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S. O. Stanley and A. H. Rose

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Bacteria and yeasts from lakes on Deception Island

By S. O. Stanley and A. H. Rose

Department of Microbiology, University of Newcastle upon Tyne

The bacterial and yeast flora of five lakes—Kroner Lake, a meltwater pool, Relict Lake and two unnamed lakes—on Deception Island in Antarctica were examined. During the sampling and isolation, special care was taken to ensure that the micro-organisms never experienced a temperature above 10 °C. Gram-negative, rod-shaped bacteria predominated in all of the lakes; Kroner Lake also contained large numbers of Gram-positive cocci. Yeasts were isolated from three of the lakes. An examination of the temperature characteristics of the predominant micro-organisms from the lakes showed that over three-quarters had optimum temperatures for growth below about 20 °C, while a third had maximum temperatures of about 20 °C. None of the thirty-one bacteria tested fermented lactose, raffinose or sucrose. Two strains fermented glucose, and one each glycerol and salicin. Several of the bacteria from Kroner Lake utilized compounds that may have been produced by the decomposition of algae in the lake.

Introduction

The presence of bacteria, yeasts and moulds in the Antarctic has been known for some years. Ekelöf (1908), who accompanied the Swedish Antarctic Expedition of 1901–03, found bacteria in the soils of the Snow Hill Islands of the Antarctic Peninsula (Graham Land). Later reports from Tsiklinsky (1908), Gazert (1912) with the German South Pole Expedition of 1901–03, Pirie during his voyage to the Weddell Sea, and McLean (1918) who accompanied Sir Douglas Mawson's Australasian Expedition of 1911–14, confirmed the widespread occurrence of bacteria in the Antarctic. These, and subsequent papers by Darling & Siple (1941), Bunt (1955), Bunt & Rovira (1955) and Boyd & Boyd (1963), were mainly concerned with the isolation of different types of bacteria, and little attention was given to the ways in which the bacteria were adapted to the rigours of the Antarctic environment. It was not until 1960 that Straka & Stokes reported that many of the bacteria that they isolated from samples of soil and glacier ice from the Antarctic were able to grow well at 0 °C, and so were psychrophilic according to the definition proposed by Ingraham & Stokes (1959).

The present paper describes the temperature characteristics and some of the nutritional requirements of bacteria isolated from lakes on Deception Island (62° 59′ S; 60° 34′ W), and shows how these bacteria are, in general, well adapted to the temperature regimes and nutritional environments that obtain in these Antarctic lakes.

Methods

(*a*) *Locations*

Samples were taken from five lakes and pools on Deception Island; the locations of these lakes are shown on figure 13. Kroner Lake, which was examined most extensively, was in a shallow circular depression in the laval plain (Hawkes 1961) and is about 350 m in

diameter. Because of fumarole activity near the northern edge, Kroner Lake is never completely frozen over in the winter. The water in Kroner Lake had a higher maximum temperature (around 10 °C) during the period of sampling (December 1963 to February 1964) than water in any of the other lakes examined. Also sampled were Relict Lake, which was partly frozen over during the period of study, two unnamed lakes (I and II) and a meltwater pool (figure 13).

