FUNGI FROM MARITIME ANTARCTIC FRESHWATER ENVIRONMENTS

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Abstract. A variety of culturing techniques were employed to isolate fungi from maritime Antarctic lakes. Although aquatic hyphomycetes were absent from all samples, several soil hyphomycetes were isolated. A variety of yeasts (mainly non-fermenting basidiomycetous forms) were also regularly encountered in all lake types investigated. Neither hyphomycetes nor yeasts were present in exceptional numbers despite the high counts previously recorded from surrounding catchment vegetation. In contrast, aquatic phycomycetes were regularly encountered and appear to be present in substantial numbers, at least in the shallow margins of the lakes. A variety of forms were identified, comprising one hyphochytridiomycete, four genera of chytridiomycete and two oomycetes. All the fungal isolates obtained appeared to be psychotrophic. Results from this preliminary study are discussed in conjunction with data from previous Antarctic studies.

Introduction

There is very little information regarding the diversity and distribution of fungi in the inland waters of either continental Antarctica (Wright and Burton, 1981) or the maritime Antarctic region. In the maritime region, Willoughby (1971) has isolated several phycomycetes from lakes at Signy Island, South Orkney Islands (60° 43′ W, 45° 36′ W), and Stanley and Rose (1967) reported yeasts and a filamentous fungus from volcanically heated water bodies at Deception Island (South Shetland Islands). Recently Maslen (1982) reported an unidentified nematode-trapping fungus from a pool on Alexander Island, the first report of this fungal group from an aquatic environment.

No quantitative study of maritime Antarctic aquatic fungi has been undertaken to date. Wynn-Williams (1982) reported that high numbers of yeasts and, to a lesser extent, filamentous fungi occurred in Signy Island terrestrial substrata and their vegetation covers during the spring/summer period when inflow streams were draining through these into various freshwater lakes and pools. It is therefore likely that, at certain times of the year, significant numbers of fungi may be washed into Antarctic lakes in addition to the indigenous mycoflora present in these systems. The lack of both qualitative and quantitative information on the composition and seasonal fluctuations of fungal populations (including yeasts) in maritime Antarctic freshwater environments prompted the preliminary work reported here which forms part of a broad-based, long-term study of Antarctic freshwater microbial ecology.

Environmental Description

The lakes of Signy Island (Fig. 1) have been fully described by Heywood and others (1980) and the seasonal cycles of biological and physico-chemical parameters are described in Light and others (1981) and Ellis-Evans (1981*a*,*b*, 1982). Moss Lake is oligotrophic, with a negligible phytoplankton but extensive benthic plant communities which are active virtually year-round despite the lake being ice-covered for nine months of each year. Heywood Lake is meso-eutrophic, with a relatively abundant phytoplankton during the summer open water period and, due to the high turbidity, has no benthic plant development except in the lake shallows. Whereas Moss Lake is surrounded by a catchment largely comprising rock, ice and fellfield, the catchment of Heywood Lake is dominated by large areas of moss carpet. Both these lakes were sampled year-round on a monthly basis.

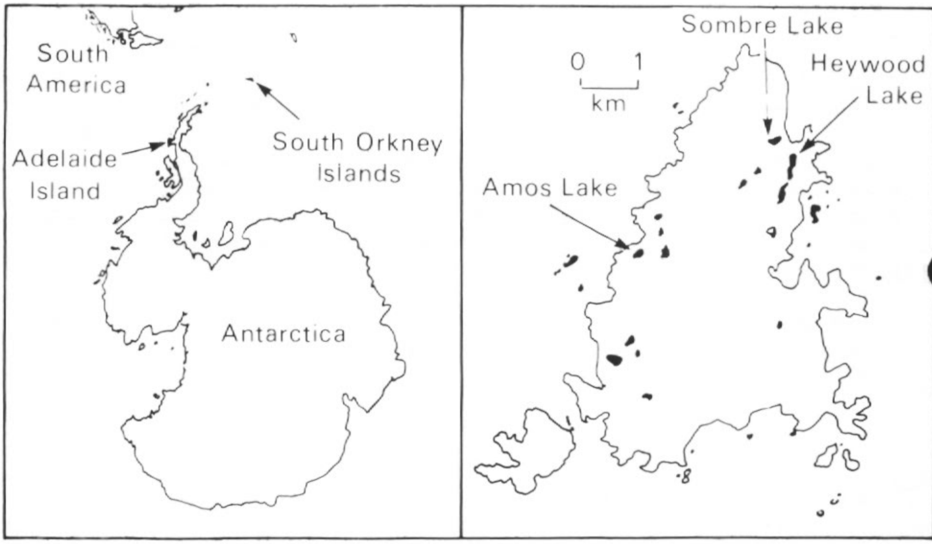

Fig. 1. Diagram showing the location of the freshwater lakes on Signy Island and the location of the South Orkney Islands and Adelaide Island with respect to the Antarctic mainland.

