FACTORS AFFECTING THE DISTRIBUTION OF YEASTS IN THE ANTARCTIC ROSS DESERT

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FACTORS AFFECTING THE DISTRIBUTION OF YEASTS

IN THE ANTARCTIC ROSS DESERT

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PREFACE

This thesis is comprised of four main parts--Chapters I, II, III, and an Appendix. The first chapter contains the Introduction and Literature Search. The remaining two chapters are manuscripts in preparation to be submitted to the Journal of General Microbiology. Chapter II is "A Comparison of Yeasts from Arid and Well-Watered Ross Desert Sites," and is a paper in preparation as "The morphology of Antarctic yeasts is correlated with aridity." This title reflects the conclusions of identifying yeasts from a well watered habitat (glacial melt stream) and comparisons with those found (as described species or biovars) in arid soil. Chapter III in this thesis is "The Effect of Soil Mineral Salts on Yeast Distribution in the Ross Desert," and is a paper in preparation as "Soil parameters limiting the distribution of yeasts in the Ross Desert." The Appendix describes in greater detail the various materials and methods referred to only briefly in the manuscripts. Approval for presenting this thesis in the above manner is based upon the Graduate College's policy of accepting a thesis in manuscript form and is subject to the Graduate College's approval of the major professor's request for a waiver of the standard format which will be submitted in March 1987.

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CHAPTER I

INTRODUCTION AND LITERATURE SEARCH

The Ross Desert of Antarctica has been described as abiotic, that is, lacking indigenous life (Horowitz et al., 1972). Since this description, yeast taxa which were both unique to the Ross Desert and adapted to the harsh conditions of this environment have been isolated from soil samples (Vishniac and Hempfling, 1979[a,b]). As these yeasts are the only known indigenous soil biota, they may serve as indicators of soil fertility. This thesis reports on the identity of additional yeasts isolated from Ross Desert sites and on some of the parameters affecting yeast population density and distribution.

Description of the Ross Desert

The Ross desert is the most extreme cold desert on earth (Tendrow and Ugolinik 1966; Schwerdtfeger, 1970; Keys and Williams, 1981). This desert, also known as the Dry Valleys of South Victoria Land, covers approximately 5000 square kilometers of mountains, glaciated and unglaciated high valleys, large barren plateau regions of rock and soil, and lowland valleys which receive drainage from glacial melt streams and lakes during the austral summer (Nov.-Jan). The Ross desert is extremely arid (< 10% relative humidity) and has a reported mean annual precipitation (usually in the form of snowfall) of < 200mm of water per

year (Webb, 1972; Keys, 1980; Wada et al., 1981). The snow which does fall either sublimes or is blown away by the wind (Cameron et al., 1976; Bull, 1976) so moisture available for living organisms is very scarce. Air temperatures range from -15° to 0°C in the summer and drop to near -60°C in the winter (Riordan, 1973). During the austral summer, microorganisms must survive the frequent freeze-thaw cycles on rock and soil surfaces which fluctuate 10 to 15°C above reported air temperatures (Friedmann and McKay, 1985). Additionally, the freeze-thaw cycles combined with high wind velocities result in freeze-drying conditions.

The Ross desert comprises three different habitats: the streams and lakes, the rocks, and the soil. Reports on the biota of these habitats are listed in Table I. The streams and lakes, free of the restricting aridity of the desert rocks and soil, are the most productive. Algal mats (often called modern stromatolites) and plankton are found growing even under ice covered lakes and streams (Parker, 1981; Wharton et al., 1982, 1983). The algal mats of Lake Vanda include the deep water moss, Bryum cf algens, providing the southernmost record of moss growth (Kaspar et al., 1982). The high salinity of these lakes keeps the lakes from completely freezing even though the temperatures remain around 0°C.

The ability of the rocks to absorb and retain heat, thereby modulating the climatic extremes (Friedmann and McKay, 1985) allow sandstones to support (ca. 1 cm below the rock surface) a moderate biomass of cryptoendolithic lichens, cyanobacteria, unidentified colorless bacteria (Friedmann, 1982), and occasionally yeasts (Cryptococcus friedmannii, Vishniac, 1985[b]). According to Friedmann, this simple community is composed only of primary producers (cyanobacteria and the phycobionts

TABLE I

REPORTS ON THE BIOTA OF ROSS DESERT HABITATS

Lakes and Streams	Rocks
Cameron, 1971	Cameron et al., 1976
Cameron et al., 1967, 1972[a], 1970	Friedmann 1977, 1980, 1982, 1984
Goto et al., 1969	Friedmann and Kibler, 1980
Heywood, 1984	Friedmann and Ocampo, 1976
Kaspar et al., 1982	Kappen and Friedmann, 1983
Llano, 1962, 1965	Vestal et al., 1984
Love et al., 1982	Vishniac, 1985[b]
Parker et al., 1977, 1981, 1982[a]	Wilson, 1970
Seaburg et al., 1981	
Sugiyama et al., 1967	
Wharton et al., 1982, 1983	
Young, 1981	

Soils

Atlas et al., 1978
Barghorn and Nichols, 1961
Baharaeen and Vishniac, 1982
Benoit and Hall, 1970
Block, 1984
Cameron, 1968, 1971, 1972, 1974
Cameron et al., 1970, 1976, 1972[a,b]
Di Menna 1960, 1966[a,b]
Horowitz et al., 1969
Rodolph, 1971
Uydess and Vishniac, 1976
Vishniac and Hempfling, 1979[a,b]
Vishniac, 1985[b,c]
Vishniac and Mainzer, 1973

of the lichens), consumers (the mycobionts of the lichens), and decomposers (colorless bacteria). No "higher order" consumers or predators have been observed.

The Soil Habitat

The soils are the least hospitable of the habitats of the Ross Desert. The "soil" is predominantly composed of coarse sand which is often interspersed with large pebbles and small rocks (Cameron, 1974). The soil does not retain as much heat as the large rocks that support the cryptoendolithic communities and, therefore, is more exposed to the effects of freezing temperatures and water loss (Nienow and Meyer, 1981). No demonstrably active photosynthetic organisms have been isolated from these soils (Friedmann and Kibler, 1980), so there is little or no evidence of any primary productivity in the soil community. The carbon content of these soils is low (usually < 0.02 wt%) (Cameron, 1974). Cameron and others have pointed out that the organic matter in the driest parts of Antarctica appears to be derived from anthracite coal (Cameron, 1974; Bauman et al., 1970; Horowitz et al., 1969). The only utilizable carbon sources appear to be those in deposited airspora and weathered cryptoendolithic lichen material. The only abundant nitrogen source appears to be nitrate (Wada et al., 1981; Parket et al., 1982). There is no evidence that nitrogen fixation is occurring in these soils (Friedmann and Kibler, 1980).

It is therefore understandable that the soils of the Ross Desert should have been considered abiotic. The harshness of this environment led to its consideration by some (Cameron et al., 1972; Horowitz et al.,

1972) as a useful martian analogue. Increased knowledge of martian conditions has led to the abandonment of this idea, though Ross Desert soils are still the closest analogue that earth can provide. This habitat does provide a unique opportunity for studying the range over which life can function on earth as well as the opportunity to study ecology at the least complex level (Heinrich, 1976; Cameron et al., 1976).

Organisms Recovered From Desert Soils

The identification of microorganisms able not only to survive, but to reproduce in these soils is crucial to understanding the ecology of the Antarctic dry valleys. The lists of organisms reported to have been recovered from Ross Desert habitats have not been reproduced here because isolating an organism from a particular habitat does not necessarily mean that it is an active component of a community. This is particularly true of the sparsely populated arid Ross Desert soils. The majority of the microorganisms recovered from this extreme environment are mesophiles of exogenous origin blown in by the polar air masses (Horowitz et al., 1972). The antarctic winds and man are responsible for widely distributing algae, bacteria, and fungi throughout the dry valleys (Cameron et al., 1976). The mesophilic bacteria recovered from these soils are common inhabitants of more temperate soils; the isolation of psychrophilic bacteria is rare (Cameron, 1974; Cameron et al., 1976). Since there are no accepted (see Skerman et al, 1980) procaryote taxa described only from Antarctic soils, it is difficult to tell whether the psychrophiles are indigenes or not.

Some antarctic bacteria do appear to be metabolically active for at least a short period of time, undergoing division in situ for a few

generations (Uydess and Vishniac, 1976; Vishniac and Mainzer, 1973) but there is no correlation of bacterial numbers with soil metabolic activity (Horowitz et al., 1972). For example, Horowitz and coworkers studied the bacterial metabolic activity in antarctic soils using a ^{14}C labeled substrate. The evolution of $^{14}C\text{-}CO_2$ was highest in the upper soil layers rather than in the deeper layers which contained the largest amount of bacteria. The recently reported recovery of 10^3 to 10^4 viable bacterial cells per gram from million year old Siberian permafrost in the laboratory (Zvyagintsev, 1985) demonstrates the longevity of dormant microbiota. At the present time, the task of unraveling bacterial roles in the Ross Desert soil ecology appears so complicated as to make bacteria worthless as indicators of soil fertility.

The algae which have been isolated from soil samples do not appear able to withstand the rigorous cold and aridity, nor the long Antarctic night (Cameron, 1972). Filamentous fungi have also been recovered from Ross Desert soils. The inability of their mycelia to survive freeze-thaw conditions, the mesophilic nature of the isolates, and their distribution suggest that, like the bacteria and algae, they are exogenous organisms which are not actively colonizing Antarctic soils.

The species of yeast reported from Antarctica are listed in Table II. The majority of those listed are not psychrophilic (i.e., failing to grow at 25°C). Of the 45 species listed, only the 11 species of the Cryptococcus vishniacii complex described by Vishniac and coworkers (Vishniac and Hempfling, 1979[a,b]; Baharaeen and Vishniac, 1982; Vishniac, 1985[b,c]) and the 8 species listed in Barnett et al. (1984) as not growing at 25°C can be regarded as psychrophiles. Of these 8

TABLE II

YEASTS REPORTED FROM ANTARCTIC HABITATS

Taxon	Habitat	Reference
Aureobasidium pullulans	Dry Valley and Brn Penin. Soils	Atlas et al., 1978
Candida spp.	Antarctic soils	Cameron et al., 1976
Candida albicans	Antarctic soils	Cameron et al., 1976
Candida antarctica	Lake sediment-Lake Vanda	Goto et al., 1969
Candida austromarina[1]	Antarctic oceans	Fell and Hunter, 1974
	Antarctic oceans	Fell, 1974.
Candida diffluens	Ross Desert lake sediment	Goto et al., 1969
	Ross Desert lake water	Goto et al., 1969
Candida famata[2]	Antarctic soils	Soneda, 1961
	glaciers	Di Menna, 1966[b]
	Antarctic continent	Tubaki, 1961
Candida humicola	Ross Desert soils	Goto et al., 1969
Candida norvegica[3]	Antarctic ocean	Fell, 1974
Candida psychrophila[4]	Penguin dung-Cape Royds	Goto et al., 1969
	Arena Valley soils	Atlas et al., 1978
Candida sake[5]	penguin dung	Goto et al., 1969
	Ross Desert lake water	Goto et al., 1969
	Antarctic waters	Fell, 1974
Cryptococcus albidus	Ross Is. soil (Moss/algae)	Di Menna, 1966[b]
	glaciers	Di Menna, 1966[b]
	Ross Desert soils	Di Menna, 1960
	Ross Desert soils	Atlas et al., 1978
	Lake Vanda water	Goto et al., 1969
	Antarctic soils	Cameron, 1971
	Antarctic soils	Cameron et al., 1976
Cryptococcus albidus var albidus[6]	Antarctic soils	Cameron et al., 1976
	Ross Desert soils	Di Menna, 1960
Cryptococcus asgardensis	Ross Desert soil	Vishniac and Baharaeen, 1982
Cryptococcus baldrensis	Ross Desert soil	Vishniac and Baharaeen, 1982
Cryptoccus consortionis	Ross Desert soil	Vishniac, 1985[c]
Cryptococcus friedmannii	lichenized rock, Ross Desert	Vishniac, 1985[b]
Cryptococcus hempflingii	Ross Desert soil	Vishniac and Baharaeen, 1982
Cryptococcus laurentii	Antarctic continent	Soneda, 1961
	Onugul Is. soils	Soneda, 1961
	Ross Desert soils	Atlas, 1978; Di Menna, 1960
	Ross Desert soils	Vishniac and Hempfling, 1979
	Ross Island soil, glaciers	Di Menna, 1966[b]
Cryptococcus luteolus	Ross Desert soils	Di Menna, 1960
	Ross Desert soils	Atlas et al., 1978
	Ross Island soils, glaciers	Di Menna, 1966[b]
	Antarctic soils	Cameron et al., 1976
Cryptococcus lupi	Ross Desert soil	Baharaeen and Vishniac, 1982
Cryptococcus macerans[7]	glaciers	Di Menna, 1966[b]
Cryptococcus rhamnovorans	Ross Deserts soils	Vishniac and Hempfling, 1979[a,b]
Cryptococcus socialis	Ross Desert soils	Vishniac, 1985[c]

TABLE II (Continued)

Taxon	Habitat	Reference
Cryptococcus tyrolensis	Ross Desert soils	Vishniac and Baharaeen, 1982
Cryptococcus vishniacii	Ross Desert soils	Vishniac and Hempfling, 1979[a,b]
	Ross Desert soils	Vishniac and Baharaeen, 1982
Cryptococcus wrightensis	Ross Desert soils	Vishniac and Baharaeen, 1982
Debaryomyces hansenii[8]	glaciers	Di Menna, 1966[b]
	Antarctic ocean waters	Fell, 1974
Leucosporidium antarcticum	Antarctic ocean	Fell, 1974; Fell et al., 1969
Leucosporidium frigidum[9a]	Ross Island soils	Di Menna, 1966[a,b]
Leucosporidium gelidum[9b]	Ross Island soils; glaciers	Di Menna, 1966[a,b]
Leucosporidium nivale[9c]	Ross Island soils; glaciers	Di Menna, 1966[a,b]
Leucosporidium scottii[10]	Ross Island soils	Atlas et al., 1978
	Ross Island soils	Di Menna, 1966[a,b]
	glaciers	Di Menna, 1966
	Ross Desert soils	Atlas et al., 1978
	Ross Desert soils	Di Menna, 1966
	Ross Desert soils	Atlas et al., 1978
	Ross Desert soils	Di Menna, 1960
	Ross Desert soil (lakeside)	Goto et al., 1969
	Lake Sediment	Goto et al., 1969
	Subantarctic waters	Fell, 1974
Leucosporidium stokesii	Antarctic soils	Sinclair and Stokes, 1965
Rhodosporidium bisporidii	Antarctic ocean waters	Fell, 1974
Rhodosporidium dacryoidum	Antarctic ocean waters	Fell, 1974
Rhodosporidium malvinellum	Antarctic ocean waters	Fell, 1974
Rhodosporidium sphaerocarpum	Antarctic ocean	Newell and Fell, 1970
Rhodotorula graminis	Campbell-Mawson glacier	Di Menna 1966[b]
	Ross Desert soil	Atlas et al., 1978; Di Menna 1960
	Antarctic soils	Cameron, 1976
Rhodotorula glutinis	Ross Desert lake water	Gogo et al., 1969
Rhodotorula minuta[11]	Ross Island soils; glaciers	Di Menna, 1966[b]
	Ross Desert soils	Di Menna, 1960
	Ross Desert lake water	Goto et al., 1969
	Antarctic soils	Cameron et al., 1976
Rhodotorula rubra[12]	Ross Island soils, glaciers	DiMenna, 1966[b]
	Ross Desert soils	Atlas et al., 1978
	Ross Desert lake sediment soil, and stream water	Goto et al., 1969
	Antarctic continent	Soneda, 1961
Sporobolomyces holsaticus	Ross Desert soils	Vishniac and Hempfling, 1979[a]
Sporobolomyces roseus	Ross Desert soils	Vishniac and Hempfling, 1979[a]
Sporobolomyces salmonicolor[13]	Antarctic soils	Cameron et al., 1976
	Dry Valley soils	Di Menna, 1960
	Brn. Peninsula, Marble Pt. soils	Atlas et al., 1978

TABLE II (Continued)

Taxon	Habitat	Reference
Sporopachydermia lactativorus[14]	Waters adj. to Ant. Peninsula	Fell and Phaff, 1967
Sympodiomyces parvus	Antarctic ocean waters	Fell, 1974
Trichosporon cutaneum	Ross Desert lake water	Gogo et al., 1969
	Ongul Island & Ant. continent soil	Soneda, 1961; Tubaki, 1961
Trichosporon pullulans	Ross Island soil	Di Menna, 1966[b]

*The names of yeast taxa given in Table II conform to the usage approved by Kreger-van Rij (1984). The taxonomic ascriptions of the yeasts as they appeared in the original papers were as follows:

[1]Candida austromarina = Torulopsis austromarina (Fell, 1974; Fell and Hunter, 1974). [2]Candida famata = Torulopsis famata (Di Menna, 1966[b]; Soneda, 1961; Tubaki, 1961). [3]Candida norvegica = Torulopsis norvegica (Fell, 1974). [4]Candida psychrophila = Torulopsis psychrophila (Goto et al., 1969) or Cryptococcus psychrophila (Atlas et al., 1978). [5]Candida sake = Candida australis (Goto et al., 1969) or Candida natalensis (Fell, 1974). [6]Cryptococcus albidus var. albidus = Cr. albidus var. diffluens (Cameron, 1976) or Cr. diffluens (Di Menna, 1960). [7]Cryptococcus macerans = Rhodotorula macerans (Di Menna, 1966[b]). [8]Debaryomyces hansenii = D. Kloeckeri and D. subglobosus (Di Menna, a 1966[b]). [9a]L. frigidum, [9b]L. gelidum, [9c]L. nivale = C. gelida, C. frigida, and C. nivalis, respectively. (Di Menna, 1966[a,b]). [10]Leucosporidium scottii = Candida scottii (Di Menna, 1966[a,b]; Goto et al., 1969; Atlas et al., 1978). [11]Rhodotorula minuta = Rhodotorula texensis (Goto et al., 1969; Di Menna, 1960) or Rh. pallida, Rh. marina (Di Menna, 1966[b]). [12]Rhodotorula rubra = Rh. mucilaginosa (Di Menna, 1966[b]; Soneda, 1961; Atlas et al., 1978). [13]Sporoblomyces salmonicolor = Sp. odorus (Di Menna, 1960). [14]Sporopachydermia lactativorus = Cryptococcus lactativorus (Fell and Phaff, 1967)

species Candida austromarina, Leucosporidium antarcticum, and Rhodosporidium malvinellum appear to be known only from ocean water; Candida psychrophila and the remaining species (all Leucosporidium spp.) appear to be associated primarily with sites of higher organic content and/or water availability than the arid Ross Desert soils. Where abundance has been reported, psychrophilic yeasts do appear to be most prominent in Antarctic microbiota (Di Menna, 1960, 1966b; Cameron et al., 1976; Atlas et al., 1978). Reported yeast population densities range from 0 to 10_5 g^{-1} of "soils" from glaciers and Ross Island (Di Menna, 1966[b]; Atlas et al., 1978). Di Menna (1966[b]) reported yeasts absent from 52% of the soil samples examined. Yeast density in arid Rodd Desert soils ranged from < 1 to 133 microcolonies (mc)g^{-1}, averaging 1 mc g^{-1} (Vishniac, 1985[a]; Vishniac and Klinger, in press).

