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Production of extracellular enzymes by Antarctic fungal strains

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Abstract Thirty-three fungal strains, isolated from different sites on Victoria Land (continental Antarctica), were plate-screened for their ability to produce twelve extracellular enzymes. Lipases were generally present and in high quantities in almost all the strains. Polygalacturonase, as well as amylase and phosphatase, was common. Glucose oxidase, protease and DNAase appeared to be generally low or absent. Many strains, producing a limited number of enzymes, appeared to have a low eco-nutritional versatility while a few, such as *Verticillium* cfr. *lecanii* no. 1, *V. cfr. lecanii* no. 3, *Aspergillus versicolor* and *Phoma* sp. no. 2, showing a diversified enzymatic competence, are probably advantaged in extreme terrestrial environments characterized by low competition. The possibility of utilizing the enzyme-producing ability of these fungi in applied research is also discussed.

Introduction

Continental Antarctica is considered an extreme terrestrial environment, which can be colonized only by microorganisms with a high level of adaptation. Terrestrial microfungal communities in the Antarctic are poorly investigated (Vincent 1988; Del Frate and Caretta 1990; Wynn-Williams 1990; Vishniac 1993) and physiological information is necessary to explain the ecological roles of microfungi in Antarctic terrestrial habitats. For instance, tests on temperature preferences for growth have shown an Antarctic microflora mainly

composed of psychrotrophic (i.e., mesophilic psychrotolerant) species (Kerry 1990; Zucconi et al. 1996).

Little information is available on the enzyme production by Antarctic fungal strains except for fermentations by yeasts (Vishniac and Hempfling 1979; Vishniac and Baharaeen 1982; Klingler and Vishniac 1988). Given that fungal species decompose the colonized organic substrata by synthesizing enzymes located at the hyphal surface or secreted into the substrate, the ability to produce enzymes related to the chemical composition of the substrata is undoubtedly adaptive and of ecological significance (Waid 1968).

The ability of 33 Antarctic strains, isolated from soil and mosses, to produce 12 extracellular enzymes has been screened with the aim of contributing to knowledge of their nutritional potential and versatility, as well as finding material for further applied research. The enzymes tested are among those related to the decomposition of common organic substrata.

Considering the limited number of microfungal species currently listed from continental Antarctica, the 22 species investigated here represent a relevant percentage of the total mycoflora of filamentous fungi from soils and mosses, comprising, to date, about 50–60 species (unpublished data).

Materials and methods

Microorganisms and culture conditions

The 33 strains tested, collection sites, and habitats and temperatures at which tests were carried out are reported in Table 1. The collection sites (Fig. 1), located in Northern Victoria Land (continental Antarctica), on the west coast of the Ross Sea, comprise different environments; Cryptogam Ridge (74°21'S, 164°42'E), near the top of Mt. Melbourne, is an active volcanic site where hot soil samples were collected (soil temperatures ranging from 14 to 43°C and air temperatures ranging from –16 to –17°C, at the site at the time of collection); Crater Cirque (72°36'S, 169°17'E), Kay Island (74°05'S, 165°17'E), the beach near Baker Rocks (74°14'S, 164°47'E) and

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Table 1 Strains screened, collection sites, habitats and temperatures (°C) at which tests were carried out