Amos Lake, a turbid, eutrophic system considerably enriched by effluent from bird colonies and seals, was sampled occasionally. Several freshwater pools at Rothera Point, Adelaide Island (67° 34′ S, 68° 08′ W) were also sampled on two occasions and all proved to be oligotrophic with an impoverished flora and fauna (Dartnall, 1980; Priddle and Belcher, 1981).

METHODS

Duplicate 500-ml water samples were obtained using the sterilizable sampling bottle described in Ellis-Evans (1981*a*) and triplicate sediment samples were obtained either by SCUBA diving or by the sediment corer described in Ellis-Evans (1982). Lake margin samples were collected by hand from approximately 10–20 cm water depth in sterile plastic containers. The margin material sampled (ten samples per site) included benthic algal mat, decomposing moss and animal remains.

All samples were kept at environmental temperatures (1–4°C) throughout examination. A variety of agar plate formulations were tested and, in the case of both filamentous fungi and yeasts, highest counts and greatest diversity (assessed visually) were found using Sabouraud Dextrose Agar or Malt Extract Agar (Oxoid). These were amended with sodium propionate (250 mg l^{-1}) to prevent excessive colony spread and aureomycin (30 mg l^{-1}) as a bacteriocide. Water moulds (Saprolegniales) prefer an osmotically inert medium and so Corn Meal Agar (Oxoid) was used here. All plates were incubated at 10°C, rather than 0–4°C (seasonal lake temperature range) to encourage fast growth. Preliminary studies indicated no major difference in isolates obtained using the higher temperature.

Direct microscopic examination of sediment material was undertaken using the agar-film technique of Jones and Mollison (1948). Additionally, glass slides were placed in surface lake sediments (ten replicates per site) or placed (in triplicate) in cork bungs attached at one-metre vertical intervals to a buoyed rope situated at the deepest point in each lake. The slides were recovered at intervals and examined microscopically by phase illumination or placed in Malt Extract Broth as an enrichment culture.

Baits were added to Petri-dishes of lake water or sediment and examined after various incubation intervals. All incubations were initially undertaken at 10°C. A variety of traditional 'baits' was tried including ball-milled chitin, cellophane, insect wings, hemp grain, snake skin (keratin) and filtered planktonic algae, all previously sterilized by autoclaving or UV irradiation.

The identification scheme of Barnett and others (1979) was used to identify yeast isolates to generic level. Other fungal groups were identified by reference to Sparrow (1960), Miller (1961), Karling (1967, 1968), Barron (1968), Onions and others (1981) and Von Arx (1981).

Results and Discussion

Baiting techniques together with direct microscopic examination proved particularly useful for the isolation and recording of phycomycetous fungi. Some differences in the fungi found by Willoughby (1971) and those found in the present study can be seen in Table I, emphasizing the need for further work. The results further show that the plate techniques (including use of Corn Meal Agar) were highly selective yielding only Hyphomycetes whereas baiting techniques, together with direct microscopic examination, proved especially useful in revealing certain parasitic chytrids. These results serve to illustrate the fact that no single method can reveal the entire diversity of a given population in an environment.

Aquatic hyphomycetes were never isolated during this study despite the group's well-known preference for low temperature (Müller-Haeckel and Maranová, 1979). This may have been due to the complete absence of suitable substrates (leaf litter, coniferous needles) but may also be linked with the observations of Maranová and Marvan (1963), who noted that low numbers of conidia coincided with large populations of benthic algae which, of course, dominated all the study sites in the present investigation.

A. Fungi from baits and submerged slides

The Oomycete, *Aphanomyces* sp. (Saprolegniales) was found in shelf margin samples from both nutrient-enriched and nutrient-poor environments in the present study (Table I). The fungus was particularly prevalent in outflow regions of lakes, where large assemblages of filamentous green algae develop each summer. However, whilst the dominant aquatic phycomycete in terms of occurrence was undoubtedly *Aphanomyces* sp., two additional filamentous forms occurred in the Signy Island lakes. *Lagenidium giganteum* (Couch, 1935), another Oomycete (Lagenidiales) previously reported from Heywood Lake margin samples by Willoughby (1971), was encountered occasionally in both study lakes during the current investigation (three samples from Heywood lake, two samples from Sombre Lake). On one occasion, resting spores were noted in association with the vegetative structure. These may have been oospores reported earlier by Willoughby.

