (Table 2).

The taxa isolated from the soil samples are listed in Table 1. The most common species was *Mortierella* sp., which was found in soil samples of three localities (S1, S2, and S3). *Cladosporium cladosporioides* was also commonly found in two localities (S1 and S2). The highest number of fungal isolates was obtained from S2, while the lowest number was from S4. Figure 2 compares the species richness of the different soil samples.

The growth-pattern studies of all isolates were conducted at different temperatures between 4˚ and 30˚C on PDA medium (Hi-Media), using Sanyo (Japan) and Bio Multi Incubator (NK systems, Japan). Colonies were measured three times at regular intervals in order to classify each fungal isolate as either psychrophilic or psychrotrophic (psychrotolerant). Microphotographs of some the dominant species have been given in Fig. 3a–i and Fig. 4a–f. *Acremonium fusidioides*, *A. roseolum*, *Emericella nidulans*, *Mortierella* sp., *Geomyces pannorum*, and *Penicillium chrysogenum* were psychrophilic in nature (temperature range 4 to 20˚C, but not above 20˚C). The remaining 13 isolates were found to be psychrotrophic (psychrotolerant) with growth between 4˚ and 30˚C (Table 1). Among the psychrophilic strains, only *Acremonium fusidioides* and *Geomyces pannorum* sporulated at 4˚C. Both *Mortierella* sp. and *Phialophora fastigiata* grew well at 4˚C, but sporulation was not observed even after prolonged incubation. The psychrotrophs *Arthrinium phaeospermum*, *Preussia* sp., and *Xylaria* sp. showed scanty sporulation at 15˚C and abundant sporulation at 20˚C. All the isolates were able to grow at 10˚C, and the optimum growth temperature varied from 15˚ to 25˚C (Table 1).

Results of the preliminary screening experiments for production of various extracellular enzymes such as amylase, cellulase, phosphatase, and pectinase (qualitatively) are given in Table 1. Out of 19 identified representative species, only two *Aspergillus* strains (*A. niger* and *A. niger* gr.) exhibited significantly positive phosphate solubilizing activity on Pikovskaya medium at 20˚C. Three isolates, *Aspergillus aculeatus*, *A. flavus*, and *Emericella nidulans*, exhibited amylolytic activity. *Aspergillus aculeatus* also exhibited pectinase activity by forming a clear zone around colonies. Cellulase activity was found positive in seven isolates (*Aspergillus aculeatus*, *Geomyces pannorum*, *Cladosporium cladosporioides*, *C. tenuissimum*, *Phialophora fastigiata*, *Preussia* sp., and *Xylaria* sp.) at both 4˚C and 20˚C, but activity was very poor at 4˚C. Promising cellulose activity was observed at 20˚C. The studies clearly revealed that fungi from Ny-Ålesund Arctic soils produced a wide range of cold-active extracellular enzymes (Table 1). Most of the isolates were cellulolytic in nature, indicating their ability to decompose dead organic material (Fig. 4f). Hence, the screened cellulolytic isolates were subjected to quantitative estimation for their β1, 4-endoglucanase activity.

TABLE 1. Fungi present in soil samples, their growth temperature, and extracellular enzyme activity.