Since, with the exception of the Cr. vishniacii complex, the species of psychrophilic yeasts reported from arid Ross Desert soils are more common elsewhere, their indigenicity may be in doubt. Population densities in these soils have never been high enough to allow evidence that centers of population were correlated with demonstrable energy sources. The only taxa which apparently occur only in Ross Desert soils are the yeasts of the Cr. vishniacii complex. The Cryptococcus vishniacii complex of yeasts are physiologically adapted to this environment; they fail to grow at 25°C, are oligotrophic, and can utilize nitrate as sole source of nitrogen (Vishniac and Hempfling, 1979[a,b]; Vishniac, 1983). The failure of previous investigators to isolate these yeasts has been attributed to inhibition by standard yeast media (Vishniac, 1983). A search of soils from similar but less

stressed habitats (sites in the Colorado Rockies and glacial melt stream sediment from the Antarctic Taylor Valley) using appropriate media, did not turn up yeasts of the Cr. vishniacii complex. The Cr. vishniacii complex may therefore consist of yeasts indigenous to arid Ross Desert soils.

It is obvious then that both characterization, to determine adaptive features, and classification, to indicate geographic and evolutionary origins, are important in studying Antarctic microbiota. The manuscript presented as Chapter II contrasts the yeasts of a glacial melt stream in the Ross Desert with yeasts isolated from arid soils.

Physico-Chemical Parameters of Ross Desert Soils

Chapter III presents the results of an examination of the correlation of soil parameters with the distribution of Antarctic yeasts. The low population density of yeasts undoubtedly reflects the absence of primary producers in Ross Desert soil. The patchiness of yeast distribution suggested that physico-chemical parameters of the soil might limit yeast growth.

Antarctic soils are usually considered to have a high mineral salt content. The major soluble salts are chlorides, nitrates, and sulfates of calcium, magnesium, potassium, and sodium. It is not always easy to compare the reported soil content of major inorganic ions (Bockheim, 1979; Claridge and Campbell, 1968, 1977; Keys and Williams, 1981; Vishniac and Hempfling, 1979[a]; Wada et al., 1981) because of the various methods employed and the difference in units of measurements reported. The number of samples studied by each investigator ranged from 4 to 177. In most of these soils sulfates and chlorides of sodium and/or calcium

predominate. The soils are clearly heterogeneous. For example, Bockheim (1979), after analyzing 40 Wright Valley (Ross Desert) soil samples, reported Na^{+} concentrations in 1:5 (w/v) soil extracts ranging from 0.13 to 554.0 meq L^{-1}, Ca^{+2} ranging from 0 to 38.8 meq L^{-1}, Mg^{+2} from 0 to 14.6, K^{+} from 0.015 to 3.9, Cl^{-} 0 to 52.7, SO_4^{-2} from 0.11 to 525.0 meq L^{-1}.

The origins of these salts have been attributed to direct marine incursions (Hendy et al., 1977,), atmospheric precipitation from global air masses and marine aerosols (Claridge and Campbell, 1968), and rock weathering (Behling, 1970). Recent evidence (Claridge and Campbell, 1968, 1977; Williams and Keys, 1981) suggests that the primary sources of magnesium, calcium, and potassium are the physical and chemical weathering of the rocks. The abundant chlorides, sulphates, and sodium minerals are primarily of marine origin (marine aerosols and incursions). Soils nearer to the coast contain a greater proportion of NaCl salts than other ions due to the effect of marine aerosols. The NaCl content of the soils usually decreases inland or at higher altitudes (> 1000 m) where sulphate salts predominate. The magnesium, calcium, and potassium content depends on the parent rock material in that area. For example, the Upper Wright Valley and Victoria Land soils are derived largely from sandstones with dolerite intrusions (Keys and Williams, 1981; Claridge and Campbell, 1977). Their relatively high magnesium and calcium content is derived from the rock weathering of the ferromagnesium minerals (e.g., augite, hornblend) in the dolerite. The high NaCl content of the Upper Wright Valley soils is due in part to its close proximity to the coast. There is also evidence that a direct marine

origin is possible. According to Webb (1972), upper Wright Valley was a fiord at the end of the Pliocene. The upper Taylor Valley soils are primarily composed of granite, do not form discrete horizons, have a relatively higher potassium content due to the weathering of potash feldspars and muscovite micas, and generally have a lower Ca^{+2} and Mg^{+2} content than those of the upper Wright Valley. The anions present are largely sulphate with some traces of chlorides and nitrates. Areas with unusually high sulphate content can be correlated with the weathering of sulphate minerals in nearby moraines, although in most cases sulphates in antarctic soils are derived primarily from atmospheric precipitation.

Inorganic ions of major nutritional importance in Antarctic soils include nitrate, ammonium, and phosphate. Of the common inorganic ions, the most important in microbial nutrition is nitrate. Nitrate concentration is of major interest because the soil content of other N-resources is low to vanishing. Soil organic nitrogen has rarely been reported. Excluding data from soils near penguin rookeries, the organic nitrogen content of these soils is negligible. Cameron (1974) reported organic-N ($\delta^{15}N$) soil contents of 0.002-0.091 $^{0}/_{00}$ (i.e. part per thousand) for soils from Mt. Oliver to Deception Island. Inorganic nitrate is usually the dominant nitrogen source in these soils, ammonia content is low (usually near the limit of detection by standard methods), and nitrite is not detected. For example, Wada et al. (1981) reported the inorganic nitrogen content of 9 Ross Desert soils from Wright Valley and Victoria Land ranged between 10-179 μA of nitrate, 0-0.08 μA of NH_4, and 0 μA of nitrite per gram of soil. Other investigators (Keys and Williams, 1981; Cameron, 1974; Claridge and Campbell, 1977) have reported similar inorganic nitrogen values.

Nitrate, the most abundant N-resource of Ross Desert soils, is primarily of abiotic origin. Delta ^{15}N values in soil from Wright Valley and Victoria Land were among the lowest detected for any terrestrial soil ($\delta^{15}N$ No_3^- from -14.1 to -23.4$^0/00$; -28.6 $\delta^{15}N$ ($^0/00$) (Wada et. al., 1981)). The natural abundance of $\delta^{15}N$ in the literature usually ranges from -17 to +20 $^0/00$. The average $\delta^{15}N$ values for nitrate are 3.2, 6.4, and -6.6 $^0/00$ for forest soils, cultivated soils, and rainwater respectively (Wada et. al., 1975; Wada, 1980). The only processes known to produce such low $\delta^{15}N$ values are abiotic: atmospheric precipitation, photochemical reactions, and aurora activity (Wada et. al., 1981; Claridge and Campbell, 1977).

Wilson and Houser (1965) reported a nitrate in fall of snow at the south pole of 5 x $10^{-8}/cm_2/yr$ (0.0045 lb/acre) and has suggested that aurora activity is the most probable source. Conversion to nitrate occurs primarily at night, and appreciable quantities are produced at high altitudes during the winter (Jones, 1974). Recent evidence by Parker et. al. (1978, 1982), shows a strong correlation of the cyclicity of solar mediated aurora activity and the NO_3^- + NO_2^- content of snow samples from South Pole and Vostok sites. Galactic cosmic radiation and giant sun flares are probably responsible for additional background and spikes in the nitrate content of the snow. The trophospheric nitrate content is extremely low (0.1 ppb, Noxon, 1978); thus the reported value of 2.7 X 10^8kg N/yr falling on the Antarctic continent must be primarily nitrate.

Atmospheric precipitation (snow) from marine aerosols and global air masses has also been proposed as a primary source of abiogenic

nitrate by Claridge and Campbell (1968, 1977). Their evidence is based on the iodate content (0.2%) of the nitrate in Antarctic soils. The only other area where nitrates and iodates are widely distributed is in the cold deserts of northern Chile (Goldschmidt, 1954), where, as in the Antarctic, lack of significant biological activity and leaching allow the nitrate salts to accumulate on such relatively large scale. According to Parker et al. (1982), however, NO_x compounds have a relatively short turnover time (days-weeks) in the atmosphere, thus the nitrate should fall out before it reaches the south pole. Regardless of the reported methods of abiotically creating the nitrate (oxidation of nitrate during global transport or by aurora activity), the effect of wind and sublimation in the absence of significant biological activity or leaching appear to be responsible for the relatively high nitrate concentration in these soils.

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CHAPTER II

A COMPARISON OF YEASTS FROM ARID AND WELL-WATERED ROSS DESERT SITES

Introduction

The microbiota of the Ross Desert of Antarctica is sparse, making it difficult to distinguish between indigenous organisms and deposited airspora (Horowitz et al., 1972; Cameron et al., 1976). The only demonstrably indigenous microbiota in Ross Desert soils are the yeasts of the Cryptococcus vishniacii complex (Vishniac and Hempfling, 1979[a,b]; Baharaeen and Vishniac, 1982[a]; Baharaeen et al., 1982; Vishniac and Baharaeen, 1982; Vishniac, 1983; Baharaeen and Vishniac, 1984), which are not only adapted to life in the cold desert habitat, but are not known to occur elsewhere. The Ross Desert includes two less stressed habitats, the rocks which shelter cryptoendolithic communities (Friedmann, 1982; 1984) and the glacial melt streams and lakes (Parker et al., 1982; Wharton et al. 1982, 1983). The communities of these habitats include primary producers (blue green algae, diatoms) supporting much denser microbial populations than are found in the soils. Several investigators have reported yeast isolations from the Ross Desert. Di Menna (1960) isolated Cryptococcus albidus, Cr. laurentii, Cr. luteolus, Leucosporidium scottii, Rhodotorula graminis, Rh. minuta, and Sporobolomyces salmonicolor from Wright Valley soil samples. With the exception of "Soil 3", from which Cr. albidus, Cr. laurentii, and

Sporobolomyces salmonicolor were isolated, all of the soil samples appear to have been associated with liquid water or organic matter. Goto et al. (1969), working with samples taken from lakes and streams in the Ross Desert, isolated Candida antarctica, C. diffluens, C. humicola, C. sake, Cryptococcus albidus, Leucosporidium scottii, Rhodotorula glutinis, Rh. minuta, Rh. rubra, and Trichosporon cutaneum. Atlas et al. (1978) reported isolating C. psychrophila and Cr. albidus from the Arena Valley; Aureobasidium pullulans, Cr. albidus and Cr. laurentii from near Don Juan Pond and Lake Vanda; and Au. pullulans, Cr. albidus, Rh. graminis, and Rh. minuta from "Asgard Range," remarking that "we did not, in fact, isolate species of yeasts from the Antarctic dry valley (Ross Desert) soils that have not been found in other areas of the Antarctic and that do not occur in other temperate or north polar regions or are even found as common contaminants in the frozen food industry." The same might have been said by earlier investigators with equal truth.

Reports of these species in the arid soils of the Ross Desert may therefore be discounted, since the very low numbers found in these soils could be deposited airspora from nearby sites better supplied with water and/or organic matter. These investigators used conventional yeast media (malt extract, yeast-malt extract, malt-yeast-glucose-peptone, glucose-peptone-yeast, or potato-glucose agar) for isolations, rather than the special (more dilute) media developed by Vishniac (1983, 1985[a]) which allow growth of the Cr. vishniacii complex from smaller inocula than do conventional yeast media. This paper reports the identity of yeasts recovered, in Dr. Vishniac's laboratory, from soil samples from Mt. Dido (an arid site), University Valley (a hanging valley with a small glacier at the closed end), and a glacial melt stream in Taylor

Valley. The yeasts identified are compared with those isolated previously from similar sites, for the purpose of correlating habitat type with yeast characteristics.

Materials and Methods

Culture Maintenance

The 26 isolates studied are listed in Table III. The isolates were originally recovered from Antarctic soil samples by H.S. Vishniac using liquid and solid enation procedures (Vishniac, 1983). Stock cultures were grown and held at 4°C, working cultures at 10°C, on YY-2 medium (Vishniac, 1985[a]) containing NaCl (50 mM), $MgSO_4$-7H20 (0.2 mM), glucose (0.5%), NH_4Cl (2 mM), Na.H. glutamate (pH 6.0, 2 mM), potassium phosphate buffer (pH 6.0, 5 mM), yeast extract (0.05%), Wickerham's vitamins (van der Walt, 1970; Appendix A), Trace minerals (Vishniac and Santer, 1957, Appendix A) and 1.8% Bacto agar (Difco Laboratories).

Methods of Characterization

The standard methods for yeast characterization (van der Walt and Yarrow, 1984) were used when possible. Test included temperatures tolerance, cell and colony morphology, diazonium blue B (DBB) reaction, carbon and nitrogen assimilation, vitamin requirements, ability to produce amylose, and anaerobically ferment sugars. Procedures differing from standard methods were performed as described below.

Temperature tolerance was determined by incubating YY-2 agar slants at 4°C, 10°C, 24°C, 30°C, and 37°C (Precision Model 815 Low Temperature Incubator, or for temperatures above ambient, Precision Thelco

TABLE III

ANTARCTIC YEAST ISOLATES[a]

Taylor Valley Stream Sediment		Mt. Dido Soil	University Valley Soil
solid phase	aqueous phase		
A823-11Y573	A823-11Y582	A834-51Y600	A834-66Y604
A823-11Y574	A823-11Y583	A834-51Y601	
A823-11Y575	A823-11Y584	A834-51Y602	
A823-11Y576	A823-11Y585	A834-51Y603	
A823-11Y577	A823-11Y586		
A823-11Y578	A823-11Y587		
	A823-11Y588		
	A823-11Y589		
	A823-11Y590		
	A823-11Y591		
	A823-11Y592		
	A823-11Y593		
	A823-11Y594		
	A823-11Y595		
	A823-11Y596		

[a]Numbers reflect the austral summer season, the site, and the yeast isolate(s) obtained from that sample respectively (i.e. yeast isolate 574 was collected from site 11 during the austral summer of 1982-1983).

Model 6 Incubator) for 7-17 days. When growth appeared absent or less than optimal, the slants were returned to more favorable temperatures to determine viability or reversibility of heat stress.

Colony morphology (Dalmau plates) was observed using YY-2 agar, yeast nitrogen base (Difco laboratories) supplemented with 0.2% glucose, and M3C agar (see Appendix A) in addition to the (Difco) standard morphology agar. Cell morphology was observed in wet mounts, using the compound microscope, and, for A823-11Y585, A823-11587, A823-11Y591, and A834-51bY600 by transmission electron microscopy, using the preparation

suggested by Baharaeen and Vishniac (1982[b]) (see Appendix B). Results were interpreted on the basis of familiarity with the procedure (see Appendix B) and with the expected characteristics. Attempts to induce sexual reproduction by salmon colored isolates were patterned on the methods which Fell et al. (1973) found successful for Rhodosporidium bisporidii (Appendix B). Parent A was multipoint inoculated onto YY-2 agar then cross streaked with parent B before being incubated at 10°C.

Diazonium blue B (DBB) tests were performed by the method of Hagler and Ahearn (1981) (see Appendix B).

Substrate assimilation tests were performed by incorporating substrates (0.2%) into YY-2 agar minus all organic ingredients, with and without vitamins as required. In some instances assimilation was also tested using yeast nitrogen base (Difco Laboratories) and M3C minus glucose. A prepared plate containing the substrate being tested was multipoint inoculated (OD_{650nm} = 1-2.5) with 14 isolates. Cross-feeding was observed only on plates containing sucrose or trehalose. These substrates were retested in individual agar slants. This procedures was used rather than the standard auxanogram technique because growth at 10°C, the incubation temperature used for psychrophiles, is so slow that substrates spotted on a uniformly inoculated plate diffuse, producing no defined growth patterns. Controls included plates without substrate added (negative control), with glucose (positive control), and inoculation with yeast known to be positive assimilators. Substrates tested were acetate, D-arabinose, L-arabinose, L-aspartate, butyrate, cellobiose, citrate, erythritol, ethanol, fumarate, galactitol, D-galactose, D-glucitol, D-gluconate, D-glucose, D-glucoseamine, L-glutamate,

glycerol, myo-inositol, inulin, 2-ketogluconate (hemicalcium salt), 5-ketogluconate, DL-lactate, lactose, L-malate, maltose, D-mannitol, melezitose, melibiose, alpha-methyl-D-glucoside, raffinose, L-rhamnose, ribitol, D-ribose, salicin, soluble starch, L-sorbose, succinate, sucrose, trehalose, D-xylose (Sigma Chemical Co.).

Nitrogen assimilation test were conducted as above, except 0.5 mls of each suspension was added to nitrogen starvation slants (YY-2 medium minus N-sources) 6 days prior to multipoint inoculation onto YY-2 solid medium supplemented with the N-source (2mM) under investigation. YY-2 plates supplemented with NH_4Cl or minus an N-source served as positive and negative controls, respectively. Compounds tested as sole sources of nitrogen were: NH_4Cl, KNO_3, cadaverine, L-lysine, creatinine, and ethylamine (Reagent grade, Sigma Chemical Company).

Results

All of the isolates studied reacted to the DBB test as basidiomycetous yeasts.