Tested strains	Collection site	Habitat	Temperature (°C)
<i>Alternaria</i> sp. no. 2	Cape Washington	Soil	25
<i>Arthrobotrys ferox</i> Onofri & Tosi no. 1	Edmonson Point	Moss	20–25
<i>A. ferox</i> no. 2	Kay Island	Moss	25–28
<i>A. ferox</i> no. 3	Kay Island	Moss	20–25
<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi	Lamplugh Island	Soil under moss	25–28
<i>Aureobasidium</i> sp.	Inexpressible Island	Mummified seal	0.5–25
<i>Chaetomium</i> sp. no. 1	Mt Melbourne	Soil	25–35
<i>Chaetomium</i> sp. no. 2	Mt Melbourne	Moss	25
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	Crater Cirque	Soil under moss	15–25
<i>C. herbarum</i> (Pers.: Fr.) Link no. 1	Crater Cirque	Moss	15–25
<i>C. herbarum</i> no. 2	Whitmer Peninsula	Soil under moss	15–25
<i>Dendryphiella salina</i> (Sutherland) Pugh & Nicot no. 1	Lake “Carezza”	Moss and soil under moss	20–25
<i>D. salina</i> no. 2	Baker Rocks	Moss	25
<i>Geomyces pannorum</i> (Link) Sigler & Carmichael var. <i>pannorum</i> no. 1	Kay Island	Soil under moss	5–25
<i>G. pannorum</i> var. <i>pannorum</i> no. 2	“Giardino”	Moss and soil under moss	15–25
<i>G. pannorum</i> var. <i>pannorum</i> no. 3	Lake “Carezza”	Moss	15–25
<i>G. pannorum</i> var. <i>pannorum</i> no. 4	“Campo Icaro”	Moss and sand under moss	15–25
<i>G. pannorum</i> var. <i>pannorum</i> no. 6	Lake “Carezza”	Moss and soil under moss	20–25
<i>P. sorghina</i> (Sacc.) Boerema, Dorenbosch & van Kesteren	Kay Island	Moss and soil under moss	25
<i>Phoma</i> sp. no. 1	Lake “Carezza”	Moss and soil under moss	20–25
<i>Phoma</i> sp. no. 2	Gondwana Station	Moss	20–25
<i>Phoma</i> sp. no. 4	Crater Cirque	Moss	25
<i>Thelebolus microsporus</i> (Berk. & Br.) Kimbrough	Vegetation Island	Moss	15–25
<i>Verticillium</i> cfr. <i>lecanii</i> (Zimm.) Viégas no. 1	Crater Cirque	Moss	25
<i>Verticillium</i> cfr. <i>lecanii</i> (Zimm.) Viégas no. 2	Mt Melbourne	Soil	25–28
<i>Verticillium</i> cfr. <i>lecanii</i> (Zimm.) Viégas no. 3	Kay Island	Moss	25
Black mycelium yeast-like	Mt Melbourne	Soil	25
Hyaline mycelium no. 1	Crater Cirque	Soil	25
Hyaline mycelium no. 2	“Campo Icaro”	Soil	25
Hyaline mycelium no. 3	Crater Cirque	Soil	25
Pink yeast no. 1	Kay Island	Moss and soil under moss	20–25
Pink yeast no. 2	“Campo Icaro”	Moss and sand under moss	15–25
White yeast no. 1	“Campo Icaro”	Moss and sand under moss	15–25

Edmonson Point (74°19'S, 165°07'E) are coastal sites very rich in mosses; the sites here referred to as Lake “Carezza” (74°43'S, 164°03'E), “Giardino” (74°41'S, 164°06'E) and “Campo Icaro” (74°43'S, 164°06'E), are close to the Italian Base, in Terra Nova Bay, delimited by Cape Washington (74°42'S, 165°25'E) in the north; Inexpressible Island (75°S, 163°45'E) is 26 km south from the Italian Base; remaining sites are Lamplugh Island (75°34'S, 162°55'E), Whitmer Peninsula (75°46'S, 162°52'E), Vegetation Island (74°47'S, 163°38'E) and Gondwana Station (74°37'S, 164°13'E); air temperatures of the above reported sites at time of collection, where recorded, ranged from 0 to –9°C, while temperatures of substrata were always above 0°C (up to 27°C).

The strains were isolated in pure culture, by direct and indirect methods, incubating both at 10 and 25°C. The isolation media used (Difco) were Potato Dextrose Agar (PDA), Czapek Agar (CzA), Czapek Yeast Agar (CzYA), Mycobiotic Agar (MBA) and Mycological Agar (MA). The cultures were subsequently maintained on PDA at 4°C and subcultured every month.

Screening procedures

All the strains were tested at 25°C; only protease activity, expressed as enzymatic gelatin liquefaction, was tested at 20°C to avoid thermal melting of the medium. Strains not having optimal growth at 25°C were also tested at their optimal temperature for growth