Accession No.	Isolated fungi	S1	S2	S3	S4	Growth temp. (°C)	Optimum temp. (°C)	Cellulase[1]	Phosphatase[1]	Amylase[1]	Pectinase[1]
NFCCI-2142	*Acremonium fusidioides* (Nicot) W. Gams	+	–	–	–	4–20	15	–	–	–	–
NFCCI-2143	*Acremonium roseolum* (G. Sm.) W. Gams	–	+	–	–	4–20	15	–	–	–	–
NFCCI-2144	*Arthrinium phaeospermum*. gene GU266274.1 (97%)	+	–	–	–	4–25	20	–	–	–	–
NFCCI-2137	*Aspergillus aculeatus* Iizuka FJ876653.1 (100%)	+	–	–	–	10–30	25	+++	–	++	++
NFCCI-2139	*Aspergillus flavus* Link ex Gray	+	–	–	–	10–30	20	–	–	+++	–
NFCCI-2140	*Aspergillus niger* van Tieghem	–	+	–	–	4–30	25	–	+++	–	–
NFCCI-2141	*Aspergillus niger* Gr.	–	+	–	–	4–30	25		+++	–	–
NFCCI-2145	*Cladosporium cladosporioides* (Fresen.) de Vries	+	+	–	–	10–25	25	+++	–	–	–
NFCCI-2146	*Cladosporium tenuissimum* Cooke	–	+	–	–	10–25	25	++	–	–	–
NFCCI-2147	*Corynespora cassiicola* (Berk. & Curt.) Wei.	–	–	–	+	4–25	25	–	–	–	–
NFCCI-2138	*Emericella nidulans* (Eidam) Vuill.	+	+	–	–	4–20	25	–	–	+	–
NFCCI-2148	*Geomyces pannorum* (Link) Sigler et J.W. Carmich.	–	+	+	–	4–20	20	++	–	–	–
NFCCI-2151	*Mortierella* sp. EF601628.1 98%	+	+	+	–	4–20	15	–	–	–	–
NFCCI-2152	*Mucor hiemalis* Wehmer	–	–	+	–	4–25	20	–	–	–	–
NFCCI-2153	*Myrothecium roridum* Tode ex Steudel	–	–	+	–	4–25	25	–	–	–	–
NFCCI-2154	*Penicillium chrysogenum* Thom.	+	–	+	–	4–20	20	–	–	–	–
NFCCI-2155	*Phialophora fastigiata* (Lagerb. & Melin) Conant	–	+	–	+	4–30	20	+++	–	–	–
NFCCI-2149	*Preussia* sp. OY2307 FJ571483.1 (86%)	–	+	–	–	4–25	25	++	–	–	–
NFCCI-2150	*Xylaria* sp. FJ884195.1 (98%)	+	–	–	–	4–25	15	+++	–	–	–
S1-J, S2-T, S3-f, S4i	Non sporulating	+	+	+	+	—	—	–	–	–	–

[1] + = weak positive, ++ = moderately positive, +++ = highly positive (above 1 mm), – = negative.

TABLE 2. Fungal count (cfu/g), soil properties, and macronutrient content in soil samples of the Ny-Ålesund region of Svalbard, Arctic.

Locality No.	GPS Position	CFU/g	Number of taxa	Depth (cm)	pH (1:2.5)	$CaCO_3$ (%)	O.C. (%)	Min-N (ppm)	P (ppm)	K (ppm)	Na (ppm)	Ca (ppm)	Mg (ppm)
S1	78°55.082 / 11°51.527 Altitude: 36 m	$2.3 \pm 1.1 \times 10^4$	10	05	7.16 ± 0.28	3.06 ± 0.36	1.00 ± 0.02	63 ± 19.8	4.75 ± 0.78	77.5 ± 18.74	97 ± 20.86	4121 ± 440	505 ± 169
S2	78°55.165 / 11°52.660 Altitude: 60 m	$2 \pm 0.5 \times 10^5$	11	05	7.71 ± 0.34	20 ± 4.5	2.61 ± 0.29	24.5 ± 19.8	3.6 ± 0.42	88.38 ± 8.66	65.38 ± 14.67	5920 ± 1089	798 ± 512
S3	78°55.254 / 11°54.256 Altitude: 30 m	$5 \pm 2.9 \times 10^4$	6	05	7.78 ± 0.01	4.84 ± 1.08	0.35 ± 0.1	26.25 ± 7.42	6.8 ± 2.97	64.75 ± 3.18	148 ± 43.13	3643 ± 1059	285 ± 53
S4	78°54.817 / 11°58.378 Altitude: 17 m	$0.5 \pm 0.2 \times 10^4$	3	05	6.66 ± 0.32	3.06 ± 0.01	0.43 ± 0.32	61.25 ± 12.37	4.25 ± 0.07	61 ± 28.64	248.25 ± 101.12	4088 ± 1246	814 ± 228