None were fermentative (Isolates A834-51bY600 through 604 were not tested for fermentative ability because they have previously been identified as yeasts belonging to the genus Cryptococcus, none of which are fermentors. Of all the basidiomycetous yeasts species described to date, only 1 species is able to ferment). The carbon assimilation and nitrogen-source utilization test results used in keying (Barnett et al. 1983 was used for primary keying, since variable species are less apt to be confounded when a greater number of characteristics are available) are shown in Table IV. These test results indicated that the isolates

TABLE IV

PHYSIOLOGICAL PROFILES

	Carbon Assimilation														
ISOLATE:	DAra	LAra	Cel	Cit	Ery	Etol	Gol	Gal	Gon	Gam	aMDG	2KG	5KG	gyol	inol
11sY574	+	+	+	-	-	-	+	+	+	-/w	-	+	+	-w	-w
11sY575	+	+	+	-	-	-	+	+	+	-/w	-	+	+	-w	-w
11sY576	+	+	+	-	-	-	w	+	+	-/w	-	+	+	-w	-w
11sY577	+	+	+	-	-	-	+	+	+	-/w	-	+	+	-w	-w
11sY578	+	+	+	-	-	-	+	+	+	-/w	-	+	+	-w	-w
11hY583	+	+	+	-	-	-	+	+	+	-/w	-	+	+	-w	-w
11hY584	+	+	+	-	-	-	+	+	+	-/w	-	+	+	-w	-w
11hY588	+	+	+	-	-	-	w	+	+	-/w	-	+	+	-w	-w
11sY573	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
11hY589	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
11hY590	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
11hY593	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
11sY582	-	-	-	-	-	-	-	-	+	-	-	+	-	+	-
11hY585	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-
11hY586	-	-	+	-	-	-	+	-	-	-	-	+	-	+	-
11hY594	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-
11hY595	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-
11hY587	-	-	+	-	-	-	-	-	+	-	-	w	-	+	-
11hY592	-	-	+	-	-	-	-	-	+	-	-	w	-	+	-
11hY596	-	-	+	-	-	-	-	-	+	-	-	w	-	+	-
11hY591	-	-	+	-	-	-	-	-	+	-	-	+	-	+	-
51bY600	-	ND	+	-	-	-	ND	-	-	ND	-	+	ND	-	+
51bY601	-	ND	+	-	-	-	ND	-	-	ND	-	+	ND	-	+
51bY602	-	ND	+	-	-	-	ND	-	-	ND	-	+	ND	-	+
51bY603	-	ND	+	-	-	-	ND	-	-	ND	w	+	ND	-	+
66bY604	-	ND	+	-	-	-	ND	-	-	ND	-	ND	ND	-	+

TABLE IV (Continued)

	Carbon Assimilation														
ISOLATE:	inu	lac	DLL	malt	man	mli	miz	raf	rha	rib	rol	sal	glol	sor	str
11sY574	-	-	+d	+	+	+	+	+	+	+	-	+	-w	-w	+
11sY575	-	-	+d	+	+	+	+	+	+	+	-	+	-w	-w	+
11sY576	-	-	+d	+	+	+	+	+	+	+	-	+	-w	-w	+
11sY577	-	-	+d	+	+	+	+	+	+	+	-	+	-w	-w	+
11sY578	-	-	+d	+	+	+	+	+	+	+	-	+	-w	-w	+
11sY583	-	-	+d	+	+	+	+	+	+	+	-	+	-w	-w	+
11sY584	-	-	+d	+	+	+	+	+	+	+	-	+	-w	-w	+
11sY588	-	-	+d	+	+	+	+	+	+	+	-	+	-w	-	+
11hY573	-	-	w	-	-	-	-	-	-	-	-	-w	-	+	-
11hY589	-	-	w	-w	-	-	-	-	-	-	-	-w	-	w	-
11hY573	-	-	w	-	-	-	-	-	-	-	-	-w	-	w	-
11hY573	-	-	w	-w	-	-	-	-	-	-	-	-w	-	w	-
11hY582	-	-	+	-	-	-	-	-	-	-	-	-w	-	w	-
11hY585	-	-	w	w	+	-	-	-	-	-	+	-	-w	+	-
11hY586	-	-	w	-	+	-	-	-	-	-	+	-w	+	-	-
11hY594	-	-	w	w	+	-	-	-	-	-	+	-w	+	-	-
11hY595	-	-	w	w	+	-	-	-	-	-	w	-w	+	-	-
11hY587	-	-	+	-	+	-	-	-	-	-	+	-w	+	-	-
11hY592	-	-	+	-	+	-	-	-	-	-	+	-w	+	-	-
11hY596	-	-	+	-	+	-	-	-	-	-	+	-w	+	-	-
11hY591	-	-	+	+d	+	-	-	-	-	-	+	-w	+	-	-
51bY600	ND	ND	-	-	-	-	ND	+	-	ND	-	+	-	-	+
51bY601	ND	ND	-	-	-	-	ND	f	ND	ND	-	+	-	-	+
51bY602	ND	ND	-	-	-	-	ND	f	ND	ND	-	+	-	-	+
51bY603	ND	ND	-	-	-	-	ND	f	ND	ND	-	+	-	-	+
66bY604	ND	ND	-w	+	+	+	ND	-	ND	ND	-	+	ND	-	+

TABLE IV (Continued)

	Carbon Assimilation				N-Utilization					Other	
ISOLATE:	sur	suc	tre	xyl	NO_3^-	LLys	cad	crn	etam	amy	w/o
11sY574	+	+	+	+	-	+	-	-	-	+	-
11sY575	+	+	+	+	-	+	-	-	-	+	-
11sY576	+	+	+	+	-	+	-	-	-	+	-
11sY575	+	+	+	+	-	+	-	-	-	+	-
11sY576	+	+	+	+	-	+	-	-	-	+	-
11sY577	+	+	+	+	-	+	-	-	-	+	-
11sY578	+	+	+	+	-	+	-	-	-	+	-
11sY583	+	+	w	+	-	+	-	-	-	+	-
11hY573	-	-	-	+	+	ND	ND	ND	ND	-	+
11hY589	-	+d	-	+	+	ND	ND	ND	ND	-	+
11hY590	-	+d	-	+	+	ND	ND	ND	ND	-	+
11hY593	-	w	-	+	+	ND	ND	ND	ND	-	+
11hY582	-	+	-	+	+	ND	ND	ND	ND	-	+
11hY585	wf	+d	-	-	+	ND	ND	ND	ND	-	+
11hY586	wf	+d	-	-	+	ND	ND	ND	ND	-	+
11hY594	wf	+d	-	-	+	ND	ND	ND	ND	-	+
11hY595	wf	+d	-	w	+	ND	ND	ND	ND	-	+
11hY587	-	w	+	w	+	ND	ND	ND	ND	-	+
11hY592	-	w	+	w	+	ND	ND	ND	ND	-	+
11hY596	-	-	+	w	+	ND	ND	ND	ND	-	+
11hY591	-	+	wf	-	-	ND	ND	ND	ND	-	+
11hY600	ND	+	+	-	-	ND	ND	ND	ND	+	+
11hY601	ND	-	+	-	-	ND	ND	ND	ND	+	+
11hY602	ND	-	+	-	-	ND	ND	ND	ND	+	+
11hY603	ND	+	+	-	-	ND	ND	ND	ND	+	+
11hY604	ND	+	+	+	+	ND	ND	ND	ND	+	+

Note: The abbreviations heading this table mean: DAra = D arabinose, LAra = L arabinose, Cel = cellobiose, Cit = citrate, Erol = erythritol, Etol = Ethanol, Gol = galactitol, Gal = galactose, Gon = gluconate, Gam = glucoseamine, MDG = alpha methyl glucoside, 2KG = 2-ketogluconate, 5KG = 5-ketogluconate, gyol = glycerol, ino = inositol, inu = inulin, lac = lactose, DLL = DL-lactate, malt = maltose, man = mannitol, mli = melibiose, mlz = melezitose, raf = raffinose, rha = L-rhamnose, rib = ribose, rol = ribitol, sal = salicin, glol = glucitol (sorbitol), sor = sorbose, str = soluble starch, sur = sucrose, suc = succinate, tre = trehalose, xyl = xylose, L-lys = L-lyline, cad = cadaverine, crn = creatinine, etam = ethylamine, amy = amylose production, w/o = growth without vitamins, N.D. = not done

could be grouped into biovars. The identification of each biovar is discussed separately below.

Biovar 46

The salmon colored isolates A823-11Y574 through 578, and 583, 584, 588 were given the biovar number 46; A823-11Y574 was then considered the type of this biovar. All were similar in cell morphology, growing as ovoid cells, so elongated as to appear rodlike (Figure 1.), and producing pseudomycelia and mycelia. On morphology agar and in mating experiments the mycelium developed clamp connections (Figure 2.), but neither teliospores nor filobasidia were seen, indicating that these yeasts were diploids or heterokaryons defective in sexual reproduction. Mating experiments were therefore unsuccessful, and would not have been undertaken if the observation of clamp connections had been made earlier. Biovar 46 was eurythermal, growing from 4°C (in the refrigerator) to 30°C, though growth was unreliable at 30°C. Biovar 46 is characterized by the production of amylose, a requirement for thiamine which makes it impossible to grow without vitamins, the utilization of ammonia, but not nitrate, and of L-lysine, but not cadaverine, creatinine, or ethylamine as N-source. In addition to the standard substrates, biovar 46 utilized as sole C-source: L-aspartate, acetate, and L-malate.

Biovar 46 has been identified as Cryptococcus hungaricus. The assimilation pattern seen in Table IV keys to and agrees with the description of Cryptococcus hungaricus in Barnett et al. (1983) in positive assimilation of L-arabinose, cellobiose, galactitol, galactose, 2-ketogluconate, maltose, mannitol, melezitose, raffinose, succinate, sucrose, trehalose, and D-xylose; in failure to assimilate inulin; and

Figure 1. Cryptococcus hungaricus isolate A823-11574. Light micrograph displaying characteristic polar budding rod shaped cells. Bar = 10 μm.

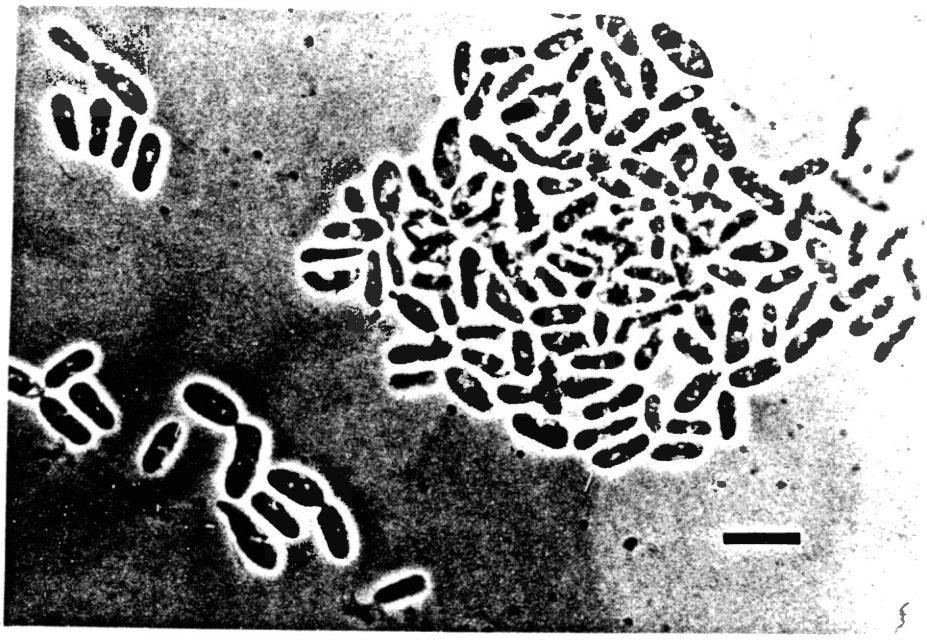
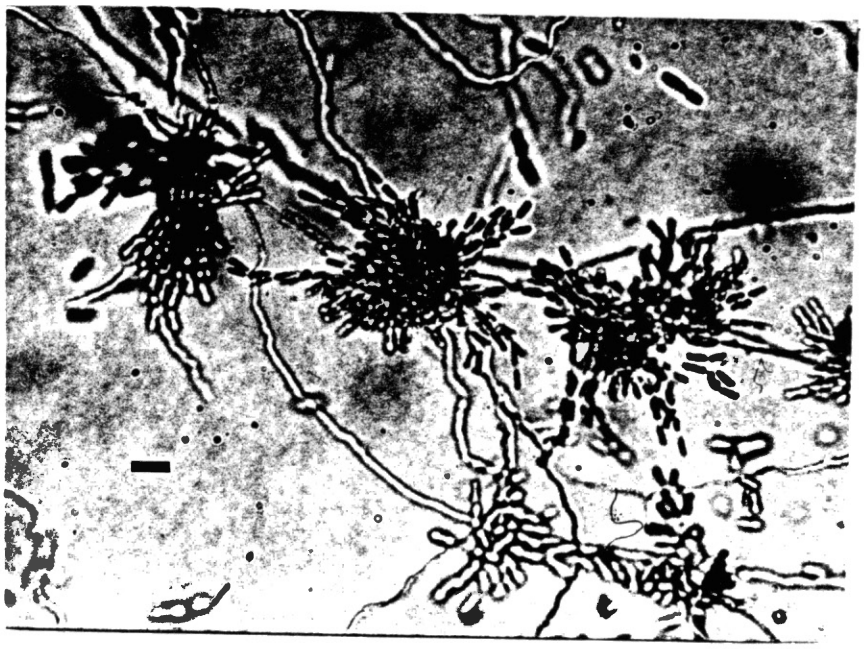
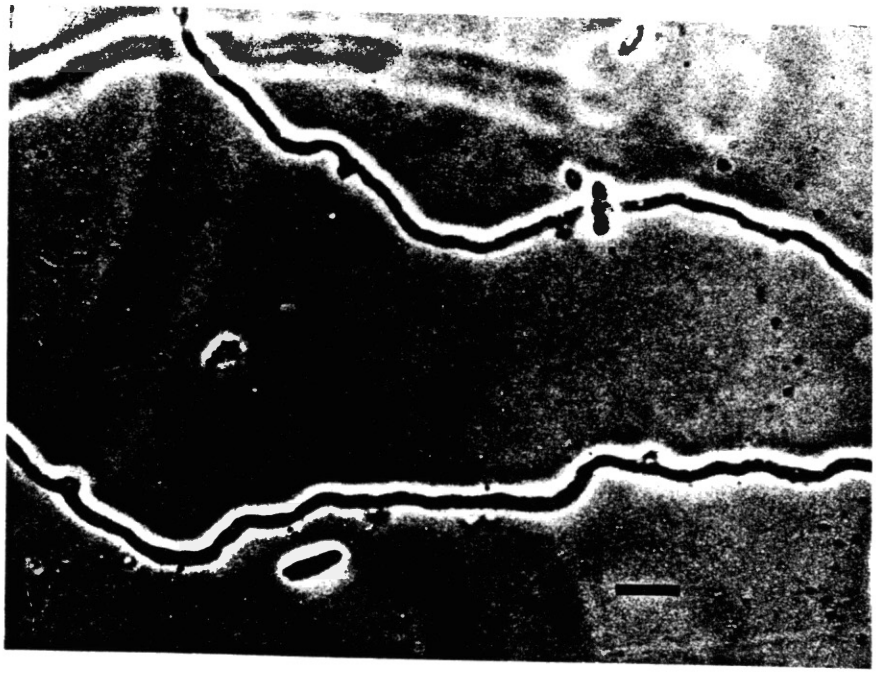


Figure 2. Cryptococcus hungaricus isolate A823-11574. (Top) pseudomycelial and mycelial development on cornmeal agar at four weeks incubation at 10°C Bar = 10 μm. (Bottom) close up of mycelia with clamp connections Bar = 10 μm.





is consistent with the description of the following as variable: D-arabinose (+), citrate (-), ethanol(-), erythritol (-), gluconate (+), D-glucoseamine (-/W), glycerol (-/W), inositol (-/W), DL-lactate (+d), lactose (-), melibiose (+), alpha-methyl-D-glucoside (-), L-rhamnose (+), ribitol (-), D-ribose (+), salicin (+), L-sorbose (-/W), and starch (+). The description of Cr. hungaricus further agrees with the characterization of biovar 46 in failure to utilize nitrate, cadaverine, or ethylamine as N-sources, in requiring vitamins (thiamine), and in producing amylose, and is consistent in being variable with the failure of biovar 46 to use L-lysine or creatinine as N-sources. The points of disagreement are weak to negative assimilation of glucitol, and some growth at 30°C by biovar 46. Cr. hungaricus is described as assimilating glucitol and failing to grow at 30°C (variable growth at 25°C). The description of the type strain CBS 4214 in Kreger-van Rij (1984) differs from that (of a number of strains) in Barnett et al. (1983) by characterizing Cr. hungaricus as assimilating raffinose variably and assimilating succinic acid weakly. Kreger-van Rij also described this species as budding multilaterally and failing to make pseudomycelium, while Barnett et al. (1983) describe it as budding polarly, and producing simple pseudohyphae. A subculture of the type was received as NRRL Y6667 (courtesy of Dr. C. P. Kurtzman) in this laboratory. The budding morphology of this biovar is identical to that described by Barnett et al. (1983). Biovar 46 differs from the type and all previously described strains of this species in producing mycelium, as well as in producing clamp connections.

Biovar 47

Biovar 47 is comprised of isolates A823-573, 582, 589, 590, and 593 which are characterized by white to cream colored colonies containing oblong shaped, biopolarly budding cells which produce pseudohyphae on corn meal agar. Chlamydospores were also observed for isolates A823-11573 and 582. This biovar is DBB positive, psychrophilic (no growth at 24°C), negative for amylose production, and does not require vitamins for growth.

Biovar 47a is typified by A823-11Y573 and includes A823-11Y589, 590, and 593. It positively assimilated D-glucose, 2-ketogluconate, glycerol, and succinate (+ to delayed) as its sole carbon source. Ammonia or nitrate could be utilized as a sole nitrogen source. Biovar 47a has been identified as Leucosporidium antarcticum using the assimilation pattern in Table IV which keys to and agrees with the description of L. antarcticum in Barnett et al. (1983) in the negative assimilation of D-arabinose, L-arabinose, cellobiose, citrate, erythritol, galactitol, D-galactose, D-gluconate, D-glucoseamine, alpha-methyl-D-glucoside, 5-ketogluconate, myo-inositol, inulin, lactose, maltose, melibiose, melezitose, raffinose, L-rhamnose, D-ribose, ribitol, salicin, and L-sorbose; and is consistent with the description of the following as variable: 2-ketogluconate (+), glycerol (+), soluble starch (-), and succinate (- to delayed). The description of L. antarcticum further agrees with the characterization of biovar 47a in the ability to use ammonia or nitrate as a sole N-source (other N-sources were not tested); in not producing amylose and in failure to grow $\geq$ 24°C. The points of disagreement are the ability to grow without vitamins, assimilation of xylose, and the inability to assimilate D-glucitol, D-mannitol, and

ethanol. The assimilation of D-glucitol, D-mannitol and ethanol are listed in Barnett et al. (1983) as delayed characteristics evident after 7 days at 19°C. Incubation of our isolates were terminated at 21 days at 10°C; delayed results were therefore not considered when keying. The description of three strains in Kreger-van Rij (1984) differs from that in Barnett et al. (1983) in not requiring vitamins for growth and the variable use (depending on strain) of D-xylose, D-mannitol, D-galactose, sucrose, maltose, trehalose, and raffinose. D-glucitol and ethanol results are not given. Excluding delayed or variable assimilation differences, this biovar does not differ significantly from the description of L. antarcticum of Barnett et al. (1983) (differences in xylose assimilation, growth without vitamins) or Kreger-van Rij (1984) (no differences) and therefore was assigned to this taxon. It should be noted that positive assimilation characteristics could not be used to identify this biovar due to the low number of substrates utilized. There are no substrates listed in Barnett et al. (1983) which are consistently used by all strains of L. antarcticum except D-glucose.

