Halotolerance of micro-organisms isolated from saline antarctic dry valley soils

KAREN J. MILLER

Department of Biochemistry
University of Massachusetts
Amherst, Massachusetts 01003

SUSAN B. LESCHINE

Department of Microbiology
University of Massachusetts
Amherst, Massachusetts 01003

ROBERT L. HUGUENIN

Department of Physics and Astronomy
University of Massachusetts
Amherst, Massachusetts 01003

The antarcic dry valley soils are generally highly saline (Cameron 1967; Hall 1968; Tedrow and Ugolini 1966). It is surprising, therefore, that previous microbiological investigations of dry valley soils have indicated a decline in halotolerance with decreasing growth temperature (Benoit and Hall 1970; Cameron 1972; Hall 1968; Ugolini 1970). We have initiated a study aimed at the examination of halotolerance as a function of temperature in strains of bacteria we have isolated from saline dry valley antarctic soil samples. Enrichment and isolation procedures were designed to select for micro-organisms capable of growth under conditions of high sodium chloride (NaCl) concentrations at relatively low temperatures (e.g., 5°C).

Several soil samples collected from a variety of locations within Wright Valley and Taylor Valley were provided to our group by Robert E. Benoit of the Virginia Polytechnic Institute. These soil samples were maintained at dry-ice temperatures in transit to our group and have subsequently been maintained at −40°C. Growth was enriched in liquid media. Media TYE-HES* contained HES salts [0.85 molar (M) NaCl, 0.04 M calcium chloride, 0.2 M magnesium sulfate], 0.01 percent Trypticase (Baltimore Biological Laboratories), and 0.01 percent yeast extract (Difco). The pH of the media was adjusted to 7.4 prior to sterilization by autoclaving. Tubes (16 × 150 millimeter) containing 10 milliliters of media were inoculated with soil (0.1 gram) and incubated at 5°C in the light (approximately 35 centimeters from a 60 watt bulb) without agitation. Initial growth was observed in enrichment cultures approximately 5 days to 4 weeks after inoculation. A variety of morphological forms were observed such as cocci, motile rods, and nonmotile rods. Micro-organisms were isolated from enrichment culture using TYE-HES media supplemented with 0.7 percent Noble agar (Difco) at 5°C. The morphologies of some of these strains are illustrated in the figure.

All isolated strains tolerated relatively high concentrations of NaCl (e.g., up to 2.0 M). The temperature optimum of all strains was between 15–25°C. One of the isolates, an orange pigmented, Gram-positive, obligately aerobic coccus (block C of the figure) was chosen for further study. This isolate, strain A4a, was capable of growth in the presence of 2.0 M NaCl at 0°C.

Growth rates of strain A4a were determined at different temperatures in media containing HES salts supplemented with 0.05 percent trypticase and 0.05 percent yeast extract. NaCl concentration was varied in these studies from 0.0 M added NaCl to 2.0 M NaCl. The final pH was 7.2. Cells were grown in 100 milliliters of medium in 250 milliliter side-arm flasks which were incubated on a rotary shaker within a Psychrotherm incubator (New Brunswick Inc.). These media were inoculated with 5 milliliters of a culture grown in medium containing 1 M NaCl at room temperature for 36 hours. Growth was monitored using a Klett-Summerson colorimeter equipped with a 660 nanometer filter.

Growth studies indicated that strain A4a was capable of growth over wide ranges of temperature (0–40°C) and NaCl concentrations (0–2.0 M). The optimal growth temperature was found to be approximately 25°C at all concentrations of NaCl examined (data not shown). Growth rate decreased with increasing concentrations of NaCl at all temperatures, and strain A4a grew in media not supplemented with NaCl. Growth in media containing high concentrations of NaCl was therefore the result of halotolerance, and a specific NaCl requirement was not detected in these studies.

To further characterize strain A4a, the guanine plus cytosine (G+C) content of the DNA (Mandel and Marmur (1968; Marmur 1961) and phospholipid composition were determined. The G+C composition was 48.7 ± 0.6 molar percent. Phospholipid analysis revealed three major phospholipids which were identified as phosphatidyl glycerol, cardiolipin, and phosphatidyl ethanolamine. Identification was based on relative mobilities in several one-dimensional thin layer chromatography solvent systems (as compared with appropriate standards) as well as reactivity with a variety of spray reagents. The presence of significant levels of phosphatidyl ethanolamine (e.g., up to 40 percent) and the DNA-base composition indicated that strain A4a was a member of the genus *Planococcus*. Planococci have been isolated from marine environments (Kocur and Schleifer 1981) however, this is the first report, as far as we are aware, of a species of *Planococcus* isolated from dry valley soils. Morphologically similar micro-organisms, however, have been described as common forms in the dry valley soils (Benoit and Hall 1970; Cameron, Honour, and Morelli 1976; Cameron, King, and David 1970; Horowitz, Cameron, and Hubbard 1972; Meyer et al. 1962). It is therefore possible that a more detailed characterization of such reported strains would indicate that the halotolerant planococci constitute a significant group of the microbial flora within the dry valley soils.

This research was supported by National Science Foundation grant DPP 81-20605.