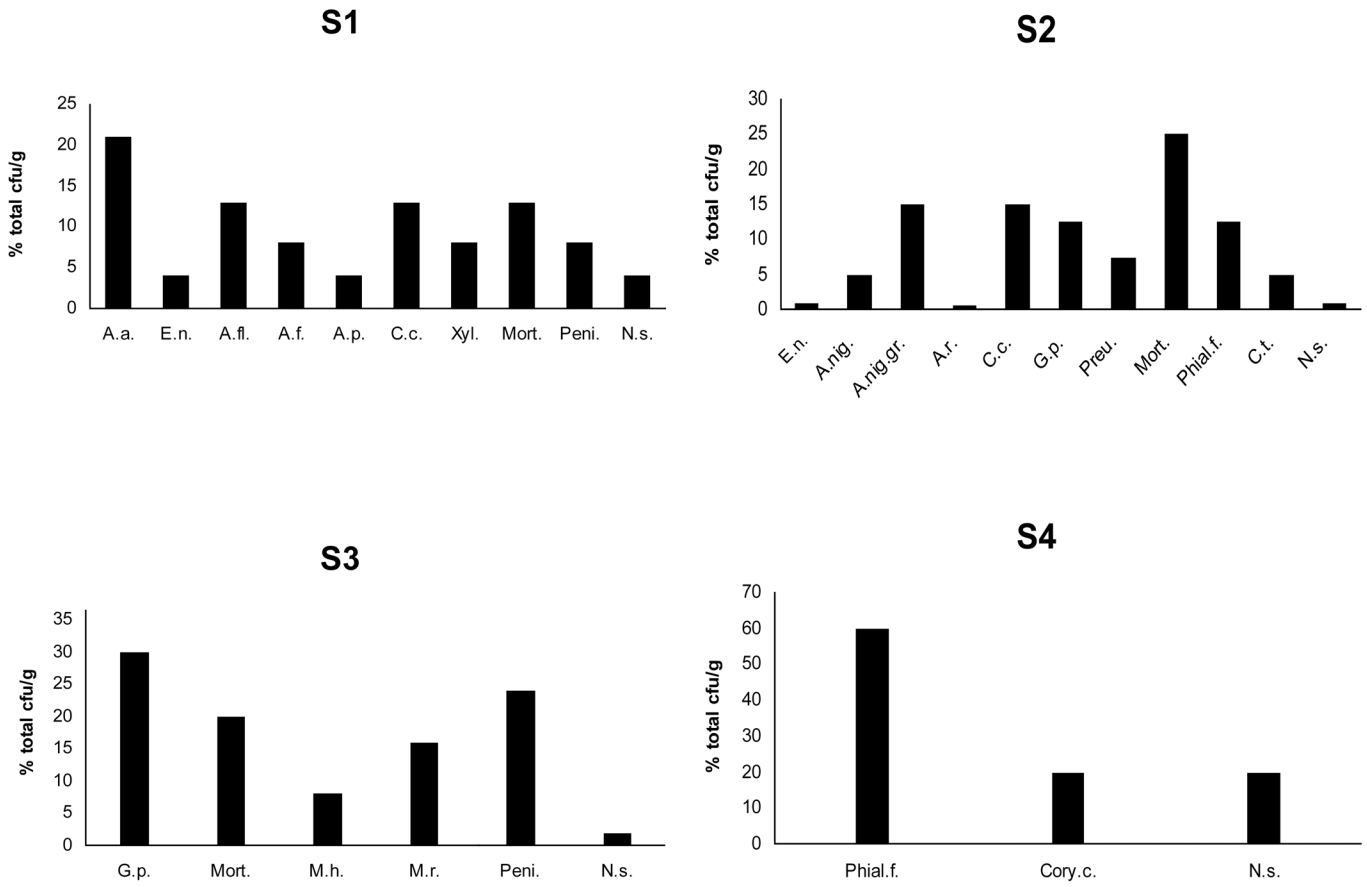

FIG. 2. Species richness (%) in soil samples of Ny-Ålesund region of Svalbard. (A.a = *Aspergillus aculeatus*, E.n = *Emericella nidulans*, A.nig = *Aspergillus niger*, A. nig.gr = *Aspergillus niger* gr., A.fl = *Aspergillus flavus*, A.f. = *Acremonium fusidoides*, A.r. = *Acremonium roseolum*, A.p = *Arthrinium phaeospermum*, G.p = *Geomyces pannorum*, C.c = *Cladosporium cladosporioides*, C.t = *Cladosporium tenuissimum*, Cory.c = *Corynespora cassiicola*, Xyl = *Xylaria* sp., Preu = *Preussia* sp., Mort = *Mortierella* sp., M.h = *Mucor hiemalis*, M.r. = *Myrothecium roridum*, Peni = *Penicillium chrysogenum*, Phial.f = *Phialophora fastigiata*, N.s = Non sporulating.) Note the different scales of the graphs.