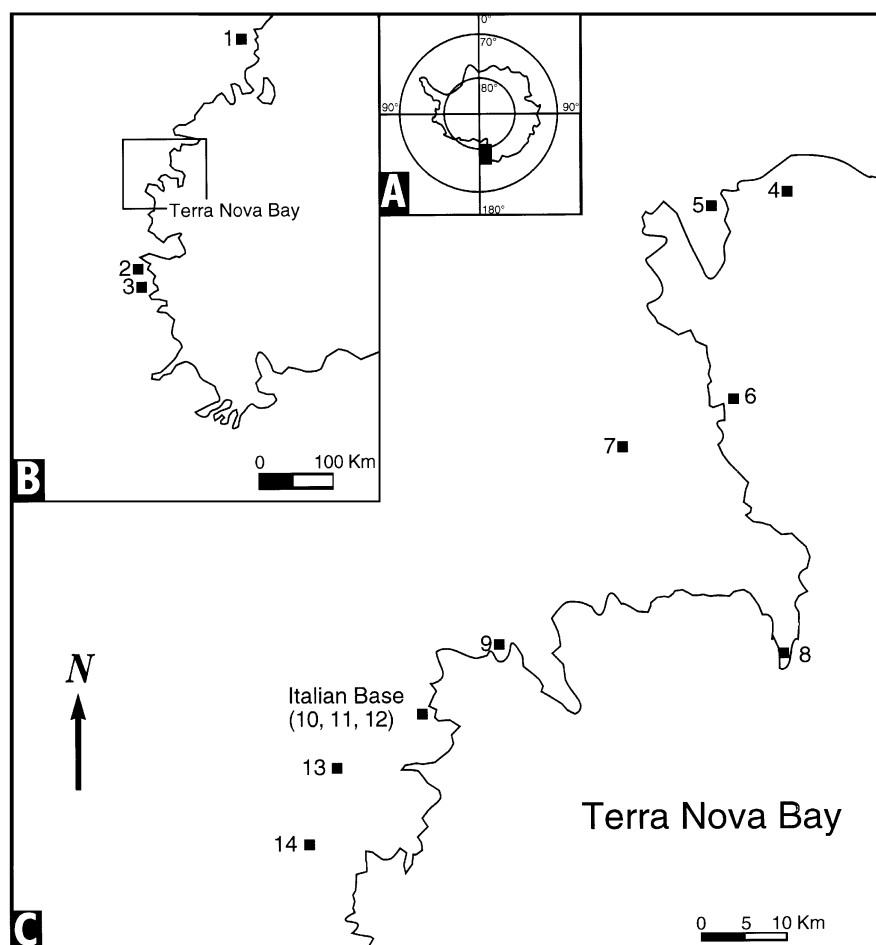
(Table 1). Optimum was determined by measuring rates of growth at different temperatures – ranging from 0 to 45°C – in duplicate. Semi-quantitative tests for enzymatic activities were performed on solid media. Media for the different enzymatic activities were prepared and activities scored using previously reported techniques (Hankin and Anagnostakis 1975). Pre-poured plates were inoculated with punctiform inocula obtained by means of sterile glass needles from 7- to 15-days-old cultures, grown on PDA (at 25°C). To avoid any interference from nearby colonies, only one strain was inoculated onto each plate (60-mm diameter). The following activities were tested, in triplicate: polygalacturonase (on pectin at pH 5.0 and polypectate at pH 5.0) (PG1, PG2); pectinylase (on pectin at pH 7.0) (PLY); amylase (AMY); cellulase (CEL); chitinase (CHI); phosphatase (PHO); glucose oxidase (GOD); urease (URE); protease (GEL); lipase (on Tween 20, 40, 60, 80) (T20, T40, T60, T80); RNAase (RNA); DNAase (DNA).

Enzymatic activity was detected after 5–15 days of incubation by measuring the diameter of halos developed around the colonies.

Results

Table 2 shows the results of the screening for enzyme activities at 25°C, and at the optimum temperature when different from 25°C.

Fig. 1A Map of Antarctica **B**, **C** insets showing the location of the collection sites: 1 Crater Cirque; 2 Lamplugh Island; 3 Whitmer Peninsula; 4 Kay Island; 5 Baker Rocks; 6 Edmonson Point; 7 Mt Melbourne; 8 Cape Washington; 9 Gondwana Station; 10 Lake "Carezza"; 11 "Giardino"; 12 "Campo Icaro"; 13 Vegetation Island; 14 Inexpressible Island



Pectolytic activities

More than 50% of the strains tested produced extracellular pectinases, with different distributions of either polygalacturonase and/or pectinlyase. The strains *Arthrobotrys ferox* no. 1, *Aureobasidium* sp. and *Cladosporium herbarum* no. 1 showed very high polygalacturonase activities and could possibly be employed in further investigations. At 35°C *Chaetomium* sp. no. 1 presented a limited pectinlyase activity, which was not detected at 25°C.

Amylase production

Unexpectedly, only 18 out of the 33 screened strains showed any amylase activity. However, the strain *Geomyces pannorum* var. *pannorum* no. 1 showed a marked activity and could possibly be considered for further applied studies.

Cellulase and chitinase production

Cellulases were detected in 12 strains only, although the activity was rather low. Chitinase activity was de-

tected in nine strains, but only four showed substantial activity (Table 2) and could be selected for further experiments.

Phosphatase and glucose oxidase production

Phosphatase production was detected in 21 strains, while glucose oxidase activity was present in 7 strains, of which *Geomyces pannorum* var. *pannorum* no. 3 and *Phoma* sp. no. 4 showed a fairly strong GOD activity.