Table I. Phycomycetes isolated from Signy Island lakes using baits and submerged slides compared with data from Willoughby (1971) and Knox and Paterson (1973).

Fungal taxa	*Rothera Pools*	*Moss Lake*	*Heywood Lake*	*Amos Lake*	*Victoria Land lakes*
Chytridiomycetes					
Phlyctochytrium acuminatum	—	—	W	—	—
Phlyctochytrium recurvastomum	—	—	—	—	K
Rhizophydium proliferum	—	—	—	—	K
Rhizophydium sp.	—	P	—	—	—
Chytriomyces willoughbyi	—	P(?)	W	—	—
Chytriomyces sp.	—	—	W	—	—
Chytridium versatile	—	P	P	P	K
Catenophlyctis variabilis	P	P	PW	—	K
Hyphochytridiomycetes					
Hyphochytrium catenoides	—	P	W	—	—
Oomycetes					
Aphanomyces sp.	P	P	PW	P	K
Lagenidium giganteum	—	P	PW	—	—
Pythium sp.	—	—	—	—	K
Zygomycetes					
Mucor sp.	—	—	—	—	K

—, absent; P, isolated in present study; W, isolated by Willoughby (1971); K, present (Knox and Paterson 1973); (?) identification uncertain.

Of even greater interest, however, was the finding of *Hyphochytrium catenoides* (Karling, 1939) in several Moss Lake margin samples. The Hyphochytridiomycetes are a very small group of uniflagellate fungi (zoospores characteristically possess an anteriorly located tinsel flagellum), which has received relatively little scientific attention (see Karling, 1967). Judging from the literature, it would appear that *H. catenoides* is reported only very infrequently. It is therefore of some significance that, whilst only a handful of studies have been made of aquatic fungi from Antarctica, *H. catenoides* has now been reported on three separate occasions from the maritime Antarctic, including the present study (Harder and Persiel, 1962; Willoughby, 1971).

In addition to the apparently ubiquitous *Aphanomyces*, Paterson and Knox (1972) also reported *Pythium* and *Mucor* spp. in shoreline samples from Victoria Land freshwater bodies. Both genera have also occasionally been isolated from Signy Island terrestrial environments (Pugh and Allsopp, 1982). Neither of these fungi, however, was found in lake material during the present study nor that of Willoughby (1971), though the latter author did find *Pythium* sp. in soil samples. Despite the limited data available, it is interesting to note that Oomycetes are represented by only one member of the Saprolegniaceae and that Biflagellateae in general are not particularly well represented in Antarctic aquatic environments in terms of either numbers or diversity.

The majority of fungi (other than yeasts) reported present in Heywood Lake (Table I) were restricted to the shallow margin of this lake, but in Moss Lake distribution was widespread. This seems to reflect the more turbid environment of Heywood Lake, which restricts benthic vegetation to the shallow regions of the lake. Contrary to this general observation, however, sediments from the deeper parts of Heywood Lake and Moss Lake on several occasions revealed the presence (on keratin bait) of a chytridiomycete, corresponding to the description of *Catenophlyctis variabilis* (Karling, 1965) previously reported from Heywood Lake by Willoughby (1971). The present study and that of Willoughby (1971) revealed several additional chytridiomycetes, *Rhizophydium* sp., *Rhizophlyctis* sp. and *Phlyctochytrium acuminatum* in the sample material examined. Knox and Paterson (1973) have identified the saprophytic chytrids *Rhizophydium proliferum*, *Phlyctochytrium recurvastomum* and, once again, *Catenophlyctis variabilis* as important members of the natural aquatic microbial flora of coastal continental Antarctica. These authors noted that, whilst chytrid numbers were high, species diversity was very low. Results from Willoughby (1971) and the present study (67 out of 84 samples yielded chytridiomycetes) support this view. Assuming that the pattern of high numbers/low diversity is not simply a function of sampling method, it would appear that the permanently low temperatures and extreme isolation of Antarctica from other continents are exerting marked selection pressures on potential colonizing chytrids. All the isolates in the present study were able to withstand at least two freeze–thaw cycles and to grow at +2°C, but were unable to grow at temperatures above 34°C. Optimum temperatures for growth around 15–20°C indicate a psychrotrophic capability similar to that already reported for bacteria in the same lakes (Ellis-Evans and Wynn-Williams, 1985).