Quantitative Estimation of β1, 4-endoglucanase at Optimum Temperature

All the tested fungi produced β1, 4-endoglucanase at 20˚C (Fig. 5). *Aspergillus aculeatus* and *Xylaria* sp. had extremely high levels of accumulated endoglucanase activity (2.9 U/ml/min), followed by *Preussia* sp. (2.7 U/ml/min). *Phialophora fastigiata* and *Cladosporium cladosporioides* also produced good amounts of β1, 4-endoglucanase (2.5 and 2.2 U/ml/min, respectively). *Cladosporium tenuissimum* produced 2 U/ml/min β1, 4-glucanase in liquid broth. The lowest level of β1, 4-gluconase activity was found in *Geomyces pannorum* (1.8 U/ml/min).

DISCUSSION

Only a few researchers have explored the fungal biota of the Svalbard region, beginning with Karsten (1872). The diversity of filamentous fungi (Tamotsu et al., 1999; Kurek et al., 2007) and yeast (Pathan et al., 2009) has been studied in the region. The Ny-Ålesund area has recently been explored for the diversity and bioprospecting potential of its bacteria (Reddy et al., 2009, Srinivas et al., 2009); however, the diversity and bioprospecting potential of the region's soil fungi have been less explored. The present study was therefore carried out to explore the fungal diversity from the soils of Ny-Ålesund, Svalbard, and to screen them for production of extracellular enzymes.

The fungal count in the soil samples studied varied from 0.5×10^4 to 2.0×10^5 cfu/g soil. The soil samples used for the study varied in altitude, chemical characteristics, and vegetation type. Fungal diversity was higher in S2 (2.0×10^5) at 60 m altitude than in S1, S3, and S4. Altitude and a diverse plant population may explain the high fungal diversity at S2. Fungal diversity was lowest at low altitudes (0.5×10^4 at 17 m). Similar observations were made by Robinson et al. (2004), who considered low altitude to be one of the reasons for lower species richness and fungal community in soil sediments. Furthermore, Robinson et al. (2004) and the present study indicate that the diversity of fungi is poor in the soils of cold regions in comparison to tropical soils. Similar results were obtained from Antarctic soils as reported by Bolter et al. (2002).

Ozerskaya et al. (2009) reported *Aspergillus niger* and *Cladosporium cladosporioides* from Arctic permafrost