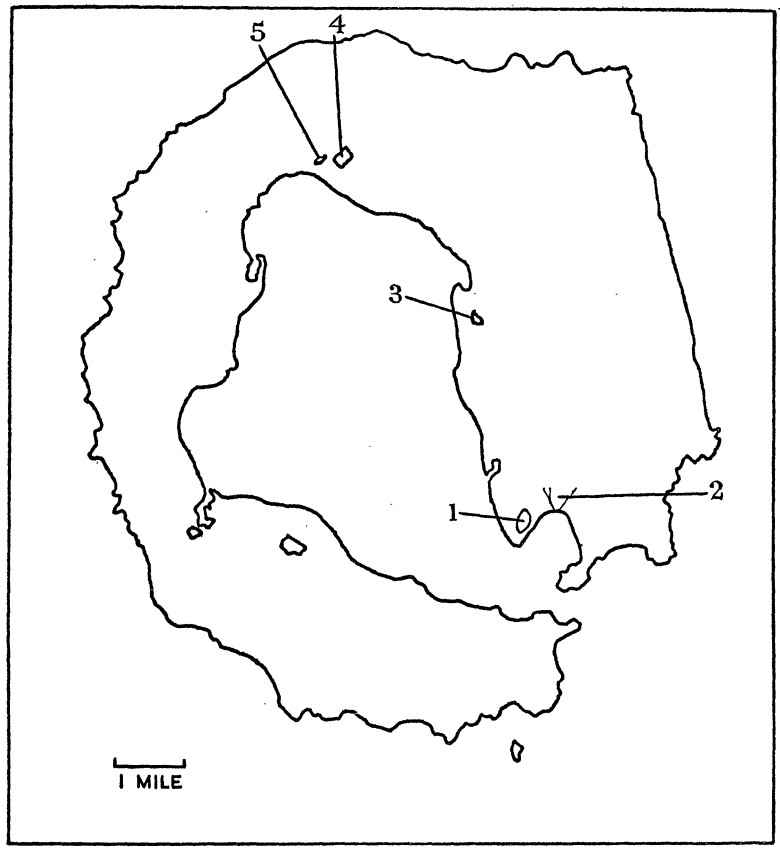

FIGURE 13. A map of Deception Island showing the locations of the five lakes tha
1, Kroner Lake; 2, meltwater pool; 3, Relict Lake; 4, unnamed lake I; 5, unn

(*b*) *Sampling technique*

Samples were taken from the lakes at a distance of about 1 m from the water's edge. Additional surface samples, and depth samples, were taken from the centre of Kroner Lake, as a boat was available. Surface samples were obtained by immersing a sterile screw-capped bottle (15 cm × 6·0 cm × 3·5 cm) approximately 1 cm below the surface, and allowing the bottle to fill by removing the cap in such a way as to avoid contamination from the hands. The screw-capped bottles were sterilized by autoclaving at 15 lb./in^2 for 15 min in a commercial pressure cooker (Prestige). The depth samples were taken with an apparatus kindly lent for the trip by Miss Vera G. Collins, of the Freshwater Biological Association, Lake Windermere. The glass bottles which were used in the depth sampler were sterilized by heating at 15 lb./in^2 for 15 min in the pressure cooker.

(*c*) *Examination of samples*

After collection, all samples were quickly returned to the field laboratory at Whalers Bay on Deception Island; this was always within 4 h of collection. Known volumes (1 to 10 ml.) of each sample were filtered aseptically through sterile membrane filters (Oxoid; Oxo Ltd., Queen Street Place, London, E.C.4; 50 mm diam.) in a Millipore filter holder (Cat no. XX1005000; Millipore Filter Corporation, Bedford, Mass., U.S.A.). Membrane filters were sterilized by autoclaving at 10 lb./in^2 for 10 min between pieces of filter paper. After the sample had been filtered, the membrane filter was transferred aseptically from the filter holder on to separate pads (5 cm diam.) of sterile Whatman no. 17 filter paper that had been soaked in 2 ml. nutrient broth (Oxoid) when isolating bacteria, or 2 ml.

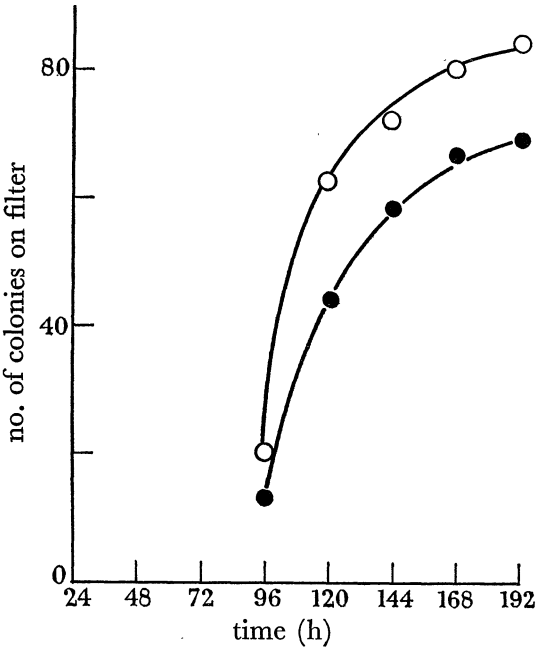

FIGURE 14. Effect of incubation time on the number of colonies that appeared on membrane filters through which samples (1 ml.) of water from Kroner Lake had been filtered. ●, sample collected on the surface at the centre of the lake; ○, sample collected at a depth of 2 m at the centre of the lake. Samples were collected on 7 January 1964.