A number of parasitic chytrids have also been found in Antarctic lakes. Willoughby (1971) reported two chytrids parasitic on *Aphanomyces*, namely *Chytriomyces* sp. and *C. willoughbyi*. Both parasites were found to attack the oogonia of *Aphanomyces*, reducing the structure to a featureless mass. Neither chytrid appeared in Heywood Lake samples during the present study, but a chytrid (*C. willoughbyi*?) was observed attacking *Aphanomyces* in a sample obtained from Moss Lake outflow.

Willoughby did not have the opportunity to examine lake water samples from Signy Island. In the present study, an important phytoplankter in Heywood Lake during the open water period (*Schroederia* sp.) was found to acquire a chytrid infection as the summer progressed. Around the time of peak infection the *Schroederia* population declined markedly but insufficient data are available to ascertain if the chytrid was the cause. The same chytrid was also found occasionally parasitizing diatoms amongst the blue-green algal mats of Moss Lake. The chytrid closely resembled *Chytridium versatile*, which has previously been reported infecting pennate diatom populations of pools in the Ross Island area of Antarctica (Paterson and Knox, 1972). It may, therefore, like *Catenophlyctis variabilis*, be of widespread occurrence in Antarctic aquatic environments.

B. Fungi isolated by spread-plating techniques

1. Filamentous fungi. A number of typical soil Hyphomycetes appeared on the spread plates. Those most frequently encountered (Table II) came under the classification Mycelia Sterilia and, judging from the variable morphology, probably included representatives of several species. The predominance of sterile mycelia in Antarctic material has been noted previously in terrestrial sites on the Antarctic Peninsula (Corte and Daglio, 1963) and at Signy Island (Pugh and Allsopp, 1982). The group was isolated from all the freshwater study sites as was *Penicillium*, which was represented by two strains, one being *P. janthinellum* and the other unidentified at present. Heal and others (1967) reported that *Penicillium* spp. formed the majority of colonies developing on agar plates exposed to the air at Signy Island, so its universal occurrence in Signy Island lakes might be anticipated.

Chrysosporium spp. (two strains) were also frequently encountered in this study and one strain, identified as *C. pannorum*, was isolated from every study site on at least one occasion. Pugh and Allsopp (1982) noted the frequent occurrence of this fungus from all their samples (other than moss material) and it was particularly prevalent in areas subject to considerable biotic influence, e.g. seals or penguins. It would appear

Table II. Filamentous fungi isolated from maritime Antarctic freshwater environments, using spread plating methods.

Fungal taxa	*Moss Lake*	*Heywood Lake*	*Amos Lake*	*Rothera Pools**
Mycelia sterilia	+++	+++	+++	+++
Chrysosporium pannorum	++	++	+++	+
Chrysosporium verrucosum	−	++	+	−
Penicillium janthinellum	−	++	++	+
Penicillium sp.	+	+	+	−
Cladosporium cladosporioides	−	+	++	−
Chloridium sp.	−	−	−	+
Gliocladium sp.	+	−	−	−
Mortierella sp.	+	+	−	−
Arthrobotrys sp.	−	+	−	−

+++, Found frequently. ++, Found in several samples. +, Found in one sample. −, Absent from sample.
* This list is probably incomplete for Rothera pools, being based on only two sample sets.

to be particularly associated with low-temperature environments (Kuthubutheen and Pugh, 1979).

The remaining taxa (Table II) were only occasionally found, usually appearing as a single colony on a plate. With the exception of *Cladosporium cladosporioides* and *Chrysosporium verrucosum*, there appeared to be little evidence that the taxa reported here could be linked with specific lake types, and this fits with the cosmopolitan occurrence of these fungi worldwide. Both of the exceptions appeared to be associated with the enriched lakes that have moss carpet-dominated catchments. Meyer and others (1967) reported that, in Antarctic materials, *Cladosporium* occurred only in association with mosses and lichens and, more recently, Pugh and Allsopp (1982) reported *Cl. cladosporioides* almost exclusively from moss and herb material at Signy Island, which links well with the findings reported here. The identification of *Chloridium* and *Arthrobotrys* has not as yet been confirmed, as the original isolates were lost in an incubator failure, but assuming the initial description to be accurate, these would be first reports for the Antarctic. It should also be noted that, with the exception of *Penicillium*, none of the taxa listed in Table II has been reported previously from Antarctic lakes or pools.