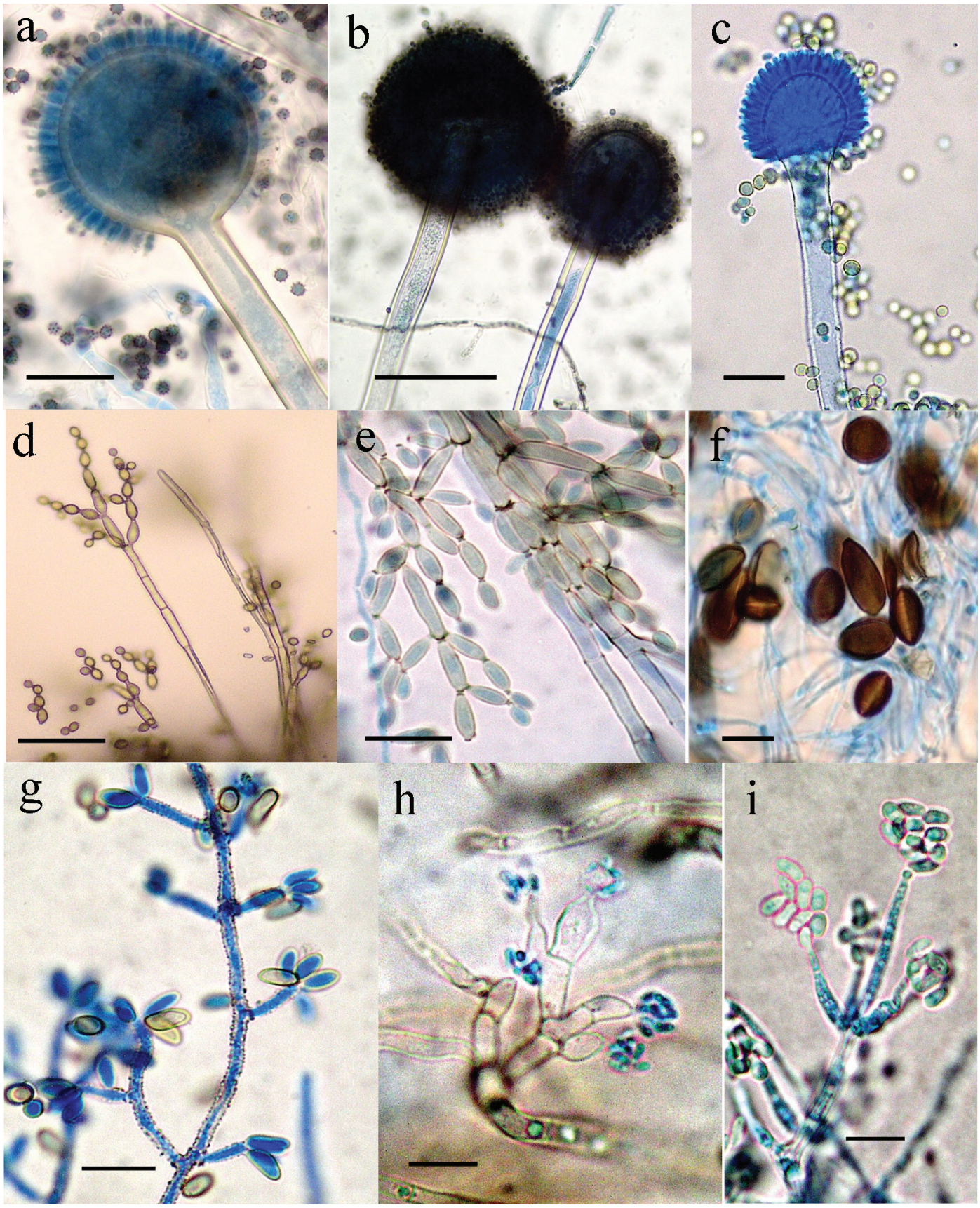

FIG. 3. a) *Aspergillus aculeatus*, b) *A. niger*, c) *A. flavus*, d) *Cladosporium cladosporioides*, e) *C. tenuissimum*, f) *Arthrinium phaeospermum*, g) *Xylaria* sp., h) *Phialophora fastigiata*, i) *Acremonium fusidioides*. Bars (a–i) = 40 μm