Sabouraud's glucose-peptone medium (Cruickshank 1962) when isolating yeasts and fungi. The filter pads were then incubated in anhydric incubators at 10 °C for 6 days or at 30 °C for 2 days. During this time, the majority of the organisms which had been retained on the filters, and which were able to grow under the conditions used, developed into discrete colonies on the filter. The data in figure 14 show that, even after 6 days incubation at 10 °C, not all of the organisms that had been retained on the filters had developed into visible colonies. The incubation period was nevertheless restricted to 6 days because of the limited availability of incubation space in the field laboratory at Whalers Bay. It is important to note that at no time between collection and filtration did the sample experience, even briefly, a temperature above 10 °C.

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At the end of the incubation periods, the membrane filters were examined and the total number of colonies on each filter counted. The characteristics of the colonies, such as their colour, shape and size, were also noted. Colonies of the predominant organisms on each filter were selected. Each colony was checked for purity by streaking out on the appropriate medium and incubating at 10 °C for 6 days. The pure culture was transferred on to a slope of the appropriate medium in a screw-capped bottle (9 cm × 2·5 cm), incubated at 10 °C for 2 days, and then stored at 0 °C. During transport of the stock cultures to Britain, they were stored at 0 °C in the low-temperature incubator which was fitted up on the R.R.S. *Shackleton*. The stock cultures were transported from Southampton to Newcastle upon Tyne by rail during which time they were stored, surrounded by ice, in a freezing cabinet. On arrival in Newcastle, the isolates were again subcultured and checked for purity; cultures of each organism were then lyophilized.

(*d*) *Examination of isolates*

The temperature and nutritional characteristics of the predominant micro-organisms isolated from the Deception Island lakes were examined by means of liquid cultures. Bacteria were grown in nutrient broth, and yeasts in Sabouraud's glucose-peptone medium (Cruickshank 1962).

Portions of medium (6 ml.) were dispensed into Samco tubes covered with anodized aluminium caps (Oxo Ltd., Queen Street Place, London, E.C. 4; Northam & Norris 1951). Organisms from a 96 h-old slope culture, grown at 10 °C, were suspended in phosphate buffer (pH 7·0; 0·1 M; Gomori 1955) to give a suspension containing 0·03 to 0·05 mg dry wt. equiv. bacteria/ml., and one drop of the suspension was added to each of the Samco tubes. The cultures were incubated at different temperatures. Growth was measured turbidimetrically with the Hilger 'Spekker' absorptiometer (Model H 760) with neutral green-grey filters (H 508) and a medium blank. The optimum and maximum temperatures for growth were determined, to the nearest 5 degC, from plots of the amount of growth against temperature for different times of incubation. Some organisms grew optimally over a wide range of temperatures; the optimum temperatures for growth recorded for these organisms are the lowest in the range.

The ability of the bacteria to utilize individual organic compounds as the sole source of carbon and energy in the presence of an inorganic source of nitrogen was determined by supplementing a mineral salts medium with the carbon compound to a concentration of 2% (w/v). The mineral salts medium had the following composition per litre of distilled water: NH_4NO_3, 2·0 g; KH_2PO_4, 0·5 g; $Na_2HPO_4 . 12H_2O$, 1·5 g; NaCl, 5·0 g; $MgSO_4 . 7H_2O$, 0·1 g; $CaCl_2 . 12H_2O$, 0·1 g; $FeCl_3 . 6H_2O$, 20 mg; $MnSO_4 . 4H_2O$, 20 mg. The pH value was adjusted to 7·4 with N KOH before the addition of the carbon source. Portions (6 ml.) were dispensed in Samco tubes which were then sterilized by autoclaving at 5 lb./in^2 for 10 min. Glucose-containing medium was sterilized by filtration through membrane filters (Millipore) before being dispensed into sterile Samco tubes. Solid medium was prepared by adding agar (1·2%, w/v; Oxoid no. 3) to liquid medium. Media for examining the sugar fermentation reactions of the bacteria, and their ability to utilize nitrate and to produce urease, lipases, amylases, and proteases, were made as described by Cruickshank (1962). Glycerol trioleate was used in the test for lipase production.

