# Biology and biotechnological potential of halotolerant bacteria from Antarctic saline lakes

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*Abstract.* The saline lakes of the Vestfold Hills, Antarctica, are unique ecosystems in which microorganisms survive and grow in cold, saline and often anaerobic conditions. A polyphasic approach to taxonomy of the microbiota has extended the known range of microbial diversity through description of nine new species including bacteria and archaea. Low temperature and reduced water activity are the major constraints on microbial growth in the lakes. Bĕlehrádek-type models provide a good description of temperature dependence and indicate the potential of the microbiota to colonise their natural habitats. Physiological traits of the microbiota selected by the physical and chemical characteristics of the saline lakes suggest biotechnological potential in areas such as polyunsaturated fatty acid production and hydrocarbon degradation.

*Key words.* Antarctica; saline lakes; polyphasic taxonomy; new species; temperature and water activity models; PUFA production; hydrocarbon degradation.

## Introduction

The Vestfold Hills region of the Australian Antarctic Territory is largely an ice-free area which contains hundreds of lakes ranging in salinity from freshwater to up to eight times that of seawater[7]. The level of microbial diversity in the lakes is related to the combined effects of low temperature and decreasing water activity[41]. Some of the lakes are meromictic (stratified with respect to salinity) and exhibit aerobic and anaerobic zones. Others are heliothermal, with deep water temperatures approaching 20 °C, although the surface waters may be frozen for much of the year.

Detailed scientific studies of the microbiota of these lakes have been underway since 1986, based on the hypothesis that the unique ecosystems represented by Antarctic saline lakes would contain novel microorganisms with unexploited biotechnological potential.

## Unique ecosystems

Wright and Burton[41] reviewed factors that limit species diversity and growth rates in Antarctic saline lakes. The ecology of the lakes depended largely on salinity which resulted in reduced water availability for microbial growth, depression of the freezing point resulting in liquid water at sub-zero temperature, reduction in ice cover which encouraged mixing, and an increase in the opacity of ice.

Combined with generally low in situ temperatures and the development of anaerobiosis in the hypolimnion of meromictic lakes, the saline lakes of the Vestfold Hills region of Antarctica offer, with increasing salinity, a wide range of increasingly harsh environments in which species diversity and potential growth rates progressively decrease. These lakes are relics of seawater catchments isolated by isostatic uplift ~6,000 years ago[34]. Since then, factors such as evaporation, freezing concentration, cooling, melt stream influx and ice melting have changed the ionic strengths of the lakes and the ratios of major cations and anions.

The lakes have variable levels of nutrients derived largely from melt streams, bird and seal excreta, feathers, and algal productivity and decline. Five lakes have been studied in detail and an outline of their characteristics is presented below.

Deep Lake (68° 33.6′ S, 78° 11.6′ E) is a hypersaline (210–280g/L salt), monomictic lake. For eight months of the year the temperature of the lake is less than 0 °C and the highest temperature recorded was 11.5 °C[2]. Deep Lake is the most ionically concentrated lake of the Vestfold Hills, with relative ionic proportions similar to that of seawater[11]. Because of the high salinity, the freezing point of Deep Lake water is depressed to the point where it always remains liquid despite winter temperatures as low as −18 °C. Because it remains ice-free year-round, it is aerated to the bottom (36 m) by mixing through wind-induced turbulence[11]. The physical and chemical characteristics of Deep Lake make it one of the most inhospitable environments on earth with minimal known biodiversity represented by one algal type (probably a *Dunaliella* sp.) and a species of *Halobacterium*.

Organic Lake (68° 27.2′ S, 78° 12.3′ E) is a meromictic, hypersaline lake with a salinity profile ranging from 0.8% to 21% and temperatures of −14 to 15 °C. The lake is only 7.5 m deep and is anoxic below 4–5 m. The anoxic zone is apparently too cold

and saline for sulphate reducers and methanogens to proliferate, but other components of the microbiota generate large amounts of dimethyl sulphide[12].