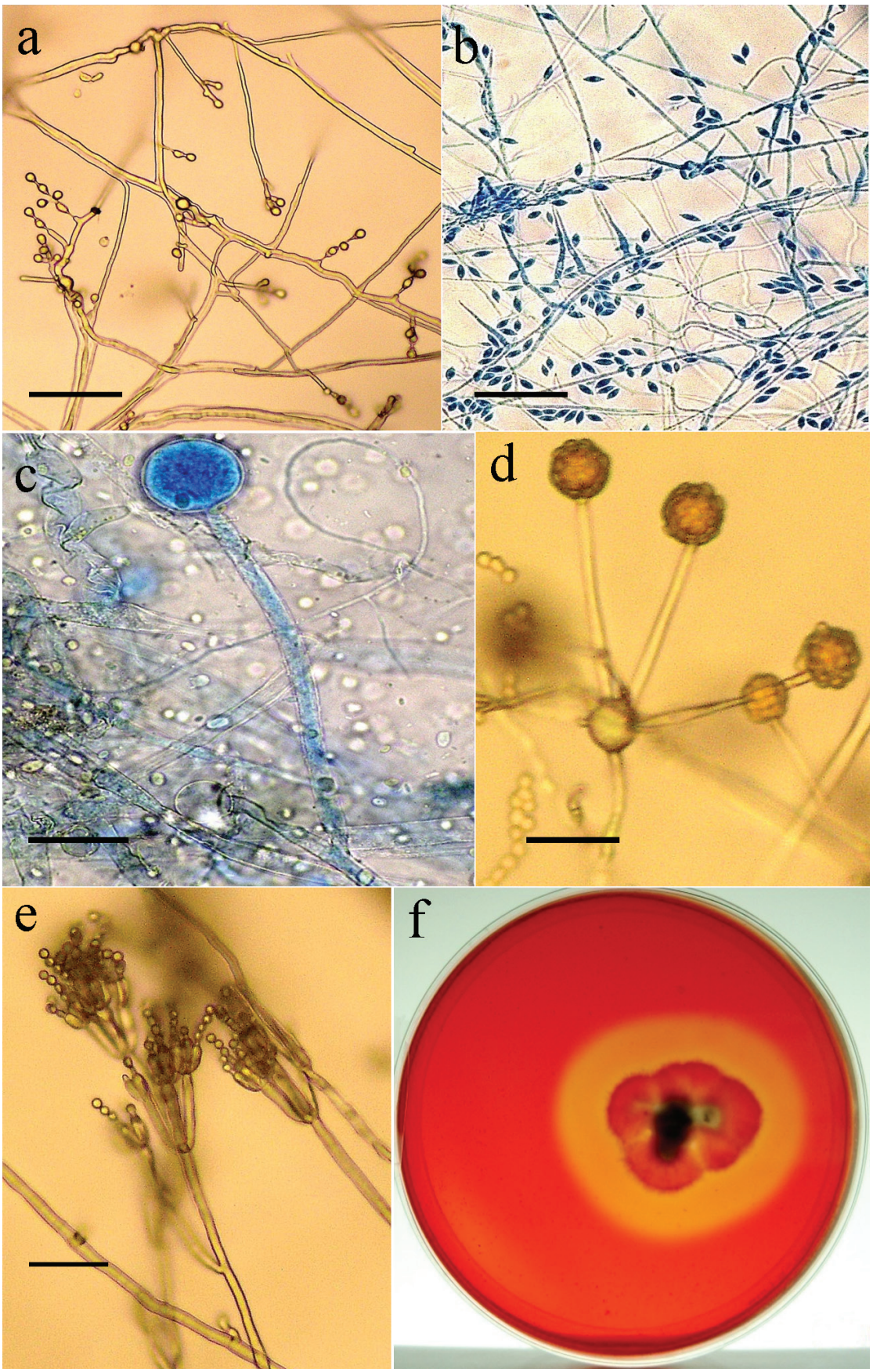

FIG. 4. a) *Geomyces pannorum*, b) *Acremonium roseolum*, c) *Mucor hiemalis*, d) *Mortierella* sp., e) *Penicillium chrysogenum*, f) Screening of cellulase activity on Congo red agar medium. Bars (a – f) = 40 μm.

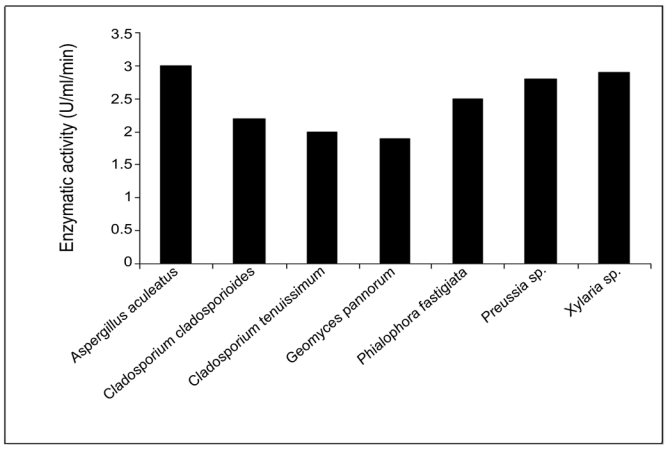

FIG. 5. Quantitative estimation of β1, 4-endoglucanase at 20˚C after 10 days.

and cryopegs. Fewer reports are available on *Arthrinium phaeospermum*, *Corynespora cassiicola*, and *Xylaria* sp. from the Arctic (Aarnæs, 2002). To the best of our knowledge from a literature survey, our records of *Acremonium roseolum* (G.Sm) W. Gams, *Aspergillus aculeatus* Iizuka, *Emericella nidulans* (Eidam) Vuill. and *Preussia* sp. are the first from Arctic soils.