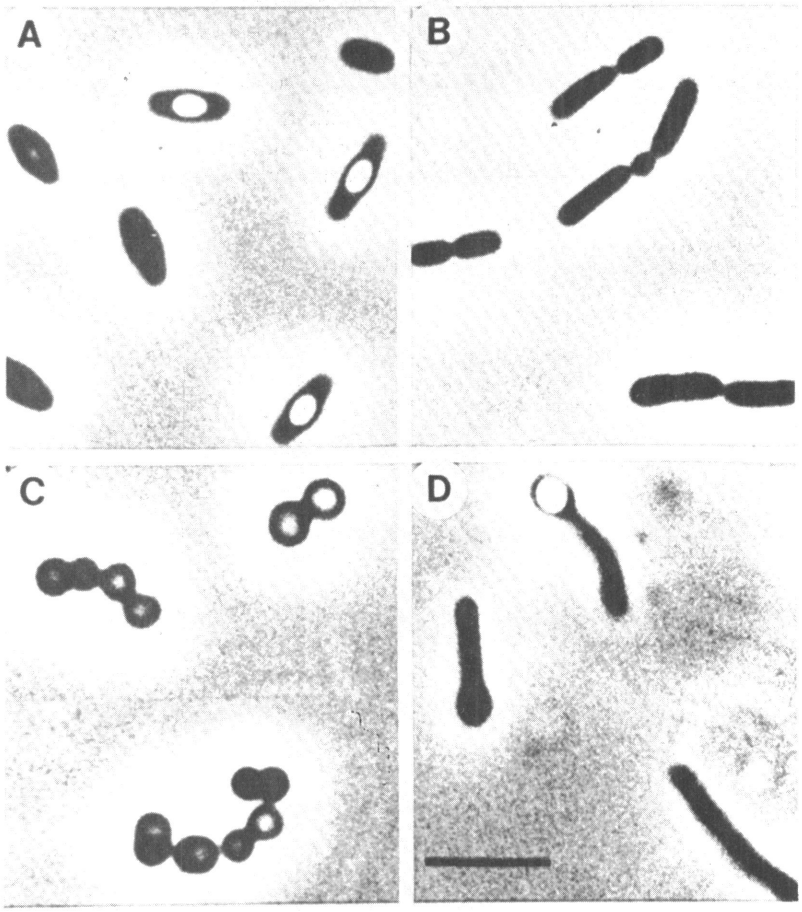
* TYE-HES is a growth medium.

References

Benoit, R. E., and C. L. Hall. 1970. The microbiology of some Dry Valley soils of Victoria Land, Antarctica. In M. W. Holdgate (Ed.) *Antarctic ecology.* New York: Academic Press.

Cameron, R. E. 1967. Soil studies—Desert microflora XIV. Soil properties and abundance of microflora from a soil profile in McKelvey Valley, Antarctica. *Jet Propulsion Laboratory Space Programs Summary,* 4(37–44), 224–236.

Cameron, R. E. 1972. Microbial and ecologic investigation in Victoria Valley, Southern Victoria Land, Antarctica. In G. A. Llano (Ed.),



(A-D). Phase contrast photomicrographs of some representative morphological types of isolated micro-organisms. All micrographs are at the same magnification. Bar = 5 micrometers.

Antarctic Terrestrial Biology, (Antarctic Research Series 20) Washington, D.C.: American Geophysical Union.

Cameron, R. E., R. C. Honour, and F. A. Morelli. 1976. Antarctic microbiology—Preparation for Mars life detection, quarantine, and back contamination. In M. P. Heinrich (Ed.), *Extreme environments.* New York: Academic Press.

Cameron, R. E., J. King, and C. N. David. 1970. Microbiology, ecology, and microclimatology of soil sites in Dry Valleys of southern Victoria Land, Antarctica. In: M. W. Holdgate (Ed.) *Antarctic ecology.* New York: Academic Press.

Hall, C. L. 1968. Isolation of psychrophilic halophiles from the Antarctic polar desert. Unpublished masters thesis, Virginia Polytechnic Institute.

Horowitz, N. H., R. E. Cameron, and J. S. Hubbard. 1972. Microbiology of the Dry Valleys of Antarctica, *Science,* 176, 242–245.

Kocur, M., and K. Schleifer. 1981. The Genus *Planococcus.* In (M. P. Starr, H. Stolp, H. G. Truper, A. Balows, H. G. Schlegel (Eds.), *The Prokaryotes,* 2. Springer-Verlag, Berlin, Heidelberg, New York: Springer-Verlag.

Mandel, M., and J. Marmur. 1968. Use of ultraviolet absorbance—temperature profile for determining the guanine plus cytosine content of DNA. In B. L. Grossman and K. Moldane (Eds.), *Methods in Enzymology,* 12. New York: Academic Press.

Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *Journal of Molecular Biology,* 3, 208–218.

Meyer, G. H., M. B. Morrow, O. Wyss, T. E. Berg, and J. L. Littlepage. 1962. Antarctica: The microbiology of an unfrozen saline pond. *Science.* 138, 1103–1104.

Tedrow, J. C. F., and F. C. Ugolini. 1966. Antarctic soils. In J. C. F. Tedrow (Ed.), *Antarctic soils and soil forming processes.* (Antarctic Research Series, Vol. 8.) Washington, D.C.: American Geophysical Union.

Ugolini, F. C. 1970. Antarctic soils and their ecology. In M. W. Holdgate (Ed.), *Antarctic ecology.* New York: Academic Press.