Biovar 47b is grouped separately in Table IV, but resembles this biovar 47a closely and will therefore be discussed here. This biovar differs from Biovar 47a in also assimilating D-gluconate, and DL-lactate. Two differences in assimilation pattern is not sufficient, per se, to define distinct species even though D-gluconate assimilation is not known to cross specific borders (Golubev, 1980). This isolate could only be identified to genus, as Vanrija (Moore, 1980) (those blastobasidiomycetes formerly placed in Candida). Whether this isolate (or other cream colored biovars from the glacial melt stream sediment) is conspecific with L. antarcticum can only be decided on the basis of

nucleic acid characteristics (Fuson et al., 1979, 1980) which are presently unknown.

Biovar 48

Biovar 48 is typified by A823-11Y585 and includes A823-11Y586, 594, and 595. This biovar differs from the profile of L. antarcticum (Barnett et al. 1983) in the assimilation of cellobiose and ribitol (excluding delayed and variable characteristics) and therefore has been keyed to and tentatively identified as Leucosporidium antarcticum. Biovar 48 differs from biovar 47 in the assimilation of cellobiose (+), D-mannitol (+), D-glucitol (+) and D-xylose (-); from biovar 47b additionally in the assimilation of gluconate (-) and in assimilating DL-lactate only weakly.

A823-11Y586 may be considered biovar 48b, since it differed from the type of biovar 48 (and from biovar 47) in the positive assimilation of galactitol and in negative assimilation (rather than weak) of maltose. Maltose assimilation is not considered significant in this case.

Biovar 49

Biovar 49 is typified by A823-11Y587 and includes A823-11Y592 and 596. This biovar is characterized by cream to white colored colonies comprised of slender, long to ovoid cells which exhibit bipolar budding and produce pseudohyphae and septate mycelia on corn meal and 3MC agar. This biovar was DBB positive, amylose negative, psychrophilic, and did not require vitamins for growth. The carbon and nitrogen sources utilized on YY-2 agar were D-glucose, cellobiose, D-gluconate, glycerol,

D-glucitol, 2-ketogluconate, DL-lactate, D-mannitol, ribitol, succinate (weak), trehalose, xylose (weak), ammonia, and nitrate. Assimilation of succinate, trehalose, and xylose appeared to be inhibited by the inclusion of Wickerhams vitamins in test media.

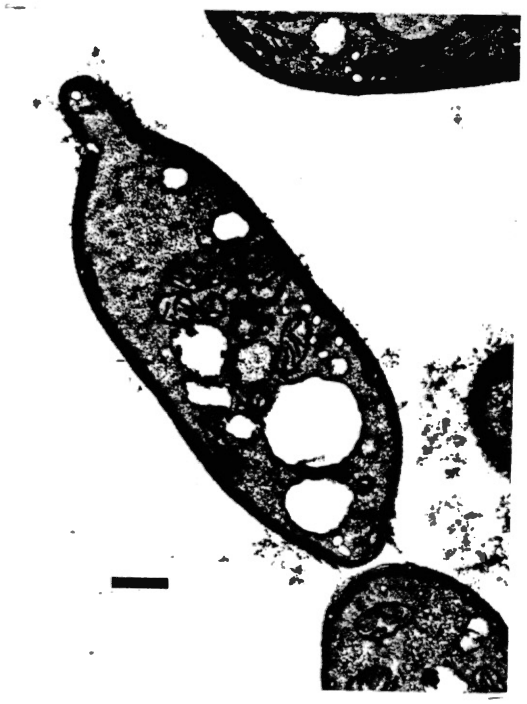
The physiological profiles listed in Barnett et al. (1983) are based on media containing vitamins. The inhibition by Wickerhams' vitamins makes this biovar unkeyable unless identification is based on results from growth on YY-2 without vitamins. Using these results, this biovar can be identified as Vanrija (Candida) foliorum from the following: positive assimilation of glucose, cellobiose, D-galactose, alpha-methyl-D-glucoside, D-glucoseamine, 5-ketogluconate, myo-inositol, inulin, maltose, melibiose, melezitose, raffinose, L-sorbose, soluble starch, and sucrose; and is consistent with the description of V. foliorum as variable in assimilating: D-arabinose (-), L-arabinose (-), L-rhamnose (-), ribose (-), ribitol (+) and salicin (- to weak). The description of V. foliorum further agrees with biovar 49 in the ability to utilize nitrate or ammonia as an N-source, DBB (+), amylose (-), and in the ability to form pseudohyphae and septate mycelia. The points of disagreement are the failure to utilize citrate or ethanol, the ability to grow without vitamins, and the inability to grow at 25°C. V. (Candida) foliorum is described in Barnett et al. (1983) as assimilating citrate and ethanol, requiring vitamins, and growth (variable) at 25°C. The description of the type strain (CBS 5234) in Kreger van Rij (1984) differs from that of strains (CBS 5234, 6370) in Barnett et al. (1983) in delayed growth (assimilation) on citrate, succinate, and D-xylose (the ethanol response is not listed) and the ability to grow in vitamin free medium. Excluding the comparisons of the delayed assimilation

results listed in Kreger-van Rij (1984), biovar 49 disagrees with the description of C. foliorum only in the failure to grow at 37°C. It has been suggested by others (Cameron et al., 1976; Di Menna 1960, 1966[a,b]; Goto et al., 1969) that some typically mesophilic yeasts collected in the Antarctic appear cold adapted to the antarctic environment. Thus this biovar has been assigned to this taxon. This biovar is listed in Kreger-van Rij (1984) and Barnett et al. (1983) as Candida foliorum. We have used the name to Vanrija foliorum according to the classification by Moore (1980) in which previously described basidioblastomycetous Candida species have been assigned to the genus Vanrija: the remaining Candida species are ascomycetous yeasts.

Biovar 50

A823-11Y591 was assigned to biovar 50 and is characterized by white to cream colored colonies containing oblong, polarly budding cells which produce pseudohyphae and septate mycelia when grown on corn meal or M3C agar. TEM micrographs (Figure 3) demonstrate a typical basidiomycete cell wall: multilayered, with a frayed collar, and repetitive budding site. This biovar differs from the previously described biovars in that it can not utilize nitrate as a nitrogen source. On YY-2 medium this isolate assimilated cellobiose, D-gluconate, D-glucitol, D-glucose, 2-ketogluconate, glycerol, D,L-lactate, maltose (delayed), D-mannitol, ribitol, and succinate as a sole carbon source. It did not assimilate the following carbon sources: D-arabinose, L-arabinose, citrate, erythritol, ethanol, galactitol, D-galactose, D-glucoseamine, alpha-methyl-D-glucoside, 5-ketogluconate, myo-inositol, inulin, lactose, melibiose,

Figure 3. TEM micrograph of biovar 50. Vanrija spp. (isolate 11Y591). Note typical basidiomycetous collar showing layered collar cell wall at the ends of the bipolarly dividing cell. Bar = 1μm.



melezitose, raffinose, L-rhamnose. Salicin (-to weak), L-sorbose, soluble starch, sucrose, or D-xylose. No conclusive results were observed for trehalose plates (feeding) or on the subsequent testing on YY-2 slants. Melibiose and inositol were also assimilated when grown on Wickerhams' but not on YY-2 media. Of the two nitrogen sources tested this biovar assimilated ammonia but not nitrate as a sole source of nitrogen. Biovar 50 was also negative for amylose production, psychrophilic, DBB positive, and did not require vitamins for growth. This biovar has been identified as a species of Vanrija based on the following characteristics (Moore, 1980): cream colored colonies, DBB positive, fermentation negative, presence of pseudohyphae and septate mycelia. There was not match with any previously described Vanrija (Candida) species (Kreger-van Rij, 1984; Barnett et al., 1983).

This biovar differs from the biovar 47b Vanrija by its ability to assimilate cellobiose, maltose (delayed), ribitol, and the failure to assimilate nitrate.

Biovar 51

A834-51bY600 typifies biovar 51 and includes A834-51bY601 through 603. This biovar was recovered from Mount Dido soils and is characterized by producing white to cream colored colonies containing round, polarly dividing cells which do not form pseudohyphae or septate mycelia. Transmission electron microscopy of the type isolate of this biovar (A834-bY600) showed monopolar budding and blastobasidiomycetous cell wall characteristics (Figure 4). Basidiomycetous cell wall characteristics have been correlated with a positive reaction to the DBB test

Figure 4. TEM micrograph of Biovar 51. Cryptococcus spp. (isolate 51bY600). Note typical basidiomycetous layered cell wall and frayed collar at the site of budding. Budding is monopolar. Bar = 1μm.

(Simmons and Ahearn, 1987). This biovar is DBB positive, psychrophilic (capable of growth at 4 to 10°C but not at 25°C), does not require vitamins for growth, and is capable of amylose production. Like biovar 50, it can utilize ammonia but not nitrate as its sole nitrogen source. This biovar is characterized by the ability to assimilate cellobiose, D-glucose, 2-ketogluconate, myo-inositol, salicin, soluble starch, and trehalose; but is unable to assimilate D-arabinose, citrate, ethanol, erythritol, galactitol, D-galactose, D-glucitol, alpha-methyl-D glucoside, glycerol, DL-lactate, maltose, D-mannitol, melibiose, ribitol, L-sorbose, and D-xylose. This biovar has been identified to the genus Cryptococcus on the following characteristics (Barnett et al., 1983): round cells, lack of filamentous growth, inositol and DBB positive. Its assimilation characteristics do not match those of any previously described species.

Biovar 52

A834-66Y604, assigned to biovar 52, was recovered from University Valley soil. The cream to white colored colonies are composed of round, polarly-budding cells which do not form pseudohyphae or septae mycelia on corn meal or M3C agar, are psychrophilic, DBB positive, and capable of amylose production and growth on medium lacking vitamins. The carbon and nitrogen sources utilized are D-glucose, cellobiose, D-gluconate, myo-inositol, maltose, D-mannitol, melezitose, raffinose, salicin, soluble starch, succinate, trehalose, D-xylose, ammonia and nitrate. The following carbon sources were not utilized: D-arabinose, citrate, ethanol, erythritol, D-galactose, alpha-methyl-D-glucoside, glycerol, D,L-lactate, L-rhamnose, ribitol and L-sorbose. (Raffinose and rhamnose

results were observed only for isolate A834-66bY600, these assimilation characteristics have not been established for the remaining isolates in this biovar.) This biovar agrees with and keys to the genus Cryptococcus (Kreger van Rij, 1984) in the positive assimilation of inositol, the nonfilamentous, round cell morphology, and DBB (+) reaction. Biovar 52 has been identified only to genus Cryptococcus and had no match with any previously described Cryptococcus species.

Biovar 52 differs from biovar 51 (recovered from the Mount Dido soil) in its ability to assimilate D-xylose, D-gluconate, malate, maltose, melezitiose, succinate, and nitrate.

This biovar and the remaining as yet unidentifiable biovars (47b, 50, 51) are currently being investigated by others in our laboratory.

Discussion

The most striking difference between the yeast biovars isolated from the comparatively rich glacial melt stream sediment and those biovars from more arid and depauperate upland soils of the Ross Desert was the ability of the former to produce mycelium and/or pseudomycelium. None of the upland isolates produced either. All of the glacial melt stream sediment isolates produced at least pseudohyphae. There was no real difference in the number or identity of carbon compounds available as sole substrates. The upland biovars 51 and 52, like the Cryptococcus spp. described previously from such sites (see Vishniac, 1985[b,c]), utilized from 6 to 15 of the standard substrates. Only Cr. hungaricus (biovar 46) utilized a greater number; the other meltstream biovars utilized 6 to 12 of the standard substrates. A vitamin requirement was seen only in Cr. hungaricus, but Cr. consortionis (Vishniac, 1985[c]) from

Linnaeus Terrace (upland) soil, also required thiamine. Both nitrate-utilizing and nitrate-non-utilizing yeasts were isolated from each habitat type.

The production of mycelium and/or pseudomycelium occurs in basidiomycetous yeasts under the following conditions: 1) as an anomaly--very short lengths of pseudohyphae (rudimentary pseudomycelium) may occur as a small percentage of cells in a culture. The description of pseudohyphae in cryptococcal lesions (Emmons et al., 1977) suggests that these were not pseudohyphae but cells which could not separate under the constraints imposed by the cells around them. 2) other descriptions of hyphae or pseudo-hyphae in the genus Cryptococcus as defined in Kreger-van Rij (1984) refer only teliomorphs, i.e., Filobasidiella, or 3) to the behavior of Cr. neoformans under extreme selection against cellular growth (Nielson et al., 1978). Such forms apparently do not occur in nature. 4) In Vanrija and its teliomorphs, as in many Candida species, hyphae and pseudohyphae appear as the colony ages or on dietetic agar, suggesting that this is a response to nutrient depletion. The ability to produce pseudohyphae or hyphae formerly separated the genus Candida Berkhout from the genus Torulopsis. Yarrow and Meyer (1978) transferred all Torulopsis spp. to the genus Candida on the grounds that the separation was arbitrary and artificial. It is true, in our experience, that the ability to produce pseudohyphae and/or hyphae may be difficult to observe. The occurrence of mycelium and pseudomycelium producing biovars only in the habitat which allowed relatively rapid growth suggests that this characteristic has ecological significance and therefore that the separation of genera on this ground is not artificial. These

yeasts, if they grew in the depauperate highlands, would presumably be frozen as mycelia and therefore die. The failure to isolate oligotrophic yeasts from the sediment suggests that they may not be able to compete with the more copiotrophic yeasts. The yeast biota of these two habitat types were quite distinct.

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CHAPTER III

THE EFFECT OF SOIL MINERAL SALTS ON YEAST DISTRIBUTION IN THE ROSS DESERT

Introduction

Arid soils from The Ross Desert (dry valleys) of south Victoria Land, Antarctica, contain a sparse population of microbiota at best. The only demonstrably indigenous microorganisms are the yeasts of the Cryptococcus vishniacii complex (Vishniac and Hempfling, 1979[a,b]). Their population density averages 1 microcolony (mc) g^{-1} (Vishniac, 1985; Vishniac and Klingler, in press). Water and substrate availability limit yeast growth (unpublished results, H. S. Vishniac). Although Horowitz et al. (1972) found that the addition of water alone invariably resulted in bacterial growth in any soil sample in which growth could be induced, the addition of water to three air dry (most of the soil samples were air dry as collected) soil samples resulted in yeast growth in only one of them, the other two required the addition of substrate as well. Yeasts were not isolated from other soil samples by any treatment. While indigenous yeasts could serve as an index of soil fertility, their distribution in Ross Desert soils appeared to be limited by factors other than nutrient and water requirements. Antarctic soils have been reported to have a high mineral salt content (Bockheim, 1979; Claridge and Campbell, 1977; Keys and Williams, 1981). In such arid soils, the resulting low water potential could be a major

factor in limiting yeast distribution. Yeasts of the Cr. vishniacii complex were reported to have rather low halotolerance (Vishniac and Hempfling, 1979[b]). We therefore examined the effect of cation and clay content on the water potential of selected soil samples, determined their inorganic nitrogen content, and correlated the results of these analyses with yeast distribution.

Materials and Methods

Soil Samples

The Ross Desert soil used in the experiments below were collected during the 1980-1981, 1981-1982, 1982-1983, and 1983-1984 summer seasons by members of the ACME (Antarctic Cryptoendolithic Microbial Ecosystem) group, under the leadership of Dr. E.I. Friedmann. Samples were collected aseptically and stored in sterile 'whirlpak' bags, shipped frozen, and stored at -80°C (Revco freezer) until time of analysis when they were shifted to -20°C. At the time of use, soils were placed on dry ice until added to various extract solutions or growth medium.

Preparation of Glassware

Glassware was routinely washed with a standard laboratory detergent (S/P detergent concentrate, American Scientific Products) and rinsed 5 times with tap water and 5 times with glass distilled reverse osmosis water. However, glassware used in cation and inorganic nitrogen resources on growth rates was acid cleaned with $4NH_2SO_4$ or 4N HCl (2-3 day soak followed by rinsing with tap water (5 times) then with glass distilled reverse osmosis water (10 times) before use.

Water Potential

The water potential of simulated soils and 6 Ross desert soils (A801-25, A812-1, A823-4, A823-6, A834-63a, A834-65b) was measured by the dew point microvoltmeter technique. Simulated soils were prepared by mixing kaolinite, montmorillonite, and sand (Wards Natural Science Corp., Rochester, N.Y.). Ten grams of each air dried soil (unless otherwise noted) was placed into a polyethylene (7 ml) scintillation vial and water was added (range 0.025-25% v/w) using a micropipettor or serological pipet. The vials were then capped, hand shaken for three minutes, and left to equilibriate overnight at room temperature. The soil samples were then poured into a weighing boat, mixed, and replaced in the vial, adding the probe midway in the process. The top of the vial was parafilmed, recapped, and again covered with parafilm. The cap was then sealed with vaspar and the vials placed in a water bath (25°C). The water potential was measured at 24 and 48 hours using a Wescor HR33-T dew point microvoltmeter (model 5103). Where possible, (when sufficient mass was available) soils were analyzed in triplicate.

Characteristics of Simulated Soils

The particle size of sand and clay (kaolinite, montmorillonite) mineral standards were determined by sieving (USDA Standard Testing Sieves: #20, 60, 100, and 200 mesh).

Rough bulk density measurements of the simulated soils were made as follows: 100 g of the simulated air dried "soil" were poured into a 100 ml glass graduated cylinder then alternately shaken and tapped gently for 15 minutes. The volume in the graduated cylinder was

recorded at 5, 10, and 15 minutes. When the volume of the packed cylinder remained stable (10-15 minutes of shaking), the bulk density was obtained by dividing the g of soil by the volume of soil in the cylinder (i.e., g/cm^3). Sand-montmorillonite (3% and 10%) and sand-kaolinite (10%) mixtures were run in duplicate. The sand was analyzed in triplicate using both 100 and 90 g samples.

The amount of water required to saturate the simulated soils was determined by adding increments of water (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 mls) to 10 g of each simulated soil until an excess appeared, and removing the excess with a pipette.