Large differences in species richness were found between the soil samples of Ny-Ålesund. Soil samples from S1 and S2 localities showed high species richness in fungal communities, with 9 and 10 species, respectively, belonging to 6 genera. Low fungal species richness was found in S3 and S4 soils. Soil sample S3 was inhabited by 5 species in 4 genera, while only 2 species in 2 genera were found in the S4 sample. Non-sporulating isolates were reported from all localities studied. Differences in species richness as reported in the present study were also observed previously by Bergero et al. (1999) during their study of different soil samples from Cape Tegetthoff, Kane Island, and Cape Flora in Franz Josef Land.

Previous studies on diversity reported fungal species such as *Cladosporium cladosporioides*, *Mortierella* sp., and *Phialophora fastigiata* to be dominant in Arctic soils (Holding, 1981; Bergero et al., 1999; Kurek et al., 2007). Our data reports *Mortierella* sp. to be the dominant taxon in Ny-Ålesund, as it occurred in three of the four soil sampling locations tested. The frequency of occurrence of *Mortierella* sp. in these samples was also comparatively high (S1: 13%, S2: 25%, and S3: 20% of total cfu/g soil). This result supports earlier reports of Ivarson (1965) and Kurek et al. (2007), who performed similar studies in other regions of the Arctic. Within soil sample S1, *Aspergillus* was a dominant genus consisting of two species, *A. aculeatus* (21%) and *A. flavus* (13%). In soil sample S2, the diversity of fungi was much higher than in the other samples studied, with *Mortierella* sp. as the most dominant (25%) species. The soil sample S3 was dominated by *Geomyces pannorum* (30%) and *Penicillium chrysogenum* (24%). Soil sample S4 had the lowest diversity, with only two taxa, of which *Phialophora fastigiata* was the most dominant (60%) (Fig. 2).

The diversity of fungi in the soil depends on the nutrient composition, organic carbon content, texture, and water-holding capacity of soil. On the basis of organic carbon content, the soils of Ny-Ålesund are broadly classified into two types: ahumic and organic. Ahumic soils contain 0.35% to 0.42% of organic carbon, while organic soils contain 1.0% to 2.61%. Details of the soil properties, macronutrient constituents, and organic carbon content of the soils studied are given in Table 2. From the soil data and from the observations related to fungal diversity, it is evident that fungi prefer to grow and proliferate in organic soils as opposed to ahumic soils. Similar observations were made by Stonehouse (1989).

Most of the soil fungi of Ny-Ålesund were capable of growing between 4˚ and 30˚C, indicating that psychrotolerant fungi are predominant in the area. This observation resembles results of earlier reports by Bergero et al. (1999) and Kytöviita (2005). The physical adaptations that help the fungi overcome low temperature and water stress include formation of chlamydospores and mycelial thickening (Robinson, 2001). Similar adaptive features have also been observed in the present study in the area of Ny-Ålesund.

A review of the literature reveals that very little information is available on enzyme production by Arctic fungal strains. Nowadays cold adaptive organisms are gaining interest because of their applications in biotechnology industries. Cold-active enzymes such as amylases, catalase, cellulases, invertase, lactase, lipases, pectinases, and proteases from psychrophilic fungal strains also find several applications in the food, medicine, and detergent industries (Feller and Gerday, 2003; Leary, 2008). Therefore, keeping this view in mind, some of the isolates were screened for a wide range of cold-active extracellular enzymes (Table 1). The extracellular enzyme activity for amylases, pectinases, and cellulases reported in this study is the first record from Spitsbergen soils. Most of the isolates were cellulolytic in nature and showed maximum cellulolytic activity at 20˚C. This finding is similar to the results of Holding (1981). These strains with cellulolytic activity and low temperatures have great potential in the future to use and recycle the large reserve (25%–33%) of organic carbon trapped in the soils of the Arctic. The assessment of biodiversity is therefore an important research area for bioprospecting in the Arctic and future biotechnology.

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