Ace Lake (68° 28.4' S, 78° 11.1' E) is one of two known methanogenic environments on continental Antarctica[7,23,39]. It is meromictic and increases in salinity with depth from 0.6% at the surface to 4.3% at the bottom depth of 24 m[7]. Oxygen is absent below 12 m and sulphate is depleted in the bottom waters to less than 0.5 mmol/L as a result of sulphate reduction[7,23]. The bottom waters are constantly between 1–2 °C, saturated with methane, and support a large population of methanogenic bacteria[23].

Ekho Lake (68° 31.4' S, 78° 16' E) is heliothermal, trapping solar energy so that the anoxic bottom waters (42 m) are at a temperature of >16 °C year-round. The salinity gradient ranges from 1.4% at the surface to 16% at the bottom, but the higher in situ temperatures provide a situation where there is a considerable increase in biodiversity as observed microscopically[1].

Burton Lake (68° 37.5 S, 78° 5' E) is a meromitic lake with a maximum depth of 18.3 m and an average depth of 7.16 m with a seasonal connection to the sea in Crooked Fjord. Water temperatures are generally close to 0 °C with salinities slightly greater than that of seawater[13].

### Novel microorganisms

The unique environments described above have yielded to date nine new species of bacteria which have been fully characterised by the polyphasic approach to taxonomy recommended by the Ad Hoc Committee[40]. Two further bacteria, with unusual morphology, have been described but not assigned to taxa, and observations on the Dunaliella-like alga from Deep Lake suggest that this is also a new species (E. S. Holdsworth, pers. comm.). This valuable resource of Antarctic biodiversity is maintained in the Australian Collection of Antarctic Microorganisms (ACAM) at the University of Tasmania. Approximately 400 strains are held by ACAM, including the type strains of the novel species listed in the table.

Novel microorganisms isolated from Antarctic saline lakes.

| Species | Origin | Reference |
|---|---|---|
| Halomonas subglaciescola | Organic Lake | 12 |
| Flectobacillus glomeratus | Burton Lake | 25 |
| Halobacterium lacusprofundi | Deep Lake | 18 |
| Halomonas meridiana | Organic Lake | 20 |
| Flavobacterium gondwanense | Organic Lake | 10 |
| Flavobacterium salegens | Organic Lake | 10 |
| Carnobacterium funditum | Ace Lake | 14 |
| Carnobacterium alterfunditum | Ace Lake | 14 |
| Anaerobic, coiled bacterium | Ace Lake | 15 |
| Wall-less spirochaete | Ace Lake | 16 |
| Methanococcoides burtonii | Ace Lake | 17 |
| Dunaliella-like psychrophile | Deep Lake | Holdsworth (pers. comm.) |

Comparison of 16S rRNA sequences of the new species with sequences available in public domain databases indicate that they are most closely related to organisms from marine habitats (P. D. Franzmann and S. J. Dobson, pers. comm.).

### Effects of temperature and water activity on microbial growth rates

Temperature and water activity are major factors that combine to determine the rate of microbial development and the biodiversity of the saline lakes of the Vestfold Hills.

Brock[4] hypothesised that microbial growth might occur wherever liquid water is present and this, not an absolute temperature, defines the environmental limits of life. At the lower end of the temperature scale, microbial growth is often limited by reduced water activity due to ice formation rather than temperature per se. One effect of increased salinity is depression of the freezing point of water so that in several of the saline lakes of the Vestfold Hills liquid water is present at sub-zero temperatures.

Most organisms isolated from low temperature environments are not obligately psychrophilic but grow optimally at well above in situ[19] temperatures. These are often referred to as psychrotrophs. Knoll and Bauld[21] speculated that the dearth of obligate psychrophiles may reflect the course of earth's history with the relatively late appearance and discontinuous presence of permanently cold environments, coupled with low selective pressure for colonisation of such environments.

The possibility of isolating obligate psychrophiles increases markedly in stable cold environments such as Burton Lake in which 50% of the easily cultivatable strains were psychrophiles[13]. These were obtained by drilling through ice during October 1984 when water temperatures were −1 °C to −2 °C. The highest temperature recorded in 1984 was 5.6 °C in February at a depth of 4 m when the surface temperature was 0.8 °C and less than 0 °C below 6 m.