Determination of Exchangeable Cations

The exchangeable cation contents (Ca^{+2}, K^{+1}, Mg^{+2}, Na^{+1}) of 30 soil samples collected from various sites in the Ross Desert were extracted with an excess of ammonium acetate and analyzed by atomic adsorption spectrometry (AAS) according to the methods of Thomas (1982) and Baker and Suhr (1982). Briefly, two and a half grams of selected air dried soils were added to 125 ml Erlenmyer flasks containing 12.5 mls of 1N ammonium acetate (ACS grade, Fisher Scientific Co.). The flasks were covered with parafilm and shaken (New Brunswick Scientific Co. Gyrotory Shaker Water Bath) for 30 minutes at room temperature. The samples were then placed into polyethylene tubes (50mls) and centrifuged (Sorvall RC-2B, 2000 rpm) for ten minutes, and filtered (Buchner funnel, Whatman #2 filter). The resulting supernatant was brought to final volume (25 ml volumetric flask) with 1N ammonium acetate, placed into 25 ml screw capped test tubes and refrigerated. The tubes were brought to

room temperature before being analyzed by atomic absorption spectrometry (Perkin-Elmer model 373). Appropriate standards were prepared for each cation analyzed. The concentration ($\mu g\ g^{-1}$ soil) of each cation was determined by setting up a standard curve (A versus ppm) then determining the concentration of each cation by comparing its absorbance (A) to that of the standard curve, and correcting for the amount of soil initially added and any subsequent dilutions that were used.

Inorganic Nitrogen Analysis

Twenty-six Ross Desert soil samples were analyzed for their inorganic nitrogen content by the Technician Autoanalyzer (Model II, Texas Instrument Corp.) according to the procedures given in "Industrial Methods No. 98-70W (NH_3) and No. 487-77A (No_3^--N;NO_2^--N). Initially, soil from frozen soil samples (held on dry ice) were aseptically transferred directly to tared Erlenmyer flasks (50 ml) for mass determination. Subsamples (in triplicate) of each soil sample were independently analyzed when a sufficient supply of soil was available. Twenty mls of 2N KCl (extracting solution) were added to each flask. The flasks were then covered with parafilm and shaken for 1 hour at room temperature. The samples were filtered (plastic funnel, Whatman #2 filter), and the resulting supernatant placed into sterile screw capped test tubes (22 mls) and refrigerated over night (4°C). Sample extracts were equilibrated to room temperature before being analyzed by the Technician autoanalyzer. The reagents (ACS grade, Fisher Scientific Corp.) used in determining the ammonia, nitrate-N and nitrite-N content of these soils by this automated procedure are listed in Appendix C.

The standards used were 1, 2 and 3 ppm NH_4Cl or KNO_3 for ammonia or nitrate-N determinations, respectively; and 0.02, 0.04 and 0.08 ppm KNO_2 for nitrite-N determinations. Two ml extracts of each soil were placed in the automatic sampler tray. A set of standards was placed at the beginning and end of each run as well as in-between every 10-15 sample extracts being analyzed. The cam was set to analyze 40 samples of the particular inorganic nitrogen source under investigation per hour.

Determination of Quantitative Nitrogen Requirement

The effect of nitrogen concentration on the growth rate of yeasts belong to the Cryptococcus vishniacii complex was determined using Cr. vishniacii var. asocialis isolate A801-30bY33 as model. The yeasts belonging to this complex utilize ammonium, nitrite, and nitrate ions as nitrogen sources. The attempt was made to determine growth rates in both liquid media and, under simulated in situ conditions, in sand. For both types of experiments, isolate A801-30bY33 was depleted of nitrogen by growth in liquid YY-2 medium without nitrogen. This medium then contained 0.4% glucose, 5 mM potassium phosphate (pH6.0), 0.2 mM magnesium sulfate heptahydrate, 50 mM sodium chloride, and a chelated trace metal solution (Vishniac and Santer, 1957) (see Appendix A). Depletion cultures and other liquid media were incubated at 10°C in a New Brunswick Scientific Co. gyrotory shaker water bath, Model G-76, shaken at 150 rpm.

Growth rates in liquid media were determined by supplementing except for the negative control) the depletion medium with KNO_3 at 0.01, 0.1, 0.2, 1.0, and 2.0 mM, or (positive control) with 2.0 mM NH_4Cl.

Growth was monitored by removing samples for spectrophotometric determination determinations of optical density at 650 nm. During exponential growth, transfers were made to secondary and sometimes tertiary cultures at the same nitrogen levels. Approximately 24 hours after the commencement of stationary phase, additional nitrogen was added to ascertain whether nitrogen limitation had caused the onset of stationary phase.

For simulated <u>in situ</u> determinations of growth rate, fine grained quartz sand was acid cleaned in 4N HCL, rinsed in distilled water (5X), and dried overnight in a drying oven (100°C). Ten grams of acid cleaned sand was added to presterilized screw cap test tubes. A solution of KNO_3 was then added to each tube, so that after drying, the addition of 0.5 mls of liquid to any particular tube would give the appropriate KNO_3 concentration in that tube (i.e. 0.2 mM, 2.0 mM, 8.0 mM, or 10 mM KNO_3). The tubes were then autoclaved for 2 hours at 121°C. In at least one experiment, the water used was freshly distilled and autoclaved. After inoculation with nitrogen-depleted cells, sets of triplicate tubes were incubated in a Precision low temperature incubator at 10°C. In at least one experiment, the incubator was freshly washed, furnished with sodium bicarbonate, and contained no other materials. Growth was followed by sprinkling 0.1 to 0.5 g portions of sand from each tube onto triplicate plates of YY-2 agar (with nitrogen sources). The sprinkler plates were incubated at 10°C (after holding at refrigerator temperature when the incubator was being reserved exclusively for the experiment) and the resulting colonies counted at intervals until the count no longer increased.

Results

The changes in water potential with soil water content are shown in Tables V and VI, and graphed in Figure 5.

The effect on water potential of adding water to the simulated soils (sand + clay mixtures) is shown separately in Figure 6. The water potentials resulting are assumed to be matric water potentials, since, although the quartz sand was washed, the kaolinite and montmorillonite were used as purchased. The particle size of the sand ranged from 250 to 850 μM. The clay standards were both ≤ 150 μM in diameter. Other characteristics of the simulated soils are given in Table VII. The predictable increase in water holding capacity produced by 10% kaolinite was most effective in reducing matric water potential.

The effect on water potential of adding water to the Antarctic soil samples is graphed separately in Figure 7. The characteristics of these soils are tabulated in Table VIII. Since the clay content of at least some of the samples investigated was insufficient to account for the low water potentials observed, it was surmised that mineral salt content had affected the osmotic water potential.

The results of analyzing 31 soil samples for the major cations of their salt content are shown in Table IX as $\mu g\ g^{-1}$ and in Table X as μAg^{-1}. Calcium or sodium salts predominate in most soils. These soils are extremely heterogeneous with respect to salt concentration. Soils from adjacent sites in the same area had significantly different concentrations of major cations (for example, see Linnaeus Terrace sites A812-24a versus A812-1, Table X). The correlation of yeast distribution (unpublished data, H. S. Vishniac) with total cation content is shown in

TABLE V

WATER POTENTIAL OF SIMULATED SOILS

% H_2O (v/w)	psi (bars)			
	Sand	3% Kaolinite	10% Kaolinite	3% Montmor'ite
0.25	-4.2±1.3[a]	ND[b]	ND	ND
0.50	-1.3±0.9	-46.9±3.5	-55.7±4.3	-69.3±4.6
1.00	-1.0±1.0	-16.3±1.3	-45.3±2.7	-51.3±1.8
1.50	ND	ND	ND	-19.0±2.7
2.00	ND	-1.2±0.3	-21.7±2.0	-11.7±4.4
3.00	-1.3±1.0	-1.0±0.3	-10.8±3.5	-5.1±1.7
4.76	-1.3±1.3	-1.3±0.3	-1.5±0.3	-1.6±0.5
0.10	-1.3±1.3	-1.4±0.8	-1.5±0.3	-1.6±0.3
15.00	-1.3±0.7	ND	ND	-1.7±0.3

[a]Average and standard deviation triplicate samples. [b]ND = not done

TABLE VI

WATER POTENTIAL OF SELECTED ANTARCTIC SOILS

% H_2O (v/w)	psi (bars)					
	A834-65b	A801-25	A812-1	A823-4	A823-6	A834-63a
0.25	-51.0 ± 6.0[a]	ND[b]	ND	ND	ND	ND
0.50	-21.0 + 1.3	ND	ND	ND	ND	ND
1.00	-5.4 + 1.0	ND	ND	-66.3 ± 4.4	ND	-19.0
3.00	ND	ND	ND	-16.4	ND	-5.3
4.76	-2.6 + 1.3	ND	-69.3	-12.0	-56.7 ± 1.3	-2.6
7.00	ND	-59.3	-49.0 ± 3.0	-5.3	ND	ND
9.10	-0.7 + 0.5	-43.3 ± 1.3	-42.7	ND	-34.7 ± 2.7	ND
10.00	ND	ND	-32.0	-3.3	ND	ND
12.00	ND	-19.7	ND	ND	ND	ND
15.00	ND	-16.0	-17.3	-4.7	-21.3	ND
20.00	ND	ND	-14.3	ND	-13.7 ± 1.7	ND
25.00	ND	ND	ND	ND	-9.0 ± 0.7	ND

[a]Average and standard deviation of samples analyzed in triplicate. [b]ND = not done

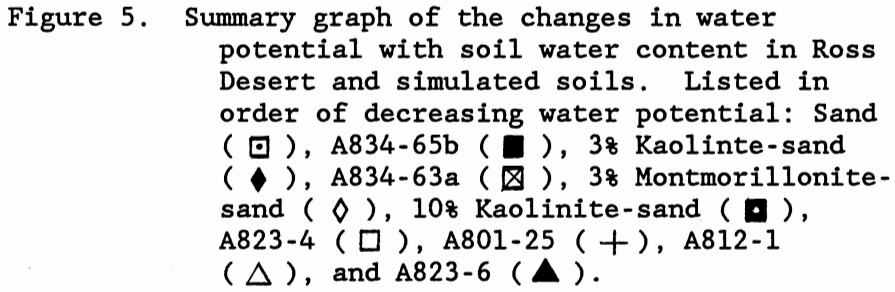


Figure 5. Summary graph of the changes in water potential with soil water content in Ross Desert and simulated soils. Listed in order of decreasing water potential: Sand (⊡), A834-65b (■), 3% Kaolinte-sand (♦), A834-63a (⊠), 3% Montmorillonite-sand (◊), 10% Kaolinite-sand (◘), A823-4 (□), A801-25 (+), A812-1 (△), and A823-6 (▲).

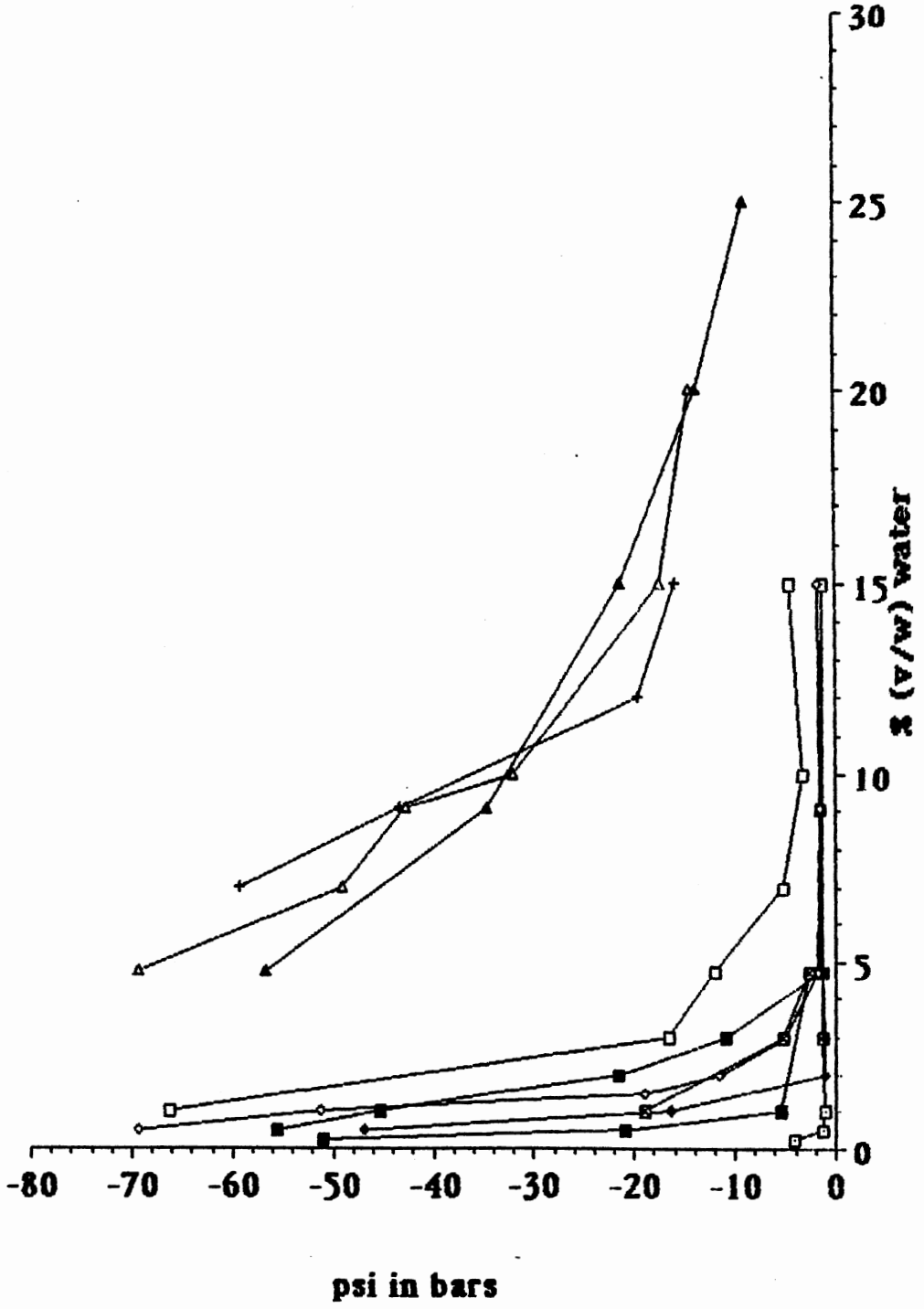

30
25
20
15
10
5
0
% (v/w) water
-80
-70
-60
-50
-40
-30
-20
-10
0
psi in bars

Figure 6. Simulated soils. Changes in water potential with soil water content. Sand (⊡), 3% Kaolinite-sand (♦), 3% Montmorillonite-sand (◊), and 10% Kaolinite-sand (■). Decreased water potential was correlated with increased clay content.

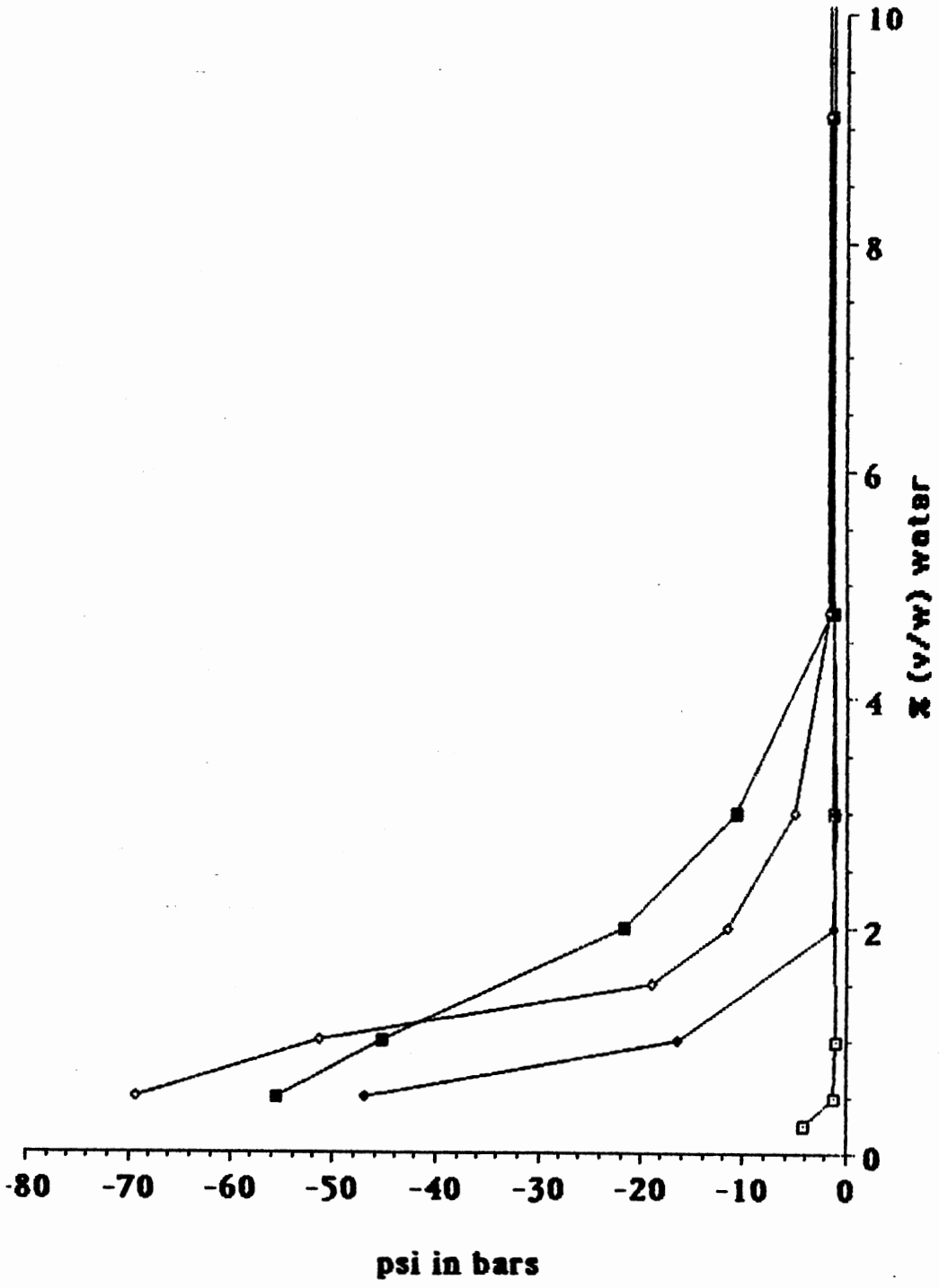

10
8
6
4
2
0
% (v/w) water
-80
-70
-60
-50
-40
-30
-20
-10
0
psi in bars

TABLE VII

CHARACTERISTICS OF SIMULATED SOILS

Material	Bulk density (g/ml)	Saturation (% H_2O)
Washed quartz sand	1.72 ± 0.02*	17
Sand + 3% montmorillonite	1.74	25
Sand + 10% montmorillonite	1.81	38
Sand + 3% kaolinite	N.D.	17
Sand + 10% kaolinite	1.76	18

*Average and standard deviation of samples analyzed in triplicate

Figure 7. Antarctic soils. Changes in water potentials with soil water content. Soil samples are listed in order of decreasing water potential. A834-65b (◊), A834-63a (■), A823-4 (♦), A801-25 (□), A812-1 (▲), and A823-6 (△).