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(*e*) *Estimation of the chloride contents of lakes*

The chloride contents of lake waters were estimated by titrating samples against a standardized solution of silver nitrate using fluorescein as an internal indicator (Vogel 1948).

Results

(*a*) *Isolation of micro-organisms*

Because of the shortage of time, and of the limited facilities available in the field laboratory at Whalers Bay, it was not possible to sample each of the lakes at frequent intervals to discover the seasonal variations that occur in the microbial flora. The data in table 8 show, however, that the total bacterial counts in the lakes differed, and that the counts for

Table 8. Total bacterial counts from Deception Island lakes

lake	date of sampling	temperature of lake water (°C)	no bacteria/ml. in samples incubated at 10 °C	30 °C
Kroner	30 Dec. 1963	8·3	71	0
	7 Jan. 1964	4·0	58	1
meltwater pool	2 Jan. 1964	0·5	13	3
	10 Jan. 1964	0·5	36	22
Relict	19 Feb. 1964	0·8	45	9
unnamed I	19 Feb. 1964	3·7	137	6
unnamed II	19 Feb. 1964	3·7	97	7
	19 Feb. 1964	3·4	52	0

Samples were taken from the surface of each lake, about 1 m from the water's edge; Kroner Lake was at the centre.

Table 9. Total bacterial counts at different depths in Kroner Lake

depth (m)	temperature of incubation: 10 °C, sample 1 (no bacteria/ml.)	10 °C, sample 2	30 °C, sample 1	30 °C, sample 2
surface	71	58	0	0
1	41	62	0	4
2	29	72	2	4
3	58	75	3	4
4	40	88	3	2
5	40	83	1	2

Samples were taken on 30 December 1963 (sample 1) and on 7 January 1964 (sample 2) at the centre of Kroner Lake at the depths stated. The total bacterial counts of the water samples were determined as described under Methods. Membrane filters were incubated at 10 or 30 °C.

each lake varied to some extent with the time of sampling. These data also show clearly that the majority of the bacteria in the lakes had maximum temperatures for growth below 30 °C. Samples were also taken at the centre of Kroner Lake, at different depths, and the total bacterial counts in these samples are given in table 9. These data show that bacteria are present in this lake at depths up to 5 m, in numbers that are not very different from those in the surface layers. The data in table 9 also show that the majority of the

bacteria isolated from each of the depth samples had maximum temperatures for growth below 30 °C.

The colony characteristics and Gram-staining reactions of the predominant organisms in the surface layers of each of the lakes were examined in the field laboratory, and the results of this examination are given in table 10. Gram-negative, rod-shaped bacteria were the predominant members of the microbial flora in each of the lakes. On the basis of their Gram-staining reaction, and on general morphological grounds, these bacteria appeared to be members of the genera *Pseudomonas*, *Flavobacterium* and *Achromobacter*. The Gram-positive, rod-shaped bacteria were all non-spore forming, and were probably members of the Micrococcaceae, some of them of the genus *Sarcina* since they were motile. A large proportion of the bacteria isolated from Kroner Lake were pigmented; pigmentation was less common in bacteria from the other lakes. No attempt was made to identify the fungus isolated from Kroner Lake. The yeast isolated from unnamed lake I was a rhodotorula, and the yeasts isolated from the other lakes were probably species of *Candida*.

TABLE 10. NUMBERS AND CHARACTERISTICS OF THE PREDOMINANT BACTERIA, YEASTS, AND FUNGI FROM DECEPTION ISLAND LAKES

		bacteria			
		Gram −ve rods	Gram +ve rods	Gram +ve cocci	
		no of isolates			
lake	chloride content (%, w/v)				yeasts and fungi
Kroner	1·4	12	1	8	1 fungus
meltwater pool	0·0	4	1	1	none
Relict	0·0	7	0	0	1 yeast
unnamed I	—	2	0	0	1 yeast
unnamed II	—	2	1	1	2 yeasts

Techniques for sampling the lakes and isolating micro-organisms were as described under Methods. Samples were taken from the edges of the lakes, with the exception of Kroner Lake which was sampled at the centre.