In general, the novel Antarctic species described show some adaptation to their environment with reduced optimum and minimum temperatures for growth compared to their taxonomic counterparts from more temperate regions. However, in situ temperatures and salinities are usually such that potential growth rates are considerably less than optimum but allow sufficient growth to account for microbial numbers observed in the lakes[27]. As an example, at the optimum temperature of 12–13 °C, the wall-less spirochaete has a generation time of 22 h which is extended to 55 h at temperatures that reflect in situ conditions at the bottom of Ace Lake[16]. Both Organic Lake and Deep Lake contain liquid water but the combined constraints of temperature and water activity prevent microbial development.

Heterotrophic bacterial growth has been confirmed at temperatures down to about $-10\,°C$[30] and may represent the point at which partial freezing of intracellular water reduces water activity beyond the limits for metabolism[24]. Knoll and Bauld[21] cite the inability of bacteria from Antarctic saline lakes to tolerate the simultaneous imposition of depressed temperature and elevated salinity conditions as support for this hypothesis. Thus the halotolerant *Halomonas subglaciescola* could grow at $-5.4\,°C$ while the halophilic *Halobacterium lacusprofundi* which could grow at 26% NaCl had a minimum temperature of $+4\,°C$.

### Models to describe microbial responses to temperature and water activity

Traditionally, models based on Arrhenius kinetics have been used to describe the temperature dependence of microbial growth rates, but more recently, relationships based on Bělehrádek power functions have been introduced[28].

Mathematical models that describe the effects of temperature and water activity may be used to predict the effect of varying these parameters on the growth rate of microorganisms[29]. Determination of the rates of growth in suitable media under varying conditions of temperature and water activity with analysis of the data by appropriate models also provides a means to estimate the ability of different species to colonise natural habitats[27]. The Arrhenius equation is often written as

$$\ln k = \ln A - E/RT \tag{1}$$

where $k$ is the specific reaction rate constant, $E$ is the 'activation energy', $A$ is the collision factor, $T$ is the absolute temperature and $R$ is the universal gas constant. For bacterial growth, microbiologists have replaced $E$ by a temperature characteristic ($\mu$) and interpreted $k$ as the specific growth rate constant. From equation 1 it is evident that a plot of $\ln k$ versus reciprocal absolute temperature ($1/T$) would yield a straight line if the activation energy ($E$) and collision factor ($A$) were unchanging with temperature. However, the evidence for biological systems is that such plots are concave downward curves indicating that activation energy ($E$) increases continually with decreasing temperature. Bělehrádek-type models can be written in the general form

$$k = b(T - T_{min})^d \tag{2}$$

where $k$ is the rate at temperature $T$, $b$ is a constant to be fitted, $T_{min}$ is 'biological zero' and $d$ is a parameter to be fitted[3].
A transformation of equation 2, the square-root equation

$$\sqrt{k} = b(T - T_{min}) \tag{3}$$

was applied successfully to describe the effect of temperature on bacterial growth rates[36] and has been verified subsequently by many authors.

There are many variants of the basic Arrhenius model that have been proposed for use in microbiology. The most widely used is the model of Schoolfield et al.[38] which assumes that growth rate is governed by a single rate-limiting enzyme-catalysed reaction with additional terms added to the basic Arrhenius equation to account for inactivation of that enzyme at high and low temperature.

Another Arrhenius-based model was proposed by Davey[9] to describe the effects of temperature and water activity on microbial growth

$$\ln k = C_0 + C1/T + C_2/T^2 + C_{3a_w} + C_{4a_w^2} \tag{4}$$

When water activity ($a_w$) is non-limiting, the $a_w$ terms may be removed, reducing equation 4 to

$$\ln k = C_0 + C1/T + C_2/T^2$$

The basic square-root model (eq. 3) describes microbial growth in the suboptimal region. Growth across the full biokinetic range can be modelled by an extension of equation 3:

$$\sqrt{r} = b(T - T_{min}\{1 - \exp[c(T - T_{max})]\} \tag{5}$$

where $T_{max}$ is the theoretical maximum temperature for growth and $c$ is the regression coefficient[35].