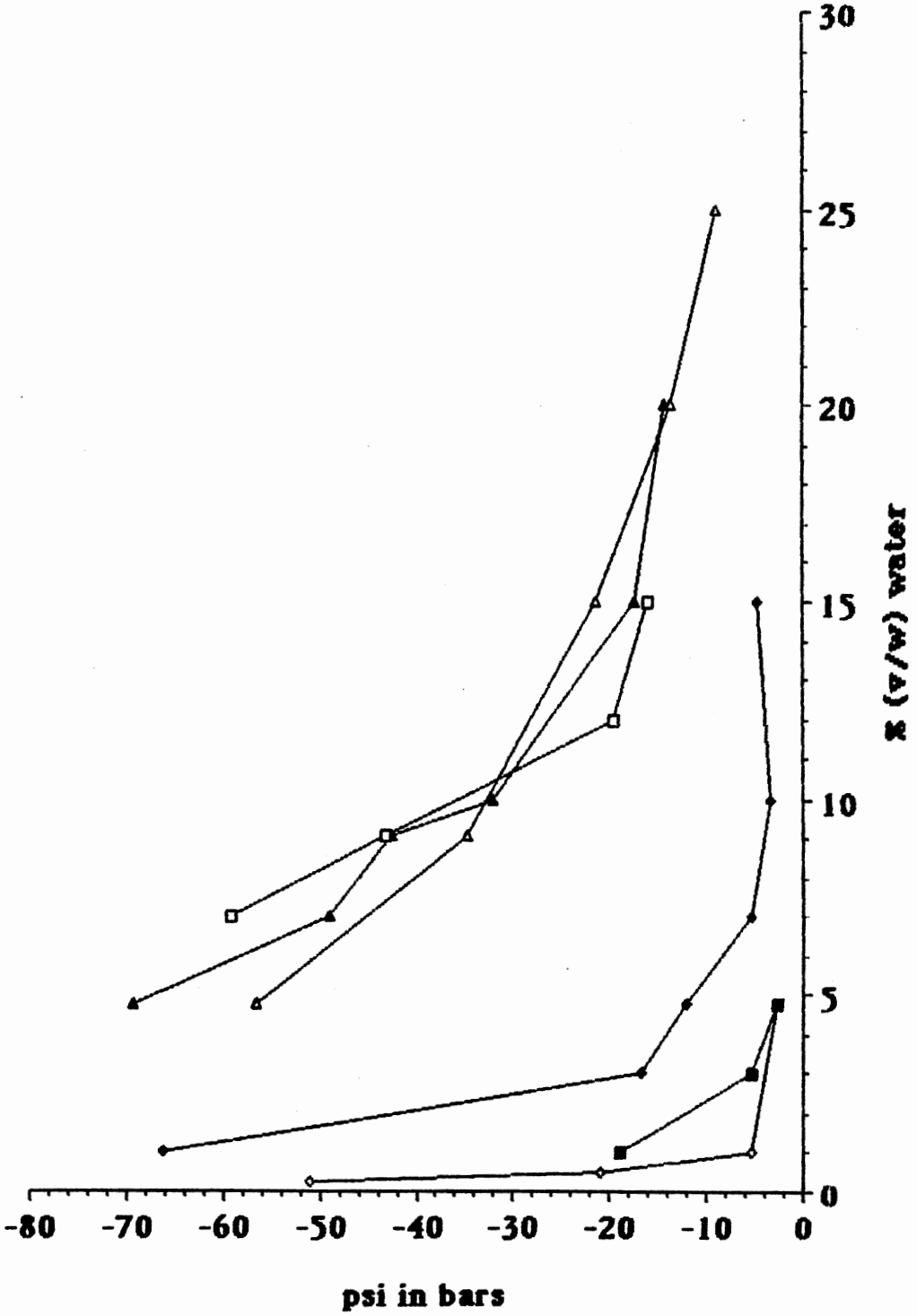

30
25
20
15
10
5
0
% (v/w) water
-80
-70
-60
-50
-40
-30
-20
-10
0
psi in bars

TABLE VIII

CHARACTERISTICS OF ANTARCTIC SOIL SAMPLES

Soil Type	Composition
A801-25	yellow clay soil, slope of Mt. Oliver, Wright Valley
A812-1	fine-grained tan sand + clay with dolerite pebbles; Linnaeus Terrace, Wright Valley
A823-4	fine-grained tan sand + clay with dolerite pebbles; East Dido Ridge, Wright Valley
A823-6	dark, variegated (black, pink, hyaline) sand with pebbles, some visibly (dissecting microscope) encrusted with mineral salts; slope above Don Juan pond, Wright Valley
A834-63	fine beige sand with some dolerite pebbles; University Valley
A834-65b	very coarse variegated sand (black, pink, hyaline); Nussbaum Riegel, Kukri Hills

TABLE IX

CATION CONTENT OF ROSS DESERT SOILS ($\mu g\ g^{-1}$)

Soil Sample	Ca^{+2}	K^{+}	Mg^{+2}	Na^{+}	Location
A801-8b[1]	37.5	39.5	172.5	456.4	Linnaeus Terrace
A801-25	210.0	99.0	400.0	771.1	Mt. Oliver
A801-28	1427.5	96.4	176.0	398.9	Mt. Odin
A801-29a	75.8	13.6	64.5	56.6	Valley W. of Mt.
-29b	101.4	19.9	76.0	59.3	Oliver
A801-30a	82.1	36.3	78.8	157.7	Tyrol Valley
-30b	235.9	40.6	96.2	141.6	
A812-1	24.5	61.2	335.0	702.2	Linnaeus Terrace
A812-20a	107.8	22.2	50.0	31.4	University Valley
-20b	56.6	16.3	27.5	24.5	
A812-22a	1254.6	140.0	152.5	927.3	Wright Valley
A812-23a	50.1	16.4	22.9	24.5	Wright Valley
-23b	152.5	27.0	40.0	47.4	
A812-24a	88.5	42.0	36.3	47.4	Arena Valley
-24b	107.7	80.0	30.0	100.3	
A823-1[2]	54.3	26.0	45.1	60.3	Linnaeus Terrace
A823-2	30.9	29.2	83.7	233.5	Linnaeus Terrace
A823-3[2]	82.7	29.0	74.1	196.0	E. Dido Ridge
A823-4	421.6	58.2	177.5	222.0	Linnaeus Terrace
A823-6a[2]	509.1	20.7	20.1	1674.0	Above Don Juan
-6b[2]	812.8	22.7	21.8	1467.0	Pond
A823-10	697.3	960.0	135.0	2209.2	Taylor Valley
A834-51b	43.8	16.7	36.7	31.4	Mt. Dido, E slope
A834-53	223.1	37.6	55.0	49.7	Wright Valley
A834-57	171.9	72.0	380.0	431.1	Linnaeus Terrace
A834-59	50.1	24.3	31.5	33.7	University Valley
A834-60	133.4	16.7	34.0	26.8	University Valley
A834-63a	255.1	42.4	140.0	268.0	University Valley
A834-65a	337.5	58.2	25.4	68.1	Nussbaum Riegel
-65b	408.8	61.2	29.0	95.7	
A834-66	517.8	144.0	172.5	213.9	University Valley

[1] a,b refer to distance from surface. "a" was usually 0-1 or 1-2 cm from the surface, "b" 2-3 cm from the surface. A823-6a was, however, collected 1-3 cm from the surface, while 6b was the 3-5 cm deep layer of soil. [2] samples run in triplicate. Averages (given) and standard deviations were: A823-1: 54.3 ± 9.55, 26.0 ± 1.95, 45.1 ± 3.90, and 60.3 ± 17.71; A823-3: 82.7 ± 7.95, 29.0 ± 3.60, 74.1 ± 6.91, and 196.0 ± 37.78; A823-6a: 509.1 ± 125.3, 20.7 ± 1.15, 20.1 ± 1.55, and 1674.0 ± 75.21; A823-6b: 812.8 ± 66.78, 22.7 ± 3.05, 21.8 ± 1.78, and 1467.0 ± 80.72.

TABLE X

CATION CONTENT OF ROSS DESERT SOILS (μA^{g-1})

Soil Sample	Ca^{+2}	K^{+}	Mg^{+2}	Na^{+}	Total
A801-8b	0.93	1.01	7.10	19.86	28.89
A801-25	5.24	2.53	16.45	33.54	57.76
A801-28	35.62	2.47	7.24	17.35	62.71
A801-29a	1.89	0.35	2.65	2.46	7.35
A801-29b	2.53	0.51	3.13	2.36	8.53
A801-30a	2.05	0.93	3.24	6.86	13.08
A801-30b	5.89	1.04	3.96	6.16	17.05
A812-1	0.61	1.57	13.78	30.54	46.50
A812-20a	2.69	0.57	2.08	1.37	6.71
A812-20b	1.41	0.42	1.13	1.07	4.03
A812-22a	31.30	3.58	6.27	40.33	81.48
A812-23a	1.35	0.42	0.94	1.07	3.78
A812-23b	3.80	0.69	1.65	2.06	8.20
A812-24a	2.21	1.07	1.49	2.06	6.83
A812-24b	2.69	2.05	1.23	4.36	10.33
A823-1	1.35	0.66	1.86	2.62	6.49
A823-2	0.77	0.75	3.44	10.16	15.12
A823-3	2.06	0.74	3.04	8.53	14.37
A823-4	10.52	1.49	7.30	9.66	28.97
A823-6a	12.70	0.53	0.83	72.81	86.87
A823-6b	20.28	0.58	0.90	63.81	85.57
A823-10	17.39	24.55	5.55	96.09	143.58
A834-51b	1.09	0.43	1.51	1.37	4.40
A834-53	5.57	0.96	2.26	2.16	10.95
A834-57	4.29	1.84	15.63	18.75	40.51
A834-59	1.25	0.62	1.27	1.47	4.61
A834-63a	6.36	1.08	5.75	11.65	24.84
A834-65a	8.42	1.49	1.04	2.96	13.91
A834-65b	10.20	1.56	1.19	4.16	17.11
A834-66	12.92	3.68	7.10	9.30	33.00

[1]a,b refer to distance from surface. "a" was usually 0-1 or 1-2 cm from the surface, "b" 2-3 cm from the surface. A823-6a was, however, collected 1-3 cm from the surface, while 6b was the 3-5 cm deep layer of soil.

Table XI. Yeasts were not isolated from soils which contained a total measured cation content greater than 42 $\mu A\ g^{-1}$.

The results of the inorganic nitrogen analysis of twenty-six soils are listed in Tables XII ($\mu g\ g^{-1}$) and XIII ($\mu A\ g^{-1}$). Nitrate was the most abundant N-source in these soils (excluding the stream sediment samples), the ammonium content was low and nitrite was detected only in trace amounts in three samples. The nitrate content was extremely variable even at sites located in the same area.

The correlation of psychrophilic yeast distribution with total inorganic nitrogen content is shown in Figure 8. Fourteen of 23 soil samples (3 samples, those from stream sediment and above Don Juan pond, were not included) analyzed for inorganic nitrogen content contained psychrophilic yeasts of which approximately 78.6% (11 samples) were capable of utilizing nitrate as an N-source. Although no yeasts were recovered from soils containing less than 0.23 μAg^{-1} of inorganic nitrogen, there was no obvious correlation of nitrate or total nitrogen content with yeast distribution.

The requirement of a model yeast (Cryptococcus vishniacii var. asocialis A801-30bY33) for nitrate-N in liquid medium is shown in Table XIV. There was no significant difference in growth rate when 1.0-2.0 mM nitrate was used as a sole source of nitrogen. When the concentration of nitrate was reduced to 0.2 mM or below, both growth rates and duration of exponential phase were reduced. Nitrogen was limiting at these concentrations, as the second addition of a nitrogen source (0.2 mM or greater) to these cultures after they reach stationary phase resulted in a second period of exponential growth.

TABLE XI

CORRELATION OF TOTAL MEASURED CATION (μAg^{-1}) CONTENT WITH YEAST RECOVERY

Soil Sample	< 13.1μAg^{-1}	Sample	13.9-41μAg^{-1}	Sample	> 42μAg^{-1}
A812-23a	3.78	A834-65a	13.91*	A812-1	46.50*
A812-20b	4.03	A823-3	14.37*	A801-25	57.56*
A834-51b	4.40	A823-2	15.12	A801-28	62.71*
A834-59	4.61	A801-30b	17.05	A812-22a	81.48*
A823-1	6.49	A834-65b	17.11*	A823-7	85.57*
A812-20a	6.71*	A834-63a	24.84*	A823-6	86.57*
A812-24a	6.83	A801-8b	28.89*	A823-10	143.58*
A801-29a	7.35	A834-4	28.97*		
A812-23b	8.20	A834-66	33.00		
A801-29b	8.53	A834-57	40.51		
A812-24b	10.33				
A834-53	10.95				
A801-30a	13.08				
Yeast recovery in above					
soil samples	92%		40%		0%
Yeast recovery in above					
Sample sites	100%		50%		0%

[1]a,b refer to distance from surface. "a" was usually 0-1 or 1-2 cm from the surface, "b" 2-3 cm from the surface. A823-6a was, however, collected 1-3 cm from the surface, while 6b was the 3-5 cm deep layer of soil. [2]samples run in triplicate. Averages (given) and standard deviations were: A823-1: 54.3 $\pm$ 9.55, 26.0 $\pm$ 1.95, 45.1 $\pm$ 3.90, and 60.3 $\pm$ 17.71; A823-3: 82.7 $\pm$ 7.95, 29.0 $\pm$ 3.60, 74.1 $\pm$ 6.91, and 196.0 $\pm$ 37.78; A823-6a: 509.1 $\pm$ 125.3, 20.7 $\pm$ 1.15, 20.1 $\pm$ 1.55, and 1674.0 $\pm$ 75.21; A823-6b: 812.8 $\pm$ 66.78, 22.7 $\pm$ 3.05, 21.8 $\pm$ 1.78, and 1467.0 $\pm$ 80.72.

* No yeasts were isolated from that sample.

TABLE XII

INORGANIC NITROGEN CONTENT ($\mu g\ g^{-1}$) OF ANTARCTIC SOILS

Soil Sample	NH_4^+	No_3^--N + NO_2^--N	No_2^--N
A801-29a	< 2.0[1]	6.73	< 0.25
A801-29b	< 2.0	3.27	< 0.25
A801-30a	2.8	48.36	< 0.25
A801-30b	4.1	59.52	< 0.25
A812-20a	2.4	7.11	< 0.25
A812-20b	4.5 ± 0.25	4.99 ± 0.32	< 0.25
A812-23a	< 2.0	6.19 ± 0.46	< 0.25
A812-23b	< 2.0	6.20 ± 0.52	< 0.25
A812-24a	2.8	5.76 ± 0.58	< 0.25
A812-24b	2.8	13.81 ± 0.47	< 0.25
A823-1	4.9 ± 0.6	8.36 ± 0.66	< 0.25
A823-2	2.8	71.28 ± 1.22	< 0.25
A823-3	3.5 ± 0.1	111.16 ± 4.22	< 0.25
A823-4	2.6	56.06 ± 1.27	< 0.25
A823-6a	4.9 ± 1.4	6.32 ± 1.33	< 0.25
A823-6b	4.3 ± 1.7	6.89 ± 1.39	< 0.25
A823-11	< 2.0	1.67	< 0.25
	7.9	< 0.25	< 0.25
	9.9	0.28	< 0.25
A834-51b	2.8	4.54 ± 0.25	0.28
A834-57	2.9	124.53 ± 3.69	0.29
A834-59	5.2 ± 2.8	4.05 ± 1.78	< 0.25
A834-61	4.6	3.74	< 0.25
A834-62	3.1	6.50	< 0.25
A834-63a	2.5	110.55	< 0.25
A834-65a	< 2.0	4.11 ± 0.54	< 0.25
A834-65b	< 2.0	3.25 ± 0.20	< 0.25
A834-66	6.6 ± 2.5	92.09 ± 8.03	0.38

[1] < implies below the limits of detection by the methods used

[2] average and standard deviation of samples analyzed in triplicate

TABLE XIII

INORGANIC NITROGEN CONTENT ($\mu A\ g^{-1}$) OF ANTARCTIC SOILS

Soil Sample	NH_4^+	No_3^--N	No_2^--N
A801-29a	< 0.11[1]	0.48	0.48
A801-29b	< 0.11	0.23	0.23
A801-30a	trace[2]	3.45	3.60
A801-30b	0.23	4.25	4.47
A812-20a	trace	0.51	0.64
A812-20b	0.25 ± 0.14	0.36 ± 0.02	0.60
A812-23a	< 0.11	0.44 ± 0.03	0.44
A812-23b	< 0.11	0.44 ± 0.04	0.44
A812-24a	trace	0.41 ± 0.04	0.55
A812-24b	trace	0.99 ± 0.03	1.11
A823-1	0.29 ± 0.03	0.606 ± 0.05	0.86
A823-2	trace	5.09 ± 0.09	5.24
A823-3	0.19 ± 0.01	7.94 ± 0.30	8.12
A823-4	trace	4.00 ± 0.09	4.14
A823-6a	0.27 ± 0.08	0.45 ± 0.09	0.71
A823-6b	0.24 ± 0.09	0.49 ± 0.10	0.72
A823-11	< 0.11	0.12	0.12
	0.43	< 0.02	0.42
	0.55	trace	0.57
A834-51b	trace	0.32 ± 0.02	0.46
A834-57	trace	8.89 ± 0.26	9.06
A834-59	0.028 ± 0.15	0.29 ± 0.13	0.58
A834-61	0.26	0.26	0.49
A834-62	trace	0.46	-0.62
A834-63a	trace	7.89	8.02
A834-65a	< 0.11	0.29 ± 0.04	0.28
A834-65[b]	< 0.11	0.23 ± 0.01	0.22
A834-66	-0.36 ± 0.14	6.57 ± 0.57	6.93

[1] < below the limits of detection; [2] trace (NH_4^+ - 0.11 to 0.16 μAg^{-1}; NO_3^--N 0.05 μAg^{-1}).