Urease and protease production

Urease activity was detected in ten strains, while gelatin liquefaction was detected in only seven strains. The strains *Alternaria* sp. no. 2 and *Arthrobotrys ferox* no. 2 showed a strong urease activity. At 15°C *Geomyces pannorum* var. *pannorum* no. 4 showed a marked urease activity that was not detected at 25°C. *Verticillium* cfr. *lecanii* no. 3 was the only strain showing a very high protease activity.

Table 2 Screening for enzymatic activities at 25°C (polygalacturonase on pectine *PGI* and polypectate *PG2*; pectinlyase *PLY*; amylase *AMY*; cellulase *CEL*; chitinase *CHI*; phosphatase *PHO*; glucose oxidase *GOD*; urease *URE*; protease *GEL*; lipase *T20*, *T40*, *T60*, *T80*; RNAase *RNA*; DNAase *DNA*). The symbols +, + + and + + + indicate mean halo diameters up to 10 mm, between 11 and 25 mm and more than 25 mm, respectively. Lack of growth and enzyme production are denoted by the symbols ? and –, respectively)

Tested strains	PG1	PG2	PLY	AMY	CEL	CHI	PHO	GOD	URE	GEL	T20	T40	T60	T80	RNA	DNA
<i>Alternaria</i> sp. no. 2	–	+	+	+	+	–	–	–	+	–	+	+	+	–	–	–
<i>Arthrobotrys ferox</i> no. 1	+	+	–	–	–	–	(+) ^b	–	+	–	–	–	–	–	–	+
<i>A. ferox</i> no. 2	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	(+) ^c
<i>A. ferox</i> no. 3	+	+	–	+	–	–	+	–	+	–	–	–	–	–	+	–
<i>Aspergillus versicolor</i>	+	+	+	+	+	–	+	–	–	–	+	+	+	+	+	–
<i>Aureobasidium</i> sp.	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–
<i>Chaetomium</i> sp. no. 1	+	+	(+) ^d	–	–	–	+	–	–	–	–	–	–	–	+	(+) ^d
<i>Chaetomium</i> sp. no. 2	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–
<i>Cladosporium cladosporioides</i>	+	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–
<i>C. herbarum</i> no. 1	+	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–
<i>C. herbarum</i> no. 2	–	+	+	–	–	–	+	–	–	+	–	–	–	–	+	–
<i>Dendryphiella salina</i> no. 1	+	+	–	+	+	–	–	–	+	–	–	–	–	–	–	–
<i>D. salina</i> no. 2	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–
<i>Geomyces pamorum</i> v. pan. no. 1	+	–	–	+	+	–	–	–	+	–	–	–	–	–	–	–
<i>G. pamorum</i> v. pan. no. 2	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–
<i>G. pamorum</i> v. pan. no. 3	–	–	+	+	+	–	–	+	+	–	–	–	–	–	–	–
<i>G. pamorum</i> v. pan. no. 4	–	–	–	–	–	–	–	–	(+) ^b	–	–	–	–	–	–	–
<i>G. pamorum</i> v. pan. no. 6	+	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–
<i>Phoma sorghina</i>	+	+	+	–	+	–	+	–	–	–	–	–	–	–	–	–
<i>Phoma</i> sp. no. 1	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–
<i>Phoma</i> sp. no. 2	+	+	+	–	–	+	+	+	–	–	–	–	–	–	–	–
<i>Phoma</i> sp. no. 4	+	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–
<i>Theloholus microsporus</i>	+	?	–	+	–	+	–	–	–	–	–	–	–	–	–	?
<i>Verticillium</i> cfr. <i>lecanii</i> no. 1	–	–	–	+	+	+	+	–	+	–	–	–	–	–	+	–
<i>Verticillium</i> cfr. <i>lecanii</i> no. 2	+	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–
<i>Verticillium</i> cfr. <i>lecanii</i> no. 3	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–
Black mycelium yeast-like	–	?	–	–	–	–	–	–	–	+	+	+	+	+	–	–
Hyaline mycelium no. 1	+	–	–	+	–	–	+	–	–	+	–	–	–	–	+	+
Hyaline mycelium no. 2	+	–	–	+	+	–	+	–	–	–	–	–	–	–	+	–
Hyaline mycelium no. 3	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–
Pink yeast no. 1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Pink yeast no. 2	–	–	+	–	–	?	–	–	–	+	–	–	–	–	–	–
White yeast no. 1	–	+	+	–	–	–	+	–	–	+	–	–	–	–	–	–
% Strains showing activity	57.6	36.4	45.4	54.5	36.4	27.3	63.6	21.2	30.3	21.2	57.6	75.7	87.9	36.4	51.5	27.3

In parentheses are the activities detected only at the optimum temperature, when different from 25°C:

^a at 15°C; ^b at 20°C; ^c at 28°C; ^d at 35°C

Lipase production

All the Antarctic strains screened, except *Arthrobotrys ferox* no. 3, were able to hydrolyse different Tweens, the highest activity generally being observed on Tween 60.