The growth rate of microorganisms in Antarctic saline lakes is also constrained by salt concentration[41] and Arrhenius-type models have been proposed to describe the combined effects of both temperature and water activity[5, 9].

The basic square-root model was also extended easily to include the effect of $a_w$ by McMeekin et al[26].

$$\sqrt{r} = d\sqrt{(a_w - a_{w\,min})}(T - T_{min}) \tag{6}$$

where $d$ is a constant and $a_{w\,min}$ is the theoretical minimum water activity for growth of the organism.

A feature of both the extended square-root model (eq. 5) and the Davey model is that their forms indicate that temperature and water activity act independently. In the square-root model, $T_{min}$ is constant across the range of water activities permitting growth and in the Davey model there are no 'cross-product' terms involving $a_w$ and temperature. This may be inferred also from the reconciliation of Arrhenius and Bělehrádek equations[28] such that

$$E_{app} = 2RT^2/(T - T_{min})$$

This indicates that the apparent activation energy ($E_{app}$) depends only on the difference between $T$ and $T_{min}$, the theoretical minimum temperature for growth. If one assumes that there is a maximum value of $E_{app}$ which the cell can overcome when $a_w$ is not limiting, an inevitable consequence of expending energy to produce

compatible solutes is to reduce the amount of energy available to overcome the temperature hurdle, i.e. the maximum value of $E_{app}$ must be less at lowered water activities and as a consequence $(T - T_{min})$ must be greater. These considerations suggest that it may not be energetically possible for an organism to combine psychrophilism and halophilism.

## Biotechnology

The systematic studies above, describing Antarctic microbial diversity have provided a sound basis from which to examine the biotechnological potential of selected strains. The rationale adopted parallels that of targeted, intelligent screening suggested by Bull et al.[6] in the excellent review 'Biodiversity as a source of innovation in biotechnology'.

Thirty eight strains of Antarctic bacteria were screened for polyunsaturated (PUFA) production[32]. Five strains (13%) contained eicosapentanoic acid (20:5ω3) in the range of trace to 3.3% of total fatty acids and up to 1.4 mg/g of cells dry weight. Thirteen strains, 34% of isolates, produced polyunsaturates including: 18:2ω6, 18:3ω3, 18:4ω3 and 20:4ω6 in the range of trace to 7% of the total fatty acids.

The proportion of Antarctic strains that produced PUFA was considerably higher than that recorded for temperate marine bacteria suggesting that the Antarctic environment has naturally selected for bacterial strains capable of maintaining membrane lipid fluidity by the production of PUFA. Furthermore, these results highlight the potential of Antarctic bacteria, particularly those inhabiting permanently cold environments, as target species for consideration in the industrial production of PUFA.

These applied studies are complementary to research on mechanisms of PUFA production and the use of fatty acid profiles to develop markers for chemotaxonomic and environmental studies. In the former area, Nichols et al.[31] demonstrated an anaerobic pathway of PUFA production in a strain of Shewanella putrefaciens (ACAM342). In the latter field, Skerratt et al.[37] demonstrated the value of lipid profiles in the identification of closely related strains in the genera Halomonas and Deleya and in Flavobacterium. Nichols et al.[33] developed a high temperature gas chromatographic technique for the analysis of diether and tetraether lipids derived from Antarctic Archaea. Lipid biomarkers (and DNA probes also under development) for several Antarctic species will allow investigation of the ecology and potential biogeochemical activities of these organisms in the Southern Ocean.

Hydrocarbon degradation is another area in which the biotechnological potential of Antarctic microorganisms is being investigated. The ecosystem targeted was Organic Lake, a nutrient-rich brine containing naturally occurring hydrocarbons. From this lake, strains have been isolated with the ability to degrade various types of hydrocarbon including hexadecane and phenanthrene[8, 22]. Current studies are assessing their efficacy at in situ temperatures and in the field.

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