(*b*) *Temperature characteristics of micro-organisms*

On return to Newcastle upon Tyne, an examination was made of the effect of temperature on growth of the principal organisms isolated from the lakes. Histograms showing the distribution of optimum and maximum temperatures for growth of the bacteria are shown in figures 15 and 16. Over three-quarters of the bacteria had optimum temperatures for growth below 20 °C (figure 15). Further evidence for the marked adaptation of the bacteria to the cold environment was seen in that about a third of the bacteria had maximum temperatures for growth of around 20 °C (figure 16).

(*c*) *Nutritional characteristics of bacteria*

The ability of a number of the bacteria to utilize certain compounds was tested, first, to assist in identifying the bacteria, and secondly to ascertain to what extent the organisms were able to utilize organic compounds that may have been present in the lakes. All of these tests were carried out at 5 °C and the cultures were incubated for 7 days. Each of thirty-one strains was tested for the ability to ferment glucose, lactose, sucrose, raffinose, glycerol and salicin. None of the strains fermented lactose, sucrose or raffinose; two strains

fermented glucose and one each glycerol and salicin. The bacteria which fermented sugars produced acid but no gas. Eleven of the thirty-one strains hydrolysed gelatin, six hydrolysed starch, and none hydrolysed triglycerides. Glucose was utilized by nineteen of the thirty-one strains, but only nine were able to hydrolyse urea; seven reduced nitrate.

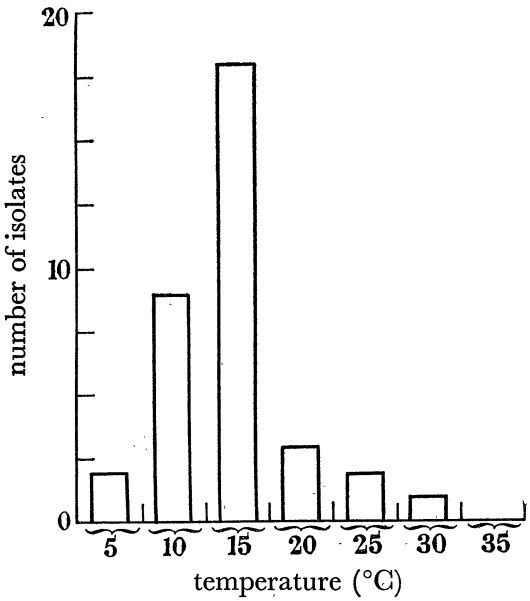

FIGURE 15. Histogram showing the distribution of optimum temperatures for growth of thirty-five bacteria isolated from Deception Island lakes.

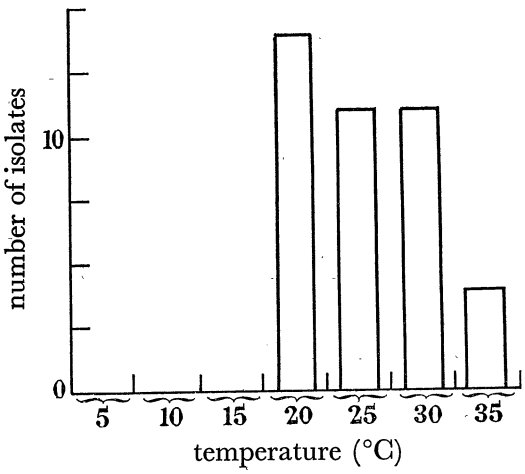

FIGURE 16. Histogram showing the distribution of maximum temperatures for growth of forty bacteria isolated from Deception Island lakes.

The ability to utilize compounds that may have been formed by the decomposition of algae in the lakes (Long 1961) was tested only with the principal strains isolated from Kroner Lake. Nine of sixteen bacteria isolated from this lake utilized glucose as the sole carbon and energy source, five utilized mannitol, three trehalose and cellobiose, and one strain utilized sodium alginate. None of the strains was able to utilize dulcitol.