Nuclease production

Ribonuclease was detected in 17 strains, *Cladosporium cladosporioides* being the highest RNAase producer. The deoxyribonuclease activity was shown by nine strains, including *Chaetomium* sp. no. 1, which showed a good activity only at 35°C.

Discussion

The strains investigated here showed diversified enzymatic patterns. As shown in Table 2, many activities were lacking in a wide number of strains, while many strains expressed few extracellular enzyme abilities.

The lipolytic activities, which are very widespread and well-documented in fungi (Trigiano and Fergus 1979; Federici 1982), were found in most of the strains screened. The general well-known ability of fungi to degrade pectin (Federici 1982; Fawole and Odunfa 1992), as well as to produce extracellular nucleases (Hankin and Anagnostakis 1975; Adams and Deploey 1978) – in particular the high pectolytic activity of *Aureobasidium* (Finkelman and Zajic 1978; Federici 1982) and *Cladosporium herbarum* (Domsch et al. 1980) – is confirmed here. However, the ability to produce amylase (Cochrane 1958), cellulase and chitinase (Knapp 1985; Sahai and Manocha 1993), which is widely reported for fungi, was little evident among the isolates studied here.

Fungi with a broad enzymatic competence possess high eco-nutritional versatility; this concept was defined by Cooke and Whipps (1980) as the ability to survive in a vegetative state, and to overcome environmental changes that might normally be harmful. Species with a wide enzymatic competence, such as *Verticillium* cfr. *lecanii* no. 1, *Verticillium* cfr. *lecanii* no. 3, *Aspergillus versicolor*, and *Phoma* sp. no. 2, possess a wide nutritional versatility – at least at the temperatures tested – that increases survival chances in unfavourable environments (Cooke and Rayner 1984). *Chaetomium* sp. no. 1, isolated from hot soil (air temperature – 16°C and soil temperature 43°C at the site at the time of collection), showed the ability to degrade pectin and DNA at 35°C only, increasing its nutritional versatility at a higher temperature. All the other strains, whether tested at 25°C or at their optimum (if different from 25°C), showed only limited enzymatic competence. A high nutritional versatility could be successful

in terrestrial Antarctic micro-habitats that are generally characterized by low competition. *Arthrobotrys ferox*, with optimal growth rates between 20 and 28°C (Zucconi et al. 1996), shows a low nutritional versatility regarding its ability to utilize carbohydrates. Satchuthananthavale and Cooke (1967) divided nematode predaceous fungi into two ecological groups: the first characterized by adhesive networks and high saprotrophic competitive ability, and the second by constricting rings and low saprotrophic competitive ability. The spring tail predaceous *Arthrobotrys ferox*, which produces adhesive vesicles, seems to fall into the second group. Moreover, *Arthrobotrys ferox* did not secrete chitinase whereas the entomopathogenous *Verticillium* cfr. *lecanii* did; this could indicate different and complementary ecological roles.

The marked ability of *Geomyces pannorum* var. *pannorum* strains to hydrolyse starch and produce extracellular chitinase, urease and lipase at temperatures lower than 25°C confirms them as psychrotolerant (Zucconi et al. 1996). *Aspergillus versicolor* showed a very diversified enzymatic pattern: the ability of this species to decompose pectin, as well as the action of amylase, cellulase and lipase (Domsch et al. 1980), is confirmed.

Some of the activities tested here, such as those for amylase, glucose oxidase and chitinase, are important for their role in several fields of practical application. Glucose oxidase is a very important enzyme in food technology and bioanalysis; it is known to be produced by only few species of *Aspergillus*, *Penicillium*, *Pleurotus*, *Talaromyces* and *Phanerochaete*, but only a few species, i.e., *Aspergillus niger*, *Penicillium amagasakiense* and *Penicillium chrysogenum*, are used for industrial-scale production (Crueger and Crueger 1990; Carlile and Watkinson 1994). High levels of chitinase production are obtained from bacterial strains, although production by moulds is widely reported (Muzzarelli 1989; Sahai and Manocha 1993). High production of fungal chitinase could have some relevant uses, such as the biological treatment of chitin-containing wastes, and mild degradation of chitin to prepare chito-oligosaccharides for medical use. The chitinase activities of the three *Verticillium* strains and *Phoma* sp. no. 2 are worthy of further study.

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