Figure 8. The number of yeast biovars recovered from Antarctic samples versus the total inorganic nitrogen content of these soils. Soil samples which did not contain yeasts are designated by an asterisk (*__*, 6 samples; *1 sample).

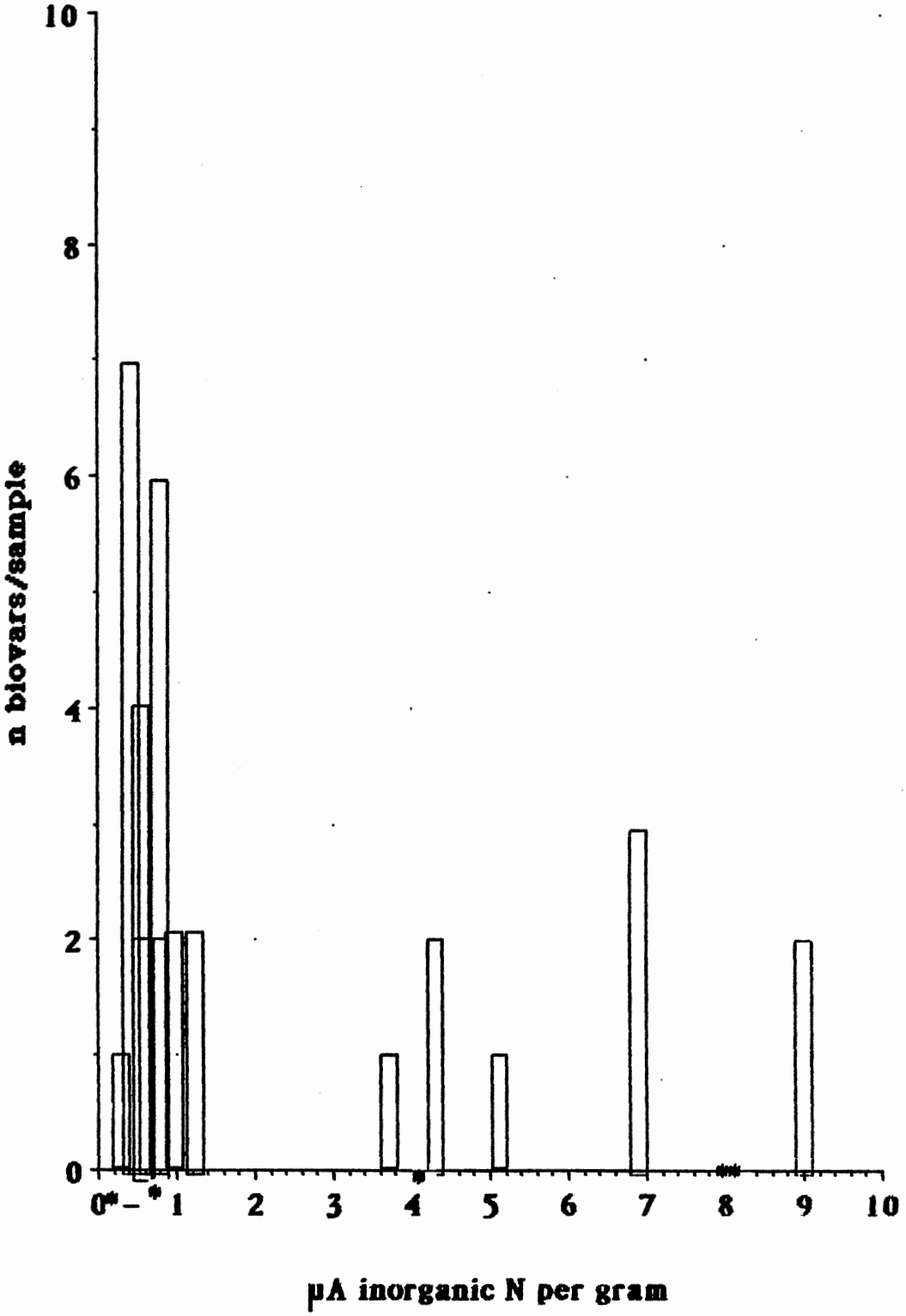

10
8
6
4
2
0
n biovars/sample
0* – *1
2
3
4
5
6
7
8
9
10
µA inorganic N per gram

TABLE XIV

NITROGEN REQUIREMENT OF CRYPTOCOCCUS VISHNIACII VAR ASOCIALIS IN LIQUID MEDIUM

N-Source	Kmax	Duration (hrs) of exponential growth
NO N-source	0.0	0.0
2.0 mM NH_4Cl	0.080	40.0
2.0 mM KNO_3 [1]	0.067	45.0
1.0 mM KNO_3 [2]	0.060 ± 0.003	44.5
0.2 mM KNO_3 [1]	0.033	24.0
0.1 mM KNO_3 [1]	0.034	24.0
0.01 mM KNO_3	0.017	26.0

[1]Average of secondary and tertiary growth curves. [2]Average and standard deviation of secondary growth curves (in triplicate).

We were unable to obtain nitrogen depletion in simulated in situ experiments in sand for reasons that escape us at present. The sand particles may have sequestered enough ammonia from the air to support growth of yeasts in sand cultures which normally lacked nitrogen sources.

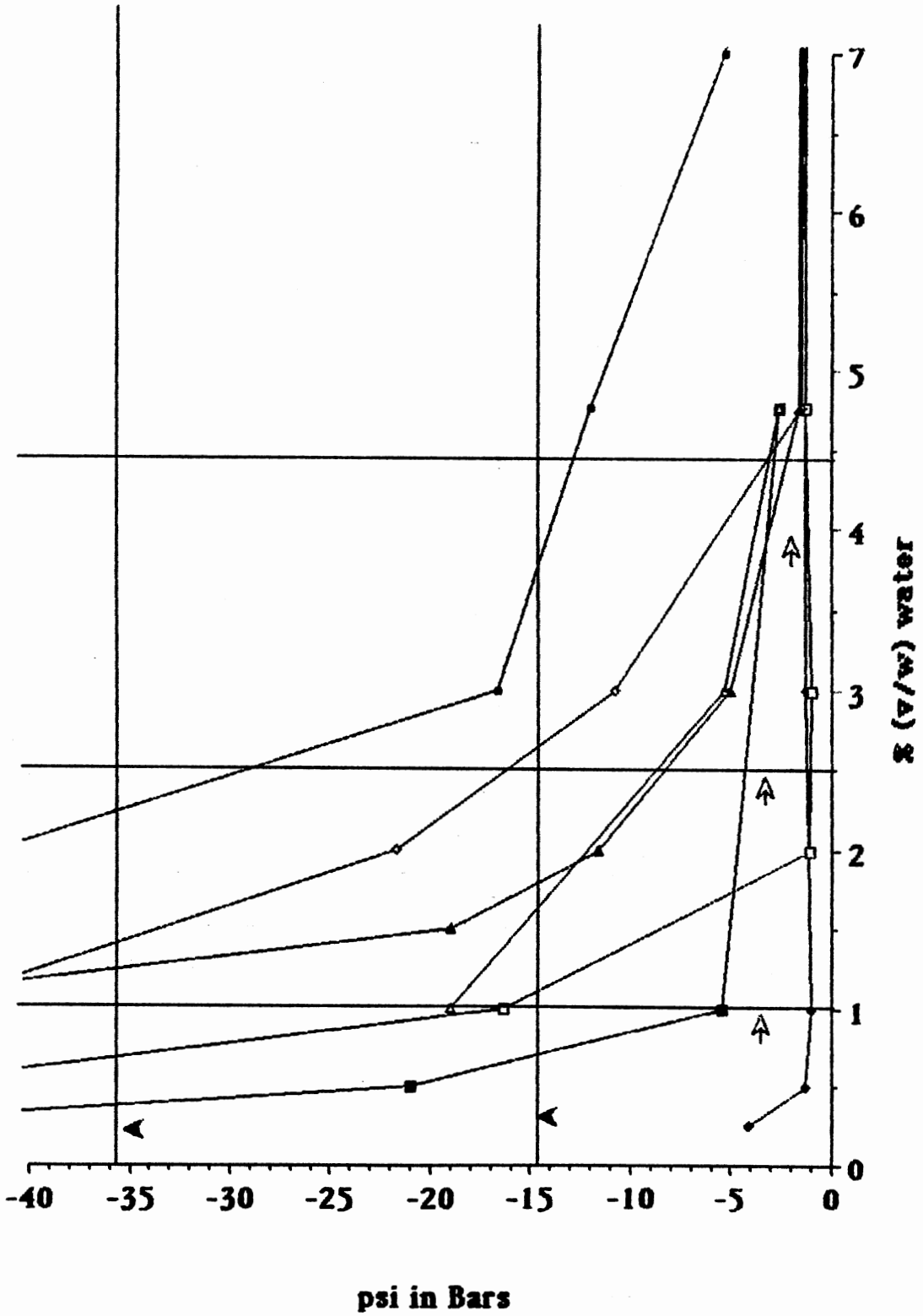
Discussion

Water potential affected yeast distribution in Ross Desert soils. It is clear from the correlation of yeast distribution with cation content that the chances of finding yeasts in soils with more than 13.1 μAg^{-1} of the cations measured are approximately halved and that yeasts do not survive in soils with greater than 42 μAg^{-1} of the cations measured. Calcium and or sodium predominate in 27 of the 31 soils analyzed (i.e. 9 samples - calcium, 15 samples - sodium, 3 samples - approximately equal sodium/calcium content). It is the total cation content, not which cation predominates, which appears to affect yeast distribution. While there was insufficient material to examine the total water potential of all soil samples, it was clear from the effect on total water potential of the high salt content of A823-6, a sandy soil in which the matric water potential should not have exceeded that of washed sand, that the effect of mineral salts on water potential was a major factor in limiting yeast distribution. A high montmorillonite content might have had equally strong effects. It is noticeable that samples A801-25 and A812-1 had higher clay contents and lower salt contents but essentially similar water potential curves. Nothing more definite can be said, because we were unable to determine the type of

clay present. Illite, kaolinite, and montmorillonite are all known to occur in Antarctic soils (Parker et al., 1982).

Figure 9 summarizes the critical areas of water potential for yeast distribution. The vertical lines marked by black arrows delimit the region of water potential in which yeast distribution declines from 100% of sites to 50%. The water potential in bars was obtained by considering the highest cation content of samples from the "100%" column and the "50%" column (Table XI) as accompanied by appropriate anions, finding the osmolarity of these salts in the CRC handbook (Weast and Astle, 1978), and calculating the resulting psi. Knowing that more than 1% water, but less than 2.5% water, was required for growth of microcolonies in simulated <u>in situ</u> experiments with sand, we have drawn horizontal lines (white arrows) to indicate the area of possible onset of growth. Any additional water would result in more positive water potentials. For microcolony growth to have occurred in A834-57, the sample with highest cation content from the "50%" column, sufficient water must have been available to raise the water potential to a more positive value than -35.6 bars. This is calculated to be 4.5% water (the third white arrow). That yeasts were not found in soil samples with higher cation content, indicates therefore that the water content of these soils never significantly exceeded 4.5% and makes this the upper boundary of the critical water potential area. It then becomes obvious that the absence of yeasts in A823-4, the sample whose water potential curve barely enters the critical area, was a matter of chance. This site must not have gotten the water required to bring it into a viable area.

Figure 9. Summary graph of the criticle areas of water potential for yeast distribution. Changes in water potential with soil water content in selected Antarctic and simulated soils in order of decreased water potential are Sand ( ), A834-65b ( ), 3% Kaolinite-sand ( ), A834-63a ( ), 3% Montmorillonite-sand ( ), 10% Kaolinite-sand ( ), and A823-4 ( ). Black arrows associated with the vertical lines indicate the limits of estimated water potential allowing yeasts isolation from 100% (14.8 bars) and 50% (35.6 bars) of the Antarctic sites. White arrows pointing toward horizontal lines indicate (from bottom to top) the water concentration at which yeast growth was not observed in simulated in situ experiments, the water concentration at which yeast growth was observed in such experiments, and the probable limit of water availability in arid Ross Desert soils as estimated from observed yeast distribution.


7
6
5
4
3
2
1
0
% (w/w) water
-40
-35
-30
-25
-20
-15
-10
-5
0
psi in Bars

Although nitrate-utilizing yeasts, as expected, predominated in Ross Desert soils, it was not possible to show that N-resources limited the growth of any psychrophilic yeasts in the Ross Desert.

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APPENDICES

APPENDIX A

MEDIA

WICKERHAM'S VITAMIN MIX (van der Walt and Yarrow, 1984)

Biotin	2.0	µg/L
Calcium pantothenate	400	µg/L
Folic acid	2.0	µg/L
Inositol	2000	µg/L
Niacin	400	µg/L
Para-aminobenzoic acid	200	µg/L
Pyridoxine HCl	400	µg/L
Riboflavin	200	µg/L
Thiamin HCl	400	µg/L

TRACE METAL SOLUTION (Vishniac, W. V. and M. Santer, 1957)

The stock solution below is added to the medium at 1.0 ml/L.

EDTA, disodium salt	171.0	mM
$CaCl_2$ (anhydrous)	49.0	mM
$CoCl_2 6H_2O$	4.9	mM
$FeSO_4 \cdot 7HY_2O$	18.0	mM
$MnCl_2 \cdot 4H_2O$	25.6	mM
$(NH_4)_6Mo_7O_{24}4H_2O$	76.5	mM

YY-2 MEDIUM (Vishniac, 1985[b])

Glucose (10% stock solution)	50	ml/L
NaCl	50.0	mM
$MgSO_4 \cdot 7H_2O$	0.2	mM
NH_4Cl	2.0	mM
NaH glutamate (ph 6.0)	2.0	mM
Potassium phospate buffer (pH 6.0)	5.0	mM
Yeast Extract	0.5	g/L
Wickerham's vitamins (100X)	1.0	ml/L
Trace mineral solution	0.1	ml/L
Difco Bacto - Agar	18.0	g/L

Y-2 Mineral Base (Vishniac and Baharaeen, 1982)

Potassium phosphate buffer	1.0	mM
NaCl	50.0	mM
$MgSO_4 \cdot 7H2O$	0.2	mM
Trace Mineral Solution	1.0	ml/L

YCA MEDIUM (Vishniac, 1983)

Glucose (10% stock solution)	40.0	ml/L
H_3BO_4	50.0	µg/l
KI	10.0	µg/L
NaCl	50.0	mM
$MgSO_4 \cdot 7H_2O$	0.2	mM
NH_4Cl	2.0	mM
NaH glutamate (pH 6.0)	2.0	mM
Potassium phospate buffer (pH 6.0)	5.0	mM
Difco Yeast Extract	0.5	g/L
Wickerham's Vitamins (100X)	1.0	ml/L
Trace mineral solution	0.1	ml/L
Difco Bacto - Agar	18.0	g/L

For slants, bring to boil (2X) stirring continuously. Aliquot 8ml per tube (25 ml volume). Autoclave 15 min., slant and cool.

FERMENTATION BASAL MEDIUM (van der Walt and Yarrow, 1984)

Peptone	4.5	g/L
Yeast Extract	7.5	g/L
Bromothymol blue	0.04	g/L
Glucose	6.0	%

Procedure: The peptone, yeast extract, and dye are dissolved in 1 liter of deionized distilled water. Ten mls of this solution is placed into large tubes with Durham inserts and autoclaved for 15 minutes at 15 lbs of pressure, 121°C. A 10% glucose solution is autoclaved separately, and added to each tube after it has cooled. Other heat labile sugars (if substituted for glucose) should be filter sterilized.

LUGOL'S IODINE SOLUTION (Cowan and Steel, 1963)

Iodine	5.0	g
Potassium iodine	10.0	g
Distilled water	100.0	mls

M3C MEDIUM (Vishniac, 1983)

Glucose (10% stock solution)	20.0	ml/L
H_3BO_4	50.0	μg/L
Kl	10.0	μg/L
MaCl	50.0	mM
$MgSO_4 \cdot 7H_2O$	0.2	mM
NH_4Cl	0.2	mM
NaH glutamate (pH 6.0)	0.2	mM
Potassium phospate buffer (pH 6.0)	1.0	mM
Yeast Extract	0.1	g/L
Wickerham's vitamins (100X)	0.1	ml/L
Trace mineral solution	0.1	ml/L
Difco Bacto - Agar	18.0	g/L

3C medium is identical to M3C except for an increased glucose (0.4%), and N-source (2.0 mM NH_4Cl and NaH glutamate) content. For TEM, suggested volume for 1 growth curve and 8 epon blocks is 300 mls of liquid 3C medium.

Sodium Cocodylate Buffer (0.2 M, pH 7.2)

(Hayat, M.A., 1970)

Na Cocodylate $3H_2O$	0.28 g
Glass distilled H_2O (dH_2O)	100 mls
1 N HCL (8.3 mls conc. HCL/91.7 mls dH_2O)	1.68 mls

This buffer is poisonous. Cover the balance with aluminum foil; keep all glassware separate and rinse immediately after use. The buffer is stored and used only in the hood.

GAP FIXATIVE (Baharaeen and Vishniac, 1982[b])

Final concentration: 3% glutaraldehyde, 1.5% acrolein, 1.5% paraformaldehyde, 0.05 M sodium cacodylate (pH 7.2)

Paraformaldehyde	0.15 g
Glass distilled water	3.4 mls
1 N NaOH	1-3 drops
Cacodylate buffer (0.2M)	2.5 mls
Glutaraldehyde (8%) Pelco Grade	3.75 mls
Acrolein (Polysciences, M Grade	0.15 mls

In a reuseable test tube, 3.4 mls of fresh dH20 is added to 0.15 g of paraformaldehyde, immersed under hot tap water (65°C) and shaken until cloudy. Add 1N NaOH until solution clears. In the hood, add the remaining solutions.

Pelco MEDCAST

One Mix System: 2A:1B - Soft Block

43%	Medcast	
41%	DDSA (dodecenyl succinic anhydride)	12.4 ml
16%	NMA (nadic methyl anhydride)	4.7 ml
1%	DMP (tri(dimethyl amino ethyl)phenol)	0.3 ml

Procedure: Mix Medcast, DDSA, and NMA, add catalyst and stir 30 minutes.

Two Mix System: 1A:2B

A mixture:		B Mixture:	
	50 ml Medcast		50 ml Medcast
	81 ml DDSA		44 ml NMA

2% Catalyst-2 ml DNP 30

Procedure: Mixtures A and B are made separately and stored in brown bottles. Before use, 1A:2B was mixed and the 2% DNP-30 (catalyst) was added. The solution was stirred for 30 minutes and then poured into a syringe. Except for small volumes (<5 mls) Medcast, DDSA, and A/B mixing should be measured in a graduated plastic beaker as pipetting is not accurate due to the viscosity of the resin.