DISCUSSION

Our data show that the majority of the micro-organisms isolated from the lakes on Deception Island were extremely well adapted to the low temperatures that occur in these lakes. All of the micro-organisms isolated from the lakes grew well at 0 °C and so were psychrophilic according to the definition proposed by Ingraham & Stokes (1959). It should be stressed that the procedures used did not select against the isolation of mesophilic micro-organisms, which have minimum temperatures for growth between 5 and 10 °C. The results also indicate that most of the bacteria isolated from the lakes had very low optimum and maximum temperatures for growth. Over three-quarters of the isolates had optimum temperatures for growth below 20 °C, and a third had maximum temperatures of around 20 °C. Such a marked adaptation to low temperatures has not previously been reported for Antarctic micro-organisms; indeed, micro-organisms with these temperature

characteristics have only occasionally been reported (Farrell & Rose 1967). Thus, a high proportion of the bacteria isolated from Macquarie Island soils by Bunt & Rovira (1955) grew optimally around 25 °C. Also, Sinclair & Stokes (1965) concluded that obligate psychrophils, which they defined as micro-organisms with optimum temperatures for growth below 20 °C, are rare, and when isolated are usually yeasts.

There would appear to be two main reasons for the differences between our findings and those reported by Bunt & Rovira (1955) and Sinclair & Stokes (1965). First, the procedures adopted during the sampling and isolation of the micro-organisms from the Deception Island lakes imposed far less temperature stress on the mirco-organisms than the procedures used by most other workers. Even freezing of the samples, as used by Straka & Stokes (1960), may have affected the viability of the micro-organisms. But probably the main reason for the differences is that bacteria isolated from Deception Island were from aquatic environments, whereas those isolated by Bunt & Rovira (1955) and Straka & Stokes (1960) were from soils. Absorption of heat by soils in Antarctica is known to be capable of causing a considerable local rise in temperature. For example, soil on the surface of rocks in the Horlick Mountains has been shown to attain a temperature of 27·8 °C on a sunny day, even though the air temperature was around 0 °C (Llano 1962). Soil micro-organisms in the Antarctic may well, therefore, become adapted to growing at these higher temperatures. The temperatures of the lakes, on the other hand, do not show such wide variations, and this could explain why micro-organisms isolated from the Deception Island lakes have, on the whole, lower optimum and maximum temperatures than those from Antarctic soils.

Certain of our data on the bacteria from Deception Island lakes are similar to those reported for bacteria from lakes in temperate regions. Thus Taylor (1942) reported a predominance of Gram-negative, rod-shaped bacteria in Thirlmere, Windermere and Esthwaite Water in the English Lake District, and similar findings have been reported for bacteria from other temperate lakes (Collins 1963). Taylor (1942) also reported the inability of the majority of the bacteria isolated from these lakes to carry out the fermentation reactions that are used to classify the bacteria. Our data confirm this finding. It is clear, however, that the bacterial counts for the Deception Island lakes are much lower than those recorded by Taylor (1940) for the English lakes. The reasons for this difference are unknown, but they clearly could have considerable implications in relation to the operation of food chains in the lakes. When comparing total bacterial counts, it should be remembered, however, that Taylor (1940) and the majority of other workers who have studied the bacterial flora of lakes, used pour-plate methods for estimating the number of viable bacteria. It is possible that even temperate lakes contain large numbers of bacteria with low maximum temperatures, though possibly not in such great numbers as the Antarctic lakes, so that one should treat with some reservation any comparisons that are made between our data on the Deception Island lakes and those reported by workers who have submitted the lake samples to temperatures around 47 °C during the preparation of poured plates.

The data obtained on the nutritional requirements of the bacteria isolated from Kroner Lake indicate that many of them are able to utilize compounds that may have been formed by the decomposition of algae (principally Phaeophyceae and Rhodophyceae) in the lake.

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These data are very limited, and before any generalizations can be made regarding the adaptation of the bacteria to the nutritional environments of the lakes, it will be necessary to extend these studies and to obtain information on the chemical composition of the lakes.

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