Quantitative analysis of population fluctuations proved impractical due to the consistently low counts returned on spread plates. Colony-forming propagules never exceeded 50 per g wet wt. of sediment and no obvious seasonality or individual species domination was observed, though *C. pannorum* and *Penicillium* sp. were invariably present. Counts in water samples rarely exceeded 5 per ml, and colonies invariably proved to be *Penicillium*. It may well be that the fungi described here, all essentially soil-related forms, are washed through the lakes and are only active in the lake sediments where, theoretically, an adequate food supply is available. More substantial numbers were recorded (30–280 propagules per g wet wt.) from the shelf margin samples of both Moss Lake and Heywood Lake, which might indicate a more significant role in this region of the lakes. However, sampling replication was very difficult in this extremely heterogeneous environment, so the significance of even these counts must be questionable.

2. Yeasts. The most common forms were basidiomycetous-type organisms (using the test of Van der Walt and Hops-Havu (1976)) corresponding to the groups

Cryptococcus, *Leucosporidium*, *Rhodotorula* and *Candida* (Table III). Rarer forms included *Hansenula* and the ascomycetous-type *Torulopsis*. Virtually all the isolates proved to be non-fermentative and nitrate-positive. With the exception of *Hansenula*, all the yeasts reported here are known to have optimum temperatures below 25°C (Davenport, 1980) or, in the case of *Leucosporidium*, are largely restricted to cold environments. With the exception again of *Hansenula*, all grew well at lake temperatures as long as the growth medium was relatively rich in organic substrates. These findings, coupled with the high percentage occurrence of basidiomycetous forms, which tend to be better suited to growth at low temperatures, conform well with the general features of cold-tolerant yeasts outlined by Davenport (1980). None of the yeasts (or filamentous fungi) isolated proved to be a true psychrophile, since all had maximum growth temperatures in excess of 20°C. But optimum temperatures in the range 15–22°C and minimum temperatures of 0°C and below indicate a psychotrophic capability.

Table III. Yeasts isolated from maritime Antarctic lakes and pools.

Taxa	*Moss Lake*	*Heywood Lake*	*Deception I. Lakes*	*Rothera Pools*
Rhodotorula	+	+	+	+
Leucosporidium	+	+	–	+
Cryptococcus	+	+	–	–
Torulopsis	–	+	–	–
Candida	+	+	+	–
Hansenula	–	+	–	–

+, Present; –, absent. Deception Island lakes data from Stanley and Rose (1967).

Table IV. Seasonal variations in yeast numbers for Moss Lake and Heywood Lake.

	Moss Lake		*Heywood Lake*	
Date	*Mid water*	*Sediment*	*Mid water*	*Sediment*
Early Nov.	0 (CGU/ml)	—	85	—
Late Nov.	40	—	50	1000
Early Dec.	72	500 (CGU/gWW)	10	1700
Late Dec.	50	2000	65	4000
Late Jan.	0	1100	0	300
Early March	0	500	0	50
April	0	750	10	55
May	0	—	0	0
June	0	—	5	0
July	15	500	0	65
Aug.	0	350	0	15
Sept.	0	100	—	—
Oct.	5	50	20	450
Nov.	10	500	70	1000

—, Not sampled. CGU/ml: Colony generating units per millilitre. CGU/gWW: Colony generating units per gram wet weight.

No attempt has been made as yet to undertake the taxonomy of terrestrial yeasts at Signy Island. However, from examination of colonial morphology, it is apparent that during the spring melt virtually identical colonial forms (often *Leucosporidium* or *Cryptococcus*) are present on agar plates inoculated with catchment, inflow-stream or lake-water sample material. Quantitative data for yeast populations in both Moss Lake and Heywood Lake are presented in Table IV. In the water column, yeasts were present in any numbers only during the spring/early summer period when populations were high in the catchment 'soil' profile (Ellis-Evans and Wynn-Williams, 1985). Bacterioplankton numbers were several orders of magnitude higher during the same period (Ellis-Evans, 1981*a,b*). Laboratory experimentation (Ellis-Evans, 1981*c*) indicates that yeasts cannot compete with bacteria for planktonic extracellular products at ambient lake temperatures, so it is likely that water-column yeasts are virtually inactive. This supports the notion that yeasts are essentially flush-through components of the plankton in these lakes. In contrast, yeasts occurred year-round in both lake sediments though numbers decreased markedly in winter, especially in Heywood Lake. Once again, bacterial populations year round were much larger than the yeast populations (Ellis-Evans, 1982). This contrasts with the terrestrial situation, where extremely high counts of yeasts relative to bacterial populations occurred during the spring melt period. In the latter environment, yeasts behaved as Ruderals, exploiting the relative abundance of nutrients that became available during the snow melt (Hurst and others, 1985). The fact that this group is present in substantial numbers in both Signy Island terrestrial and, to a lesser extent, freshwater environments would seem to merit further investigation.

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