APPENDIX B

METHODS OF YEAST CHARACTERIZATION

Multipoint Inoculation Technique

The multipoint inoculator is a hand held steel block with metal inoculation pins protruding downward in 4 rows (3 inoculating pins on the first and last rows, two sets of 4 pins in the middle rows; the rows are slightly offset from each other). In the multipoint inoculation technique, liquid inoculum is placed into sterile microtiter wells in a pattern which mirrors that of the multipoint inoculator. The sterilized (95% ethanol, shaken, flamed and cooled) multipoint inoculator is dipped into the wells and subsequently used to simultaneously inoculate up to 14 different isolates onto one plate. Fifteen plates can be inoculated before the wells need to be refilled.

Since most carbon and nitrogen assimilation, starch information and cell morphology procedures involved the use of the multipoint inoculation technique, it was necessary to determine the inoculum size which would produce repeatable results in the shortest amount of time. Choice of an inoculum size which gives (on day 0) a one cell thick yeast monolayer provides quick (14 days, 10°C), reliable results for most antarctic isolates (Vishniac, unpublished data). In order to determine the appropriate density of the cell suspension to use as inoculum, cells from working cultures of one white (11Y573) and one salmon (11Y574) isolate were each resuspended in sterile water at OD_{650nm} of 0.5, 1.0, 2-2.5, and 6.0 (Bausch and Lomb spectronic 70 spectro-photometer). Each dilution (four dilutions per isolate) was then added to a microtiter plate well and simultaneously multipoint inoculated onto modified YY-2 agar medium supplimented with 0.2% glucose, mannitol, or sterile water. Microscopic examination of the initial dried inoculum exhibited a complete monolayer when inoculated with cells at an OD_{650nm} of 2.0 or

greater. Results of subsequent incubation of these plates (15 days, 10°C), suggested that the best results were obtained when substrate-containing plates were inoculated from suspensions with an initial OD between 1.0-2.5 for both the pink and white isolates. Growth on plates inoculated at OD 0.5 or 6.0, respectively, resulted in false negative or false positive results. Subsequent observations of selected carbon assimilation tests (Chapter II) determined that OD_{650} determinations necessary in ambiguous assimilation results are slightly lower for the white isolates (OD_{650nm} = 1.0-2.0) than for the salmon isolates (OD_{650nm} = 2.0-2.5).

Cell Morphology

Cell morphology of the antarctic isolates (Chapter II) was determined by using the standard Dalmau technique (Wickerham, 1951). Cells from actively growing working slants of each isolate were longitudinally streaked onto air dried corn meal agar (BBL Co., Cockeysville, MD) contained in petri dishes. Part of the streak was overlaid with a sterile coverslip. Additionally, each isolate was also multipoint inoculated (OD_{650nm} = 2-2.5) onto corn meal agar plus Tween (BBL Co., Cockeysville, MD) and on M3C solid medium (Appendix A). Cultures were incubated at 10°C, and observed at weekly intervals for up to 8 weeks using a dissecting scope (American Optical, Model 40). When evidence of pseudohypha or mycelia was seen, a wet mount of each colony was made, stained with lactophenol cotton blue, and observed with oil immersion light microscopy (Ernst Leitz Wetzlar microscope, Germany).

Amylose Production. Vitamin Growth Requirements and Fermentative Ability

The above characteristics were determined using the methods listed in van der Walt and Yarrow (1984). Deviations from standard procedures are designated by an asterisk and involve the type of medium or the duration and temperature of incubation used. All sources of original inocula originated from working cultures grown on YY-2 slants.

Amylose production was accessed by multipoint inoculating isolates onto *YY-2, modified *YY-2 (1% glucose, 10mM NH_4Cl), *M3C agar, and yeast nitrogen base (supplemented with 0.5% glucose) plates. After *4-6 weeks incubation (10°C), plates are flooded with Lugols iodine solution (see Appendix A, Cowan and Steel, 1966). A blue color indicates a positive test for amylose production.

The ability of these antarctic isolates to grow in medium devoid of vitamins was detected by the multipoint inoculation (OD_{650nm} = 1.0-2.5) of these isolates on YY-2 medium, with and without vitamins added. The salmon isolates were also inoculated on YY-2 medium lacking only 1-3 of the following vitamins: PABA, Biotin, and/or Thiamin. Additionally, all Taylor Valley stream sediment isolates (A823-11Y573 to 596) were tested for ability to assimilate selected carbon sources (L-arabinose, cellobiose, D-galactose, D-glucitol, lactose, maltose, D-mannitol, melezitose, myo-inositol, melibiose, raffinose, L-rhamnose, sorbose, succinate, trehalose) when grown on YY-2 medium with and w/o vitamins. After the vitamin requirements were established for these

isolates, for those isolates not requiring vitamins, vitamins were no longer added when analyzing assimilation test results because the vitamins inhibited the utilization of some substrates.

The fermentative ability of these yeasts was determined by adding a heavy inoculum of each isolate into large tubes with Durham tube inserts containing 10 mls of Fermentation basal medium (see Appendix A). The tubes are then incubated for up to two months at 10°C. Evidence of fermentation is designated by the presence of gas (CO_2) in the Durham tube inserts.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy of the yeasts characterized in Chapter III was performed by another graduate student in Dr. Vishniac's laboratory. The author of this thesis examined yeast isolate A812-23bY426 by transmission electron microscopy, using the methods of Baharaeen and Vishniac (1982).

Cells from a tertiary culture in early stationary phase were harvested by centrifugation (3000 X g.5 minutes) in a Sorvall RC-2B refrigerated centrifuge, washed twice in cold glass distilled water, and resuspended in cacodylate buffer (0.2 M, pH 7.2) to a final OD_{650} of 200 [the growth curve of A812-23bY426 had been previously determined, in order to maximize the number of cells likely to show diagnostic characteristics. At the time of harvest this culture had grown through 5.6 generations, at an average rate during exponential growth of 0.08 generations per hour.] Equal volumes of glutaraldehyde-acrolein-paraformaldehyde fixative and suspended cells were placed in 1.5 mL microfuge vials which were then incubated on ice for 2 h. The fixed

cells were then centrifuged 10-15 seconds (microfuge), washed 1 X with distilled water, and resuspended to 1 mL in 6% (w/v) aqueous potassium permanganate. After a 1 hour incubation, the postfixed cells were washed 2 X with glass distilled water and dehydrated in a serious of filtered ethanol solution (30 min each in 30%, 50%, 70%, 80%, 95%, and (3X) 100%). The cells were then pelleted in the microfuge and resuspended in propylene oxide. After 15 minutes the cells were again centrifuged and overlaid with propylene oxide. After 15 minutes the propylene oxide was replaced with a 1:1 mixture of propylene oxide and Medcast Epon 812. The microvials were then allowed to sit in the hood overnight.

For embedding, a clump of fixed cells was placed in a preheated (60°C, 30 minutes) Beem capsule, overlaid with 2 mm of epoxy resin, and gently stirred to distribute the epoxy before completely filling the capsule. The Epon mixture used was prepared from the Medcast kit, using 2% catalyst, in the proportions of 1A:1B. The prepared Epon mixtures was stored overnight in an upright plastic disposable syringe before use. The filled Beem capsule was capped (it was later suggested that it should have been left uncapped) and heated in a vacuum oven at 60°C for 1-2 days.

After trimming and thick-sectioning to locate cells in the resin block, thin-sectioning was attempted with both glass and diamond knives. Knife marks made the glass cut sections unsuitable for photography. A Dupont diamond knife mounted on an MT-2 Sorvall ultramicrotome produced acceptable thin sections. Thin sections were stained with lead citrate for 7 minutes, and examined in an RCA-EMU-3G transmission electron microscope operating at an accelerating voltage of 100 KV.

The resulting micrographs (Kodak electron microscope film catalog number 1613108) showed clearly the morphological characteristics expected in a basidiomycetous yeast. Primary buds were holoblastic; secondary buds were produced repetitively through the bud scar, resulting in the formation of a frayed multilayered collar at that site, since secondary budding was enteroblastic. In enteroblastic budding only the inner layer of the parental cell wall is continuous with the developing bud. The cell wall appeared dense, lacked pits, pores, or grooves, and was visibly layered only in the collar area.

Sexual Reproduction

Initial assimilation and cell morphology results suggested that the salmon isolates (A823-11Y574, 575, 576, 577, 578, 583, 584, 588) closely resembled Rhodosporidium bisporidii. Mating experiments were conducted according to the method of Fell, et al., (1973) but substituting the multipoint inoculation technique in the initial mating step. A positive control plate was longitudally streaked with both Rhodosp. A_1B_1 (ATCC number 24496) and A_2B_2 (ATCC number 24497) to monitor normal Rhodosp. bisporidii mating behavior. The salmon isolates and the 2 ATCC Rhodosp. mating types were first simultaneously multipoint inoculated (OD_{650nm} = 5.0) onto cornmeal agar (Difco Laboratories) plates (i.e. 8 isolates plus the 2 Rhodosp. mating types per plate/11 plates total). Each inoculum on plate 1 was then horizontally streaked (sterile loop) with a isolate (i.e., 11Y574). The loop was sterilized in-between each mating. Each remaining plate was cross-streaked with a different isolate or ATTC mating type until all isolates had been mated with every other isolate and mating type. Plate

10 was not cross-streaked and was used as a negative control. When the inoculum dried, the plates were incubated (10°C), and checked at weekly intervals for evidence of clamp connections and/or teliospre development for up to 8 weeks. When mycelium or clamp connections were observed, bits of the colony was removed, stained with lactophenol cotton blue and observed with a compound microscope at 400 and 1000X.

Diazonium Blue B Procedure (Hagler and Ahearn, 1981).

Basidiomycetous yeasts were differentiated from ascomycetous ones by the diazonium blue B (DBB) procedure of Hagler and Ahearn (1981). Cells scraped from a YY-2 agar slant incubated for 2 months at 10°C were suspended in 2.0 mL of distilled water and sedimented in a clinical centrifuge (IEC Model CL) (3000 rpm. 5 minutes). After discarding the supernatant, the pellet was resuspended in 0.5 mL of 0.05 mM KOH and placed in a boiling water bath for 10 minutes. After cooling to room temperature, the cells were extracted with 2.5 mL 95% ethanol, centrifuged, and resuspended in 0.3 mL of ice cold DBB reagent [1.0 mg mL^{-1} of tetrazotized o-dianisidine (Sigma Chemical Co.) in 0.25 M Tris (hydroxymethyl)aminomethane]. When color develop, 1.0 mL of ethanol was added to stabilize it. The development of rose to purple colors indicate basidiomycetous affinity; any other colors are considered negatives.

APPENDIX C

TECHNICON AUTOANALYZER

Detection of Ammonia

The Technicon Autoanalyzer II (Technicon Industrial Systems) was used to determine the ammonium content of selected antarctic soils (Chapter III) according to "Industrial Method NO. 98-70W." This automated procedure utilizes the Berthelot reaction. The formation of a green colored compound believed to be closely related to indophenol occurs when a solution of ammonium salt is added to sodium phenoxide followed by the addition of sodium hypochlorite. A potassium sodium tartrate solution eliminates the precipitation of hydroxides of heavy metals which may be present. The reagents used in determining the ammonium content of soil extracts are listed below. All reagents are ACS certified and can be purchased form Fisher Scientific Corp.

Sodium phenoxide (Alkaline Phenol)

Sodium hydroxide	200.0	g
Liquified phenol, 88%	276.0	ml
Distilled Water q.s.	1000.0	ml
Brij-35, 30% solution	0.5	ml

Preparation: To 500 mls of distilled water, dissolve 200g of NaOH contained in a cold water bath. Slowly add while cooling, 276 mls of liquid phenol stirring continuously. Dilute to one liter with distilled water and store in a brown bottle. Add 0.5 ml of Brij-35 per liter.

Potassium Sodium Tartrate

Potassium Sodium Tartrate	150.0	g
Distilled water, q.s.	1000.0	ml
Brij-35	0.5	ml

Preparation: Dissolve 150 g of potassium sodium tartrate in 850 mls distilled water and dilute to one liter. Add 0.5 ml of Brij-35.

Stock Standard NH_4Cl(100 μg NH_4/ml)A

Ammonium cloride	0.315	g
Distilled water, q.s.	1000.0	ml

Preparation: Dissolve 0.315 g of ammonium cloride in distilled water and dilute to 1 litter in a volumetric flask.

Stock Standard B: NH_4Cl(10 μg NH_4/ml)

Stock standard A	10.0 ml
Distilled water, q.s.	100.0 ml

Preparation: Dilute 10mls of stock standard A in a volumetric flask to 100 mls with distilled water.

Working Standards

Working standards of 1,2 and 3 μg/ml were made the day of the experiment by adding 10, 20, or 30 mls, respectively, of the stock standard B in a 100 ml volumetric flask and brought to volume with 2N KCL.

Detection of Nitrate-N and Nitrite-N

The automated procedure (Industrial Method No. 487-77A) for the determination of nitrate and nitrite utilizes the procedure whereby nitrate is reduced to nitrite by a copper-cadmium reductor column. The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo blue compound which then couples with N-1 naphthylethylene-diamine dihydrochloride to form a reddish-purple azo dye. Nitrite determinations are analyzed in a separate run using nitrite standards and without the cadmium reductor column. All other reagents used in determining the nitrate and nitrite content of the soil extracts are identical. The reagents added to the technicon autoanalyzer are listed below.

Ammonium cloride reagent

Ammonium chloride	10.0	g
Alkaline water (pH 8.5)*	1000.0	ml
Brij-35, (30% solution)	0.5	ml

Preparation: Dissolve 10 g of ammonium chloride in alkaline water and dilute to one liter. Add 0.5 ml of Brij-35 per liter.

*Alkaline water is prepared by adding NaOH to distilled water to attain a pH of 8.5.

Nitrate Stock Standard B (10µg N/ml)

Stock Standard A	10.0	ml
Distilled water, q.s.	100.0	ml

Preparation: 10 mls of stock standard A is diluted in a volumetric flask to 100 mls with distilled water and stored in a dark bottle.

Working Nitrate Standard

Working nitrate standards of 1,2, and 3 µg N/ml were made the day of analysis by adding 10, 20, or 30 mls of stock nitrate solution B to a 100 ml volumetric flask and bringing it to volume with 2N KCl.

Nitrite Stock Standard A (100 µg N/ml)

Potassium nitrite	0.608	g
Distilled water, q.s.	1000.0	ml

Nitrite Stock Standard B (1µg N/ml)

Stock solution A	1.0	ml
Distilled water, q.s.	100.0	ml

Working Nitrite Standards (0.02, 0.04, 0.08 µg N/ml)

Working nitrite standards of 0.02, 0.04, and 0.08 µg N/ml were made the day of nitrite analysis of the soil extracts by adding 2, 4, or 8 mls of nitrite stock solution B, respectively, to a 100ml volumetric flask brought to volume with 2N KCL.

Color Reagent

Sulfanilamide	20.0	g
Concentrated phosphoric acid	200.0	mls
N-1-naphthylethylenediamine dihydrocloride (N-NEDD)	1.0	g
Distilled water, q.s.	2000.0	ml
Brij-35 (30% solution)	1.0	ml

Preparation: 200mls of concentrated phosphoric acid and 20g sulfanilamide of added to approximately 1500 mls of distilled water. After the solution is completely dissolved, add 1.0g of N-NEDD and dissolve again. Dilute to two liters.

Extracting solution (2N KCl)

Potassium chloride	149.1	g
Distilled water, q.s.	100.0	ml

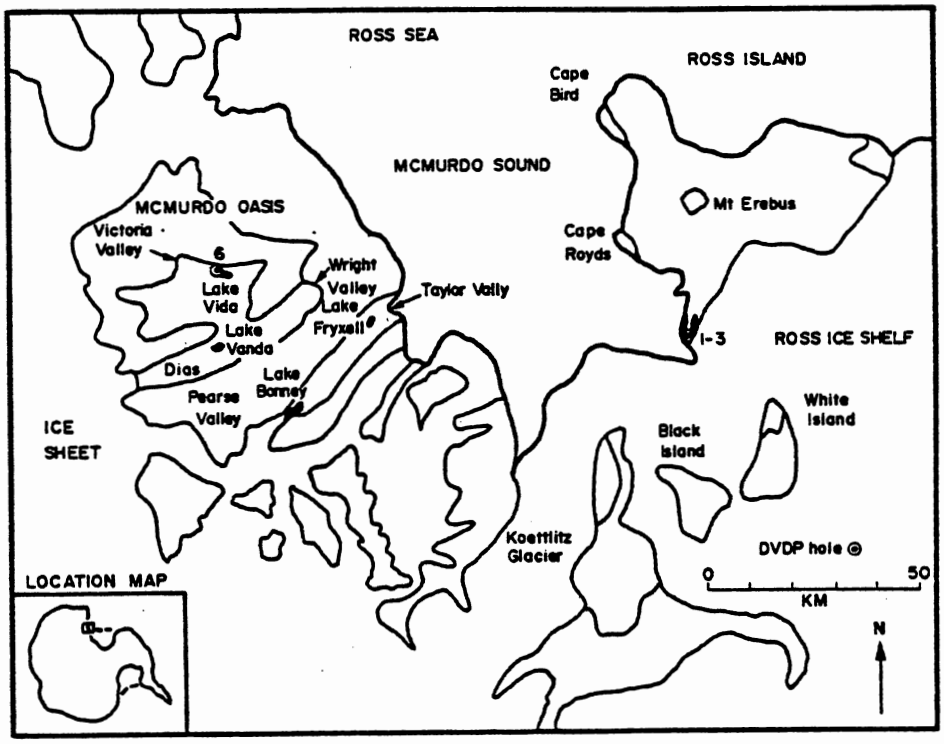
Nitrate Stock Standard A (1001g N/ml)

Potassium nitrate	0.72	g
Distilled water, q.s.	1000.0	ml
Chloroform	1.0	ml

Preparation: Potassium nitrate (0.72g) is dissolved in distilled water, diluted to one liter, and stored in a dark bottle.

APPENDIX E

MAP OF THE McMURDO REGION OF ANTARCTICA


ROSS SEA
ROSS ISLAND
Cape Bird
MCMURDO SOUND
MCMURDO OASIS
Victoria Valley
Mt Erebus
Cape Royds
6
Lake Vida
Wright Valley
Lake Fryxell
Taylor Valley
Lake Vanda
I-3
ROSS ICE SHELF
Dias
Pearse Valley
Lake Bonney
White Island
Black Island
ICE SHEET
Koettlitz Glacier
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LOCATION MAP

VITA 2

June Marie Klingler

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